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EQUINE GASTRO-INTESTINAL MOTILITY – A REVIEW

ON PROKINETICS, SPASMOLYTICS AND THEIR RECEPTORS

by

Sander DAMEN

Promotors: Prof. dr. Catherine Delesalle
Xanthippe Boulougouris

Literature review
as part of the Master's Dissertation

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PREFACE

This literature review is part of the author's Master's dissertation at Ghent University's faculty of Veterinary Medicine. It is hoped that this review provides in a thorough overview of the subject and will contribute to the reader's understanding of it. Naturally, the literature review presented here is not only the result of the author's efforts, but derives also from the useful advice and suggestions by those who assisted the author during the process of writing the review.

It is therefore appropriate to share these words of appreciation by thanking those who contributed to this review. In special, the author would like to express gratitude towards the promotors drs. Berit Boshuizen, ms. Xanthippe Boulougouris, and prof. dr. Catherine Delesalle for their dedicated assistance and constructive reviews of the manuscript.

CONTENT

ABBREVIATIONS

GI	gastro-intestinal	GPCR	G-protein coupled receptor
ENS	enteric nervous system	5-HT	5-hydroxytryptamine
Ach	Acetylcholine	ATP	Adenosine 5’-triphosphate
NO	Nitric oxide	VIP	Vasoactive intestinal peptide
NANC	Non-adrenergic, non-cholinergic	BeCh	Bethanechol
ICC	Interstitial Cells of Cajal	CRI	Constant-rate infusion
MMC	Migrating motor complex	PO	Per os
CMMC	Colonic migrating myoelectrical complex	IV	Intravenous
GD	Grass disease	AUC	Area under curve
POI	Post-operative ileus	COX	Cyclo-oxygenase
LPS	Lipopolysaccharide	NMNT	N-methylnaltrexone
NOR	Noradrenaline	HBB	Hyoscine butylbromide
ADR	Adrenaline	PPB	Propantheline bromide

ABSTRACT & KEYWORDS

Disorders of the gastro-intestinal tract are common in horses and are often associated with modifications in the normal intestinal motility pattern. Maintaining sufficient gastro-intestinal activity is essential to facilitate adequate digestion and resorption of nutrients. Disturbed motility patterns might lead to intestinal disease and clinical signs, which are to be encountered by the equine practitioner. Consequently, numerous investigations are aimed at obtaining a better understanding of the pathophysiology of gastro-intestinal motility, in order to find ways to regulate and modify gastro-intestinal motility. By doing so, therapeutics might be developed capable of modifying these motility patterns. These therapeutics often interact as ligands with receptors in the intestinal tract. This can either be a stimulating (prokinetic) or an inhibiting (spasmolytic) influence upon the intestinal motility pattern. It gives the practitioner more therapeutic possibilities in the (symptomatic) treatment of intestinal diseases. Progression has been made in recent years in this regard, but more thorough clinical studies are still desirable. Bethanechol can be used in proximal enteral pathologies (like duodenal strictures in foals), whereas neostigmine is contraindicated in case of proximal disorders of the enteral tract. Benzamides (metoclopramide, mosapride) and domperidone might be useful in disorders of the proximal segments as well. The effects of erythromycin seem more pronounced in the equine colon. Lidocaine has been observed efficient throughout the entire gastro-intestinal tract in cases of post-operative ileus and colic. The opioid antagonists attenuate the intestinal side effects of the opioids, but more research is needed to trial their clinical effectiveness. This might be a conclusion in general for the different therapeutics.

Keywords: **Horse** – **Intestinal motility** – **Prokinetics** – **Receptors** – **Spasmolytics**

SAMENVATTING

Gastro-intestinale motiliteit kan, zowel bij het paard als bij andere dieren, omschreven worden als het resultaat van een complexe interactie tussen verschillende fysiologische systemen die via hun eigen mechanismen inwerken op het maagdarmstelsel. Dit resultaat wordt onder normale omstandigheden zichtbaar als de aborale propulsie van darminhoud in de richting van het rectum. Het is juist in abnormale situaties, wanneer er een verstoring van de intestinale motoriek en diens achterliggende systemen plaatsvindt, dat er functiestoornissen in het maagdarmstelsel optreden en het paard klinische symptomen kan gaan vertonen. Het is op dit moment dat de dierenarts in de praktijk een rol krijgt toebedeeld en in staat wordt geacht om op te treden tegen het dysfunctioneren van de maagdarmtractus en wordt verwacht de motiliteitsstoornissen te kunnen tegengaan.
Motiliteitsstoornissen en de bijhorende klinische presentaties hiervan kunnen verschillende oorzaken hebben, wat voor de clinicus van belang is in het kader van een juiste etiologische therapie. Een grondige kennis van de fysiologie van de maagdarmtractus bij gezonde paarden is hierbij onontbeerlijk, om in staat te zijn de veranderingen in pathofysiologische omstandigheden te kunnen

detecteren. Zo komt men te weten dat het fenomeen ‘motiliteit’ het resultaat is van onder andere sympathische en parasympathische neuronsystemen, die een lokaal enterisch netwerk vormen maar ook in verbinding staan met het centraal zenuwstelsel. Daarnaast zorgen de interstitiële cellen van Cajal op celniveau voor een periodieke, ritmische en vooral spontane elektrische activiteit in de gladde spiercellen, naast aanvullende hormonale en paracriene invloeden en fasische contracties die optreden bovenop de basale tonus.

Een van de mogelijkheden om te interveniëren in dit stelsel van systemen dat de motiliteit reguleert, is het toespitsen op enterale receptoren in de darm. Tot nu toe zijn reeds diverse receptorsystemen bekend waarvan men heeft vastgesteld dat zij betrokken zijn bij de regulering van de intestinale motiliteit. Beïnvloeding van deze receptoren kan door hun liganden te laten binden. Zij kunnen functioneren als agonisten of antagonisten van de receptor en hierop volgend een stimulatie, respectievelijk inhibitie teweeg brengen van het signaal dat normaliter door de receptor wordt doorgegeven. Met de term ‘prokinetica’ worden deze medicamenten aangeduid, die door binding op een receptor een stimulerende invloed hebben op de darmmotiliteit, terwijl men met ‘spasmolytica’ die pharmaca bedoelt die door ligandbinding juist een inhiberende werking op het gastro-intestinaal stelsel uitoefenen. Een bespreking van beide groepen medicamenten en de receptoren waarop zij inwerken, vormt een onderdeel van deze literatuurstudie.

Bethanechol is een directe cholinerge receptor agonist met een prokinetische activiteit op zowel de maag, dunne darm als dikke darm bij paarden. De stof wordt klinisch al gebruikt bij duodenale stricturen bij veulens om de doorstroom van digesta vanuit de maag te bevorderen. Klinische studies moeten de praktische effectiviteit als prokineticum nog verder aantonen. Neostigmine is een indirecte cholinerge agonist, daar het een acetylcholinesterase-inhibitor is en daarmee indirect de concentratie aan acetylcholine in de synaptische spleet verhoogt. Neostigmine wordt verondersteld een eerder inhiberend effect te hebben op de motiliteit van de darm in het proximale deel, en is daarom niet geïndiceerd bij patiënten die hier hinder van kunnen ondervinden. De parasympathicomimetica dienen daarenboven in het algemeen met oplettendheid te worden toegediend in verband met hun parasympathische neveneffecten (speekselen en abdominaal discomfort).

Metoclopramide kan gebruikt worden om de maaglediging te bevorderen en is aangetoond een positief effect te hebben op de motiliteit bij paarden met post-operatieve ileus en koliek, maar de ernstige bijwerkingen (extrapyramidale symptomen) maken metoclopramide niet een te prefereren stof. Cisapride heeft deze bijwerkingen niet en stimuleert vooral de dunne darm peristaltiek effectief, maar is gelimiteerd verkrijgbaar omdat bij mensen cardiale stoornissen als bijwerking werden gezien. Mosapride lijkt deze effecten niet te hebben en laat desalniettemin een positieve invloed op maaglediging en darmmotiliteit zien. Mosapride zou daarom de voorkeur kunnen krijgen boven metoclopramide of cisapride.

Domperidone is een dopaminerge receptor antagonist, maar aangezien het niet de bloed-hersen barrière doorkruist (in tegenstelling tot metoclopramide), toont het niet de extrapyramidale neveneffecten van deze laatste stof. Het is een medicament met vooral een toepassing voor proximale pathologieën van het gastro-intestinaal stelsel.

Lidocaïne wordt frequent gebruikt bij patiënten met post-operatieve ileus of enteritis en hevige reflux. Het anestheticum zou ook een gereduceerd risico op post-operatieve ileus geven indien het profylactisch wordt toegediend. Erythromycine heeft door een agonistische werking op de motiline-receptor een stimulerend effect op de motiliteit van voornamelijk het colon, maar dient te worden toegediend in subtherapeutische (non-microbiële) lage bolussen.
De opioïd-antagonisten (N-methylnaltrexone, naloxone) zijn effectief in het verminderen van de inhiberende invloed van de opioïde analgetica op de gastro-intestinale motiliteit, maar hun effectiviteit als alleenstaand prokineticum (los van voorafgaande opioïden toediening) dient nog verder onderzocht te worden.

INTRODUCTION

The alimentary tract is of vital importance to the horse (*Equus caballus*). Its function is the ingestion, comminution, digestion and absorption of food, and the elimination of solid waste material[1]. By doing so, an ongoing supply of nutrients can be sustained, facilitating the energy that is required for the horse's metabolism.
The movement of the intestinal contents is produced by peristaltic waves, being the result of a complex interaction between neural, hormonal, vascular and neuromuscular pathways[2]. Major functions of these gastro-intestinal motility patterns are the transport of digesta along the tract, the mixing of the digestive juices with the food (thus facilitating close contact with digestive enzymes), and the bringing into contact of digested nutrients with the intestinal mucous membrane for subsequent absorption[1].
It stands clear that disruption of gastro-intestinal motility may cause severe health risks for the horse, comprising the digestive function of the intestinal tract as a potential result of the primary pathology by the gastro-intestinal disorder. Although a variety of primary diseases can be responsible for gastro-intestinal disorders, we often see the problem of *ileus* associated with them as a functional manifestation[3]. Ileus of the gastro-intestinal tract has been defined as the inhibition of propulsive intestinal activity, irrespective of its pathophysiology[2]. It commonly occurs after abdominal surgery in horses and is often a fatal problem, leading to an approximately 7-fold increase in postoperative euthanasia compared with horses that did not develop postoperative ileus[4,2]. Shock, electrolyte imbalances, hypoalbuminaemia, peritonitis, endotoxaemia and distention, ischaemia and inflammation of the intestinal tract have all been implicated as contributing factors to the pathophysiology of ileus in the horse[5].
Despite advances in the understanding of intestinal injury, ileus remains a significant cause of mortality and morbidity in horses[6]. Numerous investigations are thus aimed at obtaining a better understanding of the pathophysiology of disrupted gastro-intestinal motility patterns, in order to be able to develop therapeutics (prokinetic medication) capable of enhancing gastro-intestinal motility for the equine practitioner in the field. For this, a proper understanding of the healthy intestinal tract and the mechanisms, responsible for gastro-intestinal motility in physiological situations, is needed.

Prokinetics are defined as agents that facilitate or enhance the net movement of feed material down the length of the intestinal tract and do not simply produce an uncoordinated increase in local contractile activity. This can be achieved either by augmenting the pathways that stimulate motility, or by attenuating the inhibitory neurons that predominantly suppress activity[6].

The aim of this review was to provide a thorough overview of the latest information on gastro-intestinal motility, including the enteral receptors (in both the small and large intestine) that have been elucidated to play a role in the mechanism of altering and influencing gastro-intestinal motility, thus having the potential to serve as a pharmacological target. A summary of known prokinetics and spasmolytics and the corresponding receptors on which they act, will be provided and discussed. In **Appendix I**, the reader will find a table that additionally provides in a summary of these medicaments.

LITERATURE REVIEW

1. Introduction to the equine intestinal tract

The digestive tract of the horse, a monogastric, non-ruminant herbivore (**Figure 1**), can be compared with that of a pig, but with a much enlarged hind gut – especially the caecum, being responsible for the microbial fermentation of fibrous materials and therefore containing an extended population of microbiota[1]. After chewing its food and mixing it with saliva, the horse swallows and the food proceeds to the relatively small stomach. A horse's stomach is rarely empty and digesta are held only for a short time (2-6 h)[7]. The digesta then proceed to the small intestine (comprising the duodenum, jejunum and ileum), being the main organ for the absorption of dietary nutrients. The transit time in the small intestine is rapid: up to 30 cm/min. The more distal the intestinal segment is located from the gastric pylorus, the lower the frequency of contractions of the segment will be[7].

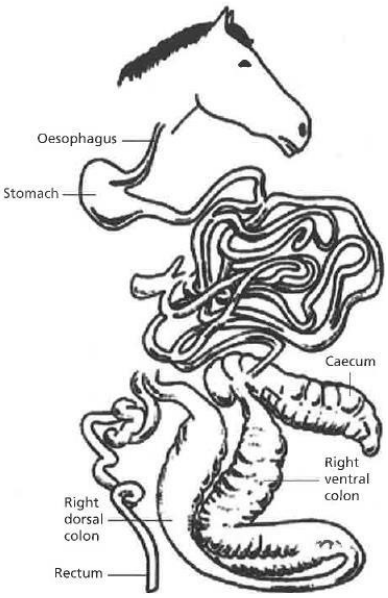

Figure 1. *The anatomy of the digestive tract of the horse. (adapted from* Reference 1*).*

From the ileum, the digesta move towards the caecum, as the first part of the large intestine, and together with the enlarged colon the caecum is responsible for the microbial fermentation in the horse. The caecum starts its contractions 12-15 cm below the caeco-colic junction[7].

The equine colon can be divided into the *colon crassum*, with its *flexura pelvina*, and the more distal *colon tenue*, caudally ending as the rectum. Contractions of the colon are complex. A first type is a rhythmic contraction in aboral direction. The second one is rhythmic as well, but propagates in the oral way, and the third type of contractions is isolated and does not propagate in either direction. The net effect of all these contractions will be a propulsive aboral movement of the digesta towards the rectum, resulting in the horse defaecating.

2. Gastro-intestinal motility

Motility patterns in the equine gastro-intestinal (GI) tract have various functions, the most important being the transport of digesta along the tract, the mixing of the digestive juices with the food, and the bringing into contact of digested nutrients with the intestinal mucous membrane for subsequent absorption[1]. These patterns are the result of rhythmic and coordinated contractions of smooth muscle cells in the wall of the intestine, especially in the *tunica muscularis*, existing of an inner circular and outer longitudinal layer. Contraction of the inner circular layer induces narrowing of the intestinal lumen, whereas contraction of the outer longitudinal layer is associated with widening of the lumen and shortening of the involved segment. Intercellular communication between smooth muscle cells is facilitated by 'gap junctions' that connect neighbouring cells with each other. Electrical potentials in

one cell can thus be send to another smooth muscle cell, enabling the muscle-layer to react as one large syncytium[3].

The impulses leading to muscular contractions are the result of a complex interaction between different systems and pathways. In this chapter, these various systems will be discussed.

2.1 Physiology in the healthy horse

2.1.1 Neuro-anatomical description

The enteric nervous system (ENS) of the horse is an intrinsic neuronal network within the gut wall, extending from the cranial oesophagus, over the entire length of the gastro-intestinal tract to the internal anal sphincter, and innervating the biliary system and the pancreatic parenchyma as well[8,9]. The ENS is a part of the autonomic (sympathetic) nervous system and integrates motility, secretions, blood flow and immune responses into organised patterns of behaviour through neuronal reflexes[10]. The extrinsic and intrinsic components of the ENS appear to be the primary mechanisms involved in GI motility regulation[11].

The ENS consists of 2 ganglionated plexuses: the submucosal plexus (Meissner's, functioning mainly as a local control of intestinal secretion and absorption) near the luminal side between the mucosa and circular muscle layer, and the *plexus myentericus* (Auerbach's, primarily responsible for the GI tract motor function) between the outer longitudinal and the inner circular muscle layer of the *tunica muscularis*[8,10]. The ENS can broadly be divided into: sensory neurons that monitor factors such as intestinal wall tension; associative neurons that link various enteric neurons; and motor neurons responsible for the smooth muscle contraction, vasomotor control and water and electrolyte transport[3].

Parasympathetic innervation of the GI tract involves the *nervus vagus* for the stomach and the upper intestines, whereas the *nervi pelvini* serve the distal intestines[12]. Transmission from vagal input neurons to enteric neurons is mediated principally by acetylcholine (ACh) acting on nicotinic cholinergic receptors, but several other transmitters are involved in these processes[10,13]. For the sympathetic system, which includes the ENS, acetylcholine is also one of the major neurotransmitters[10], together with noradrenaline, and can be found in excitatory motoneurons and interneurons, whereas nitric oxide (NO, known in mammals as a non-adrenergic, non-cholinergic (NANC) inhibitory molecule[11]) is mainly located in inhibitory motorneurons[14]. Besides these cholinergic and noradrenergic regulations, it is known that serotoninergic and peptidergic regulations are present as well[15], some of them having a facilitatory action, others having inhibitory effects upon classical neurotransmitters.

In some cases, substances released from axons seem to have minor influences on effector cells, and they can be regarded as secondary neurotransmitters. Substance P neuropeptide, for example, is a neurokinin known to stimulate smooth muscle contraction, and has proven to be present with Ach as a co-mediator in nervous structures[16].

When neurotransmitters act (either pre- or postsynaptically) to modify or to release the primary neurotransmitter, they are called neuromodulators. A transmitter can have more than one role at a neuroeffector junction[15]. These are all indications that the neuronal innervation of the GI tract is a complex and extended system with various interactions.

2.1.2. Interstitial Cells of Cajal

GI smooth muscles need to integrate all the input from different control systems and respond with an appropriate physiologic contractile reaction[17]. Apart from hormonal influences, paracrine factors and sympathetic and parasympathetic control by both the central nervous system and the ENS, local interstitial cells of Cajal (ICC, **Figure 2**) are one of the factors that contribute to the control of the GI smooth muscle contraction. The ICC are closely coupled, both with each other and with smooth muscle cells, by gap junctions (having myoid, smooth-muscle like features themselves[18]) and generate spontaneous, rhythmic electrical activity[17,18]. The ICC form a network of differentiated cells in the muscular layers of the entire intestinal tract, and through this network the electrical depolarizations spread. They are innervated by enteric neurons and can send extensions into both muscle cell layers, between muscle cells and into connective tissue layers[19].

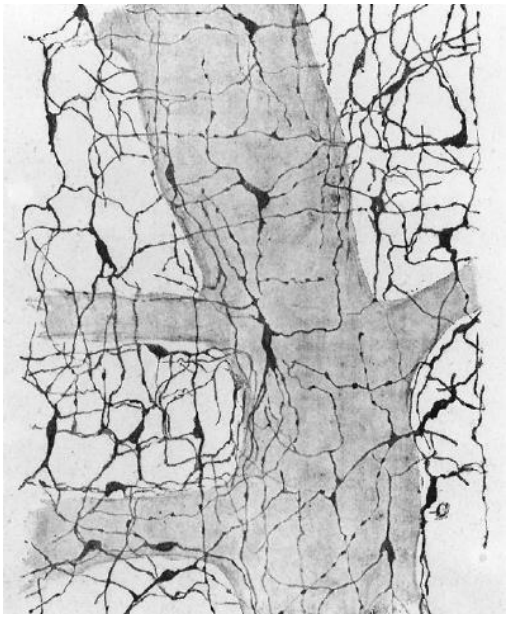

Figure 2. *Already in 1911, Ramón y Cajal described his 'interstitial neurons', here visualised using methylene blue staining (from* Reference 18*).*

This rhythmic electrical activity of the ICC is called a *slow wave*, being a periodic oscillation of the cell membrane potential (for example, varying between 5 and 15 mV in man[3]), with a frequency characteristic for each organ and for each species. Slow waves are generated continuously and independent of neural activity or any other stimulus, although these stimuli can affect the frequency or duration to a limited extent[18].

The ICC are presumed to have three roles in GI motility, being: acting as pacemaker cells that generate electrical activity (slow waves); facilitating propagation of electrical events; and mediating neurotransmission[20]. The pacemaker activity, and the resulting slow wave, provides 'conditioning' of the smooth muscle syncytium and increases the open probability of the Ca^{2+}-channels in the GI smooth muscle cell, thereby facilitating future Ca^{2+}-influx[17]. It causes a brief period of high excitability, corresponding with the plateau phase of the slow wave. On excitatory stimulation (for example, mechanical stretching by digesta, or drugs like ACh or parasympathicomimetics[21]), the plateau phase rises above the threshold for activation of the L-type Ca^{2+}-channels, leading consequently to an action potential[18], being a fast membrane potential change, the depolarization due to Ca^{2+}-influx through the L-channels. This causes contraction of smooth muscle cells. An action potential only occurs superimposed on the plateau phase of the slow wave[17,18]; slow waves therefore determine the

(maximum) frequency of contractions. The amplitude of action potentials is directly related to the force of contraction[18].

The highest densities of ICC in the equine intestinal tract have been observed in the ileum, the *flexura pelvina* and the body of the caecum[19], suggesting these sites to be predominant pacemaker sites. Further investigation is needed to confirm that high density of ICC parallels areas where there is a prominence of slow wave activity[19]. The existence of pacemaker sites in the equine tract might be evidenced by measuring the myoelectric activity of several intestinal segments during physiological circumstances, for instance *in vivo* by placing electrodes intra-abdominally in healthy living horses after laparotomy.

2.1.3 Motility patterns

Apart from a continuous 'basal tonus' in the smooth muscles of the GI tract, causing the narrowing of the intestinal lumen to a certain degree, there are the phasic contractions that are superimposed upon the basal activity. Together they provide, in a symbiotic manner, a more efficient propulsion of food material along the digestive tract. Indeed, the generally weak phasic contractions will often better succeed in occluding the intestinal lumen when a proper basal tonus is maintained[21].

In mammals, we see a spontaneous 'migrating motor complex' (MMC) occurring in the stomach and the small intestine, being a cyclic pattern of myoelectric and mechanical activity, resulting in a contraction that propagates in an oral to aboral direction[22]. The MMC is often suspected of an enteral 'housekeeping task', like propagating undigested residual material along the digestive tract. In humans, the MMC starts a few hours after ingestion of food, in contrast to the horse, where the MMC pattern is continuously expressed (irrespective of feeding). Considering this, the equine MMC is to be recognized as an active part of the digestive motor system[21].

Additionally, the equine colon can benefit from its own cyclic motor pattern, the so called 'colonic migrating myoelectrical complex' (CMMC). In contrast with the MMC, electrical activity of the CMMC will not always be followed by mechanical activity. The CMMC consists of a series of 'long spike bursts' that lasts for 3 to 6 minutes, initiating electrical waves that propagate with 3 $cm \cdot sec^{-1}$ in aboral direction[21].

2.2 Pathophysiology of disturbed gastro-intestinal motility

With GI motility being the result of a complex interaction between numerous mechanisms and pathways, as mentioned above, it stands clear that a pathologic process occurring in one of those systems potentially will have major consequences. Naturally, the more complex this interaction between systems is, the higher the risk of developing disease when failure in one of the pathways occurs. The digestive tract should therefore be considered as a vulnerable organ system, its motility being an efficient motor as long as the underlying mechanisms are thoroughly balanced.

When this balance becomes disrupted, the outcome will be a decreased or abnormal functioning of the intestinal motility, which can clinically manifest through a multitude of diseases, and likewise, be the result of a multitude of different pathogeneses. This can be explained by observing the experimental set-up of laboratory mice with mutations in the Kit-gene, the so called *W/W^v* mice. Those animals suffer from a mutation in the *white spotting (W)* locus which produces a white coat colour and sterility. Interestingly, it was found that their network of ICC in the *plexus myentericus* region was absent and, furthermore, extended defects in pacemaker activity and neurotransmission could be seen[23]. The Kit-gene codes for the Kit receptor tyrosine kinase and is expressed in ICC[24]. This Kit receptor is suspected to play a role as a signalling molecule in the development of ICC[23]. Hence, by creating Kit-negative mice the population of ICC was drastically reduced, resulting in the absence of slow waves[24,18]. This clinically caused abnormal intestinal contractions and a distended GI tract.

A reduction in ICC was also observed when studying the intestinal tract of horses suffering from *grass disease* (GD) or 'equine dysautonomia', showing significant reductions in ICC in both the ileum and *flexura pelvina* of horses suffering from GD, compared to normal animals[25,26]. Moreover, being primarily a polyneuronopathy, GD affects both the central nervous system and the ENS[26,27]. It causes degeneration of neurons in the *plexus myentericus* and submucosal Meissner's plexus, the severity of the disease varying, depending on the extent of the neuronal degeneration. In hyperacute cases, death occurs within two days, but in more 'chronic' patients (survival longer than 7 days), symptoms like colic and intestinal stasis (ileus), anorexia and weight loss can be observed. Most recent studies have postulated the role of *Clostridium botulinum* type C to be of major importance in the pathogenesis of GD[26,27].

Another disease associated with pathology concerned the ENS is called the *overo lethal white syndrome* occurring in Paint horses. This disease is the result of an autosomal inherited genetic defect, causing an unsuccessful migration of both melanocytes and neuronal cells during embryogenesis of the foal. *Post partum*, the foals will be seen mostly white (due to failed migration of the melanocytes from the neural crest), accompanied by myenteric and submucosal *aganglionosis* (due to failed migration of the neuronal crest cells), resulting in an underdeveloped and dysfunctional intestinal tract, which will lead to neurogenic functional obstruction[28,29].

In case of the formerly introduced phenomenon of *ileus*, the clinical situation of inhibited propulsive intestinal activity[2], a number of different disorders and underlying factors can be identified. One of the more frequently encountered forms of ileus, occurring after surgery (*post-operative ileus* or POI), is suggested to be associated with inflammation, neuronal sympathetic activation, and endotoxins[30]. Handling the intestines when performing a laparotomy, for example, potentially inhibits GI motility and causes acute POI[31,32]. The role of sympathetic stimulation in this process has been elucidated in studies that were able to prevent the developing of acute POI by sympathectomy in rat models[30,31].

In longer-lasting cases of POI, inflammatory infiltration is suggested to play a greater part than sympathetic stimulation, as the blocking of noradrenergic transmission in prolonged ileus does not relieve the disorder[31]. The activation of resident macrophages and the developing of a neutrophil-based inflammatory reaction are suggested to be responsible for this longer-lasting POI.

Finally, the endotoxin lipopolysaccharide (LPS) is a component of the outer membrane of Gram-negative bacteria. It causes a disruption of GI motility in horses and may thereby play a role in the occurrence of POI[30,33], apart from the fact that endotoxaemia following surgery of the intestinal tract is a common complication, due to contamination by the enteral microflora. LPS had been suspected to disrupt the gastric emptying and GI motility by the upregulation of nitric oxide synthase in resident macrophages[34]. NO is known to be an inhibitory neurotransmitter in the intestinal system, causing smooth muscles to relax.

In all the various conditions of abnormal GI motility it is of great importance to the equine practitioner to be able to intervene in the pathogenesis of the disease, or at least to improve the patient's health condition and decrease the symptoms with proper pharmacotherapy. This is where the practitioner benefits from the use of prokinetics and spasmolytics. Prokinetics, or agents that facilitate or enhance the net movement of feed material down the length of the intestinal tract[6], can be used when a patient's GI motility needs to be stimulated. Spasmolytics, on the other hand, decrease the propagation of digesta through the GI tract by reducing motility. Both groups of agents will be discussed later on in this review, but for a proper understanding of their mechanisms (when known), a short overview of the literature considering enteral receptors will first be provided. After all, several receptor systems have been observed so far to play a role in GI motility, and to be capable of functioning as targets for prokinetic and spasmolytic agents in pharmacotherapy.

2.3 Receptors

Prokinetic and spasmolytic drugs interact with receptors located in the GI tract and execute their therapeutic effects by acting as an agonist or antagonist (for example, a certain prokinetic can fulfil its stimulating effect on motility by either agonist activity on a stimulatory receptor, or by antagonist activity upon an inhibitory receptor).
In order to be able to understand the mechanisms of action of the prokinetics and spasmolytics that will be discussed later on, a brief overview of the respective receptors on which the medicaments act will first be presented in the following paragraphs.

2.3.1 Cholinergic receptors

Acetylcholine (ACh) acts as a neurotransmitter not only in the neuromuscular junction, but also in all preganglionic transmissions (both sympathetic and parasympathetic) and in parasympathetic postganglionic synapses[13]. In these synapses, ACh is supposed to bind the ACh-receptor, of which the existence of 2 subtypes is known: first, we distinguish the nicotinergic receptor (N-cholinoreceptor), to be divided in the N_N-receptor (for 'neuron', present in autonomic ganglia) and the N_M-receptor (for 'muscular', present in neuromuscular junctions). Secondly, we identify the muscarinic receptor (M-cholinoreceptor), of which 5 subtypes (M_1-M_5) are presently known.

Despite the major role of ACh as a neurotransmitter, not only in the intestinal tract but generally in the nervous system, there is little information available concerning the expression of cholinergic receptors in the GI tract of horses specifically. A study considering the expression of muscarinic receptors in circular smooth muscles of the colon in dogs provided evidence of the simultaneous presence of both M_2- and M_3-cholinoreceptors in the canine colon, with M_2 being the most predominant subtype (in a 4:1 ratio with M_3)[35]. Earlier studies in rats and man came to the same conclusions, attributing a role to both M_2- and M_3-cholinoreceptors in GI tract smooth muscle contractility, with ratios generally corresponding with the 4:1 mentioned above, and with the muscarinic M_2 believed to support contraction indirectly by inhibiting the relaxant effects of adrenergic stimulation[36,37]. Nonetheless, a possible interaction between the M_2- and M_3-cholinoreceptor subtypes, together with a lack of adequately specific muscarinic antagonists for the different subtypes, make it difficult to exclude the role of other muscarinic subtypes (M_1, M_4) in smooth muscle contractility in the GI tract[38]. Hence, more research in horses is needed to acquire more information specific for the species.

2.3.2 Adrenergic receptors

Apart from the formerly mentioned cholinergic enteric innervation, the GI tract is under control of sympathetic adrenergic neurons as well. Their effects after stimulation – typically sympathetic – result in vasoconstriction of the arteries supplying the intestinal region, reduction of the aboral propagation of digestive in the intestinal lumen, and lastly, inhibition of secretion of fluids and electrolytes to the enteral lumen[39].

Currently, nine subtypes of adrenergic receptors are commonly accepted to exist, and they can be divided at first in two subfamilies: α-adrenoreceptors (with subtypes α_1 and α_2) and β-adrenoreceptors (with subtypes β_1, β_2 and β_3). In most recent data, a further division in both the α_1- and α_2-subtype has been established[40], resulting in the subtypes α_1-A-, α_1-B-, and α_1-D-adrenergic receptor for the α_1 subfamily, whilst, on the other hand, a division in subtypes α_2-A-, α_2-B-, and α_2-C-adrenergic receptor has been postulated regarding the α_2-subfamily. The adrenergic receptors are G-coupled receptors and found in both nervous and nonnervous tissues[41].

The catecholamines noradrenaline (or norepinephrine, NOR) and adrenaline (ADR or epinephrine, synthesized from NOR in the medulla of the adrenal gland, their precursor being dopamine) are considered the major neurotransmitters in the sympathetic neuronal system, especially NOR, as it is the neurotransmitter in most of the sympathetic postganglionic synapses[13]. Furthermore, in contrast to adrenaline (which is capable of binding all subfamilies of adrenergic receptors), NOR is known to prosecute a more specific receptor selectivity, as it does not bind β_2-receptors, but can bind both the α_1- and α_2-subtype[13].

The exact distribution and expression of adrenergic receptors in the equine hindgut has been limitedly investigated. However, efforts have been made in more recent studies to investigate the distributional pattern of the adrenergic receptor throughout the GI tract. According to one study in the equine ileum[42], there is a rather large percentage of neurons in the ileal submucosal plexus (Meissner's plexus) expressing β_2-receptors (95%), whereas the percentage of neurons in the *plexus myentericus* was found to be very low (9%). Compared to laboratory species, the percentage of submucosal β_2-

expression in horses (95%) is impressively high (percentages differ from 26% in the rat to 36% in guinea pigs[41]).

Additionally, it has been postulated that post-synaptic α-adrenoceptors in the intestinal tract belong mostly to the α_1-subtype[43], whilst α_2-receptors would be located mainly pre-synaptically on cholinergic neurons of the *plexus myentericus*, thereby responsible for inhibition of ACh release upon activation[44]. The negative influence on GI motility that can be observed when using α_2-agonists (for example xylazine, romifidine, detomidine) is considered to be a side effect as a consequence of this mechanism (spasmolytics are reviewed in chapter 4, see below)[45].

2.3.3 Serotoninergic receptors

Serotonin (5-hydroxytryptamine, 5-HT) is regarded to be one of the oldest neurotransmitters. Its influence can be observed in different organ systems throughout the body, as its 5-HT receptors are distributed not only in nervous tissues (both in the central and peripheral nervous system), but can also be found in nonnervous tissues, like the intestinal tract and the cardiovascular system[46].

The classification of 5-HT receptors has proven a task of long breath, as our knowledge is still developing, and with a rather rapid pace, especially since the 1990's. This enhancement of our understanding in regard to the 5-HT receptors cannot be mentioned without appreciating the parallel development of molecular tools, giving researchers more possibilities to unravel the differences between the receptor subtypes and elucidate more precisely the patterns of distribution and levels of expression of the receptors in a tissue of interest. Since 1994, when the current nomenclature and classification was postulated[47], numerous adaptations have been made in order to measure up to the most recent discoveries.

Currently, the serotoninergic receptor family consists of at least 14 receptors, divided in several groups (5-HT_1 to 5-HT_7). Advancements in molecular research caused a further division of the groups based on biological criteria (ligand binding and second messenger systems)[46], leading to an even more complex classification system nowadays.

Excluding the 5-HT_3 group, which consists of a ligand-gated ion channel, all 5-HT receptors belong to the G-protein coupled receptor (GPCR) superfamily (in compliance with the adrenergic receptor, see above)[46].

With regard to the intestinal tract, the serotoninergic receptors 5-HT_3 and 5-HT_4 seem of primarily importance and are currently considered the receptors with the highest potential for therapeutic intervention. Studies in humans have shown a peripheral distribution of 5-HT_3 in both pre- and post-ganglionic autonomic neurons, regulating motility as well as luminal secretion throughout the entire GI tract[48]. In addition, several 5-HT_4 isoforms seem to be present in the human intestines, with 5-$HT_{4(b)}$ in specific presumed to be exclusively present in the GI tract[48]. Both 5-HT_3 and 5-HT_4 receptors have been found to play a role in the peristaltic reflex[49], but 5-HT_4 is likely to be of major importance considered GI motility[49,50].

A study in horses investigated the expression of 5-HT_4 receptors in ileum, duodenum and *flexura pelvina*, and results indicated all these regions to be positive for the receptor[50]. The 5-HT_4 receptors were detected in both the circular and the longitudinal muscle layer of the *tunica muscularis* in the

horse, but were not present in the myenteric plexus. This outcome is in contrast with earlier studies in laboratory species and human. Researchers found 5-HT_4 receptors to be present in enteric neurons (myenteric and submucosal ganglia) both in guinea pig small intestine and in rat and mice colon[51]. Additionally, another study detected 5-HT_4 receptor expression in the colonic myenteric plexus of both guinea pig and human, although the receptor density in human colon was not as high as in guinea pigs[52].

Furthermore, it is known that presynaptic 5-HT_4 receptor activation facilitates the propagation of fast excitatory action potentials (thereby increasing the release of ACh), whereas presynaptic 5-HT_{1A} receptors (found in the intestinal tract of guinea pigs) mediate inhibition of these fast excitatory potentials[51,53]. Next to this, a recent study with circular smooth muscle preparations of equine jejunum, revealed the presence of 5-HT_{1A} receptors in both muscle layers of the intestinal wall and at the level of the mucosal villi[54]. This suggests that, next to a contractile role of 5-HT_{1A} receptors in the horse's jejunum, 5-HT could be involved in regulating villus motility.

2.3.4 Dopaminergic receptors

Dopamine is not only a precursor of catecholamines in the adrenergic system (see above), but acts as a neurotransmitter itself as well, with functions both in the central and peripheral neuronal system[55]. Until now, at least 7 genetically distinct receptors for dopamine have been elucidated: D_1, D_{1C}, D_{1D}, D_2, D_3, D_4 and the D_5-receptor[56]. As for the α- and β-adrenoceptors, the dopaminergic receptors belong to the G-protein coupled receptor superfamily.

Next to this, a further division into 2 subfamilies is generally made: first the D_1-like subfamily (consisting of D_1- and D_5-receptors), because activation of these receptors stimulates adenylate cyclase, and on the other hand a D_2-like subfamily (D_2, D_3 and D_4), responsible for inhibition of adenylate cyclase upon receptor activation[56]. Consequently, dopamine receptor agonists can produce inhibitory (the relaxation or inhibition of contractions) and excitatory effects (increased contractions, observed less frequently) on GI motility throughout the full length of the intestinal tract[57], as the classical receptors D_1 and D_2 are both present in the intestinal tract. The D_1-receptor is mainly located on the effector cells (post-junctionally), whereas the D_2-receptor is present both pre- and post-junctionally[58]. In case of the pre-junctional D_2, a negative modulatory effect has been observed on ACh release from intrinsic cholinergic nerve terminals. Evidence postulating dopamine to play a role in GI motility control can also be inferred from the observation that dopamine receptor antagonists are capable of affecting the intestinal motor activity from stomach to colon[55] (see below, chapter 3 'Prokinetics' for more information).

2.3.5 Motilin receptors

Motilin is a 22 amino-acid peptide, found in the human body to be synthesized in specific endocrine cells in the epithelia of the upper small intestine, especially the jejunum and duodenum[59,60]. The peptide is released in humans after eating, by drinking water and during faster[59]. It can be regarded as an agent of importance during the interdigestive state, and in humans it is thought to induce the premature phase III contractions in the stomach during this period[60]. Beside this, other generally

accepted functions of motilin are stimulation of enzyme secretion in the stomach (in dogs, it was shown that gastric secretory and motor increase, for instance increasing levels of pepsin secretion, were at least partly regulated by motilin[61]), and stimulation of pancreas secretions[60].

Motilin receptors belong, together with ghrelin receptors (growth hormone secretagogue receptors[62]), to the same subfamily of the GPCR superfamily[59]. Motilin and ghrelin do also share some strong structural similarities, and, together with complementary observations adequately outlined in other studies[59,63], there are indications for a possible evolutionary linkage. Nevertheless, ligands of both the motilin and ghrelin receptor are not recognized by each other, according to studies using rabbit and human receptors[59,62].

Another notable phenomenon considered the motilin receptor is the observation that the intestinal tracts of mice and rats seem refractory to motilin stimulation[64], due to the absence of a functional motilin system in rodents[59,63]. A distinct species-dependent functioning of motilin is thus thought to be plausible. Research in horses has provided evidence that in this species the motilin receptor is present and can be successfully stimulated (for example by the antibiotic erythromycin, see 3.5).

2.3.6 Opioid receptors

Opioids have been used for centuries in human therapy as an antidiarrheal drug, and are also commonly known for their potent analgesic properties[65]. By inducing neuronal hyperpolarization, they indirectly inhibit ACh release from nerves that innervate smooth muscle cells, thereby causing decreased GI motility[3,66]. Morphine, as an opioid agonist, delays gastric emptying and decreases peristaltic activity, for example[66]. In humans, apart from antimotility effects, inhibition of intestinal ion and fluid secretion, and increasing intestinal fluid absorption were found additional effects upon opioid receptor stimulation[65,67]. These findings seem consistent across species, but extrapolation has to be done with caution, as the opioid agonist morphine is known to induce significant differences in behaviour between species – from excitation to sedation[68]. In horses, morphine administration in single dose has been observed to suppress borborygmus, to increase intestinal transit time and to decrease faecal water content and volume of faeces produced[66]. In contrast to this, a further interesting finding is the increase in contractile tone of small intestinal smooth muscles[69], possibly responsible for the abdominal discomfort observed in horses after prolonged parenteral morphine administration (0.5 $mg \cdot kg^{-1}$ every 12 hours, for 6 days[66]).

Three primary opioid receptor types μ, κ and δ, are responsible for the action of opiates and opioid peptides (the endogenously released opioids, being encephalins, endorphins and dynorphins) in the GI tract[68,69]. For the intestines, all three types of opioid receptors are thought to play a role in decreasing the intestinal transit time in humans, whereas in rat it is confirmed that both μ- and δ-receptor, but not κ-receptors, are responsible for inhibiting intestinal propulsion[65,66].

The hyperpolarization of enteral neurons by opioid receptor agonist binding can be evoked in excitatory as well as in inhibitory neurons in the GI tract[67]. Firstly, suppression of excitatory pathways inhibits the release of excitatory neurotransmitters like Ach, leading to blockage of peristaltic contractions. Contrarily, blocking of inhibitory pathways results in decreased release of non-adrenergic, non-cholinergic substances (NANCs) like nitric oxide, adenosine 5'-triphosphate (ATP)

and vasoactive intestinal peptide (VIP), ultimately leading to an increase in resting muscle tone and non-propulsive motility patterns[67,69].

With regard to the stomach, studies in humans and laboratory species show the μ-receptor to be the predominant receptor type in slowing the gastric emptying[70]. This slowing is associated with an increased tonic contraction in the *antrum pyloricum* and the pyloric sphincter itself in humans[69].

Given the strong influence of opioids (whether exogenously administered by an equine practitioner to ensure analgesia, or endogenously secreted after surgery) on GI motility, opioid receptor antagonists have been popular subjects of investigation, in order to gain more insights in their pharmacokinetic and pharmacodyamic properties and their potential to function as prokinetic drugs. The literature considering this research subject will be discussed in the next chapter.

3. Prokinetics

Being able to support the patient with an adequate symptomatic therapy is of great importance to the equine practitioner encountering horses with GI motility disorders, like ileus. As always, one should not forget to try elucidating the primary aetiology of the disease, but meanwhile, supporting the patient and reduce the symptoms associated with the disease – in case of this review, gastro-intestinal motility disorders – should be started immediately.

As mentioned earlier, prokinetics are defined as agents that facilitate or enhance the net movement of feed material down the length of the intestinal tract and do not simply produce an uncoordinated increase in local contractile activity. This can be achieved either by augmenting the pathways that stimulate motility, or by attenuating the inhibitory neurons that predominantly suppress activity[6]. The following chapter will discuss the most recent literature concerning investigations on these drugs that, whether in group or individual, are known to possess these prokinetic characterizations and are (potentially) of use to the equine practitioner in the field. **Appendix I** will furthermore provide in a table that summarizes the characteristics and properties of the prokinetics that will be discussed here.

3.1 Parasympathicomimetics

With ACh binding on presumably (see above) M_2- and M_3-cholinoreceptors in the GI tract, the cholinergic excitatory pathway is induced and GI motility is enhanced. The cholinergic pathway can be activated either directly by increasing the amount of ACh (or ACh agonist) in the neuromuscular synapse, or indirectly by slowing its enzymatic degradation, which can be achieved by reducing or neutralizing the acetylcholinesterase-enzyme.

3.1.1 Direct cholinergic agonists

Bethanechol (BeCh, **Figure 3**) is a methyl derivate of carbachol and acts as a ACh receptor agonist in the whole GI tract[38]. Being a parasympathicomimeticum, side effects are to be expected and include

hypersalivation and abdominal discomfort[21]. It is needless to say, therefore, that the use of cholinergic prokinetic drugs in cases like colic should be considered with caution.

Upon binding the M_2- and M_3-cholinoceptors, BeCh induces a significant, concentration-dependent increase in smooth muscle contraction, as demonstrated in *in vitro* preparations of duodenum, jejunum, caecum and *flexura pelvina* of the horse. In this specific study[38], it was observed that binding of a M_2-receptor antagonist (AF-DX 116) after high dose treatment resulted in a rightward shift of the BeCh response curve, suggesting a competitive inhibition at the M_2-receptor. Contrarily, a M_3-receptor antagonist (4-DAMP) blocked the parasympathetic action of BeCh almost completely, leading to the opinion that binding at the M_3-receptor is of a non-competitive pattern and of irreversible nature, as is postulated. Secondly, the role of the M_3-receptor in mediating the effect of BeCh seemed larger than the M_2-receptor's[38,71]. A more recent study[72] on the influence of BeCh on *in vitro* smooth muscle preparations of duodenum and jejunum of healthy dairy cows revealed observations consistent with the results obtained from equine intestinal preparations. Additionally, both studies are complementary with the data observed in human *duodenum descendens* and rectum after subcutaneous BeCh administration[73].

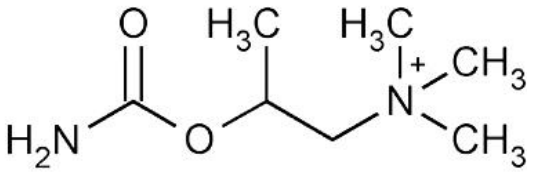

Figure 3. *Chemical structure of BeCh.*

In rat antral circular smooth muscle strips, it was shown that BeCh induced a dose-dependent contraction, through activation of M2- and M3-receptors coupled to pertussis-toxin sensitive GTP-binding proteins[74]. The presence, though, of a residual cholinergic contractile response after treatment with nifedipine (a L-type calcium channel blocker) was in this study suggestive for the involvement of an additional pathway. BeCh treatment to mice showed a progressive concentration-dependent increase in muscle contractility of longitudinal smooth muscle preparations of jejunum and ileum as well[75].

In regard to the horse's stomach and caecum, several studies have postulated an accelerating effect of BeCh on gastric and caecal emptying[38]. BeCh is commonly used in the treatment of foals in cases of duodenal strictures, in order to enhance gastric emptying[76].

The recommended therapeutic dose of BeCh is 0.02 $mg \cdot kg^{-1}$ subcutaneously (each 4-6 hours), followed by 0.35 $mg \cdot kg^{-1}$ orally, every 8 hours. Subsequently, it should be mentioned that certain researchers postulate that the combination of BeCh and yohimbine (an α_2-antagonist, used as an antidote) would be more effective than the administration of BeCh alone[77]. The clinical usability of this finding, however, is unclear.

3.1.2 Indirect cholinergic agonists

Neostigmine enhances GI motility by acting as a acetylcholinesterase-inhibitor, thereby facilitating prolonged concentrations of ACh in the neuromuscular junction. Generally, it is given in doses of 0.022-0.044 $mg \cdot kg^{-1}$ subcutaneously or intravenously, preferably every 2-4 hours, and doses can be increased by 2 mg to a total quantity of 10 mg per treatment when lacking a sufficient response[6]. A commonly known side effect in horses is abdominal discomfort shortly after treating[21].

An *in vivo* study using neostigmine constant-rate infusion (CRI) in horses showed increased faecal production and urination frequency, and gastric emptying rates were not decreased[78]. This outcome is in contrast with an older study, performed in 1985, that observed a delayed gastric emptying after neostigmine administration in horses[79]. Indeed, the opinion that neostigmine rather executes an inhibiting effect on GI motility in the proximal part of the tract, seems generally accepted[21]. As for BeCh, it is recommended to avoid administering the drug in cases where the inhibiting effect on the stomach and proximal intestinal tract could worsen the patient's health condition – although seeing the horse defaecating after neostigmine treatment will give the practitioner the false impression of a positive therapeutic effect in those cases[5].

An *in vitro* study was performed on smooth muscle strips obtained from equine's jejunum and pelvic flexure, and neostigmine was found to enhance contractile activity here in a concentration-dependent manner[78]. Of further interest in this study was, firstly, the observation that both atropine and DAU 5884 (being a nonspecific muscarinic M_1-M_5 receptor antagonist and a specific M_3 receptor antagonist, respectively) blocked the effect of neostigmine completely, suggesting that neostigmine, either directly or via ACh, acts on the M_3-cholinoceptor.

Furthermore, smooth muscle preparations were treated with neostigmine after incubation with edrophonium, a reversible acetylcholinesterase-inhibitor[78]. As a high dose of edrophonium (10^{-4} M) was used, a total blockage of the enzyme would be expected, leading to prevention of an increased contractile response in the muscle strips as a reaction to neostigmine. Surprisingly, the muscle strips did respond, although to a lower, insignificant, level. More elaborate research on this subject needs to show whether this observation can be attributed to edrophonium only partially blocking the enzyme, or to neostigmine having multiple mechanisms of action.

3.2 Benzamides

Among this group of prokinetic drugs are metoclopramide (one of the first benzamides discovered), domperidone, and newer agents like cisapride, mosapride and tegaserod. These drugs have 5-HT (serotonin) agonist activity, with the exception of the older drug domperidone, that acts on the dopaminergic D_2-receptor in the GI tract and therefore will be discussed later (see 2.3.4 'Dopaminergic antagonists').

The other agents are known to execute their effect by increasing ACh release in the intestinal tract and enhancing GI motility via 5-HT_4 receptor stimulation in the *plexus myentericus*[80,81]. The agents that will be discussed in this paragraph show different receptor selectivity and, therefore, differ significantly from each other with regard to their side effects. In general, it can be said that the more selective the agent for a certain receptor, the less side effects are expected to occur.

3.2.1 Metoclopramide

Metoclopramide (**Figure 4**) is a 5-HT_4 agonist and 5-HT_3 receptor antagonist and has, simultaneously, dopamine (D_2) receptor antagonist properties, resulting in enhanced GI motility[55,80]. The

antidopaminergic effect is unfortunately not limited to the intestinal tract (where inhibition is evoked upon both the neuronal and muscular dopamine receptors[55]), for metoclopramide is capable of penetrating the blood-brain barrier as well. By doing so, it can influence the central D_2 receptor in the brain, hence resulting in severe central side effects such as extrapyramidal symptoms (like tremor and muscular spasms, agitation and aggression)[82].

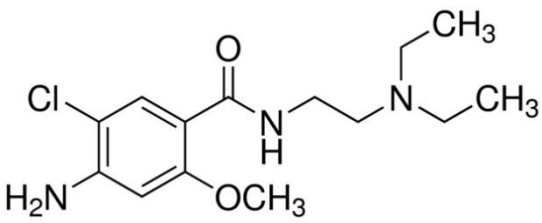

Figure 4. *Chemical structure of metoclopramide.*

Researchers have suggested that serotoninergic prokinetics (cisapride, mosapride) may be superior in prokinetic effects to antidopaminergic agents, and that the serotoninergic component of antidopaminergic drugs, like metoclopramide, might enhance their prokinetic efficacy in the gastro-intestinal tract[55,83,84].

According to a Japanese study in horses, metoclopramide (0.2mg·kg^{-1}, per os) did improve jejunal motility, but there was no effect on caecal motility, in contrast to other benzamide agents like mosapride (see below)[55]. Furthermore, when evaluating gastric emptying in this study, the researchers found metoclopramide to have a lower t_{max} (the time needed for $^{13}CO_2$ in the breath test to reach a peak) than the control group, suggesting an enhancing effect of metoclopramide on gastric emptying. Complementary to these results, another *in vitro* study on equine smooth muscle strips derived from the *antrum pyloricum*, proximal duodenum and mid-jejunum, showed a significant dose-dependent increase in contractile amplitude of the muscle strips in all three locations, caused by metoclopramide[85]. An interesting finding here, is the observation that lower concentrations of the drug were needed in the proximal parts of the GI tract to obtain a response (10^{-9} *M* in the pyloric antrum, versus 10^{-5} *M* in the mid-jejunum), this because metoclopramide is thought to work by restoring gastroduodenal coordination[85].

With regard to gastric emptying, it can be mentioned that in ponies, researchers found metoclopramide to have enhancing effects on gastric emptying, too[86]. This effect was detected using the acetaminophen-test, and the results showed a reduction in T_{max}, but not in C_{max}, when using metoclopramide, compared to the control (treating a pony with saline).

Finally, when reviewing the drug in light of motility disorders in the horse, like POI and colic, the question arises whether metoclopramide in equine practice can be considered a reliable drug of therapy. The agent has indeed been observed effective in cases of POI, both naturally occurring and experimentally induced[21,85,86]., and has been successful in countering experimentally induced colic, as well[86]. Nevertheless, its capacity to cross the blood-brain barrier and cause severe central side effects should urge equine practitioners to use this drug with caution.

Recommended dosages include 0.125-0.250 mg·kg^{-1}, diluted in 500 mL polyionic solution to be infused slowly (over 60 minutes)[82]; 0.05 mg·kg^{-1} (IM, 4 times per day); 0.1-0.25 mg·kg^{-1} (SC, 3 or 4 times per day) or 5 mg·kg^{-1} (PO, 4 times per day)[21].

3.2.2 Cisapride

Cisapride is a substituted piperidinyl benzamide with serotoninergic 5-HT_4-receptor agonist activity and 5-HT_3-receptor antagonist activity, thereby stimulating the release of ACh from postsynaptic

neurons in the ENS, and by doing so, indirectly enhancing GI motility[87]. This prokinetic activity has been mainly attributed to the agonistic activity upon the 5-HT_4-receptor, rather than cisapride's antagonistic activity on the type 3 serotoninergic receptor.

When compared to metoclopramide, cisapride can be regarded as a more potent prokinetic, for it has broader enhancing effects on contractile activity of colon, oesophagus, stomach and small intestines[82,87]. More interesting, however, is the fact that cisapride has no, or very limited, antidopaminergic activity (unlike metoclopramide), and does not cross the blood-brain barrier[82,87]. Hence, no antiemetic activity should be expected, nor any extrapyramidal effects leading to side effects that can be observed when using metoclopramide (see above).

When introduced in the 1990's, cisapride seemed a promising new prokinetic: being more potent than metoclopramide, but without the severe pyramidal complications – many regarded cisapride as the prototype prokinetic agent. Nonetheless, it was withdrawn from the market in 2000 after numerous reports of cardiac events: cisapride was shown in humans to cause prolonged QT intervals, ventricular tachyarrhythmia, *torsades de pointes*, ventricular fibrillation and sudden death[80,87,88]. The prolongation of cardiac action potential repolarisation (and thus, the QT interval) can be attributed to the blockade of hERG-encoded K^+-channels by cisapride[80,89].

Studies in horses, specifically, showed improved jejunal motility following cisapride administration (1.0 $mg \cdot kg^{-1}$), but no effect on caecal motility or gastric emptying was seen[80,90]. Another *in vitro* study evaluated jejunal smooth muscle strips as well, and results were partly consistent[91]: a concentration-dependent increase in contractile activity of the jejunal preparations was seen, but this effect persisted when the strips were incubated with atropine (a parasympathicolyticum) prior to the cisapride administration, suggesting that the increase in contractility was, at least partly, of a noncholinergic nature. An *in vivo* study concerning gastric emptying rates in horses, showed no increase after cisapride treatment, but pre-treatment of the horses with cisapride did attenuate the delay in gastric emptying induced by endotoxin treatment[92].

Cisapride has proven in the past to be effective ($0.1 mg \cdot kg^{-1}$ IM, every 8 hours) in reducing the incidence of POI and accelerating the restoration of bowel motility after surgery of the small intestines[93].

Although cisapride has been taken off the market, it is still available for veterinary use. A suggested dose regimen in horses is $0.1 mg \cdot kg^{-1}$ PO, every 8 hours[82].

3.2.3 Mosapride

Mosapride is a selective 5-HT_4 receptor agonist, known to stimulate receptors in the *plexus myentericus* of the equine intestinal tract, thereby increasing ACh release from cholinergic postsynaptic neurons[3,80,94]. In horses suffering from disturbed GI motility, indications for clinical treatment are reflux oesophagitis[95], chronic gastritis, and POI[94]. A major advantage of mosapride is the fact that it does not interact with central dopaminergic receptors (like metoclopramide), and furthermore, has not been observed to cause the severe cardiac events, like cisapride, either.

The stimulating influence on GI motility of mosapride has been demonstrated by several studies in horses. For instance, an *in vivo* study indicated that an increase in electrical activity occurred after oral

administration of mosapride (1.0, 1.5 and 2.0 mg·kg^{-1}) in both the horse's small intestine (127.0 ±12.5%, 137.7 ±22.2% and 151.1 ±24.0%, respectively) and the caecum (130.1 ±34.5% and 151.6 ±45.2% for 1.5 or 2.0 mg·kg^{-1}, respectively)[94]. The same author performed an *in vivo* study in adult Thoroughbreds and reported improved jejunal motility and enhanced gastric emptying (after mosapride administration at doses of 1.0 and 2.0 mg·kg^{-1} per os), as well as increased caecal motility (at a dose of 2.0 mg·kg^{-1})[80]. In correspondence with these results regarding gastric emptying are data obtained from an earlier *in vivo* experimental set-up by the same author, suggesting indeed a facilitating effect of mosapride on gastric emptying[96].

Mosapride has been reported to attenuate the negative influence of abdominal surgery on GI motility. When performing a laparotomy with jejunocaecostomy in healthy Thoroughbreds, all horses showed decreased electrointestinography maximum amplitudes in small intestine and caecum on postoperative day 1, but the horses that were treated with mosapride (1.5 mg·kg^{-1} in 1L water via nasogastric intubation, once daily) were observed to have significantly higher maximum amplitudes in both the jejunum and the caecum, from postoperative day 6 to 31, than the control group[97].

Mosapride has been proven to have a $t_{1/2}$ in horses twice longer than in man. Therefore, it is thought suitable to reduce the number of doses a day in horses to half[98]. At this point, a dose regimen to be recommended seems 0.5 mg·kg^{-1} for gastric indications, and 1.5 to 2 mg·kg^{-1} in the caecum (once or twice daily)[98], but further research to establish optimal dosage regimens is still needed.

3.2.4 Tegaserod

Tegaserod (or HTF 919 in literature, **Figure 5**) is a selective 5-HT$_4$ receptor agonist, like mosapride, and has been revealed to promote propulsive motility in both the upper and the lower GI tract in several species[99]. More specifically, the drug is known to be a partial 5-HT$_4$ receptor agonist, with negligible affinity for 5-HT$_3$ receptors[100].

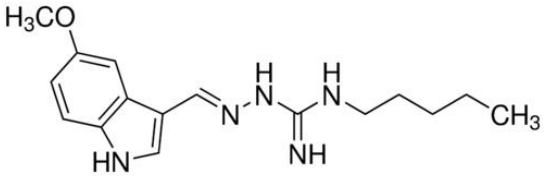

Figure 5. *Chemical structure of the selective 5-HT$_4$ receptor agonist tegaserod (HTF 919).*

Tegaserod has been reported to be effective in treatment of 'irritable bowel syndrome' in humans, and in horses, preliminary studies have indicated a promoting effect on GI motility in horses as well, but in dogs, a significant acceleration of small intestinal transit could not be observed *in vivo*[100]. A recent *in vivo* study in horses confirmed accelerating influence of tegaserod on intestinal motility, by treating with barium-filled spheres and measuring transit time, frequency of defaecation and gut score sounds, parameters which all three increased after treatment[99]. In this particular study, dosage of 0.02 mg·kg^{-1} was used, and none of the horses participating showed side effects or abnormal behaviour, suggesting tegaserod to be a safe and tolerable drug at this dose. Unlike cisapride, did tegaserod not induce prolonged QT intervals when investigating its effects on the heart after therapeutic dosage (0.1 to 10 µM) in rabbits, thus suggesting a safer cardiac profile than cisapride[101]. Nonetheless, due to suspected higher risk of heart attack in humans, it was requested to be withdrawn from the market in 2007[3]. It is, however, still limitedly available for veterinary practice.

3.3 Dopaminergic antagonists

Domperidone is a dopaminergic antagonist and structurally related to the butyrophenones. It has special affinity for the D_2-receptor subtype, present both central and in the periphery (including GI tract) of the neuronal system[102]. In contrast to metoclopramide, which crosses the blood-brain barrier easily, does domperidone cause minimal central extrapyramidal side effects, for it interacts only slightly with central dopaminergic receptors.

Studies in humans indicate that domperidone's prokinetic activity can be attributed to its ability to increase the amplitude of oesophageal motor function, to enhance antral-duodenal contractions, and better coordinated peristalsis across the pylorus region with subsequent acceleration of gastric emptying[102]. Influence on small intestines, beyond duodenum, and on the human colon could not be observed. In humans, therefore, it is often used as an antiemetic and to treat dyspepsia, gastroparesis, and gastro-oesophageal reflux disease[102,103].

Unfortunately, very little clinical information is available about domperidone's pharmacokinetics in horses. In one *in vivo* study, oral administration of the drug at 1.1 $mg \cdot kg^{-1}$ and 5.5 $mg \cdot kg^{-1}$ significantly increased laminar blood flow in the normal adult horse (starting 4 hours after treatment and up to at least 8 hours)[104]. A recent study used the same dosages to investigate, both *in vivo* and *in vitro*, the influence of domperidone treatment on gastric emptying and intestinal tract motility in horses: at a dose of 1.1 $mg \cdot kg^{-1}$ per os (PO), formerly observed to be effective in the treating of fescue toxicosis in pregnant mares[105], no effect was detected on gastric emptying rates. However, the higher dose of 5.5 $mg \cdot kg^{-1}$ PO significantly increased the *AUC* (area under curve) and C_{max} in the acetaminophen test, both parameters of the test that have been postulated to rise after administration of agents that increase gastric emptying. Furthermore, the *in vitro* set-up of the same study showed no effect on contractile response of both longitudinal and circular smooth muscle strips, obtained from equine duodenum, jejunum, ileum and colon (*flexura pelvina*)[104]. Additionally, domperidone was found to decrease the dopamine-induced contractile activity of smooth muscle strips in the mid-jejunum. Hence, more research is needed to elucidate the potential beneficial effects of domperidone *in vivo*, as well to obtain more knowledge in concern to its pharmacokinetic properties.

3.4 Sodium channel blockers

Lidocaine (or xylocaine, in Europe) is a drug frequently used in case of POI in the horse, although its mechanism of prokinetic action has not been completely discovered yet[6]. Potential pathways by which lidocaine executes its effect upon the GI tract, include suppression of sympathetic inhibitory nerves in the GI tract, anti-inflammatory effects, inhibition of free radical formation, reduction in circulating catecholamines, direct stimulation of the enteral smooth muscles, and attenuation of pain perception (by depressing spike activity, amplitude and conduction time of myelinated A fibres and unmyelinated C fibres)[106].

In horses, several studies have been performed to investigate the *in vivo* influence of intravenous (IV) lidocaine administration, but data seem not always consistent. This could be a result of studies using healthy horses instead of investigating the effect of lidocaine in clinically affected patients, suffering from GI motility disorders like POI. For instance, when treating healthy adult horses with 1.3 $mg \cdot kg^{-1}$ via infusion for 30 minutes, no effect was measured upon gastric emptying rates, small intestinal motility or caecal motility[80]. Another study observed increased *in vitro* contractile activity of the proximal duodenum in horses (suggesting lidocaine to be beneficial in case of duodenitis-jejunitis), but found no effect on contractility In the pyloric antrum or mid-jejunum[85].

Nonetheless, data obtained from several *in vivo* equine studies investigating the influence of lidocaine administration in truly affected clinical patients, do confirm the suggested prokinetic properties. This leads to the suspicion that lidocaine possibly influences the GI tract via indirect mechanisms, rather than via direct smooth muscle stimulation. Indeed, it was observed that horses suffering from POI or enteritis with severe reflux, benefited significantly from lidocaine IV administration, as the reflux in those horses stopped earlier and their hospitalization time was shorter compared to placebo treatments (0.9% NaCl, CRI for 24 hours)[107].

Of further interest in this regard, another study found data suggestive for a reduced risk of POI in horses when receiving lidocaine prophylactically[108]. Moreover, a beneficial effect upon survival rates was seen as well, thereby supporting lidocaine to be an effective prokinetic treatment after intestinal surgery.

Lidocaine has been shown to reduce secretion of inflammatory cytokines and to inhibit neutrophil function[109]. It is suggested that this inhibitory action upon neutrophil activation is likely to play an important role, like in ileus, in amelioration of reperfusion injuries[110]. For instance, it is known that flunixin meglumine (a nonselective cyclo-oxygenase (COX) inhibitor) retards repair of ischaemic-injured jejunum, and that this effect is ameliorated by simultaneous systemic administration with lidocaine[109]. In addition to this, another study investigated the effects of lidocaine intestinal contractility in ischaemic-injured smooth muscles[111]. Results showed an increase in lidocaine-stimulated contractility in the injured smooth muscles, compared to that of noninjured smooth muscle, and researchers have suggested a possible stimulating influence by lidocaine on basic cell function by cellular repair mechanisms. Furthermore, this study indicated an increase in basal contractility of the intestinal segment, but whether this influence can be assigned to ICC (responsible for spontaneous spike potentials, see 2.1.2), or to the smooth muscle cells themselves (thereby providing evidence for a direct activity of lidocaine upon smooth muscle cells in the GI tract) has yet to be determined[111].

The recommended dose regimen of lidocaine is, per-operatively, an IV loading dose of 1.3 $mg \cdot kg^{-1}$ of a 2% solution over 5-10 minutes, followed by CRI (0.05 $mg \cdot kg^{-1} \cdot min^{-1}$ in 1 L saline or Ringer's lactate), the full regimen then to be repeated post-operatively, with CRI for at least 24 hours[6,21,107]. One should be careful in order to avoid side effects like hyperhidrosis, tachypneu, and motoric dysfunction like ataxia, muscle twitching and collaps[21,107].

3.5 Macrolide antibiotics

Erythromycin has been characterized, apart from being an antibiotic, as a prokinetic agent in humans, dogs, cats, rabbits and horses[112]. The macrolide exerts this prokinetic action by direct stimulation of the motilin receptor in the GI tract[112]. It has been found that to manifest motilin agonist activity, the chemical structure of a macrolide has to meet certain requirements[60], and this has led to efforts trying to synthesize macrolide derivatives more potent than erythromycin in motilin activity, yet with less antimicrobial activity, which is desirable, for treatment with a prokinetic agent should be possible for several days to weeks without negatively affecting the intestinal microflora.

Low doses of erythromycin stimulate intestinal motility by initiating the MMC and by promoting propulsive peristalsis[85]. It was shown *in vitro* in horses, that erythromycin increased the contractile amplitude of longitudinal muscle strips from the *antrum pyloricum*, as well as that of muscle strips in the mid-jejunum[85]. Interesting results were obtained from an *in vivo* study in horses that underwent surgery to implant electrodes in smooth muscles of ileum, caecum and pelvic flexure, in order to measure the response to erythromycin administration on several occasions (post-operatively, and at least 8 days later, 'post-recovery'). The results showed a significant effect of the macrolide on myoelectric activity, but the effects were not the same when comparing post-operative data and post-recovery data, suggesting that prokinetic effects should be extrapolated carefully between healthy and diseased animals[113]. A conclusion of this study could be, that prokinetic activity by erythromycin could be evidenced at least for the colon[113].

The possibility of developing antibiotic-associated colitis after erythromycin administration is one of the concerns when using the drug as a prokinetic. Next to this, side effects include mild abdominal discomfort and passage of soft faeces within 5-10 minutes of commencement of infusion[3]. One should furthermore keep in mind, that slow, long-term infusion (for example, 60 minutes) can induce down regulation of motilin receptors, resulting in a decrease in myoelectric activity. It is therefore recommended to administer mainly low, subtherapeutic (non-microbial dose) boluses[21].

3.6 Adrenergic antagonists

Given the inhibiting effects on GI motility of adrenergic receptors in the enteral tract, it is expected that adrenergic receptor antagonists cause an attenuation of this inhibition. However, several studies have revealed surprising and unexpected results in this regard, giving rise to more and new questions. As it is suspected that adrenergic hyperactivity might play a role in the complex mechanism of triggering POI in horses[114], it is important to gain more insight into the role of the adrenergic system in the equine GI tract.

3.6.1 Yohimbine, atipamezole

α_2-agonists are very common in equine practice, due to their application as a safe sedative drug. Agents like xylazine, romifidine and detomidine are frequently administered in numerous situations

where sedation is required. In this concern are *yohimbine* and *atipamezole*, both antagonists of the adrenergic α_2-receptor, considered to be antidotes of the sedative agents, in case of toxic overdosing. Yohimbine can be regarded as a nonspecific antagonist, whereas atipamezole is highly selective for the α_2-receptor.

However, data obtained from a recent study showed interesting findings. In this *in vitro* study to the effect of α_2-agonists and the antagonising effectivity of yohimbine and atipamezole in the equine jejunum, it was observed that both these antagonists were, firstly, unable to modify the spontaneous contractility, nor the electrically-evoked contractions, in the jejunal preparations used in the study[44]. Next to this, both antagonists showed rather heterogeneous results in regard to their ability to counter the influence of the sedative α_2-agonists. For instance, yohimbine (at 10^{-7} M) was able to antagonise the sedative effects of both detomidine and medetomidine, but failed to counter (and even increased) the influence of xylazine. In addition, atipamezole (10^{-7} M) inhibited the effects of xylazine and detomidine, whilst medetomidine's effects were not significantly ameliorated. Surprisingly, at higher doses (10^{-6} M) both antagonists seemed unable to evoke any action. It is suggested that this heterogeneous interactions might exist due to variable affinity at different α_2-receptor subtypes[44], but more research in this concern is required.

Of further interest, it has been observed in mice that yohimbine was able to attenuate the inhibitory effects of endotoxin (lipopolysaccharide, LPS) on gastric emptying[115]. Indeed, LPS (through sepsis) is known to inhibit gastro-intestinal motility and gastric emptying by upregulating the expression of iNOS (inducible nitric oxide synthase), resulting in increased levels of nitric oxide, and thus smooth muscle relaxation. Complementary to the findings in mice, it was observed in horses, too, that yohimbine did prevent the inhibitory effects of LPS upon gastric emptying[116]. No data was available in this study with concern to the influence of atipamezole in this regard.

3.6.2 Tolazoline

This synthetic imidazoline derivative antagonises α-adrenoceptors nonselectively, and produces histaminergic and cholinergic effects as well[117]. Tolazoline was showed to successfully antagonise detomidine-induced sedation[118,119]. Simultaneously with tolazoline administration, blood glucose, cortisol and free fatty acid levels have been observed to increase, suggesting the activation of a stress response following tolazoline treatment. This clinical finding should be kept in mind when considering administration of the drug to vulnerable patients (for instance, post-surgery)[118]. In conclusion, this drug would not be the recommended drug of choice to use in cases of disrupted GI motility in equine veterinary practice.

3.6.3 Acepromazine

This phenothiazine molecule, with nonspecific α-adrenergic antagonist properties, has been postulated to enhance small intestinal motility in ponies in an *in vitro* study[3,120], but recent studies *in vivo* are not in correspondence with this observation: it was detected that acepromazine did not increase, but rather decreased gastric emptying (increased T_{max})[120]. This delayed action upon gastric

emptying might be due to involvement with other receptor sites, such as ACh, 5-HT or catecholamine receptors[120].

In conclusion, it stands clear that there is controversy on acepromazine being a prokinetic, apart from being a sedative. A suggested dose regimen in literature, for example in the prevention of founder laminitis (acepromazine is known to lower blood pressure), is 0.01 $mg \cdot kg^{-1}$ intramuscularly, every 4 to 6 hours[21].

3.6.4 Propranolol

The β-adrenergic receptor antagonist *propranolol* has been suggested to enhance equine GI motility[121], but clear evidence, as well as clinical studies, are not available.

3.7 Opioid antagonists

As mentioned earlier, opioids exercise their action by hyperpolarization of neurons in the GI tract. Opioid receptor agonists cause this effect by preventing the neuronal threshold for excitation to be reached, thereby indirectly decreasing ACh release, which results in decreased GI motility[66].

One of the great advantages of opioids in human medicine, is that quaternary opioid antagonists have been developed, like *N-methylnaltrexone*, a molecule that does not cross the blood-brain barrier and therefore does not attenuate the analgesic effects op opioid agonists, when administered. These antagonists might be attractive here to solve the gastro-intestinal side effects of opioids, and moreover, might have the potential to act as a prokinetic in situations of GI motility disorders. Unfortunately, a species-dependency was observed in case of N-methylnaltrexone, which undergoes demethylation in rodents, to methylnaltrexone, and then is capable of crossing the blood-brain barrier, thereby losing its peripheral selectivity[67]. In horses, however, N-methylnaltrexone (NMNT) does not undergo demethylation and is therefore able to evoke its selective action upon the equine GI tract[122]. NMNT can be regarded as a non-selective, but μ-opioid receptor preferring, antagonist[67]. One *in vivo* study in horses observed a preventive effect of NMNT upon gastro-intestinal stasis, and prevention of reduction in faecal moisture as is frequent after morphine administration. When morphine was not given, NMNT was not found to elicit any prokinetic effect at all[122]. Another study investigated the influence of NMNT *in vitro*, by incubating smooth muscle strips obtained from equine jejunum and pelvic flexure with NMNT. NMNT was found to directly affect the smooth muscles, leading to an increase in contractile response[123].

Another opioid antagonist is *naloxone*. It acts as a pan-opioid receptor antagonist and is thus nonspecific[67]. In an *in vitro* study in horses, both naloxone and naloxonazine (a selective μ-receptor antagonist) were unable to counter the inhibition of GI contraction that was induced by fentanyl (a highly selective μ-opioid receptor agonist), though naloxone did competitively antagonise a κ-opioid receptor agonist (U69593), suggesting that the κ-receptor is involved in the inhibition of GI smooth muscle contractions in horses[124].

Given the potential prokinetic benefits that can be expected from opioid antagonists, it is desirable to obtain more research data, for instance, in regard to clinical studies in patients suffering from GI motility disorders.

3.8 Other agents

With phenylbutazone being one of the most frequently used non-steroidal anti-inflammatory drugs in equine veterinary medicine, the question has risen if any prokinetic activity could be imputed to this drug. Indeed, phenylbutazone has been found *in vivo* in horses to ameliorate the LPS-induced decrease in gastric emptying and gastric secretions[33]. Phenylbutazone, as a cyclo-oxygenase (COX) inhibitor, initiates its anti-inflammatory action by decreasing prostaglandin levels. Prostaglandins have been observed to inhibit circular smooth muscle motility in the left dorsal colon of the horse[125]. Phenylbutazone has, furthermore, a protective effect by reducing the risk of the horse developing delayed faecal output after surgery[125], an effect that might be correlated to its influence upon prostaglandin levels and thus influence upon GI motility. These findings do suggest a certain prokinetic value to the drug, but more clinical data is needed.

4. Spasmolytics

An equine practitioner evidently encounters not only clinical cases where propagation of GI motility is required, but he also crosses patients where a further increase of the intestinal aboral movements is contraindicated. In these situations, the practitioner is in need of adequate spasmolytic agents to counter the hypermotility of the intestinal tract, like spasmodic colic. Next to this, in case of impaction of a certain part of the enteral tract, it is often required to counter the spasms occurring proximal of the impaction.
In this chapter, the most recent information on the subject of spasmolytic medicaments in the horse, will be discussed.

Hyoscine butylbromide (HBB, or N-butylscopolamine bromide, tradename Buscopan®) is highly effective in relieving abdominal pain in the horse, by reducing intestinal motility through blockage of muscarinic receptors, resulting in smooth muscle relaxation[126]. HBB (**Figure 6**) does not cross the blood-brain barrier and, therefore, does not induce central anticholinergic side effects. Generally, the therapeutic dose of HBB is 0.2 $mg \cdot kg^{-1}$, intravenously[126].
Molecules that contain quaternary nitrogen atoms are known to be capable of blocking nicotinergic ACh receptors. It has been suggested that this might be the case for HBB as well, but this issue has not been investigated in the past[127].

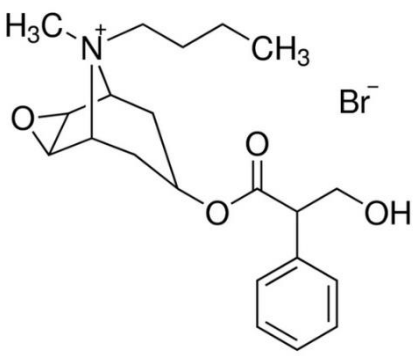

Figure 6. *Hyoscine butylbromide is a popular spasmolytic agent and mostly known under its tradename Buscopan®.*

It was shown that HBB has an immediate, short-lived reductive effect on caecum and left ventral colon contractions, and additionally, a minor but longer effect on duodenal contractility[126]. Furthermore, HBB has been used in horses as an alternative to atropine in reversing detomidine-induced and medetomidine-induced bradycardia in sedated patients[118].

Propantheline bromide (PPB) is another anticholinergic spasmolytic agent that has been proven to reduce intestinal motility and that is used widespread by equine practitioners[128]. However, a reduction in parasympathetic nervous activity (as initialised by these anticholinergic spasmolytics) can in itself be a cause of colic in horses[128]. HBB and PPB therefore need to be treated with caution in normal horses, in order to avoid this side effect.

DISCUSSION

Given the clinical importance of prokinetic and spasmolytic drugs in modern equine veterinary practice, this literature review has tried to provide an adequate overview of the latest information on the known prokinetic and spasmolytic agents, both agents frequently used by equine practitioners as well as those agents less commonly used or those whose potency to alter GI motility in horses is still questioned or controversial. Additionally, by providing a brief introduction to the physiology of gastro-intestinal motility, and by highlighting some of the frequent disorders that occur in horses in this regard, it is hoped that the reader of this review obtains new insights and knowledge considering the subject of gastro-intestinal motility in horses. This will help recognizing the gaps that still exist in our understanding of GI motility, thereby underlining the necessity of more elaborate research on these subjects. By doing so, this will hopefully contribute to finding more agents capable of interfering with the mechanisms responsible for controlling GI motility, thus giving the equine practitioner more, and qualitatively better, possibilities to treat GI motility disorders.

In general, it should be mentioned that our understanding of the normal physiology of the gastro-intestinal tract and its motility in the horse is, logically, still a limiting factor. Opportunities lie in obtaining more insight in research domains like receptor expression, distribution patterns, interference and correlations between receptors, in all different parts of the equine GI tract. Studies performed in horses are preferred in this regard, to avoid extrapolation errors. Enhancing our understanding of the physiology of the intestinal tract in healthy horses will bring researchers a step closer to understanding the pathogenesis and pathophysiology of disturbed GI motility.

With regard to parasympathicomimetic drugs, one of the major insecurities here is the lack of profound information on cholinergic receptor expression, distribution and characteristics in the equine intestinal tract. This makes interpretation of *in vitro* studies comparing the effect of BeCh on intestinal preparations of different locations more difficult. Subsequently, clinical studies in living horses (both healthy and suffering from GI motility disorders) are required as well, in order to be able to trial its clinical effectiveness in concern to its postulated prokinetic properties. The suggested synergism with yohimbine is another subject in need of more elaborate research.
The literature reviewed in concern to neostigmine have indicated some conflicting hypotheses. The opinion suggesting neostigmine to have an inhibiting effect upon GI motility in the proximal part of the GI tract, in contrast to the more distal segments, seems generally accepted in the academic world. However, not all experimental results seem to support these findings[78]. This leaves us with remaining questions. Moreover, as edrophonium was unable to block the increased contractile response induced by neostigmine in smooth muscle preparations, the question arises whether neostigmine might have multiple mechanisms of acting. Another possibility that needs to be taken into account is edrophonium being capable to block acetylcholinesterase only partially, a hypothesis that warrants further investigation as well. A possible experimental set-up *in vitro* could be the measurement of Ach concentrations before and after addition of edrophonium to a matrix with acetylcholinesterase. In addition, one could try detecting the metabolites that will be formed after Ach is degraded by the

enzyme (for example, increasing concentrations of free choline). This would give evidence to the hypothesis that edrophonium only partially blocks the enzyme.

Metoclopramide has not been found to increase caecal motility in the horse, despite significant prokinetic activity with regard to jejunal motility and gastric emptying rates. This is an interesting finding, as other benzamides (like mosapride) are known to indeed prolong caecal motility as well[55]. It would be of value to investigate how these subtle differences can be explained and what role can be attributed to 5-HT receptor expression in the equine hindgut in this concern. Subsequently, the effect of cisapride (a newer benzamide) has been observed to persist notwithstanding prior administration of atropine (a parasympathicolytic drug), suggesting a (partial)noncholinergic way of action of cisapride. Investigating the possibility of additional mechanisms of action, like here indicated for cisapride but also in general for other agents, would be an obliged subject of future research. Cisapride, additionally, has not been demonstrated to clearly enhance gastric emptying in horses, in contrast to mosapride, which has been observed to increase gastric emptying rates, and moreover, to enhance caecal motility (unlike metoclopramide). This drug therefore seems preferable above cisapride or metoclopramide. Tegaserod, on the other hand, has been shown to be effective in propagating GI motility in horses (unlike experiments in dogs, thus underlining the importance of species specific experimental set-ups), but as a higher risk of cardiac events in humans has been suspected (as for cisapride), its availability is limited, as well as its application in equine practice, explaining the lack of more clinical reports.

The prokinetic influence of domperidone, a dopaminergic antagonist, on equine GI motility remains still uncertain, as an effect on the motility of neither small intestine nor pelvic flexure could be observed, but in contrast, gastric emptying rates were measured (via the acetaminophen test) to be enhanced at an oral dose of 5.5 $mg \cdot kg^{-1}$. These results are fairly consistent with the application of domperidone in humans, where it is used particularly for proximal gastro-intestinal diseases, for its effectiveness was found not to extend beyond the human duodenum. More research into domperidone's efficacy in enhancing GI motility in the proximal parts of the equine intestinal tract is required to be able to evaluate its usefulness as a prokinetic agent.

When reviewing the literature in concern to lidocaine (xylocaine), a first observation by the critical reader might be the apparent incongruence in experimental data when using healthy horses in experimental set-ups, compared to investigations to the clinical efficacy in horses really suffering from GI motility disorders (like POI), on the other hand. Indeed, studies in healthy horses do not seem to underline the beneficial prokinetic effects that are being observed in diseased horses that are treated with lidocaine. A possible explanation for this incapacity to provoke the prokinetic properties of the drug in healthy horses might be the lack of knowledge in concern to the mechanism of action of lidocaine, although several hypotheses have been postulated (see chapter 3.4). It is evident that fundamental research is warranted to elucidate these knowledge gaps, hopefully leading to a better understanding of the drug. In the meantime, lidocaine has proven a potent prokinetic medicament for treatment after surgery and in order to reduce the risk of POI.

Interesting results were found in the formerly mentioned study that compared erythromycin administration in both post-operative and post-recovery ('healthy') horses[113]. This study showed the

inconsequent reaction to erythromycin treatment by the horse, depending on the health status of the animal at that moment: the macrolide was not able to increase myoelectric activity in the caecum during the post-operative period (at this time, the caecum did not show any spontaneous electrical activity at all), but only during the post-recovery period, whereas for the ileum it was the other way around, leading only to increased myoelectric activity in the period right after surgery. This suggests that erythromycin's effectiveness depends of the health status of the GI tract (urging researchers to be careful when comparing experimental results of healthy and diseased horses) and the location of the GI tract that is analysed. Together with the fact that slow, long-term infusions of erythromycin might down regulate the motilin receptors, thereby initiating the unwanted decrease of electrical activity, validates the suggestion to use this macrolide with caution.

In concern to the intestinal adrenergic system, it should be mentioned that our fundamental understanding of its role in GI motility needs to be improved, in order to be able to explain the effects of the adrenergic antagonists that have been observed. For instance, it is still unclear why yohimbine increases xylazine's effectiveness in low dose, but on the other hand, fails to evoke any action at higher dose. Hence, in contrast with our expectations of adrenergic antagonists (since adrenergic hyperactivity has been suggested to be involved in triggering POI in horses), these agents seem not to be capable of countering the inhibitory influence of the adrenergic system properly, although yohimbine has been demonstrated to attenuate the inhibitory effect of LPS (after sepsis) upon gastric emptying. Pending on future research, the prokinetic properties of these antagonists should be questioned, or at least be critical viewed to. Additionally, the same can be noted for tolazoline, which has been observed to induce a stress response (increases blood glucose, cortisol and fatty acid levels) in patients suggesting that administration to vulnerable patients would be contraindicated. Lastly, with regard to acepromazine, it can be mentioned that although this phenothiazine molecule has been said to evoke prokinetic effects in ponies, the usefulness of this drug in clinical conditions is questionable, as several studies are in conflict with each other where it comes to their conclusions considering its prokinetic properties.

The final group of prokinetic agents to be discussed here, is that of the opioid antagonists. N-methylnaltrexone (NMNT) directly stimulates smooth muscle strips *in vitro*, but this effect cannot be extended clinically to a direct prokinetic effect *in vivo*. Nonetheless, NMNT can be useful in inhibiting the intestinal opioid side effects when opioids are administered for analgesic purposes. One should keep in mind that (partial) selectivity for certain opioid receptors might be an important phenomenon in the group of antagonists, thus revealing a possible explanation for disappointing results in regard to GI prokinetic properties. The complexity of research in this regard is apparent, but investigating this subject is of vital importance in order to discover the potential of opioid antagonists to exert prokinetic activity *in vivo*, perhaps even without prior administration of opioids.

This literature review had tried to provide and discuss the latest information on gastro-intestinal motility, prokinetics and spasmolytics in horses. Advantages as well as disadvantages, side effects and contraindications have been discussed for the agents when relevant. It is hoped that this leaves the equine practitioner capable of a proper consideration on which prokinetic agent to be used or not

in a certain situation, thereby relying on the information as given in this review, and evenly important, relying on information obtained from the individual health status and health parameters of the patient. Meanwhile, it stands clear that none of the discussed agents can be reckoned as the ultimate drug of choice. The perfect prokinetic or spasmolytic drug has yet to be discovered, and researchers will get closer to this purpose by enhancing their understanding of the physiology of the equine intestinal tract, in general, and of GI motility, in particular. Subsequently, more clinical data and evaluations with regard to most of the current prokinetics are desirable, hence providing the practitioner with more extended information on the clinical usefulness of the current existing agents.

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APPENDICES

Appendix I. Summary of prokinetics and spasmolytics

Medicament	*Mechanism of action*	*Dose regimen*[a]	*Intestinal effects*
Bethanechol	Ach receptor agonist	0.02 mg·kg^{-1} SC q6h, then 0.35 mg·kg^{-1} PO q8h	Acceleration of gastric and caecal emptying
Neostigmine	Acetylcholinesterase-inhibitor	0.022-0.044 mg·kg^{-1} SC or IV q4h	Propagation of large intestine motility
Metoclopramide	5-HT$_4$ agonist, 5-HT$_3$ receptor antagonist	0.125-0.250 mg·kg^{-1} in saline IV, or 0.05 mg·kg^{-1} IM q6h	Attenuation of POI, counters experimentally induced colic
Cisapride	5-HT$_4$ agonist, 5-HT$_3$ receptor antagonist	0.1mg·kg^{-1} PO q8h	Improved jejunal motility, attenuation of LPS-induced delayed gastric emptying, POI prophylaxis
Mosapride	5-HT$_4$ receptor agonist	0.5 mg·kg^{-1} (gastric indications), 1.5-2.0 mg·kg^{-1} (caecal indications, q12h)	Improved jejunal and caecal motility, enhanced gastric emptying, attenuation of POI
Tegaserod	5-HT$_4$ receptor agonist	0.02 mg·kg^{-1} diluted IV q12h	Enhanced motility in small and large intestines
Domperidone	Dopaminergic D$_2$-receptor antagonist	5.5 mg·kg^{-1} PO	Increased gastric emptying
Lidocaine	Unknown	1.3 mg·kg^{-1} 2% IV (per-operative), then 0.05 mg·kg^{-1}·min^{-1} in 1 L saline or Ringer's Lactate CRI	Attenuation and reduced risk of POI, reduction of reflux (proximal enteritis)
Erythromycin	Motilin receptor agonist	Low, subtherapeutic (non-microbial) boluses	Prokinetic activity upon gastric, jejunal and (particularly) colonic motility
Yohimbine	Alpha-adrenergic receptor antagonist	0.15[b] mg·kg^{-1} IV q3h	Attenuation of LPS-induced delayed gastric emptying
Tolazoline	Alpha-adrenergic receptor antagonist	1.0-4.0[b,c] mg·kg^{-1} IV	Unknown
Acepromazine	Alpha-adrenergic receptor antagonist	0.01 mg·kg^{-1} IM q4-6h	Unknown, decreased gastric emptying
N-methylnaltrexone	Opioid antagonist	Unknown	Increased jejunal and colonic motility *in vitro*, attenuation of opioid-induced intestinal stasis
Naloxone	Pan-opioid antagonist	Unknown	Attenuation of κ-receptor induced intestinal hypomotility
Hyoscine butylbromide	Muscarinic receptor antagonist	0.2 mg·kg^{-1} IV	Intestinal smooth muscle relaxation
Propantheline bromide	Muscarinic receptor antagonist	0.2 mg·kg^{-1} IV	Intestinal smooth muscle relaxation

[a] Doses are provided per kilogram bodyweight.
[b] From Reference 3; [c] from Reference 118.