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What is the function of motilin in the rabbit gastrointestinal tract?

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Abstract

Motilin was isolated from the extract of the porcine duodenal mucosa as a gastrointestinal (GI) motility-stimulating peptide. Although responsiveness of motilin in GI tract is different depending on experimental conditions (*in vivo, in vitro*), GI regions and species, it has been demonstrated that motilin is a physiological mediator of phase III of migrating motor complexes (MMCs) in the stomach, at least in humans, dogs, house musk shrews (*suncus*), monkeys, and opossums. Since the discovery of motilin, rabbits have been used for *in vitro* research. Although small intestinal MMCs in rabbits have been observed *in vivo*, their characteristics are different from those in humans and dogs probably due to the lack of hunger periods and it is still unknown whether motilin regulates intestinal MMCs or not. The density of motilin receptors is highest in colonic enteric neurons and the responsiveness of motilin is highest in the colon. These findings are own characteristics of rabbits and it is possible that motilin regulates colonic motility related to defecation or caecotrophy, but this possibility has not been clarified at present. Further *in vivo* functional studies with measurements of colonic motility and plasma motilin are needed to understand the physiological role of motilin in regulation of GI motility in rabbits.

Key Words: colonic motility, migrating motor complex, motilin, motilin receptor, rabbit

1. Discovery of motilin

About 100 years ago, Shay and Gershon-Cohen[41)] examined the effects of injecting an alkaline solution into the human duodenal lumen and found that alkalinization increased gastric emptying. As an alkalinization-induced increase in gastric motility was also observed in a denervated stomach, it was hypothesized that a bioactive substance stimulating gastric motility was released from the duodenal mucosa by alkalinization[6)]. While observing canine gastric motility, this active substance was purified through a combination of CM cellulose column chromatography, ion exchange chromatography, and gel filtration, ultimately resulting in the isolation of a peptide hormone consisting of 22 amino acids[4,5,7)]. This substance was named motilin due to its role in increasing gastric motility, and its amino acid sequence was determined by Schubert and Brown[39)] (porcine motilin, FVPIF TYGEL QRMQE KERNK GQ).

Following the identification of the motilin sequence, motilin analogues were synthesized by Kai et al.[23)] and Wünsch[50)], and the analogues were used in both *in vivo* and *in vitro* physiological and pharmacological studies. In addition, Dryburgh and Brown[13)] established a radioimmunoassay for motilin and analyzed motilin content in various tissues and in plasma. Motilin antibodies were also used in immunohistochemical studies for

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Table 1. Presence of the motilin system and motilin-induced gastrointestinal responses both *in vivo* and *in vitro*

	Motilin	Motilin receptor	*In vitro* contraction study	*In vivo* contraction study
Pig	Yes	Yes	No response	No response
Human	Yes	Yes	Contraction (neural/myogenic)	Phase III-like activity of gastric MMCs (mediator of phase III)
Monkey	Yes	Yes	Contraction	Phase III-like activity of gastric MMCs (mediator of phase III)
Rabbit	Yes	Yes	Contraction (neural/myogenic)	Contraction (stomach, duodenum and colon)
Dog	Yes	Yes	No response	Phase III-like activity of gastric MMCs (mediator of phase III)
Rat	No	No	No response	No response
Mouse	No	No	No response	No response
Guinea pig	No	No	No response	No response
Suncus	Yes	Yes	Contraction (neural)	Phase III-like activity of gastric MMCs (mediator of phase III)

MMCs: migrating motor complexes

clarifying the tissue distribution of motilin. The presence of motilin was confirmed in the upper small intestinal mucosa of various mammals (dogs, humans, rabbits, cats, and cows), and its amino acid sequence was determined using molecular biology techniques. However, interestingly, motilin was not identified in rodents (mice and rats)[26].

Motilin was discovered as an active substance that stimulates gastric motility, and its gastrointestinal (GI) motility-stimulating action has been examined for nearly 50 years. Dogs (*in vivo*) and rabbits (*in vitro*) have been mainly used for contraction studies of motilin, and it is thought to be a physiological mediator of phase III activity of the gastric migrating motor complex (MMCs) in humans, dogs, *suncus*, monkeys, and opossums (Table 1)[26]. In this review, we first introduce the physiological role of motilin in dogs and then describe the details of motilin research in rabbits and discuss the possible role of motilin in the regulation of rabbit GI motility. Although the structure of rabbit motilin (FVPIF TYSEL QRMQE RERNRGH, with four amino acids at positions 8, 16, 20, and 22, differing from porcine motilin) was identified by Banfield et al.[2], studies in rabbits have often used porcine/human motilin due to the high homology of amino acid sequence (82%).

2. Effects of motilin on GI motility

2.1. In vivo experiments

Since the early 20th century, it has been known that the GI tract contracts spontaneously during the fasting period, a motility known as hunger contraction. Szurszewski[45] observed GI electromyograms and showed that hunger electrical activity occurred during the fasted period and shifted to a digestive pattern with feeding. As motilin was purified from porcine duodenal extracts based on its contraction-inducing activity in the canine stomach, Itoh et al.[21,22] examined the relationship between motilin and hunger contractions in dogs. They found that the stomach and duodenum contracted spontaneously at 90-150-minute intervals during fasting, similar to the cluster of electrical activity reported by Szurszewski[45]. These clusters originate in the pylorus of the stomach and migrate to the lower intestine. Known as interdigestive migrating contractions (IMCs) or migrating motor complexes (MMCs), we refer to them as MMCs here. MMCs in the stomach are classified into three phases (phase I, phase II, and phase III) based on their motility patterns and migrate to downward with time: phase I is a quiescent period with no contractions, phase II has contractions of irregular magnitude, and phase III is characterized by phasic contractions of maximum amplitude[21]. Since MMCs were also observed in a denervated stomach, it was suggested that humoral factors, rather than neural factors, are involved in MMCs regulation[21]. The presence of MMCs during fasting and their disruption during digestion have also been observed in humans[49], the house musk shrew (*suncus*)[36], monkeys[52], and opossums[46]. MMCs function as “housekeepers” for the GI

lumen, clearing the lumen to suppress bacterial overgrowth[20]. In humans, since hunger is mostly felt during phase III of gastric MMC, phase III contractions in stomach are considered a hunger signal transmitted from the periphery to the brain[9].

Itoh et al.[21] demonstrated in conscious dogs that intravenous injection of motilin during phase I induced MMC-like contractions in stomach which migrate to the lower intestine. However, motilin did not cause contractions during the postprandial phase. Itoh et al.[22] measured both plasma motilin level and gastric motility simultaneously in dogs and found that motilin concentrations fluctuated periodically during fasting periods, with motilin concentration peaks corresponding to the appearance of phase III activity of gastric MMCs[22]. Furthermore, MA-2029, a motilin receptor blocker inhibited gastric MMCs and motilin-induced contractions in dogs[33]. These findings indicate that motilin is a physiological mediator of phase III of gastric MMCs in dogs. The results of functional studies in other mammals (humans, monkeys, *suncus*, and opossums) also indicate that motilin mediates phase III of gastric MMCs (Table 1).

2.2. In vitro experiments

Segawa et al.[40] examined the effects of synthetic motilin on GI strips from various animals (pigs, guinea pigs, rabbits, dogs, and rats). Only the GI strips from rabbits responded to motilin. Furthermore, different GI regions displayed varying sensitivity to motilin in rabbits (with the duodenum and colon showing high sensitivity, followed by the gastric pylorus and jejunum, and the ileum being quite insensitive). Strunz et al.[42,43] examined the effects of motilin analogs and showed that human and rabbit GI strips were sensitive to motilin, while GI strips from rats and guinea pigs were quite insensitive. These findings highlighted a significant species-related difference in the responsiveness to motilin in GI tracts (Table 1). The lack of response to motilin in rodents is due to the pseudogenization of motilin and motilin receptor genes, meaning that they are not expressed as functional peptide/protein[17,38]. While other *in vitro* functional studies indicated that motilin could contract GI strips from the *suncus* stomach[31] and monkey duodenum[52], rabbits have been mainly used in *in vitro* studies of GI motility-stimulating action of motilin[1,24,25,44,48] and of biological assay for motilin-related peptides[53,54].

Domschke et al.[12] examined the mechanisms of motilin-induced contraction in rabbits and reported that neither tetrodotoxin nor atropine decreased the response, suggesting that motilin acts directly on the GI smooth muscle. Adachi et al.[1] also noted regional differences in responsiveness, with the duodenum being most sensitive, followed by the colon and ileum. The motilin-induced contractions in the duodenum were not inhibited by acetylcholine, 5-hydroxytryptamine (5-HT), histamine or angiotensin receptor antagonists, and motilin did not modify neural responses elicited by electrical field stimulation[1]. In rabbit pylorus, Moummi et al.[32] found that motilin induced contraction in isolated smooth muscle cells. These results confirm that motilin directly acts on smooth muscle cells to induce contraction.

Nevertheless, Kitazawa et al.[25] distinguished motilin-induced duodenal contractions as phasic (initial) and tonic (sustained) contractions and found that tetrodotoxin or atropine reduced tonic contractions but not phasic ones. This suggested that phasic contractions are due to a direct action of motilin on smooth muscle cells, while tonic contractions involve stimulation of enteric neurons as well as direct action of motilin on smooth muscles. Van Assche et al.[48] showed that low concentrations of motilin (< 10 nM) enhanced electrically stimulated cholinergic contraction in gastric pyloric strips without affecting acetylcholine-induced contraction, whereas higher concentrations (> 10 nM) caused tetrodotoxin-resistant contraction. This indicates that low concentrations of motilin excite motilin receptors in neurons, enhancing neural responses, while high concentrations activate motilin receptors in smooth muscle cells, causing tetrodotoxin-resistant contraction.

Functional analysis, using contractile

response as an index, suggests the presence of motilin receptors in both enteric neurons and smooth muscle cells, prompting biochemical and morphological research into motilin receptors.

3. Motilin receptors in the GI tract

The presence of motilin receptors in the rabbit GI tract was demonstrated through binding experiments using labeled motilin ligand. Bormans et al.[3] showed that [^{125}I]-labeled motilin bound to homogenates of the rabbit GI tract in a saturable manner, and the binding was displaced by unlabeled motilin with high affinity. Further fractionation by a density gradient showed that the degree of motilin binding was high in the cell membrane fraction, suggesting that the motilin-binding protein might be motilin receptor. In rabbits, motilin binding site density varied by GI region. Depoortere et al.[10] examined binding sites in both upper (stomach, duodenum, ileum) and lower (cecum, colon, rectum) GI regions and found that receptor density decreased from the duodenum to the ileum in the small intestine, while the colon and rectum exhibited 3-4-times higher receptor density than that in the duodenum. This receptor distribution correlates with the region-dependent action of motilin in the rabbit GI tract[1,24]. Similarly, Sakai et al.[37] identified region-dependent motilin receptor distribution via autoradiography, showing motilin binding sites abundantly in the gastric pylorus, duodenum, and colon but not in the cecum. Binding sites were more concentrated in longitudinal muscles than in circular muscles and were absent in the GI mucosa.

Since there is a neurogenic component in motilin-induced contractions, it was thought that motilin receptors might also be present on enteric neurons. Kitazawa et al.[25] used rabbit duodenal strips loaded with [^{3}H]-choline and showed that motilin increased [^{3}H] efflux, which was inhibited by tetrodotoxin and a Ca^{2+}-free solution. This indicated that motilin stimulates acetylcholine release from cholinergic neurons through activation of neural motilin receptor. Conventional GI tract homogenates contain smooth muscle cells, enteric neurons, blood vessels, and mucosa, making it unclear which cell membrane type binds motilin. Poitras et al.[34] investigated neural and smooth muscle motilin receptors in rabbits by preparing homogenates of the stomach and duodenum after removal of the mucosa. They separated smooth muscle cell membranes and synaptic terminals using markers including 5' nucleotidase activity (smooth muscle marker) and [^{3}H]-saxitoxin binding activity (a neural marker binding to Na^{+} channels). Binding of [^{125}I]-motilin was analyzed in each fraction. The results showed positive correlations between motilin binding and both 5' nucleotidase activity and [^{3}H]-saxitoxin binding in the stomach but only a positive correlation with 5' nucleotidase activity in the duodenum, suggesting that motilin receptors are present on both smooth muscle cells and enteric neurons in the stomach but only in smooth muscle cells in the duodenum. Miller et al.[30] carried out a similar study in the colon and showed that the colon had the highest density of motilin receptor in the neurons among GI regions (49.5 fmol/mg protein), the density being approximately 2.5 times higher than that of smooth muscle receptor in the colon (19.9 fmol/mg protein). The amounts of motilin receptor in the neurons of the stomach and in the smooth muscle cells of the duodenum were 6.6 fmol/mg protein and 9.4 fmol/mg protein, respectively[30]. In summary, motilin receptors are located in the enteric neurons and smooth muscle cells, and their expression levels vary by GI region in rabbits, with high levels in the colon (enteric neurons > smooth muscle cells) and low levels in the stomach (enteric neurons > smooth muscle cells) and duodenum (smooth muscle cells > enteric neurons).

McKee et al.[29] cloned two receptors from the human genomic DNA, GPR38 and GPR39, which are family of neurotensin and growth hormone secretagogue receptor (ghrelin receptor). GPR38 was encoded by a single gene and expressed in enteric neurons of human GI tract[29]. Motilin was demonstrated to be an endogenous ligand for GPR38[14]. Therefore, GPR38 is thought to be motilin receptor. GPR39 is also a member of ghrelin/neurotensin receptor family expressed in brain and intestine[29]. The function of GPR39 in

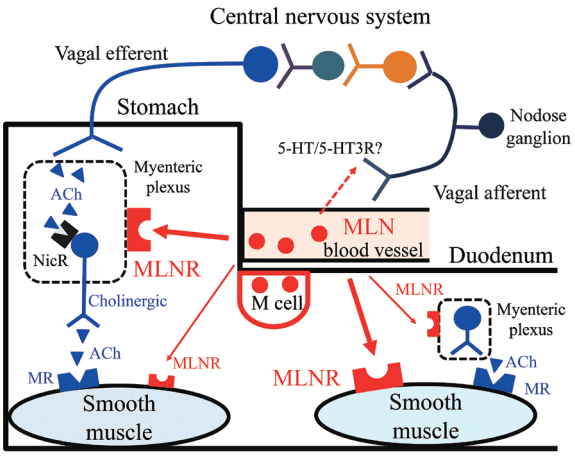

Fig. 1. Mechanisms of motilin-induced stimulation of gastric and duodenal contractility in rabbits. Motilin (MLN) is produced by M-cells in the duodenal mucosa and is transported to the stomach and duodenum by blood flow. Motilin receptors (MLNR) are expressed on enteric neurons such as cholinergic neurons and smooth muscle cells. Dominant receptors are different in the stomach (enteric neurons > smooth muscle cells) and duodenum (smooth muscle cells > enteric neurons). In the stomach, MLN causes potentiation of the neural responses through activation of MLNRs in neurons at a low concentration and at a higher concentration, activation of MLNRs in smooth muscle cells causes elevation of muscle tonus. In the duodenum, motilin acts on dominant MLNRs in smooth muscle cells at a high concentration and causes myogenic contraction. Involvement of 5-HT/5-HT_3R and vago-vagal reflex pathway in the motilin responses has not been examined in rabbits. ACh: acetylcholine, MR: cholinergic muscarinic receptor, NicR: cholinergic nicotinic receptor.

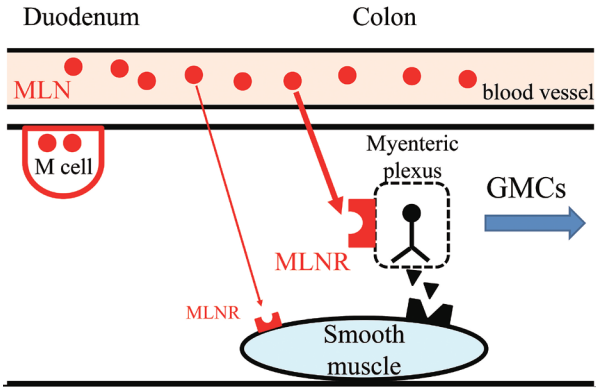

Fig. 2. Mechanisms of motilin-induced stimulation of colonic contractility in rabbits. Motilin (MLN) is produced by M-cells in the duodenal mucosa and reaches the colon by blood flow. Although MLNRs are expressed both in enteric neurons and smooth muscle cells of the colon, the amount of MLNRs in neurons is about 2.5-times larger than that of MLNRs in smooth muscle cells. Motilin acts on MLNRs in neurons and causes giant migrating contractions (GMCs) that transport feces. Motilin response in the colon was resistant to atropine and the enteric neurons excited by motilin are thought to be noncholinergic[24].

GI tract are different from those of GPR38 and GPR39 is involved in zinc-dependent signaling in intestinal epithelial tissue regulating ion transport[51]. Motilin receptors have also been cloned in several mammals including rabbits[8]. The rabbit motilin receptor is encoded by a single gene and shows 85% homology with the human motilin receptor (GPR38), and HEK293T cells expressing this receptor exhibit increased intracellular Ca^{2+} levels in response to exogenous motilin.

Figures 1 and 2 summarize the mechanism of motilin-induced contractions based on the distribution of motilin receptors in the rabbit stomach, duodenum (Fig. 1), and colon (Fig. 2). In the stomach and colon, where motilin receptors in enteric neurons are abundant, low concentrations of motilin primarily act on enteric neurons, eventually triggering the release of contractile neurotransmitters from cholinergic neurons, resulting in GI contraction. At high concentrations, motilin can stimulate motilin receptors in smooth muscle cells, causing a baseline elevation[48]. In the duodenum, where smooth muscle motilin receptors are dominant, motilin primarily induces myogenic contractions. In dogs, motilin indirectly excites the 5-HT_3 receptor on primary afferent neurons of the vagus through release of endogenous 5-HT from enterochromaffin cells and 5-HT neurons. Excitation of vago-vagal reflex pathway including the nodose ganglion, the nucleus solitary tract (NTS), and dorsal motor nucleus of the vagus (DMNV) causes gastric contraction[20,26], though it remains unclear whether this pathway exists in rabbits (Fig. 1).

4. Physiological roles of motilin in rabbits

4.1. Upper GI motility

Although motilin has been shown in *in vitro* studies to cause contractions in the rabbit GI tract in a region-dependent manner, *in vivo* contraction studies on motilin are limited. It has been demonstrated that motilin is a physiological

Table 2. Comparison of migrating motor complexes (MMCs) and motilin-induced response in the rabbit upper gastrointestinal tract.

	Origin	MMCs interval	MMCs duration	Migration	Effect of feeding	Atropine, hexamethonium	Reference
Spontaneous MMCs (*in vivo*) Electromyogram	Jejunum (3 phases)	140 min	28 min	Yes	No effects	Decrease	35
Motilin response (*in vivo*) Electromyogram	Duodenum and jejunum	—	7.5 min (motilin response)	Not	—	Not decrease	16
Spontaneous MMCs (*ex vivo*) Contraction	Duodenum (2 phases)	16 min	4 min	Yes	—	Decrease	28
Motilin response (*ex vivo*) Contraction	Stomach and duodenum	—	3-4 min (motilin response)	Yes	—	Decrease	28

mediator for phase III activity of gastric MMCs in the stomach of dogs, humans, *suncus*, monkeys, and opossums, as evidenced by *in vivo* contraction studies and measurements of plasma motilin levels[20,26].

In vivo measurements of rabbit GI activity have been conducted using electromyography (EMG) by Ruckebusch et al.[35] and Guerrero-Lindner et al.[16] Migrating myoelectric activity complexes corresponding to MMCs have been observed in rabbits but MMCs originate in the jejunum and migrate downward to lower small intestine. Unlike in dogs and humans, MMCs are not observed in the stomach and duodenum of rabbits. Rabbit MMCs occur approximately 10 times per day with a duration of 30 minutes and remain unchanged after four days of fasting, suggesting that MMCs do not vary between fasting and digestive periods. Spontaneous MMCs are suppressed by atropine and hexamethonium, indicating regulation by cholinergic neuronal factors. The characteristics of rabbit MMCs differ from those observed in dogs, humans, *suncus*, and monkeys, probably due to the unique feeding behavior of rabbits (Table 2). Rabbits eat small amounts of food frequently and are known as animals that "fast until death" due to their inability to easily empty the GI tract.

Guerrero-Lindner et al.[16] investigated the effects of motilin on GI electromyograms in conscious rabbits (*in vivo*). Spontaneous MMCs appear at the upper jejunum and migrate to the lower jejunum, but intravenously administered motilin (750 - 1,500 ng/kg) induces transient electrical responses in the duodenum, upper jejunum, and lower jejunum simultaneously, which do not migrate to the ileum. Motilin does not induce electrical responses in the stomach. While atropine and hexamethonium inhibit spontaneous MMCs in the jejunum, both antagonists do not inhibit motilin-induced responses, indicating that motilin induces contractions via direct action on smooth muscle *in vivo*, differing from spontaneous MMCs (Table 2). Consequently, it is unlikely that motilin mediates MMCs in the upper jejunum. However, since neurogenic actions of motilin appear at low concentrations and its myogenic actions appear at high concentrations *in vitro*[48], it is essential to study the action of motilin at lower doses (less than 750 ng/kg). Kitazawa et al.[24] found in anesthetized rabbits that motilin (1,000 - 10,000 ng/kg) increased intraluminal pressure in the stomach and colon without affecting the ileal contractility. Since dose of motilin is comparable in two studies, differences in experimental conditions (anesthesia vs. consciousness) and motility measurement indices (electrical activity vs. luminal pressure) could explain the discrepancies in actions of motilin on gastric contractility, though the underlying reasons remain unknown. Marzio et al.[28] excised the stomach and 60 cm of the small intestine from rabbits and incubated the organs in a large bath to record motility in the stomach, duodenum, and jejunum using force strain gauges (*ex vivo* experiment). In this setup, MMC-like activity originating from the duodenum was observed in the first 60 minutes of experiments, propagating

to the lower intestine, but MMC-like activity was not produced in the stomach. The interval between MMC-like activity was 16 minutes, markedly shorter than that observed *in vivo* (10 times/day, 140-min intervals). In 40% of the preparations, motilin caused contractions in the stomach and duodenum simultaneously, and these contractions propagated to the lower small intestine, but in the remaining 60% of the preparations, motilin triggered contractile responses in the duodenum that propagated in both directions (toward the stomach and jejunum)[28]. Since rabbit spontaneous MMCs originating in the duodenum are neurogenic[16], ability of motilin to cause atropine-sensitive migrating contractions *ex vivo* suggests the possible involvement of motilin in duodenal MMCs (Table 2). However, two questions remain unanswered: why the stomach, which is sensitive to motilin, is not a starting point for MMCs like dogs and humans and why there is a discrepancy in MMC intervals between *in vivo* and *ex vivo* studies. In *ex vivo* studies, the GI lumen is artificially emptied, differing from *in vivo* conditions, which could explain the MMC parameter differences. *In vivo* contraction studies using motilin receptor antagonists and simultaneous measurement of plasma motilin level are needed to understand the physiological significance of motilin in the regulation of upper GI motility like MMCs.

4.2. Colonic motility

Depoortere et al.[10] and Miller et al.[30] assessed motilin receptor expression levels in various parts of the rabbit GI tract and they found that receptor density in the colon and rectum was 3-4-times higher than that in the stomach, duodenum, and jejunum. High density of motilin receptors in the colon compared to that in other GI regions has not been reported in dogs[18], cats[11], humans[47], or monkeys[52], suggesting that this is a unique characteristic of rabbits. Consistent with receptor density, motilin-induced contraction was notably high in the proximal and distal colon in an *in vitro* study[10] and the largest contractions were observed in the descending colon (distal colon) in an *in vivo* study[24]. Giant migrating contractions (GMCs), responsible for colonic content transport, have been observed in various animals (humans, monkeys, rats, and mice)[15,19,27,55]. Hirabayashi et al.[19] reported that a motilin derivative induced GMCs in the colon and increased defecation in dogs. Sudo et al.[44] showed that motilin derivative application similarly increased defecation in rabbits and dogs. These findings suggest that motilin might act on neural motilin receptors to induce colonic motility, such as GMCs, facilitating defecation in rabbits. Sanger et al.[38] hypothesized that motilin regulates upper GI motility in mammals that are capable of vomiting, because vomiting involves the excitation of vago-vagal reflex pathway and gastric contractions, which is one of the mechanisms for GI motility-stimulating action of motilin (Fig. 1). However, rabbits, which are unable to vomit, are an exception; motilin might be conserved in this species to regulate colonic motility for caecotrophy, an alternative digestive function. Further functional studies on colonic motility, defecation, and plasma motilin levels are necessary to understand physiological roles of motilin in the regulation of colonic motility.

5. Conclusion

Motilin was isolated from porcine duodenal mucosa extracts 50 years ago. Following sequence determination, numerous physiological studies on GI motility have been conducted, primarily using dogs in *in vivo* studies and rabbits in *in vitro* studies. However, mice, rats, and guinea pigs lack motilin/motilin receptor genes, limiting their use in physiological research and slowing motilin research progress.

Motilin responsiveness in the GI tract varies depending on experimental conditions (*in vivo* vs. *in vitro*), species, and GI tract region. Nevertheless, it has been established that motilin is a physiological mediator of phase III of MMCs in the stomach of humans, dogs, *suncus*, monkeys, and opossums. MMCs transmit hunger signals to the brain and act as "housekeepers" by clearing the GI lumen.

Since discovery of motilin, rabbits have been widely used for *in vitro* experiments. However,

rabbits exhibit a unique feeding pattern compared to humans and dogs and do not undergo clear fasting periods as they eat small quantities of food frequently. Although MMC-like activities have been observed in the small intestine of rabbits *in vivo*, their characteristics differ from those in humans and dogs, and whether motilin regulates upper GI motility, such as MMCs, remains unknown. Motilin receptors are most abundant in enteric neurons in the colon. It is possible that motilin regulates colonic motility associated with defecation or caecotrophy, though this remains to be investigated. Further *in vivo* functional studies on colonic motility and plasma motilin levels are needed to elucidate physiological role of motilin in regulation of upper and lower GI motility in rabbits.

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