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ILEUS IN THE COLIC HORSE: PROGNOSTIC FACTORS AND THE ROLE OF SEROTONIN AND SEROTONERGIC RECEPTORS

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INTRODUCTION AND LITERATURE REVIEW

Chapter 1.I

GENERAL INTRODUCTION

Colic is still the most important cause of death in horses (White *et al*., 1990; Leblond *et al*., 1995; Hintz, 2006). The word colic is used in horses to describe a group of symptoms shown by horses suffering from predominantly abdominal pain. A colicky horse for example can kick at its flanks, lie down and roll over repeatedly, stand with stretched legs, can sweat profusely and show rapid breathing. These symptoms of colic are not always necessarily associated with gastrointestinal problems. Renal calculi, even pleuritis or a testicular torsion in a stallion can cause the horse to express exact the same symptoms. However, in the study that is described here, the word colic will only be used in the context of gastrointestinally related pathology. Surveys have shown that out of 100 horses in a general population, in the course of one year, 4 to 10 horses can be expected to have at least one bout of colic (Tinker *et al*., 1997; Kaneene *et al*., 1997). Approximately 92% of colic cases respond favorably to conservative treatment, but the remaining 8% needs surgical attention, which was far from common practice until the 60's. The days that surgical treatment of colic horses was empirical and unfortunately often fatal, have long gone. Nowadays, tremendous evolutions in anaesthetic and surgical techniques allow us to perform complex and time-consuming surgical interventions in equine patients. Advances both in physiologic understanding and in technology have greatly improved the outcome of intensive postoperative care and follow-up. Still, despite this wealth of progress, ileus or gastrointestinal stasis remains a notorious post operative complication in colic horses. Ileus is the syndrome of functional inhibition of propulsive bowel motility, most commonly arising in the immediate postoperative period after laparotomy. There is always a physiological and subsiding episode of ileus after intra-abdominal manipulation of intestines. However, the situation becomes problematic when the status of ileus lingers on, leading to small intestinal dilation and tympanism in the horse and concommitant production of large amounts (sometimes up to 15L every 4h) of GI reflux, which is the hallmark of ileus in horses. Its presence implicates the actual occurrence of

important fluid shifts in the horse's body, often aggravated by the devastating effects of endotoxemia. Although it is predominantly seen after surgical intervention for small intestinal colic, ileus can also develop as a result of or after other colic types. In comparison to humans, horses are definitely more prone to the development of life threatening postoperative ileus and the enormous fluid shift that accompanies its occurrence, significantly limits the time frame in which the metabolism can cope with such pathological condition.

The tendency for the equine small intestine to become problematically paralysed after colic surgery has prompted considerable research in equine medicine. However, despite these efforts the pathophysiology of equine ileus is far from being unraveled. Unfortunately, there is a wide disparity between the sophistication of current anaesthetic, surgical and monitoring technology and the relatively poor understanding (at least at a level at which effective intervention is possible) of the pathophysiologic mechanisms that trigger ileus. More and more data become available to help us recognize high-risk patients, which also helps us to better understand the course of events that trigger immobilization of the equine GI tract. Still there is a lot to be investigated. Monitoring of blood electrolyte levels in colic horses is gaining more and more access into the standard perioperative care. However, which electrolytes need to be monitored closely, is still subject of debate. Being able to assess ongoing intestinal ischemia in colic horses, would help clinicians to provide a better prognosis estimation to the owner in terms of postoperative survival in which ileus plays a major role.

Indeed, ileus is often fatal in horses. Once the process has started, it seems hardly possible to therapeutically intervene. The most recent human prokinetic agents modulate the enteric serotonergic system. Serotonin is an important gastrointestinal (GI) messenger neurotransmitter in humans and many animals. It has been implicated in the control of GI motility, sensitivity and secretion. This has been the basis for development of serotonergic agents for the treatment of human GI disorders (Gershon & Tack, 2007). They hold potential

as therapeutic agents by their ability to improve delayed gastric emptying, slow transit constipation, impaired gastric accommodation and visceral hypersensitivity (Kindt & Tack, 2007). Nevertheless, the use of expensive human prokinetic drugs in a 500kg colic horse represents an important additional cost whereas the benefit that can be expected from its use, is far from guaranteed.

We are desperately in need for effective promotility drugs in horses and to be able to develop them we need to get a better insight into the physiology of normal GI motility and the pathophysiology of ileus in horses, also at the ultrastructural receptor level. When entering the words “human and serotonin” in the Web of Science search engine and comparing the number of hits (7910 per 23-02-2008) with the links you can obtain when entering “equine and serotonin” (56 per 23-02-2008**)**, it becomes immediately clear that there is great need to further explore the specific properties of the serotonergic system in the equine intestine.

REFERENCES

GERSHON, M.D. & TACK, J. (2007). The serotonin signaling system from basic understanding to drug development for functional gastrointestinal disorders. *Gastroenterology* 2007; **132**, 357–414.

HINTZ (2006). Equine nutrition. *Eq Vet Sci* **20**, 861-864.

KANEENE, J.B., ROSS, W.A. & MILLER, R. (1997) The Michigan equine monitoring system. II. Frequencies and impact of selected health problems. *Prev Vet Med* **29**, 277-292.

KINDT, S. & TACK, J. (2007). Mechanisms of serotonergic agents for treatment of gastrointestinal motility and functional bowel disorders. *Neurogastroenterology and Motility* **19**, 32-39.

LEBLOND, A., VILLARD, I., LEBLOND, L., SABATIER, P. & SASKO, A.J. (1995). A Retrospective Evaluation of the Causes of Death of 448 Insured French Horses in 1995. *Vet Res Comm* **24**, 85-102.

TINKER, M.K., WHITE, N.A., LESSARD, P., THATCHER, C.D., PELZER, K.D., DAVIS, B. & CARMEL, D.K. (1997). Prospective study of equine colic incidence and mortality. *Equine Vet J* **29**, 448-453.

WHITE, N.A. Epidemiology and etiology of colic. In: White NA, ed. The equine acute abdomen. Philadelphia, PA: Lea and Febiger; 1990, 50-64

Chapter 1.II

PATHOPHYSIOLOGY OF ILEUS IN HORSES AND CURRENT TREATMENT OPTIONS

Review of pathophysiology, prevalence, predisposing factors, prognostic parameters and therapeutic options

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SUMMARY

Equine practitioners often need to address problems associated with decreased GI motility in colic horses. Likewise, ileus is a notorious complication in horses that is predominantly seen after surgical intervention for small intestinal colic.

Understanding the physiological mechanisms that are responsible for normal GI motility in horses and knowing which factors predispose horses to ileus, will help clinicians to better understand the clinical picture of a colic horse and to determine when and which prokinetic treatment should be chosen in any specific case.

However, due to the lack of fundamental research, the knowledge of pharmacological activity pathways and therapeutic efficacy of prokinetic medication in colic horses is very fragmented. Often research results in other species are extrapolated to the horse, without any pharmacological evidence that enteral receptor populations that serve as pharmacological target to induce intestinal propulsion in these species are equally important in horses. A possible discrepancy in these receptor populations between humans and horses could partially explain the inconsistent clinical efficacy of human prokinetic agents such as cisapride, metoclopramide and domperidone in equine colic cases. Furthermore, due to the lack of large, double-blind multi-center clinical studies, the evaluation of the therapeutic efficacy of many prokinetic agents that are used in colic horses is very subjective. The lack of non-invasive techniques to evaluate GI motility in healthy and colic horses contributes to this subjectivity.

As rule of thumb, it can be stated that for the treatment of stasis of the cranial part of the GI tract of horses, mainly lidocaine, metoclopramide and erythromycin should be used. In cases of colonic hypomotility, naloxone, neostigmine, erythromycin and lidocaine are the drugs of

choice. With regard to sedation of colic patients, it should be mentioned that acepromazine and xylazine both will negatively influence GI motility to a lesser extent than the alpha 2 agonists detomidine and romifidine. However, in colic cases expressing shock and endotoxemia, the use of acepromazine is hampered by its pronounced hypotensive effects.

1.II.1. INTRODUCTION

GI hypomotility or ileus in horses can manifest itself in many degrees of severity and in association with a multitude of pathologic conditions. However, the condition is mainly encountered in association with abdominal colic, intra-abdominal surgery, after extensive or repetitive use of sedatives for the purpose of orthopedic interventions, in conjunction with the use of opioid analgetics and, last but not least, as part of the clinical picture of specific intoxications. Therefore, equine practitioners tend to be confronted with the problem quite often, both under hospital and field conditions. Systemic shock, endotoxemia, ionic imbalances, intestinal dilation, ischemia, inflammation, pain, peritonitis and anesthesia are all ileus-triggering factors (French *et al.*, 2002). Which events exactly take place during the pathogenesis of ileus in horses still remains to be elucidated. Proposed viewpoints on the subject are mainly extrapolated from human medicine, due to the lack of fundamental research in horses. Afferent nociceptive input like pain and inflammatory responses to intestinal surgical manipulation can cause adrenergic hyperactivity and concomitant ileus. Also parasympathetic hypoactivity, dopaminergic hyperactivity and local entero-enteric reflexes have all been proposed as possible ileus triggering mechanisms in horses (Gering & Hunt, 1986). Many prokinetic drugs have been used in equine colic patients, each with its own specific modulation of one of the aforementioned proposed mechanisms. Up until now, however, no overall successful treatment protocol has been proposed to the equine practitioner. Depending on where in the GI tract the problem is localized, different prokinetic

agents should be used, and it has to be mentioned that the main point of action of these drugs does not always correspond in horses and humans. Furthermore, the predominant point of view currently is that the prokinetic treatment has to be part of a total therapeutic plan in which the correction of blood parameters such as ionary imbalances and the provision of anti-endotoxemia treatment are equally important (Delesalle *et al.*, 2005c; Moore & Barton, 2003). The purpose of this paper is to provide the equine practitioner with an up-to-date overview of what can be concluded about the use of prokinetic treatment in horses based on the numerous in vivo and ample in vitro studies that have been performed. A practical table with dosing regimens and indication of site of action should help optimize future decision making concerning prokinetic treatment.

1.II.2. THE MUSCULAR LAYER OF THE EQUINE GI TRACT

From mouth to anus, the wall of the GI tract consists of 4 layers (Figure 1.II.1). The inner layer is the tunica mucosa. The submucosa, which lies underneath, facilitates mobility between the elastic mucosa and the more rigid tunica muscularis, which is the third layer of the intestinal wall. Finally, the tunica serosa embraces the three aforementioned layers, creating the outer jacket of the intestine. Peroperative manual manipulation of the intestine can cause mechanical irritation and inflammation of this layer, leading to localized peritonitis and adhesions.

At the level of the tunica mucosa, numerous finger-like protrusions, called the intestinal villi, create a significant increase in the resorption capacity of the inner surface of the intestine. The tunica mucosa is provided with its own muscular tissue, which extends into each of these protrusions. However, the important outermost muscle layers of the intestine are localized in the tunica muscularis. Here, an inner circular muscle layer embracing the tunica mucosa and an outer longitudinal muscle layer, oriented in the longitudinal direction of the

intestine, can be distinguished. In each muscle layer, neighboring smooth muscle cells are connected by means of “gap junctions”. The throughput of electrical currents from one smooth muscle cell to the next is facilitated by means of these specialized contact points, which enable the muscle layer to react as one large syncitium.

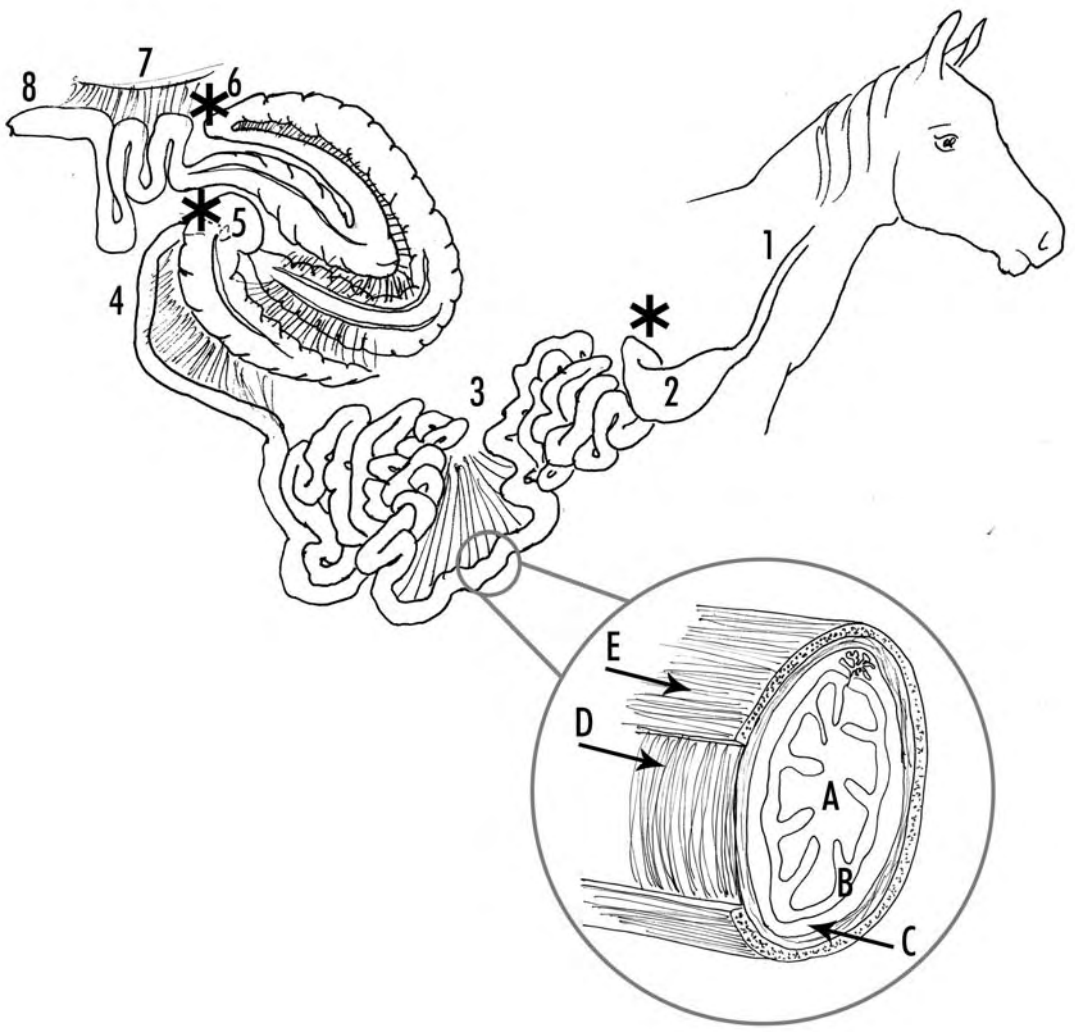

Figure 1.II.1: Anatomy of the equine GI tract; Zoom view: detail of the different layers of the intestinal wall. (1) oesophagus; (2) stomach; (3) jejunum; (4) ileum; (5) caecum; (6) pelvic flexure; (7) small colon; (8) rectum, (A) intestinal lumen; (B) mucosal layer with muscularis mucosae; (C) submucosal layer with Meissner’s neuronal plexus; (D) inner circular muscle layer; (E) outer longitudinal muscle layer (in between D and E: Auerbach’s nerval plexus); (*) enteric pacemaker nodes responsible for myoelectrical coupling between neighbouring parts of the GI tract.

The Interstitial Cells of Cajal (ICC) have to be viewed as specialized cells that are found in the gastrointestinal tract. Like the pacemaker cells of the cardiac sinus node, they show a rhythmic electrical depolarization (Figure 1.II.2). These electrical depolarizations spread like

waves in the muscular layers via the aforementioned gap junctions. As in humans, ICC cells are encountered in the horse along the full length of the GI tract. However, several specialized regions, such as the gastro-duodenal junction, the caeco-colonic junction and the pelvic flexure, are encountered with pronounced ICC densities. These regions can be viewed as "intestinal pacemaker nodes", which create myo-electrical coupling between different parts of the GI tract (Hudson *et al.*, 1999) (Figure 1.II.1).

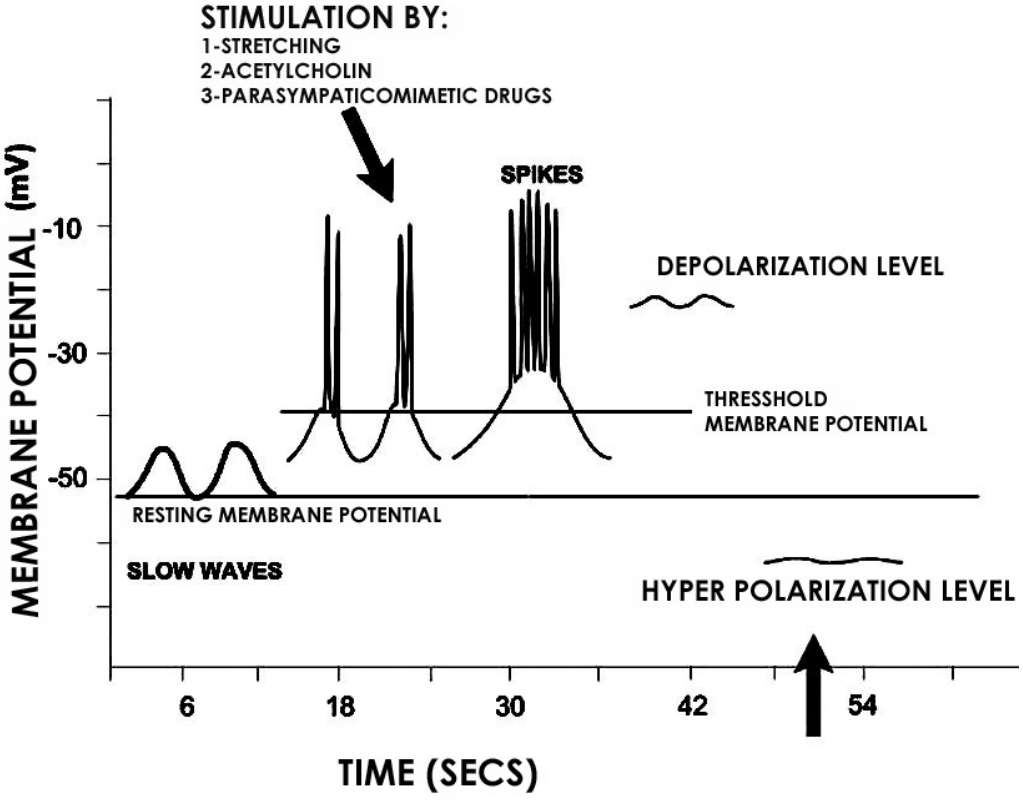

Figure 1.II.2: Slow waves and spikes: the necessary ingredients for normal enteric myoelectrical activity.

1.II.3. NEURONAL INPUT OF THE EQUINE GI TRACT

One typical feature of the GI tract is the extensive autonomy with which it regulates its own functions. For this purpose, the intestine is provided with its own enteric nervous system, the so-called "gut brain", which makes contractile activity possible, even without external input. This intrinsic enteric nervous system contains two major components: Meissner's neuronal plexus, which is embedded in the tunica submucosa, and Auerbach's plexus, which

is localized between the longitudinal and the circular muscle layers of the tunica muscularis (Figure 1.II.1, 3B).

However, too much functional autonomy would hamper the integration of the intestinal functions into the bigger picture of the body physiology. Here, the extrinsic or vegetative nervous system, represented by the sympathetic and parasympathetic nervous system, plays a modulating role. Just like the principles of Yin and Yang, the two nervous systems are each other's counterpart. By means of a complex network of interneurons, localized at the level of the neuronal plexuses of the enteric nervous system, they process all incoming information, thus making it possible for the intestine to formulate a suitable and integrated motor response (Figure 1.II.3).

Finally, there is the brain, which has an undeniable influence on GI functionality. The detrimental effects of psychological factors such as stress on the GI motility of humans are channeled by this "gut-brain axis". Likewise, in horses several stressful factors such as sudden changes in management or environment have been implicated in the pathogenesis of colic (Goncalves *et al.*, 2002).

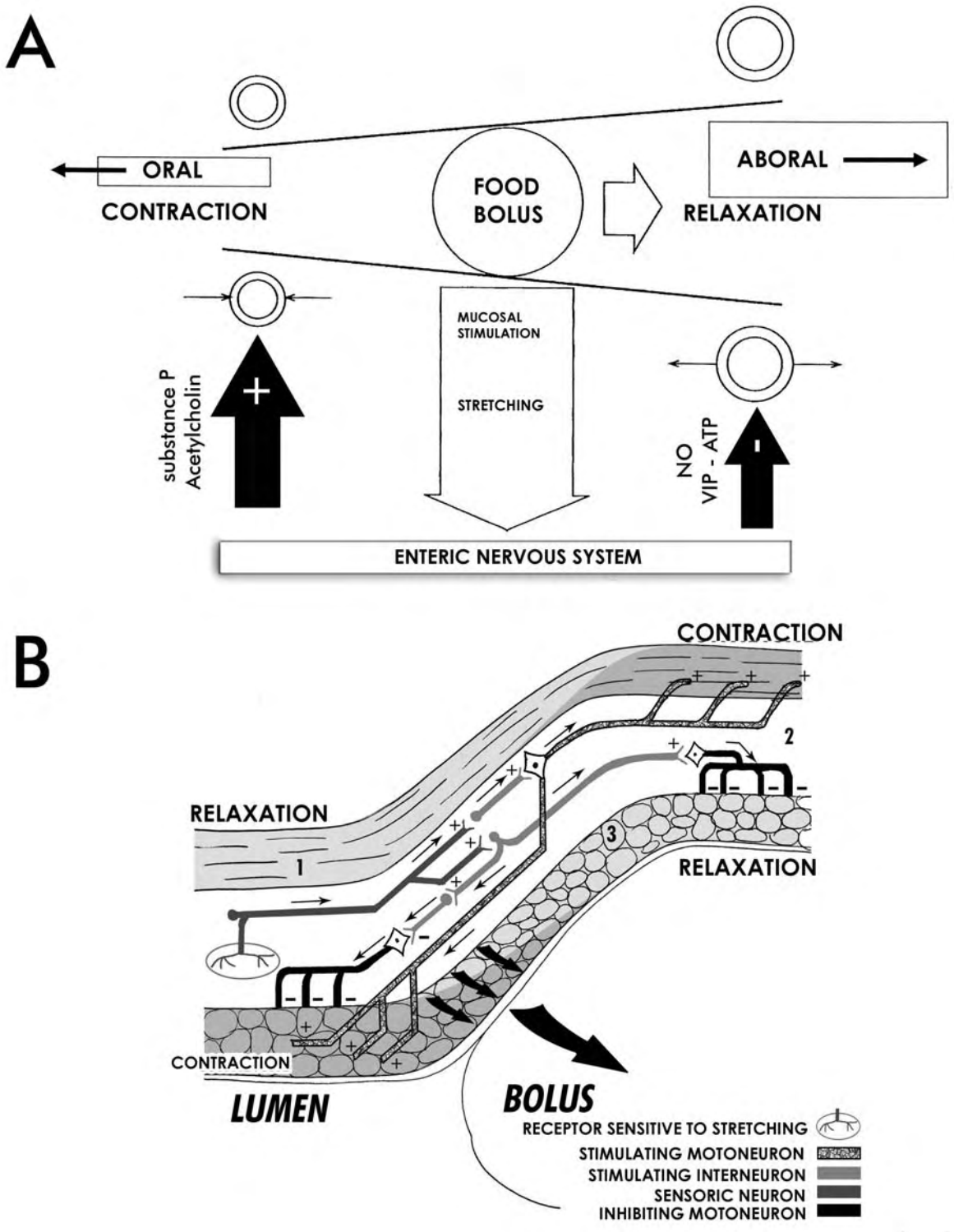

Figure 1.II.3 A,B: The peristaltic reflex: (1) outer longitudinal muscle layer; (2) Auerbach's neuronal plexus; (3) inner circular muscle layer; (+) stimulation; (-) inhibition.

It is clear that any damage to the enteric nervous system has significant implications for GI motility. For example, decreased neuronal density at the level of the myenteric plexus can be

found in some horses with acute or chronic impaction of the cecum and/or colon (Schusser & White, 1997; Schusser *et al.*, 2000). Spastic contractions at the level of these impactions can partially block vascular supply to the intestinal wall, leading to neuronal degeneration. Therefore, although they sometimes seem not to be alarming, swift and proper treatment of cecal and colonic impactions is important in order to prevent long-term damage and problems.

1.II.4. TRANSDUCTION OF MESSAGES AT THE ULTRASTRUCTURAL LEVEL

Both messengers (neurotransmitters, hormones) and receivers (different types of receptors) are equally important for orchestrating the communication that is needed to realize motor activity at the level of the intestine.

Upon electrical activation, neurons will release different kinds of neurotransmitters at their terminal endings. These released neurotransmitters can either facilitate or inhibit motor activity. Acetylcholine is an important excitatory neurotransmitter, which modulates its activity through the activation of cholinergic neurones or through direct activation of smooth muscle cells. Many prokinetic agents directly or indirectly stimulate the release of acetylcholine.

On the other hand, when noradrenalin, as a neurotransmitter, activates adrenergic receptors localized on cholinergic neurons, acetylcholine release will be suppressed, thus leading to decreased motor activity.

There is also the “NANC” or “non-adrenergic, non-cholinergic” system, which involves several non-cholinergic, non-adrenergic inhibitory and excitatory neurotransmitters. For example, the release of NO (nitric oxide), VIP (vaso-active intestinal peptide) or ATP will cause relaxation of enteric smooth muscle cells, whereas Substance P will facilitate contraction (Figure 1.II.3,A).

NO is generated not only by neuronal NO synthase, but also by inducible NO synthase produced by inflammatory cells. Indeed, histological examination of dilated and/or vascularly compromised small intestinal segments of colic horses often reveals the presence of extensive damage not visible to the naked eye, which leads to a massive influx of inflammatory cells into the mucosa, the muscularis externa and the serosa (Dabareiner *et al.*, 1993 a,b). After being activated, the attracted macrophages produce NO synthase. This leads to the production of quantities of NO which are far more significant than the quantities that are produced in response to neuronal stimulation. It has been proposed that this could lead to decreased motor activity, which in turn could trigger the condition of ileus. Furthermore, the binding of NO with superoxide anions can generate the formation of peroxynitrite, which in turn enhances tissue damage.

As mentioned previously, the neurotransmitters rely on a multitude of receptor types to transmit their messages to the target tissues. These receptors can be localized both on neurons and directly on the smooth muscle cells. Up until now, several receptor types have been shown to be involved in the regulation of intestinal motility. It is well known that the cholinergic receptors – which are activated by acetylcholine and the adrenergic receptors, which in turn are activated by noradrenalin – are capable of modulating intestinal motility. Tachykinin receptors are activated by the aforementioned NANC system and motilin receptors by the peptide motilin. Most of the receptors are located on the outer cell membrane. However, NO receptors are localized intracellulary (Domeneghine *et al.*, 2004). In conclusion, it can be stated that pharmacological modulation of GI motility can be accomplished by stimulation or inhibition of the release of specific neurotransmitters, or by direct action on enteric receptor populations.

1.II.5. BASAL MOTOR ACTIVITY OF THE EQUINE GI TRACT

The mixing and propulsion of food in the GI tract is realized by the perfect cooperation between tonic activity, phasic activity and the peristaltic reflex.

Tonic activity

Even under resting conditions, the GI tract is always contracted to a certain degree, which is known as the so-called “basal tonus”. Any rhythmic activity that occurs is always superposed upon this basal tonus. The overall narrowing of the inner intestinal lumen that is realized by the basal tonus enhances the efficiency of the intestinal propulsive and mixing contractions that occur. Indeed, the generally weak phasic contractions, which do not always succeed in occluding the intestinal lumen, will do so at times when the basal tonus is sufficiently pronounced. Many neurotransmitters and hormones exert a direct or indirect (via neurons) effect on the smooth muscle layers of the GI tract and thus help to determine the degree of basal tonus (basal degree of contraction) of the intestine.

Phasic activity

Both neuronal and non-neuronal components, such as ICC cells, cooperate to realize the regular phasic motor activity, which can be seen at the level of the stomach and the small and large intestines (Hirst & Edwards, 2004). The spontaneous fluctuations of the resting membrane potential, which are a typical feature of the ICC cells, are transmitted to the neighboring smooth muscle cells via the aforementioned gap junctions. Like the ripples that are generated by throwing a stone into a pond, these so-called “slow waves” spread themselves over the intestine. Although far from strong enough to generate actual contraction of smooth muscle cells, these slow waves change the membrane potential of the intestinal smooth muscle cells to such an extent that they become sensitive to stimulation by

neurotransmitters such as acetylcholine. When the released acetylcholine stimulates smooth muscle muscarinic receptors during the upward positive deflection of the resting membrane potential triggered by the passing slow wave, the threshold potential for contraction will be crossed, and this will trigger the opening of ion channels and a concomitant depolarization, which is better known as “action potential” or “spike”. Finally, a contraction will take place. Therefore the slow waves determine not only the timing and the direction, but also the speed with which smooth muscle contractions spread out over the intestine.

In conclusion, it can be stated that the prerequisite for the occurrence of phasic contractions is the presence of normal slow wave activity. Moreover, the release of excitatory neurotransmitters during the plateau phase of the slow waves is necessary for generating spike activity and concomitant contraction (Figure 1.II.2). In cases where both excitatory and inhibitory neurotransmitters are released at the same time, the resulting activity will be the summation of the opposing effects triggered by the two neurotransmitter types. In humans, the spontaneous electrical and motor activity that is seen at the level of the stomach and small intestine some time after the ingestion of a meal shows a typical cyclic pattern better known as the “migrating myoelectrical complex” or “MMC” or “inter-digestive enteral housekeeper” (see below). As soon as a meal is consumed, this cyclic pattern is replaced by an active enteric digestion pattern.

In horses suffering from grass disease the ICC cell density is clearly diminished (Hudson *et al.*, 2001). A comparable evolution of the ICC cell density can be observed in full-thickness biopsy specimens of the pelvic flexure of colic horses presented with acute large colon impaction or displacement. Conversely, no changes in ICC cell density could be observed in jejunal segments of horses suffering from small intestinal volvulus (Fintl *et al.*, 2004). In those acute colic cases where a decreased amount of ICC cells is encountered, this

decrease should probably be viewed merely as a cause rather than as a consequence of the colic episode.

The peristaltic reflex

The peristaltic reflex (Figures 1.II.3 A,B) is the result of a perfect coordination between the neuronal and muscular components of the intestine. The occurrence of the reflex is based on (1) the perception of the presence of a food bolus (sensor neurons/cells), (2) the transmission of this message to a neuronal network specifically designed for information integration and determination of a suitable response (interneurons), and (3) the execution of the imposed tasks (motor neurons), which implies a normal functionality of both the circular and longitudinal muscle layers of the tunica muscularis. Propulsion of food is accomplished by contractions of the circular muscle layer at the oral pole of the food bolus and a simultaneously occurring relaxation of that same muscle layer at the aboral pole. At the same time, the longitudinal muscle layer contracts at the aboral pole and relaxes at the oral pole. This results in the active movement of the food bolus in the aboral direction, which is called propulsion. It is important to realize that the induction of true propulsive activity is a prerequisite for an effective prokinetic agent. Some of these formulations will only create spastic contractions of the intestine, sometimes accompanied by the expression of abdominal pain by the treated horse. These signs of abdominal discomfort can sometimes by misinterpreted by the clinician as a sign of true and pronounced effectiveness of the administered pharmacon. However, it should always be kept in mind that the coordination with which the intestinal contractions take place is of outmost importance.

1.II.6. ENTERIC MOTILITY PATTERNS

The Migrating Myoelectrical Complex (MMC) of stomach and small intestine

As mentioned previously, the Migrating Myoelectrical Complex or MMC or “enteral housekeeper” is a typical electrical and motility activity pattern with a cyclic character that can be seen at the level of the small intestine some time after the ingestion of a meal. The colon can express a similar interdigestive electrical pattern, better known as the “Colonic Migrating Myoelectrical Complex” or “CMMC”. It is generally agreed today that spike potentials and not slow waves are responsible for contractile activity in the large bowel. Studies that try to establish a relationship between spike bursts in the human colon in vivo and actual occuring contractile activity are scanty. In a study performed by Medeiros et al. (1997), there was a good correlation between intraluminal electromyographic measurements and pressure recordings. In horses this also seems the case, although Adams and co-workers advocate the combined use of myoelectrical and myomechanical recording, to avoid errors, based on the results of their trials (Adams et al., 1984).

Phase 3 of the MMC represents a series of strong and pronounced contractile activity fronts that propagate over the stomach and small intestine in the aboral direction. It is generally accepted that these waves serve to evacuate meal residues and cellular debris that are not propulsed by the normal digestive peristaltism. Disruption of the normal MMC pattern can predispose the small intestine to bacterial overgrowth, hence the designation “enteral house-keeper” (Nieuwenhuys *et al.*, 2000).

In humans the MMC motor pattern starts a few hours after the ingestion of a meal. However, it is immediately interrupted by the active ingestion of food, to be replaced by a typical slow propulsive motor pattern, which optimizes complete digestion of the ingested food. In horses, however, the MMC pattern is continuously expressed, despite ad libitum ingestion of food (Merritt et al., 1989a; Baker & Gerring, 1994). Therefore the equine MMC

has to be viewed as an active part of the digestive motor activity. Researchers presume that the MMC in herbivorous animals predominantly serves to prevent the backflow of chyme, rather than functioning as an enteral house-keeper (Hunt, 1985; Baker & Gerring, 1994). Therefore they propose that disturbing the MMC cycle in horses can have significant implications for the active transportation of ingested food.

The motility pattern of the MMC typically consists of 4 phases, each with its own specific duration. During phase 1 – "the silent phase" –, there is no motor activity. Phase 2 is characterized by a series of irregular contractions, directly followed by phase 3, which is represented by numerous clearly defined propulsive rhythmic contractions with maximal frequency and amplitude. The MMC will first appear in the duodenum of the horse, after which it will be propagated with decreasing velocity over duodenum and jejunum. Phase 4 represents the transition phase from phase 3 to phase 1 (Ruckebusch *et al.*, 1971; Merritt et al., 1989a).

In horses, there is always a transient episode of mechanical inactivity of the stomach, at the start of phase 3 within the duodenum (Merritt et al., 1989a). Similar findings have been reported in ruminants and pigs (Ruckebusch & Bueno, 1975, 1976; Ruckebusch & Merritt, 1985).

An average of 18 to 20 MMC cycles a day can be recorded in horses. Almost all phase 3 activity will propagate all the way from the proximal duodenum, up to the terminal ileum. The long duration of phase 3 and the fact that this duration even increases going down the jejunum to the ileum, is a typical feature of the horse, which has never been found in other species (Sasaki & Yoshihara, 1999). Phase 3 is not propagated across the stomach of the horse and, as in other species, will not be the main mover of contents through the small intestine (Merritt et al., 1989b). Phase 3 has in the horse a high mean velocity of 32 cm/min, which is unique for the horse (Ruckebusch, 1981; Davies & Gerring, 1983a) (Table 1.II.1).

Table 1.II.1: Specific features of the equine "migrating myolectrical complex" or MMC (duration (min), propagation speed (cm/min). (Davies & Gerring, 1983 ; Lamar et al., 1984 ; Adams et al., 1984 ; MacHarg et al., 1986 ; Sojka et al., 1988 ; Merritt et al., 1989a,b ; **Sasaki et al., 1999**)

	Proximal jejunum	***Distal jejunum***	***ileum***
Phase 1 (min)	-	31.6 ± 17.0	22.3 ± 14.4
Phase 2 (min)	122.2 ± 26.3 (1+2)	110.8 ± 67.0	95.3 ± 63.5
Phase 3-4 (min)	7.9 ± 1.7	29.0 ± 6.1	44.6 ± 9.0
Duration MMC cycle (cm/min)	130.1±26.0	183.9 ± 72.2	160.8 ± 56.3
Propagation (cm/min)		± 32	7.3 to 12.5

The "Colonic Migrating Myoelectrical Complex" (CMMC)

In accordance with the small intestine, the colon disposes over its own typical digestive and inter-digestive myoelectrical pattern of motion, during which the activity in the ileum, cecum and colon are clearly coupled (Roger *et al.*, 1985; Ross *et al.*, 1989 & 1990; Rutkowski *et al.*, 1989; Lester *et al.*, 1992 & 1998a,b; Merritt et al., 1995). The coordination of this myoelectrical coupling between different regions of the large intestine, is probably orchestrated in pacemaker nodes, which are localized in the intestinal wall of the cecum and in the tenial bands of the colon (Burns *et al.*, 1992) (Figure 1.II.1). However whether the equine colonic motor activity is actually governed by this pacemaker system has never been confirmed either functionally or myoelectrically.

The myoelectrical activity that can be registered at the level of the pelvic flexure consists of (a) isolated "long spike bursts (LSB)" that are propagated both in the oral and the aboral directions and help with the mixing of the chyme; (b) a slowly (0.5-1.0 cm/min) aborally

migrating cluster of “short spike bursts (SSB)” and “long spike bursts (LSB)” occurring every 10 to 15 minutes, and (c) a series of pronounced and repetitive “long spike bursts (LSB)” that occurs sporadically and lasts for 3 to 6 minutes. These electrical waves, which propagate themselves with high velocity (3 cm/sec) in the aboral direction, are better known as the “Colonic Migrating Myoelectrical Complex” or “CMMC”. There is a clear increase in LSB activity in horses when food is ingested (Adams et al., 1984; Merritt et al., 1995; Lester et al., 1998b).

It is now known that these coordinated contractile colonic waves cause a pronounced back and forward movement of the pelvic flexure between the diaphragm and the pelvis. Hence the importance of free mobility of the colon and the detrimental effects of adhesions on normal colonic digestion. It is possible that colonic displacements, like nephro-splenic entrapment, are preceded by the excessive expression of such back and forward movements (Roberts & Seawright, 1983; Sellers *et al.*, 1982a).

Little is known about the possible occurrence of defective intestinal motility patterns during GI colic in horses. Up until now, no non-invasive registration techniques that are applicable in true clinical cases are available. Therefore, all information has to be extrapolated from experimental colic models in which colic is artificially mimicked in healthy horses (Sellers *et al.*, 1982b). For example, colic can be experimentally induced by the creation of an intra-or extra-luminal obstruction at the level of the small or large intestine or by occluding the mesenterial arteries (Phaneuf *et al.*, 1972; Lowe *et al.*, 1980; MacHarg *et al.*, 1986; King & Gerring, 1989; Davies & Gerring, 1985). Trauma-induced postoperative ileus is mimicked by manual mechanical irritation and serosal dehydration of parts of the small intestine (Gerring & Hunt, 1986). Endotoxemia is artificially mimicked by the IV administration of E. coli lipopolysaccharids (King & Gerring, 1992).

The “colic motor complex” is an example of a defective motility pattern that can be registered during experimental extra-luminal obstruction of the small intestine. It represents spastic contractile activity that is expressed by small intestinal segments located orally to the obstruction zone. How and why these colic motor complexes are generated by the intestine is not known, though they probably represent a local reflex of the intestine in an attempt to evacuate the obstruction (Phaneuf *et al.*, 1972; Davies & Gerring, 1985; MacHarg *et al.*, 1986). The “colic motor complex” is a motility pattern seen in all species when the small intestine is obstructed, it is not strictly an equine phenomenon. Ruchebush & Bueno (1975) described their occurrence in sheep more than 30 years ago. When these intestinal spasms linger long enough, they substantially increase the intestinal metabolism and can compromise normal intestinal blood supply, which has a detrimental effect on the viability of the intestine. Therefore it has to be mentioned that the administration of prokinetic medication to horses suspected of mechanical obstruction of the small intestine, like volvulus, is not advisable. On the contrary, it should be kept in mind that in these cases intestinal dilation and necrosis can be aggravated.

Besides the colic motor complexes that are registered during extra-luminal obstruction of the jejunum, there is also a concomitant hyperactivity to be observed at the level of the left dorsal colon, which is unique in horses and has never been found in any other species in association with small intestinal obstruction (King & Gerring, 1989). There is probably a resemblance with the human gastro-colonic reflex, where accommodation of a meal in the stomach is accompanied by an increased contractile activity in the colon (King & Gerring, 1989). In an experiment of King and Gerring (1989), dilation of the stomach was accompagnied by loss of contractile activity and onset of chaotic myoelectrical activity at the level of the stomach and small intestine. After gastric decompression, although not normalized, both myoelectrical and mechanical patterns were less disturbed. When

extrapolating this to cases of over-feeding or colic cases with accumulation of reflux, probably thorough stomach decompression and complete evacuation of reflux in colic horses is important not only to prevent rupture but also for gastro intestinal motility.

1.II.7. PHARMACOLOGICAL MODULATION OF EQUINE GASTRO-INTESTINAL MOTILITY

Imagine the enormous body fluid shift that takes place in horses during the production of reflux. Rapid dehydration and extensive ionic losses are the result. Inevitably shock and GI hypoperfusion will follow. Therefore it is important to monitor GI motility in colic horses and to start-up supportive prokinetic treatment in time (Table 1.II.2). Depending on the location of the GI problem, different types of prokinetic drugs should be used.

Table 1.II.2: A practical overview of the use of prokinetic drugs in horses

Prokinetic Drug	Route of action	Dose regimen	Disadvantages
Erythromycin lactobionate	Stimulation of motilin receptors	0,5 à 1mg/kg iv TID/QID	Colic symptoms Frequent use→ "downregulation" of motilin receptors Fatal cases of enteritis
Lidocaïne hydrochloride	Sympatic antagonism Direct stimulation of enteral smooth muscle cells Anti-inflammatory	Bolus: 1,3mg/kg iv Maintanance: 0,05mg/kg/min iv of a 2% solution	In case of overdosing: Ataxia during post-operative recovery
Metoclopramide	Alpha 2 receptor antagonist Antagonism of central and peripheral dopaminergic receptors Stimulation of cholinergic neurotransmission 5-HT_3 antagonist 5-HT_4 agonist	0,04 mg/kg/h iv 0,05 mg/kg IM QID 0,1 à 0,25 mg/kg sc TID/QID 5 mg/kg po TID	Extrapyramidal side effects
Domperidone	Peripheral dopaminergic receptor antagonist	1mg/kg po QID	In a model of POI only stimulation of the gastroduodenal junction
Neostigmine methylsulfate	Cholinesterase inhibitor	0,022 mg/kg iv or sc every 2 to 4h on effect	Pronounced signs of colic
Bethanechol chloride	Parasympaticomimetic drug Cholinergic agonist	0,025 mg/kg sc or 0,25 mg/kg iv in 10ml of 0,9%NaCl every 3 to 4h	Signs of colic Salivation Sweating
Yohimbine	Alpha 2 receptor antagonist	0,15 mg/kg iv BID/TID	No pronounced effect
Acepromazine maleate	Alpha 2 receptor antagonist	0,01 mg/kg IM every 4 to 6h	Blood pressure ↓ Peripheral vasodilation
Cisapride	Stimulates the release of ACH at the level of cholinergic neurons 5-HT_4 agonist	1mg/kg po QID	Not available
Tegaserod	Stimulates the release of ACH At the level of cholinergic neurons 5-HT_4agonist	0,02mg/kg iv BID	Not available

POI = post operative ileus; ACH = acetylcholine

1.II.7.1. Macrolids

As a member of the macrolid antibiotics, erythromycin stimulates the occurrence of MMC complexes in the horse. This is achieved through stimulation of motilin receptors localized on cholinergic neurons (Fiorucci *et al.*, 1993; Tomomasa *et al.*, 1986; Peeters *et al.*, 1989). In horses, motilin immunoreactive cells are found at the level of the duodenum, the jejunum, the cecum and the pelvic flexure. As in rabbits, there is a decreasing density of motilin receptors in the aboral direction (Kitamura *et al.*, 1984). In humans, the endogenic peptide motilin is produced by granular cells localized in the mucosal layer of the antrum, the proximal duodenum, the jejunum and the ileum (Weber *et al.*, 1993). Intravenous administration of motilin to the dog induces the occurrence of a phase 3 of the MMC, which starts in the stomach and propagates all the way to the ileum (Itoh *et al.*, 1984; Yamada *et al.*, 1997). Likewise, intravenous administration of motilin to horses (0.6 µg/kg) induces the occurrence of a phase 3 of the MMC at the level of the proximal jejunum. However, in contrast with the dog, the contractile waves do not reach the ileum (Sasaki & Yoshihara, 1999). In cases of experimentally induced postoperative ileus, the administration of motilin triggers an increased phase 3 activity of the MMC and enhances the solid phase emptying of the stomach. However, although important in horses with reflux, no acceleration of the gastric liquid phase emptying rate can be achieved (Coatney & Adams, 1988; Doherty *et al.*, 1998). This lack of effect on the liquid emptying of the stomach is probably linked to the specific prokinetic properties of erythromycin in horses, which are predominantly focused on the gastric antrum, a part of the stomach that is mainly important for the expulsion of solid food.

The prokinetic activity of erythromycin shows clear species variability. In monogastric animals like humans, cats and dogs, it is mainly the stomach and small intestine that are stimulated, whereas in horses it is also the colon that is clearly activated (Peeters, 1993; Itoh *et al.*, 1984). For example, slow intravenous administration of erythromycin (3.3 mg/kg, 20

min) to healthy horses induces the occurrence of CMMC's at the pelvic flexure (Masri *et al.*, 1991). Erythromycin doses of 0.10, 1.0 and 10 mg/kg administered to healthy ponies stimulate cecal emptying and myoelectrical spiking activity in the colon (Lester *et al.*, 1998a). However, in horses, like in other species, the rate with which the erythromycin is administered intravenously is very important. Slow infusion (60 min) will eventually lead to a pronounced suppression of myolectrical activity, despite an initial increase. As in humans, this is probably due to the reactive down regulation of motilin receptors. During bolus administration (0.10 mg/kg iv, 30 secs), these down regulating mechanisms are not triggered. Therefore, in horses it can be stated that mainly low, subtherapeutic non-anti-microbial doses given as a bolus are most suitable for obtaining prokinetic effects (Ringger et al., 1996). However, the possible detrimental effects of the repetitive use of motilin agonists in horses has not been evaluated. In humans, the earlier mentioned tachyphylaxia or receptor down regulation is thought to be responsible for the failure of clinical trials of several motilides, which clearly hampers the development of clinically useful compounds (Thielemans *et al.*, 2005). This should be kept in mind when repetitive dosing is applied in horses.

However, it is not only the amount and administration rate that are important, but also the indication for which the erythromycin is used. Research has demonstrated the expression of different gastro-prokinetic effects by erythromycin in healthy horses in comparison with horses with experimental postoperative ileus. In the latter group, the administration of erythromycin (0.5 mg/kg iv, 1 min) leads to increased myolectrical activity at the level of the ileum and the pelvic flexure, during both the post-recovery (24h) and the postoperative (8 days) periods. In the cecum, however, the stimulating effects are limited to the post-recovery period, which can be interpreted as a less favorable feature for prokinetic purposes (Roussel *et al.*, 2000). In conclusion, it can be stated that in cases of experimental postoperative ileus, the erythromycin induced prokinetic effects are mainly focused on the colon, although some

reports mention the successful treatment of clinical cases with small intestinal ileus (Van Hoogmoed *et al.*, 2000).

Last but not least, there is the alarming possibility of the occurrence of fatal colitis due to the use of erythromycin lactobionate as a gastro-prokinetic agent. Several case reports in the literature, as well as experience with the product in the Large Animal Internal Medicine Clinic of our faculty, show that administration of therapeutic doses of the antibiotic erythromycin to adult horses can lead to hyperthermia and fatal enteritis due to imbalance of the enteric flora (Prescott & Hoffman, 1993). Although the advocated prokinetic dosage of erythromycin is much lower, Coté and co-workers (2005) have reported significant changes in the fecal flora in horses treated only once with 1mg/kg erythromycin lactobionate iv. They found an increased excretion of clostridium perfringens, coliforms and anaerobes. No change in Salmonella or Clostridium difficile excretion could be found. In order to bring about a minimal antibiotic effect, a minimal receptor down-regulation and a maximal prokinetic effect, it is advised to use 0.5 to 1 mg/kg erythromycin lactobionate in 60 ml of 0.9% NaCl, administered IV three to four times a day as a bolus and not as a slow infusion.

1.II.7.2. Lidocaine hydrochloride

In human abdominal surgery, lidocaine hydrochloride is used peri-operatively as a pain killing drug and to minimize the duration of naturally occurring postoperative GI stasis (Groudine & Fisher, 1998). With respect to ileus in horses, it is believed that the activation of pain receptors localized in the peritoneum has an inhibitory effect on GI motility (Gerring & Hunt, 1986). Hence the widespread veterinary use of lidocaine in horses suffering from postoperative ileus or proximal enteritis (Malone *et al.*, 1990). It is thought that lidocaine exerts its prokinetic effects through direct stimulation of enteric smooth muscle cells and through suppression of sympathetic inhibitory reflexes (Rimback *et al.*, 1990). There are also indications that lidocaine has anti-inflammatory properties. Furthermore, it suppresses both

the net transport of fluid into the intestinal lumen and the extravasation of albumin (Nellgard *et al.*, 1996). In vitro motility tests on intestinal smooth muscle strips have demonstrated that lidocaine increases contractility, mainly at the level of the mid-jejunum (Nieto & Rakestraw, 2000a). Very few studies evaluate the in vivo efficacy of lidocaine as a gastro-prokinetic agent. Through the use of abdominal ultrasound, Brianceau and co-workers have demonstrated a decrease in both the mean jejunal diameter and the amount of abdominal fluid accumulation in colic horses that were postoperatively treated with lidocaine. However, when controls were compared with the treatment group, no significant difference in the time-interval to first defecation, production of reflux and number of small intestinal contractions per minute could be demonstrated (Brianceau *et al.*, 2002). Lidocaine is also thought to have anti-endotoxine effects, which could be interesting in countries without easy access to specialized medicines such as polymixin B and pentoxyphylline (Barton *et al.*, 2004; Piero *et al.*, 2000 a,b).

Per rectal application of lidocaine (15 ml of a 2% solution in 45 ml of tap water) can be considered to accomplish rectal relaxation, which can be a helpful tool to prevent the occurrence of rectal tears during abdominal palpation (Sanchez & Merritt, 2005). However, the sensitivity of the rectum to dilation is not influenced and IV administration of Butylscopolamine (Buscopan®) is without any doubt just as efficient and less time consuming.

In order to obtain optimum effect, lidocaine is administered per-operatively, starting with a bolus of 1.3 mg/kg of a 2% solution, followed by a continuing infusion of 0.05 mg/kg of the same 2% solution. When recovery is completed and the horse is standing stable on its feet, the bolus regimen is again applied, followed by continuous infusion during minimal 24h. Use of higher per-operative doses can lead to muscle twitching and pronounced ataxia, which can seriously hamper a safe recovery. Overdosing can lead to profuse sweating and fast breathing,

sometimes resembling the clinical picture of a horse with a ruptured viscus. Sudden collapse of the horse can also occur. Finally, it has to be mentioned that the use of lidocaine can mask early signs of founder. Therefore, careful monitoring of the colic patient is important. In some colic horses lidocaine administered in bolus dose creates enough sedation and pain relief to perform a complete clinical examination, without the need for the use of other sedatives, which often have long-term suppressing effects on GI motility.

1.II.7.3. Metoclopramide and Domperidone

One of the first benzamides discovered, **Metoclopramide** (Primperan®) **,** is widely used both in human and veterinary medicine to treat GI symptoms related to impaired motility and to tackle emesis. One of the features of the "older" drugs is that they are less specific and therefore often stimulate a wide array of enteric receptor types. The down side of this aspecificity is the numerous side effects that are often encountered with these drugs. Hence the tremendous diligence with which researchers are trying to develop receptor-specific blocking and activating agents.

Metoclopramide stimulates serotonergic 5-HT_4 receptors, which in turn enhance the release of acetylcholine at the level of postsynaptic cholinergic neurons. The drug acts on central dopaminergic receptors, where it has an antagonizing effect. Although there is little evidence for the presence of dopaminergic receptors on enteric smooth muscle cells, some researchers propose that metoclopramide has a suppressing effect on the inhibitory effect of dopamine on enteric smooth muscle cells (Tonini, 1996). Finally, it blocks serotonergic 5-HT_3 receptors and adrenergic alpha 2 receptors (Alibibi & McCallum, 1983).

Gerring (1982) classifies idiopathic and thus non-endotoxemic ileus in horses as dopaminergic mediated. Hence his advice to use metoclopramide as a gastro-prokinetic agent in these horses .

In vitro contractility tests on enteric smooth muscle strips demonstrate that metoclopramide stimulates contractile activity of the equine pylorus, the proximal duodenum and the mid-jejunum. Progressively increasing doses are required to stimulate contractile activity from proximal to distal. This specific feature of metoclopramide is probably linked with its ability to actually coordinate motility in the stimulated intestinal segments rather than simply inducing increased, but random, contractile activity (Nieto & Rakestraw, 2000).

In healthy ponies, metoclopramide (0.125 mg/kg iv) accelerates the gastric liquid phase emptying rate. When applied as an IV bolus of 0.03 mg/kg, no significant changes in the MMC and motoric activity of the jejunum and the pelvic flexure could be demonstrated (Sojka & Adams, 1988). However, used as a continuous infusion (0.5 mg/kg/h iv) in ponies with experimentally induced postoperative ileus, there is a clear increase of the contractile activity of the jejunum, the cecum and the colon. Moreover, metoclopramide is able to re-establish good coordination of disturbed MMC activity in these ponies (Gerring & Hunt, 1986). Standard postoperative administration of metoclopramide as a continuous infusion (0.04 mg/kg/h) seems to suppress the occurrence of postoperative ileus (Dart *et al.*, 1996).

However, overall clinical use of metoclopramide in colic horses is hampered by the frequently encountered extra-pyramidal side effects, which are encountered even with low dosing protocols. The reason for this is the ease with which the drug passes the blood-brain barrier. Especially skinny horses with limited body fat stores seem to be prone to showing these extra-pyramidal side effects. Although continuous low dose infusion (0.04 mg/kg/h) will create more stable blood levels of the drug than will bolus administration, nervous side effects can still occur. Even with IM administration, extrapyramidal effects have been reported. Sometimes symptoms will occur immediately after the administration of metoclopramide, and sometimes only 36 h after starting the continuous infusion. The horses are fearful and nervous. They show a continuous propensity to sit on their hindquarters. There is profuse

sweating and signs of abdominal discomfort are often seen. These symptoms can linger for more than 12h after discontinuation of the metoclopramide treatment. Sometimes walking the horse for 15 min can quickly improve the situation, though this will only be possible in those cases where safe handling is still an option. In most cases, however, the administration of sedatives will be necessary, accompanied by all the negative effects on GI motility. Other proposed dosing regimens are: 0.05 mg/kg IM QID; 0.1 - 0.25 mg/kg sc TID or QID and 5 mg/kg PO QID.

Domperidone as peripheral dopaminergic antgonist has a widespread use as gastroprokineticum and anti-emetic drug in human medicine. The veterinary use, however, is rather limited. Domperidone will not pass the blood-brain barrier and therefore is free from any extra-pyramidal side effects. Little is known about its pharmacological action. However, experimental evaluation of its efficacy as a prokinetic drug in horses is not very encouraging. In ponies with experimentally induced postoperative ileus, domperidone (0.2 mg/kg IM QID) seems only to be able to stimulate contractility at the gastroduodenal junction. No prokinetic effect could be observed at the level of the colon (Gerring *et al.*, 1991). Domperidone is only available for oral use. The proposed dose regimen is: 1 mg/kg PO QID.

1.II.7.4. Cholinergic stimulants and agonists

Neostigmine methyl sulphate antagonizes the breakdown of the neurotransmitter acetylcholine by the enzyme acetylcholinesterase. The drug can be used successfully in horses with impactions of the cecum or right dorsal colon, refractory to treatment with laxatives. When administered to healthy ponies (0.025 mg/kg sc), neostigmine methyl sulphate induces the occurrence of premature phase 3 complexes of the MMC at the level of the ileum, acceleration of the cecal emptying rate, and the occurrence of CMMC's at the level of the right dorsal colon (Lester *et al.*, 1998c). Its prokinetic activity is mainly focused on the hindgut. On the contrary, neostigmine methyl sulphate seems to have an inhibitory effect on

the proximal part of the GI tract, which is quite important for horses suffering from ileus. In healthy ponies, both the gastric liquid phase emptying rate (0.022 – 0.044 mg/kg sc) and the contractile activity of the jejunum are clearly suppressed (Adams *et al.*, 1984; Adams & Margaret, 1985). Despite these observations, there are some reports of successful treatment of horses with postoperative ileus that were refractory to treatment with lidocaine, metoclopramide and erythromycin (Van Hoogmoed & Snyder, 1997). The proposed dosing regimen is: 0.022 mg/kg iv or sc every 2 to 4 hours. Horses treated with neostigmine methyl sulphate can show signs of abdominal discomfort directly after administration of the product.

Bethanechol chloride mimics the action of the neurotransmitter acetylcholine. Acetylcholine stimulates muscarinic receptors, which are found all over the body, including the muscle that surrounds the GI tract. Bethanechol chloride has a pro-contractile effect on both stomach and small intestine. In healthy ponies it stimulates the gastric evacuation of both liquids and solids (Thompson *et al.*, 1994; Lester et al., 1998c). When administered at a dose of 0.05 mg/kg sc, there is also a clear increase of the contractile activity of the colon which lingers for more than 80 minutes (Roger *et al.*, 1985). In a model of experimentally induced postoperative ileus, there is a discrete increase of the propulsive activity of the stomach and small intestine (2.5 mg/kg sc). The co-administration of bethanechol and yohimbine triggers more distinct prokinetic effects (Gerring & Hunt, 1986). The proposed dosing regimen for bethanechol chloride is: 0.025 mg/kg sc every 3 to 4 hours. Due to the parasympaticomimetic features of bethanechol, side effects like profuse salivation and abdominal discomfort can hamper its use in colic cases. Bethanechol is not available in Belgium as registered medication, only as chemical compound.

1.II.7.5. Alpha adrenergic receptor agonists and antagonists

Of the alpha adrenergic agonists, detomidine is probably best known by the equine practitioner as sedative and analgesic. The use of this product in colic horses however seems to be somewhat contra-indicated based on the results of in vivo studies, demonstrating an inhibitory effect of detomidine on duodenal motility, which lingers on up to an hour after administration (Merritt et al., 1998; Sanchez et al., 2005).

Alpha adrenergic receptor antagonists inhibit the motility suppressing effects of the sympathetic nervous system on GI motility. As mentioned previously, adrenergic hyperactivity is thought to be part of the complex of factors that can trigger postoperative ileus in horses (Gerring & Hunt, 1986; Eades & Moore, 1993). GI stasis in horses is often complicated by the onset of endotoxemia, which in turn induces increased release of sympathetic neurotransmitters. Hence, the research that has been done on the possible use of adrenergic antagonists for treatment of ileus and endotoxemia in horses. In healthy ponies, only little if any propulsive effect could be elicited through the use of adrenergic antagonists. Administration of the alpha 2-receptor antagonist **yohimbine** (0.15 mg/kg iv) even elicits a significant slow-down of the gastric liquid phase emptying rate, both in healthy ponies and in ponies with experimentally induced endotoxemia (Doherty *et al.*, 1998; Meisler *et al.*, 1997). On the whole, this is a rather surprising observation, since one would expect an increased release of acetylcholine and a concomitant increase of contractile activity as a result of antagonizing adrenergic alpha 2 receptors localized on cholinergic neurons (Drew, 1978). On the other hand, it has been demonstrated that the use of yohimbine in cases of experimental endotoxemia specifically stimulates cecal vascularization and myoelectrical activity (Eades & Moore, 1993). In a model of experimental postoperative ileus, yohimbine (0.15 mg/kg iv) stimulates the propulsive activity of the jejunum. As mentioned previously, best results were obtained when co-administration of yohimbine (0.15 mg/kg iv) and bethanechol chloride (0.014 – 0.022 mg/kg sc) was applied (Gerring & Hunt, 1986).

Acepromazine, which is also an alpha-adrenergic blocking agent, is primarily used as a sedative drug in horses. As a neuroleptanalgetic agent, this drug is part of the chemical class known as phenothiazines. It antagonizes several neurotransmitters, including: acetylcholine, 5-hydroxytryptamin, catecholamines and histamine (Rang *et al.*, 1995). Administration of acepromazine to healthy ponies (0.05 mg/kg iv) elicits a significant suppression of the gastric liquid phase emptying rate. However, the solid phase emptying rate is not influenced (Doherty & Adams, 1999; Sutton *et al.*, 2002). Contractility experiments with experimentally intra-abdominally isolated jejunal segments (better known as "Thiry-Vella loops") show that although acepromazine suppresses the myoelectrical activity in these segments, the throughput of liquids is clearly stimulated (Davies & Gerring, 1983 b). However, these observations could not be confirmed by Lester and co-workers during in vivo experiments (Lester *et al.*, 1998c). The overall conclusion, based on all these experimental results, is that acepromazine as a sedative drug, in contrast to many other sedatives, seems to have only minor negative effects on GI motility. In some cases it even seems to stimulate contractile activity, without triggering spastic contractions. Therefore it seems to be a good choice as a sedative drug in colic horses without obstruction of the proximal part of the GI tract. However, the detrimental effects of this drug on blood pressure should always be kept in mind. Lowering blood pressure in colic horses suspected of having compromised bowel or in candidates for surgical intervention should be avoided at all times. Finally, acepromazine can be used in low doses (0.01 mg/kg IM every 4 to 6 h) as part of a founder prevention plan.

1.II.7.6. Serotonergic agonists

Cisapride stimulates presynaptic, myenterically localized 5-HT_4 receptors, which in turn trigger an increased release of the excitatory neurotransmitter acetylcholine. The drug has a blocking effect on 5-HT_2 and 5-HT_3 receptors and influences 5-HT_1 and motilin receptors (Briejer *et al.*, 1995). In humans and dogs, cisapride stimulates the release of endogenous

motilin (Song *et al.*, 1997). Whether this is also the case in horses has never been investigated. Finally, as in humans, cisapride acts on K^+-ERG channels localized in the equine GI tract. These ion channels have been identified immunohistochemically in the equine duodenum, the jejunum and the colon (Lillich *et al.*, 2003). Not surprisingly they have also been identified in the equine heart. In humans, it is known that stimulation of these cardiac ion channels is partially responsible for the cardiologic side effects of cisapride, such as life-threatening arrhythmias, that have been reported in some patients (Cools *et al.*, 2001). Whether these side effects might also occur in the horse, is not clear.

There is a lot of controversy about the therapeutic efficacy of cisapride for stimulating GI motility in horses (King & Gerring, 1988; Ruckebusch & Roger, 1988; Levy & Sojka, 1991; Baker & Gerring, 1994). The overall conclusion of the clinical trials performed is that the drug should mainly be used as part of the prophylactic plan. However, once a status of postoperative ileus and endotoxemia exists, little if any effect should be expected from the use of cisapride (Gerring & King, 1989; Gerring *et al.*, 1991; De Geest *et al.*, 1991; Van der Velden & Klein, 1993; Sasaki & Yoshihara, 2000). Although in a model of experimentally induced endotoxemia, a significant acceleration of the gastric liquid phase emptying rate was accomplished, the profound inhibiting effects of prostaglandin E_2 and E coli on jejunal contractile activity could not be antagonized (Valk *et al.*, 1998; King & Gerring, 1992). In January 2005 the decision was made to fully withdraw the licenses of cisapride because the benefits of the drug did not outweigh the cardiologic risks of treatment. In horses, we can only confirm that measured therapeutic blood levels are high enough to trigger cardiac arrhythmias (Finley *et al.*, 2002).

Tegaserod is a more specific serotonergic receptor agonist than cisapride, and it mainly stimulates myenterically localized 5-HT_4 receptors (Chey, 2004). On the basis of the results of in vitro studies, it can be concluded that this receptor is mainly found in the hindgut of the

horse (Weiss *et al.*, 2002). Tegaserod is available in the US under a regimen of restricted use as a per oral drug for the treatment of obstipation predominant irritable bowel syndrome (IBS). In vivo investigations in healthy horses (0.02 mg/kg Tegaserod iv BID) have demonstrated an acceleration of the oro-anal transit of barium filled particles, administered by naso-gastric intubation (Lippold *et al.*, 2004). Whether the drug will find any application in the treatment of postoperative ileus in horses is very questionable, since up until now, little if any evidence has been found to localize the target 5-HT_4 receptor of tegaserod in the equine stomach and small intestine (Nieto *et al.*, 2000b; Delesalle *et al.*, 2005a,b)

1.II.7.7. Other drugs

It is interesting to note that the administration of 10 to 20 x 10^6 IU ***K^+-penicillin*** to healthy horses elicits an increase of myoelectrical activity at the level of the pelvic flexure that lingers on for no longer than 15 to 45 min. The observed effect is somewhat comparable with the increased contractile activity seen after administration of erythromycin lactobionate (Roussel *et al.*, 2003). The prokinetic effects of K^+-penicillin cannot be attributed to the K^+ ions, since no myoelectrical changes are observed after the sole application of KCl. It is however not clear how the pro-motile effects of K^+ penicillin can be explained, is not clear. The mentioning of this special feature of K^+ penicillin is far from being a plea for standard use of the drug in colic horses. It should always be borne in mind that several studies have reported an increased risk for diarrhea in colic horses treated with this antibiotic (Gustafsson *et al.*, 2004).

As in humans, it is thought that also in horses the release of endogenous endorphins contributes to the onset of ileus. Therefore, efforts are being made to investigate possible beneficial effects of the use of opioid antagonists, such as ***naloxone***, in colic horses. One clinical trial reports favorable results after the treatment of colic horses with naloxone (Sciorsci *et al.*, 2000).

1.II.8. CONCLUSION

GI hypomotility in horses can be triggered by a multitude of factors and should always be considered a complex pathologic condition. Successful treatment of ileus requires a systematic approach, during which all possible problems are addressed in a swift and precise way. A thorough clinical examination is important to identify the type of ileus (mechanical or adynamic). On the basis of these findings, the possible use of prokinetic agents can be justified. The localization of the motility problem has important implications for the type of prokinetic drug that has to be used. In summary, it can be stated that for the treatment of hypomotility of the proximal part of the equine GI tract, lidocaine, metoclopramide and erythromycin are the main drugs of choice. Naloxone, neostigmine, erythromycin and lidocaine are important for the treatment of stasis of the hindgut. In cases showing signs of endotoxemia, the use of adrenergic antagonists can be a helpful tool. It should be noted that the production of reflux in horses represents an enormous loss of body fluids, leading to rapid dehydration, ionary imbalances and hypoproteinemia. The correction of these imbalances is as important as the choice of the right prokinetic drug for successful treatment of ileus in horses. Finally, it is clear that up to the present time a generally efficacious prokinetic drug for the treatment of ileus in horses has not yet become available. Clinical efficacy reports are often very contradictory, which makes it sometimes difficult to justify the use of these often very expensive agents. More fundamental research is needed to reveal enteric receptor populations that are important for GI motility and that can be used as pharmacological targets for prokinetic drug use in horses.

REFERENCES

ADAMS, S.B., LAMAR, C.H. & MASTY, J. (1984). Motility of the distal portion of the jejunum and pelvic flexure in ponies: effects of six drugs. *Am. J. Vet. Res.*, **45**, 795-799.

ADAMS, S.B. & MARGARET, A. (1985). Neostigmine methylsulphate delays gastric emptying of particulate markers in horses. *Am. J. Vet. Res*., **46**, 2498-2499.

ALIBIBI, R. & McCALLUM, P.W. (1983). Metoclopramide: pharmacology and clinical application. *Ann.Int. Med.*, **36**, 454-457.

BAKER, S.J. & GERRING, E.L. (1994). Gastric emptying of solid, non-digestible, radiopaque markers in ponies. *Res. Vet. Sci.*, **56**, 386-388.

BARTON, M.H., PARVIANEN, A. & NORTON, N. (2004). Polymixin B protects horses against induced endotoxemia in vivo. *Eq. Vet. J.*, **5**, 397-401.

BRIANCEAU, P., CHEVALIER, H. & KARAS, A. (2002). Intravenous lidocaine and small-intestinal size, abdominal fluid, and outcome after colic surgery in horses. J. Vet. Int. Med., **16**, 736-741.

BRIEJER, M.R.; AKKERMANS, L.M.A., LEFEBVRE, R.A., SCHUURKES, J.A.J. (1995). Novel 5-HT_2-like receptor mediates neurogenic relaxation of the guinea-pig proximal colon. *Eur. J. Pharmacol.*, **279**, 123-133.

BURNS, G.A. (1992). The teniae of the equine intestinal tract. *Cornell Vet.*, **82**, 187-212.

CHEY, W.D. (2004). Tegaserod, the global experience. *Aliment. Pharm. Therap. Suppl.*, **20**, 15-19.

COATNEY, R.W. & ADAMS, S.B. (1988). The effect of motilin on equine small intestinal motility during experimental postoperative ileus. *Proceedings Equine Colic research symposium.*, **3**, 12.

COOLS, F., BENATAR, A., BOUGATEF, A. & VANDENPLAS, Y. (2001). The effect of cisapride on the corrected QT interval and QT dispersion in premature infants. *J. Pediatr. Gastr. Nutr.*, **33**, 178-181.

COTE, N.M., MOHAMED, N.A., BURTON, J., KOENIG, J.B., WEESE, J.S. (2005). 8th International Colic Research Symposium, Canada, Quebec, p26-27.

DABAREINER, R.M., SNYDER, J.R. & SULLINS, K.E. (1993a). Evaluation of the microcirculation of the equine jejunum and ascending colon after ischemia and reperfusion. *Am. J. Vet. Res.* **54**, 1683-1692.

DABAREINER, R.M., SNYDER, J.R. & SULLINS, K.E. (1993b). Evaluation of the microcirculation of the equine small intestine after intraluminal distention and subsequent decompression. *Am J Vet Res.*, **54**, 1673-1682.

DART, A.J., PEAUROI, J.R. & HODGSON, D.R. (1996). Efficacy of metoclopramide for treatment of ileus in horses following small intestinal surgery: 70 cases (1989-1992). *Austr. Vet. J.*, **74**, 280-284.

DAVIES, J.V. & GERRING, E.L. (1983a). Elektromechanical activity of the equine small intestine and its correlation with transit of fluid through Thiry-Vella loops. *Res. Vet. Sci.*, **34**, 327-333.

DAVIES, J.V. & GERRING, E.L. (1983b). Effect of spasmolytic analgesic drugs on the motility patterns of the equine small intestine. *Res. Vet. Sci.*, **34**, 334-339.

DAVIES, J.V. & GERRING, E.L. (1985). Effect of experimental vascular occlusion on small intestinal motility in ponies. *Eq. Vet. J.,* **17**, 219-224.

DE GEEST, J., MUYLLE, E., VLAMINCK, K., DEPREZ, P., SUSTRONCK, B. & PICAVET, M.T. (1991). A clinical study of cisapride in horses after colic surgery. *Eq. Vet. Educ.*, **3**,138-142.

DOHERTY, T.J., ANDREWS, F.M. & PROVENZA, M.K. (1998) Acetamoniphen as a marker of gastric emptying in ponies. *Eq. Vet. J.,* **30**, 349-351.

DOHERTY, T.J. & ANDREWS, F.M. (1999). The effect of sedation on gastric emptying of a liquid marker in ponies. *Vet. Surg.,* **28**, 375-379.

DELESALLE. C., DEPREZ, P., SCHUURKES, J.A.J. & LEFEBVRE, R.A. (2005a). In vitro effects of 5-hydroxytryptamine on the jejunum of healthy and colic horses: characterization of a putative 5-HT_{1A} receptor in the equine gut. *Proceedings of the 8th International Equine Colic Research Symposium, Quebec, Canada.*

DELESALLE, C., DEPREZ, P., SCHUURKES, J.A.J. & LEFEBVRE, R.A. Contractile effects of 5-hydroxytryptamine and 5-carboxamidotryptamine in the equine jejunum. (2005b). *Br. J. Pharmacol.,* **147**, 23-35.

DELESALLE, C., DEWULF, J., LEFEBVRE, R.A., SCHUURKES, J.A.J., VAN VLIERBERGEN, B. & DEPREZ, P. Use of plasma ionized calcium levels and Ca^{2+} substitution response patterns as prognostic parameters for ileus and survival in colic horses. (2005c). *Vet Quart.,* **27**, 157-172.

DOMENEGHINI, C., RADAELLI, G., ARRIGHI, S. & BOSI, G. (2004). Cholinergic, nitrergic and peptidergic (substance P-and CGRP-utilizing) innervation of the horse intestine. A histochemical and immunohistochemical study. *Histol Histopathol., **19**,* 357-370.

DREW, G.M. (1978). Pharmacological characterization of presynaptic alpha adrenoreceptors regulating cholinergic activity in the guinea pig ileum. *Brit. J. Pharmacol.,* **64**, 293-300.

EADES, S.C. & MOORE, J.N. (1993). Blockade of endotoxin-induced cecal hypoperfusion and ileus with an alpha 2 antagonist in horses. *Am. J. Vet. Res.,* **54**, 586-590.

FINLEY, M.R., Li, Y. & HUA, F. (2002). Expression and co-association of ERG1, KCNQ1 and KCNE1 potassium channel proteins in horse heart. *Am. J. Physiol.- Heart C.,* **283**, H126-H138.

FINTL, C., HUDSON, N.P.H. & MAYHEW, I.G. (2004). Interstitial cells of Cajal (ICC) in equine colic: an immunohistochemical study of horses with obstructive disorders of the small and large intestine. *Eq. Vet. J.* **36**, 474-479.

FIORUCCI, S., SANTUCCI, L. & MORELLI, A. (1993). 5-hydroxytryptamine 3-receptor antagonist modulates gallbladder emptying and motilin release induced by erythromycin. *Dig. Dis. Sci.,* **38**, 2236-2240.

FRENCH, N.P., SMITH, J., EDWARDS, G.B. & PROUDMAN, C.J. (2002). Equine surgical colic: risk factors for postoperative complications. *Eq. Vet. J.,* **34**, 444-449.

GERRING, E.E.L. & HUNT, J.M. (1986). Pathophysiology of equine postoperative ileus: Effect of adrenergic blockade, parasympathetic stimulation and metoclopramide in an experimental model. *Eq. Vet. J.,* **18**, 249-255.

GERRING, E.L. & KING, J.N. (1989). Cisapride in the prophylaxis of equine postoperative ileus. *Eq. Vet. J. Suppl.,* **7**, 52-55.

GERRING, E.L., KING, J.N. & EDWARDS, G.B. (1991) A multicentre trial of cisapride in the prophylaxis of equine postoperative ileus. *Eq. Vet. Educ.,* **3**, 143-145.

GERRING, E.L. (1992). Management of intestinal ileus in horses. *Comp. Cont. Educ. Pract. Vet.,* **14**, 1102-1104.

GONCALVES, S., JULLIAND, V. & LEBLOND, A. (2002). Risk factors associated with colic in horses. *Vet. Res.,* **33**, 641-652.

GROUDINE, S.B. & FISHER, H.A. (1998) Intravenous lidocaine speeds the return of bowel function, decreases postoperative pain and shortens hospital stay in patients undergoing radical retropubic prostatectomy. *Anesth. Analg.,* **86**, 235-239.

GUSTAFFSON, A., BAVERUD, V. & GUNNARSSON, A. (2004) Study of faecal shedding of Clostridium difficile in horses treated with penicillin. *Eq. Vet. J.,* **2**, 180-182.

HIRST, G.D.S. & EDWARDS, F.R. (2004) Role of interstitial cells of Cajal in the control of gastric motility. *J. Pharmacol. Sci.,* **96**, 1-10.

HUDSON, N.P.H., PEARSON, G.T., KITAMURA, N. & MAYHEW, I.G. (1999). An immunohistochemical study of interstitial cells of Cajal (ICC) in the equine gastrointestinal tract. *Res. Vet. Sci.,* **66**, 265-271.

HUDSON, N.P.H., MAYHEW, I.G. & PEARSON, G.T. (2001) A reduction in interstitial cells of Cajal in horses with equine dysautonomia (grass sickness). *Auton. .Neurosci. – Basic.*, **20**, 37-44.

HUNT, J.M. (1985). The pathophysiology of post operative ileus. PhD Thesis, University of London.

ITOH, Z., NAKAYA, M. & SUZUKI, T. (1984). Erythromycin mimics exogenous motilin in gastrointestinal contractile activity in the dog. *Am. J. Physiol.,* **247**, G688-G694.

KING, J.N. & GERRING, E.L. (1988). Actions of the novel gastrointestinal prokinetic agent cisapride on equine bowel motility. *J. Vet. Pharmacol. Ther.,* **11**, 314-321.

KING, J.N. & GERRING, E.L. (1989). Observations on the colic motor complex in a pony with a small intestinal obstruction. *Eq. Vet. J. Suppl.,* **7**, 43-45.

KING, J.N. & GERRING, E.L. (1992) Cisapride does not modify equine gastrointestinal motility disrupted by E. coli endotoxin or prostaglendin E_2. *J. Gastrointest. Mot.,* **4**, 261-269.

KITAMURA, N., YAMADA, J. & CALINGASAN, N.Y. (1984). Immunocytochemical distribution of endocrine cells in the gastrointestinal tract of the horse. *Eq. Vet. J.*, **16**, 103-107.

LAMAR, C.H., MASTY, J., ADAMS, S.B. (1984). Impedance monitoring of equine intestinal motility. *Am. J. Vet. Res.* **45**, 810-812.

LESTER, G.D., BOLTON, J.R., THURGATE, S.M. Computer-based collection and analysis of myoelectric activity of the intestine in horses. *Am. J. Vet. Res.* **53**, 1548-1552

LESTER, G.D., MERRITT, A.M. & NEUWIRTH, L. (1998, a). Effect of erythromycin lactobionate on myoelectric activity of ileum, cecum, and right ventral colon, and cecal emptying of radiolabeled markers in clinically normal ponies. *Am. J. Vet. Res.* **59**, 328-334.

LESTER, G.D., MERRITT, A.M. & NEUWIRTH, L. (1998, b). Myoelectric activity of the ileum, cecum, and right ventral colon, and cecal emptying of radiolabeled markers in clinically normal ponies. *Am. J. Vet. Res.*, **59**, 313-319.

LESTER, G.D., MERRITT, A.M. & NEUWIRTH, L. (1998, c). Effect of alpha2-adrenergic, cholinergic, and nonsteroidal anti-inflammatory drugs on myoelectric activity of ileum, cecum, and right ventral colon and on cecal emptying of radiolabeled markers in clinically normal ponies. *Am. J. Vet. Res.*, **59**, 320-327.

LEVY, M. & SOJKA, J. (1991) Control of gastric emptying in the horse: effect of cisapride. *Proceedings 4th Equine Colic Research Symposium*, **17**.

LILLICH, J.D., RAKESTRAW, P.C., ROUSSEL, A.J., FINLEY, M.R., GANTA, S. & FREEMAN, L. (2003). Expression of the ether-a-go-go (ERG) potassium channel in smooth muscle of the equine gastrointestinal tract and influence on activity of jejunal smooth muscle. *Am. J. Vet. Res.*, **64**, 267-272.

LIPPOLD, B.S., HILDEBRAND, J. & STRAUB, R. (2004) Tegaserod (HTF 919) stimulates gut motility in normal horses. *Eq. Vet. J.*, **36**, 622-627.

LOWE, J.E., SELLERS, A.F. & BRONDUM, J. (1980) Equine pelvic flexure impaction: a model used to evaluate motor events and compare drug response. *Cornell Vet.*, **70**, 401-412.

MACHARG, M.A., ADAMS, S.B., LAMAR, C.H. & BECHT, J.L. (1986) Electromyographic, myomechanical, and intraluminal pressure changes associated with acute extraluminal pressure changes associated with acute extraluminal obstruction of the jejunum in conscious ponies. *Am. J. Vet. Res.* **47**, 7-11.

MALONE, E.D., TURNER, T.A. & WILSON, J.H. (1990). Intravenous lidocaine for treatment of equine ileus. *J. Vet. Int. Med.*, **13**, 229.

MASRI, M.D., MERRITT, A.M. & BURROW, J.A. (1991) Effect of erythromycin on equine colonic motility. *Proceedings 4th Equine Colic Research Symposium*., **47**.

MEDEIROS, J.A.S., PONTES, F.A., MESQUITA, A.R.O. (1997). *Dis. Colon. Rectum*. **40**, 93-99.

MEISLER, S.D., DOHERTY, T.J. & ABRAHA, T. (1997). The effects of yohimbine on endotoxin-induced delayed gastric emptying in horses. *Proceedings 15th American College Veterinary Internal Medicine Forum.*

MERRITT, A.M., CAMPBELL-THOMPSON, M.L., LOWREY, S. (1989a). Effect of xylazine treatment on equine proximal gastrointestinal tract myoelectrical activity. *Am. J. Vet. Res.* **50**, 945-949.

MERRITT, A.M., CAMPBELL-THOMPSON, M.L., LOWREY, S. (1989b). Effect of butorphanol on equine antroduodenal motility. Eq. Vet. J. Suppl. **7**, 21-23

MERRITT, A.M., PANZER, R.B., LESTER, G.D., BORROW, J.A. (1995). Equine pelvic flexure myoelectric activity during fed and fasted states. *Am. J. Physiol.* **269**, G262.

MERRITT, A.M., BURROW, J.A., HARTLESS, C.S. (1998). Effect of xylazine, detomidine, and a combination of xylazine and butorphanol on equine duodenal motility. *Am. J. Vet. Res.* **59**, 619-623.

MOORE, J.N. & BARTON, M.H. (2003). Treatment of endotoxemia.*Vet. Clin. North. Am. Eq. Pract.*, **19**, 681 – 695.

NELLGARD, P., JONSSON, A. & BOJO, L. (1996). Small-bowel obstruction and the effects of lidocaine, atropine and hexamethonium on inflammation and fluid losses. *Acta. Anaesth. Scand.*, **40**, 287-292.

NIETO, JE. & RAKESTRAW, P.C. (2000) In vitro effects of erythromycin, lidocaine, and metoclopramide on smooth muscle from the pyloric antrum, proximal portion of the duodenum, and middle portion of the jejunum of horses. *Am. J. Vet. Res.* **61**, 413-419.

NIETO, J.E., SNYDER, J.R., KOLLIAS-BAKER, C. & STANLEY, S. (2000b). In vitro effects of 5-hydroxytryptamine and cisapride on the circular smooth muscle of the jejunum of horses. *Am. J. Vet. Res.*, **61**, 1561-1565.

NIEUWENHUYS, V.B., VAN DIJK, J.E. & GOOSZEN, H.G. (2002). Obstructive Jaundice, Bacterial Translocation and Interdigestive Small-Bowel Motility in Rats. *Digestion.*, **62**, 255-261.

PEETERS, T.L., MATTHYS, G. & DEPOORTERE, I. (1989) Erythromycin is a motilin receptor agonist. *Am. J. Physiol.,* **257**, G469-G474.

PEETERS, T.L. (1993). Erythromycin and other macrolids as prokinetic agents. *Gastroenterology.,* **105**, 1886-1899.

PHANEUF, L.P., GRIVEL, M.L. & RUCKEBUSCH, Y. (1972). Electromyoenterography during normal gastrointestinal activity, painful or non-painful colic and morphine analgesia in the horse. *Can. J. Comp. Med.,* **36**, 138-144.

PIERO, J.R., BARNABE, P.A. & CADIOLI, F.A. (2000a). Clinical and laboratory effects of lidocaïne continuous infusion during experimental endotoxaemia in horses. *Seventh International Equine Colic Research Symposium. Manchester, UK,* **133**.

PIERO, J.R., CUNCHA, F.Q. & VALADAO, C.A.A. (2000b). Serum and peritoneal tumour necrosis factor levels after lidocaine continuous infusion during experimental endotoxaemia in horses. In: *Seventh international equine colic research symposium. Manchester, UK,* **108**.

PRESCOTT, J.F. & HOFFMANN, A.M. (1993). Rhodococcus equi. *Vet. Clin. North. Am. Eq. Pract.*, **9**, 375-384.

RANG, H.P., DALE, M.M. & RITTER, J.M. (1995). Neuroleptic drugs. In: Rang H.P., Dale M.M. (Eds). *Pharmacology*, 2nd ed. Churchill Livingstone, New York. 576-594**.**

RIMBACK, G., CASSUTO, J. & TOLLESSON, P.O. (1990). Treatment of postoperative paralytic ileus by intravenous lidocaine infusion.. An*esth. Analg.*, 414-419.

RINGGER, N.C., LESTER, G.D., NEUWIRTH, L., MERRITT, A.M., VETRO, T., HARRISON, J. (1996). Effect of bethanecol or erythromycin on gastric emptying in horses. *Am. J. Vet. Res.*, ***57***, 1771-1775.

ROBERTS, M.C. & SEAWRIGHT, A.A. (1983). Experimental studies of drug-induced impaction colic in the horse. *Eq. Vet. J.,* **15**, 222-228.

ROGER, T., BARDON, T. & RUCKEBUSCH, Y. (1985). Colonic motor responses in the pony: relevance of colonic stimulation by opiate antagonists. *Am. J. Vet. Res.* **46**, 31-35.

ROSS, M.W., RUTKOWSKI, J.A. & CULLEN, K.K. (1989). Myoelectric activity of the cecum and right ventral colon in female ponies. *Am. J. Vet. Res.,* **50**, 374-379.

ROSS, M.W., CULLEN, K.K. & RUTKOWSKI, J.A. (1990) Myolectric activity of the ileum, cecum, and right ventral colon during the interdigestive, nonfeeding, and digestive periods. *Am. J. Vet. Res.,* **51**, 561-566.

ROUSSEL, A.J., HOOPER, R.N. & COHEN, N.D. (2000). Prokinetic effects of erythromycin on the ileum, cecum, and pelvic flexure of horses during the postoperative period. *Am. J. Vet. Res.,* **61**, 420-424.

ROUSSEL, A.J., HOOPER, N.R. & COHEN, N.D. (2003). Evaluation of the effects of penicillin G potassium and potassium chloride on the motility of the large intestine in horses. *Am. J. Vet. Res.* **64**, 1360-1363.

RUCKEBUSCH, Y., GRIVEL, M.L. & HATLEY, F. (1971). Electromyographic study on motor profile of small intestine in horse, Equus-Caballus. *Comptes rendus des séances de la société de biologie et de ses filiales.,* **165**, 1454.

RUCKEBUSCH, Y. & BUENO, L. (1975). Electrical activity of the ovine jejunum and changes due to disturbances. *Dig. Dis. Sci.*, **20**, 1027-1034.

RUCKEBUSCH, Y.& BUENO, L. (1976). The effect of feeding on the motility of the stomach and small intestine in the pig. *Brit. J. Nutr.*, **35**, 397-405.

RUCKEBUSCH, Y. (1981). Motor functions of the intestine. *Adv. Vet. Sci. Comp. Med.*, **25**, 345-369.

RUCKEBUSCH, Y. & MERRITT, A.M. (1985). Pharmacology of the ruminant gastroduodenal junction. *J. Vet. Pharmacol. Ther.*, **8**, 339-351.

RUCKEBUSCH, Y. & ROGER, T. (1988). Prokinetic effects of cisapride, naloxone, and parasympathetic stimulation on the equine ileo-caeco-colonic junction. *J. Vet. Pharmacol. Ther.*,. **11**, 322-329.

RUTKOWSKI, J.A., ROSS, M.W. & CULLEN, K. (1989) Effects of xylazine and/or butorphanol or neostigmine on myoelectric activity of the cecum and right ventral colon in female ponies. *Am. J. Vet. Res.*, **50**, 1096-1101.

SANCHEZ, L.C., ELFENBEIN, J., MOCK, J., ROBERSTON, S., KOLLIAS-BAKER, C. (2005) Effect of detomidine on somatic and visceral nociception and duodenal motility in conscious horses. 8th International Equine Colic Research Symposium, Canada Quebec.

SASAKI, N.L. & YOSHIHARA, T. (1999). The effect of motilin on the regulation mechanism of intestinal motility in conscious horses. *J. Vet. Med. Sci.* **61**, 167-170.

SASAKI, N. & YOSHIHARA, T. (2000). The effect of orally administered cisapride on intestinal motility of conscious horses. *J. Vet. Med. Sci.*, **62**, 211-213.

SANCHEZ, L.C. & MERRITT, A.M. (2005). Colorectal distention in the horse: visceral sensitivity, rectal compliance and effect of iv xylazine or intrarectal lidocaïne. *Eq. Vet. J.*, **37**, 70-74.

SCHUSSER, G.F. & WHITE, N.A. (1997). Morphologic and quantitative evaluation of the myenteric plexus and neurons in the large colon of horses. *JAVMA.*, **210**, 928-934.

SCHUSSER, G.F., SCHIEDEMANN, W. & HUSKAMP, B. (2000). Muscle thickness and neuron density in the caecum of horses with chronic recurrent caecal impaction. *Eq. Vet. J. Suppl.*, **32**, 69-73.

SCIORSCI, R.L., LACALANDRA, G.M. & MINOIA, P. (2000). Opioid involvement in equine colic syndrome: clinical observations and therapy. *Seventh International Equine Colic Research Symposium. Manchester UK*, **130**.

SELLERS, A.F., LOWE, J.E. & DROST, C.J. (1982, a). Retropulsion-propulsion in equine large colon. *Am. J. Vet. Res.*, **43**, 390-396.

SELLERS, A.F., LOWE, J.E. & RENDANO, V.T. (1982, b). The reservoir function of the equine cecum and ventral large colon: its relation to chronic nonsurgical obstructive disease with colic. *Cornell Vet.*, **72**, 233-241.

SOJKA, J.E. & ADAMS, S.B. (1988). Effect of butorphanol, pentazocine, meperidine, and metoclopramide on motility in female ponies. *Am. J. Vet. Res.*, **49**, 527-529.

SONG, C.W., LEE, K.Y., KIM, C.D., CHANG, T.M. & CHEY, W.Y. (1997). Effect of cisapride and renzapride on gastrointestinal motility and plasma motilin concentration in dogs. *J. Pharmacol. Exp. Ther.*, **281**, 1312-1316.

SUTTON, D.G.M., PRESTON, T. & CHRISTLEY, R.M. (2002). The effects of xylazine, detomidine, acepromazine and butorphanol on equine solid phase gastric emptying rate. *Eq. Vet. J.*, **34**, 486-492.

THIELEMANS, L., DEPOORTERE, I., PERRET, J., ROBBERECHT, P., LIU, Y., THIJS, T. & CARRERAS, C. (2005) Desensitization of the Human Motilin Receptor by Motilides. *J. Pharmacol. Exp. Ther.*, **313**, 1397-1405.

THOMPSON, L.P., MERRITT, A.M. & MADISON, J.B. (1994). Effect of bethanechol on equine gastric motility and secretion. *Proceedings Equine Colic Research Symposium* **5**, 12.

TOMOMASA, T., KUROUME, T. & ARAI, H. (1986). Erythromycin induces migrating motor complex in human gastrointestinal tract. *Dig. Dis. Sci.*, **31**, 157-161.

TONINI, M. (1996). Recent advances in the pharmacology of gastrointestinal prokinetics. *Pharmacol. Res.* **33**, 217-226.

VALK, N., DOHERTY, T.J. & BLACKFORD, J.T. (1998). Effect of cisapride on gastric emptying in horses following endotoxin treatment. *Eq. Vet. J.*, **30**, 344-348.

VAN DER VELDEN; M.A. & KLEIN, W.R. (1993). The effects of cisapride on the restoration of gut motility after surgery of the small intestine in horses: a clinical trial. *Vet. Quart.* **15**, 175-179.

VAN HOOGMOED, L.M. & SNYDER, J.R. (1997). Adjunctive methods in equine gastrointestinal surgery. *Vet. Clin. North. Am. Eq. Pract.* **13**, 221-242.

VAN HOOGMOED, L.M., NIETO, J.E., SNYDER, J.R., HARMON, F.A. (2004). Survey of prokinetic use in horses with gastrointestinal injury. *Vet. Surg*. **33**, 279-285.

WEBER, F.H., RICHARDS, R.D. & McCALLUM, R.W. (1993). Erythromycin: a motilin agonist and gastrointestinal prokinetic agent. *Am. J. Gastroenterol.*, **88**, 485-490.

WEISS, R., ABEL, D., SCHOLTYSIK, G., STRAUB, R. & MEVISSEN, M. (2002). 5-hydroxytryptamine mediated contractions in isolated preparations of equine ileum and pelvic flexure: pharmacological characterization of a new 5-HT4 agonist. *J. Vet. Pharmacol. Ther.*, **25**, 49-58.

YAMADA, T., MIZUMOTO, A. & SATOH, M. (1997). Muscarinic control of phase 3 contractions and motilin release in dogs. *Peptides.*, **18**, 673-680.

Chapter 1.III

SEROTONIN AND GI MOTILITY

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SUMMARY

In the GI tract, serotonin is predominantly encountered at the level of the enterochromaffin cells and the enteric nervous system, where it exerts its effects through activation of a wide array of specific serotonergic receptors. Up until now very little is known about the equine serotonergic enteric receptor population. The pattern of the equine enteric serotonergic receptor population that has been identified up until now doesn't correspond with the human pattern. Despite this knowledge, expensive human prokinetic agents are still used in colic horses, however with inconsistent to poor results. More research is needed into the specific serotonergic receptor population in the horse that can function as pharmacological target for potential motility mediating medication.

1.III.1. INTRODUCTION

Some 30 years ago, the chemical structure of serotonin was discovered by Rapport and his co-workers (Rapport *et al*. 1974). They identified the substance as a potent vasoconstricting agent, circulating in the blood, hence its name: "sero-tonin". Shortly after, serotonin and enteramine were synthesized and found to be both 5-HT (Erspamer & Asero, 1952). The amino-acid trypthophan is used as main ingredient for the build-up of the serotonin molecule. Serotonin can be found in men, animals and even plants.

In drug development, serotonin is without any doubt the most targeted neurotransmitter of all. Medication that is designed to modulate the serotonergic nervous system is used in a wide array of pathological conditions like depression, anxiety, obsessive compulsive disorders, schizophrenia, hypertension, pulmonary hypertension, eating disorders, blood clotting disorders, migraine, nausea and GI problems like gastro-oesophageal reflux disease, bloating, irritable bowel syndrome, chronic constipation, etc. If one realizes that the human body contains on average only 10mg of serotonin, it can easily be comprehended that this is a very

potent substance indeed. For more than two decennia, researchers know that important amounts of this potent serotonin can be found in the human GI system. It is estimated that as much as 90% of the total amount of 5-HT in the body is located here (Erspamer, 1966; Thompson, 1971).

As a neurohumoral messenger, serotonin is synthesized and stored in different celltypes. About 80% is stored at the level of the enteric enterochromaffin (EC) cells (Erspamer, 1966), whereas about 10% can be encountered in the enteric neurons, like the myenteric plexus(Costa *et al*., 1982). An important amount of 5-HT can also be found in the circulating blood platelets, that function as free 5-HT scavengers. Finally, 5-HT is also found in the central nervous system, hence its wide array of applications in psychopharmacology (Barnes *et al*., 1999; Spiller, 2002).

Serotonin fulfills as neurotransmitter (Gershon & Erde, 1981) important tasks at the level of the GI system. It takes part in secretion processes (Cussato *et al*. 1982) and in the regulation of GI motility and sensitivity (Gershon *et al*., 1990). Furthermore it is an important modulator of the blood circulation and vascular permeability (Jandu *et al*., 2001). The fact that 5-HT also is found in enteric mast cells, supports the idea that it also interacts with the immune system (Kraneveld, 1994). Bulbring was the first researcher to associate serotonin with GI motility. It took over 40 years of intensive research to get some insight into the complex properties of this potent substance (Bulbring & Crema, 1959). No doubt that the wide array of existing 5-HT receptor subtypes, the diversity of their localizations and intracellular signal transduction systems, are causes of the complexicity of research in this area. Until now 7 classes of 5-HT receptors, encompassing 14 different 5-HT receptor subtypes, have been identified. Each of these receptors with their own specific affinity and intracellular second messenger system. The fairly recent development of specific 5-HT

receptor agonists and antagonists has greatly improved the efficacy with which the different 5-HT receptor subtypes can be studied (Sarna *et al*. 2000).

Currently, most newly developed enteral motility modulating agents use the serotonergic nervous system as their target. Several of these expensive human prokinetic agents are used in colic horses. Despite this evolution, very little is known about the equine serotonergic enteric nervous system. A systematic characterization of the equine enteric serotonergic receptor population through application of a wide array of specific 5-HT receptor antagonists, has not been performed until now.

The following report gives an overview of what is currently known about the interactions of 5-HT with the human GI tract and what is known about the serotonergic system in the horse.

1.III.2. ENTERIC DISTRIBUTION OF 5-HT

In the human intestine, 5-HT is found in the mucosal enterochromaffin cells (EC cells) and in the neurons of the myenteric and submucosal plexuses (Costa *et al*., 1982; Fujimiya *et al*., 1997a). The bulk-load of 5-HT is found in the secretory granules of the EC cells, which are part of the entero-endocrine cell population of the intestine (Fujita *et al*., 1988). These specialized cells are very important regulators of the digestive physiology. The EC cells are embedded between the epithelial mucosal cells in an average ratio of 1 on 100 and are predominantly found at the base of the mucosal crypts. Up until now, as many as 20 different types of EC cells have been identified. They have small (200 to 400 nm) pleomorphic secretory granules, which are localized in the apical and basolateral cytoplasm (Fujimiya *et al*., 1997b). Besides 5-HT, these secretory granules can also contain peptides like cholecystokinine (CCK), neurotensine, glucagon-like peptide 1 (GP-1) and peptide YY (PYY) (Cristina *et al*., 1978). In humans, the entero-endocrine cells are found along the full

length of the GI tract. About 50% of these cells contain 5-HT (Sjolund *et al*., 1983) and are also called enterochromaffin cells, since they can be visualized histologically through use of silver staining.

EC cells are equipped with apical microvilli to detect luminal processes, which on their turn can trigger the release of 5-HT from the secretory granules. Not only mechanical stimulation of these apical villi, but also bacterial toxins, like cholera toxin and cytotoxic pharmaca, used to treat cancer, can stimulate 5-HT exocytosis (Minami *et al*., 2003). This process of exocytosis takes place at the basolateral membrane of the EC cells. During exocytosis, 5-HT is released directly into the portal circulation, where it can be scavenged by the circulating blood platelets. In this way the amount of free circulating 5-HT is controlled. It has been demonstrated that some cases of human depression are related with disturbances in the 5-HT uptake capacity from these platelets (Muller-Oerlinghausen *et al*., 2004). Besides this direct portal release of 5-HT, the EC cells can also secrete a small amount of 5-HT directly into the intestinal lumen via the apical or mucosal membrane (Beubler, 1995; Fujimiya *et al*., 1998). The mucosal cells that line the intestine have a specific transmembrane transport system at their disposal, named “SERT” or “serotonin reuptake transporter” to inactivate this luminally released 5-HT (Chen *et al*., 1998). Some prokinetic agents stimulate the aforementioned luminal 5-HT release, but whether this 5-HT actually participates in the regulatory mechanisms controlling GI motility, is still subject of debate (Kojima, 1999; Fukumoto *et al*., 2003).

Bearcroft and his co-workers found an abnormally large EC cell population and an increased tendency to postprandial 5-HT release in patients suffering from irritable bowel syndrome (IBS). They suggest that these findings can partly explain diarrhea and disturbances in GI motility, found in these patients (Bearcroft *et al*., 1998).

In contrast to humans, the EC cell population in horses can only be found in the proximal part of the GI tract, with a clear tendency of decrease in distribution density from oral to aboral (Ceccarelli *et al*., 1995). The equine EC cells can be found in large amounts at the level of the gastric pylorus, to a lesser extend at the level of the gastric fundus and finally only a small amount in the equine duodenum. No EC cells could be identified in the equine jejunum, caecum and colon. A more recent study however, performed by Fink et al. (2006) reports the presence of EC cells in the mucosal epithelium of the whole equine GI tract (Fink *et al.* 2006), which is in accordance with findings in humans and other mammals (Rizzotti *et al*., 1979; Peranzi & Lehy, 1984; Krause *et al*., 1985; Ceccarelli *et al*., 1991).

1.III.3. EFFECTS OF 5-HT ON THE GI SYSTEM

5-HT modulates GI motility, secretion and sensitivity. The well known “migrating myoelectrical compex” or “MMC”, the typical enteral motility pattern that can be distinguished in between uptake of meals, needs 5-HT for a normal functionality (Pineiro-Carrero *et al*., 1991).

5-HT mediates its wide array of GI effects most often through direct activation of specific 5-HT receptors localized in the GI system. These receptors can reside on neurons but can also be localized directly on smooth muscle cells. Acetylcholine acts as an important contractile neurotransmitter in the intestine. Activation of 5-HT_4 receptors localized on cholinergic neurons, facilitates the release of acetylcholine. The action of many human prokinetic agents is based on this effect. A direct myogenic receptor localization implicates the occurrence of contraction or relaxation, upon activation, depending on the 5-HT receptor subtype that has been activated (Cfr. Infra) Besides these peripheral 5-HT-receptor effects, many in vivo studies have identified interactions of 5-HT with receptors localized in the central nervous system, leading to effects in the GI system. Rouzade *et al.*, demonstrated that stimulation of 5-

HT_{1A} receptors in the rat brain leads to gastric contractions (Rouzade *et al.*, 1998). In the mid-1980s it was discovered that serotonin is at least partially responsible for producing chemotherapy-induced nausea and vomiting, predominantly through activation of 5-HT_3 receptors localized in the chemoreceptor trigger zone of the central nervous system (Gregory & Ettinger, 1999). Hence the development of 5-HT_3 blocking agents like tropisetron, to counter-act chemotherapy induced nausea and vomiting.

1.III.4. 5-HT RECEPTOR SUBTYPES AND "SECOND MESSENGER SYSTEMS"

Up until now 7 major serotonergic receptor classes, containing 14 different 5-HT receptor subtypes have been identified and characterized in mammals. In the past, all 5-HT receptors were classified as M or D receptors (Gaddum & Picarelli, 1997). Those found on neurons were termed M receptors because they were blocked by morphine, while the smooth muscle receptors were termed D receptors because they were inhibited by phenoxybenzamine (Gaddum & Picarelli, 1957). Some 30 years ago, the 5-HT_1, 5-HT_2 and 5-HT_3 receptors were proposed, of which the latter two correspond with the formerly termed D and M receptors (Bradley *et al.*, 1986). Nowadays this classification is out of date and a new classification has been proposed by Hoyer *et al.*, which up until now serves as a base for nomenclature of newly discovered 5-HT receptors (Hoyer *et al.*, 1994). Indeed, up to 7 populations of 5-HT receptors, 5-HT_1 to 5-HT_7 have been reported. Briefly, the currently known and characterized 5-HT receptor subtypes are: 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-ht_{1E}, 5-ht_{1F}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_3, 5-HT_4, 5-ht_{5a}, 5-ht_{5b}, 5-HT_6 en 5-HT_7. Capitals are used to indicate those receptor subtypes for which a clear in vivo physiological role has been identified and proven. In other words, these receptors trigger upon activation in their natural environment a clear and quantifiable process, like induction of enzymatic processes, changes in permeability of the

cell membrane or smooth muscle contraction (Hoyer & Martin, 1997). The aforementioned receptor classification is certified and approved by “The Receptor Nomenclature Committee of the International Union of Pharmacology” or “NC-UIPHAR”. The 5-ht_{1p} receptor is somewhat an exception, as its presence at the level of the enteric nervous system is still subject of debate. Since some research groups still feel that this newly proposed 5-HT_{1p} receptor, actually is the 5-HT_{1B} receptor, it is currently not included in the IUPHAR classification.

When localized neuronaly, the 5-HT receptors can modulate neurotransmitter release upon activation. However, as mentioned previously, they can also reside directly upon the smooth muscle cell membrane, as has been described for the 5-HT_{2A}, the 5-HT_{2B}, the 5-HT_4 and the 5-HT_7 receptor.

The aforementioned 7 5-HT receptor classes can be subdivided into two main families on pharmacological bases: there are the G-protein coupled receptors (5-$HT_{1,2,4,5,6,7}$) and the ligand-gated ion channel receptor (5-HT_3). G-protein stands for guaninenucleotide-binding protein. G-protein coupled receptors contain 7 transmembrane α-helices, each of them provided with 3 extra-cellular and 3 intra-cellular amino-acid arches (Figure 1.III.1).

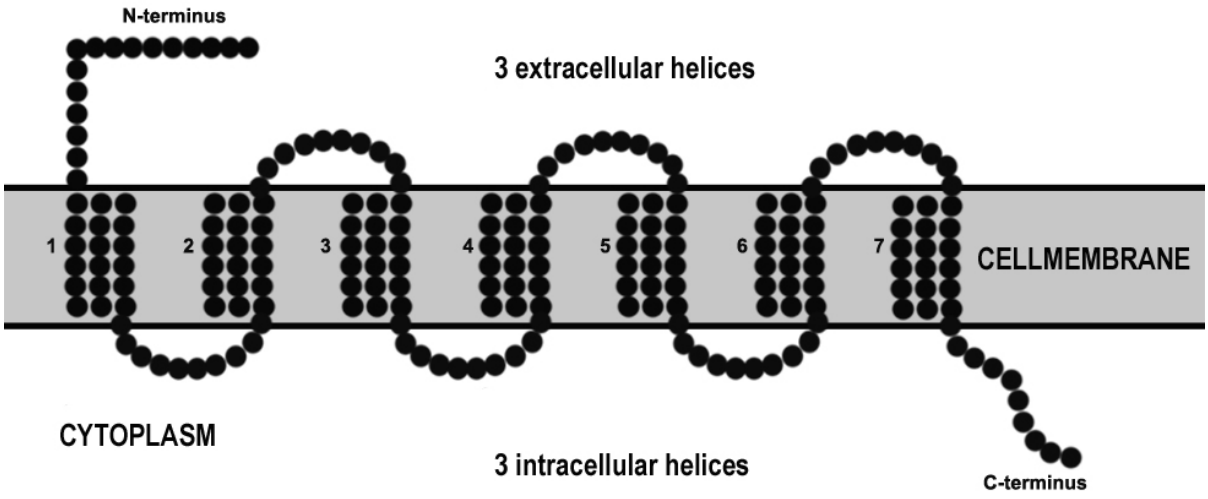


Figure 1.III.1: Classical conformation of a G-protein coupled receptor.

The G-protein binds to the third intra-cellular arch. The receptor agonist binding zone is most often found at the level of the transmembrane crossings of the α-helices. The membrane-bound G-proteins link their receptor to a specific intracellular "second messenger" system, like adenylate cyclase or phospholipase C. This coupling can have stimulating (s) or inhibiting (i) purposes. G_s stimulates the enzyme adenylate cyclase, whereas G_i inhibits it. G_q stimulates phospholipase C, which triggers on its turn the conversion of phosphatidyl inositol (PIP_2) into diacylglycerol (DAG).

The following cascade of events takes place when 5-HT binds to a G-protein coupled receptor that uses adenylate cyclase as second messenger (Figure 1.III.2):

Upon binding of an agonist, the G-protein is coupled to the receptor. This leads to the conversion of guanosine-di-phosphate (GDP) to guanosine-tri-phosphate (GTP) at the level of the G_α subunit of the G-protein. The G-protein breaks down into a βγ and an α-subunit. The latter, can activate (α_s), or inhibit (α_i) the conversion of ATP to cAMP, by influencing the enzyme adenylate cyclase. What takes place thereafter, depends upon the specific localization of the receptor. At the level of the myocardium, cAMP increases mediate positive inotropic effects. On the other hand, cAMP increases in vascular and GI smooth muscle cells will mediate relaxation. 5-HT_4, 5-HT_6 en 5-HT_7 receptors are positively coupled to adenylate cyclase, whereas a negative coupling is found for the 5-HT_1 en 5-ht_5 receptors.

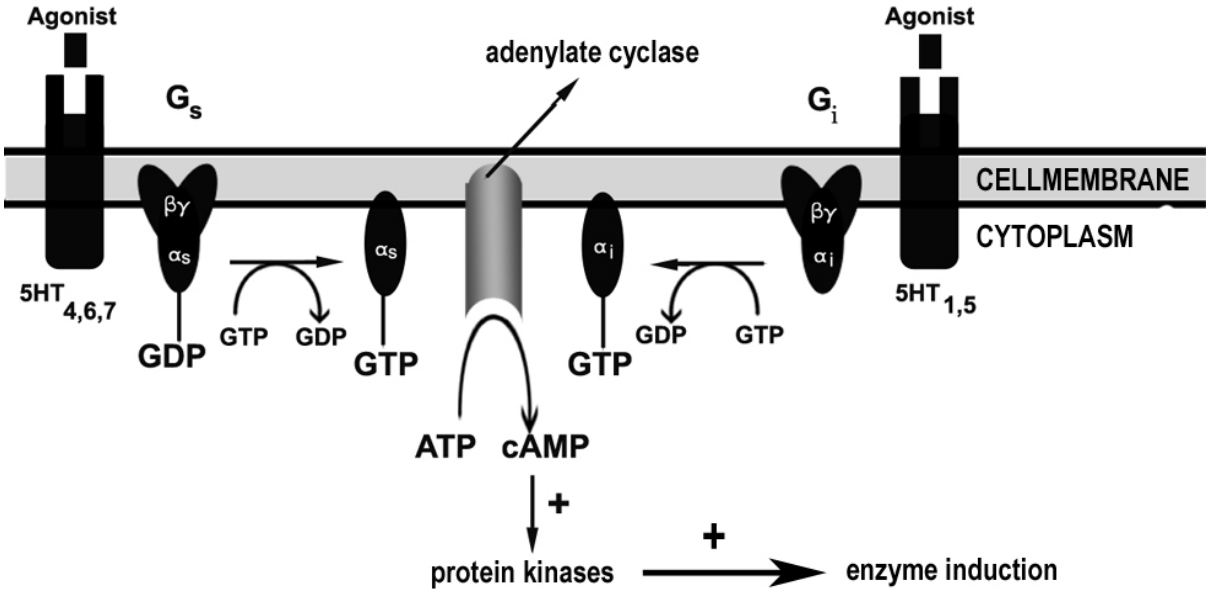


Figure 1.III.2: Signaling cascade of G-protein-coupled serotonergic receptors, positively (G_s) or negatively (G_i) coupled to adenylate cyclase.

The cascade of events that takes place when 5-HT binds to a G-protein coupled receptor that uses phospholipase C as second messenger, is in its initial steps comparable with the aforementioned cascade (Figure 1.III.3). However, here the α-subunit of the G-protein stimulates phospholipase C. This leads to the break down of PIP_2 into IP_3 and DAG. The increased intra-cellular IP_3 levels will stimulate the release of Ca^{2+} out of the endoplasmatic reticulum through stimulation of specific Ca^{2+} channels. DAG on its turn will activate protein kinases, which can modulate a wide array of biological processes. 5-HT_2 receptors are positively coupled to phospholipase C.

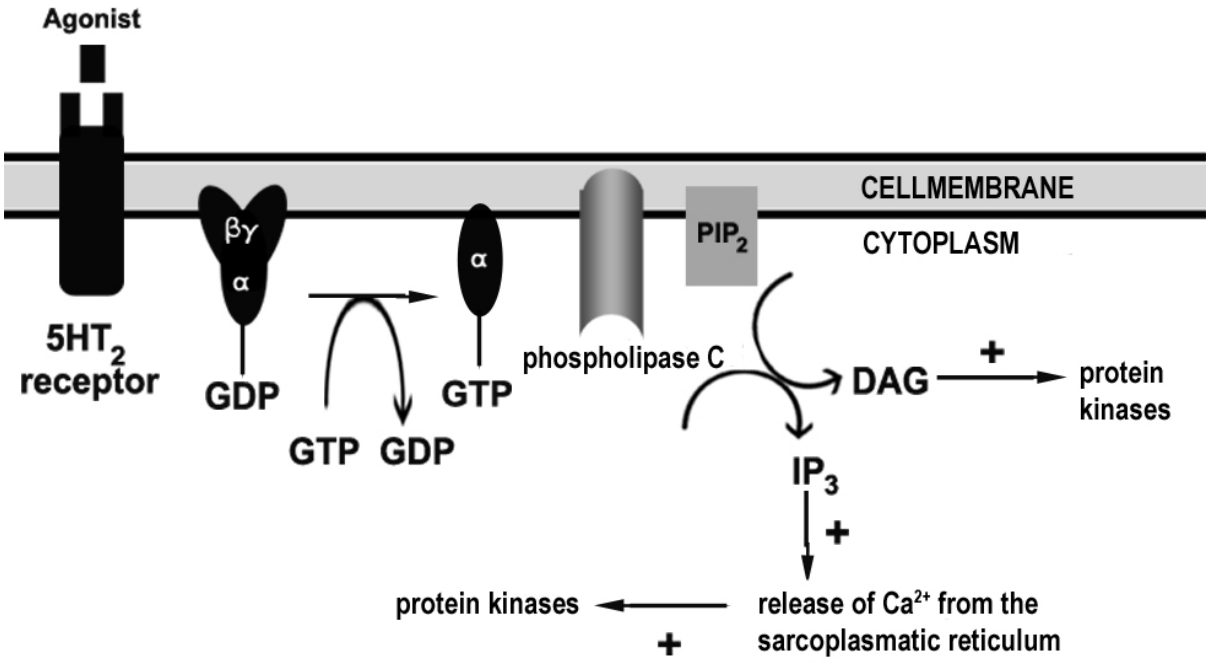


Figure 1.III.3: Signaling cascade of G-protein-coupled serotonergic receptors, coupled to phospholipase C.

Amplification of the receptor mediated effects is achieved by activation of numerous G-proteins upon receptor stimulation. For example, one 5-HT receptor can be coupled to as many as 100 G-proteins. Some G-proteins are coupled to more than one "second messenger" system at the same time and some G-proteins are not always linked with the same type of second messenger system. Not surprisingly, disturbances in the G-protein activity can lead to pathological processes. The toxin that is produced by Vibrio cholerae, irreversibly activates G_s-protein. This leads to an enormous accumulation of cAMP in the enterocytes, with important enteral water loss as a consequence (Farfel *et al*. 1999).

The second group of receptors encompasses the ligand gated ion channels. Of all 5-HT receptor subtypes, the 5-HT_3 receptor is the only one that can be classified in this group (Figure 1.III.4).

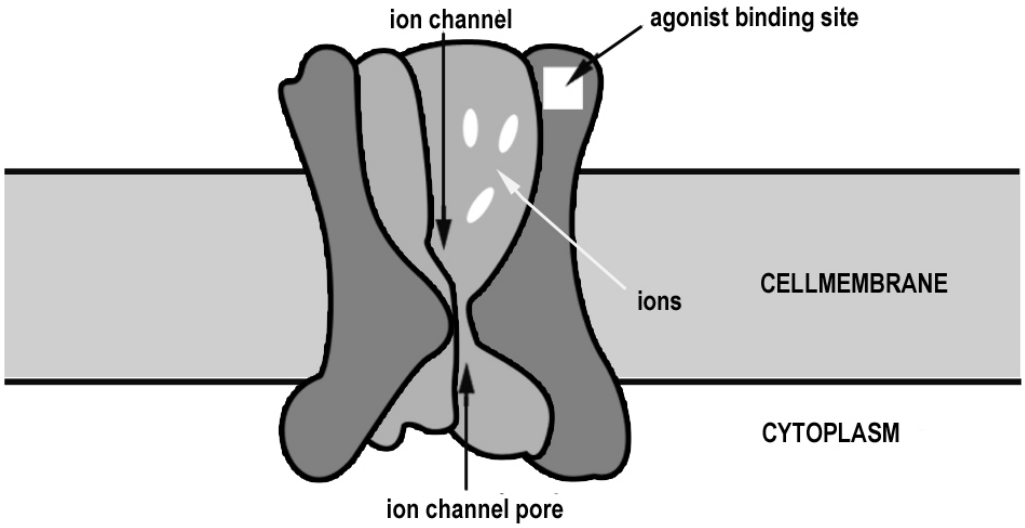


Figure 1.III.4: Conformation of a ligand gated ion channel receptor

Up until now only neuronal localizations have been described for the 5-HT_3 receptor. Here, the agonist binds directly to the ion channel, typically leading to swift transmission of neural impulses at the level of the synapse. Receptor activation triggers rapid influx of Na^+ and Ca^{2+} into the neuronal cell, with depolarization as a consequence.

The 5-HT_3 receptor has a typical pentamere structure of which the subunits rotate upon receptor activation. This leads to opening of the ion channel (Mott *et al*., 2001; Unwin, 2000).

Desensitization or internalization are both features that apply for all 5-HT receptors. These two negative feed-back mechanisms enable the 5-HT receptors to regulate the cellular response upon persisting presence of agonists. In the case of desensitization, the receptor reshapes itself into an inactive conformation, whereas internalization encompasses an actual process of endocytosis leading to the intracellular uptake of the receptor itself. Some 5-HT receptor subtypes are more prone to the aforementioned negative feedback processes than others. Also the inclination towards desensitization can be influenced by factors that affect the binding of agonists upon the receptor (Lalley *et al*., 1994).

1.III.5. 5-HT RECEPTOR SUBTYPES AND GI MOTILITY

Up until now following 5-HT receptor subtypes have been related with regulation of GI motility: 5-HT_{1A}, 5-ht_{1p}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_3, 5-HT_4 en 5-HT_7. These receptors can reside in the central nervous system, or at the level of the intestine itself.

5-HT_{1A} receptors are distributed throughout the CNS, while their presence is recognized in the vascular and GI system as well. Stimulation of 5-HT_{1A} receptors in the rat brain, triggers gastric contractions (Rouzade *et al*., 1998). In dogs 5-HT_{1A} receptor agonists induce gastric relaxation, which can be blocked by vagotomy. Whether these receptors are localized on vagal afferents or in the central nervous system could not be discerned (Janssen *et al*., 2003). In the GI tract, 5-HT_{1A} receptors are found on EC cells and neurons of both the submucous and myenteric plexus (Kirchgessner *et al*., 1996). No enteric direct smooth muscle localization has been described for this receptor. Moreover, up until now, the enterally identified 5-HT_{1A} receptors only are involved in relaxation of smooth muscle cells. In the guinea pig ileum, for example, they reduce electrically induced contraction (Forster *et al*., 1995). Indeed, 5-HT_{1A} receptor activation has been found to induce inhibition of acetylcholine release from the guinea-pig myenteric plexus (Dietrich & Kilbinger, 1996). In the isolated guinea-pig whole stomach preparation the non-selective 5-HT_1 receptor agonist sumatriptan induces relaxation, through activation of 5-HT_{1A} receptors on cholinergic motor neurons (Meulemans *et al*., 1996).

In humans, sumatriptan is used to treat migraine. Here, in vivo tests have demonstrated the occurrence of gastric relaxation and a decreased gastric sensitivity to distention in human subjects treated with this drug. Phase 3 of the MMC was clearly suppressed at the level of the stomach and occurred prematurely in the jejunum (Tack *et al.* 1998; Tack *et al*., 2000). These effects were attributed to the putative 5-ht_{1p} receptor.

Although, several in vitro studies point out that 5-HT_2 receptors can influence GI motility, only limited in vivo proof supports these observations (Matsuda *et al*. 2000). In vitro stimulation of smooth muscle cell 5-HT_2 receptors, triggers contraction. In guinea-pigs, rats and dogs, 5-HT mediates GI contractions through activation of enterally localized 5-HT_{2A} and 5-HT_{2B} receptors (Briejer *et al*.,1995a; Baxter *et al*., 1994; Prins *et al*., 1997; Prins *et al*., 2001). Borman and his co-workers demonstrated the presence of contractile 5-HT_{2A} receptors localized on human small intestinal smooth muscle cells (Borman & Burleigh, 1997). 5-HT_{2B} receptors are identified immunohistochemically on smooth muscle cells and enteric neurons of human fetal stomach and small intestinal tissue. Stimulation of these 5-HT_{2B} receptors facilitates development and differentiation of enteric neurons (Fiorica-Howells *et al*., 2000).

With respect to the 5-HT_3 receptor, there is substantial proof that both the central nervous system and enteric localization are involved in regulation of GI motility. As mentioned previously, the 5-HT_3 receptor antagonist ondansetron inhibits chemotherapy induced nausea and vomiting by blocking 5-HT_3 receptors localized on vagal afferents and in the central chemoreceptor trigger zone. Hence its use in cancer patients (de Wit & Alphen, 2003). Further research with specific 5-HT_3 blocking agents in humans, revealed that 5-HT stimulates gastric emptying and transit via these 5-HT_3 receptors (Akkermans *et al*., 1988; Houghton *et al*., 2000). Similarly in rats, 5-HT_3 agonists, dose dependently stimulate the occurrence of small intestinal MMCs (Lördal & Hellström, 1999). Inhibition of visceral afferent nociceptive neurons by 5-HT_3 blocking agents suppresses visceral nociception by rectal distention in dogs and by distention of the colon in patients suffering from irritable bowel syndrome (IBS) (Balfour *et al*., 2000). These findings have led to the therapeutic use of 5-HT_3 blocking agents in IBS patients (De Ponti *et al*., 2001).

Of all 5-HT receptor subtypes, the GI effects mediated by the 5-HT_4 receptors are probably most studied. Numerous in vitro and in vivo studies illustrate the motility stimulating effects

upon 5-HT_4 receptor activation at the level of the human stomach and small and large intestine. Up until now, only peripheral serotonergic effects have been attributed to this receptor in the GI tract.

Reviewing all reported effects is beyond the scope of this article. However, as rule of thumb it can be stated that the enteral 5-HT_4 receptors are predominantly localized on enteric cholinergic neurons, where they stimulate acethylcholine release upon activation (Prins *et al*., 2000a). At the level of the ileum and colon, they have also been identified directly on smooth muscle cells, where they mediate relaxation (Prins *et al*., 2000b).

Finally, also the 5-HT_7 receptor subtype is involved in regulation of GI motility. This receptor is positively coupled to an adenylate cyclase second messenger system and its greatest abundance is found in the brain (Neumaier *et al*., 2001). In the rat, 5-HT_7 receptor mRNA was isolated in the myocard and the GI system (Plassat *et al*., 1993). Different in vitro studies on peripheral tissues have demonstrated the receptor on smooth muscle cells, mediating relaxation. It is shown that smooth muscle 5-HT_7 receptors mediate relaxation of isolated canine antrum and human colonic muscle strips (Prins *et al.,* 2001). The peristaltic reflex in guinea-pigs is suppressed by 5-HT_7 receptor activation (Tuladhar & Naylor, 2003). To our knowledge, no in vivo studies have demonstrated the involvement of 5-HT_7 receptors in GI motility.

In the horse Nieto and co-workers report that the contractile effects of 5-HT in vitro in the jejunal circular smooth muscle are suppressed by the 5-HT_2 receptor antagonist ketanserin and the 5-HT_3 receptor antagonist tropisetron. No effect could be elicited by the 5-HT_4 receptor antagonist SDZ 205 557, atropine and tetrodotoxin (TTX), a neurotoxic agent that inhibits neuronal conduction (Nieto *et al*., 2000). These study results suggest that 5-HT mediates its promotile effects on the jejunal circular muscle through activation of 5-HT_2 and 5-HT_3 receptors. Since both atropine and TTX have no effect, a non-cholinergic, non-

neuronal pathway is proposed for these effects. This is very peculiar, since up until now no other study reports a non neuronal localization for the 5-HT_3 receptor.

When the 5-HT_4 receptor antagonist SDZ 205 557 was applied on ileal and pelvic flexure circular smooth muscle strips, some inhibition could be detected. This suggests the presence of 5-HT_4 receptors at this level. However, only clear contractile effects could be elicited in the pelvic flexure segments, when the specific 5-HT_4 receptor agonist HTF 919 was used. Also 5-HT_3 receptor antagonists showed inhibiting effects in these segments, suggesting that 5-HT_3 receptors are also involved in the 5-HT induced contractions (Weiss *et al*., 2002).

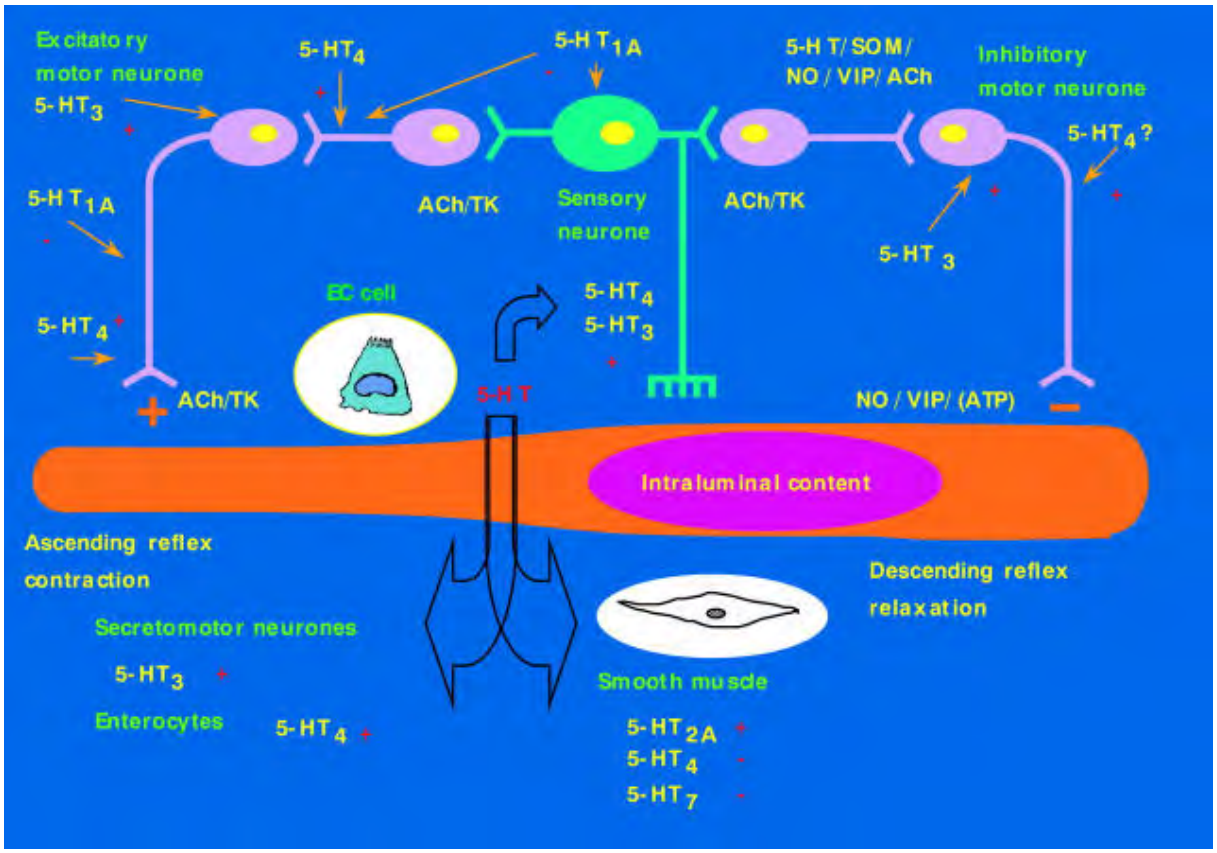
It is clear that the equine enteric serotonergic receptor population shows distinct features. When compared with the human situation, many differences are found. Therefore no success or even any therapeutic effect can be guaranteed when these expensive human prokinetic agents are used in horses. Also, in analogy with human research, only the presence of 5-HT_2, 5-HT_3 and 5-HT_4 receptors has been investigated. Maybe other 5-HT receptor subtypes are important for regulation of GI motility in horses. Identification of these receptors could lead to development of new motility modulating therapies.

Table 1.III.1 and addendum: Reported effects of 5-HT on the human and equine GI system, through activation of serotonergic receptors.

	5-HT$_{2A}$	**5-HT$_3$**	**5-HT$_4$**
HUMANS			
Stomach	contraction[1]	contraction[2]	contraction[3]
Small Intestine			
Colon		contraction[2]	contraction[3] relaxation[4]
HORSES			
Jejunum	contraction[5]	contraction[5]	
Pelvic Flexure		contraction[6]	contraction[6]

1. Via 5-HT$_{2A}$-receptors localized directly on smooth muscle cells
2. Probably through activation of 5-HT$_3$-receptors localized on vagal afferent neurons
3. Via 5-HT$_4$-receptors localized on cholinergic enteric neurons
4. Via 5-HT$_4$-receptors localized directly on smooth muscle cells
5. The effect can not be blocked by atropine and TTX, and thus is probably mediated through activation of receptors localized directly on smooth muscle cells
6. TTX was not tested, therefore no differentiation can be made between a neuronal or muscular receptor localization.

Addendum: Modulation of human intestinal function by serotonin (5-hydroxytryptamine or 5-HT) receptors.



Distension by intraluminal contents stimulates sensory neurones (intrinsic primary afferent neurones) which trigger an ascending excitatory reflex (leading to contraction) and a descending inhibitory reflex (leading to relaxation). Transmitters released by interneurones in the ascending reflex include acetylcholine (ACh) and substance P (a tachykinin) whereas descending interneurones belonging to different subpopulations may use 5-HT, somatostatin (SOM), vasoactive intestinal polypeptide (VIP), nitric oxide (NO), ACh, and other mediators as transmitters. Excitatory motor neurones release ACh and tachykinins (TK) at the neuromuscular junction whereas inhibitory motor neurones may release NO, VIP, or ATP depending on the gut level and on the animal species. 5-HT released from enterochromaffin (EC) cells may affect several subtypes of enteric neurones (sensory, motor, and secretomotor neurones) as well as final effector cells (smooth muscle cells and enterocytes). +, stimulation;−, inhibition;?, circumstantial evidence.
Source: De Ponti F et al., 2004 : Pharmacology of serotonin : what a clinician should know. *Gut* 2004; **53**: 1520-1535.

1.III.6. APPLICATION OF 5-HT RECEPTOR AGONISTS AND ANTAGONISTS IN THE TREATMENT OF GASTROINTESTINAL MOTILITY DISORDERS

All the aforementioned research results illustrate the therapeutic potential that 5-HT receptor agonists and antagonists have in the pathophysiology of the GI tract. No wonder that most newly marketed prokinetic drugs use the serotonergic system as their target. Some of these agents are used to treat ileus in horses, however with inconsistent to poor results.

In human medicine cisapride (Prepulsid®) is used to treat reflux oesophagitis, gastroparesis and chronic intestinal pseudo-obstruction. Nowadays the use of this drug is greatly restricted due to cardiac side effects, namely a prolongation of the QT interval. Cisapride activates presynaptic 5-HT_4 receptors, which on their turn facilitate the release of acetylcholine, leading to promotile activity. Cisapride is known as a partial 5-HT_4 receptor agonist and a partial 5-HT_3 receptor antagonist (Taniyama *et al.*, 1991). Recently also agonistic effects have been reported on 5-HT_1 receptors and blocking effects on 5-HT_2 receptors (Briejer *et al.*, 1995b). In the dog gastric antrum, cisapride also mediates non-serotonergic effects (de Ridder & Schuurkes, 1993). Cisapride shows many inter species differences, when its effects are evaluated and within one species the effects can clearly differ depending on which segment of the GI tract is observed (Goldhill *et al.*, 1997; Tonini *et al.*, 1989). Both atropine and TTX have no effect on the in vitro response of equine jejunal circular smooth muscle to cisapride. This suggests a non cholinergic, non neuronal pathway for the actions of cisapride. Nieto and

co-workers propose the 5-HT_2 receptor as possible target, since ketanserin could antagonize the cisapride induced contractile response (Nieto *et al.*, 2000). This is a very peculiar observation since up until now only blocking effects of cisapride at the level of 5-HT_2 receptors have been described. The in vitro study results of Weiss and his co-workers show some evidence for the presence of 5-HT_4 receptors at the level of the ileum and pelvic flexure. Therefore it is possible that in these intestinal segments cisapride interacts with 5-HT_4 receptors (Weiss *et al.*, 2002). Another possibility is that cisapride triggers the release of another effector like motilin, which on its turn modulates contractions. Such a cascade of events has already been demonstrated in humans and dogs (Song *et al.*, 1997). In horses however, it has never been investigated whether uptake of serotonergic agonists leads to increases in plasma motilin levels. A third possible action pathway for cisapride, is the blocking of ether-a-go-go K^+ channels (ERG-K^+ channels). By blocking these K^+ specific channels, cisapride prevents the inward influx of K^+ ions, leading to depolarization and subsequently contraction of smooth muscle cells (Lillich *et al.*, 2003). Since these ion specific channels are also present in the human myocard, they are also responsible for the aforementioned cardiac side effects of cisapride (Mohammad *et al.*, 1997). The ERG-K^+ channels have been identified in the equine GI tract from duodenum to colon. When ERG-K^+ channel blockers are applied on circular jejunal muscle strips of horses, a contractile effect similar to the effect of cisapride can be elicited. However as in humans, the ERG-K^+ channels are also found in the equine myocard. Therefore one has to proceed with caution when using this product in horses (Finley *et al.*, 2002). In view of the identified equine serotonergic enteric receptor population and the reported side-effects, cisapride seems not to be the ideal prokinetic agent for the horse.

Further research has led to the development of the more specific 5-HT_4 receptor agonist tegaserod. This drug facilitates colon transit in humans and is used to treat obstipation

dominant irritable bowel syndrome (IBS) (Appel *et al*., 1997). In vitro, this drug had only limited effects on the equine ileum and pelvic flexure (Weiss *et al*., 2002). In vivo there was evidence of a shortening of the oro-anal transit time in healthy horses, which was however thought to be predominantly mediated by an increased colonic acitivity (Lippold *et al*., 2004). This observation renders tegaserod as less suitable to treat small intestinal ileus.

Also the use of the recently developed 5-HT_3 antagonists ondansetron, granisetron, alosetron and cilansetron, seems contra-indicated in horses, based on the in vitro study results that identify the 5-HT_3 receptor as important for intestinal contractile activity.

It is clear that the use of human prokinetic drugs in equine colic patients is no guarantee for therapeutic success. Moreover, a judicious use of these therapeutics requires a thorough knowledge of their action pathways and of the equine enteral serotonergic receptor population.

1.III.7. SEROTONIN AND ENDOTOXEMIA

Another important factor which has to be taken into account, when evaluating clinical therapeutic efficacy of certain promotility drugs in horses, is endotoxemia. A lot of horses suffering from ileus, also show signs of endotoxemia. The classical scenario is this of the newly developed human prokinetic agent, that gets introduced as the promising anti-ileus therapy in horses, based on in vitro and in vivo study results in healthy horses. However, when these drugs are then used in real clinical cases, therapeutic efficacy is disappointing. Disturbed intestinal myoelectrical and myomechanical patterns have been described in horses during iv infusion of endotoxin. However, iv infusion of LPS doesn't completely mimic the clinical picture of the endotoxaemic colic horse suffering from ileus. Also there is no non-invasive technique to evaluate objectively small intestinal coordinated propulsive activity, which then could be applied in real colic cases. Therefore clinical studies use all kinds of

rough parameters, like time to first defaecation, or amount of produced reflux, to evaluate therapeutic efficacy of promotility drugs in real colic patients. This leads inevitably to subjectivity and therefore most equine clinical studies can not be considered as truly double-blind.

It is possible that during colic or ileus large amounts of 5-HT are released into the circulation of the horse. Both clotting of circulating blood platelets and EC cells of necrotizing intestinal segments could serve as a 5-HT source in colic horses.

It is known in horses that intestinal ischemia renders the mucosa more permeable. This leads to an important translocation of endotoxins and dietary amines, amongst which 5-HT, out of the intestinal contents into the systemic circulation (Snyder, 1989; Morris, 1991; Turnage *et al*., 1994). 5-HT is widely known as a prostaglandine synthase independent activator of blood platelets, capable of even reinforcing the effects of other platelet activators (Holmsen *et al*., 1985).

Research into the ethiopathogenesis of laminitis in horses has revealed that iv infusion of E. coli lipopolysacharide, to mimic endotoxemia, leads to a distinct increase of 5-HT and thromboxane β_2 levels in these horses. The release of thromboxane β_2 is a clear sign of platelet activation (Bailey *et al*., 2000; Elliott *et al*., 2003; Menzies-Gow *et al*., 2004).

As mentioned previously, circulating blood platelets function as free 5-HT scavengers to regulate plasma 5-HT levels. Therefore, a decreased 5-HT uptake capacity also could lead to important increases in plasma 5-HT levels. It has already been demonstrated that this 5-HT uptake capacity is not a constant and that it can be influenced by many factors. In humans, for example, the platelet 5-HT uptake capacity is clearly suppressed after abdominal surgery (Naesh *et al*., 2001).

As mentioned previously, also the EC cells of necrotizing intestinal segments can serve as 5-HT source. Indeed, in dogs and rats, intestinal ischemia and reperfusion is accompagnied by

a distinct increase in the plasma 5-HT levels (Teramoto *et al*., 1998; Nakamura *et al*., 2001). This increase in 5-HT has even been described in humans suffering from simple intestinal obstruction (Prasad *et al*., 1977). Also the effects on the enterocytes and EC cells by chemotherapy, triggers the release of large amounts of 5-HT in cancer patients, leading to nausea and vomiting (Tan *et al*., 2003).

Increases in plasma 5-HT levels can have important consequences for colic horses. It has been demonstrated many times, both in vitro and in vivo that 5-HT is an important and very potent vaso-constricting agent in horses (Elloitt *et al*., 2003, Bailey *et al*., 2003, Menzies-Gow *et al*., 2004). Accumulation of 5-HT in the systemic circulation of colic horses could reinforce ongoing intestinal ischemia. In rats it has been demonstrated that 5-HT stimulates the enteral translocation of endotoxins (Yamada *et al*. 2003). This could be true for the horse as well.

Also, increased mucosal and plasma 5-HT levels can influence the enteral expression of certain 5-HT receptor subtypes. As mentioned previously, both a decrease in sensitivity of the receptors for their agonists or an actual receptor internalization can take place, in answer to the presence of large amounts of agonist. Both processes serve as a natural defense mechanism of the receptor against an overstimulation of the receptor and are known as “receptor desensitization”. Chen and co-workers identified an aberrant GI motility pattern in SERT (Serotonin Reuptake Transporter) knock-out mice. These mice miss the serotonin re-uptake system, leading to large amounts of free 5-HT. Typically episodes of diarrhea, during which the serotonergic transmission is potentialized by the missing SERT, are alternated with episodes of pronounced waning of the GI motility. This waning is than caused by 5-HT receptor desensitization in the presence of an excess of 5-HT (Chen *et al*., 2001).

Doe-Young *et al.* (2002) even suggest that excess amounts of 5-HT have a negative effect on the clinical efficacy of applied serotonergic prokinetic agents.

5-HT is metabolized mainly in the intestines (Legay et al., 1983), kidneys (Adler et al., 1977) and liver (Cheifetz et al., 1980). In Beagle dogs it has been demonstrated that systemic 5-HT release due to intestinal ischemia leads to hepatic hypoperfusion and hepatocellular dysfunction. This was contributed to the platelet aggregating and vasoconstrictive properties of 5-HT (Nakamura et al., 2000). It could be that on its turn disturbed liver function leads to decreased 5-HT metabolism. Davis and co-workers (2003) have reported hepatic injury in horses suffering from duodenitis-proximal jejunitis. Besides injury caused by ascending infection from the common bile duct, they also propose the absorption of endotoxin or inflammatory mediators from the portal circulation, or hepatic hypoxia resulting from systemic inflammation and endotoxemic shock as possible causes of hepatic injury in these horses. The fact that markedly fewer horses suffering from small intestinal strangulation showed biochemical evidence of hepatic injury in this study, probably underlines the importance of endotoxins and inflammatory mediators.

1.III.8. CONCLUSION

It is clear that although many serotonergic prokinetic agents are used, the knowledge of the role of serotonin as an enteral neurotransmitter in horses is very scanty. Despite the lack of efficacious equine prokinetic therapies for horses suffering from ileus, only little fundamental research has been performed. This would be necessary in order to identify equine enteral receptors that can function as pharmacological target for development of prokinetic therapies. The empirical use of human promotility drugs has proven to be an expensive adventure with inconsistent to poor results. It is clear that there are distinct differences between the human and equine enteric serotonergic receptor population. More research into this area is needed.

Prokinetic therapy is most needed to treat horses after surgical resection of several meters of necrotized intestine or after surgical correction of tympanism due to carbohydrate overload.

Unfortunately, endotoxemia is quite often a distinct feature in these horses. And more research is needed here as well, if we are to find a scientifically well-founded treatment protocol for horses suffering from ileus.

REFERENCES

ADLER, S. (1977). Serotonin and the kidney, vol 4. New York: Spectrum Publications Inc; 99-137.

AKKERMANS, L.M.A., VOS, A., HOEKSTRA, A., ROELOFS, J.M. & HOROWITZ, M. (1988). Effect of ICS 205-930 (a specific 5-HT_3 receptor antagonist) on gastric emptying of a solid meal in normal subjects. *Gut.*, **29**, 1249-1252.

APPEL, S., KUMLE, A. & MEIER, R. (1997). Clinical pharmacodynamics of SDZ HTF 919, a new 5-HT4 receptor agonist, in a model of slow colonic transit. *Clin. Pharmacol. Ther.*, **62**, 546-55.

BAILEY, S.R., CUNNINGHAM, F.M. & ELLIOTT, J. (2000). Endotoxin and dietary amines may increase plasma 5-hydroxytryptamine in the horse. *Eq. Vet. J.*, **32**, 497-504.

BAILEY, S.R., WHEELER-JONES, C. & ELLIOTT, J. (2003). Uptake of 5-hydroxytryptamine by equine digital vein endothelial cells: inhibition by amines found in the equine caecum. *Eq. Vet. J.*, **35**, 164-169.

BALFOUR, J.A., GOA, K.L. & PERRY, C.M. (2000). Alosetron. *Drugs.*, **59**, 511-518.

BARNES, N.M. & SHARP, T. (1999). A review of central 5-HT receptors and their function. *Neuropharmacol.*, **38**, 1083-1152.

BAXTER, G.S., MURPHY, O.E. & BLACKBURN, T.P. (1994). Further characterization of 5-hydroxytryptamine receptors (putative 5-HT_{2B}) in rat stomach fundus longitudinal muscle. *Br. J. Pharmacol.*, **112**, 323-331.

BEARCROFT, C.P., PERRETT, D. & FARTHING, M.J. (1998). Postprandial plasma 5-hydroxytryptamine in diarrhoea predominant irritable bowel syndrome: a pilot study. *Gut.*, **42**, 42-46.

BEUBLER, E. (1995). Serotonin as an internal secretagogue. In Gaginella T.S. and Galligan J.J. (Eds). *Serotonin and gastrointestinal function*, Boca Raton, CRC Press, 85-108.

BORMAN, R.A. & BURLEIGH, D.E. (1997). 5-HT1D and 5-HT2B receptors mediate contraction of smooth muscle in human small intestine. *Ann. NY. Acad. Sci.*, **812**, 222-223

BRADLEY, P.B., ENGEL, G., FENIUK, W., FOZARD, J.R., HUMPHREY, P.P., MIDDLEMISS, D.N., MYLECHARANE, E.J., RICHARDSON, B.P. & SAXENA, P.R. (1986). Proposals for the classification and nomenclature of functional receptors for 5-hydroxytryptamine. *Neuropharmacol.*, **25**, 563-576.

BRIEJER, M.R., AKKERMANS, L.M.A. & SCHUURKES, J.A.J. (1995a). Interactions of serotonin with multiple receptors and neurotransmitters in the guinea-pig isolated colon. *Archives Internationales de Pharmacodynamie et de Therapie 329*, 121-133.

BRIEJER, M.R., AKKERMANS, L.M. & SCHUURKES, J.A. (1995b). Gastrointestinal prokinetic benzamides: the pharmacology underlying stimulation of motility. *Pharmacol. Rev.*, **47**, 631-51.

BULBRING, E. & CREMA, A. (1959). The release of 5-hydroxytryptamine in relation to pressure exerted on the intestinal mucosa. *J. Physiol.-London.*, **146**, 18-28.

CECCARELLI, P., PEDINI, V. & GARGIULO, A.M. (1991). Enteroendocrine cells in sheep fetuses. *Small Ruminant Res.,* **6**, 85-93.

CECCARELLI, P., PEDINI, V. & GARGIULO, M. (1995). Serotonin-containing cells in the horse gastrointestinal tract. *Anat, Histol, Embryol.,* **24**, 97-99.

CHEIFETZ, S., WARSH, J.J. (1980). Occurrence and distribution of 5-hydroxytryptophol in the rat. Journal of Neurochemistry 34, 1093-1099.

CHEN, J.X., PAN, H., ROTHMAN, T.P., WADE, P.R. & GERSHON, M.D. (1998). Guinea pig 5-HT transporter: cloning, expression, distribution, and function in intestinal sensory reception. *Am. J. Physiol.,* **275**, G433-G448.

CHEN, J.J., ZHISHAN, L., PAN, H., MURPHY, D.L., TAMIR, H., KOEPSELL, H. & GERSHON, M.D. (2001). Maintenance of serotonin in the intestinal mucosa and ganglia of mice that lack the High-Affinity Serotonin Transporter: Abnormal intestinal motility and the expression of cation transporters. *J. Neurosci.,* **21**, 6348-61.

COSTA, M., FURNESS, J.B., CUELLO, A.C., VERHOFSTADT, A.A.J., STEINBUSCH, H.D.J. & ELDE, R.P. (1982). Neurons with 5-hydroxytryptamine-like immunoreactivity in the enteric nervous system: their vizualisation and reactions to drug treatment. *Neurosci.,* **7**, 351-363.

CRISTINA, M.L., LEHY, T., ZEITOUN, P. & DUFOUGERAY, F. (1978). Fine structural classification and comparative distribution of endocrine cells in normal human large intestine. *Gastroenterol.,* **75**, 20-28.

CUSSATO, J., JODAL, M., TUTTLE, R. & LUNDGREN, O. (1982). 5-hydroxytryptamine and cholera secretion. *Scand. J. Gastroentero.,* **17**, 695-703.

DAVIS, J.L., BLIKSLAGER, A.T., CATTO, K., JONES, S.L. (2003). A retrospective analysis of hepatic injury in horses with proximal enteritis (1984-2002). *J. Vet. Intern. Med.* **17**, 896-901.

DE PONTI, F. & TONINI, M. (2001). Irritable Bowel Syndrome. New agents targeting Serotonin Receptor Subtypes. *Drugs.,* **61**, 317-32.

DE RIDDER, W.J. & SCHUURKES, J.A. (1993). Cisapride and 5-hydroxytryptamine enhance motility in the canine antrum via separate pathways, not involving 5-hydroxytryptamine 1, 2 , 3, 4 receptors. *J. Pharmacol. Exp. Ther.,* **264**, 79-88.

DE WIT, R. & VAN ALPHEN, M.M. (2003). New developments in the treatment of nausea and vomiting caused by chemotherapy. *Ned. Tijdschr. Genees.,* **147**, 690-694.

DIETRICH, C. & KILBINGER, H (1996). 5-HT1A receptor-mediated inhibition of acetylcholine release from guinea pig myenteric plexus: potential mechanisms. *Neuropharmacol*., **35**, 483-488.

DOE-YOUNG, K. & CAMILLERI, M. (2000). Serotonin: A mediator of the Brain-Gut connection. *Gut.,* **95**, 2698-2709.

ELLIOTT, J., BERHANE, Y. & BAILEY, S.R. (2003). Effects of monoamines formed in the cecum of horses on equine digital blood vessels and platelets. *Am. J. Vet. Res.*, **64**, 1124-1131.

ERSPAMER, V. & ASERO, B. (1952). Identification of enteramine, the specific hormone of the enterochromaffin cell system, as 5-hydroxytryptamine. *Nature*, **169**, 800-801.

ERSPAMER, V. (1966). Occurence of indolealkylamines in nature. In: Erspramer V. (editor). *Handbook of Experimental Pharmacology 5-Hydroxytryptamine and Related Indolealkylamines*, New York, Springer-Verlag, 132-181.

FARFEL, Z., BOURNE, H.R. & IIRI, T. (1999). The expanding spectrum of G protein diseases. *New. Engl. J. Med.,* **340**, 1012-20.

FINK, C., TATAR, M., FAILING, K., HOSPES, R., KRESSIN, M., KLISCH, K. (2006). Serotonin-Containing cells in the gastrointestinal tract of newborn foals and adult horses. *Anat. Histol. Embryol.* **35**, 23-27

FINLEY, M.R., LI, Y., HUA, F., LILLICH, J., MITCHELL,K.E., GANTA, S., GILMOUR, R.F. Jr. & FREEMAN, L.C. (2002). Expression and coassociation of ERG1, KCNQ1, and KCNE1 potassium channel proteins in horse heart. *Am. J. Physiol.- Heart C.*, **283**, H126-38.

FIORICA-HOWELLS, E., MAROTEAUX, L. & GERSHON, M.D. (2000). Serotonin and the 5-HT(2B) receptor in the development of enteric neurons. *J. Neurosci.,* **20**, 294-305.

FORSTER, E.A., CLIFFE, I.A., BILL, D.J., DOVER, G.M., JONES, D. & REILLEY, Y. (1995). A pharmacological profile of the selective silent 5-HT_{1A} receptor antagonist, WAY-100635. *Eur. J. Pharmacol.,* **281**, 81-88.

FUJIMIYA, M., OKUMIYA, K., YAMANE, T. & MAEDA, T. (1997a). Distribution of serotonin-immunoreactive nerve cells and fibers in the rat gastrointestinal tract. *Histochem. Cell. Biol.,* **107**, 105-14.

FUJIMIYA, M., OKUMIYA, K. & KUWAHARA, A. (1997b). Immunoelectron microscpoc study of the luminal release of serotonin from rat enterochromaffin cells induced by high intraluminal pressure. *Histochem. Cell. Biol.,* **108**, 105-113.

FUJIMIYA, M., YAMAMOTO, H. & KUWAHARA, A. (1998). Effect of VIP and PACAP on basal release of serotonin from isolated vascularly and luminally perfused rat duodenum. *Am. J. Physiol.*, **275**, G731-G739.

FUJITA, T., KANNO, T. & KOBAYASHI,S. (1988). In: *The paraneuron.* Springer Verlag, Tokyo.

FUKUMOTO, S., TATEWAKI, M., YAMADA, T., FUJIMIYA, M., MANTYH, C., VOSS, M., EUBANKS, S., HARRIS, M., PAPPAS, T.N. & TAKAHASHI, T. (2003). Short-chain fatty acids stimulate colonic transit via intraluminal 5-HT release in rats. *Am. J. Physiol. Reg. I.,* **284**, R1269-1276.

GADDUM, J.H. & PICARELLI, Z.P. (1997). Two kinds of tryptamine receptor. *Br. J. Pharmacol.,* **120** (4 Suppl):134-139.

GERSHON, M.D. & ERDE, S.M. (1981). The nervous system of the gut. *Gastroenterol.,* **80**, 1571-1594.

GERSHON, M.D., WAGE, P.R., KIRCHGESSNER, A.L. & TAMIR, H. (1990). 5-HT receptor subtypes outside the central nervous system. Roles in the physiology of the gut. *Neuropsychopharmacol.,* **3**, 385-395.

GOLDHILL, J., PORQUET, M.F. & ANGEL, I. (1997). Post-synaptic 5-HT4 receptor modulation of tachykinergic excitation of rat oesophageal tunica muscularis mucosae. *Eur. J. Pharmacol.,* **4**, 229-233.

HOLMSEN, H. (1985). Platelet activation and serotonin. In: Van Houtte P.M. (Ed). *Serotonin and the cardiovascular system*. New York, Raven Press: p. 75-86.

HOUGHTON, L.A., FOSTER, J.M. & WHORWELL, P.J. (2000). Alosetron, a 5-HT_3 receptor antagonist, delays colonic transit in patients with irritable bowel syndrome and healthy volunteers. *Aliment. Pharm. Therap.,* **14**, 775-782.

HOYER, D., CLARKE, D.E., FOZARD, J.R., HARTIG, P.R., MARTIN, G.R., MYLECHARANE, E.J., SAXENA, P.R. & HUMPHREY, P.P.A. (1994). International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (Serotonin). *Pharmacol. Rev.,* **46**, 157-203.

HOYER, D. & MARTIN, G. (1997). 5-HT receptor classification and nomenclature: towards a harmonization with the human genome. *Neuropharmacol.,* **36**, 419-28.

JANDU, K.S., BARRETT, V., BROCKWELL, M., CAMBRIDGE, D., FARRANT, D.R., FOSTER, C., GILES, H., GLEN, R.C., HILL, A.P., HOBBS, H., HONEY, A., MARTIN, G.R., SALMON, J., SMITH, D., WOOLLARD, P. & SELWOOD, D.L. (2001). Discovery of 4-[3-(trans-3-dimethylaminocyclobutyl)-1H-indol-

5-ylmethyl]-(4S)-oxazolidin-2-one (4991W93), a 5HT(1B/1D) receptor partial agonist and a potent inhibitor of electrically induced plasma extravasation. *J. Med. Chem.*, **44**, 681-93.

JANSSEN, P., PRINS, N.H., MOREAUX, B., MEULEMANS, A.L. & LEFEBVRE, R.A. (2003). In vivo characterization of 5-HT1A receptor-mediated gastric relaxation in conscious dogs. *Br. J. Pharmacol.*, **140**, 913-920.

KING, J.N. & GERRING, E.L. (1991). The action of low dose endotoxin on equine bowel motility. *Eq. Vet. J.*, **23**, 11.

KIRCHGESSNER, A.L., LIU, M.T., RAYMOND, J.R. & GERSHON, M.D. (1996). Identification of cells that express 5-hydroxytryptamine1A receptors in the nervous system of the bowel and pancreas. *J. Comp. Neurol*., **364**, 439-455.

KRAUSE, W.J., YAMADA, J. & CUTTS, J.M. (1985). Quantitative distribution of enteroendocrine cells in the gastrointestinal tract of the adult opossum, Didelphis Virginiana. *J. Anat.*, **140**, 591-605.

KOJIMA, S. (1999). KW-5092, a novel gastrokinetic agent, facilitates luminal serotonin release from the guinea-pig colon. *Eur. J. Pharmacol.*, **374**, 113-115.

KRANEVELD, A.D. (1994). Neuroimmune regulation of macrovascular permeability. Central role of the mast cell. PhD Thesis, Faculty of Pharmacy, Utrecht University, The Netherlands.

LALLEY, P.M., BISCHOFF, A.M. & RICHTER, D.W. (1994). 5-HT-1A receptor-mediated modulation of medullary expiratory neurones in the cat. *J. Physiol.*, **476**, 117-30.

LEGAY, C., FAUDON, M., HERY, F., RERNAUX, J.P. (1983). 5-HT metabolism in the intestinal wall of the rat-I. The mucosa. *Neurochemistry International* **5**, 721-7.

LILLICH, J.D., RAKESTRAW, P.C., ROUSSEL, A.J., FINLEY, M.R., GANTA, S. & FREEMAN, L.C. (2003). Expression of the ether-a-go-go (ERG) potassium channel in smooth muscle of the equine gastrointestinal tract and influence on activity of jejunal smooth muscle. *Am. J. Vet. Res.*, **64**, 267-72.

LIPPOLD, B.S., HILDEBRAND, J. & STRAUB, R. (2004). Tegaserod (HTF 919) stimulates gut motility in normal horses. *Eq Vet J*, **36**, 622-627.

LORDAL, M. & HELLSTROM, P.M. (1999). Serotonin stimulates migrating myoelectric complex via 5-HT_3-receptors dependent on cholinergic pathways in rat small intestine. *Neurogastroent. Motil.*, **11**, 1-10.

MATSUDA, H., LI, Y. & YOSHIKAWA, M. (2000). Possible involvement of 5-HT and 5-HT2 receptors in acceleration of gastrointestinal transit by escin Ib in mice. *Life. Sci.*, **66**, 2233-2238.

MENZIES-GOW, N.J., BAILEY, S.R., KATZ, L.M., MARR, C.M. & ELLIOTT, J. (2004). Endotoxin-induced digital vasoconstriction in horses: associated changes in plasma concentrations of vasoconstrictor mediators. *Eq. Vet. J.*, **36**, 273-278.

MEULEMANS, A.L., HELSEN, L.F., DE RIDDER, W.J.E. & SCHUURKES, J.A.J. (1996). Effects of sumatriptan on the guinea-pig isolated stomach. *Neurogastroenterol. Motil*., **8**, A183.

MINAMI, M., ENDO, T. & HIRAFUJI, M. (2003). Pharmacological aspects of anticancer drug-induced emesis with emphasis on serotonin release and vagal nerve activity. *Pharmacol. Therapeut.*, **99**, 149-165.

MOHAMMAD, S., ZHOU, Z., GONG, Q. & JANUARY, C.T. (1997). Blockage of the HERG human cardiac K+ channel by the gastrointestinal prokinetic agent cisapride. *Am. J. Physiol.*, **273**, H2534-2538.

MORRIS, DD. (1991). Endotoxemia in horses-A review of cellular and humoral mediators involved in ITS pathogenesis. *J. Vet. Int. Med.*, **5**, 167-181.

MOTT, D.D., ERREGER, K., BANKE, T.G. & TRAYNELIS, S.F. (2001). Open probability of homomeric murine 5-HT3A serotonin receptors depends on subunit occupancy. *J. Physiol.*, **535**, 427-443.

MULLER-OERLINGHAUSEN, B., ROGGENBACH, J. & FRANKE, L. (2004). Serotonergic platelet markers of suicidal behavior--do they really exist? *J. Affect. Disorders.,* **79**, 13-24.

NAESH, O, HINDBERG, I. & BRUUN, A.B. (2001). Decreased reuptake of serotonin in human platelets after surgery. *Clin. Physiol.,* **21**, 39-43.

NAKAMURA, N., HAMADA, N., MURATA, R., KOBAYASHI, A., ISHIZAKI, N., TAIRA, A. & SAKATA, R. (2001). Contribution of serotonin to liver injury following canine small-intestinal ischemia and reperfusion. *J. Surg. Res.,* **99**, 17-24.

NEUMAIER, J.F., SEXTON, T.J., YRACHETA, J., DIAZ, A.M. & BROWNFIELD, M. (2001). Localization of 5-HT_7 receptors in rat brain by immunocytochemistry, in situ hybridization, and agonist stimulated cFos expression. *J. Chem. Neuranat.,* **21**, 63-73.

NIETO, J.E., SNYDER, J.R., KOLLIAS-BAKER, C. & STANLEY, S. (2000). In vitro effects of 5-hydroxytryptamine and cisapride on the circular smooth muscle of the jejunum of horses. *Am. J. Vet. Res.,* **61**, 1561-5.

PERANZI, G. & LEHY, T. (1984). Endocrine cell population in the colon and rectum of cat, dog and monkey: fine structure, immunocytochemistry and distribution. *Anat. Rec.,* **210**, 87-100.

PINEIRO-CARRERO, V.M., CLENCH, M.H., DAVIS, R.H., ANDRES, J.M., FRANZINI, D.A. & MATHIAS, J.R. (1991). Intestinal motility changes in rats after enteric serotonergic neuron destruction. *Am. J. Physiol.,* **260**, G232-G239.

PLASSAT, J.L. & AMLAIKY, N. (1993). Molecular cloning of a mammalian serotonin receptor that activates adenylate cyclase. *Mol. Pharmacol.,* **44**, 229-236.

PRASAD, C.N., ROY, S.K., DEENATH, P.K. & SANYAL, A.K. (1977). Importance of plasma serotonin in intestinal obstruction. *Indian. J. Pharmacol.,* **9**, 11-16.

PRINS, N.H., BRIEJER, M.R. & SCHUURKES, J.A.J. (1997). Characterisation of the contraction to 5-HT in the canine colon longitudinal muscle. *Br.J. Pharmacol.,* **120**, 714-720.

PRINS, N.H., AKKERMANS, L.M., LEFEBVRE, R.A. & SCHUURKES, J.A.J. (2000a). 5-HT(4) receptors on cholinergic nerves involved in contractility of canine and human large intestine longitudinal muscle. *Br. J. Pharmacol.,* **131**, 927-932.

PRINS, N.H., SHANKLEY, N.P., WELSH, N.J., BRIEJER, M.R., LEFEBVRE, R.A., AKKERMANS, L.M. & SCHUURKES, J.A.J. (2000b). An improved in vitro bioassay for the study of 5-HT(4) receptors in the human isolated large intestinal circular muscle. *Br. J. Pharmacol.,* **129**, 1601-1608.

PRINS, N.H., AKKERMANS, L.M., LEFEBVRE, R.A. & SCHUURKES, J.A.J. (2001). Characterization of the receptors involved in the 5-HT-induced excitation of canine antral longitudinal muscle. *Br. J. Pharmacol.,* **134**, 1351-1359.

RAPPORT, M.M., GREEN, A.A. & PAGE, I.H. (1947). Purification of the substance which is responsable for vasoconstrictor activity of serum. *Fed. Proc.,* **6**, 184.

RIZZOTTI, M., DOMENEGHINI, C. & CASTALDO, L. (1979). The endocrine cells of the rectum of adult ox. *Basic. Appl. Histochem.,* **23**, 235-240.

ROUZADE, M.L., FIORAMONTI, J. & BUENO, L. (1998). Decrease in gastric sensitivity to distension by 5-HT1A receptor agonists in rats. *Dig. Dis. Sci.,* **43**, 2048-2054.

SARNA, S.K., BRIEJER, M.R. & SCHUURKES, J.A. (2000). 5-HT_3/5-HT_4 receptors in motility disorders. In: Gaginella TS, Guglietta A. (editors). *Drug Development: Molecular Targets for GI diseases,* Humana Press, Totowa (NJ), 177-203.

SJOLUND, K., SANDEN, G., HAKANSON, R. & SUNDLER, F. (1983). Endocrine cells in human intestine: an immunocytochemical study. *Gastroenterol.,* **85**, 1120-1130.

SNYDER, J.R. (1989). The pathophysiology of intestinal damage: effects of luminal distention and ischemia. *Vet. Clin. North. Am. Eq. Pract.,* **5**, 247-270.

SONG, C.W., LEE, K.Y., KIM, C.D., CHANG, T.M. & CHEY, W.Y. (1997). Effect of Cisapride and Renzapride on Gastrointestinal Motility and Plasma Motilin Concentration in Dogs. *J. Pharmacol. Exp. Ther.,* **281**, 1312-1316.

SPILLER, R. (2002). Serotonergic modulating drugs for functional gastrointestinal diseases. *Br. J. Clin. Pharmacol.,* **54**, 11-20.

TACK, J., COULIE, B., WILMER, A., PEETERS, T. & JANSSENS, J. (1998). Actions of the 5-hydroxytryptamine 1 receptor agonist sumatriptan on interdigestive gastrointestinal motility in man. *Gut.,* **42**, 36-41.

TACK, J., COULIE, B., WILMER, A., ANDRIOLI, A. & JANSSENS, J. (2000). Influence of sumatriptan on gastric fundus tone and on the perception of gastric distention in man. *Gut,* **46**, 468-473.

TAN, M. (2003). Granisetron: new insights into its use for the treatment of chemotherapy-induced nausea and vomiting. *Expert Opin Pharmaco.,* **4**, 1563-1571.

TANIYAMA, K., NAKAYAMA, S., TAKEDA, K., MATSUYAMA, S., SHIRAKAWA, J., SANO, I. & TANAKA, C. (1991). Cisapride stimulates motility of the intestine via the 5-hydroxytryptamine receptors. *J. Pharmacol. Exp. Ther.,* **258**, 1098-1104.

TERAMOTO, Y., URANO, T., NAGAI, N., TAKADA, Y., IKEDA, K. & TAKADA, A. (1998). Plasma levels of 5-HT and 5-HIAA increased after intestinal ischemia/reperfusion in rats. *Jap. J. Physiol.,* **48**, 333-339.

THOMPSON, J.H. (1971). Serotonin and the alimentary tract. *Res. Commun. Chem. Path.,* **2**, 687-781.

TONINI, M., GALLIGAN, J.J. & NORTH, R.A. (1989). Effects of cisapride on cholinergic neurotransmission and propulsive motility in the guinea pig ileum. *Gastroenterol.,* **96**, 1257-1264.

TULADHAR, B.R., G.E., L. & NAYLOR, R.J. (2003). 5-HT7 receptors mediate the inhibitory effect of 5-HT on peristalsis in the isolated guinea-pig ileum. *Br. J. Pharmacol.,* **138**, 1210-1214.

TURNAGE, R.H., GUICE, K.S. & OLDHAM, K.T. (1994). Endotoxemia and remote organ injury following intestinal reperfusion. *J. Surg. Res.,* **56**, 571-578.

UNWIN, N. (2000). The Croonian Lecture 2000. Nicotinic acetylcholine receptor and the structural basis of fast synaptic transmission. *Philosophical Transactions of the Royal Society of London. Series B. Biological Sciences 355*: 1813-29.

WEISS, R., ABEL, D., SCHOLTYSIK, G., STRAUB, R. & MEVISSEN, M. (2002). 5-Hydroxytryptamine mediated contractions in isolated preparations of equine ileum and pelvic flexure: pharmacological characterization of a new 5-HT(4) agonist. *J. Vet. Pharmacol. Ther.,* **25**, 49-58.

YAMADA, T., INUI, A., HAYASHI, N., FUJIMURA, M. & FUJIMYA, M. (2003). Serotonin stimulates endotoxin translocation via 5-HT3 receptors in the rat ileum. *Am. J. Physiol.-Gastr.L.,* **284**, G782-788.

AIMS

Postoperative ileus (POI) in humans is defined as a transient impairment of GI motility and function, characterized by abdominal distension and pain, nausea and vomiting, reduced desire to eat, and an inability to pass flatus (gas) or stool. However, besides patient discomfort, a prolonged hospital stay and additional costs, reports of postoperative ileus in humans are not as alarming as in horses. An overall prevalence rate as low as 0.2% is reported, and fatalities are rare (Carter, 2006; Saclarides, 2006; Goettsch *et al*., 2007). This contrasts sharply with the equine situation, where an overall prevalence of life-threatening postoperative ileus in small and large intestinal colic cases is around 18 to 21% (Blikslager *et al*., 1994; Roussel *et al*., 2001). Apparently, the horse is more prone to the development of this postoperative complication. An important reason for the significantly higher fatality rate of this condition in horses compared to humans, is the important fluid shift that takes place towards the equine GI system during development of postoperative ileus. Typically large amounts of reflux, up to 12 to 15 liters every 4 hours, have to be evacuated out of the stomach. On top of that, large amounts of endotoxins are often absorbed through the intestinal wall and released into the systemic circulation of colic horses, exerting devastating effects on the horse's cardiovascular and other organ systems (Moore & Barton, 2003). Clearly, such a drastic pathologic condition cannot be protracted for days.

Research into the physiological mechanisms that are responsible for normal GI motility in horses and knowing which factors predispose horses to ileus are very important to better understand the clinical picture of a colic horse and to develop case specific treatment options for horses suffering from ileus. Calcium and calcium channels are known for their importance in smooth muscle and neuronal function in the digestive system. With the development of modern ion specific electrodes, ionized calcium levels are carefully monitored in human Intensive Care Units (ICU), because low plasma calcium levels are reportedly associated with

hypotension and bad prognosis. Most haematological parameters require a clinical setting for their analysis and implicate the need for round the clock access to laboratory personnel, which is obviously no option for the veterinarian in the field. With the development of the hand held lactic acid analyzer for condition training of human athletes, field analysis of blood lactic acid levels has become common practice in human sports medicine. This opens interesting opportunities not only for equine sports medicine, but also for monitoring colic horses in the field. Blood lactic acid analysis is historically one of the most important golden standards to evaluate acidosis and poor tissue perfusion in an intensive care setting (Fall et al., 2005). Recently, intra-abdominal on line monitoring of peritoneal fluid lactic acid levels is performed in human ICU for early detection of bowel ischemia. It would be interesting for the equine veterinarian to be able to rapidly evaluate the presence of bowel ischemia in a colic patient and to use the lactic acid level as a prognostic parameter.

Despite the obvious importance of postoperative ileus in horses, there are currently no commercial veterinary prokinetics available to treat this troublesome syndrome. Hence the veterinary use of expensive human prokinetic agents, such as metoclopramide, erythromycin, lidocaine and cisapride. Unfortunately therapeutic results are disappointing and some of these products have even potentially fatal side effects. A possible explanation for these poor results can be found in the lack of fundamental research into the pharmacological activity pathways and therapeutic efficacy of different prokinetic medications in horses. Research results in other species are often extrapolated to the horse, without any pharmacological evidence that enteral receptor populations that serve as pharmacological target to induce intestinal propulsion in these species are equally important in horses. All recently developed prokinetic agents such as cisapride, tegaserod and prucalopride activate 5-HT_4 serotonergic receptors to induce their prokinetic effects. Despite the wide spread use of these agents in horses, up until now only little, if any evidence of the presence of 5-HT_4 receptors in the equine small

intestine is found. There is even no clear view on which contractile serotonergic receptor types are present in the equine small intestine.

The purpose of the present study was therefore to provide the clinician with extra clinical parameters, for the identification of high risk patients for development of postoperative ileus and for prognostic guidance. A second purpose was to investigate the contractile serotonergic receptor population of the equine small intestine. These objectives were met by investigating:

1. the role of ionized calcium in the pathophysiology of ileus in colic horses
2. the use of blood and peritoneal fluid lactic acid levels as a prognostic parameter in colic horses
3. the role and source of increased plasma and peritoneal fluid 5-HT levels in colic horses with compromised bowel
4. the presence and distribution of the contractile serotonergic receptor population in the longitudinal and circular smooth muscle layer of the equine small intestine.

REFERENCES

BLIKSLAGER, A.T., BOWMAN, K.F., LEVINE, J.F., BRISTOL, D.G. & ROBERTS, M.C. (1994). Evaluation of factors associated with postoperative ileus in horses: 31 cases (1990–1992). *J Am Vet Med Assoc.* 1994;205:1748–1752.

CARTER, S. (2006). The Surgical team and outcomes management: Focus on postoperative ileus. *Journal of PeriAnaesthesia Nursing* **21**, S2-S6

FALL, P.J., SZERLIP, H.M. (2005). Lactic acidosis: from sour milk to septic shock. *J. Intensive Care Med.* **20**, 255-271.

GOETTSCH, W.G., SUKEL, M.P., VAN DER PEET, D.L., VAN RIEMSDIJK M.M., & HERINGS, R.M. (2007). In-hospital use of opioids increases rate of coded postoperative paralytic ileus. *Pharmacoepidemiol Drug Saf* **16**, 668-674.

MOORE, J.N., BARTON, M.H. (2003). Treatment of endotoxemia. *Vet. Clin. North. Am. Equine Practice* **19**, 681-695.

ROUSSEL, A.J., COHEN, N.D., HOOPER, R.N. & RAKESTRAW, P.C. (2001). Risk factors associated with development of postoperative ileus in horses. *J Am Vet Med Assoc.* **219**, 72–78.

SACLARIDES, T.J. (2006). Current choices-good or bad-for the proactive management of postoperative ileus: A surgeon's view. *Journal of PeriAnaesthesia Nursing* **21**, S7-S15

PROGNOSTIC FACTORS

Chapter 3.I

USE OF PLASMA IONIZED Ca^{2+} LEVELS AND Ca^{2+} SUBSTITUTION RESPONSE PATTERNS AS PROGNOSTIC PARAMETERS FOR ILEUS AND SURVIVAL IN COLIC HORSES

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SUMMARY

Hypocalcaemia is a common finding in horses with enterocolitis and severe GI disorders. The aims of this study were to investigate in colic horses 1) the parameters related to hypocalcaemia, 2) the influence of hypocalcaemia on outcome and 3) the possible beneficial effect of Ca^{2+} substitution. The survey was designed as a randomized controlled trial. One hundred fourthy-four horses who were admitted with an acute abdomen during a 1.5 year period were enrolled and evaluated for clinical criteria and whole blood ionized Ca^{2+} levels. The control group consisted of 25 (10 females, 5 sexually intact males, 10 castrated males) clinically healthy Belgian Warmblood horses from the Ghent University Faculty of Veterinary Medicine teaching herd. It was attempted to assign the hypocalcaemic colic horses randomly as much as possible to receive Ca^{2+}. In accordance with standard protocol in human critical care, the amount of infused Ca^{2+} was restricted to the strict minimum in order to attain lower limit reference range values of normocalcemia ($\geq$ 1.61 mmol/l). PCV and BE were always stabilized as much as possible, before Ca^{2+} substitution was performed and Ca^{2+} substitution was always postponed until after abdominal surgery. Part of the treated (36 out of 48 treated hypocaelcemic colic horses) and untreated (29 out of 61) hypocalcaemic colic horses were rechecked for calcaemia for 7 consecutive days, to line out a time profile of calcaemia in untreated hypocalcaemic colic horses and to construct a time course of responsiveness to Ca^{2+} substitution in treated hypocalcaemic colic horses.

Analysis for ionized Ca^{2+} levels was performed on heparinised whole blood samples. Horses that were assigned to be treated received 400 mEq Ca^{2+} diluted in 10L of Ringer's Lactate solution every 24h until low reference range limits ($\geq$1.61 mmol/l) were obtained or until death.

The relation between several potential risk factors and the probability of developing ileus and the probability of survival, was analyzed using logistic regression. For this, the calcemia levels of the study population were arbitrarily subdivided into four quartiles, creating four calcemia intervals, graded as: 1) values lower than 1.27 mmol/l: “very low”; 2) 1.28-1.45 mmol/l: “low”; 3)1.46-1.61 mmol/l: “moderate”; 4) > 1.61 mmol/l: “normal”.

88% of all colic patients (126 out of 144 colic horses) showed blood ionized Ca^{2+} levels below the reference range (1.61-1.85 mmol/l) at the time of admission. 18 horses were subjected to euthanasia, either intra-or postoperatively and were all part of the hypocalcaemia group. Although no statistical difference could be demonstrated between colic groups in our study, it seems that horses with small intestinal related colic and duodenitis-proximal jejunitis are most prone to severe hypocalcaemia; calcium levels were lowest in the horses among the different groups that showed signs of endotoxemia. Multivariable analysis revealed that the presence of reflux, signs of endotoxemia, an increased PCV, alkalinisation of pH and the interaction PCV/pH all predispose colic horses to low ionized Ca^{2+} levels at the time of admission. The Odds for developing ileus during hospitalization are ± 11.94 times larger for horses in the “very low” calcemia interval (< 1.27 mmol/l), in comparison with normocalcemic horses. The Odds for fatal outcome are respectively ± 9.82 and 8.33 times larger for horses in the “very low” and “low” calcemia interval. Production of reflux coincided with a further decrease in blood ionized calcium levels. Ca^{2+} substitution increased the probability of survival, provided that Ca^{2+} levels could be normalized. 48 hypocalcaemic colic horses were Ca^{2+} treated, 36 of them were rechecked for calcaemia for 7 consecutive days, 20 horses were responsive to Ca^{2+} substitition, the remaining 16 horses were unresponsive and eventually died. The lack of an upward calcemia response, despite repetitive Ca^{2+} substitutions, can be guarded as a poor ominous sign.

Hypocalcaemia in colic horses is of prognostic relevance both with regard to survival as to the probability of development of ileus during hospitalization. This study shows the importance of routine measurement of ionized calcium levels in colic horses. Moreover, correction of hypocalcaemia seems to improve clinical outcome.

3.I.1. INTRODUCTION

The calcium ion is a highly versatile regulator of numerous physiological processes, such as muscle contraction (Tupling, 2004), blood coagulation (Spronk *et al*., 2003), hormonal secretion, cell division (Kahl & Means, 2003), maintenance of cell integrity, permeability of cell membranes, control of enzymatic reactions and last but not least GI smooth muscle motility and excitability (Hirst, 1999; Malykhina & Akbarali, 2004). Calcium circulates in the extracellular fluid in three distinct fractions: about 50% is the biologically important ionised fraction, 40% is protein-bound, and 10% is complexed to anions such as bicarbonate, citrate, sulphate, phosphate and lactate. Most of the protein-bound calcium is bound to albumin, the remainder being complexed to globulins. This binding to proteins is pH dependent. Alkalinisation of blood stimulates the binding of Ca^{2+} to proteins and thus lowers the ionized Ca^{2+} concentration (Bushinsky & Monk, 1998; Scenci *et al*., 1991). Despite earlier reports mentioning a lack of correlation between blood pH and ionized calcium in horses (Kohn & Brooks, 1990), the research groups of Toribio and Van der Kolk clearly defined a distinct correlation between both parameters and determined the regression equations to describe the relationship (Toribio *et al*., 2001a; Van der Kolk *et al*., 2002). Because the ionized calcium is the biologically active form, direct measurement of this fraction is most appropriate to evaluate calcium homeostasis in human and equine patients. Due to the limited availability of ion-selective electrodes in human Intensive Care (IC) units to measure the ionized form, until some years ago it was common practice to determine total serum or plasma calcium contents

and to correct these values for the actual concentration of serum or plasma albumin (Van der Wiel, 2001; White *et al*., 1986). Considerable debate as to the suitability of this technique to evaluate calcemia in critically ill IC unit patients has led to the advice of direct measurement of ionized calcium levels in these patients (Koch *et al*., 2002; Slomp *et al*., 2003; Ward *et al*., 2004). Likewise, several equine studies have demonstrated that determination of ionized calcium fractions is a far more sensitive method to identify horses with hypocalcaemia than determination of total calcium concentrations (Garcia-Lopez *et al*., 2001; Toribio *et al*., 2001a). In horses, ionized calcium levels can be assessed in serum or plasma, but the recently described technique of measuring ionized calcium in heparinised whole blood, by means of an ion selective electrode, offers the advantages of a rapid and practical applicable analysis method both in healthy horses and horses suffering from colic or diarrhoea (Van der Kolk *et al*., 2002).

Hypocalcaemia has been reported in several pathological conditions and can have important consequences. Decreased calcium levels are associated with impaired cardiovascular performance, hypotension, arrhythmias, muscle cramps, laryngospasms, tetany and seizures (Bushinsky & Monk, 1998; Desai *et al*., 1988; Kovacs *et al*., 1998). Hypocalcaemia is encountered in 88% of all human IC unit admitted patients who need a prolonged 48 hours stay (Zivin *et al*., 2001). On top of that, unrelenting hypocalcaemia in human IC unit patients seems to represent an ominous sign for poor outcome and high patient mortality (Hästbacka & Pettilä, 2003; Ward *et al*., 2004; Zivin *et al*., 2001).

Although hypocalcaemia seems to be a common finding in horses with GI disease, the diagnostic and therapeutic implications of this finding are not clearly defined. (Dart *et al*., 1992; Garcia-Lopez *et al*., 2001; Toribio *et al*., 2001a, 2005; Van der Kolk *et al*., 2002). Hypocalcaemia is most pronounced in horses suffering from large or small intestinal colic associated with vascular compromise of the bowel (Dart *et al*., 1992). Low serum ionized

calcium levels are measured in horses with postoperative ileus (Garcia-Lopez *et al*., 2001). These findings lead to the question as to what prognostic implications low ionized calcium levels have in colic horses and whether correction of this ionic imbalance in colic horses has a beneficial effect. Porcine endotoxaemia models have indeed indicated that calcium infusion can promote cellular damage and so calcium substitution could have a detrimental effect on patient outcome (Carlstedt *et al*., 2000).

The purpose of this study is to investigate the possible use of blood ionized calcium levels as a prognostic parameter in colic horses in terms of development of postoperative ileus and survival. Also the possible use of response patterns to calcium substitution as a possible aid in monitoring the clinical progress of colic horses, was examined. Finally, it was tested whether calcium substitution has a detrimental effect on patient outcome.

3.I.2. MATERIALS AND METHODS

Horses

The control group consisted of 25 (10 females, 5 sexually intact males, 10 castrated males) clinically healthy Belgian Warmblood horses from the Ghent University Faculty of Veterinary Medicine teaching herd, aged between 4 and 15 years (mean ± SD, 9.61 ± 4.62). Horses were housed in individual stalls, with straw bedding and they were fed twice daily with grass hay and a commercial feed, which met nutrient requirements. All horses had free access to water.

The study population consisted of 144 horses with GI colic, that were presented to the Large Animal Internal Medicine Clinic of Ghent University, Belgium over a 1.5 year period. Horses in this group were between 1 and 23 years of age (8.35 ± 7.12) and comprised several breeds (74 Belgian Warmblood horses, 40 French Warmblood horses, 12 trotters, 6 Belgian Draft horses and 12 other breeds) and both sexes (72 females, 63 castrated males, 9 sexually

intact males). 16 mares were carrying a foal (mean duration of gestation ± s.e.m = 6.81 ± 0.44 months); 7 horses were lactating (foals aged between 4 days and 1.5 months). Mean duration of colic at the time of arrival in the clinic was 15.59 ± 14.74 hours.

Experimental design

In order to evaluate the relation between abdominal pathologic conditions and different treatment protocols on calcemia, several categories were created (Table 3.I.1).

Table 3.I.1. Population Characteristics

	Small Intestinal Colic	Large Intestinal Colic	Undeter-mined Colic	Gastro-duodenitis	Total
Horses (n)	62 (43%)	53 (37%)	12 (8%)	17 (12%)	144 → **Pre-operative euthanasia (n=9) Per-operative euthanasia (n=9) → 126 hospitalized horses**
Reflux at time of admission[a]	39/62 (63%)	12/53 (23%)	7 (58%)	17 (100%)	75 (52%)
Endotoxemia	21/62 (34%)	9/53 (17%)	5 (42%)	14 (82%)	49 (34%)
Ileus during hospitalisation[b]	22/62 (35%)	5	4	15	46/126 (36%)
Left clinic alive	28	35	6	5	74/126 (59%)
Died 1 to 4 D post clinic admittance	17	5	2	7	31/126 (25%)
Died > 4 D post clinic admittance	9	4	3	5	21/126 (17%)
Conservative treatment	19	24	3	9	55/126 (44%)
Surgery without resection	11	16	6	7	40/126 (32%)
Surgery with resection	26	4	0	1	31/126 (25%)
Prokinetic use	30	7	4	16	57/126 (45%)
Ringer's lactate solution	46	20	6	17	89/126 (71%)
Single calcium substitution	18	5	3	3	29/126
Multiple calcium substitutions	9	1	2	7	19/126

[a]Gastric intubation at the time of admission yielded > 1.5 L reflux
[b]Reflux continued > 24h after admission time or reflux started to show post-operatively

The horses were allotted to 4 colic categories on the basis of clinical and, if performed, surgery findings: (1) small intestinal colic: with the exception of duodenitis-proximal jejunitis, all cases of strangulating and non-strangulating obstruction of the small intestine (62 horses); (2) large intestinal colic: all cases of strangulating and non-strangulating obstruction or displacement of the colon (53 horses); (3) colic of undetermined origin: most often presented as mixed tympanic distension of small and large intestine (12 horses); (4) duodenitis-proximal jejunitis: an inflammatory condition affecting the upper small intestine and resulting in gastric and small intestinal distension with reflux, resulting in a clinical picture that sometimes closely resembles small intestinal obstructive colic (17 horses). Horses that were euthanized without surgery, were subjected to autopsy, in order to confirm the clinical diagnosis. Horses with known systemic diseases in addition to colic were excluded from the study. None of the colic horses had received parenteral perfusions before admission.

Some of the hospitalized colic horses (19/55) that were treated conservatively received intravenous lactated Ringer's solution. In all horses that underwent surgery (n=71; 37 small intestinal colic cases; 20 horses suffering from large intestinal colic; 6 horses suffering from colic of undetermined origin and 8 horses suffering from duodenitis-proximal jejunitis) intravenous administration of lactated Ringer's solution was commenced during the surgical procedure and continued during 4 or more consecutive days, depending on the occurrence of postoperative complications such as diarrhoea or ileus. When Packed Cell Volume (PCV) was above 50% or Base Excess (BE) was below -5 mEq/l at the time of admission or during hospital stay, this was corrected by the administration of hypertonic saline (7.2 % NaCl) and bicarbonate respectively.

Prior to surgery, all horses received antibiotic treatment consisting of potassium penicillin[a] (20 000IU/kg BW iv) or sodium ceftiofur[b] (2 mg/kg BW iv), gentamycin sulphate[c] (3 mg/kg BW iv SID), and flunixin meglumine[d] (1.1 mg/kg BW iv). Antibiotic treatment (20

000 IU/kg BW potassium penicillin iv TID or sodium ceftiofur 2 mg/kg BW iv SID; gentamicin sulphate 8 mg/kg BW iv SID) and flunixin meglumine (0.5 mg/kg iv TID), and fluid therapy were continued in all horses recovered from surgery.

Horses that were treated with prokinetic medication (n=57), received either metoclopramide[e] (0.04 mg/kg/h iv; n=53) or erythromycin lactobionate[f] (1g iv TID; n=4).

Horses were categorised as endotoxemic if the following criteria were fulfilled: heart rate > 60 bpm, PCV > 50%, congested mucous membranes with "toxic line" and capillary refill time > 3 secs (Barton et al., 2004; Moore et al., 2007). Horses were categorized as having reflux at the time of admission, when more than 1.5 L of reflux could be retrieved by stomach intubation on arrival (n=75). Horses that continued producing reflux during > 24h after admission time or that started to show reflux postoperatively, were allotted to the "ileus during hospitalisation" group (n=46). Incoming colic horses were randomly assigned to the study (144 colic horses) and when hypocalcaemic (127 colic horses; 18 euthanized pre-or intraoperatively, 109 remaining hypocalcemic hospitalized colic horses), were randomly assigned to receive calcium or not (48/109 hospitalized colic horses with hypocalcaemia received Ca^{2+}). Whenever a surgical intervention was needed, Ca^{2+} substitution was postponed until after recovery. Correction of hypovolemia and acid-base imbalances was realised as much as possible before administration of Ca^{2+}, in order to reduce possible promotion of cell death and tissue damage. When Ca^{2+} substitution was performed, blood ionized Ca^{2+} levels were monitored and repetitive corrections were performed whenever necessary, limited to a maximal dose of 400 mEq Ca^{2+} per 24h, until normalisation or death. Correction was performed by slow intravenous administration of 400 mEq Ca^{2+}[, g] diluted in 10L of a Ringer's lactate solution Hypocalcaemic colic horses were randomly assigned to receive Ca^{2+}. In accordance with standard protocol in human critical care, the amount of

infused Ca^{2+} was restricted to the strict minimum in order to attain lower limit reference range values of normocalcemia (≥ 1.61 mmol/l).

Sample collection and analysis

At the time of admission two venous blood samples were collected anaerobically from the jugular vein in tubes containing lithium heparin. In one tube Na^{+}, K^{+}, Ca^{2+} levels and in the second tube PCV and BE were determined by use of an ion-selective electrode[h] and blood gas analyser[i] respectively.

All reported ionized Ca^{2+} levels are the actual measured ionized calcium concentrations, without standardization to pH = 7.4.

29 out of 61 hospitalized hypocalcaemia colic horses (127 hypocalcaemic colic horses – 18 pre and intra-operative deaths-48 Ca^{2+} treated hypocalcaemic colic horses) that were assigned to not receive calcium were rechecked for calcemia on a daily basis during 7 consecutive days, to line out a time profile of calcemia in untreated hypocalcaemic colic horses.

In another 36 hospitalized hypocalcaemic colic horses that were assigned to the calcium treatment group (n=48), calcemia was followed-up during seven consecutive days or until death. In this way a time course of responsiveness to Ca^{2+} substitution could be constructed for survivors (S) versus non-survivors (NS). Blood was drawn from the jugular vein opposite to the catheter side. The heart rate, PCV, pH and BE were recorded at the time of collection of each blood sample. Onset of complications such as ileus or diarrhoea were recorded. All samples were processed within 10 min of collection.

For determination of a standard laboratory reference range, ionized Ca^{2+} levels measured in 25 healthy horses were adjusted to pH=7.4 by application of the multiple regression equation that was proposed by Aguilera-Tejero and co-workers (23):

Adjusted Ca^{2+} mmol/l at $pH_{7.4}$= -6.4570 + 0.8739 x (measured pH) + 0.9944 x (measured Ca^{2+} mmol/l)

A normal reference range was then determined, using the mean ± 2 SD.

Statistical analysis

Results of continuous variables are presented as mean ± standard deviation (SD).

The Ca^{2+} levels in the different colic groups of the study population were compared with the control group using a one way ANOVA with a LSD post hoc test.

The relation between several potential risk factors and the blood ionized Ca^{2+} levels at the time of admission was studied using linear regression analysis (SPSS 11.0) [j]. First a univariable analysis was performed in order to identify all potential factors related with calcemia in colic horses at admission. All variables with $p<0.2$ were then entered in a multivariable model. In this way, the results of the multivariable analysis, represent the relation between each examined factor and calcemia, corrected for the effect of the other variables in the model. The multivariable model was build following a stepwise backward procedure. All interactions between the significant variables were evaluated.

The Ca^{2+} levels at the time of admission in colic horses that remained without ileus during hospitalization and those who developed ileus were compared using an independent samples t-test. Daily Ca^{2+} levels were respectively compared between untreated survivors and non-survivors and between calcium-treated survivors and non-survivors, using an independent samples t-test.

The calcemia time profile was compared between survivors and non-survivors with and without reflux, using a one-way ANOVA, followed by a Scheffé post-hoc test.

Finally, using an independent samples t-test, it was analysed whether the effect of Ca^{2+} substitution and the effect of ileus on subsequent blood ionized calcium measurements was significantly different from 0.

The relation between several potential risk factors and the probability of developing ileus and the probability of survival, was analyzed using logistic regression (SPSS 11.0) [j]. For this, in accordance with results of human clinical trials, where a non linear relation has been demonstrated between blood ionized Ca^{2+} levels and survival (Hästbacka et al 2003), the calcemia levels of the study population were arbitrarily subdivided into four quartiles, creating four calcemia intervals, graded as: 1) values lower than 1.27 mmol/l: “very low”; 2) 1.28-1.45 mmol/l: “low”; 3)1.46-1.61 mmol/l: “moderate”; 4) > 1.61 mmol/l: “normal”.

First a univariable analysis was performed to select all risk factors indicating a hint of possible association with ileus or survival. Secondly, all parameters with a *p* value below 0.2 in the univariate analysis, were combined in a multivariable model. This model was developed following a stepwise backward procedure. In this way the effect of calcemia, corrected for the effects of all other significant variables, on ileus and survival, was evaluated.

Odds ratios are presented in combination with the 95% CI and corresponding *p* value.

3.I.3. RESULTS

3.I.3.1. Assessment of heparinised whole blood ionised Ca2+ reference range in healthy horses (n=25)

Whole blood ionized Ca^{2+} was 1.73 ± 0.05 mmol/l and corresponding pH was 7.40 ± 0.03. Adjusted whole blood ionized Ca^{2+} was 1.73 ± 0.06 mmol/l. Based on these data, a normal reference range for our clinical laboratory was determined as 1.61-1.85 mmol/l (expressed as mean ± 2SD).

3.I.3.2. Baseline patient characteristics (Table 3.I.1)

62 horses (43%) had small intestinal related colic (volvulus, hernia, epiploic foramen entrapment,…), 53 horses (37%) had large intestinal related colic (large colon displacement, torsion, obstipation,…), 12 horses (8%) showed colic of undetermined origin and 17 horses (12%) suffered from duodenitis-proximal jejunitis.

It was possible to retrieve > 1.5 L reflux during naso-gastric intubation in 75 horses (52%) at the time of admission. 47 (63%) of these horses underwent surgery. 56 of the 69 horses (81%) that were free of reflux at arrival, did not develop ileus during their stay in the clinic. A mortality rate of 54.7% was determined in the group of horses showing reflux at the time of admission. The group of horses free of reflux at the time of arrival, had a much lower mortality rate of 18.8%.

49% (71 horses) of the studied colic cases needed surgical intervention (Table 3.I.1).

Prokinetic medication was used in 57 horses, including 30 small intestinal colic, 7 large intestinal colic, 4 undetermined colic and 16 duodenitis-proximal jejunitis cases. 49 horses showed signs of endotoxaemia at the time of admission.

Pre-operative euthanasia was performed in 9 colic cases. Peroperative euthanasia was performed in another 9 colic cases, leaving 126 horses to be hospitalized. Most colic horses died during the first 4 days of hospitalization (n=31).

3.I.3.3. Frequency of hypocalcaemia in colic horses

Figure 3.I.1 shows the differences in Ca^{2+} distribution among the different study groups. 88% of all colic patients showed blood ionized Ca^{2+} levels below the reference range of 1.61-1.85 mmol/l at the time of admission.

Mean ionized Ca^{2+} levels of all colic categories were significantly different from the control group. Although no significant difference could be demonstrated between colic categories, there is a clear tendency of horses with small intestinal colic and horses suffering

from duodenitis-proximal jejunitis to present lower ionized Ca^{2+} levels than horses with large intestinal colic and undetermined digestive colic. Horses with signs of endotoxaemia tended to have very low blood ionized calcium levels.

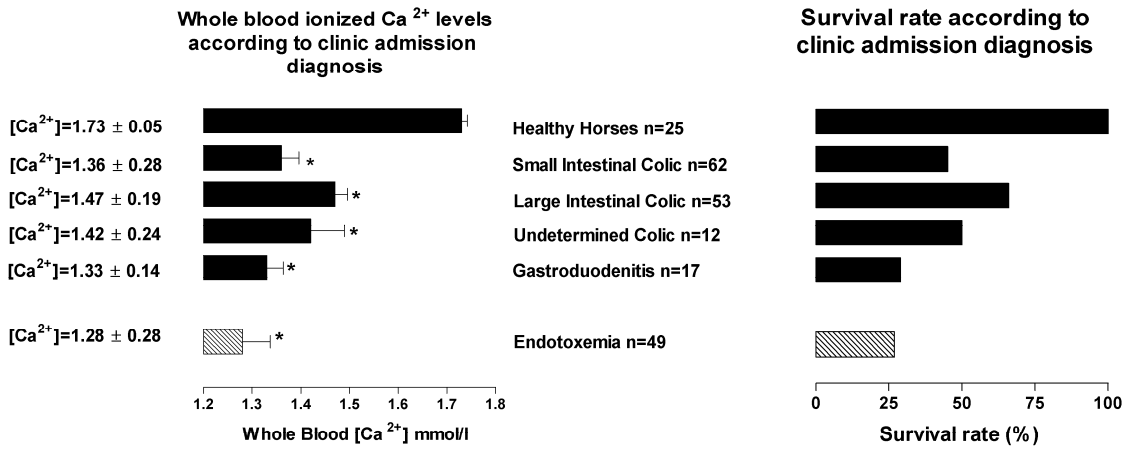

Figure 3.I.1, panel A (left): Mean ionized Ca^{2+} levels (± SD) in 25 healthy control horses and 144 colic horses, according to the clinic admission diagnosis. The Ca^{2+} levels in the colic horses showing signs of endotoxemia are also shown.
*significant difference between colic and healthy horses at $p<0.001$.
Figure 3.I.1, panel B (right): Corresponding survival rates expressed in %

3.I.3.4. Factors influencing blood ionized calcium levels

Univariable linear regression analysis revealed that the presence of reflux, signs of endotoxaemia, pH, BE, PCV and heart rate, were all significantly related with the blood ionized Ca^{2+} level at the time of admission (Table 3.I.2). In the multivariable analysis the presence of reflux at arrival, signs of endotoxemia, an increased PCV and acidification of blood pH remained significant; also the interaction PCV/pH was significant (Table 3.I.2).

Table 3.I.2:The relation between several potential risk factors and the blood ionized Ca^{2+} levels at the time of admission according to univariable and multivariable linear regression analysis

	Whole blood ionized Ca^{2+} at admission	
	Univariable	Multivariable
Breed	NS (p=0.42)	_
Age	NS (p=0.39)	_
Gender	NS (p=0.88)	_
Gestation	NS (p=0.13)	*
Duration of colic at admission	NS (p=0.79)	_
Type of colic	NS (p=0.06)	*
pH	p<0.01	p=0.01
BE	p<0.01	*
PCV	p<0.01	p<0.01
Na^+	NS (p=0.62)	-
K^+	NS (p=0.84)	-
Heart rate	p<0.01	*
Reflux at time of admission	p<0.01	p=0.01
Signs of endotoxemia	p<0.01	p<0.01
Interaction pH/PCV	-	p<0.01

Significance is set at p<0.05; * since only significant variables are present in the final multivariable model it is impossible to present a p value here.

3.I.3.5. Factors influencing the probability of ileus

According to the univariable logistic regression analysis the blood ionized Ca^{2+} level at the time of admission in the “very low” and “low” calcemia intervals, the type of colic, PCV, Ca^{2+} administration, heart rate, the presence of reflux, the presence of signs of endotoxaemia and intestinal resection, are all factors that influence the probability of development of ileus during hospitalization (Table 3.I.3).

In the multivariable analysis the blood ionized Ca^{2+} level at the time of admission in the "very low" calcemia interval remained significantly related to the probability of development of ileus during hospitalization. The Odds for horses in the "very low" calcemia interval, for developing ileus during hospitalization, are ± 11.94 times larger in comparison with normocalcemic horses. The "low calcemia interval" shows a clear tendency of relation with the probability of ileus, although not statistically significant. Also the presence of reflux at arrival and the presence of signs of endotoxemia were significantly related with the probability of developing ileus during the hospitalisation period (Table 3.I.3).

Calcemia levels, measured at the time of admission in horses that remained without ileus during their hospital stay (1.50 ± 0.12 mmol/l; n=80) were significantly higher (p<0.01) than the admission levels in horses who developed reflux during their hospitalization (1.31 ± 0.22 mmol/l; n=46).

Table 3.I.3: The relation between the blood ionized Ca^{2+} levels at the time of admission and the probability of developing ileus during hospitalization in the respective calcemia intervals graded as "normal", "moderate", "low" and "very low", according to univariable and multivariable logistic regression analysis.

	Ileus Probability	
	Univariable	Multivariable
Overall	$p<0.01$	$p=0.03$
Very low versus normal	$p<0.01$	$p=0.03$; OR 11.94 (1.23-115.93)
Low versus normal	$p=0.02$	$p=0.05$; OR 1.93 (0.27-14.01)
Moderate versus normal	$p=0.24$	$p=0.92$; OR 0.90 (0.14-6.02)
Breed	NS ($p=0.47$)	–
Age	NS ($p=0.19$)	*
Gender	NS ($p=0.09$)	*
Gestation	NS ($p=0.15$)	*
Duration of colic	NS ($p=0.80$)	*
Type of colic	$p<0.01$	*
pH	NS ($p=0.54$)	–
BE	NS ($p=0.13$)	*
PCV	$p<0.01$	*
Na^+	NS ($p=0.95$)	–
K^+	NS ($p=0.16$)	*
Heart rate	$p<0.01$	*
Reflux at time of admission	$p<0.01$	$p<0.01$; OR 21.84 (6.43-74.16)
Signs of endotoxemia	$p<0.01$	$p<0.01$; OR 5.51 (1.81-16.77)
Ca^{2+}-administration	$p<0.01$	*
Number of Ca^{2+}-administrations	$p=0.84$	*
	No versus single $p=0.55$	*
	No versus multiple $p=0.99$	*
Resection of intestine	$p=0.04$	*
Need for surgery	NS ($p=0.50$)	–

Significance is set at $p<0.05$; * since only significant variables are present in the final multivariable model it is impossible to present a p value here; OR>1 corresponds with an increased probability of developing ileus; OR's are expressed with their 95% confidence interval and corresponding p value.

3.I.3.6. Factors influencing the probability of fatal outcome

Univariable logistic regression analysis revealed that the “very low” and “low” calcemia intervals are significantly related to the probability of fatal outcome. Also the type of colic, BE, PCV, heart rate, the presence of reflux at admission, the presence of signs of endotoxaemia, Ca^{2+} administration and the number of Ca^{2+} administrations, necessity for resection of intestine and development of ileus during hospitalization, all are significantly associated with the probability of survival (Table 3.I.4).

In the multivariable model the “very low” and “low” calcemia intervals remained significantly related with the probability of fatal outcome. The Odds for fatal outcome are ± 9.82 times larger for horses in the “very low” calcemia interval than in horses with normocalcemia. For horses in the “low” calcemia interval the Odds for fatal outcome are ± 8.33. Also the presence of signs of endotoxemia and the administration of Ca^{2+} remained significantly related to the probability of survival. When normalization of Ca^{2+} levels could be obtained with one administration, this was associated with a significant decrease of the probability of fatal outcome (Table 3.I.4). Conversely, the effect of multiple corrections on the probability of fatal outcome, was not significantly different from no administration.

Table 3.I.4: The relation between the blood ionized Ca^{2+} levels at the time of admission and the probability of fatal outcome in the respective calcemia intervals graded as “normal”, “moderate”, “low” and “very low”, according to univariable and multivariable logistic regression analysis. Table continues on next page.

	Probability of fatal outcome	
	Univariable	Multivariable
Overall	$p<0.01$	$p=0.01$
Very low versus normal	$p<0.01$	$p=0.02$ OR 9.82 (1.41-68.27)
Low versus normal	$p=0.02$	$p=0.03$ OR 8.33 (1.23-56.28)
Moderate versus normal	$p=0.29$	$p=0.60$ OR 1.58 (0.29-8.99)
Breed	NS ($p=0.43$)	–
Age	NS ($p=0.28$)	–
Gender	NS ($p=0.23$)	–
Gestation	NS ($p=0.35$)	–
Duration of colic	NS ($p=0.96$)	–
Type of colic	$p=0.03$	*
pH	NS ($p=0.14$)	*
BE	$p=0.03$	*
PCV	$p<0.01$	*
Na^+	NS ($p=0.50$)	–
K^+	NS ($p=0.49$)	–
Heart rate	$p<0.01$	*
Reflux at time of admission	$p<0.01$	*
Signs of endotoxemia	$p<0.01$	$p<0.01$ OR 9.62 (3.88-23.87)
Ca^{2+}-administration	$p<0.01$	$p<0.01$
Number of Ca^{2+}-administrations	$p<0.01$	$p<0.01$
	No versus single: $p=0.06$	$p<0.01$ No versus single: OR 0.10 (0.03-0.38)

	No versus Multiple p=0.02	No versus p=0.63 Multiple OR 0.72 (0.20-2.65)
Resection of intestine	p=0.03	*
Prokinetic medication	NS (p=0.06)	*
Ileus	p<0.01	*
Need for surgery	NS (p=0.36)	–
IV Ringer's Lactate	NS (p=0.10)	*

Significance is set at p<0.05; * since only significant variables are present in the final multivariable model it is impossible to present a p value here; OR>1 corresponds with an increased probability of death; OR's are expressed with their 95% confidence interval and corresponding p value.

3.I.3.7. Time profile of blood ionized calcium levels in survivors versus non-survivors

Mean blood ionized calcium levels (mmol/l) ± SD for survivors (S) and non-survivors (NS) are graphed in function of time in Figures 3.I.2, 3.I.3 and 3.I.4. Non-survivors tended to be admitted with lower blood ionized calcium levels than survivors.

The time profile of calcemia in survivors and non-survivors, not treated with calcium, is significantly different, showing a progressive decline in blood ionized Ca^{2+} levels in non-survivors, which is especially pronounced on the third day of hospitalisation (Figure 3.I.2). In non-calcium-treated survivors, there is a slow but gradual increase in blood ionized calcium to low normal levels (Figure 3.I.2). It takes for these horses approximately 72 hours to obtain spontaneous normalization of calcemia.

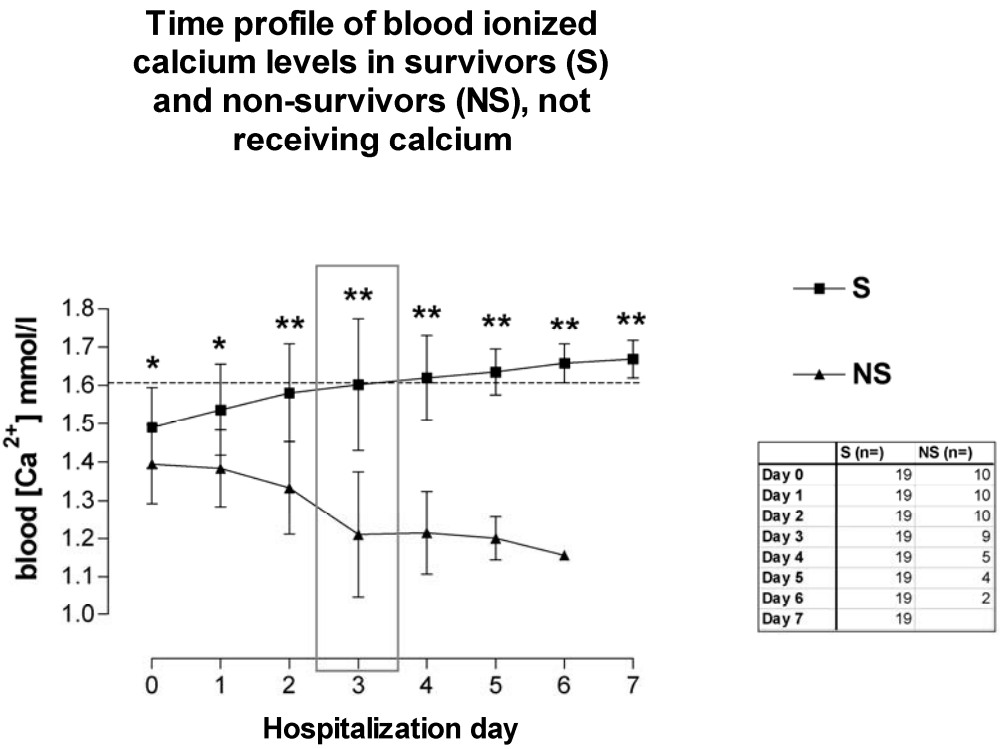

Figure 3.I.2: Time profile of whole blood ionized calcium levels in survivors (S) and non-survivors (NS) that did not receive calcium. The dotted line depicts the lower limit of the reference interval for normocalcemia. *significant difference at $p<0.05$; ** significant difference at $p<0.001$

Response patterns to Ca^{2+} substitution are significantly different ($p<0.01$) between survivors and non-survivors (Figure 3.I.3). In survivors there is a gradual and steady upward course of calcemia. Whereas in non-survivors, a very capricious response pattern to Ca^{2+} substitution was seen, responsible for the rather large SD values depicted in figure 3. Despite repetitive administration of Ca^{2+}, there is no gradual increase in mean blood ionized Ca^{2+} levels in these horses. On the contrary, there is even a tendency of progressive decline. This progressive decline of calcemia in calcium-treated non-survivors, although indeed less pronounced, follows the same course as in non-calcium-treated non-survivors (Figures 3.I.2, 3.I.3).

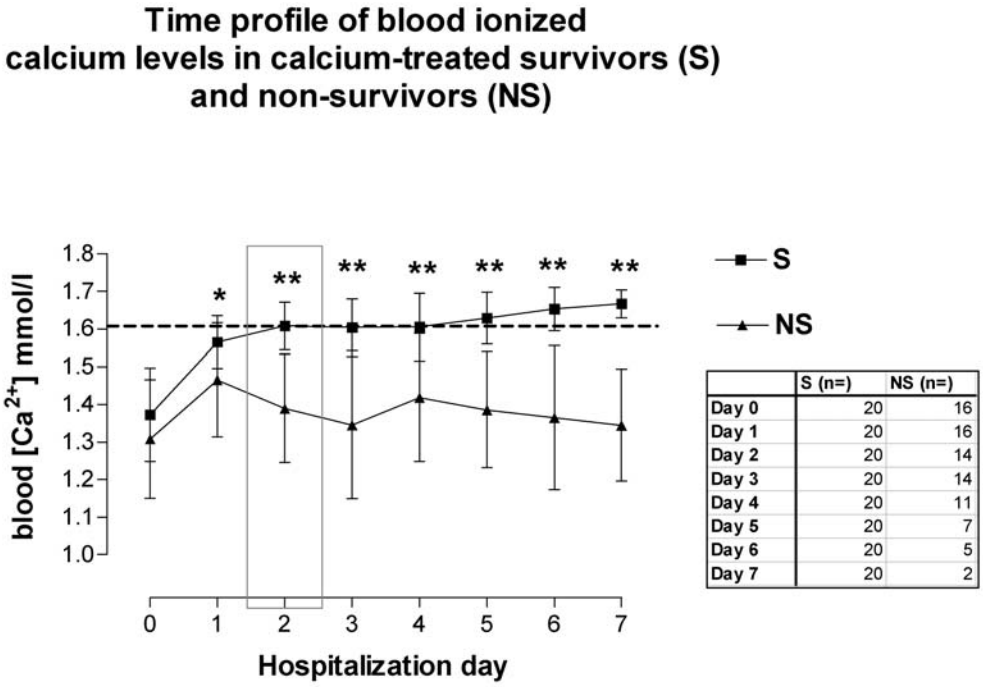

Figure 3.I.3: Time profile of whole blood ionized calcium levels in calcium-treated survivors (S) and non-survivors (NS). The dotted line depicts the lower limit of the reference interval for normocalcemia. * significant difference at $p<0.05$; ** significant difference at $p<0.001$

In 29 horses a single Ca^{2+} substitution was sufficient to normalize the calcium levels within 50 ± 8 hours. This corresponded with 60% of all hospitalized hypocalcemic colic horses that were assigned to the treatment group (n=48). Most horses in this group survived (80%). Horses, where hypocalcaemia was not corrected by one Ca^{2+} administration (n=19), received several Ca^{2+} doses and had a mortality rate of 65%. Those horses that did survive, obtained normalization of Ca^{2+} levels after 102.3 ± 54 hours.

3.I.3.8. Time profile of blood ionized calcium levels in horses with reflux

Production of reflux has a suppressing effect on calcemia. Figure 3.I.4 depicts the time profiles of mean blood ionized Ca^{2+} levels in 8 horses with reflux, not treated with calcium.

During the first two days of hospitalization, there is a significant difference in the time profile of calcemia in survivors with and without reflux (Figure 3.I.5; **). As of the third day of hospitalization there is a significant difference in the calcemia time profile of survivors and non-survivors with reflux (Figure 3.I.5; ××).

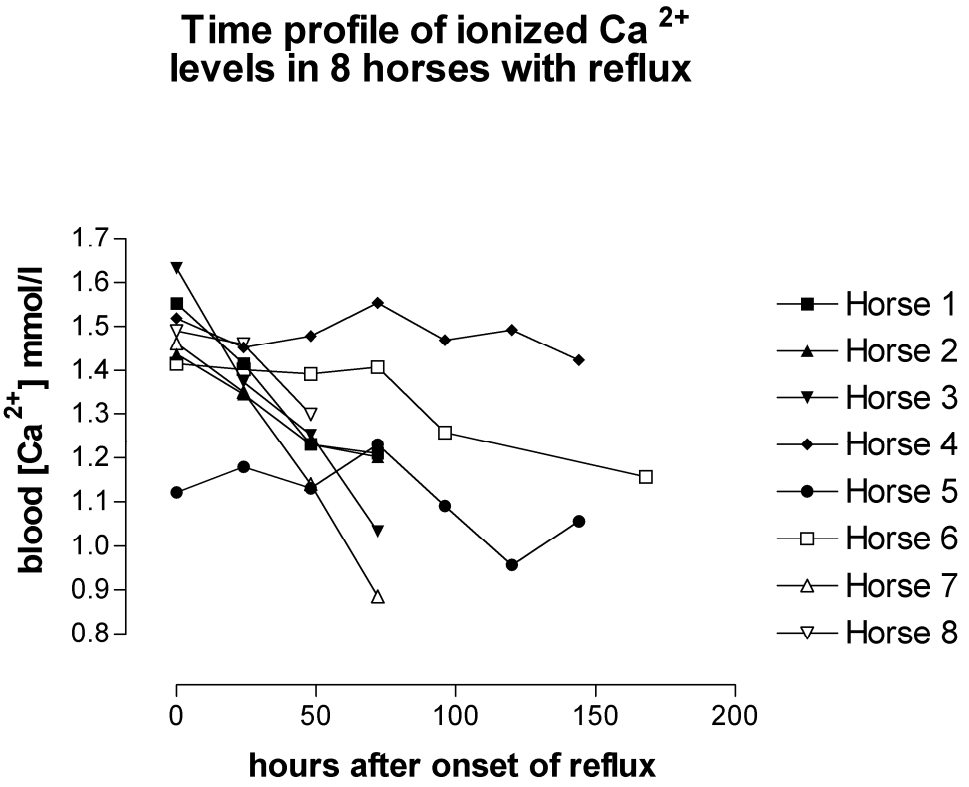

Figure 3.I.4: Time profile of whole blood ionized calcium levels in 8 horses with reflux, starting at the onset of reflux production until the last day of reflux production or death. None of the horses was substituted with Ca^{2+}.

In non-calcium-treated horses, the onset of development of reflux coincided with a mean decrease in blood ionized Ca^{2+} levels of 0.07 ± 0.18 mmol/l. Whenever reflux production continued, a further progressive decrease in calcemia was observed. When reflux subsided in these horses, a mean increase of calcemia with 0.14 mmol/l ± 0.12 could be observed.

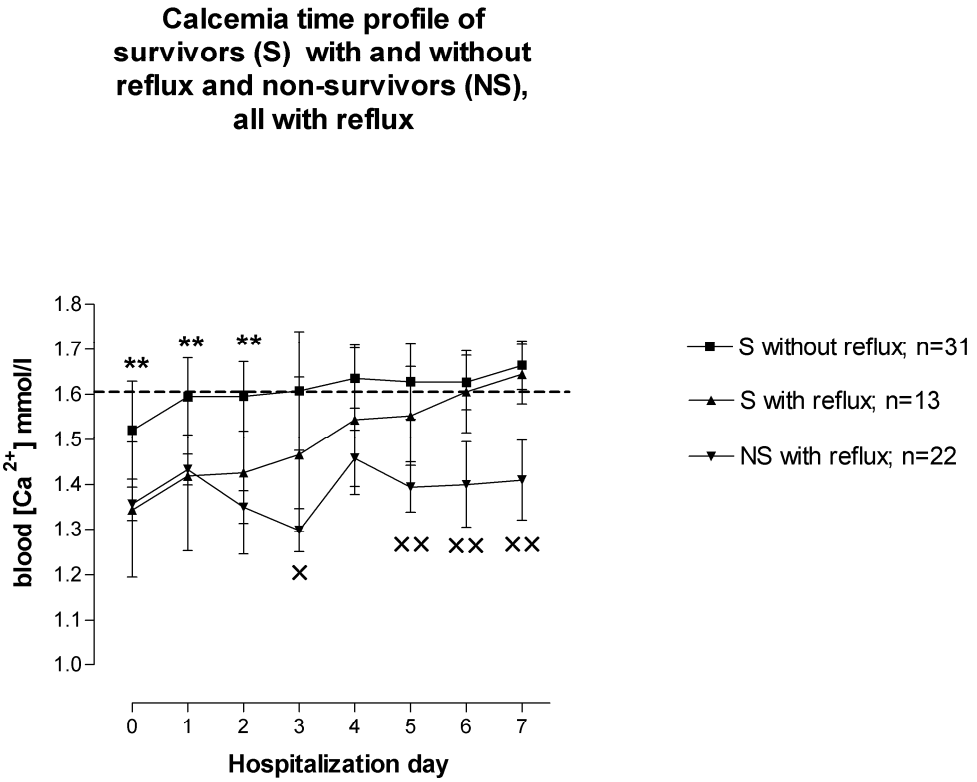

Figure 3.I.5: Time profile of whole blood ionized calcium levels in survivors (S) with and without reflux and non-survivors (NS), all with reflux. The dotted line depicts the lower limit of the reference interval for normocalcemia.
** significant difference at $p<0.01$ (Survivor without reflux versus survivor with reflux)
× significant difference at $p<0.05$; ×× significant difference at $p<0.01$ (Survivor with reflux versus non-survivor with reflux)

3.I.4. DISCUSSION

The normal range for heparinised whole blood ionized calcium of 1.61-1.85 mmol/l in our study, is somewhat higher than the ranges determined in previous studies (1.53-1.61 mmol/l, (Aguilera-Tejero *et al*., 1998); 1.45-1.75 mmol/l, (Van der Kolk *et al*., 2002); 1.51-1.55 mmol/l, (Hess *et al*., 2005). In these 3 studies a different type of analyzer then in our study was used. It has been reported that marked analyzer-specific variations exist. In a study where the AVL 988, a former model of the analyzer used in our study, was compared with two other analyzers designed by other manufacturers, the AVL 988 analyzer showed results for iCa^{2+}

that were significantly higher (Hristova *et al*., 1995). Many variables can introduce biases among the commercial analyzer systems, such as deviations in the calibration solutions of various analyzers, as well as the design of the liquid junction between the reference electrode and the sample. For this reason we established an analyzer-specific reference range, based on the analysis results of 25 healthy horses.

Factors influencing the occurrence of hypocalcaemia

As in previous studies, our study demonstrates that hypocalcaemia is a common finding in colic horses (Dart *et al*., 1992; Garcia-Lopez *et al*., 2001; Toribio *et al*., 2001a; Van der Kolk *et al*., 2002). Although no statistical difference could be demonstrated between colic groups in our study, it seems that horses with small intestinal related colic and duodenitis-proximal jejunitis are most prone to severe hypocalcaemia (Figure 3.I.1); calcium levels were lowest in the horses among the different groups that showed signs of endotoxemia. In humans, the correlation of severity of illness, defined by patient groups and APACHE II scores, with the incidence of hypocalcaemia, strongly implies that severity of illness rather than a particular illness per se is responsible for the hypocalcemic state (Zivin *et al*., 2001). This statement seems to be appropriate for the horse as well, where in the multivariable analysis, besides the presence of reflux at the time of admission, also pH, PCV, the interaction pH/PCV, and the presence of signs of endotoxemia all were significantly related with the whole blood ionized Ca^{2+} levels.

Endotoxaemia can be a complicating factor in horses suffering from colic. Due to intestinal ischemia or necrosis in colic, important amounts of endotoxins produced by gut flora can translocate to the systemic circulation, triggering all kinds of effects related to the inflammatory cascade, which in turn can affect calcium homeostasis. Pro-inflammatory mediators, which are released into the systemic circulation of colic horses, such as IL-1, IL-6

and TNF-α have been shown in vitro to suppress equine PTH secretion and lower the physiological set-point at which PTH production is induced (Barton & Collatos, 1999; Nielsen *et al*., 1997; Toribio *et al*., 2003a). Toribio and co-workers suggest that some horses with endotoxaemia and hypocalcaemia have an impaired parathyroid gland function associated with an abnormally low Ca^{2+} set-point (Toribio *et al*., 2003b). Those horses show low ionized calcium levels, together with low PTH levels, classified as so-called non-responders. However, in the majority of horses with enterocolitis and hypocalcaemia, normal to increased levels of PTH are measured, classified as so-called mid-to high-responders (Toribio *et al*., 2001a). In these horses end-organ resistance to the action of PTH in the bone and kidney could result in an attenuated and reduced bone resorption with concomitant urinary leakage of calcium. However, Toribio and co-workers already demonstrated that renal calcium loss has to be discarded as possible cause of hypocalcaemia in horses with enterocolitis (Toribio *et al*., 2001a). The "PTH resistant peripheral tissue theory" was first proposed in human critically ill patients with pronounced ionized hypocalcaemia and concomitant high PTH levels. However, in these cases, the administration of parenteral parathyroid extract, has been shown to increase serum ionized calcium similar to controls, indicative of parathyroid hormone-responsive target organs (Sibbald *et al*., 1977). Other investigators propose a possible hypocalcaemia inducing effect of calcitonin precursors, which are measured in high levels in the plasma of these patients (Müller *et al*., 2000). Calcitonin precursors, also referred to as "procalcitonin" are acute phase proteins that are markedly increased in human patients with infection, and have been shown to be the most reliable markers for the diagnosis of sepsis in critically ill patients in a medical IC unit (Carrol *et al*., 2005; Müller *et al*., 2000a; Müller *et al*., 2000b; Whang *et al*., 1998). The exact pathobiological activities of these calcitonin precursors still remain to be elucidated, but they possibly promote the influx of calcium from the extracellular to the intracellular compartment,

which on turn contributes to cellular damage (Nylen *et al*., 1998). Attempts are made to develop horse-specific immunoassays for calcitonin and procalcitonin to investigate their role in the pathogenesis of hypocalcaemia associated with enterocolitis (Toribio *et al*., 2001b). However, to our knowledge no clinical study investigating the possible association between hypocalcaemia in colic horses and increased levels of procalcitonin is yet available.

Finally, several studies have demonstrated a significant adrenocortical response in colic horses (Boatwright *et al*., 1996; Milne *et al*., 1990; Pritchett *et al*., 2003). The elevations in catecholamine levels can contribute to disturbances in calcium homeostasis (Wuster, 1997).

In the multivariable analysis, also the presence of reflux was retained as an independent factor influencing calcemia in colic horses. That production of reflux represents an important factor for Ca^{2+} loss in horses is emphasized by the significant difference that can be seen between the time profiles of calcemia in survivors with and without reflux (Figure 3.I.5). A status of ileus is associated with significantly lower levels of ionized calcium and these levels tend to progressively decline as long as the reflux episode continues (Figure 3.I.4). Hypocalcaemia in horses suffering from ileus represents an imbalance between efflux of calcium from the body not met by concomitant influx. The cause for defective calcium influx is multifactorial. Proper motility of the digestive tract is essential for absorption of adequate amounts of calcium (Jorgensen *et al*., 1998). Horses suffering from ileus are often restricted to a fasting protocol and GI motility is clearly disturbed. So, dietary intake and GI uptake are often negligible in ileus horses. Nevertheless it has to be mentioned that dietary calcium intake is not required to maintain normal circulating calcium concentrations, because the skeleton represents an inexhaustible source for adequate calcium mobilization to the blood stream. Ileus in horses is also associated with the sequestration of an important amount of fluid in the stomach and small intestines, referred to as reflux. This fluid is a mix of saliva, gastric juice, pancreatic secretions, bile and predominantly in cases suffering from duodenitis-

proximal jejunitis also inflammatory exudate. The pancreatic fluid, although not rich in enzymatic substances, is produced in large amounts in comparison to humans (Alexander & Hickson, 1970). An average of 12L/24h for a pony with a body weight of 100kg represents an important fluid shift with concomitant electrolyte losses. Additionally, bile fluids (pony 100 kg body weight: ± 150 ml/h) and saliva (± 12L/24h), which tends to contain high Ca^{2+} levels, will accumulate in the gastric compartment. This, together with possible presence of exudate due to an inflammatory response at the level of the GI tract in cases of duodenitis-proximal jejunitis, probably accounts for an important loss of calcium ions in horses suffering from ileus. Finally, acid-base status in horses with prolonged ileus follows an alkalotic trend, which can contribute to a further decline of ionized Ca^{2+} concentration (Lopez *et al*., 2004).

Factors influencing the occurrence of ileus and death

Although the presence of reflux at the time of admission and the presence of signs of endotoxemia are clearly associated with an increased probability of developing ileus during hospitalization, our study clearly indicates that this association also accounts for low ionized Ca^{2+} levels. The Odds for horses in the "very low" calcemia interval, for developing ileus, are 11.94 times larger in comparison with normocalcemic horses (Table 3.I.3). In human ICU's the possible association between hypocalcaemia and a tendency of GI stasis is commonly assumed. However, to our knowledge there is no clinical study that demonstrates this association in horses, nor in humans. The association between both findings, however is physiologically not surprising. At the cellular level Ca^{2+} is an important ion for normal GI smooth muscle motility (Hirst, 1999). The fact that it is one of the most tightly regulated ions in the human and equine body, illustrates the importance of even small disbalances. In humans, Ogilvy's syndrome or colonic pseudo-obstruction tends to be associated with hypocalcaemia (Jetmore *et al*., 1992). In dairy cows, a prolonged atony of the ruminant

digestive tract is a noticeable symptom in the well known syndrome of milk fever, induced by pronounced hypocalcaemia. The aforementioned association underlines the importance of follow-up of the calcemia status in colic horses.

The evidence of correlation between low ionized calcium levels and an increased probability of death is an interesting finding. Several studies have demonstrated low ionized calcium levels in colic horses and horses suffering from enterocolitis (Dart *et al.*, 1992; Toribio *et al.*, 2001a). However, the association of hypocalcaemia with survival was not examined. Garcia-Lopez and co-workers demonstrated that the predictive value of pre-operative serum ionized Mg^{2+} and Ca^{2+} concentrations for identifying horses with or without strangulating lesions of the intestine, is unsatisfactory (Garcia-Lopez *et al.*, 2001). Conversely, our study demonstrates that pronounced hypocalcaemia in colic horses at the time of admission has to be treated as a poor ominous sign. The association between decreased ionized calcium levels and poor outcome in critical illness has been well documented in human medicine (Burchard *et al.*, 1990; Chernow *et al.*, 1982; Desai *et al.*, 1988). In humans, ischemia-reperfusion injury, sepsis, multisystem organ failure, endocrine dysfunction and fatty acid liberation have all been implicated as causes of hypocalcaemia in critical illness (Ward *et al.*, 2004). The fact that our study shows a clear association between low blood ionized calcium levels and both the probability of death and ileus, demonstrates that calcium is an important ion for normal function of equine physiology. In humans, plasma ionized calcium levels between 1.16 and 1.27 mmol/l are appreciated as normal. In comparison to studies on calcemia in horses this seems to be a fairly low value. Indeed, serum total and ionized calcium concentrations in horses are higher than in humans (Van der Wiel, 2001). Toribio and co-workers have demonstrated that the Ca^{2+} set-point in the horse is higher than in other domestic animals and man (Toribio *et al.*, 2003). The Ca^{2+} set-point can be interpreted as an indicator of the serum Ca^{2+} concentration at which PTH secretion is

stimulated (Felsenfeld & Llach, 1993). Apparently, in horses, corrective PTH secretion is already stimulated at fairly high levels of Ca^{2+}. They speculate that horses are not as sensitive as other species to the suppressive effects of Ca^{2+} on PTH secretion. All the aforementioned observations tend to indicate once more that routine monitoring of Ca^{2+} levels in colic patients seems more than appropriate.

Calcium administration

The fact that hypocalcaemia is related to an increased probability of mortality, does not necessarily mean that calcium should be supplemented. It is possible that lowering of ionized Ca^{2+} levels is part of the host defence mechanisms against endotoxemia and sepsis, in order to limit cellular oxygen requirements and cellular damage (Vincent *et al*., 1995). In a rat model, where sepsis is mimicked by cecal ligation and perforation, supplementation of Ca^{2+} has detrimental effects (Malcolm *et al*., 1989). Ca^{2+} administration to pigs, subjected to endotoxin infusion, has no effect on survival (Carlstedt *et al*., 2000). Conversely, it has been shown that correction of hypocalcaemia in critically ill patients significantly improves hemodynamic parameters (Erdmann & Reuschel-Janetschek, 1991; Kovacs *et al*., 1998; Vincent *et al*., 1995). Little is known about the effects of correcting low ionized Ca^{2+} levels in critically ill human ICU patients. Due to the lack of studies, investigating possible beneficial or detrimental effects of Ca^{2+}-substitution on patient outcome and due to the alarming results of Ca^{2+}-substitution in animal models of endotoxemia and sepsis, the overall advise is to perform a correction in critical ill patients, but to limit the amount of infused Ca^{2+} to the strict minimum in order to attain lower limit reference range values of normocalcemia in these patients. Our study, clearly confirms this advice, by demonstrating a beneficial effect from slow and gradual Ca^{2+} substitution on survival of colic horses (Table 3.I.4). Bearing the

results of the animal endotoxemia models in mind, it was decided to optimize PCV and BE as much as possible, before calcium substitution was performed in the colic horses. As a consequence of this decision, substitution was always postponed until after abdominal surgery. In this way, per-operative possible beneficial haemodynamic effects due to Ca^{2+} administration are missed. It would be interesting to evaluate the pro's and con's of pre-operative calcium substitution in colic horses without prior stabilisation of acid-base and PCV disturbances.

When mean blood ionized calcium levels are graphed in function of time in survivors and non-survivors, not receiving calcium, a clear difference can be seen between both. This emphasises not only that appreciating the ionized Ca^{2+} level of a colic patient at the time of admission delivers interesting information concerning the probability of survival and development of ileus during hospitalization, but also a follow-up in time helps to evaluate the clinical progress of a colic patient (Figure 3.I.2). The same conclusion can be made for calcium-treated horses, where a striking difference can be seen between response patterns to calcium substitution in survivors versus non-survivors (Figures 3.I.3). It seems that when normocalcemia can be obtained by means of a single Ca^{2+} substitution, this can be guarded as a positive sign. However, when multiple corrections are needed, and more specifically when a progressive decrease is seen in calcemia, despite repetitive Ca^{2+} administrations, this warrants for poor outcome. Apparently survivors and non-survivors exhibit different response patterns to Ca^{2+} substitution: survivors show a gradual but steady increase in calcemia, while non-survivors tend not to do so and they lack an upward Ca^{2+} response. This progressive declining calcemia pattern follows the same course in non-calcium-treated and calcium-treated non-survivors (Figure 3.I.2 & 3.I.3). One might argue that in these cases a larger amount than 400 mEq Ca^{2+}/24h had to be supplemented. However, it is more probable that in these horses no normal physiological response to Ca^{2+} substitution was possible due to multi-organ failure

and that increasing the dose would not have made any difference. A similar response pattern has been identified in human patients in an ICU setting where follow-up of calcemia was performed until death or hospital discharge (Ward *et al*., 2004). Probably this downward response is due to calcium wasting as seen in multisystem organ failure of non survivors after major trauma or after toxic cell injury (Henderson *et al*., 1992; Trump & Berezesky, 1990).

3.I.5. CONCLUSION

Follow-up of whole blood ionized calcium levels in colic horses seems to be warranted for several reasons. The value at admission, gives the clinician an appraisal in terms of short term survival and probability of development of ileus. Correction of hypocalcaemia is associated with an increased probability of survival. Serial Ca^{2+} measurements during several consecutive days are advised, because the response pattern of the horse to Ca^{2+} substitution represents an additional factor to monitor its clinical progress. Correction of low Ca^{2+} levels should be performed whenever possible, however further research should be done in order to clarify whether prior optimalization of PCV and BE is necessary.

Footnotes

[a]Benzylpenicillinum natricum 1.000.000 IU, Kela N.V. Hoogstraten, Belgium.
[b]Excenel, sodium ceftiofur 1g, Pfizer Animal Health, Zaventem, Belgium.
[c]Alfamycine 5%, gentamicin sulphate, Eurovet, Sint Martens Latem, Belgium.
[d]Finadyne, flunixine meglumine 83 mg/ml, Shering-Plough Animal Health, Brussels, Belgium.
[e]Primperan®, Sanofi-Synthelabo, Brussels, Belgium.
[f]Erythrocine®, Abbott, Saint Remy/Avre, France.
[g]Calcii-Borogluconas® Eurovet, Belgium
[h]AVL 9180 Electrolyte Analyzer, AVL Scientific Corporation, Roswell, GA, US.
[i]248 pH/Blood Gas Analyzer, Chiron Diagnostics Ltd., Essex, UK.
[j]SPSS, Statistical Analysis Software, SPSS Inc, Chicago, US.

REFERENCES

AGUILERA-TEJERO, E., GARFIA, B., ESTEPA, J.C., LOPEZ, I., MAYER-VALOR, R. & RODRIGUEZ, M. (1998). Effects of exercise and EDTA administration on blood ionised calcium and parathyroid hormone in horses. *Am. J. Vet. Res.,* **59**, 1605-1607.

ALEXANDER, F. & HICKSON, J.C.D. (1970). The salivary and pancreatic secretions of the horse. *In*: Physiology of digestion and metabolism in the ruminant. Phillipson AT (Ed). Newcastle upon Tyne: Oriel Press, 375-389.

BARTON, M.H. & COLLATOS, C. (1999). Tumor necrosis factor and interleukin-6 activity and endotoxin concentration in peritoneal fluid and blood of horses with acute abdominal disease. *J. Vet. Int. Med.,* **13**, 457-64.

BARTON, M.H., PARVIAINEN, A., NORTON, N. (2004). Polymixin B protects horses against induced endotoxaemia in vivo. *Equine Vet J* **36**, 397-401.

BOATWRIGHT, C.E., FUBINI, S.L., GROHN, Y.T. & GOOSSENS, L.(1996). A comparison of N-butylscopolammonium bromide and butorphanol tartrate for analgesia using a balloon model of abdominal pain in ponies. *Can. J. Vet. Res.,* **60**, 65-68.

BURCHARD, K., GANN, D., COLLITON, J. & FORSTER, J. (1990). Ionized calcium, parathormone, and mortality in critically ill surgical patients. *Ann. Surg.,* **212**, 543-550.

BUSHINSKY, D.A. & MONK, R.D. (1998). Calcium. *The Lancet*., **352**: 306-311.

CARLSTEDT, F., ERIKSSON, M., KIISKI, R., LARSSON, A. & LINDL, L. (2000). Hypocalcemia during porcine endotoxemic shock: effects of calcium administration. *Crit. Care. Med.,* **28**: 2909-2914.

CARROL, E.D., NEWLAND, P., THOMSON, A.P. & HART, C.A. (2005). Prognostic value of procalcitonin in children with meningococcal sepsis. *Crit. Care. Med.,* **33**, 224-225.

CHERNOW, B., ZALOGA, G., MCFADDEN, E., CLAPPER, M., KOTLER, M., BARTON, M. & RAINEY, T.G. (1982). Hypocalcemia in critically ill patients. *Crit. Care. Med.*, **10**, 848-851.

DART, A.J., SNYDER, J.R., SPIER, S.J. & SULLIVAN, K. (1992). Ionized calcium concentration in horses with surgically managed gastrointestinal disease: 147 cases (1988-1990). *JAVMA.,* **15**, 1244-1248.

DESAI, T., CARLSON, R. & GEHEB, M. (1988). Prevalence and clinical implications of hypocalcemia in acutely ill patients in a medical intensive care unit. *Am. J. Med.,* **84**, 209-214.

DESAI, T., CARLSON, R., THILL-BAHAROZIAN, M. & GEHEB, M.A. (1998). A direct relationship between ionized calcium and arterial pressure in an intensive care unit. *Crit. Care. Med.,* **16**, 578-582.

ERDMANN, E. & REUSCHEL-JANETSCHEK, E. (1991). Calcium for resuscitation? *Brit. J. Anaesth.,* **67**, 178-184.

FELSENFELD, A.J. & LLACH, F. (1993). Parathyroid gland function in chronic renal failure. *Kid. Int.,* **43**, 771-789.

GARCIA-LOPEZ, J.M., PROVOST, P.J., RUSH, J.E., ZICKER, S.C., BURMASTER, H. & FREEMAN, L.M. (2001). Prevalence and prognostic importance of hypomagnesemia and hypocalcemia in horses that have colic surgery. *Am. J. Vet. Res.*, **62**, 7-12.

HENDERSON, S.A., GRAHAM, H.K. & MOLLAN, R.A. (1992). Serum and other calcium fractions in patients after severe musculoskeletal trauma. *Clin. Orthop.,* **275**, 306-311.

HESS, T.M., KRONFELD, D.S., WILLIAMS, C.A., HESS, T.M., KRONFELD, D.S., WILLIAMS, C.A., WALDRON, J.N., GRAHAM-THIERS, P.M., GREIWE-CRANDELL, K., LOPES, M.A. & HARRIS, P.A.

(2005). Effects of oral potassium supplementation on acid-base status and plasma ion concentrations of horses during endurance exercise. *Am. J. Vet. Res.,* **66**, 466-473.

HIRST, G.D. (1999). A calcium window to the gut. *Nature*., **399**, 16-17.

HRISTOVA, E.N., CECCO, S., NIEMELA, J.E., REHAK, N.N. & ELIN, R.J.(1995). Analyzer-dependent differences in results for ionized calcium, ionized magnesium, sodium, and pH. *Clin. Chem.*, **41**, 1649-1653.

HASTBACKA, J. & PETTILA, V.(2003). Prevalence and predictive value of ionized hypocalcemia among critically ill patients. *Acta. Anaesth. Scand.*, **47**, 1264-1269.

JETMORE, A.B., TIMMCKE, A.E., GATHRIGHT, J.B., HICKS, T.C., RAY, J.E. & BAKER, J.W. (1992). Ogilvies Syndrome-Colonoscopic decompression and analysis of predisposing factors. *Dis. Colon. Rectum.*, **35**, 1135-1142.

JORGENSEN, R.J., NYENGAARD, N.R., HARA, S., ENEMARK, J.M. & ANDERSEN, P.H. (1998). Rumen motility during induced hyper- and hypocalcaemia. *Acta. Vet. Scand.,* 39, 331-338.

KAHL, C.R. & MEANS, A.R. (2003). Regulation of cell cycle progression by calcium/calmodulin-dependent pathways. *Endocr. Rev.,* **24**, 719-736.

KANEKO, J.J., HARVEY, J.W. & BRUSS, M.L. (1997). Appendixes. *In*: Clinical Biochemistry of Domestic Animals. Kaneko JJ, Harvey JW, Bruss ML (Eds). San Diego, Academic Press, 885-905.

KOCH, S.M., WARTERS, R.D. & MEHLHORN, U. (2002). The simultaneous measurement of ionized and total calcium and ionized and total magnesium in intensive care unit patients. *J. Crit. Care.*, **17**, 203-205.

KOHN, C.W. & BROOKS, C.L. (1990). Failure of pH to predict ionized calcium percentage in healthy horses. *Am. J. Vet. Res.*, **51**, 1206-1210.

KOVACS, A., COURTOIS, M.R., BARZILAI, B., KARL, I.E., LUDBROOK, P.A. & HOTCHKISS, R.S. (1998). Reversal of hypocalcemia and decreased afterload in sepsis. *Am. J. Resp. Crit. Care. Med.,* **158**, 1998-1998.

LOPEZ, I., AGUILERA-TEJERO, E., ESTEPA, J.C., RODRIGUEZ, M. & FELSENFELD, A.J. (2004). Role of acidosis-induced increases in calcium on PTH secretion in acute metabolic and respiratory acidosis in the dog. *Am. J. Physiol.,* **286**, E780-E785.

MALCOLM, D.S., ZALOGA, G.P. & HOLADAY, J.W. (1989). Calcium administration increases the mortality of endotoxic shock in rats. *Crit. Care. Med.,***17**, 9.

MALYKHINA, A.P. & AKBARALI, H.I. (2004) Inflammation-induced "channelopathies" in the gastrointestinal smooth muscle. *Cell Biochem. Biophys.,* **41**, 319-330.

MILNE, E.M., DOXEY, D.L. & GILMOUR, J.S. (1990). Serum lipids and lipoproteins in equine colic and grass sickness. *Res. Vet. Sci.,* **48**, 170-174.

MOORE, J.N., NORTON, N., BARTON, M.H., HURLEY, D.J., REBER, A.J., DONOVAN, D.C., VANDENPLAS, M.L., PARKER, T.S., LEVINE, D.M. (2007). Rapid infusion of a phospholipid emulsion attenuates the effects of endotoxaemia in horses. *Equine Vet J.* **39**, 243-248.

MULLER, B., BECKER, L., KRANZLIN, H., SCHAGINGER, H., HUBER, P.R., NYLEN; E.S., SNIDER, R.H., WHITE, J.C., SCHMIDT-GAYK, H. & ZIMMERLI, W. (2000a). Disordered calcium homeostasis of sepsis: association with calcitonin precursors. *Eur. J. Clin. Invest.*, **30**, 823-831.

MULLER, B., BECKER, K.L., SCHAGINGER, H., RICKENBACHER, P.R., HUBER, P.R., ZIMMERLI, W. & RITZ, R. (2000b). Calcitonin precursors are reliable markers of sepsis in a medical intensive care unit. *Crit. Care Med.*, **28**, 977-983.

NIELSEN, P.K., RASMUSSEN, A.K., BUTTERS, R., FELDT-RASMUSSEN, U., BENDTZEN, K., DIAZ, R., BROWN, E.M. & OLGAARD, K. (1997). Inhibition of PTH secretion by interleukin-1 beta in bovine parathyroid glands in vitro is associated with an up-regulation of the calcium-sensing receptor mRNA. *Biochem. Bioph. Res. Co.,* **238**, 880-885.

NYLEN, E.S., WHANG, K.T., SNIDER, R.H., STEINWALD, P.M., WHITE, J.C. & BECKER, K.L. (1998). Mortality is increased by procalcitonin and decreased by an antiserum reactive to procalcitonin in experimental sepsis. *Crit. Care. Med.,* **26**, 1001-1006.

PRITCHETT, L.C., ULIBARRI, C., ROBERTS, M.C., SCHNEIDER, R.K. & SELLON, D.C. (2003). Identification of potential physiological and behavioural indicators of postoperative pain in horses after exploratory celiotomy for colic. *Appl. Anim. Behav. Sci.,* **80**, 31-43.

SIBBALD, W.J., SARDESAI, V. & WILSON, R.F. (1977). Hypocalcemia and nephrogenous cyclic AMP production in critically ill or injured patients. *J. Traum.,* **17**, 677-684.

SLOMP, J., VAN DER VOORT, P.H., GERRITSEN, R., BERK, J.A.M. & BAKKER, A.J. (2003). Albumin-adjusted calcium is not suitable for diagnosis of hyper-and hypocalcemia in the critically ill. *Crit. Care Med* ., **31**, 1389-1393.

SPRONK, H.M., GOVERS-RIEMSLAG, J.W. & TEN CATE, H. (2003). The blood coagulation system as a molecular machine. *Bioessays*., **25**, 1220-1228.

SZENCI, O., BRYDL, E. & BAJCSY, C.A. (1991). Effect of storage on measurement of ionized calcium and acid-base variables in equine, bovine, ovine, and canine venous blood. *JAVMA.,* **199**, 1167-1169.

TORIBIO, R.E., KOHN, C.W., CHEW, D.J., SAMS, R.A. & ROSOL, T.J. (2001a) Comparison of serum parathyroid hormone and ionized calcium and magnesium concentrations and fractional urinary clearance of calcium and phosphorus in healthy horses and horses with enterocolitis. *Am. J. Vet. Res.,* **62**, 938-947.

TORIBIO, R.E., KOHN, C.W., LEONE, G.W., CAPEN, C.C. & ROSOL, T.J. (2001b). Cloning of Equine calcitonin, calcitonin gene-related peptide-I, and calcitonin gene-related peptide-II cDNAs. *International Bone and Mineral Society/ the European Society for Calcified Tissue,* Abstracts.

TORIBIO, R.E., KOHN, C.W., SAMS, R.A., CAPEN, C.C. & ROSOL, T.J. (2003a). Hysteresis and calcium set-point for the calcium parathyroid hormone relationship in healthy horses. *Gen. Comp. Endocin.,* **130**, 279-288.

TORIBIO, R.E., KOHN, C.W., CAPEN, C.C. & ROSOL, T.J. (2003b). Parathyroid hormone (PTH) secretion, PTH mRNA and calcium-sensing receptor mRNA expression in equine parathyroid cells, and effects of interleukin (IL)-1, IL-6, and tumor necrosis factor-α on equine parathyroid cell function. *J. Mol. Endocrinol.,* **31**, 609-620.

TORIBIO, R.E., KOHN, C.W., HARDY, J., ROSOL, T.J. (2005). Alterations in serum parathyroid hormone and electrolyte concentrations and urinary excretion of electrolytes in horses with induced endotoxemia. J. Vet. Intern. Med. 19, 223-231.

TRUMP, B.F. & BEREZESKY, I.K. (1990). The importance of calcium regulation in toxic cell injury. *Clin. Lab. Med.,* **10**, 531-547.

TUPLING, A.R. (2004). The sarcoplasmic reticulum in muscle fatigue and disease: Role of the sarco(endo)plasmic reticulum Ca2+-ATPase. *Can. J. Appl. Physiol.,* **29**, 308-329.

VAN DER KOLK, J.H., NACHREINER, R.F., REFSAL, K.R., BROUILLET, D. & WENSING, T.H. (2002). Heparinised blood ionised calcium concentrations in horses with colic or diarrhoea compared to normal subjects. *Eq. Vet. J.,* **34**, 528-531.

VAN DER WIEL, H.E. (2001). Changes in calcium homeostasis in the critically ill. *Ned. Tijdschr. Int. Care.*, **16**, 108-113.

VINCENT, J.L., BREDAS, P., JANKOWSKI, S. & KAHN, R.J. (1995). Correction of hypocalcaemia in the critically ill: what is the haemodynamic benefit? *Int. Care. Med.*, **21**, 838-841.

WARD, R.T., COLTON, D.M., MEADE, P.C., HENRY, J.C., CONTRERAS, L.M., WILSON, O.M. & FLEMING, A.W. (2004). Serum levels of calcium and albumin in survivors versus nonsurvivors after critical injury. *J. Crit. Care*., **19**, 54-64.

WHANG, K.T., STEINWALD, P.M., WHITE, J.C., NYLEN, E.S., SNIDER, R.H., SIMON, G.L., GOLDBERG, R.L. & BECKER, K.L. (1998). Serum calcitonin precursors in sepsis and systemic inflammation. *J. Clin. Endocrinol. Metabol.*, **83**, 3296-3301.

WHITE, T.F., FARNDON, J.R., CONCEICAO, S.C., LAKER, M.F., WARD, M.K. & KERR, D.N. (1986). Serum calcium status in health and disease: a comparison of measured and derived parameters. *Clin. Chim. Acta*., **157**, 199-213.

WUSTER, C. (1997). Parathyroid hormone – a new prognostic marker of mortality? *Eur. J. Clin. Invest.*, 27, 982-983.

ZIVIN, J.R., GOOLEY, T., ZAGER, R.A. & RYAN, M.J. (2001). Hypocalcemia: A pervasive metabolic abnormality in the critically ill. *Am. J. Kidney. Dis.*, **37**, 689-698.

Chapter 3.II

DETERMINATION OF LACTATE LEVELS IN BLOOD PLASMA AND PERITONEAL FLUID IN HORSES WITH COLIC BY AN ACCUSPORT®-ANALYSER

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SUMMARY

Intestinal hypoperfusion can lead to increased lactate concentrations in plasma and peritoneal fluid of horses with colic.

The purposes of this study were to: a) evaluate the reliability of the Accusport® analyser to assess peritoneal fluid lactate (PFL) concentrations in healthy horses and those with colic, b) identify clinical features associated with abnormal blood plasma lactate (BPL) and PFL concentrations and c) evaluate the prognostic value of BPL and PFL.

BPL and PFL were determined in 20 healthy horses and in 106 horses with colic.

The Accusport® was reliable for determination of BPL concentrations 0.70-13 mmol/l and PFL concentrations 0.70-20 mmol/l. Multivariate analysis indicated that PCV and need for intestinal resection were independently associated with the BPL. Pulse, PCV, venous pO_2, presence of necrotic intestine, increased amount of peritoneal fluid and fluid total protein content were independently associated with PFL. Per 1 mmol/l increase in BPL or PFL, the respective Odds ratios for the need of abdominal surgery increase to 1.23 (BPL) and 1.58 (PFL), Odds ratios for the need of an intestinal resection increase to 1.20 (BPL) and 1.41 (PFL), and Odds ratios for developing ileus increase by 1.33 (BPL) and 1.36 (PFL). PFL concentrations of 1, 6, 12 and 16 mmol/l correspond to a probability of death of 11%, 29%, 63% and 82%, respectively in horses without strangulating obstruction and of 25%, 52%, 82% and 92%, respectively in horses with strangulating obstruction.

It can be concluded that PFL is more useful and sensitive than BPL for prognostic purposes in horses with colic.

3.II.1. INTRODUCTION

Several studies indicate that blood and peritoneal fluid lactate concentrations can be used as prognostic parameters in horses with colic (Donawick *et al*., 1975; Genn & Hertsch, 1982; Parry *et al*., 1983; Von Braun *et al*., 2002). Inadequate intestinal perfusion and ischemia lead to anaerobic glycolysis and an increase in lactate concentrations in the peritoneal fluid and blood of horses suffering from colic. However, this increase does not occur simultaneously in both body compartments. Typically, in horses with visceral ischemia, the first increases in lactate concentration occur in peritoneal fluid, after which a gradual increase is seen in the systemic circulation (DeLaurier *et al*., 1989; Liao *et al*., 1995). Similar findings in human patients have led to postoperative direct intra-abdominal on-line monitoring of peritoneal fluid lactate concentrations by means of microdialysis catheters for the early detection of visceral ischemia as a predictive sign of shock and multi-organ failure (Jansson *et al*., 2003; Sommer & Larsen, 2004).

In both field and hospital conditions, veterinarians frequently are confronted with owners asking for the prognosis of a horse with colic, before making important financial decisions. The assessment of several blood and peritoneal fluid variables such as blood coagulation tests, blood ionized calcium concentration, peritoneal fluid inorganic phosphorus concentration, alkaline phosphatase activity, lactate dehydrogenase activity, and haemoglobin concentration, have been shown to have prognostic value (Johnstone & Crane, 1986; Delesalle *et al*., 2005; Arden & Stick, 1988; Saulez *et al*., 2004; Van Hoogmoed *et al*., 1999; Weimann *et al*., 2002). However, determination of these variables requires direct access to permanently accessible laboratory analysis. Previously, Latson *et al*. (2005) found that peritoneal fluid lactate concentrations could be used as a marker of intestinal ischemia in horses with colic. Stall-side analysis of blood and peritoneal fluid lactate concentrations, by means of simple and

affordable hand-held equipment would add an important prognostic tool to field and hospital diagnosis and the evaluation of colic patients.

The aims of this study were to evaluate the reliability of a hand-held blood lactate analyser and to assess peritoneal fluid lactate concentrations in healthy horses and those with colic. Subsequently, we evaluated which clinical variables of horses suffering from colic are correlated with blood plasma and peritoneal fluid lactate concentrations. Finally, comparisons were made between blood plasma and peritoneal fluid lactate concentrations with respect to case outcome.

3.II.2. MATERIAL AND METHODS

Horses

The control group consisted of 20 (12 females, 8 castrated males) clinically healthy Belgian Warmblood horses from the Ghent University Faculty of Veterinary Medicine teaching herd, aged 1-22 years (mean ± SD, 9.61 ± 4.62). Horses were housed in individual boxes with straw bedding, and they were provided twice daily with grass hay and concentrate feed to meet all nutritional needs. All horses had free access to water.

The study population consisted of 106 horses with GI colic, that were presented to the Large Animal Internal Medicine Clinic of Ghent University, Belgium over a period of 1.5 years. All patients were divided into 2 categories on the basis of clinical and, if performed, surgical findings: (1) horses with non-strangulating obstruction of the small and large intestine and; (2) horses with small and large intestinal strangulating obstruction. When euthanasia was performed without surgery, an autopsy was performed to confirm the clinical diagnosis. Horses subjected to euthanasia without treatment due to financial limitations and horses with known systemic diseases in addition to colic were excluded from the study. None of the horses with colic received IV fluids before admission.

Study design and blood sample collection

20 healthy horses were used to determine the reference range for blood plasma (BPL) and peritoneal fluid (PFL) lactate concentrations. For this purpose, analyses were performed in duplicate with the use of a laboratory applied analysis technique and the Accusport® analyser (see below).

Clinical examination of horses with colic consisted of assessment of pulse quality, capillary refill time, and appearance of mucous membranes, respiratory rate, heart rate, lung and gut sounds and rectal temperature. Rectal examination always was followed by transabdominal ultrasonography by means of a 2.5 mHz sectorial probe[a] with which small and large intestinal wall thickness and the amount of peritoneal fluid were evaluated. Scanning the complete abdomen of each horse with colic and comparing suspicious intestinal parts with normal parts of the intestine was used to evaluate intestinal wall thickness. Small and large intestines were classified as being oedematous when wall thickness clearly was visually increased. The amount of peritoneal fluid was assessed and was designated as “moderately increased” when free fluid could be visualized at the ventral part of the spleen (left cranial portion of the abdomen, cranially and adjacent to the stomach). The amount was designated as “severely increased” when free fluid could be visualized in the ventral part of the abdomen and in between intestinal loops. Upon arrival, horses were categorized as having reflux, when > a net amount of 1.5 L of reflux fluid was collected by gastric decompression. Horses that continued producing refluxed fluid for > 24 h after admission or that needed repeated gastric decompression during the first 24 h or longer postoperatively were categorized in the “ileus during hospitalization” group.

At the time of admission, 2 venous blood samples were collected anaerobically from the jugular vein. One sample was placed in a tube containing lithium heparin and a second sample in a tube containing Na^+-fluoride/ potassium oxalate. After clinical examination and

abdominal ultrasound examination, paracentesis was performed and peritoneal fluid was collected in 3 separate tubes. The first tube contained Na^+-fluoride/potassium oxalate, the second EDTA, and the third did not contain any anti-coagulant and was used for determination of total protein content.

Heparinized whole blood samples were used to determine PCV; pH and base excess (BE) were determined by use of a bloodgas analyser[b]. Analyses were performed within 5 min after blood collection. Na^+-fluoride-containing blood and peritoneal fluid samples were used to measure lactate concentrations. In 20 healthy horses and 50 with colic, BPL or PFL analyses were performed in duplicate using an enzymatic colorimetric method[c] as a reference and the hand-held Accusport® analyser[d]. In 15 horses with colic, blood samples for laboratory BPL analysis were not drawn or handled as prescribed by the study protocol and therefore were not available for analysis. The Accusport® consists of a small portable battery-powered hand-held analyser and disposable test strips. After placing a drop of whole blood on the chemistry strip, plasma seeps through a membrane filter into the measuring chamber. Here, in time course of 60 secs the plasma lactate concentration is determined by reflectance photometry via a colorimetric lactate-oxidase reaction using a built-in algorithm. The instrument converts plasma concentrations to whole blood concentrations by means of an internal conversion formula. However, previous research has demonstrated that this conversion factor, which is based on human data, is not suitable for horses (Evans *et al*., 1996). Therefore, in this study, the meter was set on the plasma lactate mode, which has a measuring range of 0.70 – 26.00 mmol/l. In 5 randomly picked horses with colic, PFL and BPL concentrations were determined 5 times consecutively with the Accusport® analyser in order to determine the intra-assay coefficient of variation. All hand-held analyses were performed within 5 min after blood or peritoneal fluid collection by placing a drop of whole blood or peritoneal fluid on the test pad of the Accusport® analyser. Samples that were used for consecutive enzymatic

colorimetric lactate analysis[c] were centrifuged within 10 min after collection, and the top fraction was stored at 4°C and sent to the clinical laboratory within 4 h.

All peritoneal samples also were analysed for total protein content (sample without anti-coagulant) by use of a refractometer[e] and white blood cell (WBC) count by use of a coulter counter[f]. Gross appearance was compared visually with samples from control horses. In this way, the peritoneal fluid samples were categorized as being clear or turbid (turbid, sero-hemorrhagic or fecal contaminated).

Statistical analysis

Associations between the lactate concentrations measured with the 2 devices were visualized by plotting a limits of agreement plot (Bland & Altman, 1986). The repeatability of the Accusport® was determined on 5 replicate Accusport® analyses per horse (performed in a total of 5 colic patients) by calculating the intra-assay coefficient of variation which is defined as the within-horse standard deviation divided by the within-horse mean.

The relationship between potential risk factors and the BPL and PFL concentration at the time of admission was analysed with a generalized linear model (SPSS 12.0). Both BPL and PFL were not normally distributed and therefore results were log transformed wherever they served as dependent variables. Duration of symptoms before entry into the clinic was categorized into 4 intervals: 0-6 h (20 horses); 7-12 h (52 horses); 13-24 h (19 horses); and 25-240 h (15 horses).

First, the relation of each variable with the log BPL and the log PFL was evaluated univariably. All variables with a univariable *p* value below 0.2 were included in the multivariable model that was constructed using a stepwise backward procedure. If 2 highly correlated variables were both significant in the univariable analysis, only the variable with the smallest *p* value was included in the multivariable model. The advantage of the

multivariable model is that the effect of all variables determined as significant by this model is corrected for the levels of influence of all other outcome-related variables incorporated in this model. This approach insures that each variable that is determined to be significant by the multivariable analysis, has a significant effect on the outcome, independent of the level (high or low) of the other variables. Also, in the multivariable model, all possible interactions were tested among the significant variables.

A paired sample t-test was used to compare the log BPL and log PFL concentrations in horses with colic.

The relationship between BPL and PFL and the need for surgery, the need for intestinal resection, the probability of developing ileus during hospitalization and the probability of survival was evaluated using logistic regression (SPSS 12.0). For the latter, the type of colic lesion (strangulating VS non-strangulating obstruction) was included as a co-variable.

3.II.3. RESULTS

3.II.3.1. Assessment of BPL and PFL reference range in healthy horses (n=20)

The mean BPL concentration in 20 healthy horses measured by means of the enzymatic colorimetric analysis[b] was 0.59 ± 0.22 mmol/l (mean ± 1SD). The Accusport® determined mean BPL concentrations in the same 20 horses could not be used to determine a reference range because 12 of 20 healthy horses had a BPL concentration below the minimum detectable concentration of the Accusport® analyser (0.70 mmol/l). However, for concentrations > 0.70 mmol/l, the BPL concentrations obtained with the Accusport® analyser were in accordance with the laboratory method. All results below the threshold limit of the Accusport® also were < 0.70 mmol/l with the laboratory method. Mean PCV and pH values of the control population were 37% ± 7% and 7.40 ± 0.03, respectively.

The PFL reference range in the 20 healthy horses measured with the laboratory method was set at 0.49 ± 0.27 mmol/l. Again, no reference range could be established with the Accusport® due to the lower threshold limit (only 5 horses with PFL concentration < 0.70 mmol/l). As for BPL, both measurement techniques showed good correlation for determination of PFL concentrations.

3.II.3.2. Assessment of the ability of the hand-held lactate analyser to determine blood plasma and peritoneal fluid lactate concentrations in horses with colic

The BPL concentrations in 55 horses (20 healthy; 35 colic) were measured in duplicate. Only results from 36 (n=8 healthy; n=28 colic) were above the threshold of the Accusport®. All BPL concentrations that were below the threshold limit of detection with the Accusport® (n=12 healthy; n=7 colic) were excluded from analysis. The limits of agreement are shown in Figure 3.II.1. The mean difference between both tests was 0.14 mmol/l and in 95% of the cases the difference between test results was between –0.61 and +0.89 mmol/l. Below a BPL concentration of 13 mmol/l, the difference between the values for the 2 methods was small. Results from 54 (n=5 healthy; n=49 colic) of the 70 horses in which the PFL concentrations were analysed in duplicate, were above the threshold of the Accusport®. All PFL concentrations that were below the threshold limit of detection with the Accusport® (n=15 healthy; n=1 colic) were excluded from analysis. The limits of agreement are illustrated in Figure 3.II.2. The mean difference between both tests is 0.48 mmol/l. In 95% of cases the difference between the test results was between –2.70 and +3.66 mmol/l. The higher the PFL concentrations are, the larger the difference between both tests (see Figure 3.II.2). Below a PFL concentration of 20 mmol/l, the difference between the values for the 2 methods is small. The intra-assay coefficient of variation was between 5.40 and 7.00 % for determination of BPL concentrations, and between 5.90 and 7.20 % for the PFL analyses.

For further statistical analyses in the horses with colic, Accusport® measurements below the threshold level were replaced by the results obtained using the laboratory analysis technique (n=7 for BPL; n=1 for PFL).

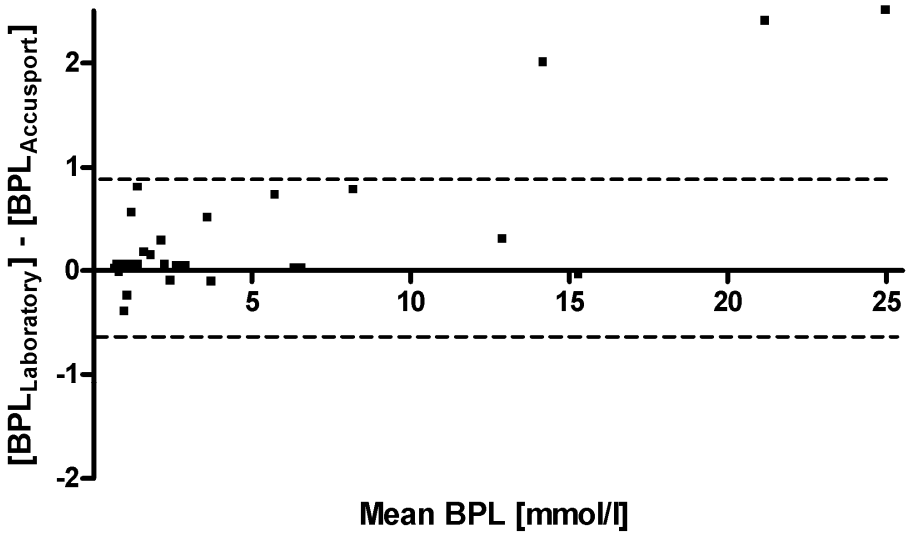

Figure 3.II.1: Difference between laboratory method[c] and Accusport[d] determined BPL concentrations in 36 horses (n=8 healthy; n=28 colic). Points represent paired measurements on the same sample.

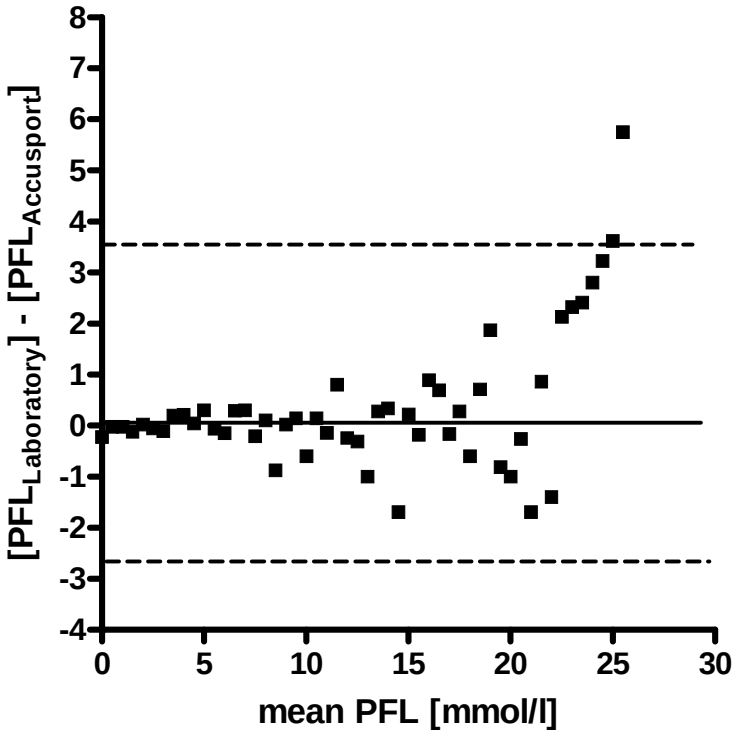

Figure 3.II.2: Difference between laboratory method[c] and Accusport[d] determined PFL concentrations in 54 horses (n=5 healthy; n=49 colic). Points represent paired measurements on the same sample.

3.II.3.3. Baseline patient characteristics

Horses in the study population were between 1 and 23 years of age (9.83± 5.68 years) and consisted of several breeds (45 Belgian Warmblood horses, 29 French Warmblood horses, 3 trotters and 29 other breeds) and both sexes (53 females, 37 castrated males, 16 sexually intact males). The duration of colic at the time of arrival in the clinic varied between 3.0 and 240.0 hours with a median of 12.0 hours and a mean of 19.4 ± 28.8 hours. Baseline patient characteristics are presented in Tables 3.II.1, 3.II.2 and 3.II.3. The duration of clinical signs before admission was quite variable among horses. Horses in the non-strangulating colic group were admitted to the clinic after a mean duration of colic of 20.1 ± 14.3 hours

(minimum, 3.0 h; maximum, 240.0 h; median, 10.5 h). Horses with a strangulating lesion were presented after a mean duration of 18.4 ± 16.7 h (minimum, 6.0 h; maximum, 72.0 h; median, 12.0 h).

Table 3.II.1: Study population characteristics per colic group.

	No strangulation *n*=66	**Strangulation** *n*=40	**Total** *n*=106
Reflux at admission	*n*=15	*n*=14	*n*=29
Surgical intervention	*n*=24	*n*=40	*n*=64
Intestinal necrosis √ No necrosis √ Necrosis √ Inflammation	 *n*=56 *n*=0 *n*=10	 *n*=0 *n*=40 *n*=0	*n*=40
Resection	*n*=1	*n*=37	*n*=38
Increased intestinal wall thickness	*n*=4	*n*=13	*n*=17
Amount of peritoneal fluid √ Absent √ Moderate √ Severe	 *n*=25 *n*=22 *n*=19	 *n*=1 *n*=10 *n*=29	 *n*=26 *n*=32 *n*=48
Ileus during hospitalization	*n*=10	*n*=9	*n*=19
Duration of colic before admission √ 0-6h √ 7-12h √ 13-24h √ 25-240h	 *n*=17 *n*=29 *n*=11 *n*=9	 *n*=3 *n*=23 *n*=8 *n*=6	 *n*=20 *n*=52 *n*=19 *n*=15
Outcome √ Survival √ Peroperative euthanasia √ Death < 3 days post surgery √ Death > 3 days post surgery	 *n*=49 *n*=3 *n*=2 *n*=5	 *n*=18 *n*=20 *n*=7 *n*=2	 *n*=67 *n*=23 *n*=9 *n*=7

3.II.3.4. Clinical features of horses with colic that are related to the lactate concentration in blood plasma and peritoneal fluid

Univariable linear regression analysis indicated that duration of clinical signs (p=0.046), pulse (p<0.001), PCV (p<0.001), base excess (p=0.001), pCO_2 (p=0.035), HCO_3^- (p=0.001),

and capillary refill time (p=0.001) were significantly related with log BPL and log PFL concentrations in horses with colic. In horses without strangulating obstruction, there was no significant increase in both log BPL and log PFL concentrations with an increasing symptom duration before admission. In horses with strangulating obstruction, a marked increase in log PFL concentrations was observed with increasing symptom duration before admission. However, this increase was not significant. For the log BPL concentration, the increase was less pronounced and also not significant (Table 3.II.3).

Table 3.II.2: Clinical and laboratory data of the colic horses

	No strangulation			**Strangulation**		
	Min	Max	Median (Interquartile range)	Min	Max	Median (Interquartile range)
Age (years)	1.00	22.00	7.00 (5.00)	1.00	20.00	8.00 (10.00)
Pulse (beats/min)	32.00	116.00	48.00 (30.00)	40.00	132.00	80.00 (24.00)
PCV (%)	29.00	65.00	40.00 (11.50)	34.00	72.00	45.00 (15.50)
BE (mEq/l)	-7.90	10.70	2.60 (5.95)	-14.60	6.40	0.30 (5.60)
PH	7.26	7.48	7.40 (0.13)	7.15	7.45	7.36 (0.12)
pCO_2 (mmHg)	38.50	73.60	45.50 (6.70)	25.60	65.80	45.30 (6.15)
HCO_3^- (mmol/l)	17.60	34.40	25.40 (5.25)	12.80	29.20	24.20 (5.95)
pO_2 (mmHg)	29.30	62.60	40.10 (11.45)	25.60	49.30	41.30 (11.05)
Capillary refill (secs)	1.00	4.00	2.00 (0.50)	1.00	5.00	3.00 (2.00)
Resected intestine(m)	0.00	0.20	0.20	0.50	12.00	2.44 (4.12)
Blood plasma lactic acid[d] (mmol/l)	0.25	9.70	1.20 (1.52)	0.90	23.00	4.90 (4.98)
Peritoneal fluid lactic acid[d] (mmol/l)	0.63	23.60	2.60 (2.45)	2.30	25.50	12.50 (10.30)
Log blood plasma lactic acid	-0.52	0.99	0.08 (0.46)	- 0.05	1.36	0.69 (0.46)
Log peritoneal fluid lactic acid	-0.23	1.37	0.42 (0.48)	0.36	1.41	1.10 (0.36)
Total protein peritoneal fluid (g/l)	6.00	56.00	21.00 (18.00)	20.00	56.00	35.00 (12.50)
Peritoneal fluid WBC/mm³	620	50000	3200 (4390)	600	190000	9200 (31000)

Univariable linear regression analysis also indicated that presence of reflux at the time of admission (p=0.016), visualization of an increased amount of peritoneal fluid (p<0.001) by means of abdominal ultrasound, gross appearance of the peritoneal fluid (p=0.020) and its total protein content (p<0.001) all were significantly related with both the log BPL and log PFL concentrations in horses with colic. Horses with strangulating intestinal lesions had higher log BPL and log PFL concentrations than those without strangulating lesions (p<0.001). Mean log PFL and log BPL concentrations were higher in horses with colic that needed surgery in comparison to horses that were treated conservatively (p<0.001). In accordance with this observation, horses with necrotic intestine (p<0.001) and horses subjected to intestinal resection (p<0.001) had significantly higher log BPL and log PFL concentrations. Mean log PFL and log BPL concentrations in colic cases with clear abdominal fluid were significantly lower than in horses in which the fluid was categorized as turbid (p<0.001).

The length of intestine to be resected was uniquely related to the log BPL concentration in the univariable model (p=0.036). Factors uniquely related with the log PFL concentration in the univariable model were venous blood pO_2 (p=0.025), peritoneal fluid white blood cell count (p=0.007) and increase in intestinal wall thickness (p=0.022).

In the multivariable analysis, only PCV (p<0.001) and need for intestinal resection (p<0.001) remained independently associated with log BPL in colic patients. Both variables account for 49% of the variability present in the dataset. No significant interactions between these variables could be identified. For the log PFL concentration in the multivariable model, pulse (p<0.001), venous pO_2 (p=0.030), presence of necrotic intestine (p<0.001), visualization of an increased amount of peritoneal fluid (p=0.017) and peritoneal fluid total protein content (p<0.001) were independently related to the log PFL concentration. Here, the model accounted for 73% of the variability present in the dataset.

Table 3.II.3: BPL and PFL levels (Accusport®) (Median (Interquartile Range)) according to the presence or absence of compromised bowel, gross appearance of peritoneal fluid, the need for surgery or intestinal resection, survival, the presence or absence of strangulating obstructions and duration of colic signs before admission.

		BPL (mmol/l)	PFL (mmol/l)
Necrotic intestine	No (*n=56*)	1.18 (0.95)	2.65 (2.38)
	Yes (*n=50*)	4.60 (4.90)	11.93 (11.95)
Abdominal fluid	Clear (*n=58*)	1.20 (1.32)	2.60 (2.30)
	Turbid (*n=48*)	3.98 (4.14)	10.50 (11.80)
Surgery	No (*n=42*)	1.12 (1.03)	2.40 (4.43)
	Yes (*n=64*)	3.86 (5.16)	10.12 (12.80)
Intestinal resection	No (*n=69*)	1.13 (1.40)	2.90 (5.65)
	Yes (*n=37*)	4.70 (4.04)	12.05 (12.50)
Death	No (*n=67*)	1.13 (1.31)	2.80 (4.31)
	Yes (*n=39*)	6.30 (3.47)	14.00 (8.75)
Strangulation	No (*n=66*)	1.20 (1.52)	2.60 (2.45)
	0-6h	1.20 (0.85)	2.41 (2.05)
	7-12h	1.10 (1.74)	2.65 (2.78)
	13-24h	1.20 (0.71)	2.90 (5.10)
	25-300h	1.45 (1.53)	5.00 (3.93)
	Yes (*n=40*)	4.90 (4.98)	12.50 (10.30)
	0-6h	1.95 (1.10)	8.33 (1.12)
	7-12h	4.35 (5.81)	12.50 (10.10)
	13-24h	6.00 (3.10)	15.50 (7.50)
	25-300h	5.90 (-)	21.95 (-)

BPL indicates blood plasma lactate; PFL, peritoneal fluid lactate.

3.II.3.5. Relationship between plasma and peritoneal fluid lactate concentrations and patient outcome

Logistic regression indicated that BPL and PFL are significantly related with the need for surgery. Per 1 mmol/l increase in BPL concentration, the respective Odds ratios (95% CI) describing the need for surgical intervention and intestinal resection increased to 1.23 (1.10-1.37) and to 1.20 (1.11-1.30), respectively. Per 1 mmol/l increase in the PFL concentration, the aforementioned Odds ratios increased to 1.58 (1.17-2.13) and 1.41 (1.15-1.72), respectively. Considering the probability of developing ileus during hospitalization, it was

demonstrated that for every 1 mmol/l increase in the BPL and PFL concentration, the Odds ratio for ileus development increased to 1.33 (1.14-1.54) for BPL and to 1.36 (1.06-1.74) for PFL, respectively. Finally, a clear association was demonstrated between BPL and PFL concentrations and the probability of death. The Odds for fatal outcome were 1.33 (1.18-1.50) and 1.46 (1.16-1.82) times greater for every 1 mmol/l increase in BPL and PFL, respectively. In the multivariable logistic regression model, the presence of strangulation also was an explanatory variable. In horses without strangulating obstruction, the Odds ratios for fatal outcome were 1.26 (1.00-1.59) and 1.27 (1.11-1.45) for every 1 mmol/l increase in BPL and PFL, respectively. In the group of horses with strangulating obstruction, the Odds ratios for fatal outcome were 2.62 (0.74-9.24) (for BPL) and 5.51 (1.57-19.32) (for PFL), respectively. Mean (Interquartile Range) BPL and PFL concentrations in non-surviving horses were 6.30 (3.47) mmol/l and 14.00 (8.75) mmol/l, respectively. Eventually, all horses with a PFL concentration > 16.90 mmol/l died. No horse with a BPL concentration > 8.60 mmol /l survived. In the majority of colic cases, the log PFL was significantly higher than the log BPL (85/106 horses; $p<0.001$). In only 4% of the colic cases, was BPL higher than PFL. The average ratio was 0.55. Within horses, the average concentrations of log BPL and log PFL differed significantly ($p<0.001$). In the reference population, 55% of the horses had BPL higher than PFL. The average ratio was 1.61.

PFL concentrations of 1, 6, 12 and 16 mmol/l in horses without strangulating obstruction corresponded to a probability of death of 11%, 29%, 63% and 82%, respectively. Similar PFL concentrations in horses with strangulating obstruction corresponded to a probability of death of 25%, 52%, 82% and 92%, respectively. The BPL and PFL concentrations of the study population and corresponding probabilities of death are presented in Figure 3.II.3.

Multivariable logistic regression also indicated that calculation of the BPL on PFL ratio provides no additional information on survival in comparison to both results determined seperately.

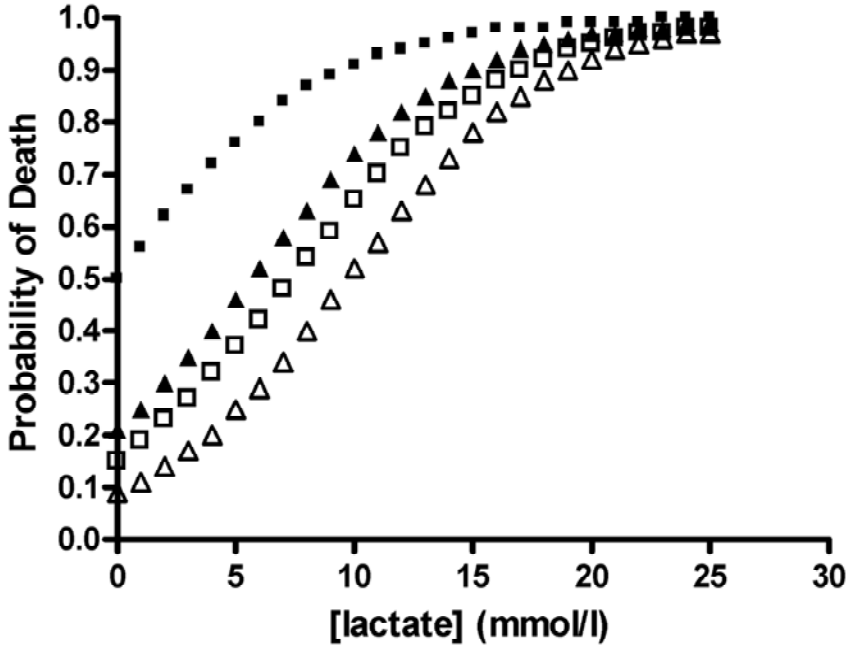

Figure 3.II.3: BPL and PFL levels with their corresponding probability of death, in horses with and without strangulating obstruction. (■ BPL strangulation; ▲ PFL strangulation; □ BPL no strangulation; Δ PFL no strangulation).

3.II.4. DISCUSSION

Evaluation of the Accusport® analyser

The accuracy of the Accusport® analyser for lactate measurements in blood and plasma of human athletes and patients has been studied many times with varying results (Fell *et al*., 1998; Nordstrom *et al*., 1998; Yam *et al*., 1998; Brinkert *et al*., 1999; Bishop, 2001). Likewise, varying findings have been reported in horses. Lindner *et al* (1996) reported a strong correlation between lactate determination in a drop of heparinized whole blood measured with the Accusport® in plasma mode and the laboratory wet chemistry method

applied on centrifuged plasma samples (Lindner, 1996). Williamson *et al*. (1996) confirmed these findings, using Na^+-fluoride as an anti-coagulant (Williamson *et al*., 1996). Both studies used blood samples of horses drawn during training trials. Reported PCV's ranged from 45 to 60%. However, the previous findings were not supported by Evans *et al*. (1996), who demonstrated that in cases in which lactate concentrations were > 10 mmol/l and PCV > 53%, the Accusport® tended to underestimate heparinized whole blood lactate concentrations in comparison with the laboratory wet chemistry method applied on centrifuged plasma samples (Evans & Golland, 1996). Similar findings were reported by Schulman and co-workers (2001) who found that the Accusport® can be used reliably in colic horses for measurement of blood lactate concentrations < 10 mmol/l (Schulman *et al*., 2001). In these cases, it was proposed that high PCV could have prevented the plasma fraction of a drop of whole blood from seeping accurately into the measuring chamber of the Accusport® analyser. This latter argument however was refuted by Simmons and co-workers (1999), because they demonstrated that the accuracy of the Accusport® also was disturbed with heparinized plasma samples at concentrations > 13 mmol/l (Simmons *et al*., 1999). In our study, a strong correlation was identified between measurements of plasma lactate concentration in a drop of whole blood with the Accusport® meter in plasma mode and the laboratory analysis applied on centrifuged plasma samples, both drawn in Na^+-fluoride blood collection tubes, even with values well > 10 mmol/l. Still, only 6 horses with colic had plasma lactate concentrations > 10 mmol/l and in 3 of these cases the difference between both test results was > 1.90 mmol/l. A discrepancy between both measurement methods at higher lactate concentrations may have been detected more readily, if additional data in these ranges had been available.

Concerning the peritoneal fluid analyses, a strong correlation was identified between both measuring techniques for values < 20 mmol/l, which encourages the use of the Accusport® meter for rapid peritoneal fluid lactate analysis. For measurement of PFL concentrations > 20

mmol/l the Accusport® was less reliable (Figure 3.II.2). To our knowledge, ours is the first clinical study demonstrating the suitability of the Accusport® for this purpose.

The tendency for positive bias between both analysis techniques that can be seen for the BPL concentrations (Figure 3.II.1) is absent in the PFL analyses (Figure 3.II.2). We have no explanation for this observation.

Correlation of clinical variables with blood plasma and peritoneal fluid lactate concentrations

Although only a relatively small population was used to determine the reference range, reference ranges for both whole blood and peritoneal fluid lactate concentrations, are in accordance with previously published data (Table 3.II.4). In colic patients, these concentrations tend to increase in horses that needed surgery or intestinal resection, had compromised bowels or turbid peritoneal fluid . Similar findings were reported by Latson *et al* (2005).

As mentioned previously, no horse in our study with a blood lactate concentration > 8.60 mmol/l survived. PCV values of these horses ranged between 44% and 72%. This finding is in accordance with the studies of Genn *et al*. (1982), Moore *et al*. (1976) and Schulman *et al*. (2001) who found that horses with colic and plasma lactate concentrations > 8.30 and 8 mmol/l, respectively, had an extremely low chance of survival (Genn & Hertsch, 1982; Schulman *et al*., 2001; Moore *et al*., 1976). However, these are specific study population results and must be interpreted as such. That is, not every horse with colic and a lactate concentration > 8 mmol/l necessarily has a fatal outcome.

Table 3.II.4: Reference values for whole blood or plasma (sampled at the jugular vein) and peritoneal fluid lactic acid levels (mmol/l)

Blood			
Reference	Sample type	Reference range	Number of horses
Donawick et al. 1975	plasma	± 0.81	11
Moore et al. 1976	plasma	0.4-1.33	50
Lumsden et al. 1980	Whole blood	0.28-1.72	60
Eikmeier et al. 1982	plasma	± 0.89	?
Genn et al. 1982	plasma	≤ 0.49	5
Williamson et al. 1996	whole blood	< 0.70	6
Piccione et al. 2004	plasma	0.75-1	12
Latson et al. 2005	whole blood	0.37-1.43	20
Peritoneal fluid			
Moore et al. 1977		0.30-1.47	15
Nelson et al. 1979		< 1.64	13
Moore et al. 1980		0.66 ± 0.43	?
Parry et al. 1991		0.7 ± 0.2	?
Mair et al. 2002		0.4-1.2	?
Latson et al. 2005		0.22-0.98	20

In accordance with the results of previous studies, the BPL/PFL ratio in healthy horses is > 1 (Latson *et al*., 2005; Moore *et al*., 1977). In horses with colic, however, this trend does not persist and log PFL concentrations are on average significantly higher than log BPL concentrations. A plausible explanation for this finding is that the time point at which abdominocentesis is performed during the colic episode is important. PFL concentrations first exceed BPL concentrations, after which blood concentrations will increase due to circulatory collapse. Therefore, abdominal fluid lactate concentrations sampled early in the colic process will give more accurate and timely information to the veterinarian and owner as compared to blood lactate concentrations, measured simultaneously.

A statistical relationship between the presence of an ischemic intestine and lactate concentrations could only be demonstrated for peritoneal fluid and not for blood plasma. This finding confirms the idea that lactate increments in blood plasma of horses with colic are less specific with respect to the presence of a compromised bowel. Moreover, lactate concentrations tend to increase as the disease process progresses and are influenced by many other pathologic processes, such as hemoconcentration, shock and endotoxemia, which can

occur in horses with colic. How fast diffusion of produced lactate occurs from a necrotic intestinal segment into the peritoneal fluid and from there into the systemic circulation is not documented. One study used 8 pigs in which occlusive intestinal ischemia was established by clamping 20 cm of the mesentery of the small intestine (Sommer & Fromholt, 2003). Microdialysate peritoneal lactate concentrations started to increase within 1 h. Systemic arterial lactate concentrations however did not increase during the entire experimental preparation that encompassed 250 min. In accordance with the Latson study, additional analysis indicates a lack of correlation between meters of intestine to be resected in surgical colic cases and peritoneal fluid lactate concentration (Latson *et al*., 2005). This observation can be explained by the fact that, in those cases, the resected portion of the intestine is not the only important source of lactate production due to anaerobic glycolysis. Distended intestine located orally to the compromised bowel also is often subject to dilatation and decreased vascular supply (Dabareiner *et al*., 2001; Snyder *et al*., 1990). Intestinal ischemia can be the cause of gut barrier failure. Depending on the degree of resulting endotoxemia, overall GI blood flow will be suppressed, stimulating intestinal ischemia and lactate accumulation (Moore & Barton, 2003).

Both BPL and PFL concentrations are associated with venous pO_2, presence of necrotic intestine, visualization of increased amount of peritoneal fluid and peritoneal fluid total protein content in the univariable model. In the multivariable model, these factors remain independently associated with only the PFL concentration. This observation again emphasizes the fact that PFL concentrations provide the clinician with more accurate information on the presence or absence of necrotic intestine as compared to BPL concentrations. The interaction between visualization of an increased amount of peritoneal fluid by means of abdominal ultrasound and pulse (identified in the multivariable model applied to PFL results) indicates that both variables have a synergistic effect on PFL concentration.

Prognostic value of blood plasma and peritoneal fluid lactate concentrations

When BPL and PFL concentrations are compared for their usefulness as potential prognostic variables, based on the Odds ratio and Nagelkerke coefficient of variation, PFL is more suitable than BPL for predicting need for surgery, intestinal resection, probability of development of ileus during hospitalization and probability of death. The BPL determination should be viewed primarily as a tool to provide additional information on the cardio-vascular status of the colic patient, with clearly increased results in cases of advanced circulatory failure. PFL concentrations however are more suitable and sensitive for early recognition of intestinal ischemia and concomitant prediction of outcome. As Thoefner *et al*. previously demonstrated, however, results of these analyses cannot be separated from information gained by a thorough clinical examination to assess the colic patient (Thoefner *et al*., 2000). The PFL cut-off value for survival of 16.90 mmol/l is in accordance with the study of Genn and Hertsch who had 1 surviving horse in the 16-18 mmol/l range (Genn & Hertsch, 1982). In the Latson study, no cut-off value for survival was mentioned (Latson *et al*., 2005). However, caution should be exercised in applying the outcome curves (Figure 3.II.3) to a different clinical population. Many factors such as lesion type, distribution, and colic duration can differ significantly among populations and can influence the relationship between outcomes and lactate concentration.

3.II.5. CONCLUSION

Assessment of BPL and PFL concentrations can be used as a prognostic variable in horses with colic. PFL concentrations are more sensitive and suitable for the early recognition of intestinal ischemia and the need for intestinal resection as compared to BPL concentrations. We do not necessarily recommend abdominocentesis in all colic patients. However, in some cases, assessment of PFL concentrations can provide the owner with a quick and sensitive

assessment of prognosis and ultimately help evaluate the benefits of financial investment in an operative procedure. This study confirms that the Accusport® meter can be reliably used for this purpose.

Footnotes

[a]HP Sonos 100; 2,5 Mhz sectorial probe
[b]248 pH/Blood Gas Analyser, Chiron Diagnostics Ltd., Essex, U.K.
[c]Cobas Integra Lactate®, Roche Diagnostics, Belgium.
[d]Accusport Analyser®, Boehringer Mannheim, Germany.
[e]Bausch and Lomb optical Co, Rochester, USA.
[f]Coulter Electronics Ltd, Harpenden, Herts, UK.
[g]Boehringer Monotest®, Boehringer Mannheim, Germany

REFERENCES

ARDEN, W.A. & STICK, J.A. (1988). Serum and peritoneal fluid phosphate concentrations as predictors of major intestinal injury associated with equine colic. *JAVMA*., **193**, 927-931.

BISHOP, D. (2001). Evaluation of the Accusport® lactate analyser. *Int. J. Sports. Med*., **22**, 525-530.

BLAND, M. & ALTMAN, D.G. (1986). Statistical Methods for Assessing the Difference Between Two Methods of Measurement. *Lancet i.*, 307-310.

BRINKERT, W., ROMMES, J. & BAKKER, J. (1999). Lactate measurements in critically ill patients with a hand-held analyser. *Int. Care. Med*., **25**, 966-969.

DABAREINER, R.M., WHITE, N.A. & DONALDSON, L.L. (2001). Effects of intra-luminal distention and decompression on microvascular permeability and hemodynamics of the equine jejunum. *Am. J. Vet. Res*., **62**, 225-236.

DELAURIER, G.A., CANNON, R.M., JOHNSON, R.H., SISLEY, J.F., BAISDEN, C.R. & MANSBERGER, A.R. (1989). Increased peritoneal fluid lactate values and progressive bowel strangulation in dogs. *Am. J. Surg* ., **158**, 32-35.

DELESALLE, C., DEWULF, J., LEFEBVRE, R.A., SCHUURKES, J.A.J., PROOT, J., LEFERE, L. & DEPREZ, P. (2005). Use of plasma ionized Ca^{2+} levels and Ca^{2+} substitution response patterns as prognostic parameters for ileus and survival in colic horses. *Vet. Quart*., **27**, 157-172.

DONAWICK, W.J., RAMBERG, C.F., PAUL, S.R. & HIZA, M.A. (1975). The diagnostic and prognostic value of lactate determinations in horses with acute abdominal crisis. *J. S. Afr. Vet. Assoc*., **46**, 127.

EIKMEIER, H. (1982). Uniform values for the laboratory diagnosis in horses. *Berl. Munch. Tierarztl. Wochenschr* ., **95**, 85-86.

EVANS, D.L. & GOLLAND, L.C. (1996). Accuracy of Accusport for measurement of lactate concentrations in equine blood and plasma. *Eq. Vet. J*., **28**, 398-402.

FELL, J.W., RAYFIELD, J.M., GULBIN; J.P. & GAFFNEY, P. (1998). Evaluation of the Accusport® lactate analyser. *Int. J. Sports. Med*., **19**, 199-204.

GENN, H.J. & HERTSCH, B (1982). Die diagnostische und prognostische bedeutung des laktatwertes im blut sowie in der bauchhöhlenflüssigkeit bei der kolik des Pferdes. *Dtsch. Tierärztl. Wschr.*, **89**, 261-312.

JANSSON, K., REDLER, B., MAGNUSSON, A. & NORGREN, L. (2004). Postoperative on-line monitoring with intraperitoneal microdialysis (IPM) detects early visceral ischemia and correlates to the current intraperitoneal cytokine response. *Crit. Care*., **7**, P196.

JOHNSTONE, I.B. & CRANE, S. (1986). Haemostatic abnormalities in horses with colic--their prognostic value. Eq. Vet. J., **18**, 271-274.

LATSON, K.M., NIETO, J.E., BELDOMENICO, P.M. & SNYDER, J.R. (2005). Evaluation of peritoneal fluid lactate as a marker of intestinal ischaemia in equine colic. *Eq. Vet. J*., **37**, 342-346.

LIAO, X.P., SCHE, Y.X. & LI, M. (1995). Changes in body fluid markers in intestinal ischemia. *J. Ped. Surg.*, **30**, 1412-1415.

LINDNER, A. (1996). Measurement of plasma lactate concentration with Accusport®. *Eq. Vet. J*., **28**, 403-405.

LUMSDEN, J.H., ROWE, R. & MULLEN, K. (1980). Hematology and biochemistry reference values for the light horse. *Can. J. Comp. Med*., **44**, 32-42.

MAIR, T. (2002). Additional diagnostic procedures. In : Manual of Equine Gastroenterology, 1st edn., Eds: Mair T, Divers T, Ducharme N. W.B. Saunders, London, 16-17.

MOORE, J.N., OWEN, R.R. & LUMSDEN, J.H. (1976) Clinical evaluation of blood lactate levels in equine colic. Eq. Vet. J., **8**, 49-54.

MOORE, J.N., TRAVER, D.S., TURNER, M.F., WHITE, F.J., HUESGEN, J.G. & BUTERA, T.S. (1977). Lactate concentration in peritoneal fluid of normal and diseased horses. *Res. Vet. Sci*., **23**, 117-118.

MOORE, J.N., GARNER, H.E., SHAPLAND, J.E. & HATFIELD, D.G. (1980) Lactic acidosis and arterial hypoxemia during sublethal endotoxemia in conscious ponies. *Am. J. Vet. Res*., **41**, 1696-1698.

MOORE, J.N. & BARTON, M.H. (2003). Treatment of endotoxemia. *Vet. Clin. North. Am., Equine Practice*., **19**, 681-695.

NELSON, A.W. (1979). Analysis of equine peritoneal fluid. *Vet. Clin. North. Am: Large Animal Practice*., **1**, 267-274.

NORDSTROM, L., CHUA, R., ROY, A., NAKA, K., PERSSON, B. & ARULKUMARAN, S. (1998). Quality assessment of two lactate test strip methods suitable for obstetric use. *J. Perinat. Med*., **26**, 83-88.

PARRY, B.W., ANDERSON; G.A. & GAY, C.C. (1983). Prognosis in equine colic: A study of individual variables used in case assessment. *Eq. Vet. J*., **15**, 337-344.

PARRY, B.W. & CRISMAS, M.V. (1991). Serum and peritoneal fluid amylase and lipase reference values in horses. *Eq. Vet. J*., **23**, 390-391.

PICCIONE, G., CAOLA, G. & REFINETTI, R. (2004). Feeble weekly rhythmicity in hematological, cardiovascular, and thermal parameters in horses. *Chronobiol. Int*., **21**, 571-589.

SAULEZ, M.N., CEBRA, C.K. & TOMQUIST, S.J. (2004). The diagnostic and prognostic value of alkaline phophatase activity in serum and peritoneal fluid from horses with acute colic. J. Vet. Intern. Med., **18**, 564-567.

SCHULMAN, M.L., NURTON, J.P. & GUTHRIE, A.J. (2001). Use of the Accusport semi-automated analyser to determine blood lactate as an aid in the clinical assessment of horses with colic. *Tydskr. S. Afr. Vet. Ver*., **72**, 12-17.

SIMMONS, D., STEWART, A., STEWART, C., PEDLER, P. & ROBERTS, P. (1999). A comparative evaluation of the Accusport vs conventional assay method for the determination of lactate in equine plasma. *J. Eq. Vet. Sci*., **19**, 402-407.

SNYDER, J.R., PASCOE, J.R., OLANDER, H.J., HINDS, D.M., YOUNG, R. & TYLER, W.S. (1990). Vascular injury associated with naturally occurring strangulating obstructions of the equine large colon. *Vet. Surg*., **19**, 446-455.

SOMMER, T. & FROMHOLT, L. (2003). Detection of intestinal ischemia using a microdialysis technique in an animal model. *World. J. Surg*., **27**, 416-420.

SOMMER, T. & LARSEN, J.F. (2004). Intraperitoneal and intraluminal microdialysis in the detection of experimental regional intestinal ischemia. *Br. J. Surg*., **91**, 855-861.

THOEFNER, M.B., ERSBOLL, A.K. & HESSELHOLT, M. (2000). Prognostic indicators in a Danish hospital-based population of colic horses. *Eq. Vet. J. Suppl*., **32**, 11-18.

VAN HOOGMOED, L., RODGER, L.D., SPIER, S.J., GARDNER, I.A., YARBROUGH, T.B. & SNYDER, J.R. (1999). Evaluation of peritoneal fluid pH, glucose concentration, and lactate dehydrogenase activity for detection of septic peritonitis in horses. *JAVMA*., **214**, 1032-1036.

VON BRAUN, U., SCHOBERL, M., BRACHER, V., GEYER, H. & FEIGE, K. (2002). Untersuchungen zur prognostik bei Pferden mit Kolik. *Tierärztl. Umschau*, **57**, 15-22.

WEIMANN, C.D., THOEFNER, M.B. & JENSEN, A.L. (2002). Spectrophotometric assessment of peritoneal fluid haemoglobin in colic horses: an aid to selecting medical vs. surgical treatment. *Eq. Vet. J*., **34**, 523-527.

WILLIAMSON, C.C., JAMES, E.A., JAMES, M.P., MAY, C.D. & CASEY, P.J. (1996). Horse plasma lactate determinations: comparison of wet and dry chemistry methods and the effect of storage. Eq. Vet. J., **28**, 406-408.

YAM, J., CHUA, S., RAZVI, K. & ARULKUMARAN, S. (1998). Evaluation of a new portable system for cord lactate determination. *Gynecol. Obstet. Invest.*, **45**, 29-31.

CHAPTER 4

SEROTONIN AND SEROTONERGIC RECEPTORS

Chapter 4.I

DETERMINATION OF FACTORS CONTRIBUTING TO INCREASED SEROTONIN (5-HT) LEVELS IN BLOOD AND PERITONEAL FLUID OF COLIC HORSES WITH COMPROMISED BOWEL.

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SUMMARY

Contractile serotonergic (5-HT) receptors have been identified in the equine GI tract and digital circulation. Increased plasma 5-HT levels are reported in horses predisposed to develop laminitis and after iv infusion of endotoxins. In the equine jejunum contractile 5-HT_{1A}-like receptors show tachyphylaxia upon prolonged activation with 5-HT. Therefore, increased systemic 5-HT release in colic horses with compromised bowel could play a possible role in the pathophysiology of equine ileus.

The aim of the study was to investigate possible increased systemic release of 5-HT in colic horses with compromised bowel, predisposed to develop ileus and to identify the source of the increased 5-HT release.

5-HT levels were determined in plasma and peritoneal fluid (PF) of healthy horses and colic horses with compromised bowel. It was attempted to identify the source of 5-HT overload, by comparing the blood and peritoneal fluid 5-HT levels within horses and by assessing the *in vivo* platelet activation in these horses through determination of the β-thromboglobulin (β-TG)/platelet factor 4 (PF4) ratio.

All horses in the strangulating small intestinal colic group had plasma (365.6 ± 217.1 nmol/l; p=0.006) and peritoneal fluid (138.8 ± 111.5 nmol/l; p=0.01) 5-HT levels well above those found in the control group (plasma: 39.1 ± 19.3 nmol/l; PF: 37.3 ± 20.9 nmol/l;). Plasma β-TG/PF4 ratio in these horses exceeded 2 in all cases, indicating in vivo platelet activation. 5-HT levels in peritoneal fluid of colic horses with compromised bowel were significantly lower than the corresponding plasma levels (p=0.005).

Apparently in colic horses with compromised bowel, important amounts of 5-HT can be released into the systemic circulation, through massive release of platelet stored 5-HT upon platelet activation. 5-HT is a very potent pro-inflammatory, vasoconstrictive and

immunomodulatory agent. In view of the rapid and prolonged tachyphylaxia, shown for the jejunal 5-HT_{1A}-like receptors, this increased systemic 5-HT release could play a role in the pathophysiology of ileus in horses.

4.I.1. INTRODUCTION

5-hydroxytryptamine (5-HT, serotonin) is a very important messenger in the digestive tract; it is essential to the brain-gut connection and related to GI motility and visceral sensation. Contractile 5-HT receptors have been identified in several parts of the equine GI-tract (Sellers *et al*. 1985; Nieto *et al*. 2000; Weiss *et al*. 2002; Delesalle *et al*. 2006; Delco *et al*. 2007) and also in the equine digital circulation (Bailey *et al*. 1998). For the equine mid-jejunum, our group (Delesalle *et al.* 2006; 2007b), identified the presence of contractile 5-HT_{1A} like receptors, both in the jejunal longitudinal and circular smooth muscle layer. The 5-HT_{1A} serotonergic receptor subtype is known for its features of tachyphylaxia, also known as receptor desensitization; the latter can be viewed as a natural defence mechanism of 5-HT receptors against protracted and excessive receptor activation in the presence of an overload of agonist, such as endogenous 5-HT. This typical characteristic of rapid and prolonged receptor desensitization opens interesting considerations on the possible role of 5-HT in the pathophysiology of equine ileus; excessive stimulation of the 5-HT_{1A} like receptor might contribute to the hypomotility during ileus.

Bailey and his co-workers already demonstrated seasonal changes in plasma concentrations of cecum-derived amines, amongst which 5-HT, in clinically normal ponies and ponies predisposed to laminitis (Bailey *et al*. 2003a). They suggest that the release of these amines into the systemic circulation may contribute to hemodynamic disturbances in horses and ponies with acute laminitis (Menzies-Gow *et al*. 2004; Elliott and Bailey 2006). Likewise,

release of excessive amounts of 5-HT in colic horses, could play a role in the pathophysiology of ileus (Delesalle *et al*. 2006).

In the body, serotonin (5-HT) is stored in blood platelets and in the enterochromaffin cells (EC cells) of the GI tract. These EC cells are found in the mucosal epithelium of the whole GI tract of numerous vertebrates, including the horse (Fink *et al.* 2005). Taking into consideration two important features which are predominantly found in colic horses predisposed to develop ileus, namely intestinal necrosis and endotoxemia, both platelets and enterochromaffin cells of necrotising bowel segments, could serve as a source of 5-HT overload in colic horses. The aim of this study was to determine the 5-HT levels in blood and peritoneal fluid (PF) of healthy horses and colic horses with compromised bowel. It was attempted to identify the source of 5-HT overload, by comparing the blood and peritoneal fluid 5-HT levels within horses and by assessing the *in vivo* platelet activity in these horses.

4.I.2. MATERIALS AND METHODS

4.I.2.1. Study population characteristics and sampling time

Table 4.I.1 gives an overview of the study population characteristics. The control group consisted of clinically healthy horses, where blood and peritoneal fluid were collected at the slaughterhouse. The study population consisted of horses that were presented to the Large Animal Internal Medicine Clinic of Ghent University, Belgium and was divided into 3 categories. A first category consisted of cryptorchid stallions that underwent planned castration; blood was sampled 2h before and 2h after surgery. This category was used to evaluate the influence of anaesthesia and intra-abdominal exploration on plasma 5-HT levels and platelet activity. A second category consisted of surgical colic cases with strangulating lesions of the small intestine. Blood was sampled at arrival, as well as peritoneal fluid after

clinical examination and abdominal ultrasound[k]. In 5 horses that survived after development of postoperative ileus, blood was also obtained 1 day before discharge. Finally, there was a third category of non-surgical colic horses which responded well to conservative treatment, where a blood sample was obtained at arrival.

Table 4.I.1: Study population characteristics and sampled body fluids.

Category	Blood	Peritoneal fluid
Controls (n=10),(3-16 years)	At the slaughterhouse *4 samples additionally handled according to the activation protocol	At the slaughterhouse
Small intestinal strangulation (n=18); (2-18 years) 5 peroperative euthanasia 5 recovered uneventfully	At arrival	At arrival
8 postoperative ileus →5 survived	At arrival & 1day before discharge (i.e. 18.5 ± 6 days post surgery)	
Non surgical colic (n=10); (4-20 years) Large colon obstruction (n=5) Dilated small intestines (n=5)	At arrival	ND
Cryptorchid stallions (n=6) (1.5-4 years)	2h before surgery 2h post-surgery	ND*

*ND: not done

4.I.2.2. Sample Collection and Analyzis

Seven venous blood samples were collected anaerobically from the jugular vein. Table 4.I.2 gives a detailed overview of used tubes and performed analyses. Briefly, 3 consecutive EDTA samples were taken of which the last two were immediately stored on ice and provided with stabilizing agents as proposed by Bailey *et al*. 2003b: clomipramine and phenelzine (to inhibit uptake and metabolism, respectively, of 5-HT and other amines by platelets) and acetyl salicylic acid (to stabilize the blood platelets). Thereafter 4 additional tubes, each with

different anticoagulants were drawn to determine electrolytes, packed cell volume (PCV), base excess (BE), blood lactic acid level and the blood coagulation profile. D-dimer analysis was performed using a latex agglutination kit[j], which has been validated previously for use in horses (Stokol *et al*. 2005). The reference value of D-dimer <250 ng/ml for healthy horses reported by Heidmann *et al*. (2005) was used. *In vivo* platelet activity was assessed by means of ß-thromboglobulin (ß-TG) and platelet factor 4 (PF4) analysis (cf. Infra). Peritoneal fluid was harvested in 5 separate tubes as shown in Table 4.I.2. Electrolyte, BE and lactic acid analysis were performed within 5 min after collection. The remaining analyses were performed within 12h after collection, with the exception of the D-dimer, 5-HT, ß-TG and PF4 assays. The samples for D-dimer analysis were centrifuged at 1500g, after which the supernatant was transferred and stored at -80°C until assayed. All 5-HT, ß-TG and PF4 samples were centrifuged following an adapted protocol, proposed by Bailey *et al.*, to obtain platelet poor plasma (Bailey *et al*. 2003b). Briefly, the tubes were centrifuged at 2000g and 4°C during 15 min and the middle part of the supernatant was transferred with a pipette to a second plain 10 ml polypropylene tube. This fraction was centrifuged once again at 10 000g and 4°C for 10 min. Again the middle part of the supernatant was transferred to a plain polypropylene tube, and was then stored at -80°C until analyzed.

Table 4.I.2: Performed analyses in blood/plasma and peritoneal fluid

Blood		
EDTA	1st tube	Platelet count[a] & WBC count[b] and cell differentiation
	2nd tube Stabilizing agents added: 1 µmol/l clomipramine 10 µmol/l phenelzine 1 mmol/l acetyl salicylic acid	5-HT, β-TG and PF4
	3rd tube Idem as 2nd tube	Idem as 2nd tube
Lithium heparin	1 tube	(Na^+, K^+, iCa^{2+})[c]; PCV, BE[d]
Na^+-fluoride/potassium oxalate	1 tube	Blood lactic acid[e]
Na^+-citrate	1st tube	aPTT[f], PT[g], fibrinogen[h,i]
	2nd tube	D-dimer[j]
Peritoneal fluid		
EDTA	1st tube	WBC count[b]
	2nd tube Stabilizing agents added (cfr. Supra)	5-HT, β-TG and PF4
Na^+-fluoride/potassium oxalate	1 tube	Peritoneal fluid lactic acid[e]
Lithium heparin	1 tube	(Na^+, K^+, iCa^{2+})[c], PCV, BE[d]
Plain tube	1 tube	Total protein[l]

WBC (White Bloodcell count); β-TG (β-thromboglobulin); PF4 (platelet factor 4); iCa^{2+} (ionized Ca^{2+}); PCV (packed cell volume); BE (base excess); aPTT (partial thromboplastin time; PT (prothrombin time)

4.I.2.3. 5-HT analysis

For the analysis of serotonin (5-HT) in platelet-poor plasma, a solid-phase extraction (SPE) method for plasma sample preparation was used (Raggi *et al.* 1999). Waters Oasis HLB extraction plates (5 mg, 1 mL) (Milford, MA, USA) were used. All standards and samples were prepared working on ice and with dimmed light to prevent degradation by light and temperature. 10 µL of 5-HT standard or milliQ, 100 µL of internal standard (dihydroxybenzylamine) and 90 µL of buffer were added to aliquots of 200 µL of reconstituted plasma or plasma sample. The resulting mixtures were loaded onto previously conditioned cartridges. Elution of the cartridge was performed by passing 200 uL of citric

acid. Next, the elutes were subjected to a pre-column derivatisation procedure with benzylamine (BA) and 1,2-diphenylethylenediamine (DPE), which yielded highly fluorescent derivatives of the monoamines (Fujino *et al.* 2003,).

The derivatised standards and samples were analyzed by HPLC using a Waters Alliance HT 2795 system equipped with a Waters Fluorescence detector 2475. Chromatographic separation of the amino acids was achieved on a X-terra (Waters) reversed-phase column (C18, 2.1 mm ID x 100 mm, 3 um) with a X-terra (Waters) guard-column (C18, 2.1 mm ID x 10 mm, 3 µm). Separation of benzylamine-DPE derivatives of 5-hydroxyindolamines and catecholamines was achieved using a mixture of acetonitrile and 15 mM acetate buffer (pH 4.5) containing 1 mM octanesulfonic acid sodium salt. The flow rate was 0.7 mL/min. The column temperature was 60 °C. The samples were injected by means of a partial injection of 20 µL in a 50 uL PEEK loop.

4.I.2.4. βTG and PF4 analyzis

Because serotonin is highly concentrated in platelets, its concentration in plasma may be overestimated owing to an artificial platelet contribution, such as platelet activation after sampling or leakage due to errors in blood collection and preparation. Both ß-TG and PF4 are stored in similar quantities in the α-granules of platelets and are released extracellularly upon platelet activation (Kaplan and Owen, 1981). As PF4 has a very short half life *in vivo* (< 3 min), probably because of rapid binding of released PF4 to vascular endothelial cells (Walz *et al*. 1985), it is frequently used as an indicator for undesired *in vitro* platelet activation due to sample handling. In vivo platelet activation is characterized by concurrent presence of increased levels of β-TG and normal to slightly increased PF4 levels, whereas in vitro release can be recognized by increased levels of both βTG and PF4 levels in combination with a β-TG/ PF4 ratio < 2. To assess the *in vivo* activation of equine platelets, the plasma levels of

both β-TG and PF4 were measured and the β–TG/PF4 ratio was determined. Hereby, an increased in vivo platelet activation will be reflected by an increased β-TG/PF4 ratio in comparison to controls, whereas *in vitro* platelet activation will decrease the ratio, because of increased levels of both β-TG and PF4 (Kaplan and Owen, 1981).

To determine the ratio of equine β-TG and PF4 in horse plasma, based on their respective optical density (OD), a sandwich ELISA was used. As no antibodies against equine β-TG and PF4 are commercially available, we developed a sandwich ELISA using anti-human β-TG and PF4 antibodies. Wells of ninety-six-well microtiter plates (Greiner, Frickenhausen, Germany) were coated overnight (ON) at 4 °C with 5µg/ml rabbit anti-human β-TG or PF4 antibodies (Nordic Immunological Laboratories, Tilburg, The Netherlands) in phosphate-buffered saline (PBS). Wells were blocked with PBS containing 3% milk powder for 2 h at room temperature (RT) and incubated for 2 h at 37°C with a dilution series of horse plasma in PBS containing 0.3% milk powder. Bound equine β-TG or PF4 was detected for 1h at 37°C with 1µg/ml biotin-labeled anti-β-TG or anti-PF4 polyclonal antibodies respectively (Nordic Immunological Laboratories, Tilburg, The Netherlands). The biotinylation was performed with EZ-Link Sulfo-NHS-LC-LC-Biotin from Pierce, following the manufacturer's instructions (Perbio Science N.V., Aalst, Belgium). Bound biotinilyated antibodies were detected for 45 min at 37°C with a 1/10 000 dilution of streptavidin-HRP (Roche Molecular Biochemicals, Mannheim, Germany). Visualization was obtained with ortho-phenylenediamine (Sigma) and H_2O_2, and the colouring reaction was stopped with 4 mol/ml H_2SO_4, after which the absorbance was determined at 490 nm.

To validate this assay and to evaluate if there is cross-reactivity of the human antibodies with our equine proteins of interest, we used serum from a healthy human donor and compared the optical density (OD) values with sera obtained from healthy horses. In serum samples, allowed to cloth, platelet activation is induced *in vitro* and hence, β-TG and PF4

should be detected in such samples. Both human and horse sera were positive for β-TG and PF4, with horse sera showing a slightly higher OD than human sera (an OD of 0.1 and 0.14 for β-TG and PF4 respectively in human serum versus an OD of 0.3 and 0.24 for horse serum). The β-TG/PF4 ratio's in all sera tested, however, were <2, indicating that *in vivo* platelet activation did not occur in these healthy individuals as expected. A second validation was performed by application of an additional aberrant centrifugation protocol in 4 control plasma samples (see Table 4.I.1), referred to as "the activation protocol". Samples were centrifuged at 1500g during 10 min, which is known not to retrieve platelet poor plasma in horses (Bailey *et al.* 2003b). The plasma fraction was then frozen at -80°C and again thawed. Mean β-TG (0.63 ± 0.35 OD) and PF4 (0.50 ± 0.22 OD) levels in these samples were significantly higher than these in control samples, centrifuged according to the protocol for obtaining platelet poor plasma (see Table 4.I.3), with a mean β-TG/PF4 ratio of 1.2 ± 0.22, corresponding with *in vitro* platelet activation. All further plasma samples were tested in a blinded fashion with the sandwich ELISA.

4.I.2.5. Statistics

Laboratory data of colic horses and controls were compared by means of an unpaired *t*-test[m]. Data of "the activation protocol" samples and controls were compared through use of a paired *t*-test. Similarly, pre-surgical and post-surgical parameters in cryptorchid stallions were compared through use of a paired *t*-test. Plasma and peritoneal fluid 5-HT levels were compared within horses by means of a paired *t*-test. All values are expressed as mean ± s.e.m.; *n* denotes the number of tissues obtained from different horses. Significance was set at a value of $p<0.05$.

4.I.3. RESULTS

Table 4.I.3 shows an overview of the results of the performed laboratory analyses. Mean plasma 5-HT level in control horses was 39.1 ± 19.3 nmol/l. Mean plasma β-TG and PF4 levels were respectively 0.12 ± 0.11 OD and 0.14 ± 0.12 OD. The 5-HT and ion levels were comparable in plasma and peritoneal fluid (PF) samples of control horses. None of the samples showed D-dimer levels above 200 ng/ml.

The 5-HT level in the 4 control plasma samples which were handled according to "the activation protocol", was significantly higher (p=0.003) than in not activated samples from control horses (190.9 ± 180.8 nmol/l). The D-dimer level in these samples was 159 ± 25 ng/ml. Pre-surgical plasma 5-HT, β-TG and PF4 levels in cryptorchid stallions were not significantly different from control values and from those determined in post-recovery samples. Likewise, coagulation profile parameters and β-TG/PF4 ratios were comparable before and after surgery and not different from those in controls.

All horses in the strangulating small intestinal colic group had 5-HT levels in plasma and peritoneal fluid well above those found in the control group (respectively p=0.006 and p=0.01). Plasma β-TG/PF4 ratio in these horses exceeded 2 in all cases. Most coagulation profile parameters were significantly different from controls, showing a trend towards increased coagulation activity (p=0.002 for aPTT; p=0.22 for PT; p<0.001 for fibrinogen and p=0.004 for D-dimer). 5-HT levels in peritoneal fluid of colic horses with compromised bowel were significantly lower than the corresponding plasma analyses (p=0.005). There was no significant difference in Na^+ (p=0.07) and iCa^{2+} (p=0.65) levels between plasma and peritoneal fluid of the small intestinal strangulating colic horses. A significantly lower K^+ (p=0.01) and higher lactic acid level (p=0.05) could be found in the peritoneal fluid of these horses in comparison to the peritoneal fluid of control horses. The 5-HT, β-TG and PF4 levels that were determined in blood of strangulating small intestinal colic cases, drawn one day

before hospital discharge were no longer significantly different from the control values (5-HT: 27.9 ± 16.56 nmol/l; ß-TG: 0.08 ± 0.05 OD; PF4: 0.15 ± 0.15 OD; ß-TG/PF4 ratio: 0.89 ± 0.28).

The non-surgical colic cases, which served as a “within colic control”, showed all low to mid-high (never exceeding 135 nmol/l) 5-HT levels. In none of these cases the β-TG/PF4 ratio exceeded 2.

	Reference Ranges		**Controls** (n=10)		**Cryptorchid stallions** (n=6)		**Small intestinal** (n=18)		**Non surgical colic** (n=10)
					Before Surgery (n=6)	***After Surgery*** (n=6)			
	Blood	PF	Blood	PF	Blood	Blood	Blood	PF	Blood
WBC count (/mm³)	4-9 x 10^{9}[a]	< 3500[a]	8.4 ± 1.9	1296 ± 722	7.9 ± 2.3	9.8 ± 0.6	9.8 ± 5.3	17 386 ± 27 974	9.5 ± 3.3
Cell Differentiation				ND					
% segments	NA	NA	56.5 ± 12.1	ND	55.5 ± 12.2	60.7 ± 3.7	78.5 ± 13.3	ND	65.4 ± 13.4
% lymphocytes	NA	NA	44.3 ± 12.5	ND	44.5 ± 12.2	36 ± 5.9	21.4 ± 13.3	ND	34.6 ± 13.4
PCV (%)	35-40[a]	NA	35.7 ± 2.8	ND	35.3 ± 3.5	38.9 ± 1.08	44.8 ± 8.8	ND	39.5 ± 7.0
BE (mEq/l)	(-5) –(+5)[a]	NA	1.4 ± 2.7	ND	1.1 ± 1.9	2.0 ± 1.4	0.7 ± 5.4	ND	3.0 ± 4.9
Platelets (/µl)	100-350 x 10^{3}[a]	NA	206 ± 63	ND	241 ± 35	269 ± 47	179 ± 69	ND	226 ± 102
aPTT (sec)	<50.0[a]	NA	44.1 ± 3.16	ND	43.3 ± 3.9	44.4 ± 4.3	51.3 ± 6.1$^{\bullet\bullet}$	ND	46.1 ± 5.9
PT (sec)	8-10[a]	NA	11.7 ± 1.0	ND	10.7 ± 0.5	11.6 ± 0.9	12.8 ± 0.9	ND	11.6 ± 0.7
Fibrinogen (mg/dl)	200-375[a]	NA	262.6 ± 70.2	ND	172.3 ± 9.4	201.7 ± 68.6	143.6 ± 32.7$^{\bullet\bullet\bullet}$	ND	225.5 ± 34.8
D-dimer (ng/ml)	< 250[b]	NA	159.2 ± 35.6	ND	165.8 ± 18.1	219.3 ± 96.3	1516.7 ± 634.1$^{\bullet\bullet}$	ND	180.2 ± 94.3
βTG	NA	NA	0.12 ± 0.11	ND	0.08 ± 0.05	0.06 ± 0.05	0.20 ± 0.17	ND	0.18 ± 0.14
PF 4	NA	NA	0.14 ± 0.12	ND	0.10 ± 0.08	0.08 ± 0.08	0.09 ± 0.07	ND	0.14 ± 0.12
βTG/PF4 ratio	NA	NA	1.52 ± 1.35	ND	1.62 ± 2.10	1.00 ± 0.66	2.29 ± 0.21$^{\bullet}$	ND	1.24 ± 0.48
5-HT (nmol/l)	NA		39.1 ± 19.3	37.3 ± 20.9	50.8 ± 35.5	49.4 ± 21.6	365.6 ± 217.1$^{\bullet\bullet}$	138.8 ± 111.5$^{\bullet\bullet,\dagger}$	84.2 ± 40.2
Na^{+} (mEq/l)	132-146[a]	134.2 ± 2.14[c]	135.9 ± 2.4	135.7 ± 3.3	136 ± 1.5	132 ± 1.2	137.1 ± 4.2	133.9 ± 4.1	136.2 ± 2.7
K^{+} (mEq/l)	2.4-4.7[a]	3.6 ± 0.3[c]	3.6 ± 0.5	3.6 ± 0.3	3.62 ± 0.7	3.6 ± 0.2	3.2 ± 0.4	2.9 ± 0.3$^{\bullet\bullet}$	3.7 ± 0.4
iCa^{2+} (mmol/l)	1.61 – 1.85[d]	NA	1.65 ± 0.06	1.64 ± 0.05	1.6 ± 0.14	1.51 ± 0.09	1.32 ± 0.15	1.29 ± 0.12	1.55 ± 0.09
Lactic acid (mmol/l) detection limit of 0.7 mmol/l	0.59 ± 0.22[e]	0.49 ± 0.54[e]	below detection limit in 3 samples	below detection limit in 6 samples	below detection limit in 4 samples	below detection limit in 4 samples	5.71 ± 1.63$^{\bullet\bullet\bullet}$	8.52 ± 5.45$^{\bullet}$	below detection limit in 5 samples
Total Protein (g/l)	NA	< 25[a]	ND	17.1 ± 5.2	ND	ND	ND	35.3 ± 14.7	ND

PF : peritoneal fluid
NA : not available (want van referentiewaarden zegt men toch niet : not done)
ND*: Not done
Paired t-test versus blood : $^{\dagger}P\leq0.01$
Unpaired t-test versus control: $^{\bullet}P\leq0.05$, $^{\bullet\bullet}P\leq0.01$: $^{\bullet\bullet\bullet}P\leq0.001$
[a]Equine Internal Medicine, 2nd Edition
[b]Heidmann *et al.*, 2005
[c]Latson *et al.*, 2005 ; [d]Delesalle *et al.*, 2005 ; [e]Delesalle *et al.*, 2007a

4.I.4. DISCUSSION

The mean plasma 5-HT levels in healthy horses, measured by means of the solid-phase extraction (SPE) method, were in close accordance with those reported by Bailey *et al*. (2003).

Increased plasma and peritoneal fluid 5-HT levels were most apparent in the small intestinal strangulating colic group, with values exceeding 200 nmol/l. All conservatively treated colic horses, showed low to mid-high (never exceeding 135 nmol/l) plasma 5-HT levels. Apparently, presence of compromised bowel in colic horses leads to the release of important amounts of 5-HT into the systemic circulation. Several mechanisms could be involved in causing these increased plasma 5-HT concentrations. Elliott and Bailey (2006) already proposed the resorption of dietary amines, amongst which 5-HT, from the GI tract into the systemic circulation. Furthermore, 5-HT can be released by enterochromaffin cells of compromised bowel and subsequently absorbed into the systemic circulation. Based on this knowledge, it has been shown that increased 5-HT concentrations in plasma or increased concentrations of its breakdown product 5-hydroxyindole-3-acetic acid (5-HIAA) in urine, can be used as a sensitive indicator for the presence of acute appendicitis in children and adult humans (Rordam *et al* 1987; Apak *et al.* 2005). Teramoto et al. (1998) reported increased plasma 5-HT and 5-HIAA concentrations in rats subjected to intestinal ischemia/reperfusion. The 5-HIAA/5-HT ratio was significantlty increased in these rats, suggesting that elevated 5-HT was quickly metabolized in the systemic circulation. Indeed, in the aforementioned studies, the reported increases in plasma 5-HT levels, never exceeded a 4-fold increase of control values. Similar results have been reported by Nakamura et al. (2000), who measured a less than 4-fold increase of plasma 5-HT levels in dogs subjected to intestinal ischemia in comparison to controls. Apparently, serotonin is quickly metabolized in the gastrointestinal system, probably to avoid toxic effects and desensitization of the receptors (Chen *et al.* 1998

and 2001). The maximal 4-fold increase is in contrast with our study, where up to a 9-fold increase in plasma 5-HT levels was found in strangulating small intestinal colic horses. The fact that in our study peritoneal fluid 5-HT levels increase to a much lesser extent than corresponding plasma 5-HT levels, also makes it less plausible that the enterochromaffin cells are the main source of the measured increased plasma 5-HT levels in the studied colic horses. To our knowledge up until now no one has measured changes in peritoneal fluid 5-HT levels in the presence of bowel ischemia. However, it can be expected that 5-HT, released from enterochromaffin cells of compromised bowel, readily traverses the damaged intestinal barrier, to enter the peritoneal fluid, as has been described for intestinal endotoxin resorption in colic horses with compromised bowel (Barton *et al.* 1999). Therefore, we hypothesized that the association between the presence of compromised bowel and high plasma 5-HT levels in colic horses could be caused by increased platelet degranulation activity. Blood platelets are known to store important amounts of 5-HT (Czabanka *et al.* 2007), which can be released upon platelet activation. In the present study, we could link the increased systemic release of 5-HT to a pronounced *in vivo* platelet activity, and hence degranulation, in colic horses with compromised bowel. Only few analysis methods have been described for detection of *in vivo* platelet activation in horses. One study describes the detection of equine platelet activation through use of flowcytometry with fluorescent-labelled annexin-V or anti-human fibrinogen antibody (Kingston *et al.*, 2002). More recently, Segura *et al.* (2006) reported that an increase in P-selectin, as measured by using a specific monoclonal anti-human antibody, is a useful indicator of equine platelet activation. However, it is difficult to interpret an observed elevation when studying only one protein released upon platelet activation, because there is no control for *in vitro* platelet activation. In the present study, we developed a sandwich ELISA to determine both ß-TG and PF4 levels, and the ß-TG/PF4 ratio was shown to be a useful tool to differentiate between *in vivo* and *in vitro* platelet degranulation in horses. In the

studied population, all cases with a ß-TG/PF4 ratio >2 also showed increased D-dimer levels and concomitant increased plasma 5-HT levels. Increased D-dimer levels, as a specific fibrin breakdown product, are indicative of fibrinolysis *in vivo*. The samples that were handled according to "the activation protocol" showed high PF4 levels, indicative for *in vitro* platelet activation, but normal D-dimer values. Disturbed coagulation test results (plasma fibrinogen concentration < 150 mg/dl, PT>12,5 secs, aPTT > 50 secs, D-dimer > 550 mg/dl) were found in most (n=13/18) small intestinal colic cases with compromised bowel. D-dimer levels were also above normal in all of these cases, indicating *in vivo* platelet degranulation in these patients.

"Disseminated intravascular coagulation" (DIC) and coagulopathy have been demonstrated in colic horses on several occasions (Stokol *et al*. 2005; Dolente *et al*. 2002, Feige *et al*. 2003; Armengou *et al*. 2005). Its occurrence is associated with a negative prognosis. The exact cascade that takes place during DIC is still subject of research, however absorption of bacterial endotoxins from devitalized, ischemic intestine followed by platelet activation, is generally viewed as a key event. Using the Limulus Amoebocyte Lysate assay system to detect the presence of endotoxins in biological fluids, data from a variety of clinical studies indicate that approximately 25 to 35% of horses presented with colic are endotoxemic (Fessler *et al*. 1989; Meyers *et al*. 1982; Steverink *et al*. 1995, Moore *et al*. 2003). Amongst a wide array of detrimental effects, the process of endotoxemia is characterized by vascular damage, thrombosis and platelet activation (Lalko *et al*. 2003, Czabanka *et al.* 2007). Iv administration of bacterial endotoxins for experimental induction of endotoxemia in horses, leads to a clear increase in plasma 5-HT, thromboxane β_2 and p38 MAPK, an important platelet associated pro-inflammatory signalling molecule (Elliott *et al*. 2003; Menzies-Gow *et al*. 2004, Brooks *et al*. 2007). In addition, 5-HT is a very potent prostaglandin-synthetase independent activator of blood platelets and is able to enhance the effect of other blood

platelet activators (Holmsen *et al*. 1983). The 5-HT-induced cascade therefore seems to be a self-supporting system. Whether the increased plasma 5-HT levels in colic horses are solely the result of an increased 5-HT release from platelets, or, are also the result of concomitant disturbances in the metabolism and/or reuptake mechanisms of 5-HT, can not be concluded from this study. In this respect, it would be interesting to determine plasma and urine 5-HT degradation products such as 5-hydroxyindole-3-acetic acid (5HIAA) and 5-hydroxytryptophol (5HTOL) in colic horses (Some *et al*. 2002). Based on the normal plasma 5-HT levels measured in surgical colic cases one day before hospital discharge, the events causing increased plasma 5-HT levels in colic horses with compromised bowel, most probably have no long term character. Because 5-HT levels were also slightly elevated (ie above 80 nmol/l) in some horses (n=5/10) of the conservative treatment group, without concurrent elevation of any of the other factors known to identify DIC, the determination of plasma 5-HT levels could be a useful tool as a prognostic parameter for DIC that is going to develop.

When taking into consideration not only the potency, but also the involvement of 5-HT in a wide array of both physiological and pathological conditions such as inflammation, GI motility disturbances and immunity (Smith *et al*. 1974; Gershon *et al*. 2007; Csaba G *et al*. 2007), the increased 5-HT levels can undoubtedly trigger many undesired side effects. It is known that free circulating plasma 5-HT influences GI motility (Hansen *et al*. 2007); the rapid and prolonged desensitization of the contractile 5-HT_{1A}-like receptor in both muscle layers of the equine jejunum described before (Delesalle *et al.* 2006; 2007b), opens interesting considerations on the possible role of this increased systemic 5-HT release in colic horses with compromised bowel, in the pathophysiology of ileus. Recently it was demonstrated that specific serotonin receptor antagonists can reduce the detrimental effects of endotoxemia in rats (Walther *et al*. 2007). Likewise, the 5-HT_3 receptor antagonists ondansetron and

granisetron have proven to therapeutically antagonize nausea and vomiting in humans, caused by chemotherapy-induced increased release of 5-HT (Gershon *et al.* 2007). Being able to counteract in the massive release of 5-HT, which is known to be a highly potent pro-inflammatory and vaso-constrictive substance, would probably open interesting opportunities in the management of the colic horse.

The increased 5-HT levels in the peritoneal fluid of horses with compromised bowel, are probably caused by diffusion and leakage out of the systemic circulation. At which rate this takes place is not known. Electrolyte levels tend to balance out quite rapidly, as has been demonstrated by Saulez and co-workers (Saulez *et al*. 2005) and is confirmed by our data, where no significant differences could be found between Na^+, iCa^{2+} and K^+ levels in corresponding blood and peritoneal fluid of the examined horses. The decreased K^+ and increased lactic acid peritoneal fluid levels found in colic horses with compromised bowel, in comparison with controls are in accordance with other reports. Hypokalemia and hypocalcemia are common findings with equine colic of any etiology and compromised bowel can cause lactic acid levels to increase in the peritoneal fluid, early in the disease process (Johnson *et al.* 1995; Delesalle *et al.* 2005 & 2007). Reported side effects from increased peritoneal fluid 5-HT levels are congestion and an increased permeability of the blood vessels in the peritoneal cavity (Nagy *et al*. 1989; Wang *et al*. 1993, McHale *et al.* 2000) and modulation of immune cells in peritoneal fluid (Csaba *et al.* 2007).

By sampling the cryptorchid stallions both pre and postoperatively we wanted to investigate whether intra-abdominal surgical manipulation and general anaesthesia can cause additional systemic 5-HT release. It has been demonstrated both in rabbits and humans, that volatile anaesthetics, such as isoflurane, which was also used during surgery in the colic horses presented in this study, can increase peripheral 5-HT release, leading to postoperative nausea and vomiting. (Cook *et al*. 1982; Läer *et al*. 2001). However, no significant difference

in plasma 5-HT levels before and after surgery was found in the cryptorchid stallions. Apparently, anaesthesia and abdominal exploration did not result in a significant increase in plasma 5-HT levels in the studied cryptorchid horses.

4.I.5. CONCLUSION

Our study demonstrates that in colic horses with compromised bowel, important amounts of 5-HT can be released into the systemic circulation. Not only direct absorption of enteral bioactive amines, but also the massive release of platelet stored 5-HT contributes to this 5-HT increase. The results of our study underline the importance for more research into platelet stabilizing therapies for colic horses.

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Footnotes

[a]platelet count: QBC details

[b]Coulter Electronics Ltd, Harpenden, Herts, UK.

[c]AVL 9180 Electrolyte Analyzer, AVL Scientific Corporation, Roswell, GA, US.

[d]248 pH/Blood Gas Analyser, Chiron Diagnostics Ltd., Essex, U.K.

[e]Accusport Analyser®, Boehringer Mannheim, Germany.

[f]APTT (Dade® Actin® FS Activated PTT Reagent; Dade Behring Marburg, Germany

[g]PT (Dade® Innovin®Test; Dade Behring Marburg, Germany

[h]Sysmex® CA-1500, Dade Behring Marburg, Germany

[i]Dade® fibrinogen detection reagent

[j]Accu-Clot™ D-dimer test kit, Trinity Biotech, Bray, Ireland.

[k]HP Sonos 100; 2,5 Mhz sectorial probe

[l]Bausch and Lomb optical Co, Rochester, USA.

[m]SPSS, Statistical Analysis Software, SPSS Inc, Chicago, US.

REFERENCES

ARMENGOU, L., MONREAL, L., SEGURA, D. & NAVARRO, M. (2005). Plasma D-dimers in horses with colic. *8th Equine Colic Research Symposium, Quebec, Canada.* 1

BAILEY, S.R. & ELLIOTT, J. (1998). Plasma 5-hydroxytryptamine constricts equine digital blood vessels in vitro: implications for pathogenesis of acute laminitis. *Equine vet. J.* **30**, 124-130.

BAILEY, S.R., KATZ, L.M., BERHANE, Y., SAMUELS, T., DE BRAUVERE, N., MARR, C.M. & ELLIOTT, J. (2003a). Seasonal changes in plasma concentrations of cecum-derived amines in clinically normal ponies and ponies predisposed to laminitis. *Am. J. Vet. Res*. **64**, 1132-1138.

BAILEY, S.R., MARR, C.M. & ELLIOTT, J. (2003b). Identification and quantification of amines in the equine caecum. *Res. Vet. Sci.* **74**, 113-118.

BROOKS, A.C., MENZIES-GOW, N.J., WHEELER-JONES, C., BAILEY, S.R., CUNNINGHAM, F.M. & ELLIOTT, J. (2007) Endotoxin-induced activation of equine platelets: evidence for direct activation of p38 MAPK pathways and vasoactive mediator production. *Inflamm. Res.* **56**, 154-161.

COOK, D.R. & BRANDOM, B.W. (1982). Enflurane, halothane, and isoflurane inhibit removal of 5-hydroxytryptamine from the pulmonary circulation. *Anaesth. Analg*. **61**, 671-678.

CSABA, G., KOVACS, P. & PALLINGER, E. (2007). In vitro effect of biogenic amines on the hormone content of immune cells of the peritoneal fluid and thymus. Is there a hormonal network inside the immune system? *Cell Biol Int.* **31**, 224-228.

CZABANKA, M., PETER, C. & WALTHER, A. (2007). Microcirculatory endothelial dysfunction during endotoxemia--insights into pathophysiology, pathologic mechanisms and clinical relevance. *Curr. Vasc. Pharmacol.* ***5***, 266-275.

DELCO, M., NIETO, J., CRAIGMILL, A.L., STANLEY, S.D. & SNYDER, J.R. (2007). Pharmacokinetics and in vitro effects of tegaserod, a serotonin 5-hydroxytryptamine 4 ($5\text{-}HT_4$) receptor agonist with prokinetic activity in horses. *Veterinary Therapeutics*. **8**, 77-87.

DELESALLE, C., DEWULF, J., LEFEBVRE, R., SCHUURKES, J.A.J., VANVLIERBERGEN, B. & DEPREZ, P. (2005). Use of plasma ionized Ca^{2+} levels and Ca^{2+} substitution response patterns as prognostic parameters for ileus and survival in colic horses. *Vet. Quart*. **27**, 157-172.

DELESALLE, C., DEPREZ, P., SCHUURKES, J.A.J. & LEFEBVRE, R.A. (2006). Contractile effects of 5-hydroxytryptamine and 5-carboxamidotryptamine in the equine jejunum. *Br. J. Pharmacol.* **147**, 23-35.

DELESALLE, C., DEWULF, J., LEFEBVRE, R.A., SCHUURKES, J.A.J., PROOT, J. & DEPREZ, P. (2007a). Use of Accusport® determined L-lactic acid levels in blood and peritoneal fluid of colic horses as a prognostic parameter. *J. Vet. Intern. Med.* **21**, 293-301.

DELESALLE, C., VAN ACKER, N., CLAES, P., DEPREZ, P., De SMET, I., Dewulf, J., Lefebvre, R. (2007b). Contractile effects of 5-Hydroxytryptamine (5-HT) in the equine jejunum circular muscle: functional and immunohistochemical identification of a 5-HT_{1A}-like receptor. EVJ file 07/148, Accepted for publication 18-12-2007.

DOLENTE, B.A., WILKINS, P.A. & BOSTON, R.C. (2002). Clinicopathologic evidence of disseminated intravascular coagulation in horses with acute colitis. *J. Am Vet. Med. Ass*. **220**, 1034-1038.

ELLIOTT, J., BERHANE, Y. & BAILEY, S.R. (2003). Effects of monoamines formed in the cecum of horses on equine digital blood vessels and platelets. *Am. J. Vet. Res.* **64**, 1124-1131.

ELLIOTT, J. & BAILEY, S.R. (2006). Gastrointestinal derived factors are potential triggers for the development of acute equine laminitis. *The Journal of Nutrition* **136**, 2103S-2107S

FEIGE, K., KASTNER, K., DEMPFLE, C.E. & BALESTRA, E (2003). Changes in coagulation and markers of fibrinolysis in horses undergoing colic surgery. *J. Vet. Med*. **50**, 30-36.

FESSLER, J.F., BOTTOMS, G.D. & COPPOC, G.L. (1989). Plasma endotoxin concentrations in experimental and clinical equine subjects. *Equine. vet. J. Suppl*. **7**, 24-28.

FINK, C., TATAR, M., FAILING, K., HOSPES, R., KRESSIN, M. & KLISCH, K. (2006). Serotonin-Containing cells in the gastrointestinal tract of newborn foals and adult horses. *Anat. Histol. Embryol.* **35**, 23-27

FUJINO, K., YOSHITAKE, T., KEHR, J., NOHTA, H. & YAMAGUCHI, M. (2003). Simultaneous determination of 5-hydroxyindoles and catechols by high-performance liquid chromatography with fluorescence detection following derivatization with benzylamine and 1,2-diphenylethylenediamine. *J. Chromatogr. A.* **19**, 169-177.

GERSHON, M.D. & TACK, J. (2007). The serotonin signaling system: from basic understanding to drug development for functional GI disorders. *Gastroenterology* **132**, 397-414

HANSEN, M.B., ARIF, F., GREGERSEN, H., BRUUSGAARD, H. & WALLIN, L. (2007). Effect of serotonin on small intestinal contractility in healthy volunteers. *Physiological Research*, May 16, epub

HEIDMANN, P., TORNQUIST, S.J., QU, A. & CEBRA, C.K. (2005). Laboratory measures of hemostasis and fibrinolysis after intravenous administration of ε-aminocaproic acid in clinically normal horses and ponies. *Am. J. Vet. Res*. **66**, 313-318.

HOLMSEN, H. (1983). Platelet activation and serotonin. In: Van Houtte P.M., ed. Serotonin and the cardiovascular system. New York, NY: Raven Press; 75-86.

JOHNSON, P.J. (1995). Electrolyte and acid-base disturbances in the horse. *Vet. Clin. North. Am. Equine. Pract.* **11**, 491-514.

KAPLAN, K.L. & OWEN, J. (1981). Plasma levels of beta-thromboglobulin and platelet factor 4 as indices of platelet activation in vivo. *Blood.* **57**, 199-202.

KINGSTON, J.K., BAYLY, W.M., SELLON, D.C., MEYERS, K.M. & WARDROP, K.J. (2002) Measurement of the activation of equine platelets by use of fluorescent-labeled annexin V, anti-human fibrinogen antibody, and anti-human thrombospondin antibody. *Am. J. Vet. Res*. **63**, 513-519.

LALKO, C.C., DEPPE, C.C., ULATOWSKI, D., LU-TGEN, A., HART, A.P., PATTON, E.A., LUNN, D.P., SURESH, M. & DARIEN, B.J. (2003). Equine platelet CD62P (P-selectin) expression: a phenotypic and morphologic study. *Vet. Immunol. Immunop* **91**, 114-134.

LAER, S., SCHOLZ, J., RITTERBACH, C., LAER, C., RAHME, E. & SCHULTE AM ESCH, J. (2001). Association between increased 5-HIAA plasma concentrations and vomiting in patients undergoing general anaesthesia for surgery. *Eur. J. Anaesth*. **18**, 833-838.

LATSON, K.M., NIETO, J.E., BELDOMENICO, P.M. & SNYDER, J.R. (2005). Evaluation of peritoneal fluid lactate as a marker of intestinal ischemia in equine colic. *Equine. vet. J*. **37**, 342-346.

MCHALE, N.G., THORNBURY, K.D. & HOLLYWOOD, M.A. (2000). 5-HT inhibits spontaneous contractility of isolated sheep mesenteric lymphatics via activation of 5-HT_4 receptors. *Microvascular Research* **60**, 261-268.

MENZIES-GOW, N.J., BAILEY, S.R. & KATZ, L.M. (2004). Endotoxin-induced digital vasoconstriction in horses: associated changes in plasma concentrations of vasoconstrictor mediators. *Equine. vet. J*. **36**, 273-278.

MEYERS, K.M., REED, S.M. & KECK, M. (1982). Circulating endotoxin-like substance(s) and altered hemostasis in horses with gastrointestinal disorders. *Am. J. Vet. Res.* **43**, 2233-2238.

MOORE, J.N. & BARTON, M.H. (2003). Treatment of endotoxemia. *Vet. Clin. North. Am. Eq. Practice*. **20**, 77.

NAGY, J.A., HERZBERG, K.T., MASSE, E.M., ZIENTARA, G.P. & DVORAK, H.F. (1989). Exchange of macromolecules between plasma and peritoneal cavity in ascites tumor-bearing, normal, and serotonin-injected mice. *Cancer Res*. **49**, 5448-5458.

NIETO, J.E., SNYDER, J.R., KOLLIAS-BAKER, C. & STANLEY, S. (2000) In vitro effects of 5-hydroxytryptamine and cisapride on the circular smooth muscle of the jejunum of horses. *Am. J. Vet. Res.*, **61**, 1561-1565.

RAGGI, M.A., SABBIONI, C., CASAMENTI, G., GERRA, G., CALONGHI, N. & MASOTTI, L. (1999). Determination of catecholamines in human plasma by high-performance liquid chromatography with electrochemical detection. *J. Chromatogr. B. Biomed. Sci. Appl.* **9**, 201-211.

SAULEZ, M.N., CEBRA, C.K. & DAILEY, M. (2005). Comparative biochemical analyses of venous blood and peritoneal fluid from horses with colic using a portable analyser and an in-house analyser. *Vet. Rec*. **157**, 217-223.

SEGURA, D., MONREAL, L., PEREZ-PUJOL, S., ALONSO, A., DIAZ-RICART, M., BRUGUES, R., ORDINAS, A. & ESCOLAR, G. (2006). Effects of etamsylate on equine platelets: In vitro and in vivo studies. *Vet J*. **9**, Epub ahead of print.

SELLERS, A.F., LOWE, J.E. & CUMMINGS, J.F. (1985). Trials of serotonin, substance P and alpha 2-adrenergic receptor effects on the equine large colon. *Cornell. Vet.* **75**, 319-323.

SMITH, M.J.H., FORD-HUTCHISON, A.W., ELLIOTT, P.N.C. & BOLAM, J.P. (1974). Prostaglandins and the anti-inflammatory activity of a human plasma fraction in carrageenan-induced paw oedema in the rat. *J. Pharm. Pharmacol* ., **26**, 692-698.

SOME, M. & HELANDER, A. (2002). Urinary excretion patterns of 5-hydroxyindole-3-acetic acid and 5-hydroxytryptophol in various animal species: implications for studies on serotonin metabolism and turnover rate. *Life. Sci.* **71**, 2341-2349.

STEVERINK, P.J.G.M., STURK, A. & RUTTEN, V.P.M.G. (1995). Endotoxin, interleukin-6 and tumor necrosis factor concentrations in equine acute abdominal disease: relation to clinical outcome. *J. Endotoxin. Res.* **2**, 289-299.

STOKOL, T., ERB, H.N., DE WILDE, L., TORNQUIST, S.J. & BROOKS, M. (2005). Evaluation of latex agglutination kits for detection of fibrin(ogen) degradation products and D-dimer in healthy horses and horses with severe colic. *Vet. Clin. Pathol.* **34**, 375-382.

TERAMOTO, Y., URANO, T. & NAGAI, N. (1998). Plasma levels of 5-HT and 5-HIAA increased after intestinal ischemia/reperfusion in rats. *Jpn. J. Physiol*. **48**, 333-339.

WALTHER, A., PETRI, E., PETER, C., CZABANKA, M. & MARTIN, E. (2007). Selective Serotonin-Receptor Antagonism and Microcirculatory Alterations During Experimental Endotoxemia. *J. Surg. Res*., **5**, Epub ahead of print

WALZ, D.A. & HUNG, G.L. (1985). In vivo studies on the binding of heparin and its fractions with platelet factor 4. *Semin. Thromb. Hemost*. **11**, 40-47

WANG, Z.H. (1993). Correlation between serotonin and prostaglandins in peritoneal fluid collected from patients with pelvic congestion. *Chinese Journal of Obstetrics and Gynecology* **28**, 337-339.

WEISS, R., ABEL, D., SCHOLTYSIK, G., STRAUB, R. & MEVISSEN, M. (2002). 5-hydroxytryptamine mediated contractions in isolated preparations of equine ileum and pelvic flexure: pharmacological characterization of a new 5-HT_4 agonist. *J. Vet. Pharmacol. Therap.* **25**, 49-58.

Chapter 4.II

CONTRACTILE EFFECTS OF 5-HYDROXYTRYPTAMINE AND 5-CARBOXAMIDOTRYPTAMINE IN THE EQUINE LONGITUDINAL JEJUNUM

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SUMMARY

The use of human prokinetic drugs in colic horses leads to inconsistent results. This might be related to differences in GI receptor populations. The motor effects of 5-hydroxytryptamine (5-HT; serotonin) on the equine mid-jejunum were therefore studied. Longitudinal muscle preparations were set up for isotonic measurement.

5-HT induced tonic contractions with superimposed phasic activity; these responses were not influenced by tetrodotoxin and atropine, suggesting a non-neurogenic, non-cholinergic pathway.

The 5-HT receptor antagonists GR 127935 (5-$HT_{1B,D}$), ketanserin (5-HT_{2A}), SB 204741 (5-HT_{2B}), RS 102221 (5-HT_{2C}), granisetron (5-HT_3), GR 113808 (5-HT_4) and SB 269970 (5-HT_7) had no influence on the 5-HT induced response; the 5-HT_{1A} receptor antagonists NAN 190 (pK_b=8.13 ± 0.06) and WAY 100635 (pK_b=8.69 ± 0.07), and the 5-$HT_{1,2,5,6,7}$ receptor antagonist methysergide concentration-dependently inhibited the 5-HT-induced contractile response.

The 5-$HT_{1,7}$ receptor agonist 5-carboxamidotryptamine induced a contractile response similar to that of 5-HT; its effect was not influenced by tetrodotoxin and atropine, and SB 269970 but antagonized by WAY 100635. 8-OHDPAT, buspiron and flesinoxan, that are active at rat and human 5-HT_{1A} receptors had no contractile influence.

These results suggest that the contractile effect of 5-HT in equine jejunal longitudinal muscle is due to interaction with muscular 5-HT receptors, that cannot be characterized between the actually known classes of 5-HT receptors.

4.II.1. INTRODUCTION

Postoperative ileus is a notorious complication in horses that is predominantly seen after surgical intervention for small intestinal colic. Ileus in horses is characterized by a loss of

adequate and coordinated intestinal motility and propulsion leading to the production of large amounts of gastric reflux and small intestinal distention. This complication is responsible for as many as 86% of equine deaths following abdominal surgery (Roussel *et al.*, 2001). The pathogenic mechanisms which have been implicated as possible causes are sympathetic inhibitory reflexes, parasympathetic hypoactivity, dopaminergic hyperactivity and inhibitory mediators of the inflammatory response (Gerring & Hunt, 1986; Morris, 1991).

The goals of postoperative treatment are maintenance of adequate hydration, correction of electrolyte imbalance, pain relief, control of infection and last but not least, restoration of normal intestinal propulsion. The latter however often poses a real therapeutic challenge. The mainstays of currently used prokinetic treatments are extra-polated from human medicine by use of cisapride, metoclopramide, domperidone and erythromycin (Van Hoogmoed *et al.*, 2004). Also postoperative iv administration of lidocaine is inspired by human use (Brianceau *et al.*, 2002). Up until now however, application of these prokinetic treatments is invariably associated with inconsistent to poor results in horses with ileus.

An overview of the literature shows the lack of fundamental in vitro research on the equine intestine to justify the routine use of these human prokinetic drugs in colic horses. There is insufficient scientific evidence that the already established enteral receptor populations that serve as pharmacological target to induce intestinal propulsion in humans, are equally important in horses. A possible discrepancy in these receptor populations between humans and horses could partially explain the inconsistent clinical efficacy of human prokinetic agents in equine colic cases.

Recently, increasing scientific interest in the role of serotonin (5-hydroxytryptamin; 5-HT) in human GI motility has led to the development of several compounds of potential interest for the treatment of functional GI tract disorders. The gastroprokinetic effect of the recently introduced tegaserod in humans is, as for cisapride, related to the activation of 5-HT_4

receptors on cholinergic neurons, facilitating release of the contractile neurotransmitter acetylcholine (Talley, 2001). In healthy horses, tegaserod administered intravenously, was shown to accelerate gastrocolonic transit of barium-filled particles given via a stomach tube and identified radiographically in the collected faeces; it increased the frequency of defaecation and the gut sounds at the caecal base (Lippold *et al.*, 2004). Little information is available on the in vitro characterization of the 5-HT receptor population in the equine gut. In equine jejunum circular muscle, Nieto *et al.* (2000) reported that the stimulatory effect of 5-HT was antagonised by a 5-HT_2 and a 5-HT_3 receptor antagonist, but not by a 5-HT_4 receptor antagonist. Both atropine and tetrodotoxin (TTX) had no effect on the 5-HT-induced contractions, which suggests that in this part of the intestine 5-HT mediates its effect through 5-HT_2 and 5-HT_3 receptors, active via a non neurogenic, non-cholinergic pathway. This is very peculiar, since up until now a solely neuronal localisation has been ascribed to the 5-HT_3 receptor. The stimulatory effect of cisapride, which was less pronounced than that of 5-HT, was not influenced by atropine plus TTX and was attributed to 5-HT_2 receptor activation, based on the antagonistic effects of the specific 5-HT_2 receptor antagonist ketanserin. Again this observation is surprising, since cisapride has only been characterized as a 5-HT_2 receptor antagonist. For equine ileum and pelvic flexure circular and longitudinal muscle, Weiss *et al.* (2002) reported stimulatory effects of 5-HT that were reduced by 5-HT_4 receptor antagonism, but still more by 5-HT_3 receptor antagonism, so that an interaction with 5-HT_3 and 5-HT_4 receptors was proposed; tegaserod had a stimulatory effect that was less pronounced than that of 5-HT.

The aim of this study was to identify the contractile serotonergic receptor population in the small intestine of the horse, taking into account all serotonergic receptor-type possibilities, and thus not limiting the study to the testing of the presence of 5-HT receptor populations identified in human intestine, being mainly 5-HT_2, 5-HT_3 and 5-HT_4 receptors. The rationale

to investigate primarily small intestine is the fact that postoperative ileus is predominantly located in this intestinal segment. The jejunal longitudinal smooth muscle was elected because up until now no 5-HT receptor population characterization has been performed in this muscle layer.

4.II.2. MATERIALS AND METHODS

4.II.2.1. Tissue collection and smooth muscle strip preparation

The study population was comprised of horses of various breeds and either sex, with an age range of 2 to 20 years. Ponies, foals and draft horses were excluded from the study.

Segments of the middle part of the equine jejunum were collected at the slaughterhouse, using the ileum as point of orientation. Shortly after stunning, the GI tract was removed from the carcasses and a jejunal segment of 20 cm was dissected at a distance of 8 m proximal to the jejunoileal junction. The segments were then rinsed with oxygenated Krebs-Henseleit solution (composition in mM: glucose 11.1, $CaCl_2$ 2.51, $NaHCO_3$ 25, $MgSO_4$ 1.18, KH_2PO_4 1.18, KCl 4.69 and NaCl 118) at 4°C, to remove bowel contents and were subsequently immersed in the same oxygenated solution during transportation to the laboratory.

Within one hour after tissue collection, the intestinal segments were opened along the mesenteric border and were carefully cleared of mucosa, submucosa and mesenterium. Strips (maximum 32 per horse) of approximately 1.5 cm length and 4-5 mm width were then prepared in the direction of the longitudinal muscle layer and mounted onto tissue holders. These were placed in a set-up of 16 organ baths, containing Krebs-Henseleit solution (20 ml) at 37°C, continuously gassed with 95% O_2 and 5% CO_2. The mechanical activity of the preparations was recorded via isotonic transducers (Harvard apparatus) coupled to a 16 channel PowerLab (ADInstruments, Melbourne, Australia), under a load of 2g. The load of 2g

was determined as optimal by preliminary testing on strips of 10 horses, measuring maximal carbachol-induced contraction under loads ranging from 1 up to 10g.

A one hour stabilisation period was allowed before the start of the experiment, during which the organ baths were flushed with Krebs-Henseleit solution at 30 and 60 min. After this period, regular spontaneous activity was observed in all preparations. Subsequently the tissue was challenged twice with 1 µM carbachol at an interval of 30 min. This induced in all preparations two tonic contractions of similar size, illustrating complete equilibration of the tissue.

4.II.2.2. Experimental protocols

Preliminary experiments with 5-HT

In preliminary experiments, the responses to cumulative administration of 5-HT (0.1 nM to 1 µM) within the same tissue were compared with those to administration of 8 increasing concentrations of 5-HT (0.1 nM to 3 µM) in 8 parallel jejunal strips of the same horse (one concentration per tissue). This learned that the cumulative concentration-response curve to 5-HT was clearly depressed at the higher concentrations of 5-HT in comparison to the isolated one (see results), so that only isolated concentration-response curves were obtained in further experiments with 5-HT and other 5-HT receptor agonists. Preliminary experiments also indicated that repeated administration of 0.1 µM 5-HT at 15 min interval (with washout once the contractile response was obtained) led to a decreasing response to 5-HT already at the 2nd administration. When the interval was increased to 30 min, the response to repetitive administration of 0.1 µM 5-HT (up to 7 times) remained stable.

Influence of tetrodotoxin (TTX) and atropine, N^G-nitro-L-arginine (L-NNA) and 5-HT receptor antagonists on the response to 5-HT

TTX (0.3 µM) plus atropine (0.3 µM), and L-NNA (100 µM) were tested versus 5-HT as follows. An isolated concentration-response curve to 5-HT was constructed by administering 8 increasing concentrations of 5-HT to 8 jejunal strips of a horse (thus each preparation only receiving one concentration of 5-HT), and a parallel curve to 5-HT was obtained after incubation for 20 min with TTX plus atropine, or L-NNA in 8 strips of the same horse. A series of 5-HT receptor antagonists was tested versus 5-HT in the same way: ketanserin (5-HT_{2A}; 0.3 µM), granisetron (5-HT_3; 0.3 µM); GR 113808 (5-HT_4; 0.1 µM); SB 269970 (5-HT_7; 0.3 µM); methysergide (5-$HT_{1,2,5,6,7}$; 1, 10 and 100 nM), NAN 190 (5-HT_{1A}; 0.1, 0.3 and 1 µM) and WAY 100635 (5-HT_{1A}; 3, 30 and 300 nM).

TTX (3 µM) and atropine (1 µM) were also tested separately versus 1 µM 5-HT. 5-HT was added twice at 30 min interval; 20 min before the second administration, TTX (3 µM) and/or atropine (1 µM) were added to the organ bath; a third tissue was used as a control. The following 5-HT receptor antagonists were also tested in the same way: GR 127935 (5-$HT_{1B,D}$; 0.1 µM), ketanserin (5-HT_{2A}, 0.3 µM), SB 204741 (5-HT_{2B}; 0.3 µM), RS 102221 (5-HT_{2C}, 0.3 µM), granisetron (5-HT_3; 0.3 µM) and GR 113808 (5-HT_4; 0.1 µM).

The above described experiments showed that WAY 100635, NAN 190 and methysergide were the only 5-HT receptor antagonists, with a clear-cut influence on the effect of 5-HT. Therefore, they were also tested in the following way. 5-HT (0.1 µM) was added 7 times at 30 min interval with washout after the contractile response was obtained in 4 tissues of the same horse; 20 min before the 2^{nd} to 6^{th} administration of 5-HT, increasing concentrations of WAY 100635, NAN 190 or methysergide were added; the 7^{th} administration of 5-HT was done after washout of the antagonists. The fourth tissue of the same horse was used as a control.

Influence of other 5-HT receptor agonists

Isolated concentration-response curves were also constructed for the 5-HT_{1A} receptor agonists flesinoxan, 8-OH-DPAT and buspiron, and for 5-carboxamidotryptamine (5-CT; 5-$HT_{1,7}$). TTX (0.3 µM) plus atropine (0.3 µM), SB 269970 (0.3 µM) and WAY 100635 (3, 30 and 300 nM) were tested versus isolated concentration-response curves of 5-CT, as described for 5-HT. The influence of TTX (3 µM) and atropine (1 µM) was also tested separately versus 1 µM 5-CT, as described versus 1 µM 5-HT. The possible antagonistic effect of flesinoxan (0.1 µM), 8-OH-DPAT (0.1 µM) and buspiron (1 µM) versus 5-HT was tested by adding 0.1 µM 5-HT twice at 30 min interval; 20 min before the second administration, flesinoxan, 8-OH-DPAT or buspiron was added. The concentrations of flesinoxan, 8-OH-DPAT and buspiron in these experiments were chosen to be at least 100 times higher than their affinity values determined from competition binding with [^{3}H]8-OH-DPAT in CHO (Chinese Hamster Ovary) cells expressing the human 5-HT1A-receptor (Newman-Tancredi *et al.*, 2001).

4.II.2.3. Drugs

The following drugs were used (abbreviations and respective suppliers in parentheses): carbachol (Merck, Germany), 5-hydroxytryptamine (5-HT; Janssen Research foundation, Belgium), atropine sulphate (Merck, Germany), methysergide maleate, ketanserin tartrate, 1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl]piperazine HCl (NAN-190), N-(1-methyl-5-indolyl)-N'-(3-methyl-5-isothiazolyl)urea(SB204741), [1-[2-[(methylsulphonyl)amino]ethyl]-4-piperidinyl]methyl-1-methyl-1H-indole-3-carboxylate (GR 113808), 2-methyl-4-(5-methyl-[1,2,4]oxadiazol-3-yl)-biphenyl-4-carboxylic acid [4-methoxy-3-(4-methyl-piperazin-1-yl)-phenyl]amide HCl (GR 127935), N^G-nitro-L-Arginine (L-NNA), granisetron HCl, 8-[5-(2,4-dimethoxy-5-(4-trifluoromethylphenylsulfon-amido)phenyl-5-oxopentyl)]-1, 3, 8- triazaspi-

ro[4.5]decane-2,4-dione hydrochloride (RS 102221), (R)-3-(2-(2-(4-methylpiperidin-1-yl) ethyl)pyrrolidine-1-sulfonyl) phenol (SB 269970) (Janssen Research foundation, Belgium), tetrodotoxin (TTX; Serva, Germany), 5-carboxamidotryptamine (5-CT; Tocris Cookson, UK), 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT), flesinoxan, buspiron (Janssen Research foundation, Belgium); N-2-4-(2-methoxyphenyl)-1-piperazinylethyl-N-(2-pyridinyl)cyclohexane carboxamide trihydro-chloride (WAY 100635; Tocris Cookson, UK). All compounds were dissolved in distilled water, except for NAN 190 that was dissolved in distilled water with 10% cyclodextrin, and SB 204741 that was dissolved in distilled water with 20% cyclodextrin. The solvents had no effect on the muscle strips per se and did not affect the agonist and antagonist concentration-response curves. All stock solutions were prepared freshly on the day of the experiment and dilutions were prepared using distilled water.

4.II.2.4. Data Analysis

Data collection was performed using Chart for Windows (v4.12, ADInstruments, Oxfordshire, UK).

The amplitude of contractions induced by 5-HT and 5-HT receptor agonists, is expressed as % of the second carbachol-induced contraction. In the experiments where increasing concentrations of the antagonists methysergide, WAY 100635 and NAN 190 were tested versus 0.1 µM 5-HT, the amplitude of contractions is normalized by expressing them as % of the blanco 5-HT-induced contraction before administration of these antagonists, used as reference.

Concentration-response curves to 5-HT and other agonists were individually fitted to the Hill equation using a computerized iterative non-linear curve fitting procedure, obtaining curve parameter estimates for upper asymptote E_{max}, midpoint location pEC_{50} and Hill slope n_H. Curve parameters in the presence of an antagonist were compared to those in its absence

by unpaired t-test, accepting competitive antagonism when the pEC_{50} was significantly decreased but E_{max} and slope were not significantly altered. In case of competitive antagonism, the pK_b of the antagonist was calculated according to the Schild equation: log K_b= log B-log (DR-1) (In which B represents the used concentration of antagonist; DR is defined as the concentration ratio of the EC_{50} in the presence and absence of [B]). When the influence of a single concentration of antagonist was tested versus a single concentration of 5-HT (1 µM), within the same tissue, the responses to 5-HT in the absence and presence of the antagonist were compared by a paired t-test. When several concentrations of a 5-HT receptor antagonist (NAN 190, WAY 100635) were tested in one single strip, versus a fixed dose of 5-HT (0.1 µ), K_b values of the antagonists were calculated using the logistic function described by Lazareno & Birdsall (1993), which represents a modification of the Cheng-Prusoff equation for analysing antagonist inhibition curves in functional experiments:

$$K_b = \frac{IC_{50'}}{\frac{[A_f]}{EC_{50'}} - 1}$$

where K_b is the antagonist dissociation constant and $[A_f]$ is the fixed agonist concentration (in this case 5-HT 0.1 µM). For reasons of accuracy and convenience, when using this method, it is necessary to constrain the agonist and antagonist concentration-effect curves to have the same maximum (in this case 0.1 µM). So, in the above described logistic function IC_{50}' is derived from the antagonist inhibition curves, that were constructed by non linear regression. The EC_{50}' value was obtained by fitting the control concentration-response curve to 5-HT in 24 horses (Figure 4.II.2b) to a maximum of 0.1 µM 5-HT and constraining the Hill slope to 1.

All values are expressed as mean ± s.e.m.; *n* denotes the number of tissues obtained from different horses. Significance was set at a value of $p<0.05$.

4.II.3. RESULTS

4.II.3.1. Concentration-response curves to 5-HT

The equine jejunal longitudinal muscle strips showed spontaneous phasic activity. 5-HT induced mainly a tonic contraction with superimposed phasic activity (Figure 4.II.1a). The amplitude and the frequency of these phasic contractions tended to be increased in comparison to the spontaneous activity before the administration of 5-HT, but this effect did not show concentration-dependency. Only the tonic response was therefore measured for calculation.

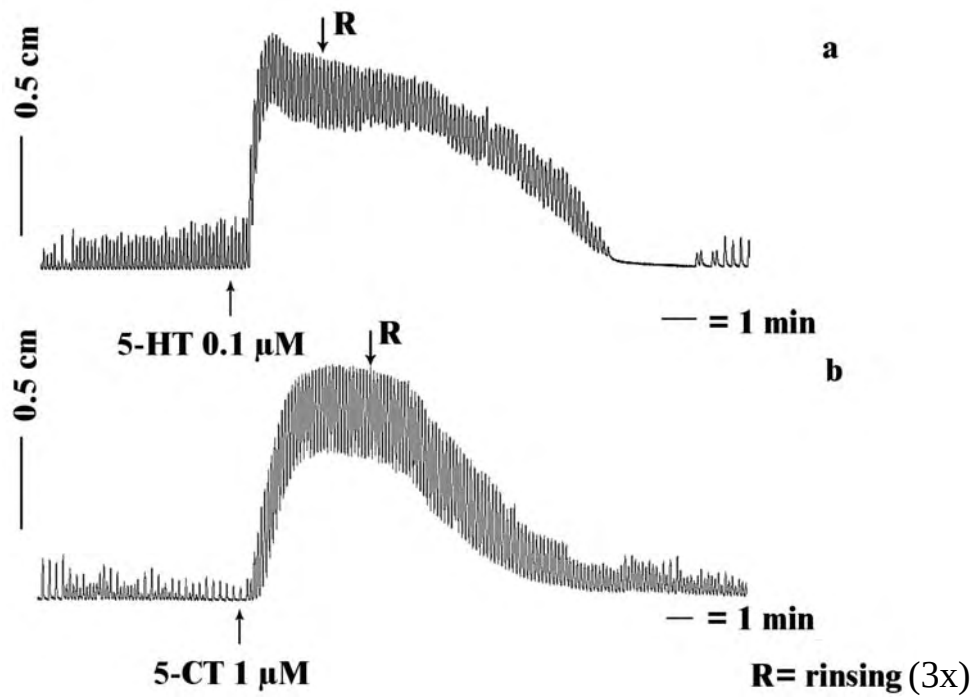


Figure 4.II.1: Representative tracings of the response to 0.1 µM 5-HT (panel a) and 1 µM 5-CT (panel b) in isolated equine jejunal longitudinal muscle strips.

Figure 4.II.2a shows the concentration-response curves obtained by cumulative administration of 5-HT in the same strip and by administration of 8 increasing concentrations of 5-HT to 8 parallel strips. The cumulative concentration-response curve was bell shaped and the maximal effect was clearly decreased compared to that of the isolated curve. Accordingly, the cumulative administration protocol was not used to investigate the effect of 5-HT in equine jejunum longitudinal muscle.

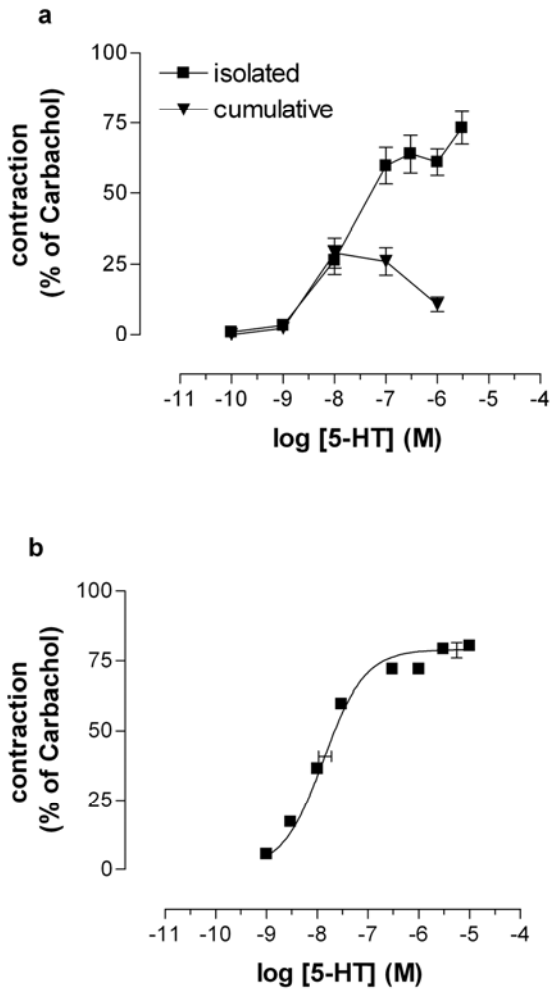


Figure 4.II.2:

a. Mean (± s.e.m) concentration-response curves to 5-HT, when added cumulatively or in an isolated way (8 increasing concentrations in 8 different tissues) in equine jejunal longitudinal muscle (n=6).
b. Mean isolated concentration-response curve to 5-HT in equine jejunal longitudinal smooth muscle strips (n=24). The curve shown represents a simulation using the Hill equation; the estimates of E_{max} (with vertical error bars) and pEC_{50} (with horizontal error bars) are shown.

Figure 4.II.2b shows the constructed mean isolated 5-HT (1 nM – 10 µM) concentration-response curve of 24 horses. It has the features of a monophasic sigmoidal concentration-response curve, consistent with a single-site interaction. The iterative fitting procedure of the

individual curves yields a mean upper asymptote E_{max} of 79.04 ± 2.47%, a mean midpoint location pEC_{50} of 7.88 ± 0.07 and a mean Hill slope of 1.07 ± 0.08.

4.II.3.2. Effect of TTX, atropine and L-NNA on the response to 5-HT

Addition of TTX and atropine to the organ baths had no effect on frequency or amplitude of spontaneous activity or base-line tonus. The midpoint location, slope and upper asymptotes of the isolated concentration-response curves to 5-HT that served as a control were not significantly influenced by the combination of TTX (0.3 µM) and atropine (0.3 µM), (Table 4.II.1). The contractile response to 1 µM 5-HT in the presence of TTX (3 µM) or atropine (1 µM) or the combination of both, was also not changed in comparison to the response induced by 1 µM 5-HT before adding TTX and/or atropine (Table 4.II.2).

L-NNA (100 µM), a nitric oxide synthase inhibitor, did not influence spontaneous activity or base-line tonus of the tissues. There was no significant effect on the concentration-response curve to 5-HT (Table 4.II.1).

Table 4.II.1: Curve parameters for the isolated concentration-response curves to 5-HT and 5-CT in the absence and presence of the antagonists indicated (Values are expressed as mean ± s.e.m.)

Agonist	**In the absence of antagonist**			*In the presence of antagonist*		
5-HT	$E_{max\ (\%)}$	pEC_{50}	n_H	$E_{max\ (\%)}$	pEC_{50}	n_H
TTX plus atropine (n=4) (Both 0.3 µM)	90.44 ± 2.31	7.56 ± 0.23	0.88 ± 0.14	89.29 ± 2.88	7.66 ± 0.11	0.83 ± 0.19
L-NNA (n=4) (100 µM)	65.80 ± 6.00	7.85 ± 0.13	0.90 ± 0.05	73.00 ± 7.82	7.52 ± 0.26	0.90 ± 0.05
Ketanserin (n=4) (0.3 µM)	80.64 ± 3.55	7.83 ± 0.05	0.81 ± 0.06	86.91 ± 4.46	7.63 ± 0.14	0.70 ± 0.12
Granisetron (n=4) (0.3 µM)	85.40 ± 2.57	8.26 ± 0.08	1.30 ± 0.15	82.26 ± 0.84	8.15 ± 0.11	1.04 ± 0.13
GR 113808 (n=4) (0.1 µM)	75.46 ± 4.12	7.92 ± 0.08	1.01 ± 0.16	76.98 ± 4.16	7.87 ± 0.10	0.84 ± 0.07
SB 269970 (n=4) (0.3 µM)	70.94 ± 7.60	7.72 ± 0.11	0.91 ± 0.15	72.22 ± 8.18	7.52 ± 0.07	0.74 ± 0.07
5-CT						
TTX plus atropine (n=4) (Both 0.3 µM)	86.90 ± 7.48	6.19 ± 0.26	0.74 ± 0.06	86.16 ± 7.71	6.34 ± 0.31	0.73 ± 0.09
SB 269970 (n=4) (0.3 µM)	86.90 ± 7.48	6.19 ± 0.26	0.74 ± 0.06	83.99 ± 3.19	6.31 ± 0.27	0.69 ± 0.06

Table 4.II.2: Contractile responses to 1 µM 5-HT and 1 µM 5-CT before and in the presence of the antagonists indicated (Values are expressed as mean ± s.e.m.)

Antagonist	*Response to 1 µM 5-HT*	
	Before	In the presence
TTX (n=4) (3 µM)	73.32 ± 1.02	72.44 ± 1.75
Atropine (n=4) (1 µM)	74.03 ± 2.37	74.93 ± 1.81
TTX plus Atropine (n=4) (3 µM and 1 µM)	73.33 ± 1.64	74.38 ± 1.34
GR 127935 (n=5) (0.1 µM)	49.26 ± 2.07	49.65 ± 2.16
Ketanserin (n=6) (0.3 µM)	49.35 ± 6.44	39.08 ± 5.63
SB 204741 (n=4) (0.3 µM)	52.38 ± 2.18	41.33 ± 6.27
RS 102221 (n=6) (0.3 µM)	52.29 ± 2.88	51.79 ± 3.32
Granisetron (n=6) (0.3 µM)	57.72 ± 3.16	52.94 ± 4.17
GR 113808 (n=6) (0.1 µM)	58.08 ± 3.91	56.99 ± 5.15
	Response to 1 µM 5-CT	
TTX (n=4) (3 µM)	68.42 ± 3.71	68.54 ± 2.71
Atropine (n=4) (1 µM)	66.98 ± 0.35	69.61 ± 1.58
TTX plus Atropine (n=4) (3 µM and 1 µM)	65.91 ± 2.68	65.97 ± 2.16

4.II.3.3. *Effect of 5-HT-receptor antagonists on the response to 5-HT*

Neither the selective 5-HT_{2A}-receptor antagonist ketanserin (0.3 µM; Hoyer *et al.*, 1994), nor the selective 5-HT_3-receptor antagonist granisetron (0.3 µM; Sanger & Nelson, 1989) altered the concentration-response curve to 5-HT (Table 4.II.1). The same observation was made for the highly selective 5-HT_4-receptor antagonist GR 113808 (0.1 µM; Johnson *et al.*, 1993) and the 5-HT_7-receptor antagonist SB 269970 (0.3 µM; Hagan *et al.*, 2000).

In further experiments, antagonists were tested by studying the response to 1µM 5-HT before and in the presence of a given antagonist within the same tissue. These experiments confirmed that the 5-HT_{2A}-receptor antagonist ketanserin (0.3 µM), the 5-HT_3-receptor antagonist granisetron (0.3 µM), and the 5-HT_4-receptor antagonist GR 113808 (0.1 µM) had no significant influence on the response to 5-HT; they further showed that also the 5-$HT_{1B,D}$-receptor antagonist GR 127935 (0.1 µM; Terron, 1996), the 5-HT_{2B}-receptor antagonist SB 204741 (0.3 µM; Forbes *et al.*, 1995) and the 5-HT_{2C}-receptor antagonist RS 102221 (0.3 µM; Bonhaus *et al.*, 1997) did not significantly influence the response to 5-HT (Table 4.II.2).

The 5-HT_{1A} receptor antagonists NAN 190 (0.1, 0.3 and 1 µM; Cao & Rodgers, 1997) and WAY 100635 (3, 30 and 300 nM; Khawaja *et al.*, 1995) inhibited the contractions to 5-HT in a concentration-dependent fashion. Figure 4.II.3 shows the results for NAN 190.

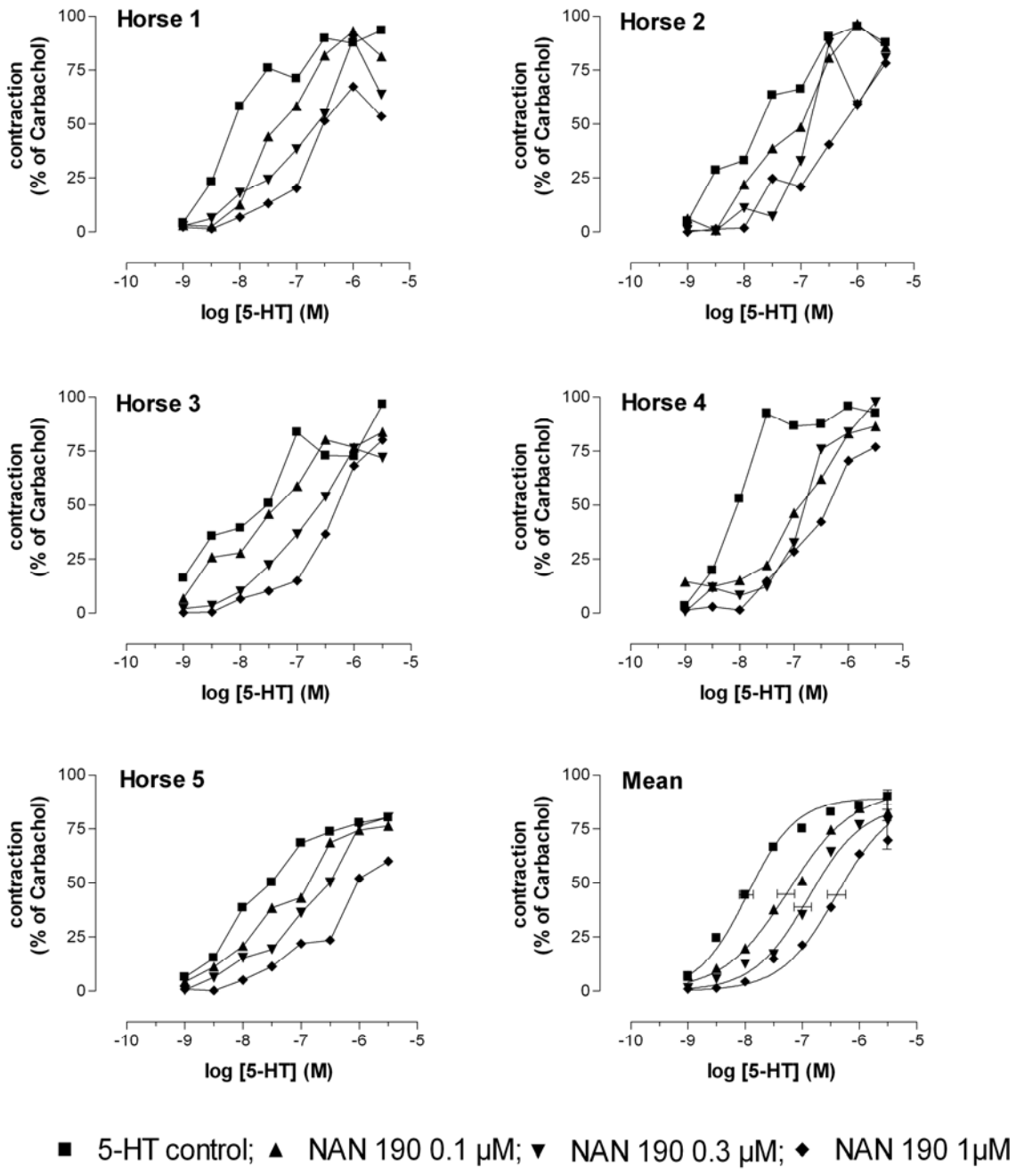


Figure 4.II.3: Influence of increasing concentrations of NAN 190 on the 5-HT-induced contraction of equine jejunal longitudinal muscle strips. The individual responses in 5 different horses (horse 1-5) are shown, as well as the mean curve simulations using the Hill equation; in the latter panel, the estimates for E_{max} (with vertical error bars) and pEC_{50} (with horizontal error bars) are given.

Although the concentration-response curves of 5-HT in an individual horse showed a somewhat capricious shape, the mean results illustrate a parallel rightward shift of the

concentration-response curve to 5-HT in the presence of increasing concentrations of NAN 190. The slopes and upper asymptotes of the concentration-response curves to 5-HT in the presence and the absence of NAN 190 were indeed not significantly different, while the pEC_{50} significantly decreased (Table 4.II.3). The pK_b values calculated on the basis of the results with 0.1, 0.3 and 1 µM NAN 190 were 7.58 ± 0.51, 7.54 ± 0.24 and 7.55 ± 0.19 respectively. WAY 100635 (3 nM) shifted the concentration-response curve to 5-HT to the right in a parallel way without a change in E_{max}, but the higher concentrations of WAY 100635 (30 nM and 300 nM) significantly depressed the E_{max} of 5-HT (Table 4.II.3; Figure 4.II.4). Apparently, WAY 100635 behaves as a non-competitive antagonist in these higher concentration ranges. The pK_b value calculated for the lowest concentration of WAY 100635 was 8.83 ± 0.44.

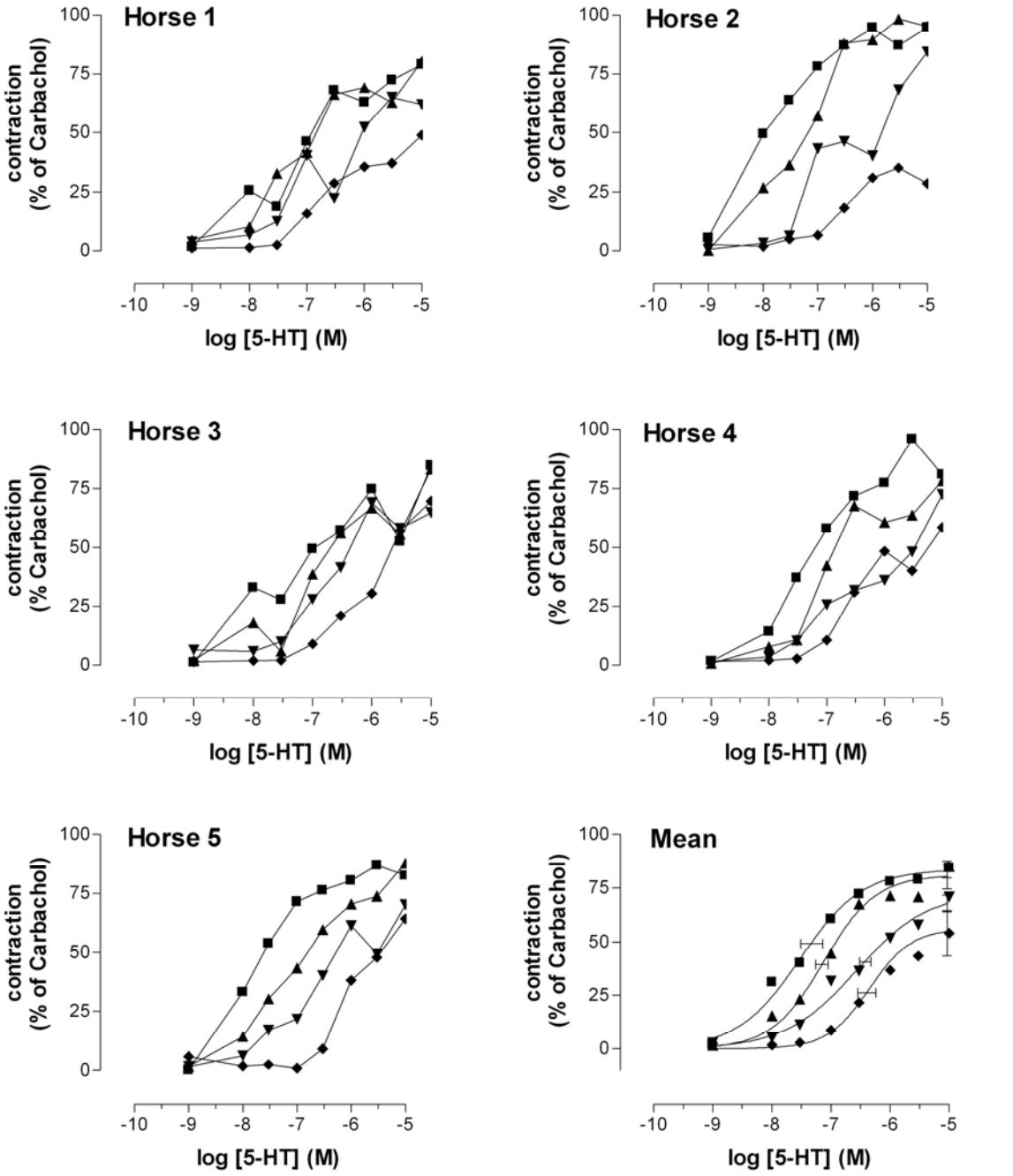
In view of the capricious shape of the isolated concentration-response curves to 5-HT in the individual horses in figures 4.II.3 and 4.II.4, and the fact that different antagonist concentrations were tested in different tissues in these experiments, the antagonists NAN 190 and WAY 100635 were also tested in different concentrations versus 0.1 µM 5-HT within the same tissue (Figures 4.II.5, 4.II.6). Both antagonists concentration-dependently reduced the response to 0.1 µM 5-HT; their effect was easily rinsed out. From these experiments, a pK_b value of 8.13 ± 0.06 was estimated for NAN 190 and a pK_b value of 8.69 ± 0.07 for WAY 100635, using the 'functional' version of the Cheng Prusoff equation proposed by Lazareno & Birdsall (1993).

Table 4.II.3: Curve parameters for the isolated concentration-response curves to 5-HT and 5-CT in the absence and presence of increasing concentrations of NAN 190 (5-HT) or WAY 100635 (5-HT, 5-CT).

	E_{max}	pEC_{50}	n_H
5-HT control	89.32 ± 3.00	7.96 ± 0.09	1.02 ± 0.23
NAN 190 (0.1 µM)	92.74 ± 4.74	7.24 ± 0.16*	0.76 ± 0.11
NAN 190 (0.3 µM)	86.41 ± 5.39	6.89 ± 0.08**	0.90 ± 0.11
NAN 190 (1 µM)	88.92 ± 9.18	6.40 ± 0.17**	0.90 ± 0.16
5-HT control	83.55 ± 3.04	7.53 ± 0.17	0.83 ± 0.10
WAY 100635 (3 nM)	80.96 ± 6.28	7.13 ± 0.08*	0.99 ± 0.21
WAY 100635 (30 nM)	73.00 ± 3.53*	6.60 ± 0.10**	0.72 ± 0.12
WAY 100635 (300 nM)	56.10 ± 10.47*	6.35 ± 0.21**	1.22 ± 0.16*
5-CT control	85.86 ± 3.78	6.20 ± 0.13	0.63 ± 0.09
WAY 100635 (3 nM)	84.22 ± 1.90	5.81 ± 0.09*	0.76 ± 0.11
WAY 100635 (30 nM)	66.22 ± 5.44*	5.78 ± 0.14**	1.10 ± 0.07*
WAY 100635 (300 nM)	33.35 ± 6.85**	5.48 ± 0.21**	1.30 ± 0.13*

*$p<0.05$; **$p<0.001$

The 5-HT_1, 5-HT_2, 5-HT_5, 5-HT_6 and 5-HT_7-receptor antagonist methysergide (1, 10 and 100 nM; Gommeren *et al.*, 1998) antagonised non-surmountably the 5-HT-induced concentration-response curve as shown in figure 4.II.7. When tested in different concentrations (0.2 nM to 3.2 nM) versus 0.1 µM 5-HT in the same tissues, methysergide induced a concentration-dependent reduction of the response to 5-HT; this effect was only partially washed out (Figure 4.II.6).



▲ WAY 100635 3nM; ▼ WAY 100635 30 nM; ◆ WAY 100635 300 nM

■ 5-HT Blanco

Figure 4.II.4: Influence of increasing concentrations of WAY 100635 on the 5-HT-induced contraction of equine jejunal longitudinal muscle strips. The individual responses in 5 different horses (horse 1-5) are shown, as well as the mean curve simulations using the Hill equation; in the latter panel, the estimates for E_{max} (with vertical error bars) and pEC_{50} (with horizontal error bars) are given.

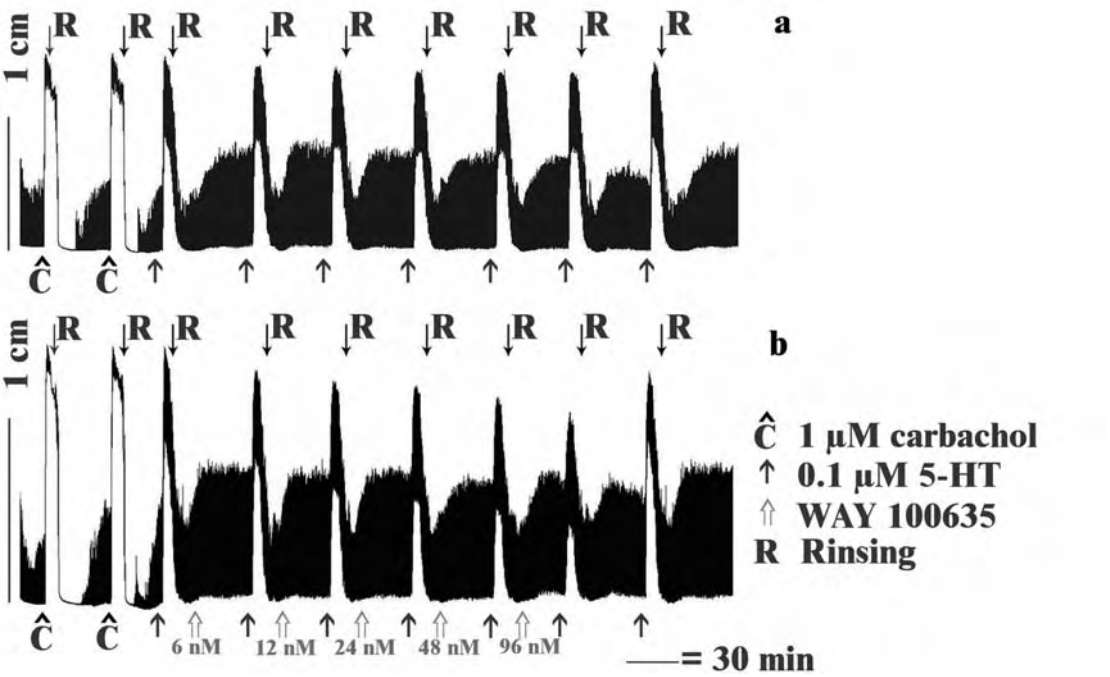


Figure 4.II.5: Representative traces showing the influence of 1 µM carbachol and 0.1 µM 5-HT in 2 equine jejunum longitudinal muscle strips. In the upper panel (a), 5-HT was studied 7 times consecutively without adding an antagonist (control); in the lower panel (b), the response to 5-HT was studied in the presence of increasing concentrations of WAY 100635.

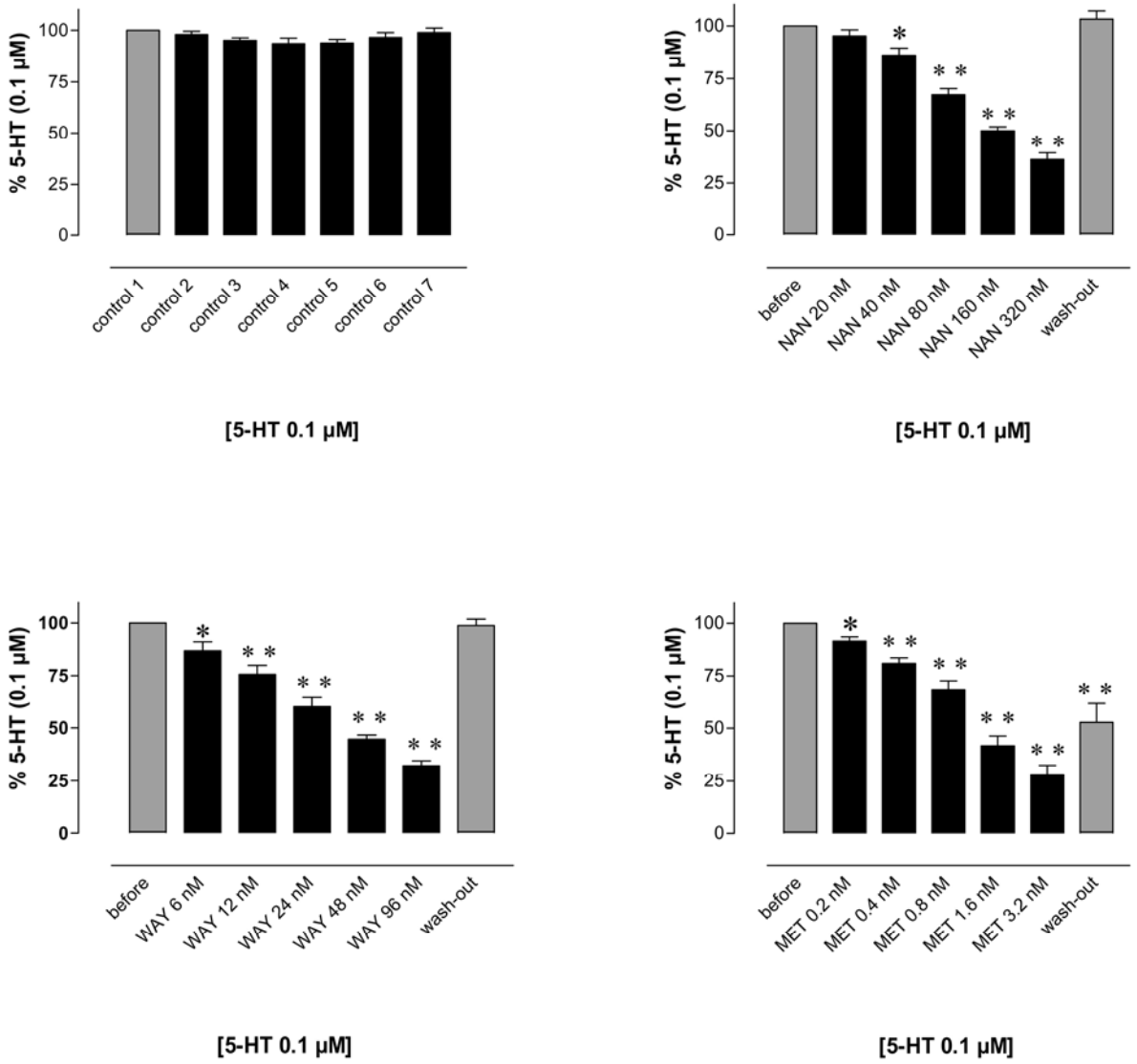


Figure 4.II.6: Influence of increasing concentrations of NAN 190 (NAN), WAY 100635 (WAY) and methysergide (MET) on the 5-HT-induced (0.1 µM) response of equine jejunal longitudinal muscle strips. 5-HT was administered 7 times at 30 min interval; antagonists were added 20 min before the 2nd to 6th administration. Tissues were rinsed after each 5-HT-induced contraction; the 7th administration was performed to check for wash out of the antagonists. In control strips, 5-HT was tested 7 times without adding antagonist (left upper panel). Data are expressed as mean values ± s.e.m (n=8). $^{*}p<0.05$; $^{**}p<0.001$ vs. 5-HT before antagonist.

4.II.3.4. *Influence of other 5-HT receptor agonists*

In accordance with the 5-HT response, it was shown in preliminary experiments, that the cumulative concentration-response curve to the 5-HT_1, 5-HT_7 receptor agonist 5-CT (Hoyer *et al.*, 1994) was clearly depressed at the higher concentrations of 5-CT in comparison to the isolated one, so that only isolated concentration-response curves were obtained in further experiments with 5-CT.

Addition of 5-CT to the organ baths elicited a response similar to that of 5-HT (Figure 4.II.1b). The contractile responses to 5-CT are concentration-dependent, yielding curve parameters for E_{max} of 85.86 ± 3.78%, pEC_{50} of 6.02 ± 0.18 and a mean Hill slope of 0.76 ± 0.09.

In contrast, the 5-HT_{1A} receptor agonists 8-OH-DPAT (Sanger & Schoemaker, 1992) and flesinoxan (Hadrava *et al.*, 1995), and the partial 5-HT_{1A} receptor agonist buspiron (Sharif *et al*., 2004) had no effect in the muscle strips (tested concentrations: 1 nM to 3 µM). When 5-HT (1 µM) was added on top of 8-OH-DPAT, flesinoxan or buspiron, this immediately induced muscle strip contraction. 8-OH-DPAT, flesinoxan and buspiron did not antagonize the response to 5-HT. The contractile response to 0.1 µM 5-HT was 66.16 ± 6.20% before and 70.99 ± 8.69 % in the presence of 0.1 µM 8-OH-DPAT, 64.43 ± 5.56% before and 68.70 ± 5.14% in the presence of 0.1 µM flesinoxan, and 68.38 ± 4.88% before and 63.15 ± 7.47% in the presence of 1µM buspiron (n=6 for each series); the response to 5-HT in the parallel control tissues not receiving antagonist was 72.23 ± 3.24% and 79.57 ± 4.68% (n=6).

4.II.3.5. *Effect of antagonists on the response to 5-CT*

Curve parameters of the concentration-response curves to 5-CT were not influenced by application of TTX plus atropine (both 0.3 µM), nor by the selective 5-HT_7 receptor antagonist SB 269970 (0.3 µM) (Table 4.II.1). Likewise, the contractile response to 1 µM 5-

CT in the presence of TTX (3 µM) or atropine (1 µM) or the combination of both, was also not changed in comparison to the response induced by 1 µM 5-CT before adding TTX and/or atropine (Table 4.II.2).

The specific 5-HT_{1A} receptor antagonist WAY 100635 in its lowest concentration (3 nM) produced a parallel rightward shift of the concentration-contraction curve to 5-CT, without influence on the maximum response (Figure 4.II.8; Table 4.II.3). When WAY 100635 was applied at a concentration of 30 nM and 0.3 µM, the concentration-response curve to 5-CT was further shifted to the right but there was a clear concomitant suppression of the maximum effect elicited by 5-CT. The pK_b value calculated for the lowest concentration of WAY 100635 was 8.63 ± 0.34.

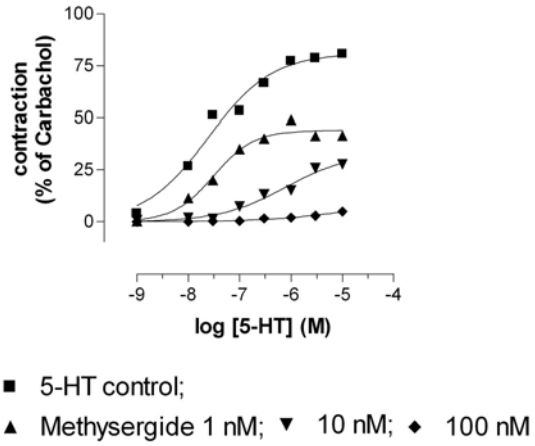


Figure 4.II.7: Concentration-response curves to 5-HT in the absence and the presence of increasing concentrations of methysergide in equine jejunal longitudinal muscle strips (n=5). The curves shown represent simulations using the Hill equation; the estimates of E_{max} (with vertical error bars) and pEC_{50} (with horizontal error bars) are shown.

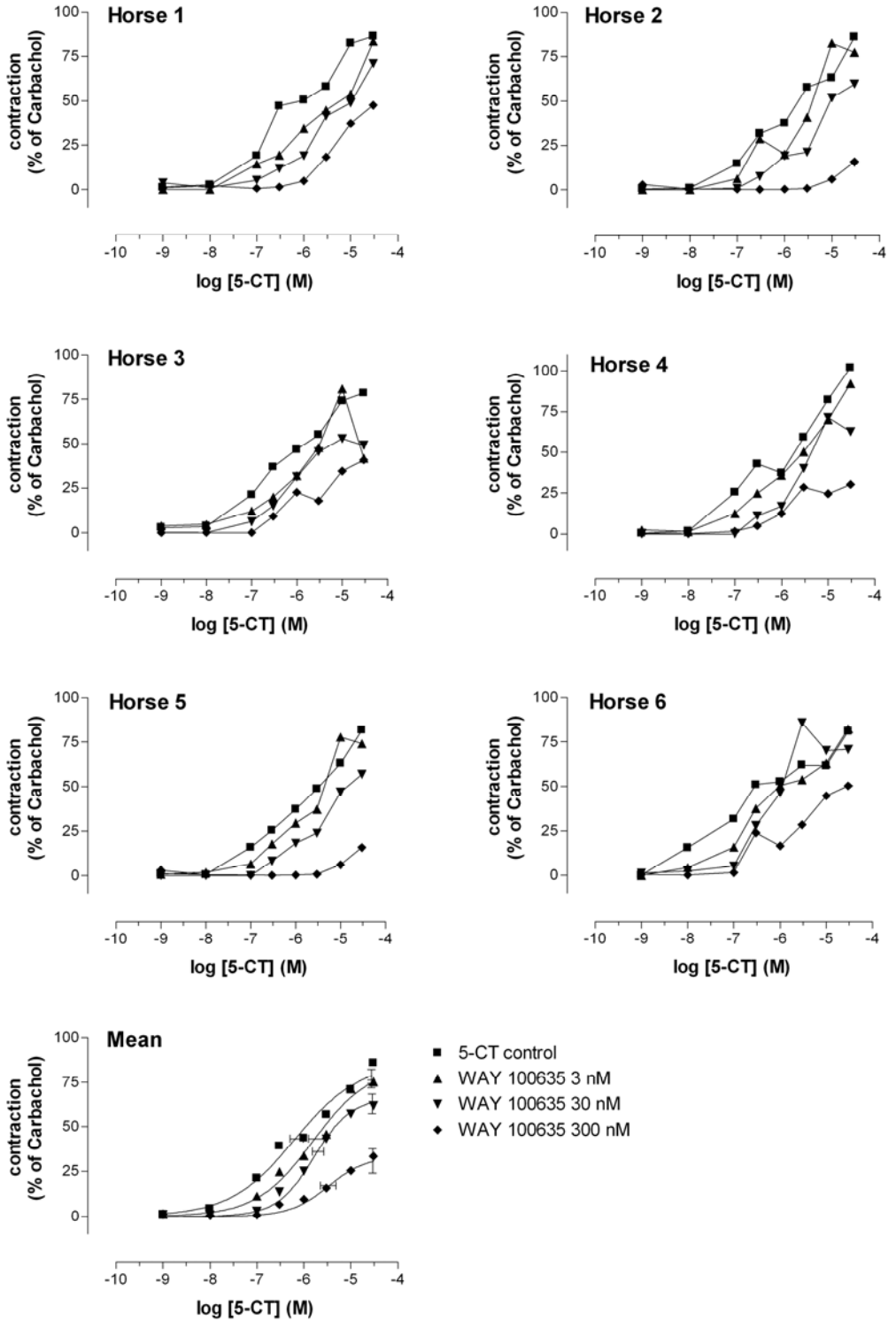


Figure 4.II.8: Influence of increasing concentrations of WAY 100635 on the 5-CT-induced contraction of equine jejunal longitudinal muscle strips. The individual responses in 6 different horses (horse 1-6) are shown, as well as the mean curve simulations using the Hill equation; in the latter panel, the estimates for E_{max} (with vertical error bars) and pEC_{50} (with horizontal error bars) are given.

4.II.4. DISCUSSION

Interaction of 5-HT with muscular 5-HT receptors, antagonized by the 5-HT_{1A} receptor antagonists NAN 190 and WAY 100635

The inability of TTX and atropine, even in the higher concentrations tested, to affect the 5-HT-induced contractile response in equine jejunal longitudinal smooth muscle suggests that 5-HT mediates its effects through non-neurogenic, non-cholinergic pathways. A similar mechanism of action was observed in the circular smooth muscle of the equine jejunum (Nieto *et al.*, 2000). Also a possible interference of NO release by 5-HT was excluded by the lack of effect of the NO synthase inhibitor L-NNA on the 5-HT-induced contractile response. From the experiments in which several antagonists were tested versus a full 5-HT concentration-response curve, or versus a single nearly maximal concentration of 5-HT, the participation of 5-$HT_{1B,1D}$, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_3, 5-HT_4 and 5-HT_7 receptors in the 5-HT-induced contractile response can be excluded. These findings on longitudinal muscle are in contrast with those on 5-HT-induced responses in equine jejunal circular smooth muscle, where interaction with 5-HT_2 and 5-HT_3 receptors has been proposed (Nieto *et al.*, 2000).

Of all tested antagonists, only the 5-HT_{1A} receptor antagonists NAN 190 and WAY 100635, and the 5-$HT_{1,2,5,6,7}$ receptor antagonist methysergide elicited a clear-cut inhibitory effect on the 5-HT-induced contractile response of equine jejunum longitudinal smooth muscle. The specific 5-HT_{1A} receptor antagonist NAN 190 fulfilled all requirements of pure competitive antagonism. The pK_b calculated from the experiments, where increasing concentrations of NAN 190 were tested versus a fixed concentration of 5-HT (8.13 ± 0.06) is in good accordance with the affinity of NAN 190 for the 5-HT_{1A} receptor, reported in the literature (Ahlers *et al.*, 1992: pigeon brain, pK_b=8.12; Sharif *et al.*, 2004: human cloned 5-HT_{1A} receptors, pK_b=8.5). The pK_b calculated from the experiments with concentration-

response curves of 5-HT was more than a half unit lower (7.54-7.58). We have no explanation for this difference.

The second 5-HT_{1A} receptor antagonist WAY 100635 (3, 30 and 300 nM) also concentration-dependently antagonised the contractile responses to 5-HT but from 30 nM on, it behaved as a non-competitive antagonist, decreasing the maximal effect of 5-HT. A pK_b estimate was calculated from the experiments where 5 concentrations of WAY 100635 were tested versus 0.1 µM 5-HT. It should be realized that the pK_b calculated in this way can be to some extent an overestimation of the antagonizing effect of WAY 100635 as the decrease in the 5-HT-induced response by WAY 100635 is not solely determined by competitive antagonism. Still the pK_b estimate obtained (8.69 ± 0.07) was similar to that calculated for the lowest concentration of WAY 100635 versus the concentration-response curve of 5-HT (8.83 ± 0.44); these values correspond to pK_b values reported before for WAY 100635 at 5-HT_{1A} receptors (Fletcher *et al.*, 1994: rat hippocampal 5-HT_{1A} receptors, pIC_{50}= 8.87 ± 0.14; Khawaja *et al.*, 1997: CHO cell line transfected with human recombinant 5-HT_{1A} receptors, pIC_{50}= 8.39 ± 0.12; Hall *et al.*, 1997: human brain, pK_b=8.60). All these cited in vitro studies were performed in brain tissue, the principal location of 5-HT_{1A} receptors. In these tissues, WAY 100635 behaves as a pure competitive antagonist. However, in one study on a GI myenterically localized 5-HT_{1A} receptor, WAY 100635 behaved as a competitive antagonist of 5-CT when tested in electrically stimulated guinea-pig ileum up to a concentration of 0.3 nM, but showed insurmountable antagonism at higher concentrations (Forster *et al.*, 1995). The results with NAN 190 and WAY 100635 thus seem to point to an interaction of 5-HT with 5-HT_{1A} receptors in equine jejunal longitudinal smooth muscle. This seems corroborated by the results with the 5-$HT_{1,7}$ receptor agonist 5-CT.

As for the 5-HT-induced contractile response, it was observed that TTX and atropine did not influence the effect of 5-CT. Because of the lack of effect of the 5-HT_7 receptor antagonist

SB 269970, the 5-CT-induced motor effects point to activation of 5-HT_1 receptors, located directly on the smooth muscle cells. The influence of WAY 100635 on the concentration-response curve of 5-CT was similar to its effect on 5-HT and the pK_b calculated for the lowest concentration of WAY 100635, that influenced the concentration-response curve of 5-CT in a competitive way, was similar to that obtained for 5-HT (8.63 versus 8.83), supporting the interaction of 5-CT and 5-HT with the same receptor.

The presence of a GI muscular 5-HT_{1A} receptor would be exceptional. The 5-HT_{1A} receptor is found predominantly in the central nervous system, the hippocampus and neocortex (Pazos & Palacios, 1985; Moller *et al.*, 2004). 5-HT_{1A} receptors are only occasionally described in the GI tract and when a GI localisation was identified, they reside in neuronal tissue where they mediate inhibitory functions. In the myenteric plexus of the isolated guinea-pig ileum and stomach, the neuronally localized 5-HT_{1A} receptors mediate inhibition of electrically evoked twitch contractions (Bill *et al.*, 1990; Lepard & Galigan, 2004; Buchheit & Buhl, 1994). In situ hybridization reveals that many submucosal and myenteric neurons of the rat and guinea-pig small intestine express mRNA encoding the 5-HT_{1A} receptor (Kirchgessner *et al.*, 1993 and 1996). The response of enteric neurons to 5-HT that has been attributed to 5-HT_{1A} receptors is a hyperpolarisation, accompanied by an increase in input resistance caused by an increase in K^+ conductance (Galligan *et al.*, 1988). Inhibitory enteric 5-HT_{1A} receptors have also been located on nerve terminals releasing the mediators of fast and slow excitatory postsynaptic potentials. Inhibition of synaptic transmission in the myenteric plexus is likely to account for 5-HT-induced inhibition of the peristaltic reflex in some studies (Galligan, 1996). Indeed, 5-HT_{1A} receptor activation has been found to induce inhibition of acetylcholine release from the guinea-pig myenteric plexus (Dietrich & Kilbinger, 1996). In contrast, a GI muscular 5-HT_{1A} receptor is expected to induce an excitatory contractile response, when coupled to inhibition of adenylate cyclase.

This is indeed the primary coupling mechanism of this receptor, although also other coupling mechanisms are described (Raymond *et al.*, 1999).

Differences between the receptor mediating the contractile effect of 5-HT in equine jejunum and the 5-HT$_{1A}$ receptor

Although the results with NAN 190 and WAY 100635 versus 5-HT and 5-CT suggest the presence of a 5-HT$_{1A}$ receptor in equine jejunum, several observations do not fit with this conclusion.

1. 5-CT is expected to be equipotent with 5-HT or even more potent than 5-HT at 5-HT$_{1A}$ receptors (Newman-Tancredi *et al.*, 1998; Cowen *et al.*, 2005). But in equine jejunum longitudinal muscle, 5-CT was at least ten fold less potent than 5-HT.

2. Methysergide has been shown to possess agonist activity (Pauwels *et al.*, 1993; Hoyer *et al.*, 1994) and to have a low affinity (Kilpatrick *et al.*, 1989) at 5-HT$_{1A}$ receptors. However, in equine jejunum, methysergide had no contractile effect per se and seemed to have a high affinity at the receptor involved, having a pronounced antagonizing effect at 1 nM. It can be mentioned that methysergide was shown to antagonize the inhibitory effect of 5-HT via 5-HT$_{1A}$ receptors on electrically induced GABA release from GABAergic neurones in the guinea-pig ileum, but in a concentration of 300 nM (Shirakawa *et al.*, 1989).

3. Three specific 5-HT$_{1A}$ receptor agonists, ie 8-OH-DPAT, buspiron and flesinoxan, did not elicit any contractile effect in the equine jejunum. They also did not antagonize the effect of 5-HT. In a system with low efficacy reserve, a partial 5-HT$_{1A}$ receptor agonist such as buspiron (Pauwels *et al.*, 1993; Sharif *et al.*, 2004) might stay without effect per se, but it should antagonize the effect of the full agonist 5-HT, which was not the case.

It is thus clear that the receptor involved in the contractile effect of 5-HT and 5-CT in equine jejunal longitudinal muscle does not correspond with a classic 5-HT_{1A} receptor. This might be related to the presence of another 5-HT receptor subtype, not yet described. Alternatively, a possible explanation could be found in interspecies differences in the specific structure of the 5-HT_{1A} receptor. As a member of the 5-HT_1 family of serotonin receptors, the 5-HT_{1A} receptor is a seven-transmembrane spanning receptor, composed of 422 amino acids. The rat and human 5-HT_{1A} receptor nucleic acid sequences are 88% homologous with each other and accordingly there appears to be a similar pharmacological profile observed between these species (Raymond *et al.*, 1999). The 5-HT_{1A} receptor has one antagonist binding site and 5 different agonist binding sites (Raymond *et al.*, 1999). Restricted mutations can lead to very important changes in the effect of a given substance. When Guan *et al.* (1992) mutated Asn^{386} in the seventh transmembrane domain of the human 5-HT_{1A} receptor, this caused a 100 fold decline in the affinity of the antagonist pindolol binding to the 5-HT_{1A} receptor. Ho *et al.* (1992) rendered the 5-HT_{1A} receptor refractory to 5-HT stimulation in several ways by introducing various point mutations. The substitution of a conserved asparagine at position 396 (localised in the 7^{th} transmembrane region) with either alanine, phenylalanine or valine, results in a 5-HT_{1A} receptor that is refractory to 8-OH-DPAT activation (Chanda *et al.*, 1993).

It can be concluded that the muscular contractile 5-HT receptor in equine jejunal longitudinal muscle can not be characterized between the actually known classes of 5-HT receptors with the experimental data provided, but is sensitive to the 5-HT_{1A} receptor antagonists NAN 190 and WAY 100635.

Desensitization of the equine muscular 5-HT receptor

In the former studies concerning in vitro characterization of 5-HT-induced responses in the equine gut it is not mentioned whether it was tested that the applied cumulative administration

protocol of 5-HT yielded the same contractile responses as isolated administration (Nieto *et al.*, 2000; Weiss *et al.*, 2002). In our study apparently a fast desensitization of the muscular 5-HT receptors takes place. It can be mentioned that desensitization is a typical feature of the 5-HT_{1A} receptor (Raymond *et al.*, 1999; Serres *et al.*, 2000; Hensler & Durgam, 2001). Acute treatment with 5-HT_{1A} agonists leads to rapid desensitization of central 5-HT_{1A} autoreceptors (Beer *et al.*, 1990; Seth *et al.*, 1997; Riad *et al.*, 2001). Rapid desensitization of 5-HT_{1A} receptors by agonists has also been described in various transfected cell lines (Nebigil *et al.*, 1995; Rotondo *et al.*, 1997; Della Rocca *et al.*, 1999). Whether we are dealing with an "equine" 5-HT_{1A} receptor or another not yet characterized 5-HT receptor, our observation of a rapidly desensitizing muscular 5-HT receptor in the equine jejunum opens interesting considerations concerning the possible role of this receptor in the complex pathophysiology of ileus in colic horses, where several factors can serve as a possible source of 5-HT overload. Bailey and co-workers already identified the presence of bioactive amines formed by bacterial decarboxylation of amino acids in the caecum and colon of healthy and colic horses (Bailey *et al.*, 2003). It is known that the permeability of intestinal mucosa in horses is increased during intestinal ischemia, which promotes translocation of endotoxins and possibly dietary amines, amongst which 5-HT, from the chyme into the systemic circulation (Snyder, 1989; Morris, 1991; Bailey *et al.*, 2000; Bailey *et al.*, 2004; Vatistas *et al.*, 2003). Within the scope of research into the ethiopathogenesis of laminitis in horses, it was shown that during iv administration of E coli lipopolysacharids for experimental induction of endotoxemia, a clear increase in plasma 5-HT and thromboxane beta 2 levels is seen. Both substances are released during activation of blood platelets (Elliott *et al.*, 2003; Vatistas *et al.*, 2003; Menzies-Gow *et al.*, 2004). Therefore, important amounts of 5-HT can be released into the blood stream in colic horses with ischemic or necrotic intestinal segments. These increased 5-HT levels in ileus horses might lead to desensitization of the muscular 5-HT receptor, meaning that 5-HT

can no longer stimulate the smooth muscle cells via these receptors. In how far the muscular contractile 5-HT receptor might contribute to hypomotility in ileus has to be further investigated.

4.II.5. CONCLUSION

This study shows the presence of muscular 5-HT receptors, inducing contraction in equine jejunal longitudinal muscle. The receptor does not belong to the 5-$HT_{1B,1D}$, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_3, 5-HT_4 and 5-HT_7 receptor class. Although blocked by the 5-HT_{1A} receptor antagonists NAN 190 and WAY 100635, the receptor cannot be classified as a classic 5-HT_{1A} receptor since the 5-HT_{1A} receptor agonists 8-OH-DPAT, flesinoxan and buspiron were not active. Whether a horse specific 5-HT_{1A} receptor or a not yet described 5-HT receptor subtype is involved needs further investigation. More research is also needed to clarify whether these muscular contractile 5-HT receptors play a role in the pathophysiology of ileus and/or can serve as pharmacological target for possible prokinetic medication in horses.

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REFERENCES

AHLERS, S.T., WEISSMAN, B.A. & BARRETT, J.E. (1992). Antagonism studies with BMY-7378 and NAN 190 – effects on 8-hydroxy-2-(di-normal-propylamino)tetralin-induced increases in punished responding of pigeons. *J. Pharmacol. Exp. Ther.*, **260**, 474-481.

BAILEY, S.R., CUNNINGHAM, F.M. & ELLIOTT, J. (2000). Endotoxin and dietary amines may increase plasma 5-hydroxytryptamine in the horse. *Eq. Vet. J.*, **32**, 497-504.

BAILEY, S.R., MARR, C.M. & ELLIOTT, J. (2003). Identification and quantification of amines in the equine caecum. *Res. Vet. Sci.*, **74**, 113-118.

BAILEY, S.R., MENZIES-GOW, N.J., MARR, C.M. & ELLIOTT, J. (2004). The effects of vasoactive amines found in the equine hindgut on digital blood flow in the normal horse. *Eq. Vet. J.*, **36**, 267-272.

BEER, M., KENNETH, G.A. & CURZON, G. (1990). A single dose of 8-OH-DPAT reduces raphe binding of [^{3}H]8-OH-DPAT and increases the effect of raphe stimulation on 5-HT metabolism. *Eur. J. Pharmacol.*, **178**, 179-187.

BILL, S.J., DOVER, G.M. & RHODES, K.F. (1990). Demonstration of 5-HT_{1A} agonist actions of 5-carboxamidotryptamine in the isolated transmurally stimulated ileum of the guinea-pig. *Br. J. Pharmacol.*, **100**, 483 P.

BONHAUS, D.W., WEINHARDT, K.K., TAYLOR, M., DESOUZA, A. & McNEELEY, P.M. (1997). RS-102221: a novel high affinity and selective, 5-HT2C receptor antagonist. *Neuropharmacology*, **36**, 621-629.

BRIANCEAU, P., CHEVALIER, H., KARAS, A., COURT, M.H., BASSAGE, L. & KIRKER-HEAD, C. (2002). Intravenous Lidocaine and small-intestinal size, abdominal fluid, and outcome after colic surgery in horses. *J. Vet. Intern. Med.*, **16**, 736-741.

BUCHHEIT, K.H. & BUHL, T. (1994). Stimulant effects of 5-hydroxytryptamine on guinea-pig stomach preparations in-vitro. *Eur. J. Pharmacol.*, **262**, 91-97.

CAO, B.J. & RODGERS, R.J. (1997). Influence of 5-HT1A receptor antagonism on plus-maze behaviour in mice. II. WAY 100635, SDZ 216-525 and NAN-190. *Pharmacol. Biochem. Behav.*, **58**, 593-603.

CHANDA, P.K., MINCHIN, M.C., DAVIS, A.R., GREENBERG, L. & REILLY, Y. (1993). Identification of residues important for ligand binding to the human 5-hydroxytryptamine1A serotonin receptor. *J. Exp. Pharmacol. Ther.*, **43**, 516-520.

COWEN, D.S., JOHNSON-FARLEY, N.N. & TRAVKINA, T. (2005). 5-HT_{1A} receptors couple to activation of Akt, but not extracellular-regulated kinase (ERK), in cultured hippocampal neurons. *J. Neurochem.*, **93**, 910-917.

DELLA ROCCA, G.J., MUKHIN, Y.V., GARNOVSKAYA, M.N., DAAKA, Y., CLARK, G.J., LUTTRELL, L.M., LEFKOWITZ, R.J., & RAYMOND J.R. (1999). Serotonin 5-HT1A receptor-mediated Erk activation requires calcium/calmodulin-dependent receptor endocytosis. *J. Biol. Chem.*, **274**, 4749-4753.

DIETRICH, C. & KILBINGER, H. (1996). 5-HT_{1A} receptor mediated inhibition of of acetylcholine release from guinea-pig myenteric plexus: potential mechanisms. *Neuropharmacology*, **35**, 483-488.

ELLIOTT, J., BERHANE, Y. & BAILEY, S.R. (2003). Effects of monoamines formed in the cecum of horses on equine digital blood vessels and platelets. *Am. J. Vet. Res.*, **64**, 1124-1131.

FLETCHER, A., BILL, D.J., CLIFFE, I.A., FORSTER, E.A., JONES, D. & REILLY, Y. (1994). A pharmacological profile of WAY-100635, a potent and selective 5-HT_{1A} receptor antagonist. *Br. J. Pharmacol.*, **122**: 91P.

FORBES, I.T., JONES, G.E. & MURPHY, O.E. (1995). N-(1-methyl-5-indolyl)-N'-(3-methyl-5-isothiazolyl)urea: a novel, high affinity 5-HT2B receptor antagonist. *J. Med. Chem.*, **38**, 855-857.

FORSTER, E.A., CLIFFE, I.A., BILL, D.J., DOVER, G.M., JONES, D., REILLY, Y. & FLETCHER, A. (1995). A pharmacological profile of the selective silent 5-HT_{1A} receptor antagonist WAY 100635. *Eur. J. Pharmacol.*, **281**, 81-88.

GALLIGAN, J.J. (1996). Electrophysiological studies of 5-hydroxytryptamine receptors on enteric neurones. *Behav. Brain Res.*, **73**, 199-201.

GALLIGAN, J.J., SUPRENANT, A., TONINI, M. & NORTH, A. (1988). Differential localization of 5-HT_1 receptors on myenteric and submucosal neurons. *Am. J. Physiol.*, **255**, 603-611.

GERRING, E.E.L. & HUNT, J.M. (1986). Pathophysiology of equine postoperative ileus: Effects of adrenergic blockade, parasympathetic stimulation and metoclopramide in an experimental model. *Eq. Vet. J.*, **18**, 249-255.

GOMMEREN, W., RENDERS, J., VAN GOMPEL, P., LESAGE, A., LEYSEN, J.E. & JURZAK, MT. (1998). Extensive pharmacological study of the G-protein coupled fraction of human 5-HT receptors using agonist radioligand binding. *Naunyn Schmiedebergh's Arch. Pharmacol.*, **358**, 8-42.

GUAN, X.M., PERTOUKA, S.J. & KOBILKA, B.K. (1992). Identification of a single amino acid residue responsible for the binding of a class of ß-adrenergic receptor antagonists to 5-hydroxytryptamine$_{1A}$ receptors. *Mol. Pharmacol.*, **41**, 695-698.

HADRAVA, V., BLIER, P., DENNIS, T., ORTEMANN, C. & DE MONTIGNY, C. (1995). Characterization of 5-hydroxytryptamine(1A) properties of flesinoxan-in-vivo electrophysiology and hypothermia study. *Neuropharmacology*, **34**, 1311-1326.

HAGAN, J.J., PRICE, G.W., JEFFREY, P., DEEKS, N.J., STEAN, T., PIPER, D., SMITH, M.I., UPTON, N., MEDHURST, A.D., MIDDLEMISS, D.N., RILEY, G.J., LOVELL, P.J., BROMIDGE S.M. & THOMAS, D.R. (2000). Characterization of SB-269970-A, a selective 5-HT7 receptor antagonist. *Br. J. Pharmacol.*, **130**, 539-548.

HALL, H., LUNDKVIST, C., HALLDIN, C., FARDE, L., PIKE, V.W., McCARRON, J.A., FlETCHER, A., CLIFFE, I.A., BARF, T., WIKSTRÖM, H. & SEDVALL, G. (1997). Autoradiographic localization of 5-HT1A receptors in the post-mortem human brain using [H-3]WAY-100635 and [C-11]WAY-100635. *Brain Res.*, **745**, 96-108.

HENSLER, J.G. & DURGAM, H. (2001). Regulation of 5-HT1A receptor-stimulated [S-35]-GTP gamma S binding as measured by quantitative autoradiography following chronic agonist administration. *Br. J. Pharmacol.*, **132**, 605-611.

HO, B.Y., KARSCHIN, A., BRANCHEK, T., DAVIDSON, N. & LESTER, H.A. (1992). The role of conserved aspartate and serine residues in ligand binding and in function of the 5-HT_{1A}receptor: a site-directed mutagenesis study. *FEBS Lett.*, **312**, 259-262.

HOYER, D., CLARKE, D.E., FOZARD, J.R., HARTIG, P. R., MARTIN, G. R., MYLECHARANE, E. J., SAXENA, P. R. & HUMPHREY, P. P. A. (1994). International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (Serotonin). *Pharmacol. Rev.*, **46**, 157-203.

JOHNSON, M.P., AUDIA, J.E., NISSEN, J.S. & NELSON, D.L. (1993). N(1)-substituted ergolines and tryptamines show species differences for the agonist-labeled 5-HT2 receptor. *Eur. J. Pharmacol.*, **239**, 111-118.

KHAWAJA, X., ENNIS, C. & MINCHIN, M.C.W. (1997). Pharmacological characterization of recombinant human 5-HT_{1A} receptors using a novel antagonist radioligand, [^{3}H] WAY 100635. *Life Sci.*, **60**, 653-665.

KHAWAJA, X., EVANS, N., REILLY, Y., ENNIS, C. & MINCHIN, M.C.W. (1995). Characterization of the binding of (^{3}H)WAY-100635, a novel 5-hydroxytryptamine$_{1A}$ receptor antagonist, to rat brain. *J. Neorochem.*, **64**, 2716-2726.

KILPATRICK, A.T., BROWN, C.M., MACKINNON, A.C. & SPEDDING, M. (1989). The α_2-adrenoceptor antagonist SK&F 104078 has high affinity for 5-HT_{1A} and 5-HT_2 receptors. *Eur. J. Pharmacol*., **166**, 315-318.

KIRCHGESSNER, A.L., LIU, M.T., HOWARD, M.J. & GERSHON, M.D. (1993). Detection of the 5-HT_{1A} and 5-HT_{1A} receptor mRNA in the rat bowel and pancreas: comparison with 5-HT_{1p} receptors. *J. Comp. Neurol.*, **327**, 233-250.

KIRCHGESSNER, A.L., LIU, M.T. & RAYMOND, J.R. (1996) Identification of cells that express 5-hydroxytryptamine$_{1A}$ receptors in the nervous systems of the bowel and pancreas. *J. Comp. Neurol.*, **364**, 439-455.

LAZARENO, S. & BIRDSALL, N.J.M. (1993). Estimation of antagonist K_b from inhibition curves in functional experiments: alternatives to the Cheng-Prusoff equation. *TiPS*, **14**, 237-239.

LEPARD, K.J. & GALLIGAN, J.J. (2004). Presynaptic modulation of cholinergic and non-cholinergic fast synaptic transmission in the myenteric plexus of guinea pig ileum. *Neurogastroenterol. Motil.*, **16**, 355-364.

LIPPOLD, B.S., HILDEBRAND, J. & STRAUB, R. (2004). Tegaserod (HTF 919) stimulates gut motility in normal horses. *Eq. Vet. J.*, **36**, 622-627.

MENZIES-GOW, N.J., BAILEY, S.R., KATZ, L.C., MARR, C.M. & ELLIOTT, J. (2004). Endotoxin-induced digital vasoconstriction in horses: associated changes in plasma concentrations of vasoconstrictor mediators. *Eq. Vet. J.*, **36**, 273-278.

MOLLER, M., CUMMING, P., ANDERSEN, G. & GJEDDE, A. (2004). Parametric mapping of serotonin 5HT1A receptors in healthy human brain. *Neuroimage*, **22**, T157-T157.

MORRIS, D.D. (1991). Endotoxemia in horses. A review of cellular and humoral mediators involved in its pathogenesis. *J. Vet. Intern. Med.*, **5**, 167-181.

NEBIGIL, C.G., GARNOVSKAYA, M.N., CASANAS, S.J., MULHERON, J.G., PARKER, E.M., GETTYS, T.W. & RAYMOND, J.R. (1995). Agonist-induced desensitization and phosphorylation of human 5-HT1A receptors expressed in SF9 insect cells. *Biochemistry.*, **34**, 11954-11962.

NEWMAN-TANCREDI, A., VERRIELE, L. & MILLAN, M.J. (2001). Differential modulation by GTP gamma S of agonist and inverse agonist binding to h5-HT1A receptors revealed by [H-3]-WAY100635. *Br. J. Pharmacol.*, **132**, 518-524.

NIETO, J.E., SNYDER, J.R., KOLLIAS-BAKER, C. & STANLEY, S. (2000) In vitro effects of 5-hydroxytryptamine and cisapride on the circular smooth muscle of the jejunum of horses. *Am. J. Vet. Res.*, **61**, 1561-1565.

NEWMAN-TANCREDI, A., GAVAUDAN, S., CONTE, C., CHAPUT, C., TOUZARD, M., VERRIELE, L., AUDINOT, V. & MILLAN, M.J. (1998). Agonist and antagonist actions of antipsychotic agents at 5-HT_{1A} receptors: a [^{35}S] GTPγS binding study. *Eur. J. Pharmacol*., **355**, 245-256.

PAUWELS, P.J., VAN GOMPEL, P. & LEYSEN, J.E. (1993). Activity of serotonin (5-HT) receptor agonists, partial agonists and antagonists at cloned human 5-HT_{1A} receptors that are negatively coupled to adenylate cyclase in permanently transfected HeLa cells. *Biochem. Pharmacol*., **45**, 375-383.

PAZOS, A. & PALACIOS, J.M. (1985) Quantitative autoradiographic mapping of serotonin receptors in the rat brain. *Brain Res.*, **346**, 205-230.

RAYMOND, J.R., MUKHIN, Y.V., THOMAS, W.G. & GARNOVSKAYA, M.N. (1999). The recombinant 5-HT_{1A} receptor: G protein coupling and signalling pathways. *Br. J. Pharmacol.*, **127**, 1751-1764.

RIAD, M., WATKINS, K.C., DOUCET, E., HAMON, M. & DESCARRIES, L. (2001). Agonist-induced internalization of serotonin-1A receptors in the dorsal raphe nucleus (autoreceptors) but not hippocampus (heteroreceptors). *J. Neurosci.*, **21**, 8378-8386.

ROTONDO, A., NIELSEN, D.A., NAKHAI, B., HULIHAN-GIBLIN, B., BOLOS, A., & GOLDMAN, D. (1997). Agonist-promoted down-regulation and functional desensitization in two naturally occurring variants of the human serotonin(1A) receptor. *Neuropsychopharmacology*, **17**, 18-26.

ROUSSEL, A.J., COHEN, N.D., HOOPER, R.N. & RAKESTRAW, P.C. (2001). Risk factors associated with development of postoperative ileus in horses. *J. Am. Vet. Med. Assoc.*, **219**, 72-78.

SANGER, G. & NELSON, D.R. (1989). Selective and functional 5-hydroxytryptamine 3 receptor antagonism by BRL 43694 (granisetron). *Eur. J. Pharmacol.*, **159**, 113-124.

SANGER, D.J. & SCHOEMAKER, H. (1992) Discriminative stimulus properties of 8-OH-DPAT: relationship to affinity for 5HT1A receptors. *Psychopharmacology.*, **108**, 85-92.

SERRES, F., MUMA, N.A., RAAP, D.K., GARCIA, F., BATTAGLIA, G. & VAN DE KAR, L.D. (2000). Coadministration of 5-hydroxytryptamine (1A) antagonist WAY-100635 prevents fluoxetine-induced desensitization of postsynaptic 5-hydroxytryptamine (1A) receptors in hypothalamus. *J. Pharmacol. Exp. Ther.*, **294**, 296-301.

SETH, P., GAJENDIRAN, M. & GANGULY, D.K. (1997). Desensitization of spinal 5-HT_{1A} receptors to 8-OH-DPAT: an in vivo spinal reflex study. *Neuro Report*, **8**, 2489-2493.

SHARIF, N.A., DRACE, C.D., WILLIAMS, G.W. & CRIDER, J.Y. (2004). Cloned human 5-HT1A receptor pharmacology determined using agonist binding and measurement of cAMP accumulation. *J. Pharm. Pharmacol.*, **56**, 1267-1274.

SHIRAKAWA, J., TAKEDA, K., TANIYAMA, K. & TANAKA, C. (1989). Dual effects of 5-hydroxytryptamine on the release of G-aminobutyric acid from myenteric neurones of the guinea-pig ileum. *Br. J. Pharmacol.*, **98**, 339-341.

SNYDER, J.R. (1989) The pathophysiology of intestinal damage: effects of luminal distention and ischemia. *Vet. Clin. North. Am. Equine Practice*, **5**, 247-270.

TALLEY, N.J. (2001). Serotoninergic neuroenteric modulators. *The Lancet*, **15**, 2061-2068.

TERRON, J.A. (1996) GR 127935 is a potent antagonist of the 5-HT1-like receptor mediating contraction in the canine coronary artery. *Eur. J. Pharmacol.*, **300**, 109-112.

VAN HOOGMOED, L.M., NIETO, J.E. & SNYDER, J.R. (2004). Survey of prokinetic use in horses with gastrointestinal injury. *Vet. Surg.*, **33**, 279-285.

VATISTAS, N.J., NIETO, J.E., VAN HOOGMOED, L., GARDNER, I. & SNYDER, J.R. (2003). Use of an isolated intestinal circuit to evaluate the effect of ischemia and reperfusion on mucosal permeability of the equine jejunum. *Vet. Surg.*, **32**, 52-61.

WEISS, R., ABEL, D., SSCOLTYSIK, G., STRAUB, R. & MEVISSEN, M. (2002). 5-Hydroxytryptamine mediated contractions in isolated preparations of equine ileum and pelvic flexure: pharmacological characterization of a new 5-HT_4 agonist. *J. Vet. Pharmacol. Ther.*, **25**, 49-58.

Chapter 4.III

CONTRACTILE EFFECTS OF 5-HYDROXYTRYPTAMINE (5-HT) IN THE EQUINE JEJUNUM CIRCULAR MUSCLE: FUNCTIONAL AND IMMUNOHISTOCHEMICAL IDENTIFICATION OF A 5-HT_{1A}-LIKE RECEPTOR

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SUMMARY

The use of several human prokinetic drugs to treat GI ileus in horses yields equivocal results. Although many human prokinetic drugs have 5-HT_4 receptors as their pharmacological target, little is known about the 5-HT-receptor subtypes present in the equine small intestine.

The aim of this study was to identify pharmacologically and immunohistochemically the serotonin receptor subtype(s) responsible for the 5-HT induced contractile response in the equine circular jejunum.

Isometric organ-bath recordings were carried out to assess spontaneous and drug-evoked contractile activity of equine circular jejunum. Histological investigations by immunofluorescence analyses were performed to check for presence and localization of this functionally identified 5-HT receptor subtype.

5-HT induced tonic contractions in horse jejunal circular muscle. Tetrodotoxin, atropine and N^G-nitro-L-arginine did not modify this response. A set of 5-HT receptor subtype selective antagonists excluded interaction with 5-$HT_{1B, 1D, 2A, 3, 4 \text{ and } 7}$ receptors. The selective 5-HT_{1A} receptor antagonists WAY 100635 and NAN 190 caused a clear rightward shift of the concentration-response curve to 5-HT. The contractile effect of 5-CT, that can interact with 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_5 and 5-HT_7 receptors was also antagonized by WAY 100635, identifying the targeted 5-HT receptor as a 5-HT_{1A}-like receptor.

Immunohistology performed with rabbit polyclonal anti-5-HT_{1A} receptor antibodies confirmed the presence of muscular 5-HT_{1A} receptors in the muscularis mucosae, and both longitudinal and circular smooth muscle layers of the equine jejunum.

5-HT_{1A}-like receptors are involved in the 5-HT-induced contractile responses in equine jejunal circular smooth muscle.

The lack of evidence for presence of 5-HT_4 receptors in the equine small intestine further brings into question the use of human prokinetic drugs acting at 5-HT_4 receptors in horses with small intestinal ileus. Tachyphylaxia is a typical 5-HT_{1A} receptor property, which renders the receptor refractory to further stimulation in the presence of an overload of 5-HT. More research is needed to further investigate the possible role of these 5-HT_{1A} receptors in the pathophysiology of ileus in horses.

4.III.1. INTRODUCTION

Postoperative ileus is a notorious complication in horses that is predominantly seen after surgical intervention for small intestinal colic. The lack of potent and therapeutically efficient prokinetic drugs hampers a straightforward approach to the management of this troublesome syndrome. In human medicine several potent prokinetic drugs, acting via the serotonergic system, are available. Before using those expensive drugs in horses, more knowledge is needed about the equine enteric serotonergic receptor population. The gastroprokinetic effect of human gastroprokinetic agents such as cisapride, and the more recent drugs tegaserod and prucalopride is related to activation of 5-HT_4 receptors on cholinergic neurons, enhancing acetylcholine release (Gershon & Tack 2007). As far as documented, the evidence for the presence of 5-HT_4 receptors in the equine GI tract is weak. In circular and longitudinal muscle strips of equine ileum and pelvic flexure, 5-HT enhanced the frequency and amplitude of spontaneous contractile activity; the effect was reduced by a 5-HT_4 receptor antagonist but also by a 5-HT_3 receptor antagonist suggesting that the effect of 5-HT might be mediated by 5-HT_4 and 5-HT_3 receptors (Weiss *et al.* 2002). Delco *et al.* (2007) reported the lack of activity of the specific 5-HT_4 receptor agonist tegaserod in equine mid jejunum circular muscle, while contractions of pelvic flexure circular muscle only started to show in the higher molar ranges, above 10^{-5}M.

Nieto *et al.* (2000) reported that 5-HT has a contractile effect per se in equine distal jejunal circular muscle, that was reduced by 5-HT_2- and 5-HT_3-receptor antagonism. We recently reported that 5-HT also induces contraction in equine mid jejunum longitudinal muscle, but this effect is mediated via a muscular 5-HT_{1A} like receptor (Delesalle *et al.* 2006). As mediators and receptors can differ between the circular and longitudinal smooth muscle layer at a given location in the GI tract (Corleto *et al.* 2006; El-Yazbi *et al.* 2007) and as the circular muscle layer is the most important for enteral propulsive contractions (Grider 1998), we now investigated both functionally and immunohistochemically, the influence of 5-HT and the receptors involved in circular muscle of equine jejunum.

4.III.2. MATERIAL AND METHODS

4.III.2.1. Organ Bath studies

Tissue collection and smooth muscle strip preparation

The study population was comprised of horses of various breeds and either sex, with an age range of 4 to 20 years. Ponies, foals and draft horses were excluded from the study.

Briefly, segments of the middle part of the equine jejunum, 8 m proximal to the jejunoileal junction were collected at the slaughterhouse. The segments were then rinsed with oxygenated Krebs-Henseleit solution at 4°C, to remove bowel contents and were subsequently immersed in the same oxygenated solution during transportation to the laboratory.

Mucosa-stripped jejunum was cut into strips (maximum 32 per horse) of approximately 1.5 cm length and 4-5 mm width, in the direction of the circular muscle layer and mounted onto tissue holders as previously described (Delesalle *et al.* 2006). The mechanical activity of the preparations was recorded via isometric transducers (Harvard apparatus) coupled to a 16 channel PowerLab (ADInstruments, Melbourne, Australia), under a load of 2g. Preliminary experiments in 32 strips from 8 horses showed that the contractile response to 1 µM carbachol

was not different under a load of 1, 2, 3, 4 or 5g. A load of 2g was selected for further experiments in analogy with previously published in vitro work on equine jejunal circular muscle (Nieto *et al.* 2000). After 1h of stabilization with rinsing at 30 min interval, regular spontaneous activity was observed in all preparations. Triple challenging with 1 µM carbachol at an interval of 30 min induced three tonic contractions, the last two being of similar size, illustrating complete equilibration of the tissue.

Experimental protocols

Concentration-response curve to 5-HT; reproducibility of the response to a single concentration of 5-HT

The response to cumulative administration of 5-HT (3nM-10µM) within the same tissue (concentrations increased at 2 min intervals) was compared with the response to the same concentrations of 5-HT added in 8 parallel jejunal strips (one concentration per tissue) of the same horse. In an additional series, the isolated concentration-response curve obtained in 8 parallel jejunal strips, was compared with the concentration-response curve obtained when adding the increasing concentrations in 1 strip of the same horse at 30 min interval (2 min contact time per concentration, followed by repetitive rinsing).

The reproducibility of the response to a single concentration of 5-HT (1µM) was tested by adding it repetitively at 15, 30 or 60 min intervals (2 min contact time, followed by rinsing).

Influence of tetrodotoxin (TTX) and atropine, $N^{(G)}$-nitro-L-arginine methyl ester (L-NAME) and 5-HT receptor antagonists on the response to 5-HT

TTX (3 µM), atropine (3 µM) and L-NAME (300 µM) were tested versus 5-HT as follows. An isolated concentration-response curve to 5-HT was constructed by administering

8 increasing concentrations of 5-HT to 8 jejunal strips of a horse (thus each preparation only receiving one concentration of 5-HT), and three parallel curves to 5-HT were obtained after incubation for 20 min with respectively TTX (3 µM), atropine (3 µM) or L-NAME (300 µM) (in 8 strips of the same horse per inhibitor). A series of 5-HT receptor antagonists was tested versus 5-HT in the same way: GR 127935 (5-$HT_{1B,D}$; 0.3 µM); ketanserin (5-HT_{2A}; 0.3 µM), granisetron (5-HT_3; 0.3 µM); GR 113808 (5-HT_4; 0.1 µM); SB 269970 (5-HT_7; 0.3 µM); methysergide (5-$HT_{1,2,5,6,7}$; 1 nM), NAN 190 (5-HT_{1A}; 0.3 µM) and WAY 100635 (5-HT_{1A}; 0.3 µM).

Influence of other 5-HT receptor agonists

Isolated concentration-response curves were also constructed for 5-carboxamidotryptamine (5-CT) alone and in the presence of the 5-HT_7 receptor antagonist SB 269970 (0.3 µM) or the 5-HT_{1A} receptor antagonist WAY 100635 (0.3 µM), as described for 5-HT. In these experiments 2 separate strips of the same horses were challenged with the 5-HT_{1A} receptor agonists buspiron (3 µM) and flesinoxan (3 µM).

Drugs

The following drugs were used: atropine sulphate and carbachol (Merck, Germany), 5-hydroxytryptamine (5-HT); methysergide maleate, ketanserin tartrate, [1-[2-[(methylsulphonyl)amino]ethyl]-4-piperidinyl]methyl-1-methyl-1H-indole-3-carboxylate (GR 113808), 2-methyl-4-(5-methyl-[1,2,4]oxadiazol-3-yl)-biphenyl-4-carboxylic acid [4-methoxy-3-(4-methyl-piperazin-1-yl)-phenyl]amide HCl (GR 127935), $N^{(G)}$-nitro-L-arginine methyl ester (L-NAME), 1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl]piperazine HCl (NAN 190), granisetron HCl, (R)-3-(2-(2-(4-methylpiperidin-1-yl) ethyl)pyrrolidine-1-sulfonyl) phenol (SB 269970), flesinoxan and buspiron (Janssen Research Foundation, Belgium), tetrodotoxin (TTX; Serva, Germany), 5-carboxamidotryptamine (5-CT) and N-2-4-

(2-methoxyphenyl)-1-piperazinylethyl-N-(2-pyridinyl)cyclohexane carboxamide trihydrochloride (WAY 100635; Tocris Cookson, UK). All compounds were dissolved in desionized water. All stock solutions and dilutions were prepared in advance and stored at -20°C until use.

Data analysis

Data collection was performed using Chart 5 for Windows (v5.3, ADInstruments, Oxfordshire, UK).

The area under the curve (AUC) of the contractile response to a given concentration of 5-HT and 5-CT was determined for 2 min (g.s).

Cumulative and isolated concentration-response curves to 5-HT were compared through use of an un-paired t-test. The response to repetitive administration of a single concentration of 5-HT (1 µM) was compared through use of a paired t-test.

Concentration-response curves to 5-HT and 5-CT were individually fitted to the Hill equation using a computerized iterative non-linear curve fitting procedure, obtaining curve parameter estimates for upper asymptote E_{max}, midpoint location pEC_{50} and Hill slope n_H. Curve parameters in the presence of an antagonist were compared to those in its absence by unpaired t-test, accepting competitive antagonism when the pEC_{50} was significantly decreased but E_{max} and slope were not significantly altered. In case of competitive antagonism, the pK_b of the antagonist was calculated according to log K_b= log B-log (DR-1).

All values are expressed as mean ± s.e.m.; *n* denotes the number of tissues obtained from different horses. Significance was set at a value of $p<0.05$.

4.III.2.2. Immunohistochemistry

A fresh non mucosa-stripped jejunal sample of 5 healthy horses, also used in the organ-bath studies, was collected to perform immunohistochemistry. The samples were taken at the anti-mesenteric border, right next to the tissue used for the functional study. Three 1cm x 1cm pieces of jejunum were dissected-out and immediately cryopreserved in OCT (Tissue Tec; Sakura, Zoeterwoude, Netherlands) by freezing in liquid nitrogen. Storage of the samples was performed at -80°C until immunohistochemistry was performed.

Tissue processing

Frozen samples were sectioned at 10 µm with a cryostat (HM 500 OM; Microm, Heidelberg, Germany) and placed on SuperFrost Plus glass slides (Menzel Gläser, Braunschweig, Germany).

Immunofluorescence staining on air dried cryosections of horse jejunum require paraformaldehyde 4% fixation for 2 minutes at room temperature. Prior to incubation with primary rabbit polyclonal rat anti-$5HT_{1A}$ receptor antibody (ab44635; Abcam plc, Cambridge UK)(1/100, overnight at room temperature) sections were blocked with 10% normal goat serum (DakoCytomation, Denmark) for 30 minutes at room temperature. Following anti-$5HT_{1A}$ receptor incubation, sections were rinsed and incubated with Cy3 conjugated goat anti-rabbit IgG (Jackson Immunoresearch Laboratories, Inc., PA, USA) (1/500 in PBS) for 2 hours at room temperature and counterstained with Hoechst 33342, to label the nuclei (Invitrogen Corporate, Molecular Probes, California, USA) (1/500 in AD). The specificity of the immunohistochemical reactions in the equine tissue was checked in a series of control experiments. For proper localization of $5HT_{1A}$ receptor immunoreactivity, selected sections were subsequently incubated with Alexa-488 conjugated phalloidin (Invitrogen Corporate, Molecular Probes, California, USA)(1/40 in PBS/BSA) for 20 minutes at room temperature, to concurrently stain smooth muscle cells. All samples were stained in one batch. Rat

hippocampus was used as a positive control (Patel *et al.*, 2005; Saigal *et al.*, 2006). No immunoreactivity was detectable if the primary antisera were omitted and replaced with either PBS or non-immune rabbit serum. Adjacent slides incubated with the blocking solution alone in the absence of the primary antibodies were used as negative controls.

Data analysis

All morphological analyses and data acquisition were performed on an Axioplan-2 system (Carl Zeiss, Germany) with integrated image analysis software package (Axiovision Release 4.6). For imaging of $5HT_{1A}$-receptor immunoreactivity an exposure time of 550 ms was used for all cases in Cy3-channel.

4.III.3. RESULTS

4.III.3.1. Organ Bath Studies

Concentration-response curve to 5-HT; reproducibility of the response to a single concentration of 5-HT.

Circular smooth muscle strips of the equine mid-jejunum showed spontaneous activity (Figure 4.III.1). 5-HT mainly induced a tonic contraction with an initial peak, that declined to a stable level within 1 min; superimposed on this tonic response, phasic activity was visible (Figure 4.III.1). Cumulative administration of 5-HT yielded responses that were significantly lower than when the same concentrations were added to 8 parallel strips (one concentration per tissue; Figure 4.III.2A).

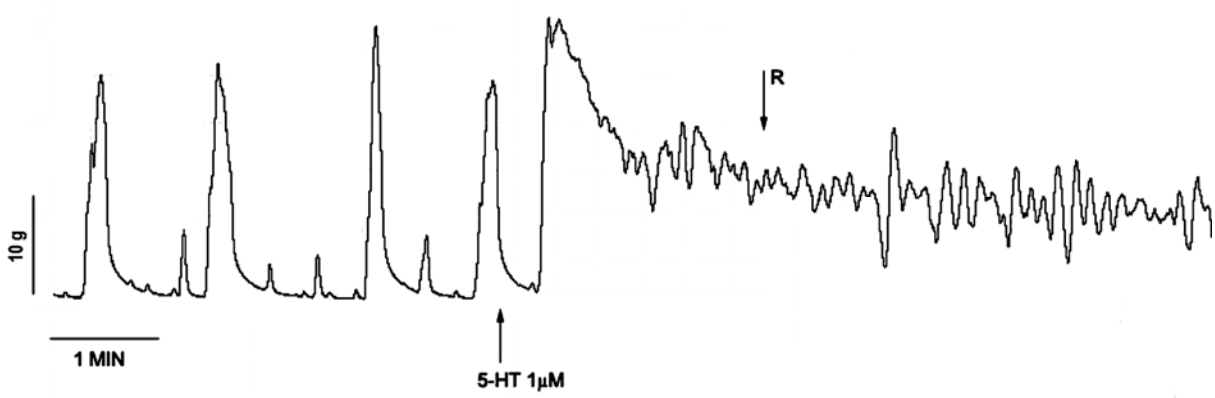

Figure 4.III.1: Representative tracing of the response of an equine mid-jejunum circular smooth muscle strip to the administration of 1 µM 5-HT. R denotes rinsing.

Also when concentrations were not administered cumulatively, but at 30 min intervals in the same strip, the responses were significantly lower than when added to separate strips (Figure 4.III.2B). When a single concentration of 5-HT (1 µM) was repetitively administered in the same strip at 15, 30 or 60 min intervals, a clear progressive fading of the contractile response was seen. E.g., at 60 min interval, the response to 1 µM 5-HT declined from 2160 ± 121 g.s at the 1st administration, to 1579 ± 285 g.s (p=0.04) at the 2nd administration, 1405 ± 287 g.s (p=0.03) at the 3rd administration and 1350 ± 294 g.s (p=0.03) at the 4th administration (n=3). Accordingly, only isolated concentration-response curves were obtained in further experiments with 5-HT and 5-carboxamidotryptamine (5-CT), which entails a single application of agonist (5-HT or 5-CT) per strip.

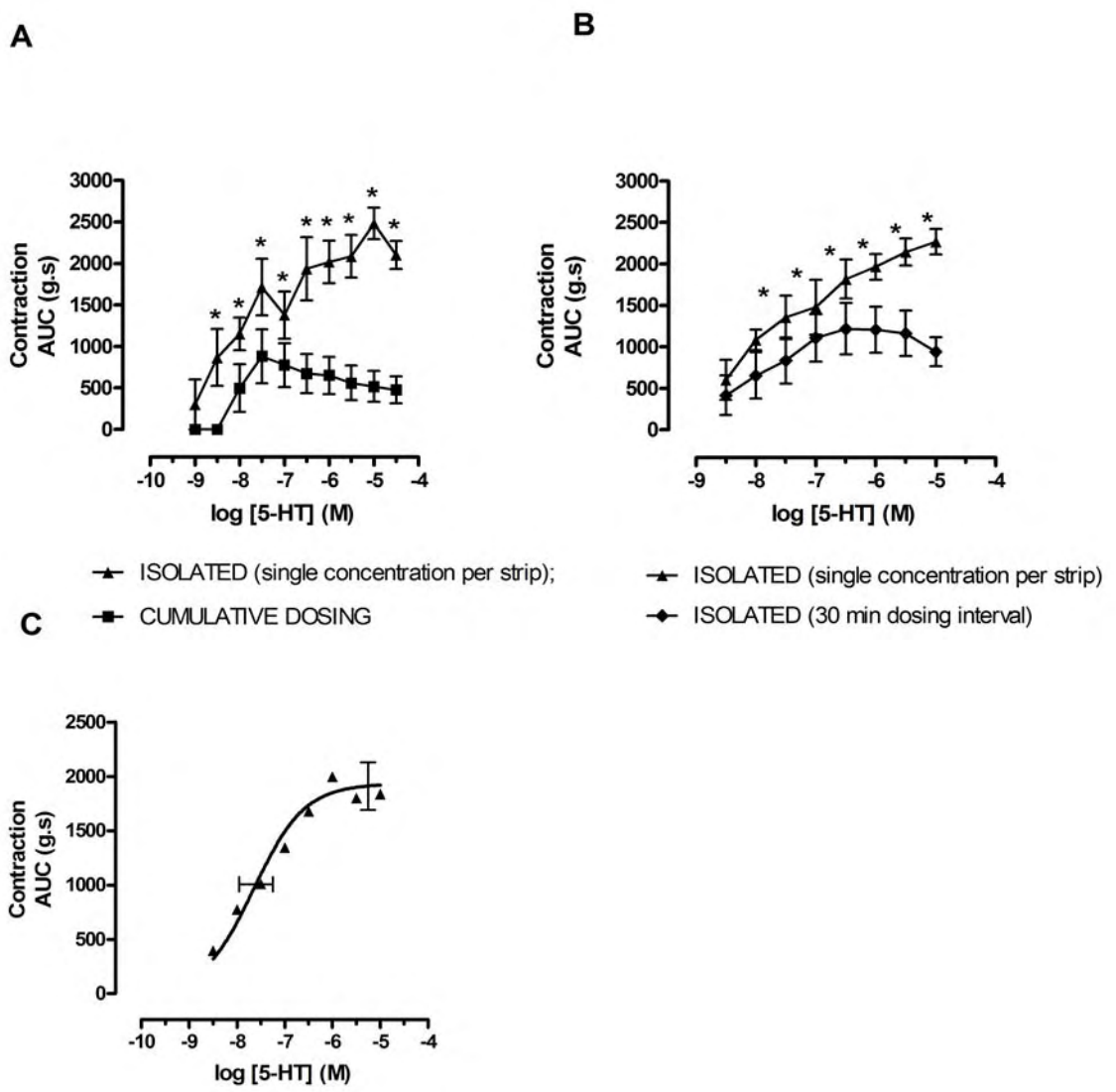

Figure 4.III.2:

A. Mean (± s.e.m) concentration-response curves to 5-HT, when added cumulatively (■) or in an isolated way (▲) using the non repetitive dosing protocol (8 increasing concentrations in 8 different tissues) in equine jejunal circular muscle (n=4).
B. Mean (± s.e.m) concentration-response curves to 5-HT, when added using the 30 min repeated dosing protocol (♦) or in an isolated way (▲) using the non repetitive dosing protocol (8 increasing concentrations in 8 different tissues) in equine jejunal circular muscle (n=7).
C. Mean isolated concentration-response curve to 5-HT in equine jejunal circular smooth muscle strips (n=34). The curve shown represents a simulation using the Hill equation; the estimates of E_{max} (with vertical error bars) and pEC_{50} (with horizontal error bars) are shown.
* $P<0.05$: significantly different from the response with cumulative dosing (A) or with isolated dosing within the same strip at 30 min interval (B).

Figure 4.III.2, panel C shows the constructed mean isolated 5-HT (3nM-10μM) concentration-response curve obtained in tissues of 34 horses. It has the features of a monophasic sigmoidal concentration-response curve, consistent with a single-site interaction. The iterative fitting procedure of the individual curves yields a mean upper asymptote E_{max} of 1932.6 ± 600.7 g.s, a mean midpoint location pEC_{50} of 7.64 ± 0.54 and a mean Hill slope n_H of 0.82 ± 0.35.

Effect of TTX, atropine and L-NAME on the response to 5-HT

Addition of TTX, atropine or L-NAME had no effect on frequency or amplitude of spontaneous activity or base-line tone. The midpoint location, slope and upper asymptotes of the isolated concentration-response curves to 5-HT were not significantly changed in the presence of atropine (3μM), TTX (3 μM) or the nitric oxide synthase inhibitor L-NAME (300 μM) (Table 4.III.1).

Tabel 4.III.1: Curve parameters for the isolated concentration-response curves to 5-HT in the absence and presence of the antagonists indicated

	E_{max}	pEC_{50}	n_H
5-HT control (n=5)	2211 ± 126	7.92 ± 0.13	1.04 ± 0.33
Atropine (n=5) (3 µM)	1993 ± 159	7.71 ± 0.19	0.83 ± 0.34
TTX (n=5) (3 µM)	1934 ± 163	7.91 ± 0.34	0.83 ± 0.31
5-HT control (n=4)	2135 ± 592	7.53 ± 0.57	0.60 ± 0.27
L-NAME (n=4) (300 µM)	1588 ± 250	7.06 ± 0.34	0.70 ± 0.29
5-HT control (n=4)	1556 ± 248	7.12 ± 0.35	0.69 ± 0.30
GR 127 935 (n=4) (0.3 µM)	1552 ± 173	7.47 ± 0.24	1.01 ± 0.49
5-HT control (n=4)	1481 ± 132	7.83 ± 0.21	0.96 ± 0.43
ketanserin (n=4) (0.3 µM)	1394 ± 157	7.80 ± 0.26	0.87 ± 0.46
5-HT control (n=4)	1227 ± 168	7.98 ± 0.33	0.89 ± 0.64
granisetron (n=4) (0.3 µM)	1170 ± 177	7.84 ± 0.36	0.73 ± 0.48
5-HT control (n=4)	1753 ± 166	8.13 ± 0.24	0.63 ± 0.30
GR113808 (n=4) (0.1 µM)	1611 ± 178	7.99 ± 0.27	0.84 ± 0.48
5-HT control (n=4)	1458 ± 181	7.37 ± 0.27	0.85 ± 0.39
SB 269970 (n=4) (0.3 µM)	1538 ± 126	7.22 ± 0.15	1.23 ± 0.40
5-HT control (n=4)	2015 ± 472	7.56 ± 0.35	1.28 ± 0.14
NAN 190 (n=4) (0.3 µM)	1547 ± 341	6.77 ± 0.38**	1.55 ± 0.43
5-HT control (n=6)	1599 ± 90	7.69 ± 0.15	0.82 ± 0.12
WAY 100635 (n=6) (0.3 µM)	864 ± 57**	6.70 ± 0.12**	2.00 ± 0.32**
5-HT control (n=3)	1576 ± 116	7.71 ± 0.19	1.12 ± 0.32
Methysergide (n=3) (0.001 µM)	1055 ± 129**	6.29 ± 0.15**	2.46 ± 1.38**

Values are expressed as mean ± s.e.m. ** $p<0.001$: significantly different vs 5-HT in the absence of antagonist

Effect of 5-HT receptor antagonists on the response to 5-HT

Application of the 5-$HT_{1B/1D}$ receptor antagonist GR 127 935 (0.3 µM; Terron 1996), the 5-HT_{2A} receptor antagonist ketanserin (0.3 µM; Hoyer *et al.* 1994), the 5-HT_3 receptor antagonist granisetron (0.3 µM; Sanger and Nelson 1989), the 5-HT_4 receptor antagonist GR 113808 (0.1 µM; Johnson *et al.* 1993) and the 5-HT_7-receptor antagonist SB 269970 (0.3 µM; Hagan *et al.* 2000) did not influence the concentration-response curve to 5-HT.

Of all tested antagonists, only the 5-HT_{1A} receptor antagonists NAN 190 (0.3 µM; Cao and Rodgers 1997) and WAY 100635 (0.3 µM; Khawaja *et al.* 1995) and the 5-$HT_{1,2,5,6,7}$ receptor antagonist methysergide (1 nM) elicited a clear-cut inhibitory effect on the 5-HT-induced contractile response of equine mid-jejunum circular smooth muscle. Figure 4.III.3 illustrates the parallel rightward shift of the concentration-response curve to 5-HT in the presence of NAN 190 (0.3 µM). The slopes and upper asymptotes of the concentration-response curves to 5-HT in the presence and the absence of NAN 190 were indeed not significantly different, while the pEC_{50} significantly decreased, corresponding with competitive antagonism (Table 4.III.1). The calculated pK_b for NAN 190 was 7.24 ± 0.38.

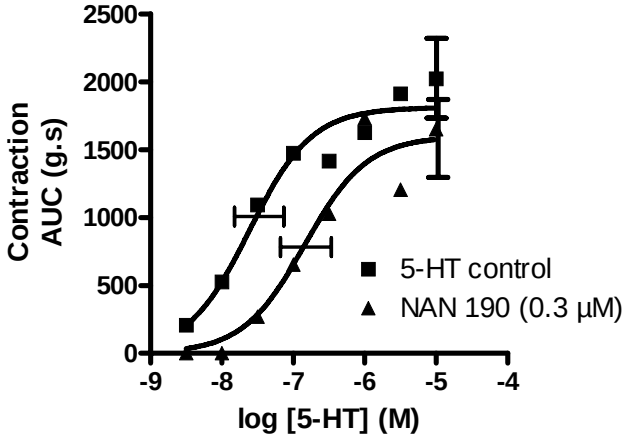

Figure 4.III.3: Mean curve simulations using the Hill equation of the 5-HT-induced contraction of equine jejunal circular muscle in the absence (■) and the presence (▲) of 0.3 µM of NAN 190 (n=4). (The estimates for E_{max} (with vertical error bars) and pEC_{50} (with horizontal error bars) are given.)

At the concentration used, the 5-HT_{1A} receptor antagonist WAY 100635, behaved as a non-competitive antagonist, since it caused a clear decrease in the E_{max} of the 5-HT induced concentration-response curve (Figure 4.III.4A). Therefore no pK_b value was calculated for this antagonist.

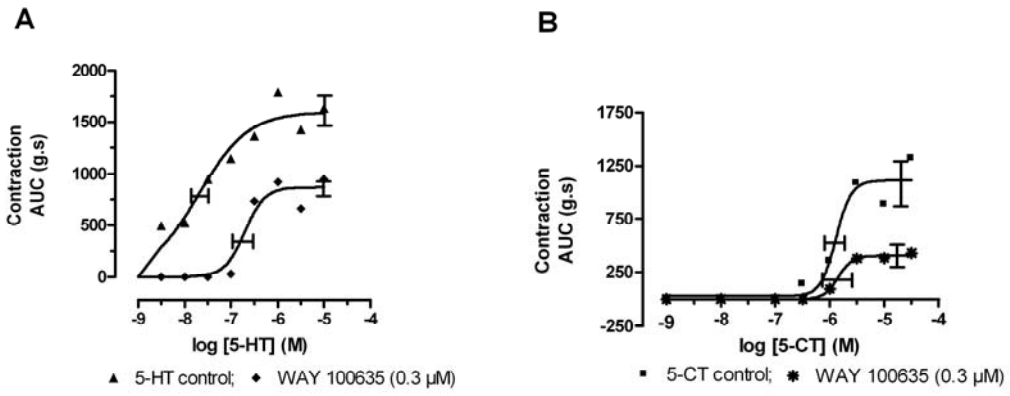

Figure 4.III.4: Mean curve simulations using the Hill equation of the 5-HT-induced (A, n=6) or 5-CT-induced (B, n=4) contraction of equine jejunal circular muscle in the absence (▲) and the presence (♦) of 0.3 µM of WAY 100635. (The estimates for E_{max} (with vertical error bars) and pEC_{50} (with horizontal error bars) are given.)

Influence of other 5-HT receptor agonists

Because cumulative dosing of 5-HT was proven to yield decreased responses, it was decided to solely construct isolated concentration-response curves to 5-CT. Addition of 5-CT to the organ baths elicited a response similar to that of 5-HT. The contractile responses to 5-CT are concentration-dependent, yielding curve parameters for E_{max} of 1115 ± 199 g.s, pEC_{50} of 5.89 ± 0.13 and a mean Hill slope of 0.50 ± 0.23.

However, the 5-HT_{1A} receptor agonist flesinoxan, and the partial 5-HT_{1A} receptor agonist buspiron had no effect (tested concentration: 3 µM, n=3). When 5-HT (1 µM) was added on top of flesinoxan or buspiron, this immediately induced muscle strip contraction.

Curve parameters of the concentration-response curves to 5-CT were not influenced by application of the 5-HT_7 receptor antagonist SB 269970 (0.3 µM). The 5-HT_{1A} receptor antagonist WAY 100635 (0.3 µM) however, clearly reduced the contractile responses to 5-CT (Figure 4.III.4B, Table 4.III.2).

Table 4.III.2: Curve parameters for the isolated concentration-response curves to 5-CT in the absence and presence of the antagonists indicated.

	E_{max}	pEC_{50}	n_H
5-CT control (n=6)	1115 ± 199	5.89 ± 0.13	0.50 ± 0.23
SB 269970 (n=4) (0.3 µM)	1267 ± 321	5.71 ± 0.87	0.57 ± 0.40
WAY 100635 (n=4) (0.3 µM)	407 ± 69**	5.79 ± 0.38	2.95 ± 0.76**

Values are expressed as mean ± s.e.m. ** $p<0.001$: significantly different vs 5-CT in the absence of antagonist.

4.III.3.2. Immunohistochemistry

5-HT_{1A}-receptor immunoreactivity is detected in both the longitudinal and circular smooth muscle layer of the equine jejunum (Figure 4.III.5 a,b).

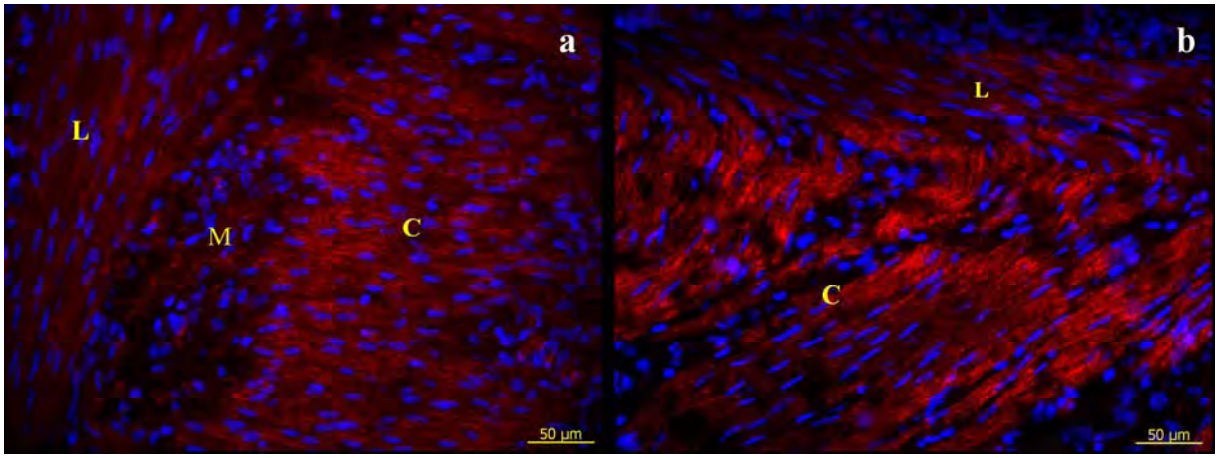

Figure 4.III.5 a,b: 5-HT_{1A} immunoreactivity (red) in equine jejunum circular (C) and longitudinal (L) smooth muscle layer. No 5-HT_{1A} immunoreactivity was seen in myenteric and submucosal ganglia. L=longitudinal muscle; C=circular muscle; M=Myenteric plexus; blue=Hoechst 33342 staining cell nuclei.

5-HT_{1A}-receptor labelling appears in a granular way at smooth muscle cells as revealed by colabelling using Phalloidin (Figure 4.III.6 a,b,c).

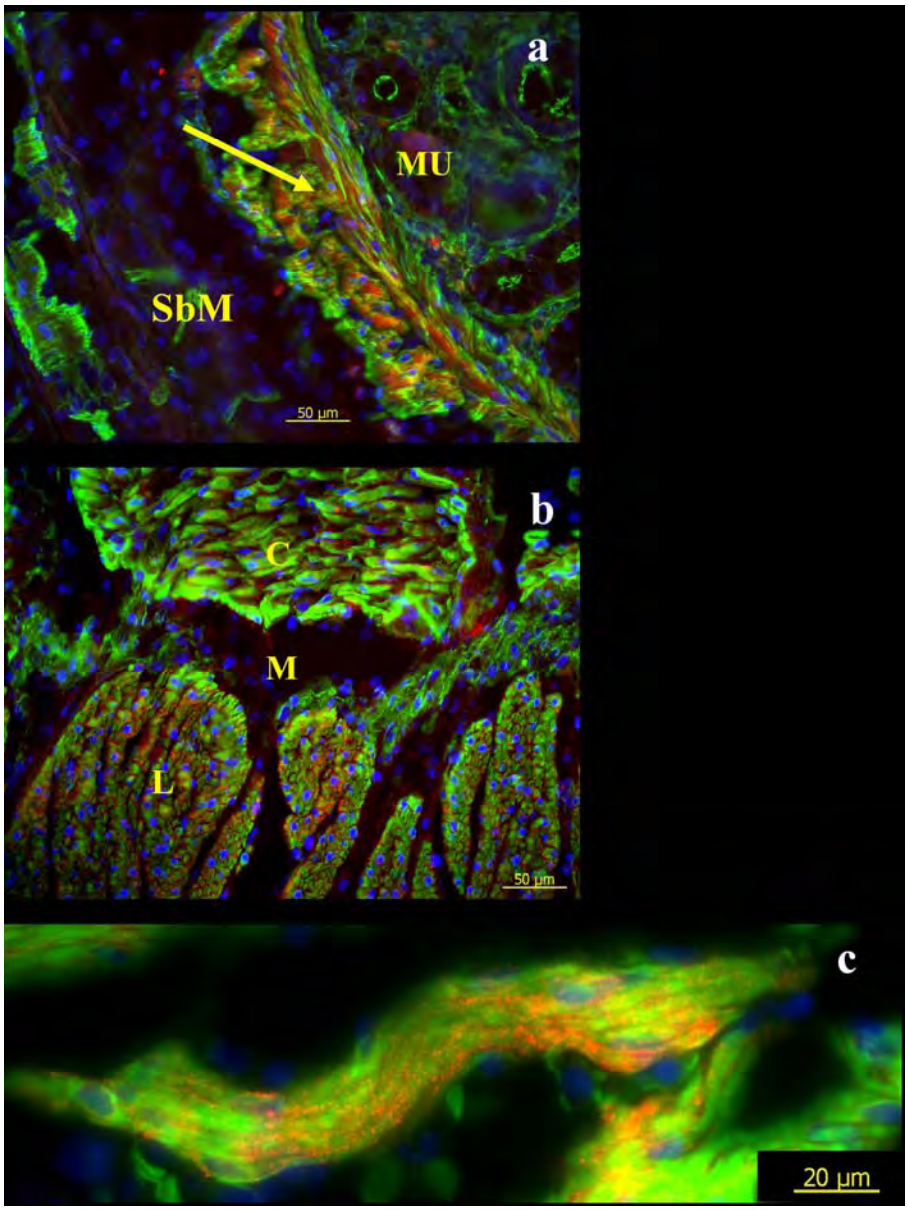

Figure 4.III.6 a,b,c: 5-HT_{1A} immunoreactivity-Alexa 488-Phalloidin co-staining present in jejunal muscularis mucosae (arrow) (a) and longitudinal and circular smooth muscle layer of equine jejunum (b). Red=5HT_{1A} immunoreactivity; green=Alexa-488-Phalloidin, blue=Hoechst. (c) detail of co-staining of smooth muscle cells (green) and 5-HT_{1A} receptor (red). (MU=mucosa; SbM=submucosa; C: circular muscle; L: longitudinal muscle.

The 5-HT_{1A}-receptor seems more abundant in circular compared to longitudinal muscle layer and seems absent in myenteric and submucosal plexuses. In addition, 5-HT_{1A}-receptor immunoreactivity is also observed in muscularis mucosae (Figure 4.III.6 a) and smooth muscle cells located in the mucosal villi (Figure 4.III.7 a,b).

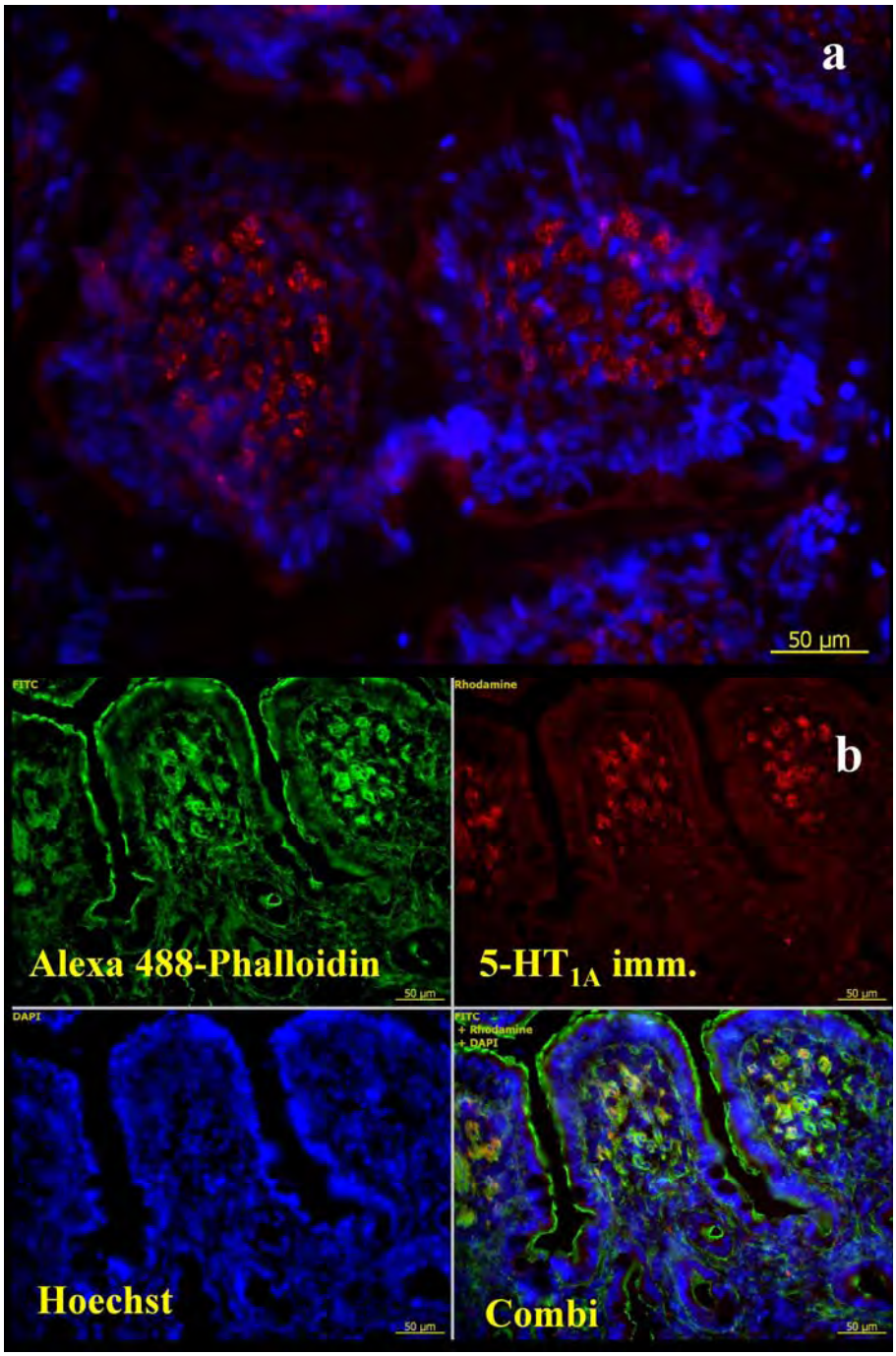

Figure 4.III.7 a,b: 5-HT_{1A} immunoreactivity in enteric mucosal villi (a). b. Detail of a mucosal villus. Left upper panel: Alexa 488-Phalloidin staining; right upper panel: 5-HT_{1A} immunostaining; Left lower panel: Hoechst staining (cell nuclei); Right lower panel: superposition of stainings. Note the co-localization of Phalloidin and 5-HT_{1A} immunoreactivity.

4.III.3. DISCUSSION

4.III.4. DISCUSSION

The spontaneous baseline phasic contractile activity shown by the circular smooth muscle strips of the equine mid-jejunum had a lower frequency and more irregular amplitude than seen in the longitudinal smooth muscle strips of the same region (Koehne 2000; Delesalle *et al.* 2006). 5-HT induces a tonic contractile answer, also comparable with the contractile answer seen in the longitudinal muscle layer of the same region, though more irregularly shaped (Delesalle *et al.* 2006). Relaxation by 5-HT, secondary to activation of inhibitory neurons releasing NO, has been demonstrated in the guinea-pig colon ascendens and stomach (Briejer *et al.* 1992; Meulemans *et al.* 1993). As relaxant nitrergic neurotransmission has also been shown in the equine jejunum (Rakestraw *et al.* 1996), we investigated the effect of the NO-synthase inhibitor L-NAME. However, its lack of effect in our study, together with the non-effect of TTX indicates that such a mechanism is not counteracting the 5-HT-induced contraction in equine mid-jejunum.

The non-effect of TTX and atropine on the tonic contractions with 5-HT, suggests interaction of 5-HT with a muscular receptor. Possible activation of the 5-$HT_{1B/1D}$, 5-HT_{2A}, 5-HT_3, 5-HT_4 or 5-HT_7 receptor was excluded based on the lack of effect of either of the corresponding specific 5-HT receptor antagonists. Only the 5-HT_{1A} receptor antagonists NAN 190 and WAY 100635, and the 5-$HT_{1,2,5,6,7}$ receptor antagonist methysergide elicited a clearcut inhibitory effect on the 5-HT-induced contractile response of equine jejunum circular smooth muscle. These results are in agreement with our findings in the equine jejunum longitudinal smooth muscle (Delesalle *et al*. 2006). The pK_B value determined for NAN 190

versus 5-HT in the actual study was 7.24 which is one log unit or more lower than the affinity at the 5-HT_{1A} receptor, reported for NAN 190 in the literature (Ahlers *et al.* 1992: pigeon brain, pK_B=8.12; Schoeffter *et al.* 1993: calf hippocampus, pK_B=8.70). This difference might at least be partially due to the protocol used, as concentration-response curves for 5-HT had to be constructed by adding one concentration of 5-HT to 8 different muscle strips, yielding a more capricious shape of the concentration-response curve. When using this protocol to determine the pK_B of NAN 190 in our previous study on equine jejunal longitudinal muscle, we obtained pK_B values of 7.54-7.58; however, when testing the influence of increasing concentrations of NAN 190 versus a fixed concentration of 5-HT, a pK_B of 8.13 was obtained (Delesalle *et al.* 2006). The latter protocol could not be used in circular muscle as even at an interval of 60 min, contractions to a fixed concentration of 5-HT declined. The results with NAN 190 and WAY 100635 thus seem to point to an interaction of 5-HT with a 5-HT_{1A} receptor. This is corroborated by the results with the 5-$HT_{1A,7}$ receptor agonist 5-CT. 5-CT has highest affinity at 5-HT_{1A}, 5-HT_{5A} and 5-HT_7 receptors and about 10 fold less affinity at 5-HT_{1B} and 5-HT_{1D} receptors (Hoyer *et al.* 1994). 5-HT_{5A} receptors have not been described in the GI tract (Nelson 2004). 5-HT_7 receptors are present in the GI tract but they are positively coupled to adenylate cyclase and induce relaxation (Thomas & Hagan 2004; Janssen *et al*. 2004); the contractile effect of 5-CT was thus not influenced by the 5-HT_7 receptor antagonist SB 269970. The 5-HT_{1A} receptor antagonist WAY 100635 (0.3 µM) had a similar influence as versus 5-HT. When the calculated pEC_{50} values for the 5-HT and 5-CT-induced contractile response in the actual study are compared with those obtained before in longitudinal muscle of the equine mid jejunum (Delesalle *et al.* 2006), one can find a remarkable accordance, with 5-HT (pEC_{50} jejunum longitudinal 7.88 ± 0.07; pEC_{50} jejunum circular 7.64 ± 0.54) and 5-CT (pEC_{50} jejunum longitudinal 6.20 ± 0.13; pEC_{50} jejunum circular 5.89 ± 0.13) pEC_{50} values not even differing half a log unit between both muscle

layers. This corroborates that 5-HT interacts in both muscle layers with a similar muscular 5-HT_{1A}–like receptor. Indeed, obvious differences with a classic 5-HT_{1A} receptor were observed as the specific 5-HT_{1A} receptor agonist flesinoxan and the partial 5-HT_{1A} receptor agonist buspiron had no effect. Structural interspecies differences at the agonist binding sites of the 5-HT_{1A} receptor could be responsible for this observation. The 5-HT_{1A} receptor has only one antagonist binding site, whereas 5 different agonist binding sites have been identified (Raymond *et al.* 1999). Restricted mutations at the level of these binding sites could account for the agonist-linked observations in the horse. It has been reported that point mutations in the 5-HT_{1A} receptor sequence can lead to complete refractoriness towards 5-HT or the specific 5-HT_{1A} receptor agonist 8-OH DPAT (Ho *et al.* 1992; Chanda *et al.* 1993).

The finding of a GI muscular 5-HT_{1A} receptor is very exceptional, since this receptor is predominantly found in the central nervous system (Pazos & Palacios 1985; Moller *et al.* 2004). 5-HT_{1A} receptors are only occasionally described in the GI tract and when a GI localisation was identified, they reside in neuronal tissue where they mediate inhibitory functions (Lepard & Galigan, 2004; Buchheit & Buhl, 1994). But when located on smooth muscle cells, a 5-HT_{1A} receptor is expected to induce contraction as it is negatively coupled to adenylate cyclase (Raymond *et al.* 1999). The muscular localization of the 5-HT_{1A} receptor in the equine intestine is confirmed by our immunohistological study in which specific rabbit polyclonal anti-rat 5-HT_{1A} receptor antibodies stain the receptor at the same level as phalloidin, used to highlight the smooth muscle cell membranes, indeed in both the circular and longitudinal muscle layer. The gene localization and amino-acid sequence of the equine 5-HT_{1A} receptor was recently revealed and shows a 98% homology with the rat sequence (Momozawa *et al.*, 2006). This pronounced homology, together with the intense staining of the positive control tissue of rat hippocampus and the fact that the immunohistochemical staining was encountered in our samples at the exact localization as was expected based upon

the organ-bath studies, confirms the identification of an equine GI 5-HT_{1A} receptor. The finding of 5-HT_{1A} receptors at the level of the mucosal villi, opens interesting considerations. It could be, that besides small intestinal contraction, the 5-HT_{1A} receptor plays a role in villus motility. Still, the functional characteristics of the equine jejunal 5-HT_{1A} receptor are not fully similar to those of a classic 5-HT_{1A} receptor as the 5-HT_{1A} receptor agonists flesinoxan and buspiron had no effect (see above); it is thus preferable to indicate the receptor for now as a 5-HT_{1A}-like receptor.

Our observation of rapid fading of the 5-HT induced contractile response, in both intestinal muscle layers, upon cumulative administration of 5-HT, opens interesting considerations concerning the possible role of this receptor in the complex pathophysiology of ileus in colic horses. Rapid and persistent desensitization upon prolonged contact with an excessive amount of 5-HT, is a typical feature of 5-HT_{1A} receptors. In colic horses, several factors can serve as a possible source of 5-HT overload. Bailey *et al.* (2004), already suggested the translocation of bioactive amines, amongst which 5-HT, through the intestinal wall into the systemic circulation of horses. Since ischaemia promotes intestinal mucosal permeability, colic horses with compromised bowel would even be more prone to such an event (Vatistas *et al.* 2003). Another possible source are degranulating blood platelets, which are known to be an important body store for 5-HT. I.V. administration of *Escherichia Coli* lipopolysacharids is shown to cause a clear increase in plasma 5-HT levels in horses (Menzies-Gow *et al.* 2004). It would be interesting to investigate the 5-HT_{1A}-like receptor density in small intestine of colic horses suffering from several types of colic, such as strangulating and non-strangulating or fermentation colic, and to combine these immunohistological studies with determination of plasma 5-HT levels in these horses.

4.III.4. CONCLUSION

This study shows that 5-HT induces its contractile responses in the equine jejunum circular smooth muscle via a muscular 5-HT_{1A}-like receptor. Immunohistologically the receptor can be found at the level of the muscularis mucosae and both muscle layers. The maintained desensitization encountered during functional studies, is a striking feature of the studied receptor. Further research is needed to examine the possible role of this receptor in the pathophysiology of ileus in horses.

REFERENCES

AHLERS, S.T., WEISSMANN, B.A. & BARRETT, J.E. (1992). Antagonism studies with BMY-7378 and NAN 190 – effects on 8-hydroxy-2-(di-normal-propylamino)tetralin-induced increases in punished responding of pigeons. *J. Pharmacol. Exp. Ther.* **260**, 474-481.

BAILEY, S.R., MENZIES-GOW, N.J., MARR, C.M. & ELLIOTT, J. (2004). The effects of vasoactive amines found in the equine hindgut on digital blood flow in the normal horse. *Equine vet. J.* **36**, 267-272.

BRIEJER, M.R., AKKERMANS, L.M.A., MEULEMANS, A.L., LEFEBVRE, R.A. & SCHUURKES, J.A.J. (1992). Nitric oxide is involved in 5-HT-induced relaxations of the guinea-pig colon ascendens in vitro. *Br. J. Pharmacol.* **107**, 756-761.

BUCHHEIT, K.H. & BUHL, T. (1994). Stimulant effects of 5-hydroxytryptamine on guinea-pig stomach preparations in-vitro. *Eur. J. Pharmacol.* **262**, 91-97.

CAO, B.J. & RODGERS, R.J. (1997). Influence of 5-HT1A receptor antagonism on plus-maze behaviour in mice. II. WAY 100635, SDZ 216-525 and NAN-190. *Pharmacol. Biochem. Behav.* **58**, 593-603

CHANDA, P.K., MINCHIN, M.C., DAVIS, A.R., GREENBERG, L. & REILLY, Y. (1993). Identification of residues important for ligand binding to the human 5-hydroxytryptamine1A serotonin receptor. *J. Exp. Pharmacol. Ther.*, **43**, 516-520.

CORLETO, V.D., SEVERI, C., ROMANO, G., TATTOLI, I., WEBER, H.C., STRIDSBERG, M., RINDI, G, CAMPANINI, N., TOMASSONI, F., PAGOTTO, U., COY, D.H., JENSEN, R.T. & DELLE FAVE, G. (2006). Somatostatin receptor subtypes mediate contractility on human colonic smooth muscle cells. *Neurogastroenterol. Motil.* **18**, 217-225.

DELCO, M., NIETO, J., CRAIGMILL, A.L., STANLEY, S.D. & SNYDER, J.R. (2007). Pharmacokinetics and in vitro effects of tegaserod, a serotonin 5-hydroxytryptamine 4 (5-HT_4) receptor agonist with prokinetic activity in horses. *Veterinary Therapeutics*. **8**, 77-87.

DELESALLE, C., DEPREZ, P., SCHUURKES, J.A.J. & LEFEBVRE, R.A. Contractile effects of 5-hydroxytryptamine and 5-carboxamidotryptamine in the equine jejunum. (2006). *Br. J. Pharmacol.* **147**, 23-35.

EL YAZBI, A.F., SCHULZ, R. & DANIEL, E.E. (2007). Differential inhibitory control of circular and longitudinal smooth muscle layers of Balb/C mouse small intestine. *Autonomic Neuroscience Basic & Clinical*. **131**, 36-44.

GERSHON, M.D. & TACK, J. (2007). The serotonin signaling system: from basic understanding to drug development for functional GI disorders. *Gastroenterology*. **132**, 397-414.

GRIDER, J.R. (1998). Reciprocal activity of longitudinal and circular muscle during intestinal peristaltic reflex. *Am. J. Physiol. Gastrointest. Liver. Physiol*. **284**, 768-775.

HAGAN, J.J., PRICE, G.W., JEFFREY, P., DEEKS, N.J., STEAN, T., PIPER, D., SMITH, M.I., UPTON, N., MEDHURST, A.D., MIDDLEMISS, D.N., RILEY, G.J., LOVELL, P.J., BROMIDGE, S.M. & THOMAS, D.R. (2000). Characterization of SB-269970-A, a selective 5-HT7 receptor antagonist. *Br. J. Pharmacol.* **130**, 539-548.

HOYER, D., CLARKE, D.E., FOZARD, J.R., HARTIG, P. R., MARTIN, G. R., MYLECHARANE, E. J., SAXENA, P. R. & HUMPHREY, P. P. A. (1994). International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (Serotonin). *Pharmacol. Rev.* **46**, 157-203.

HO, B.Y., KARSCHIN, A., BRANCHEK, T., DAVIDSON, N. & LESTER, H.A. (1992). The role of conserved aspartate and serine residues in ligand binding and in function of the 5-HT_{1A}receptor: a site-directed mutagenesis study. *FEBS Lett.*, **312**, 259-262.

JANSSEN, P., PRINS, N.H., PEETERS, P.J., ZUIDEVELD, K.P., LEVEBVRE, R.A. (2004). 5-HT7 receptor efficacy distribution throughout the canine stomach. *Br. J. Pharmacol* **143**, 331-342.

JOHNSON, M.P., AUDIA, J.E., NISSEN, J.S. & NELSON, D.L. (1993). N(1)-substituted ergolines and tryptamines show species differences for the agonist-labeled 5-HT2 receptor. *Eur. J. Pharmacol.* **239**, 111-118.

KHAWAJA, X., EVANS, N., REILLY, Y., ENNIS, C. & MINCHIN, M.C.W. (1995). Characterization of the binding of (^{3}H)WAY-100635, a novel 5-hydroxytryptamine$_{1A}$ receptor antagonist, to rat brain. *J. Neorochem.* **64**, 2716-2726.

KOEHNE, E. (2000). Dissertation: Dünn-und Dickdarmmotilität des Pferdes. Tierärztliche Hocgschule, Hannover. 111-114.

LEPARD, K.J. & GALLIGAN, J.J. (2004). Presynaptic modulation of cholinergic and non-cholinergic fast synaptic transmission in the myenteric plexus of guinea pig ileum. *Neurogastroenterol. Motil.* **16**, 355-364.

MENZIES-GOW, N.J., BAILEY, S.R., KATZ, L.C., MARR, C.M. & ELLIOTT, J. (2004). Endotoxin-induced digital vasoconstriction in horses: associated changes in plasma concentrations of vasoconstrictor mediators. *Equine vet. J.* **36**, 273-278.

MEULEMANS, A.L., HELSEN, L.F. & SCHUURKES, J.A.J. (1993). The role of nitric oxide (NO) in 5-HT-induced relaxations of the guinea-pig stomach. *Naunyn-Schmiedebergh's Arch. Pharmacol.* **348**, 424-430.

MOLLER, M., CUMMING, P., ANDERSEN, G. & GJEDDE, A. (2004). Parametric mapping of serotonin 5HT1A receptors in healthy human brain. *Neuroimage*. **22**, T157-T157.

MOMOZAWA, Y., TAKEUCHI, Y., TOZAKI, T., KIKUSUI, T., HASEGAWA, T., RAUDSEPP, T., CHOWDHARY, B.P., KUSUNOSE, R., MORI, Y. (2006). SNP detection and radiation hybrid mapping in horses of nine candidate genes for temperament. *Animal Genetics*, **38**, 81-91.

NELSON, D.L. (2004). 5-HT_5 receptors. *Current Drug Targets-CNS & Neurological Disorders* **3**, 53-58.

NIETO, J.E., SNYDER, J.R., KOLLIAS-BAKER, C. & STANLEY, S. (2000) In vitro effects of 5-hydroxytryptamine and cisapride on the circular smooth muscle of the jejunum of horses. *Am. J. Vet. Res.* **61**, 1561-1565.

PATEL, T.D. & ZHOU, F.C. (2005). Ontogeny of 5-HT1A receptor expression in the developing hippocampus. *Brain. Res. Dev. Brain. Res.* **9**, 42-57.

PAZOS, A. & PALACIOS, J.M. (1985) Quantitative autoradiographic mapping of serotonin receptors in the rat brain. *Brain Res.* **346**, 205-230.
Rakestraw, P.C., Snyder, J.R., Woliner, M.J., Sanders, K.M. and Shuttleworth, W.R. (1996). Involvement of nitric oxide in inhibitory neuromuscular transmission in equine jejunum. *AJVR*. **57**, 1206-1213.

RAYMOND, J.R., MUKHIN, Y.V., THOMAS, W.G. & GARNOVSKAYA, M.N. (1999). The recombinant 5-HT_{1A} receptor: G protein coupling and signalling pathways. *Br. J. Pharmacol.* **127**, 1751-1764.

SAIGAL, N., PICHIKA, R., EASWARAMOORTHY, B., COLLINS, D., CHRISTIAN, B.T., SHI, B., NARAYANAN, T.K., POTKIN, S.G. & MUKHERJEE, J. (2006). Synthesis and biologic evaluation of a novel serotonin 5-HT1A receptor radioligand, 18F-labeled mefway, in rodents and imaging by PET in a nonhuman primate. *J. Nucl. Med.* **47**, 1697-706.

SANGER, G. & NELSON, D.R. (1989). Selective and functional 5-hydroxytryptamine 3 receptor antagonism by BRL 43694 (granisetron). *Eur. J. Pharmacol.* **159**, 113-124.

SCHOEFFTER, P., FOZARD, J.R., STOLL, A., SIEGL, H., SEILER, M. & HOYER, D. (1993). SDZ 216-525, a selective and potent 5-HT_{1A} receptor antagonist. *Eur. J. Pharmacol.* **244**, 251-257.

TERRON, J.A. (1996) GR 127935 is a potent antagonist of the 5-HT1-like receptor mediating contraction in the canine coronary artery. *Eur. J. Pharmacol.* **300**, 109-112.

THOMAS, D.R. & HAGAN, J.J. 5-HT7 Receptors. *Current Drug Targets-CNS & Neurological Disorders* **3**, 81-90.

VATISTAS, N.J., NIETO, J.E., VAN HOOGMOED, L., GARDNER, I. & SNYDER, J.R. (2003). Use of an isolated intestinal circuit to evaluate the effect of ischemia and reperfusion on mucosal permeability of the equine jejunum. *Vet. Surg.* **32**, 52-61.

WEISS, R., ABEL, D., SCHOLTYSIK, G., STRAUB, R. & MEVISSEN, M. (2002). 5-Hydroxytryptamine mediated contractions in isolated preparations of equine ileum and pelvic flexure: pharmacological characterization of a new 5-HT_4 agonist. *J. Vet. Pharmacol. Ther.* **25**, 49-58.

CHAPTER 5

GENERAL DISCUSSION & CONCLUSION

Recent studies indicate that the prevalence of ileus amongst colic horses taken to surgery for abdominal exploration, ranges from 10% to over 40%, depending upon the study population and the criteria for defining cases of ileus (Blikslager *et al.*, 1994; Freeman *et al.*, 2000; Roussel *et al.*, 2001; French *et al.*, 2002; Proudman *et al.*, 2002; Cohen *et al.*, 2004; Mair and Smith, 2005). Ileus is the syndrome of functional inhibition of propulsive bowel motility, most commonly arising in the immediate postoperative period after laparotomy (Turnage *et al.*, 1998). In horses the manifestation of proximal GI reflux is the hallmark of ileus. Its presence implicates the actual occurrence of important fluid shifts in the horse's body, often aggravated by the devastating effects of endotoxemia. Not surprisingly, ileus is a highly fatal pathological condition in the horse. In a retrospective study of 259 horses that had undergone abdominal surgery for colic, postoperative ileus accounted for 43% of the 84 postoperative deaths (Hunt *et al.*, 1986). There are two important categories of factors that place horses at risk of ileus: those related to the degree of circulatory shock, and those related to the extent and location of the intestinal damage. Several studies confirm that an elevated heart rate and packed cell volume in the preoperative period place horses at risk of postoperative ileus (Edwards and Hunt, 1985; Gerring and Hunt, 1986; King and Gerring, 1989; King and Gerring, 1991; Blikslager *et al.*, 1994). Also the nature and degree of the intestinal lesion plays a key role. Most studies, investigating the ileus-triggering effect of several factors, through use of multiple logistic regression, come to the main conclusion that the more severely damaged the small intestine is, the less likely the GI system is to function normally after surgery. Need for extensive peroperative bowel manipulation, occurrence of reperfusion injury, resorption of endotoxins through the wall of compromised bowel: each factor pulls its weight on the process of ileus. Since we depend upon the referring veterinarian for the state in which a colic patient is admitted to the clinic, strategies have to be developed aiming at the timely identification of patients at risk for developing postoperative ileus. In that

way, the horse owner can be served with a better prognosis estimation, before important financial decisions are made. The aim of this thesis was to investigate 1) the value of ionized calcium in blood and lactic acid levels in blood and peritoneal fluid as prognostic factors in colic horses and 2) the possible role of 5-HT in the pathogenesis of ileus.

The value of ionized calcium levels in blood and lactic acid levels in blood and peritoneal fluid as prognostic factors in colic horses

Our results indicate that both ionized calcium levels in blood and lactic acid levels in blood and peritoneal fluid can help us to assess the tendency of a colic case to develop ileus. 88% of all studied colic patients showed blood ionized Ca^{2+} levels below the reference range at the time of admission. The Odds for developing ileus during hospitalization were ± 11.94 times larger for horses in the "very low" calcemia interval, in comparison with normocalcemic horses. The Odds for fatal outcome were ± 9.82 times larger for horses in the "very low" calcemia interval. Ca^{2+} substitution increased the probability of survival, provided that Ca^{2+} levels could be normalized. The lack of an upward calcemia response, despite repetitive Ca^{2+} substitutions, can be considered as a poor ominous sign.

Multivariable analysis demonstrated that PCV and the need for intestinal resection are independently associated with the blood plasma lactic acid level and the pulse, PCV, venous pO_2, presence of necrotic intestine, an increased amount of peritoneal fluid and total protein content are independently associated with peritoneal fluid lactic acid level. Per 1 mmol/l increase of the blood plasma lactic acid or peritoneal fluid lactic acid levels, the respective Odds ratios for the need of abdominal surgery increased with 1.23 (blood plasma lactic acid (BPL)) and 1.58 (peritoneal fluid lactic acid (PFL)), those for the need of an intestinal resection increased with 1.20 (BPL) and 1.41 (PFL), and those for developing ileus increased with 1.33 (BPL) and 1.36 (PFL). PFL levels of 1, 6, 12 and 16 mmol/l corresponded to a

probability of death of 11%, 29%, 63% and 82%, respectively, in horses without a strangulating obstruction and of 25%, 52%, 82% and 92%, respectively, in horses with strangulating obstruction. Apparently, the peritoneal fluid lactic acid level is even more useful and sensitive than the blood plasma lactic acid level for prognostic purposes in colic patients.

Beside blood ionized Ca^{2+} levels and blood and peritoneal fluid lactic acid levels, many other parameters such as TNFα, IL-6, endotoxin, D-dimer and alkaline phosphatase activity have already been identified in the past, as significantly related with a propensity to develop ileus (Barton *et al.*, 1995; Sandholm *et al.*, 1995; Horney, 2005). It is true that use of each of these values on its own does not provide an accurate prognosis estimation. As a clinician we need to have an overview of the full clinical picture and it is imperative to place laboratory results in that context. To try and improve the accuracy of assessment of prognosis of individual cases of colic, several investigations have attempted to use a combination of several of these ileus-predicting laboratory values and put them into complex formulas or scoring systems to predict the Odds of survival (Reeves *et al.*, 1990; Furr *et al.*, 1995; Proudman *et al.*, 2002). However, none of these formulas provide a clear-cut separation between survivors and non-survivors. So, none of these formulas can predict exactly which horse will live and which one will develop ileus or eventually die. They have to be viewed as ancillary tools in the clinical judgement of a colic case. Nonetheless, identification of individual "predictive" parameters, also helps unravelling the complex pathophysiology of ileus in horses. The results of the ionized Ca^{2+} study open interesting considerations. Ca^{2+} is an important ion for enteral motility (De Ponti *et al.*, 1993; Bushinsky *et al.*, 1998). According to our results, colic horses with low ionized Ca^{2+} levels in blood are more prone to develop ileus and correction of these values seems rewarding, at least in some cases. So called non-responders (horses remaining with low ionized Ca^{2+} levels, despite substitution) have to be viewed as high risk ileus patients, often with fatal outcome. Similar results are reported in

human ICU patients with septic shock and are attributed to systemic inflammatory response syndrome (SIRS) and multiple organ dysfunction syndrome (MODS) (Carlstedt *et al.*, 1998; Ward *et al.*, 2004). Therefore the results of our study underline once more the importance of endotoxemia in the ileus cascade. The study also highlights that production of GI reflux coincides with a further decline in blood ionized Ca^{2+} levels. Hence the importance of regular monitoring of blood ionized Ca^{2+} levels in refluxing horses. In future research, it would be interesting to determine ionized Ca^{2+} levels in reflux samples of colic horses. Likewise, it would be interesting to determine ionized Ca^{2+} levels in peritoneal fluid of colic horses, to check for the possibility of Ca^{2+} sequestration into abdominal fluid as an additional cause of low blood ionized Ca^{2+} levels in colic horses. Such mechanism has been described in pigs with endotoxemia (Carlstedt *et al.*, 2000). Also, the fact that production of GI reflux aggravates hypocalcemia, invites to investigate the pharmacological inhibition of the exocrine pancreas function of the horse, in order to decrease the amount of fluid that can reflux. The study group of Pierzynowski *et al.* (2007), demonstrated that pancreatic juice, bile and bile salts infused into the ileum, inhibited exocrine pancreas function in pigs. This is referred to as "the ileal brake". It would be interesting to evaluate whether this applies for the horse as well, and whether the receptors responsible for this inhibitory mechanism can be modulated medically. This could be performed by peroperative intraluminal infusion of certain compounds.

The conclusions that were drawn from the results of the lactic acid study, follow the same line of thoughts as the Ca^{2+} study. Namely: the degree of circulatory shock, endotoxemia and bowel ischemia are important predisposing factors for development of postoperative ileus. However, the measurement of lactic acid levels, both in blood and peritoneal fluid can be done outdoors, as has been confirmed in our study, in which the suitability of a hand-held lactic acid analyzer was evaluated for this purpose. In this way, the

equine practitioner in the field is provided with an extra, easy to apply analysis method, to provide horse owners with more accurate prognosis estimations of colic cases. Interestingly, the results of our study confirm results of human studies in which abdominal fluid lactic acid levels of ICU patients provide early and more accurate information on splanchnic ischemia than blood lactic acid levels. Hence the continuous direct intra-abdominal on-line monitoring of peritoneal fluid lactate levels in these patients, by means of microdialysis catheters, for the early detection of visceral ischemia as a predictive sign of shock and multi-organ failure (Jansson *et al.*, 2003; Sommer *et al.*, 2004). It would be interesting to deploy such a device in the research into the pathophysiology of ileus in horses. Questions such as at which point does small intestinal luminal distension, but also onset of endotoxemia, affects intestinal blood flow and oxygen uptake, could be investigated.

Role of 5-HT in the pathogenesis of postoperative ileus.

If analysis of 5-HT levels in blood and peritoneal fluid would not be so time consuming and would not require expensive and highly specific analysis techniques, this determination in blood and/or peritoneal fluid could probably also be used as a prognostic aid. Our study demonstrates that high 5-HT levels can be found in blood and peritoneal fluid of colic horses with compromised bowel, which are prone to develop ileus. Because 5-HT levels were also slightly elevated in some colic horses that responded well to conservative treatment, without concurrent elevation of any of the other factors needed to identify Disseminated Intravascular Coagulation (DIC) and endotoxemia, the determination of plasma 5-HT levels could be a useful tool to diagnose pre-clinical DIC in a very sensitive fashion. More research is needed to evaluate this.

5-HT is an important signalling molecule in the GI tract. Because 5-HT is such an important contributor to normal and abnormal function of the GI tract, there has been a

significant amount of work performed in human medicine to unravel its role in both physiological and pathological conditions of GI motility. This has led to the development of several human prokinetic agents, with proven therapeutic efficacy, both in experimental and clinical settings (Kindt *et al.*, 2007). Unfortunately, their success rate in equine ileus patients is low and their use is predominantly a costly business (Smith et al, 2005; Koenig *et al.*, 2006). A solid explanation for these disappointing results cannot be provided since very little research on serotonin has been performed in horses. The results of our study are interesting because they show that the 5-HT_3 and 5-HT_4 receptor subtypes, the predominant serotonergic receptor subtypes involved in the regulation of human GI motility and targeted by several human anti-emetic and prokinetic agents, were not found in the equine small intestine. Therefore one can argue on the use of expensive human prokinetic agents, targeting 5-HT_3 and 5-HT_4 receptors, in horses suffering from ileus. It must however be mentioned that our study protocol does not allow to completely rule out the presence of 5-HT_4 receptors in equine small intestine. The prokinetic effect of 5-HT_4 receptor agonists in humans is related to activation of 5-HT_4 receptors on cholinergic nerve endings, increasing the release of acetylcholine upon cholinergic nerve activation. To observe these receptors in vitro, cholinergic contractions must be induced by electrical field stimulation of the cholinergic nerve endings; these contractions are enhanced by agents acting at 5-HT_4 receptors if the latter are present on the cholinergic nerves under study (Talley, 2001; Kindt *et al.*, 2007). Our study demonstrates that the 5-HT-induced contractile response in both smooth muscle layers of the equine small intestine can be inhibited by the specific 5-HT_{1A} receptor antagonists NAN 190 and WAY 100635. It was shown that the calculated pK_b values for NAN 190 in both circular and longitudinal muscle correspond with those published earlier in literature for NAN 190 at 5-HT_{1A} receptors. However, the non-effect of some specific 5-HT_{1A} receptor agonists, have led us to classify the identified receptor as a 5-HT_{1A} like receptor. Indeed, of all

tested specific 5-HT_{1A} receptor agonists, only 5-carboxamidotryptamine (5-CT) elicited a clear-cut contractile response, comparable with the 5-HT-induced contraction. It was proposed that structural differences between classic and equine 5-HT_{1A} receptors could be responsible for this observation. Apparently even very restricted mutations can change drastically the affinity of agonists at the binding sites of the 5-HT_{1A} receptor (Guan *et al.* 1992; Ho *et al.,* 1992; Chanda *et al.,* 1993; Raymond *et al.,* 1999).

Recently, a Japanese study group has unravelled the protein sequence of the equine 5-HT_{1A} receptor (Momozawa *et al.,* 2006). Apparently there is a 98 % homology with the human 5-HT_{1A} receptor, which is even higher than the reported homology of 88% between human and rat 5-HT_{1A} receptors (Raymond *et al.,* 1999). This, together with the results of our immunohistochemistry study, supports that it is a 5-HT_{1A} receptor that has been identified in our functional in vitro experiments as the contractile serotonergic receptor in both smooth muscle layers of the equine small intestine. The immunohistochemical results are in complete accordance with the in vitro studies, localizing the equine 5-HT_{1A} receptors on enteric smooth muscle cells. In both functional in vitro studies, the identified 5-HT_{1A} receptor reacts with rapid desensitization upon cumulative administration of 5-HT and 5-CT. This is not surprising, since tachyphylaxia is a well known typical feature of 5-HT_{1A} receptors (Raymond *et al.,* 1999; Serres *et al.,* 2000; Hensler & Durgam, 2001). It has to be viewed as a natural defence mechanism of 5-HT_{1A} receptors against protracted and excessive receptor activation in the presence of an overload of 5-HT. Our finding that important amounts of 5-HT can be found in the plasma of horses with compromised bowel, opens interesting considerations on the possible role of 5-HT in causing intestinal hypomotility in colic horses. One can expect also other than solely GI related effects, such as cardiovascular or even psychosomatic effects from these elevated plasma 5-HT levels. Bailey and his co-workers already demonstrated seasonal changes in plasma concentrations of cecum-derived amines, amongst which 5-HT, in

clinically normal ponies and ponies predisposed to laminitis (Bailey *et al.* 2003a). They suggest that the release of amines from the cecum into the systemic circulation may contribute to hemodynamic disturbances in horses and ponies with acute laminitis (Menzies-Gow *et al.* 2004; Elliott and Bailey, 2006). In humans, elevated plasma 5-HT levels in carcinoid syndrome lead to cutaneous flushing, bronchospasms and diarrhoea (Saslow *et al.*, 1997). Cisplatinum-induced release of 5-HT in patients undergoing chemotherapy, induces profound nausea and vomiting (Castejon *et al.*, 1999). Increased plasma 5-HT levels and increased urine excretion of the 5-HT metabolite, 5-hydroxy indole acetic acid (5-HIAA), are found in patients with coeliac disease (Challacombe *et al.*, 1972; Sjolund *et al.*, 1985; Coleman *et al.*, 2006). Diarrhea predominant irritable bowel syndrome patients are reported to have elevated concentrations of plasma 5-HT under fasting and fed conditions compared with controls. Likewise, they exhibit increased sigmoid-colonic motility under fasting and fed conditions compared with controls (Houghton *et al.*, 2003; Houghton *et al.*, 2007). Platelet stored 5-HT concentrations were not increased in these patients, suggesting a concomitant disturbance in SERT (serotonin transporter) dependent platelet uptake of free plasma 5-HT (Bellini *et al.*, 2003; Houghton *et al.*, 2003; Dunlop *et al.*, 2005). This serotonin uptake by platelets is, besides breakdown of 5-HT in lung and liver, the most important pathway to control plasma 5-HT levels. Platelet 5-HT levels and urinary levels of 5-hydroxy indole acetic acid (5-HIAA) or 5-hydroxytryptophol (5-HTOL), two important 5-HT breakdown products, were not determined in our study on colic horses with compromised bowel. It would be interesting to do so and to get more insight into 5-HT metabolism in these horses.

Through comparison of plasma and peritoneal fluid 5-HT levels and through determination of D-dimere, and ß-thromboglobulin on PF4 ratio, we identified the blood platelets as an important source of increased systemic 5-HT release in colic horses. It would be interesting to investigate the effect of platelet stabilizing treatments on plasma 5-HT levels

and on outcome in colic horses. Platelet stabilizing treatments are rewarding in patients with Crohn's disease and IBS (Carty *et al.,* 2000). Compounds such as acetyl salicylic acid, aminosalicylates, clopidogrel and ticlopidine are currently used as antiplatelet therapy in human patients (Troxler *et al.,* 2007). Use of Low Molecular Weight heparin or fractionated heparin in colic horses is shown to be beneficial in the prophylaxis of coagulation-linked disorders such as Disseminated intravascular coagulation (DIC) and laminitis (Feige *et al.,* 2003). Because increased 5-HT levels could be detected in the plasma and peritoneal fluid of several colic horses, even before clinical manifestation of DIC, platelet stabilizing agents should probably be given early in the disease process to prevent a systemic 5-HT overload. However, similar to human medicine, administering anticoagulants in the perioperative period, also brings horses at risk for sudden drops in PCV, hemoglobin concentration, RBC count and can result in an increased propensity for bleeding. In this era of cardiovascular diseases, a lot of effort has been done in the recent years to develop new platelet stabilizing drugs and anticoagulants with reduced bleeding induction. (Stone *et al.,* 2007). Maybe this opens new opportunities for equine medicine as well.

Conclusion

Much progress has been made in reducing the development and duration of postoperative ileus in humans (Person & Wexner, 2006). In horses however, knowledge about the pathophysiology of ileus still seems to be in its infancy. We have learned a lot in the past from human disorders to inform research in equine GI motility. However, there is still an ocean of truth to be discovered. Obviously, we have to leave the habit of blind extrapolation of human research results to horse pathology. Turning thoughts around, the horse, being so sensitive to develop ileus and Disseminated intravascular coagulation (DIC), could deliver interesting information for human GI motility disorders and platelet-stabilizing therapy.

Although irritable bowel syndrome, currently a hot topic in human GI research, has not been documented in the horse, its existence in the horse seems plausible as has been suggested recently by Hudson *et al.* (2005). Considering the large number of equine colic and chronic diarrhoea cases that remain unelucidated, this proposition probably is not totally unfounded. We should also leave the idea that ileus is practically untreatable in horses. Similar to human medicine, optimization of peroperative intensive care protocols and anesthesia, surgical and recovery techniques will render the ileus problem less fatal in horses. It is clear that education towards the equine practitioner in the field, should emphasise the importance of early referral of colic cases. Still, we are desperately in need for efficacious GI motility stimulating drugs in horses. As always, it will take time, money and effort to unravel the secrets of the equine gut. But with enough researchers, passionate about the problem, and with joined forces no doubt we will get there. Aude audenda.

REFERENCES

BAILEY, S.R., KATZ, L.M., BERHANE, Y., SAMUELS, T., DE BRAUVERE, N., MARR, C.M. & ELLIOTT, J. (2003a). Seasonal changes in plasma concentrations of cecum-derived amines in clinically normal ponies and ponies predisposed to laminitis. *Am. J. Vet. Res*. **64**, 1132-1138.

BARTON, M.H. & COLLATOS, M.C. (1999). Tumor necrosis factor and interleukin-6 activity and endotoxin concentration in peritoneal fluid and blood of horses with acute abdominal disease. *J. Vet. Int. Med.* **13**, 457-464.

BELLINI, M., RAPPELLI, M., BLANDIZZI, C., COSTA, F., STASI, C., COLLUCI, L., GIANNACCHINI, G., MARAZZITI, D., BETTI, L., BARONI, S., MUMOLO, M.G., MARCHI, S. & DEL TACCA, M. (2003). Platelet serotonin transporter in patients with diarrhea-predominant irritable bowel syndrome both before and after treatment with alosetron. *Am. J. Gastroenterol.* **98**, 2705–2711.

BLIKSLAGER, A.T., BOWMAN, K.F., LEVINE, J.F. BRISTOL, D.G. & ROBERTS, M.C. (1994). Evaluation of factors associated with postoperative ileus in horses: 31 cases (1990-1992). *J. Am. Vet. Med. Assoc*. **205**, 1748-1752.

BUSHINSKY, D.A. & MONK, R.D. (1998). Calcium. *The Lancet* **352**, 306-311.

CARLSTEDT, F., LIND, L., RASTAD, J., STJERNSTROM, H., WIDE, L. & LJUNGHALL, S. (1998). Parathyroid hormone and ionized calcium levels are related to the severity of illness and survival in critically ill patients. *European Journal of Clinical Investigation* **28**, 898-903.

CARLSTEDT, F., ERIKSSON, M., KIISKI, R., LARSSON, A. & LIND, L. (2000). Hypocalcemia during porcine endotoxemic shock: effects of calcium administration. *Crit. Care. Med.* **28**, 2909-2914.

CARTY, E., MACEY, M. & RAMPTON, D.S. (2000). Inhibition of platelet activation by 5-aminosalicylic acid in inflammatory bowel disease. *Aliment. Pharmacol. Ther.* **14**, 1169-1179.

CASTEJON, A.M., PAEZ, X., HERNANDEZ, L. & CUBEDDU, L.X. (1999). Use of intravenous microdialysis to monitor changes in serotonin release and metabolism induced by cisplatin in cancer patients: comparative effects of granisetron and ondansetron. *J Pharmacol Exp Ther* **291**, 960-6.

CHALLACOMBE, D.N., BROWN, G.A., BLACK, S.C. & STORRIE, M.H. (1972). Increased excretion of 5-hydroxyindoleacetic acid 55-HIAA) in urine of children with untreated coeliac disease. *Arch Dis Child* **47**, 442-445.

CHANDA, P.K., MINCHIN, M.C., DAVIS, A.R., GREENBERG, L. & REILLY, Y. (1993). Identification of residues important for ligand binding to the human 5-hydroxytryptamine1A serotonin receptor. *J. Exp. Pharmacol. Ther*., **43**, 516-520.

COHEN, N.D., LESTER, G.D., SANCHEZ, L.C., MERRITT, A.M. & ROUSSELL, A.J.Jr. (2004). Evaluation of risk factors associated with development of postoperative ileus in horses. J Am Vet Med Assoc 2004; 225, 1070-1078.

COLEMAN, N.S., FOLEY, S., DUNLOP, S.P., WHEATCROFT, J., BLACKSHAW, E., PERKINS, A.C., SINGH, G., MARSDEN, C.A., HOLMES, G.K. & SPILLER, R.C. (2006). Abnormalities of serotonin metabolism and their relation to symptoms in untreated celiac disease. *Clin. Gastroenterol. Hepatol.* **4**, 874-881.

DE PONTI, F., GIARONI, C., COSENTINO, M., LECCHINI, S. & FRIGO, G. (1993). Calcium-channel blockers and gastrointestinal motility: basic and clinical aspects. *Pharmac. Ther.* **60**, 121-148.

DUNLOP, S.P., COLEMAN, N.S., BLACKSHAW, E., PERKINS, A.C., SINGH, G., MARSDEN, C.A. & SPILLER, R.C. (2005). Abnormalities of 5-hydroxytryptamine metabolism in irritable bowel syndrome. *Clin. Gastroenterol. Pathol.* **3**; 349-357.

EDWARDS, G.B. & HUNT, J.M. (1985). An analysis of the incidence of equine postoperative ileus (POI) and an assessment of the predisposing factors. *Proc. Eq. Colic. Res. Symp.* **2**, 307-312.

ELLIOTT, J. & BAILEY, S.R. (2006). Gastrointestinal derived factors are potential triggers for the development of acute equine laminitis. *The Journal of Nutrition* **136**, 2103S-2107S

FEIGE, K., SCHWARZWALD, C.C. & BOMBELI, T. (2003). Comparison of unfractioned and low molecular weight heparin for prophylaxis of coagulopathies in 52 horses with colic: a randomised double-blind clinical trial. *Eq. Vet. J.* **35**; 506-513.

FRENCH, N.P., SMITH, J., EDWARDS, G.B., PROUDMAN, C.J. (2002). Equine surgical colic: risk factors for postoperative complications. *Eq. Vet. J.* **34**, 444-449.

FREEMAN, D.E., HAMMOCK, P., BAKER, G.J., GOETZ, T., FOREMAN, J.H., SCHAEFFER, D.J., RICHTER, R.A., INOUE, O. & MAGID, J.H. (2000). Short –and long-term survival and prevalence of postoperative ileus after small intestinal surgery in the horse. *Equine Vet J Suppl*., 42-51.

FURR, M.O., LESSARD, P. & WHITE, N.A. (1995). Development of a colic severity score for predicting the outcome of equine colic. *Veterinary Surgery* **24**, 97-101.

GERRING, E.L. & HUNT, J.M. (1986). Pathophysiology of equine postoperative ileus: effect of adrenergic blockade, parasympathetic stimulation, and metoclopramide in an experimental model. *Equine Vet J.* **18**, 249-255.

GREENBERG, B.D., TOLLIVER, T.J., HUANG, S.J., LI, Q., BENGEL, D. & MURPHY, D.L.(1999). Genetic variation in the serotonin transporter promoter region affects serotonin uptake in human blood platelets. *Am. J. Med. Genet.* **88**, 83–87.

GUAN, X.M., PERTOUKA, S.J. & KOBILKA, B.K. (1992). Identification of a single amino acid residue responsible for the binding of a class of ß-adrenergic receptor antagonists to 5-hydroxytryptamine$_{1A}$ receptors. *Mol. Pharmacol.*, **41**, 695-698.

HENSLER, J.G. & DURGAM, H. (2001). Regulation of 5-HT1A receptor-stimulated [S-35]-GTP gamma S binding as measured by quantitative autoradiography following chronic agonist administration. *Br. J. Pharmacol.*, **132**, 605-611.

HORNEY, B. (2005). The diagnostics and prognostic value of alkaline phosphatase activity in serum and peritoneal fluid from horses with acute colic. *J. Vet. Int. Med.* **19**, 783-784.

HOUGHTON, L.A., ATKINSON, W., WHITAKER, R.P., WHORWELL, P.J. & RIMMER, M.J. (2003). Increased platelet depleted plasma 5-HT concentration following meal ingestion in symptomatic female subjects with diarrhoea predominant irritable bowel syndrome. *Gut* **52**, 663-670.

HOUGHTON, L.A., ATKINSON, W., LOCKHART, C., WHORWELL, P.J. & KEEVIL, B. (2007). Sigmoid-colonic motility in health and irritable bowel syndrome: a role for 5-hydroxytryptamine. *Neurogastroenterol. Motil.* **19**, 724-731.

HO, B.Y., KARSCHIN, A., BRANCHEK, T., DAVIDSON, N. & LESTER, H.A. (1992). The role of conserved aspartate and serine residues in ligand binding and in function of the 5-HT$_{1A}$receptor: a site-directed mutagenesis study. *FEBS Lett.*, **312**, 259-262.

HUDSON, N.P.H. & DUNLOP, M.G. (2005). Horses for courses. Comparative gastroenterology: common ground and collaborative potential. *British Medical Journal* **331**, 1248-1251.

HUNT, J.M., EDWARDS, G.B. & CLARKE, K.W. (1986). Incidence, diagnosis and treatment of postoperative complications in colic cases. *Equine Vet J* **18**, 264-270.

JANSSON, K., REDLER, B., TRUEDSSON, L., MAGNUSON, A., UNGERSTEDT, U. & NORGREN, L. (2003). Postoperative on-lin e monitoring with intraperitoneal microdialysis (IPM) detects early visceral ischemia and correlates to the current intraperitoneal cytokine response. *Critical Care* **7**, P196.

KINDT, S. & TACK, J. (2007). Mechanisms of serotonergic agents for treatment of gastrointestinal motility and functional bowel disorders. *Neurogastroenterol Motil* **19**, 32-39.

KING, J.N. & GERRING, E.L. (1989). Antagonism of endotoxin-induced disruption of bowel motility by flunixin and phenylbutazone. *Eq. Vet. J. Suppl.* **7**, 38-42.

KING, J.N. & GERRING, E.L. (1991). The action of low dose endotoxin on equine bowel motility. *Eq. Vet. J.* **23**, 11-17.

KOENIG, J. & COTE, N. (2006). Equine gastrointestinal motility--ileus and pharmacological modification. *Can. Vet. J.* **47**, 551-557.

LEMBO, A., ZAMAN, M., JONES, M. & TALLEY, N.J. (2007). Influence of genetics on irritable bowel syndrome, gastro-oesophageal reflux and dyspepsia: a twin study. *Aliment. Pharmacol. Ther.* **25**, 1343-1350.

MAIR, T.S. & SMITH, L.J. (2005). Survival and complication rates in 300 horses undergoing surgical treatment of colic. Part 4: Early (acute) relaparotomy. *Eq. Vet. J.* **37**, 315-318.

MENZIES-GOW, N.J., BAILEY, S.R. & KATZ, L.M. (2004). Endotoxin-induced digital vasoconstriction in horses: associated changes in plasma concentrations of vasoconstrictor mediators. *Equine. vet. J.* **36**, 273-278.

MOMOZAWA, Y., TAKEUCHI Y., TOZAKI, T., KIKUSUI, T., HASEGAWA, T., RAUDSEPP, T., CHOWDHARY, B.P., KUSUNOSE, R. & MORI, Y. (2006). SNP detection and radiation hybrid mapping in horses of nine candidate genes for temperament. *Animal Genetics*, **38**, 81-91.

PERSON, B. & WEXNER, S.D. (2006). In brief. Curr Probl Surg 43, 6-10.

PIERZYNOWSKI, S.G., SILEIKIENE, V., VALVERDE, P.J.L., SZYMANZYK, S., GREGORY, P.C., KRUSZEWSKA, D., MOSENTHIN, R., RZASA, A., KOWALIK, S., ZABIELSKI, R. & WESTROM, B. (2007). Ileal exposure to pig pancreatic juice and bile inhibit exocrine pancreatic secretion in pigs. *Livestock Science* **108**, 53-56.

PROUDMAN, C.J., SMITH, J.E., EDWARDS, G.B., FRENCH, N.P. (2002). Long-term survival of equine surgical colic cases. Part 2: modelling postoperative survival. *Equine Vet J.* **34**, 438-443.

SOMMER, T. & LARSEN, J.F. (2004). Intraperitoneal and intraluminal microdialysis in the detection of experimental regional intestinal ischemia. *Br J Surg* **91**, 855-861.
RAYMOND, J.R., MUKHIN, Y.V., THOMAS, W.G. & GARNOVSKAYA, M.N. (1999). The recombinant 5-HT_{1A} receptor: G protein coupling and signalling pathways. *Br. J. Pharmacol.*, **127**, 1751-1764.

REEVES, M.J., CURTIS, C.R., SALMAN, M.D., REIF, J.S. & STASHAK, T.S. (1990). A multivariable prognostic model for equine colic patients. *Preventive Veterinary Medicine* **9**, 241-257.

ROUSSEL, A.J.Jr., COHEN, N.D., HOOPER, R.N. & RAKESTRAW, P.C. (2001). Risk factors associated with development of postoperative ileus in horses. *J Am Vet Med Assoc* **219**, 72-78.

SANDHOLM, M., VIDOVIC, A., PUOUNEN-REINERT, A., SANKARI, S., NYHOLM, K. & RITA, H. (1995). D-dimer improves the prognostic value of combined clinical and laboratory data in equine gastrointestinal colic. *Acta. Vet. Scand.* **36**, 255-272.

SASLOW, S.B., O'BRIEN, M.D., CAMILLERI M, VON DER OHE, M., HOMBURGER, H.A., KLEE, G.G., PITOT, H.C. & RUBIN, J. (1997). Octreotide inhibition of flushing and colonic motor dysfunction in carcinoid syndrome. *Am J Gastroenterol* **92**, 2250-2256

SERRES, F., MUMA, N.A., RAAP, D.K., GARCIA, F., BATTAGLIA, G. & VAN DE KAR, L.D. (2000). Coadministration of 5-hydroxytryptamine (1A) antagonist WAY-100635 prevents fluoxetine-induced desensitization of postsynaptic 5-hydroxytryptamine (1A) receptors in hypothalamus. *J. Pharmacol. Exp. Ther.*, **294**, 296-301.

SIMPSON, J.W. (1998). Diet and large intestinal disease in dogs and cats. *J. Nutr.* **128**, 2717-2722.

SJOLUND, K. & NOBIN, A. (1985). Increased levels of plasma 5-hydroxytryptamine in patients with coeliac disease. *Scand J Gastroenterol* **20**, 304-308.

SMITH, M.A., EDWARDS, G.B., DALLAP, B.L., CRIPPS, P.J. & PROUDMAN, C.J. (2005). Evaluation of the clinical efficacy of prokinetic drugs in the management of post-operative ileus: can retrospective data help us? *Vet. J.* **170**, 230-236.

STONE, W.M., TONNESSEN, B.H. & MONEY, S.R. (2007). The new anticoagulants. *Perspect Vasc Surg Endovasc Ther* **19**, 332-335.

TALLEY, N.J. (2001). Serotoninergic neuroenteric modulators. *The Lancet*, **15**, 2061-2068.

TROXLER, M., DICKINSON, K. & HOMER-VANNIASINKAM, S. (2007). Platelet function and antiplatelet therapy. *Brit. J. Surg*. **94**, 674-682.

TURNAGE, R.H. & BERGEN, P.C. (1998). Intestinal obstruction and ileus. In: Feldman MD, Scharschmidt BC, Sleisenger MH, eds. Gastrointestinal and liver disease. Philadelphia: WB Saunders, 1799-810.

WARD, R.T., COLTON, D.M., MEADE, P.C., HENRY, J.C., CONTRERAS, L.M., WILSON, O.M. & FLEMING, A.W. (2004). Serum levels of calcium and albumin in survivors versus nonsurvivors after critical injury. *J. Crit. Care*. **19**, 54-64.

SUMMARY-SAMENVATTING

6.I. SUMMARY

Postoperative ileus is a notorious and highly fatal complication in horses that is predominantly seen after surgical intervention for small intestinal colic. Reported prevalences range from 10% to over 40%, depending upon the study population and the criteria for defining cases of ileus. The goals of postoperative treatment are maintenance of adequate hydration, correction of electrolyte imbalance, pain relief, control of infection and last but not least, restoration of normal intestinal propulsion. The latter however often poses a real therapeutic challenge. The use of human prokinetic agents in horses suffering from ileus has unfortunately little therapeutic success. This could be due to important differences in the human and equine enteric receptor population.

As a general introduction (Chapter 1.I) a review is given on the current knowledge of equine GI motility and the application of human prokinetic agents in equine medicine (Chapter 1.II). Furthermore, a review is given on the role of serotonin in GI motility, which receptors are targeted in the human GI system and what is known about the equine enteric serotonergic receptor population (Chapter 1.III).

In Chapter 2 the aims of the study are formulated. It was investigated whether ionized Ca^{2+} is an important ion for GI motility and can be used as a factor to prognosticate the propensity of a colic patient to develop postoperative ileus. Furthermore it was investigated whether correction of low ionized Ca^{2+} levels is rewarding and whether production of reflux represents a source of Ca^{2+} loss.

The use of blood and peritoneal fluid lactic acid levels for evaluation of intestinal ischemia and the risk for development of postoperative ileus in a colic horse was evaluated.

Also the suitability of Is a hand held lactic analyzer to determine peritoneal fluid lactic acid levels in horses was examined.

Our next aim was to investigate the role and source of increased plasma and peritoneal fluid 5-HT levels in colic horses with compromised bowel, to identify the contractile serotonergic receptor population in the equine small intestine, in order to evaluate the usefulness of human prokinetic agents targeting 5-HT_4 receptors to treat equine ileus.

Chapter 3 gives an overview of the investigation into prognostic factors to identify colic patients at risk for developing postoperative ileus. The results of the investigation on ionized Ca^{2+} are presented in Chapter 3.I. Low blood ionized Ca^{2+} levels were identified in 88% of all colic patients at the time of admission. Multivariable analysis revealed that the presence of reflux, signs of endotoxemia, an increased PCV, alkalinisation of pH and the interaction PCV/pH all predispose colic horses to low ionized Ca^{2+} levels at the time of admission. The Odds for developing ileus during hospitalization were ± 11.94 times larger for horses in the "very low" calcemia interval, in comparison with normocalcemic horses. The Odds for fatal outcome were ± 9.82 times larger for horses in the "very low" calcemia interval. Ca^{2+} substitution increased the probability of survival, provided that Ca^{2+} levels could be normalized. The lack of an upward calcemia response, despite repetitive Ca^{2+} substitutions, was found to be a poor prognostic sign. It was concluded that hypocalcaemia in colic horses is of prognostic relevance both with regard to survival as to the probability of development of ileus during hospitalization. The study results also showed the importance of routine measurement of ionized Ca^{2+} levels in colic horses and the beneficial effects of correction of hypocalcaemia.

The results of the investigation on use of blood and peritoneal fluid lactic acid levels as prognostic parameters are presented in Chapter 3.II. It was shown that intestinal

hypoperfusion can lead to increased lactate concentrations in plasma and peritoneal fluid in horses with colic. The Accusport® was reliable for determination of blood plasma lactate (BPL) concentrations < 13 mmol/l and peritoneal fluid lactate (PFL) concentrations < 20 mmol/l. Multivariate analysis indicated that PCV and need for intestinal resection were independently associated with the BPL and that pulse, PCV, venous pO_2, presence of necrotic intestine, increased amount of peritoneal fluid and fluid total protein content were independently associated with PFL. Per 1 mmol/l increase in BPL or PFL, the respective Odds ratios for the need of abdominal surgery increased to 1.23 (BPL) and 1.58 (PFL), Odds ratios for the need of an intestinal resection increased to 1.20 (BPL) and 1.41 (PFL), and Odds ratios for developing ileus increased by 1.33 (BPL) and 1.36 (PFL). PFL concentrations of 1, 6, 12 and 16 mmol/l corresponded to a probability of death of 11%, 29%, 63% and 82%, respectively in horses without strangulating obstruction and of 25%, 52%, 82% and 92%, respectively in horses with strangulating obstruction. It was concluded that PFL is more useful and sensitive than BPL for prognostic purposes in horses with colic.

In Chapter 4 the results of the in vivo and in vitro investigation on the role of serotonin (5-HT) in equine GI motility are presented.

Chapter 4.I describes the results of the determination of 5-HT levels in plasma and peritoneal fluid of colic horses with compromised bowel. In the body, serotonin (5-HT) is stored in blood platelets and in the enterochromaffin cells (EC cells) of the GI tract. Taking into consideration two important features which are predominantly found in colic horses predisposed to develop ileus, namely intestinal necrosis and endotoxemia, both platelets and enterochromaffin cells of necrotising bowel segments, could serve as a source of 5-HT overload in colic horses.

All colic cases with strangulating small intestinal lesions had plasma and peritoneal fluid 5-HT levels well above those found in healthy horses (p=0.006). Plasma β-TG/PF4 ratio, used as a marker to distinguish between in vivo and in vitro platelet degranulation, exceeded 2 in all cases, indicating in vivo platelet activation. 5-HT levels in peritoneal fluid of colic horses with compromised bowel were significantly lower than the corresponding plasma levels (p=0.005). Apparently in colic horses with compromised bowel, important amounts of 5-HT can be released into the systemic circulation, through massive release of platelet stored 5-HT upon platelet activation. 5-HT is a very potent pro-inflammatory, vasoconstrictive and immunomodulatory agent. In view of the rapid and prolonged tachyphylaxia, shown for the jejunal 5-HT_{1A}-like receptors (cfr. Infra), this increased systemic 5-HT release could play a role in the pathophysiology of ileus in horses.

In Chapter 4.II the in vitro characterization of the contractile serotonergic receptor population of the equine jejunum longitudinal smooth muscle is given. 5-HT induced tonic contractions with superimposed phasic activity. These responses were not influenced by tetrodotoxin and atropine, suggesting a non-neurogenic, non-cholinergic pathway. The 5-HT receptor antagonists GR 127935 (5-$HT_{1B,D}$), ketanserin (5-HT_{2A}), SB 204741 (5-HT_{2B}), RS 102221 (5-HT_{2C}), granisetron (5-HT_3), GR 113808 (5-HT_4) and SB 269970 (5-HT_7) had no influence on the 5-HT induced response; the 5-HT_{1A} receptor antagonists NAN 190 (pK_b=8.13 ± 0.06) and WAY 100635 (pK_b=8.69 ± 0.07), and the 5-$HT_{1,2,5,6,7}$ receptor antagonist methysergide concentration-dependently inhibited the 5-HT-induced contractile response. The 5-$HT_{1,7}$ receptor agonist 5-carboxamidotryptamine induced a contractile response similar to that of 5-HT; its effect was not influenced by tetrodotoxin and atropine, and SB 269970 but antagonized by WAY 100635. 8-OHDPAT, buspiron and flesinoxan, that are active at rat and human 5-HT_{1A} receptors had no contractile influence. These results

suggest that the contractile effect of 5-HT in equine jejunal longitudinal muscle is due to interaction with muscular 5-HT receptors, characterized as 5-HT_{1A} like. A similar in vitro characterization was performed in the equine jejunum circular smooth muscle, the results of which are presented in Chapter 4.III. Similar to the observations in the longitudinal smooth muscle layer, 5-HT and the specific 5-$HT_{1A,7}$ receptor agonist 5-CT induced tonic contractions. Tetrodotoxin, atropine and N^G-nitro-L-arginine did not modify this response. A set of 5-HT receptor subtype selective antagonists excluded interaction with 5-$HT_{1B,1D,2A,3,4}$ and 5-HT_7 receptors. Again, the selective 5-HT_{1A} receptor antagonists WAY 100635 and NAN 190 caused a clear rightward shift of the concentration-response curve to 5-HT and 5-CT, identifying the targeted 5-HT receptor as a 5-HT_{1A} like receptor. The results of both in vitro studies were confirmed immunohistochemically through use of specific rabbit polyclonal anti-5-HT_{1A} receptor antibodies. Numerous 5-HT_{1A} receptors, localized on smooth muscle cells, were identified in the t. submucosa, and both longitudinal and circular smooth muscle layers of the equine jejunum.

We can conclude that the lack of evidence for the presence of 5-HT_4 receptors in the equine small intestine further brings into question the use of human prokinetic drugs acting at 5-HT_4 receptors in horses with small intestinal ileus. The 5-HT_{1A} like receptor, identified as the main receptor responsible for the 5-HT induced contractile response of the equine small intestine, shows clear features of tachyphylaxia. The increased plasma and peritoneal fluid 5-HT levels that are found in colic horses with compromised bowel, open interesting considerations on that matter. More research is needed to further investigate the possible role of these 5-HT_{1A} receptors in the pathophysiology of ileus in horses.

6.II. SAMENVATTING

Chirurgie van de dunne darm wordt bij het koliekpaard beschouwd als één van de meest risicovolle ingrepen. Het beduidend lagere slaagpercentage ten opzichte van chirurgie van de dikke darm wordt vooral veroorzaakt door het frekwent optreden van vaak fataal verlopende postoperatieve ileus bij deze patiënten. Studies melden prevalenties van 10 tot meer dan 40%, afhankelijk van de studiepopulatie en de gebruikte criteria om ileus te definiëren. Tot op de dag van vandaag blijft de behandeling van postoperatieve ileus een echte uitdaging. Naast het corrigeren van verlies aan vocht en electrolyten en het bestrijden van pijn, ontsteking en infectie, tracht men vooral een herstel te bekomen van de normale darmmotiliteit. Dat laatste blijkt echter bij het paard tot op heden een quasi onmogelijke taak. Het is alom bekend dat behandeling van paarden met ileus met humane prokinetica geen garantie biedt op succes. Een van de mogelijke verklaringen daarvoor is dat de enterale receptorpopulatie die een belangrijke rol speelt in contracties van de menselijke darm in belangrijke mate verschilt van deze van het paard.

Als algemene inleiding (Hoofdstuk 1.I) wordt een overzicht gegeven van de huidige kennis over de mechanismen die verantwoordelijk zijn voor de gastro-intestinale motiliteit bij het paard en over het gebruik van humane prokinetica bij het paard (Hoofdstuk 1.II). In Hoofdstuk 1.III wordt de rol besproken die serotonine speelt in gastro-intestinale motiliteit, welke serotonerge receptoren belangrijk zijn voor de motiliteit van het humane maagdarm-stelsel en wat er tot op heden geweten is over de enterische serotonerge receptorpopulatie van het paard.

In Hoofdstuk 2 worden de doelstellingen van de respectievelijke studies geformuleerd. Kunnen bloedspiegels van geïoniseerd Ca^{2+} (iCa^{2+}) bij koliekpaarden gehanteerd worden als

parameters om ileus te voorspellen? Heeft het corrigeren van lage iCa^{2+} spiegels een gunstig effect? Gaat de produktie van reflux gepaard met een verlies van iCa^{2+}?

Kunnen de melkzuurspiegels in bloed en peritoneaal vocht van koliekpaarden gehanteerd worden als parameters om enerzijds darmischemie te evalueren en anderzijds het optreden van postoperatieve ileus te voorspellen? Kan de Accusport®, een draagbare melkzuurmeter van zakformaat, gebruikt worden als handig alternatief om op betrouwbare wijze melkzuurspiegels in buikvocht van paarden te meten?

Verder werd een antwoord gezocht op de vraag wat de rol en de bron is van gestegen serotoninespiegels in bloed en peritoneaal vocht van koliekpaarden. Tot slot werd een antwoord gezocht op de vraag welke serotonerge receptoren specifiek bij het paard een belangrijke rol spelen in contracties van de dunne darm.

In Hoofdstuk 3 worden de studies voorgesteld waarin de meting van iCa^{2+} in bloed en van melkzuur in bloed en buikvocht geëvalueerd worden als prognostische parameters voor het optreden van postoperatieve ileus bij koliekpaarden. In Hoofdstuk 3.I worden de resultaten van het onderzoek naar iCa^{2+} weergegeven. 88% van de bestudeerde koliekpatiënten hadden een iCa^{2}-bloedspiegel lager dan de referentielimiet. Via multivariabele statistische analyse werd aangetoond dat de aanwezigheid van reflux in de maag van het koliekpaard bij aankomst in de kliniek, tekenen van endotoxemie, een gestegen hematocriet, een alkalische pH in bloed, alsook de interactie PCV/pH allen geassocieerd zijn met een daling van het iCa^{2+}-gehalte in het bloed van koliekpaarden. Zo werd aangetoond dat koliekpaarden die worden aangeboden met een zeer laag iCa^{2+}-gehalte in het bloed (er werden in de studie 4 calcemie-intervallen gecreëerd: normaal, middelmatig, laag en zeer laag), maar liefst 11.94 maal meer kans maken om postoperatieve ileus te ontwikkelen, in vergelijking met normocalcemische koliekpaarden. De kans op een fatale afloop was 9.82 maal groter

voor koliekpaarden behorend tot het zeer laag calcemie-interval. Er werd ook aangetoond dat correctie van deze lage calcemiewaarden aanleiding geeft tot een grotere kans op overleven, op voorwaarde dat de calcemiespiegels blijvend konden gecorrigeerd worden. Bij sommige koliekpaarden kon namelijk, ondanks repetitieve Ca^{2}-substitutie, geen normalisatie van de spiegels bekomen worden. Bij de overgrote meerderheid van deze paarden was de afloop uiteindelijk fataal. Er kan dus gesteld worden dat iCa^{2+}-spiegels in bloed bij koliekpaarden kunnen gehanteerd worden om zowel de kans op overleven als de kans op ontwikkeling van postoperatieve ileus bij een koliekpaard te evalueren. Aangezien produktie van reflux bij ileus-paarden aanleiding geeft tot een steeds verder dalende calcemiespiegel, wordt een dagelijkse meting en, indien nodig, correctie van iCa^{2+}-spiegels bij koliekpaarden aangeraden.

In Hoofdstuk 3.II worden de resultaten weergegeven van het onderzoek naar melkzuur. Er werd aangetoond dat intestinale ischemie bij koliekpaarden aanleiding geeft tot een stijging van het melkzuurgehalte in achtereenvolgens het buikvocht en het bloed. De Accusport®, een handige melkzuurmeter van zakformaat, bleek geschikt om melkzuurgehalten te meten tot 13 mmol/l in bloed en tot 20 mmol/l in buikvocht. Door middel van multivariabele statistische analyse werd aangetoond dat de hematocriet en de noodzaak voor een intestinale resectie elk onafhankelijk geassocieerd waren met de melkzuurspiegels in bloed van koliekpaarden. Zowel de pols, de hematocriet, de veneuze $pO_{2,}$ aanwezigheid van genecrotiseerde darmsegmenten, een toegenomen hoeveelheid buikvocht, alsook het eiwitgehalte in het buikvocht, bleken elk onafhankelijk geassocieerd te zijn met het melkzuurgehalte in het buikvocht. Voor elke toename van het melkzuurgehalte in bloed of buikvocht met 1 mmol/l, stijgt de probabiliteit voor noodzaak van abdominale chirurgie met een factor 1,23 (bloed) en 1,58 (buikvocht), de probabiliteit voor noodzaak van uitvoering van een intestinale resectie met een factor 1,20 (bloed) en 1,41 (buikvocht), en de probabiliteit voor ontwikkeling van ileus met een factor 1,33 (bloed) en 1,36 (buikvocht). Een lactaatgehalte in buikvocht van

respectievelijk 1, 6, 12 en 16 mmol/l correspondeert met een probabiliteit op sterfte van respectievelijk 11%, 29%, 63% en 82% bij koliekpaarden zonder strangulerende obstructie en van respectievelijk 25%, 52%, 82% en 92%, bij paarden met een strangulerende obstructie. Er kan gesteld worden dat het gebruik van melkzuurgehalten in het buikvocht meer betrouwbaar is dan melkzuurgehalten in bloed voor prognostische doeleinden in verband met koliek.

Hoofdstuk 4 geeft een overzicht van de resultaten van de in vivo en in vitro studies die onderzoek verrichten naar de rol van serotonine (5-HT) in de gastro-intestinale motiliteit van het paard.

In Hoofdstuk 4.I werden 5-HT-spiegels bepaald in plasma en buikvocht van koliekpaarden met ischemische darmsegmenten. In het lichaam wordt 5-HT hoofdzakelijk opgeslagen in circulerende bloedplaatjes en in de enterochromaffiene cellen van het maagdarmkanaal. Aangezien zowel intestinale necrose als endotoxemie worden aangetroffen bij koliekpaarden die gepredisponeerd zijn voor ontwikkeling van ileus, kunnen zowel degranulerende bloedplaatjes, als enterochromaffiene cellen van necrotiserende darmsegmenten dienst doen als bron van overmatige systemische vrijstelling van 5-HT bij deze koliekpaarden.

De 5-HT-spiegels in buikvocht en plasma van koliekpaarden met strangulerende obstructies van de dunne darm waren allen beduidend hoger dan deze van gezonde paarden (p=0,006). De βTG/PF4 ratio in plasma, in deze studie gehanteerd als een merker om het onderscheid te maken tussen in vivo en in vitro bloedplaatjesdegranulatie, was groter dan 2 in al deze patiënten. Dit is een duidelijke indicatie dat een toegenomen in vivo degranulatie van bloedplaatjes plaatsgrijpt bij koliekpaarden met strangulerende obstructie van de dunne darm. De 5-HT-spiegels die werden aangetroffen in het buikvocht waren steeds significant lager dan deze in het bloed (p=0,005). Blijkbaar vindt bij koliekpaarden met strangulerende koliek, een

massale vrijstelling plaats van 5-HT, opgeslagen in circulerende degranulerende bloedplaatjes. 5-HT wordt beschouwd als een uiterst potente pro-inflammatoire, vasoconstrictieve en immunomodulatorische molecule. De verhoogde 5-HT-spiegels in plasma en buikvocht, die worden aangetroffen bij koliekpaarden gepredisponeerd tot ontwikkeling van ileus, zetten aan tot nadenken. Gedurende functionele in vitro studies werd al aangetoond dat de enterale 5-HT_{1A} receptor, die bij het paard een belangrijke rol speelt in contracties van de dunne darm, een snelle en langdurige tachyphylaxie vertoont wanneer deze langdurig in aanraking komt met verhoogde spiegels van 5-HT (cfr. Hoofdstuk 4.II). Het zou kunnen dat de gestegen 5-HT-spiegels in plasma en buikvocht een rol spelen in de pathofysiologie van ileus bij koliekpaarden.

In Hoofdstuk 4.II worden de resultaten weergegeven van de functionele in vitro studies op de longitudinale spierlaag van het jejunum van het paard. 5-HT induceert in deze spierlaag tonische contracties die gesuperponeerd worden op een spontane fasische activiteit. De contractiele antwoorden geïnduceerd door 5-HT konden niet beïnvloed worden door het zenuwgif tetrodotoxine en het parasympaticolyticum atropine, wat suggereert dat 5-HT zijn contractiele effecten medieert via niet-neurogene, niet cholinerge weg. De 5-HT receptor antagonisten GR 127935 (5-$HT_{1B,1D}$), ketanserin (5-HT_{2A}), SB 204741 (5-HT_{2B}), RS 102221 (5-HT_{2C}), granisetron (5-HT_3), GR 113808 (5-HT_4) en SB 269970 (5-HT_7) hadden geen invloed op de contractiele respons geïnduceerd door 5-HT. De 5-HT_{1A} receptor antagonisten NAN 190 (pK_b=8,13 ± 0,06) en WAY 100635 (pK_b=8,69 ± 0,07) en de 5-$HT_{1,2,5,6,7}$ receptor antagonist methysergide inhibeerden concentratie-afhankelijk de contractiele respons geïnduceerd door 5-HT. De 5-$HT_{1,7}$ receptor agonist 5-carboxamidotryptamine induceerde een contractiele respons die vergelijkbaar was met de contractiele respons op 5-HT. Ook deze contractie kon niet beïnvloed worden door tetrodotoxine en atropine. De 5-HT_7 receptor antagonist SB 269970 had geen effect op de contractiele respons geïnduceerd door 5-CT. De

5-HT_{1A} receptor antagonist WAY 100635 antagoniseerde echter wel op concentratie-afhankelijke wijze de contractiele respons geïnduceerd door 5-CT. 8-OHDPAT, buspiron en flesinoxan, farmaca met duidelijk agonistische effecten ter hoogte van 5-HT_{1A} receptoren van de mens en de rat, hadden geen enkel effect op de longitudinale spierlaag van het jejunum van het paard. De resultaten van de functionele in vitro studie geven aan dat 5-HT zijn contractiel effect op de longitudinale spierlaag van het jejunum van het paard medieert via niet klassieke 5-HT_{1A} receptoren, rechtstreeks op de gladde spiercellen gelokaliseerd. De resultaten van een gelijkaardige functionele in vitro studie, dit maal op de circulaire spierlaag van het jejunum van het paard, worden weergegeven in Hoofdstuk 4.III. Weerom kon via 5-HT en de 5-$HT_{1,7}$ receptor agonist 5-CT een tonische contractie uitgelokt worden. Zowel tetrodotoxine, atropine als N^G-nitro-L-arginine konden geen invloed uitoefenen op deze contractiele respons. Door gebruik te maken van een waaier van specifieke 5-HT receptor antagonisten, kon een interactie met 5-$HT_{1B, 1D, 2A, 3, 4 en 7}$ receptoren worden uitgesloten. Opnieuw vertoonden de specifieke 5-HT_{1A} receptor antagonisten NAN 190 en WAY 100635 een duidelijk antagonistisch effect op de contractiele respons geïnduceerd door 5-HT en 5-CT. De 5-HT_{1A} receptor werd bijgevolg weerom geïdentificeerd als de receptor verantwoordelijk voor de contractiele respons geïnduceerd door 5-HT in de circulaire spierlaag van het jejunum van het paard. Aangezien zowel in de longitudinale als in de circulaire spierlaag de betrokken 5-HT_{1A} receptor enkel kon beïnvloed worden door de specifieke 5-HT_{1A} antagonisten NAN 190 en WAY 100635 en door de specifieke 5-$HT_{1A,7}$ receptor agonist 5-CT, maar niet door de specifieke 5-HT_{1A} receptor agonisten 8-OHDPAT, buspiron en flesinoxan, verkiezen we te spreken van een niet-klassieke 5-HT_{1A} receptor.

De resultaten van beide functionele in vitro studies werden immunohistochemisch bevestigd, door visualisatie van 5-HT_{1A} receptoren in zowel de longitudinale als de circulaire spierlaag van het jejunum van het paard. Door middel van specifieke antistoffen werden

talrijke 5-HT_{1A} receptoren aangetoond in de de tunica submucosa, en de longitudinale en circulaire spierlaag van het jejunum van het paard.

Het gebrek aan evidentie voor aanwezigheid van 5-HT_4 receptoren ter hoogte van de dunne darm van het paard, doet terecht vragen rijzen over het gebruik van dure humane prokinetica, die hun werking mediëren via 5-HT_4 receptoren, bij paarden met ileus. De uitgevoerde in vitro studies hebben aangetoond dat de geïdentificeerde 5-HT_{1A} receptor een snel optredende en langdurige tachyphylaxie vertoont wanneer hij in aanraking wordt gebracht met hoge concentraties van 5-HT. In dat opzicht vormen de verhoogde 5-HT-spiegels die werden aangetroffen in plasma en buikvocht van koliekpaarden, gepredisponeerd om ileus te ontwikkelen, een interessante bevinding. Er is meer onderzoek nodig om de rol van de 5-HT_{1A} receptor in de pathofysiologie van ileus bij het paard te onderzoeken.

CURRICULUM VITAE

Cathérine Delesalle werd op 25 april 1972 geboren te Diest. Na het behalen van het diploma hoger secundair onderwijs bij de Zusters Van De Voorzienigheid te Diest, begon zij in 1991 met de studie Diergeneeskunde aan de Universiteit Gent en behaalde het diploma van Dierenarts in 1997, magna cum laude.

Onmiddellijk daarna trad zij in dienst bij de vakgroep Inwendige ziekten en Klinische Biologie van de Grote Huisdieren. In 1997 werd zij assistent op dezelfde vakgroep. Naast klinisch onderwijs in de inwendige ziekten te geven aan de studenten heeft ze zich vooral toegelegd op het probleem van koliek bij paarden. Het eigenlijke onderzoek werd gestart in 2002 en heeft uiteindelijk tot dit proefschrift geleid.

Catherine Delesalle is auteur of mede-auteur van 16 publicaties in internationale en nationale tijdschriften.

PAPERS

DELESALLE, C., VAN LOON, G., NOLLET, H., DEPREZ, P. (2002) Tumor-induced ventricular arrhythmia in a horse. *Journal of Veterinary Internal Medicine* **16**, 612-617.

DELESALLE, C., DEPREZ, P., VANBRANTEGEM, L., VANEECHOUTTE, M., DECOSTERE, A., DUCATELLE, R. (2003). Emphysematous gastritis in a horse associated with Clostridium septicum. *Journal of Veterinary Internal Medicine* **17**, 115-118.

DELESALLE, C., LEFEBVRE, R.A., SCHUURKES, J.A.J., CLAES, P., DEPREZ, P. (2004). Serotonin: the unknown for the horse? A review. *Flemish Veterinary Journal* **73**, 407-419.

DELESALLE, C., DEPREZ, P., SCHUURKES, J.A.J., LEFEBVRE, R.A. (2006). Contractile effects of 5-hydroxytryptamine and 5-carboxamidotryptamine in the equine jejunum. *British Journal of Pharmacology* **147**, 23-35.

DELESALLE, C., DEWULF, J., LEFEBVRE, R.A., SCHUURKES, J.A.J., VAN VLIERBERGEN, B., DEPREZ, P. (2005). Use of plasma ionized Ca^{2+} levels and Ca^{2+}

substitution response patterns as prognostic parameters for ileus and survival in colic horses. *Veterinary Quarterly* **27**, 157-172.

DELESALLE, C., LEFEBVRE, R.A., SCHUURKES, J.A.J., LEFERE, L., VANSCHANDEVIJL, K., DEPREZ, P. (2006). Gastro-intestinal motility in horses, a practical overview of the therapeutic use of prokinetic agents. *Flemish Veterinary Journal*, **75**; 122-139.

DELESALLE, C., DEWULF, J., LEFEBVRE, R.A., SCHUURKES, J.A.J., PROOT, J., DEPREZ, P. (2007). Use of Accusport® determined L-lactic acid levels in blood and peritoneal fluid of colic horses as a prognostic parameter. *Journal of Veterinary Internal Medicine* **21**, 293-301.

DELESALLE, C., VAN ACKER, N., CLAES, P., DEPREZ, P. DESMET, I., DEWULF, J., LEFEBVRE, R.A. (2007). Contractile effects of 5-hydroxytryptamine (5-HT) in the equine jejunum circular muscle: functional and immunohistochemical identification of a 5-HT_{1A}-like receptor. *Equine Veterinary Journal*, accepted.

DELESALLE, C., VAN DE WALLE, G., NOLTEN, C., VER DONCK, L., VAN HEMELRYCK, A., DRINKENBURG, W., DE BOSSCHERE, H., CLAES, P., DEPREZ, P., LEFERE, L., TORFS, S., LEFEBVRE, R. (2008). Determination of the source of increased serotonin (5-HT) levels in blood and peritoneal fluid of colic horses with compromised bowel. *Equine Veterinary Journal*, accepted.

TORFS, S., DELESALLE, C., VANSCHANDEVIJL, K., DE CLERCQ, D., VAN LOON, G., NOLLET, H., DEPREZ, P. (2007). Anti-inflammatory phytotherapeutics: a valuable alternative for NSAID treatment in horses? A closer look into the world of herbal medicine. *Flemish Veterinary Journal*, accepted.

DEVISSCHER, L., DELESALLE, C., DEWULF, J., DEPREZ, P., TORFS, S., SCHAUVLIEGE, S., MARTENS, A., GASTHUYS, F. (2008). Pre-and per-operative parameters related to hypotension and hypoxaemia during anaesthesia in colic horses: a retrospective study. *Equine Veterinary Journal*, submitted.

TORFS, S., DELESALLE, C., DEWULF, J., DEVISSCHER, L., DEPREZ, P. (2008). Determination of risk factors associated with development of postoperative ileus and short-term survival, focussing on effectiveness of lidocaine treatment. *Equine Veterinary Journal*, submitted.

VERSCHOOTEN, F., DEPREZ, P., DE CLERCQ, T., NOLLET, H., VAN LOON, G., DELESALLE, C., LEFERE, L., SAUNDERS, J. (2000). The inspection of stallions, a practical overview. *Flemish Veterinary Journal*, 441-445.

DE CLERCQ, D., VAN LOON, G., NOLLET, H., DELESALLE, C., LEFERE, L., DEPREZ, P. (2003). Percutaneous puncture technique for treating persistent retropharyngeal lymph node infections in seven horses. *Veterinary Record*, **152**, 169-172.

VERSCHOOTEN, F., DEPREZ, P., NOLLET, H., LEFERE, L., VAN LOON, G., VAN ZEVEREN, A., DEVRIESE, L., VERVAET, P., VAN SCHANDEVIJL, K., VANDENBERGHE, F., DELESALLE, C., DE CLERCQ, D., SAUNDERS, J.,

VANDEVELDE, B., TAEYMANS, O., HOEGAERTS, M, De GRAEF, E., PILLE, F., GASTHUYS, F., PEREMANS, K. (2003). Stallion Inspection of the Belgian Draft Horse: 1995-2002. *Flemish Veterinary Journal* **72**, 224-232.

POHLMANN, J., VAN LOON, G., LEFERE, L., VANSCHANDEVIJL, K., NOLLET, H., DE CLERCQ, D., DELESALLE, C., DEPREZ, P. (2005). Hepatoencephalopathy caused by Senecio Jacobaea intoxication in five horses. *Flemish Veterinary Journal* **74**, 440-445.

ORAL PRESENTATIONS & POSTERS

Postgraduate education: An update on respiratory diseases in horses. Presentation with Syllabus. 8 february 2002.

Postgraduate specialization course for equine practitioners. Inflammatory airway disease. Presentation with Syllabus. 7 may 2003.

Assemblé nationale de l'UPECT 2004. L'immunodéficience chez le poulain

Annual Meeting of the VPP.
Presentation with Syllabus. Ileus in horses and efficacy of prokinetic drugs. 5th February 2005.

13th International Equine Colic Research Symposium, Canada, Quebec, 2005. 2 presentations:
C. Delesalle, P. Deprez, J. Schuurkes, R. Lefebvre. In vitro effects of 5-hydroxytryptamine on the jejunum of healthy and colic horses: characterization of a putative 5HT1A receptor in the equine gut &
C. Delesalle, J. Dewulf, J. Schuurkes, B. Vanvlierbergen, H. Nollet, D. Declerq, P. Deprez. Ionized calcium as a prognostic parameter in colic horses.

Belgian week of Gastroenterology, 26th-28th February 2005. Presentation of research results financially supported by an Interuniversity Attraction Poles Programme-Belgian Science Policy. Contractile response to 5-hydroxytryptamine in the equine jejunum. C. Delesalle, P. Deprez, A. Schuurkes, R. Lefebvre. Acta Gastro-enterologica Belgica 2006; 69: B11.

Hippos 2007, Luik, Belgium. Poster presentation: determination of the source of increased serotonin (5-HT) levels in blood and peritoneal fluid of colic horses with compromised bowel.

Hippos 2008, Luik, Belgium. Poster presentation. Ultrasound-guided pervaginal drainage of rectal tear-associated abscesses in 4 mares.

May 2001:practical training at the University of Pennsylvania, School of Veterinary medicine, George D. Widener Hospital for Large Animals: Connelly Intensive Care Unit and the Graham French Neonatal section. Tutors: Prof. Dr. J. Palmer and Prof. Dr. Wilkins.

C. Delesalle, P. Deprez, H. Nollet, I. De Smet, J. Dewulf, R. Lefebvre.
Contractile effects of 5-hydroxytryptamine (5-HT) in the equine jejunum circular muscle: identification of a 5-HT_{1A}-like receptor. WEVA 2008, Moskou.

Belgian week of Gastroenterology 2008. C. Delesalle, P. Deprez, N. Van Acker, G. Van de Walle, C. Nolten, L. Ver Donck, A. Van Hemelrijck, W. Drinkenburg, I. De Smet, J. Dewulf, H. De Bosscher, P. Claes, R.A. Lefebvre. Functional and immunohistochemical identification of a 5-HT_{1A} like receptor responsible for 5-H-induced contraction of the equine jejunum; differences in 5-HT_{1A}-like receptor density in healthy and colic horses. Acta Gastro-enterologica Belgica 2008.

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Ik herinner mij nog het prille begin, waar Dr. Kathleen Vlaminck van Janssen Pharmaceutica mij introduceerde bij Dr. Jan Schuurkes, toenmalig vice-president en hoofd van de GI-research afdeling in Beerse. Jan, jij hebt mij echt onder je vleugels genomen en in het labo bij Walter De Ridder leerde ik de kneepjes van het vak. Ik mocht proeven van de fantastische sfeer in jouw team. Iedereen was doordrongen van de gedrevenheid en vakkundigheid waarmee jij jouw afdeling leidde. Ideeën over de "stilligende paardendarm" werden gespuid. We zochten naar mogelijkheden en technieken om een tipje van de sluier op te lichten en staken van wal. Menig nachtelijk uurtje heb ik doorgebracht in het oude GI research gebouw in Beerse. Op en af pendelend tussen het slachthuis in Rekkem, waar ik dankzij Donald Kemzeke van de firma Chevideco, toegang had tot een onuitputtelijke bron gezond research materiaal, vervolgens karrend naar Beerse om proeven te doen tot s'avonds laat. Pieter Janssen, Walter De Ridder, Luc Hoskens en Joris De Mayer lieten mij kennis maken met de wereld van de agonisten en antagonisten en dosis-respons curven. Hun inbreng was mij goud waard.

We zaten dan nog met de vraag hoe we in de toekomst, zo min mogelijk invasief bij een levend paard de beweeglijkheid van de darm konden onderzoeken. De koppen werden weerom samen gestoken en zo ontstond het idee van de "impedantiecatheter". Met

engelengeduld en een zee van technische ervaring werd een 8m lange catheter vervaardigd, voorzien van impedantie electroden, om in de paardendarm via de maag te laten afdalen. Patrik Claes stond mij bij met praktische tips om het ontwerp vorm te geven; Johan Vekeman realiseerde het bijna onmogelijke om 8 electroden en een centraal drukkanaal te stoppen in een amper halve cm dikke catheter van 8m lang. Jullie waren fantastisch! In het vervolg onderzoek van mijn Phd zal deze catheter noch menig uurtje in de paardendarm doorbrengen.

De maanden verstreken en hervormingen waren op til in Beerse. Een moeilijke periode voor Jan en zijn team. Jan trok weg uit Beerse en twee jaar later hield hij als Chief Scientific Officer, de firma Movetis, boven de doopvont. Beste Jan, opnieuw nam je me onder je vleugels en je troonde me mee naar het Heymans Instituut in Gent, naar een echte “crack” in GI research, naar Professor Lefebvre, al even enthousiast over de paardendarm als jij. In de paardenvrachtwagen van onze faculteit werd de peperdure volledige in vitro proefopstelling, inclusief meetkasten en printers vanuit Beerse getransporteerd naar het Heymans instituut in Gent. Op 14 dagen tijd hebben we een heel in vitro labo geïnstalleerd. Iedereen droeg zijn steentje bij: Tony Dossche, Roger Vanhoecke, Norbert Lippens, Gratien Janssen. En dan waren er de reddende engelen in nood: Luc Hoskens en Joris De Mayer. Onbaatzuchtig als jullie zijn, kwamen jullie gezwind van Beerse om in een handomdraai de hele proefopstelling in elkaar te steken en alle meetapparatuur vakkundig geijkt aan de praat te krijgen, terwijl ik de muren stond te verven. We hebben mekaar denk ik goed leren kennen en jullie hebben alletwee een hart van goud. Zonder al jullie steunende hulp vanuit Beerse, had ik dit alles nooit kunnen realiseren.

We staken opnieuw van wal met de in vitro proeven, dit maal in het Heymans Instituut in Gent. Ik werd schitterend opgevangen in het team van het Heymans Instituut: Gwen Vanneste, Koen Van Crombruggen, Ole De backer, Inge Dhaese, Inge Van Colen, Valere Geers, Bart Blanckaert en niet te vergeten Annie De Smet wiens deur altijd openstond voor

een gezellige babel en wijze levensraad. De gestructureerdheid en vakkundigheid waarmee Professor Lefebvre me begeleide was een openbaring voor mij. Professor Lefebvre, woorden schieten mij te kort om U te bedanken voor de ondersteuning die U mij hebt gegeven (nu ik ga dat tussen pot en pint (WestVleeteren dan) zeker nog eens uit de doeken doen). Uw kwinkslagen, de gevatheid en scherpte waarmee U de dingen leest, uw talent om met engelengeduld de champagne in een mens naar boven te halen, afkortingen als PEPLR, het zijn allemaal dingen waaraan ik met een warme lach terugdenk terwijl ik dit schrijf. In de loop der jaren is er een vriendschapsband ontstaan die mij nauw aan het hart ligt. Ik heb het U vaak niet makkelijk gemaakt: half afgewerkte manuscripten, met 10 dingen tegelijk bezig zijn, een aardige dosis legendarische verstrooidheid…U hebt ze allemaal de revue zien passeren. Maar U bracht ons steeds “back on track” en dat is uniek aan U. Ik hoop dat in de toekomst nog veel jonge mensen van uw professionele begeleiding mogen genieten.

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