Motilin controls cyclic release of insulin through vagal cholinergic muscarinic pathways in fasted dogs

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Suzuki, Hideki, Erito Mochiki, Norihiro Haga, Minoru Satoh, Akiyoshi Mizumoto, and Zen Itoh. Motilin controls cyclic release of insulin through vagal cholinergic muscarinic pathways in fasted dogs. Am. J. Physiol. 274 (Gastrointest. Liver Physiol. 37): G87–G95, 1998.—The effect of motilin on insulin release has not been studied in the interdigestive state. Adult mongrel dogs were chronically implanted with force transducers in the stomach and duodenum to monitor contractile activity, and the plasma motilin and insulin concentrations were measured by a specific radioimmunoassay and enzyme immunoassay, respectively. The concentration of insulin in plasma was found to fluctuate in close association with that of motilin and phase III of the interdigestive migrating contractions in the stomach. This spontaneous release of insulin was mimicked by intravenous administration of motilin at a dose of 0.3 µg·kg⁻¹·h⁻¹. Exogenous motilin (0.01–0.3 µg/kg) dose dependently stimulated insulin release, which was abolished by atropine, hexamethonium, ondansetron, and truncal vagotomy. Phentolamine significantly enhanced, whereas propranolol inhibited, motilin-induced insulin release. In a perfusion system using islet cells from the canine pancreas, motilin did not affect insulin release. In conclusion, motilin stimulates insulin release through vagal cholinergic, muscarinic receptors on pancreatic β-cells, and the effect appears to be modulated by adrenergic nerves.

In Vivo Study

Preparation of animals. Normal dogs. Normal dogs were prepared according to the method described previously (24). Briefly, in five healthy mongrel dogs weighing 10–15 kg, force transducers were chronically implanted onto the serosal surface in the direction of the circular muscle in the gastric antrum and midduodenum. A silicone tube was chronically inserted into the superior vena cava through the right jugular vein, and another was placed into the inferior vena cava through a branch of the femoral vein. The jugular tube was used for withdrawal of blood samples, and the femoral tube was used for injection of test drug solutions. During the experimental period, drinking water was given ad libitum.

Truncally vagotomized dogs. Five additional dogs underwent bilateral thoracic vagotomy at the subcardiac level through a right thoracotomy. After thoracic surgery, Heineke-Mikulitz type pyloroplasty was added.

Experimental procedures. Normal intact dogs. Experiments were started after the regular cyclic occurrence of interdigestive phase III contractions had been confirmed in the stomach; these contractions usually became established ~1 wk after the operation. As a preliminary study to determine the relationship between plasma motilin, glucose, and insulin concentrations, random blood samples were obtained, mainly during phase II and phase III periods. Thereafter, we decided to take blood samples two times during phase I and II, and in early, middle, and late phase III. The timing of blood collections was guided by visual inspection of contractile activity recordings from the gastric antrum. After the preliminary study, we carried out experiments to confirm whether exogenous motilin mimics the spontaneous release of insulin.

Materials and Methods

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For this, an intravenous continuous infusion of motilin at a dose of 0.3 \( \mu g \cdot kg^{-1} \cdot h^{-1} \) was initiated 20 min after the termination of phase III and was continued for 30 min. Blood samples were collected before and during motilin infusion in a similar way.

In the study to examine the mechanism of stimulation of insulin release by motilin, a dose-response study was first conducted. Canine motilin was given as a slow single intravenous injection at doses of 0.01, 0.03, 0.1, and 0.3 \( \mu g/kg \) after starting a background intravenous infusion of normal saline at a rate of 20 ml/h. The saline infusion was started 10 min after the end of phase III contractions in the stomach. From the dose-response study, a submaximal dose of 0.1 \( \mu g/kg \) was chosen for use in subsequent experiments with antagonists.

In the study with antagonists, intravenous continuous infusion of an antagonist was initiated 10 min after the end of phase III and was continued for 40 min. Ten minutes after the start of the infusion, canine motilin at a dose of 0.1 \( \mu g/kg \) was given as a slow intravenous injection.

The antagonist doses, determined from our previous study, were as follows: atropine was given intravenously as a single bolus of 0.05 mg/kg followed by intravenous infusion at 0.05 mg/kg-1·h-1, and hexamethonium was given as a single bolus dose of 3.0 mg/kg followed by a continuous infusion at 7.0 mg·kg-1·h-1. Because ondansetron [a 5-hydroxytryptamine (5-HT3) receptor antagonist] has been reported to be active for 30 min after intravenous injection, it was given as a single bolus dose of 1.0 mg/kg. \( \alpha \)- and \( \beta \)-adrenergic blockers were each given as an intravenous infusion of 2.0 mg·kg-1·h-1.

Each experiment was repeated twice in each of the five dogs, and the results are expressed as means ± SE.

**TRUNCALLY VAGOTOMIZED DOGS.** In the vagotomized dogs, the typical contractile pattern of the interdigestive state was lost, and the phases were obscured. Experiments were therefore carried out during the quiescent period in the stomach to determine whether exogenous motilin stimulates endogenous insulin release.

**Measurements, Monitoring of Gastrointestinal Contractions.** Gastroduodenal contractions were monitored on a multichannel pen-writing recorder (WT685G; Nihon Kohden Kohyo, Tokyo, Japan) by connecting the lead wires from the force transducers to the cable leads of the amplifiers from 0900 to 1700. These recordings were used to identify the phases of interdigestive contractile activity to determine the times of injection for exogenous motilin and inhibitors and of blood sampling.

**In Vitro Study**

Isolation of pancreatic islets. The isolation of pancreatic islets was carried out according to the method of van der Burg et al. (32). Three adult mongrel dogs were anesthetized with a single intravenous dose of pentobarbital sodium (30 mg/kg body wt Nembutal; Abbott Laboratories, Chicago, IL), and the abdominal cavity was opened. After the pancreatic duct had been cannulated with a 3-Fr tube, the splenic segment of the pancreas was removed and immediately perfused with 150 ml ice-cold collagenase solution for 10 min. The gland was covered with collagenase solution, transported on ice within 40 min of excision, and incubated at 38°C for 20 min in a water bath. The collagenase solution was decanted, and adhering nondigested tissue was dispersed in ice-cold University of Wisconsin solution (UWS) by gentle aspiration and flushing through a blunt 14-gauge needle, then filtering through a 400-µm steel mesh. Trapped tissue was syringed and sieved one or two times, and the trapped tissue was finally discarded. The suspension was centrifuged at 200 g for 2 min at 4°C in a Beckman centrifuge in 50-ml screw-capped conical tubes, and the sediments were pooled into one tube. After resuspension in ice-cold UWS, 1-ml samples for assessment were transferred to Microfuge tubes on ice, and digest aliquots were transferred into tubes for density gradient purification. After centrifugation and decantation, the pellet digest was resuspended by gentle passage through a 10-ml pipette using 12 ml Percoll (Pharmacia, Uppsala, Sweden) solution (density 1.055 g/ml). The Percoll solutions were underlaid with 4 ml and overlaid with a further 6 ml each of these solutions with densities of 1.085, 1.075, and 1.045 g/ml. The gradients were centrifuged at 40 g for 5 min at 4°C and then at 500 g for 12 min, without braking. Purified islets were aspirated from the first (1.045/1.075) and second (1.075/1.085) layers of the gradients. After the purified fractions were washed in ice-cold UWS, the purified fractions and aliquots were taken for assessment.

Perfusion studies. The perfusion system that we recently developed for motilin release (27) was used to examine insulin release from the islet tissue. The dispersed cells were mixed with preswollen Bio-Gel P-2 (Bio-Rad Laboratories, Richmond, NY) and separated into columns (1.5-ml volume). Cell columns were perfused continuously using a peristaltic pump at a flow rate of 0.3 ml/min. The perfusion medium was maintained at 37°C and continuously gassed with 95% O2-5% CO2. Each experiment consisted of an initial 90-min equilibration period with 2.5 mM glucose, followed by a 10-min period with 16.7 mM glucose, 10-2 M acetylcholine, or 10-6 M motilin. The fractions were collected at 2-min intervals, and the insulin concentration in the perfusion media was determined by enzyme immunoassay (EIA). Insulin secretion data were expressed as percentages relative to the average of two basal secretion values obtained from -10 to -5 min.

**Measurement of blood glucose.** Blood samples were taken at 0, 5, and 10 min after intravenous injection of motilin, and blood glucose was determined in 200-µl aliquots of plasma by means of an automatic glucose analyzer (Glucoroder-E; Analytical Instruments, Tokyo, Japan) that uses the glucose oxidase technique. The sensitivity of the analyzer is 1.0 mg/ml glucose.

**Measurement of insulin.** The method used in the present study was a microparticle capture EIA for high-molecular-mass analysis and fluorescence polarization immunoassay for hapten, as reported by Flore et al. (5). Insulin was specifically recognized with a monoclonal mouse antibody against insulin, and bound/free separation was achieved using microparticles as a solid phase. When the same samples were measured by this method and a radioimmunoassay, the correlation coefficient between the two measurements was very high.
0.982 with a regression line; \( y = 0.87x - 0.66 \). The actual assay procedures were carried out using an automated bench-top immunochemistry analyzer system (IMx; Abbott Korea, Ansan, Korea), and the minimum detectable concentration was 0.1 \( \mu U/\text{ml} \) in dog plasma.

Measurement of motilin. The radioimmunoassay employed for motilin has been described previously (24). The minimum detectable concentration was 6.25 pg/ml in dog plasma.

Drugs. Synthetic canine motilin and its fragments were purchased from the Peptide Institute (Osaka, Japan). Kits for insulin EIA were purchased from Dainabot (Tokyo, Japan), and those for glucose measurement were from Analytical Instruments. Other materials used were obtained from the following sources: collagenase XI from Sigma Chemical; UWS (ViaSpan) from Du Pont Pharmaceuticals; bovine serum albumin (fraction V) from Miles Laboratories (Elkhart, IN); Percoll from Pharmacia; atropine sulfate from Tanabe Pharmaceutical (Osaka, J apan); hexamethonium bromide and propranolol from Wako Pure Chemicals (Osaka, J apan); phentolamine from Ciba-Geigy (Hyogo, J apan); Ondansetron was a gift from Glaxo Wellcome (Herts, UK).

Statistics

The experiments were conducted two times in each dog, and the results are expressed as means \( \pm \) SE. Differences between the groups were compared by Student's t-test and repeated-measures analysis of variance where appropriate. The level of significance was \( P < 0.05 \).

RESULTS

Interrelationship of Plasma Insulin and Motilin Concentrations and Gastric Contractions in Normal Intact Dogs

Figure 1A shows a typical example of the relationship between changes in the plasma motilin, insulin, and glucose concentrations and contractile activity in the gastric antrum and duodenum of a conscious dog. Random blood samples were taken from phase I to the subsequent phase II. The plasma motilin concentration started to increase at the beginning of phase II in the stomach and duodenum, reached a peak at the end of phase III, as reported previously by us (14), and then declined. As the motilin concentration started to increase, the plasma insulin concentration rose quickly, reached a peak during early phase III, and then gradually decreased to the control level, exhibiting small fluctuations. The glucose concentration did not fluctuate significantly during phase I, II, or III. The mean concentrations of insulin and motilin in each phase of gastric contractile activity are shown in Fig. 1B and indicate a significant increase in insulin level (from 3.2 \( \pm \) 0.2 \( \mu U/\text{ml} \) during phase I to 6.7 \( \pm \) 0.9 \( \mu U/\text{ml} \) during early phase III; \( P < 0.05 \)) as well as in motilin level (from 58.3 \( \pm \) 12.5 pg/ml in phase I to 311.5 \( \pm \) 60.0 pg/ml in late phase III; \( P < 0.05 \)).

Effect of Exogenous Motilin on Insulin Release

A continuous intravenous infusion of motilin at a dose of 0.3 \( \mu g/\text{kg} \cdot \text{h}^{-1} \), which was initiated 20 min after the termination of spontaneous phase III contractions in the stomach, reproduced a series of contractions that were quite similar to the spontaneous phase

Fig. 1. A: spontaneous changes in plasma insulin, glucose, and motilin concentrations, and contractile activity in the stomach and duodenum in a fasted conscious dog. Plasma motilin concentration started to increase with phase II in the duodenum and reached a peak at the end of phase III. Insulin concentration started to rise with the increase in motilin concentration and quickly reached a peak at the beginning of phase III. B: mean changes in plasma insulin, glucose, and motilin concentrations during phase I, II, and III of the interdigestive state in conscious dogs. Mean duration of phase III was 25.6 \( \pm \) 3.1 min. Mean plasma concentrations of insulin in early phase III and motilin in late phase III were significantly (\( P < 0.05 \)) elevated from the respective values during phase I. Values are means \( \pm \) SE; \( n = 5 \).

* \( P < 0.05 \).
Dose-Response Study of the Effect of Motilin

Endogenous insulin release was significantly stimulated by an exogenous single bolus dose of motilin and reached a peak 5 min after that of motilin. Figure 3A shows the dose-dependent increase in peak insulin release values produced by exogenous motilin over the range 0.01–0.3 µg/kg. Peak insulin values obtained in response to 0.03, 0.1, and 0.3 µg/kg motilin were significantly (P < 0.05) different from those observed with the saline control. Glucose concentrations after motilin injection did not change significantly in comparison with the basal level, as shown in Fig. 3B. A typical contractile response of the gastric antrum to 0.01–0.3 µg/kg motilin is shown in Fig. 4 in which it can be seen that contractile activity in the gastric antrum was not stimulated by exogenous motilin at 0.01 µg/kg, whereas 0.1 and 0.3 µg/kg motilin induced phase III-like contractions.

Effects of Cholinergic and Serotonergic Antagonists on Motilin-Induced Insulin Release

The cholinergic blockers atropine and hexamethonium completely suppressed the insulin response to exogenous motilin, as shown in Fig. 5 (P < 0.05 vs. saline control). The contractile response of the stomach and duodenum was also inhibited by pretreatment with atropine and hexamethonium (data not shown). On the other hand, a 5-HT₃ receptor antagonist, ondansetron, at a dose of 1.0 mg/kg, strongly (P < 0.05 vs. saline control) inhibited the insulin response to exogenous motilin, but the insulin concentration gradually increased, as shown in Fig. 6.

Effects of Adrenergic Antagonists on Motilin-Induced Insulin Release

In contrast to the cholinergic antagonists, basal insulin secretion was significantly affected by treatment with adrenergic antagonists. Phentolamine, an α-adrenergic antagonist, significantly (P < 0.05 vs. saline control at -5 and 0 min) stimulated basal insulin release, as shown in Fig. 7A, whereas propranolol, a β-adrenergic antagonist, tended to inhibit the
basal secretion of insulin. With continued infusion of phentolamine, intravenous injection of motilin enhanced insulin secretion ($P < 0.01$, $33.2 \pm 5.5 \mu U/ml$ vs. saline control, $12.4 \pm 3.1 \mu U/ml$) about threefold in comparison with the control, and motilin-induced insulin release did not return to the premotilin level, even by 30 min after motilin injection, as shown in Fig. 7A. The insulin level 30 min after motilin injection was still significantly ($P < 0.05$, $11.8 \pm 2.5$ vs. saline control, $4.5 \pm 1.0 \mu U/ml$) elevated. The enhancement of motilin-induced insulin release by phentolamine was, however, completely eliminated by atropine, as shown in Fig. 7B, and, in comparison with the situation when no motilin was given, motilin-induced insulin release was significantly stimulated during the infusion of phentolamine (Fig. 7B). Pretreatment with propranolol clearly suppressed the insulin response to motilin, but the response was not significantly different from the saline control, as shown in Fig. 7A.

**Effect of Vagotomy on Motilin-Induced Insulin Release**

In fasted vagotomized dogs, the mean insulin concentration was $4.2 \pm 0.6 \mu U/ml$. Under these conditions, endogenous release of insulin in response to $0.1 \mu g/kg$ motilin was suppressed, as shown in Fig. 8.

**In Vitro Study**

Figure 9 shows the results obtained upon perifusion of isolated pancreatic islets. At a high glucose level (16.7 mM), the rise in the insulin concentration was similar to that obtained by van der Burg et al. (32). In response to glucose and acetylcholine, a significant ($P < 0.05$ vs. saline control) release of insulin was observed, but addition of motilin ($10^{-6}$ M) to this system did not stimulate insulin release.

**DISCUSSION**

During the interdigestive state, the plasma concentrations of motilin and PP are known to fluctuate at ~100-min intervals (19), and the increase in the motilin concentration initiates phase III contractions in the stomach and duodenum. These interdigestive contractions move the intraluminal contents to the large intestine (31), but the role of PP in the fasted state remains unknown. In our previous study (24), we confirmed a concomitant increase in the plasma concentrations of motilin and PP and found that exogenous motilin at doses lower than the physiological range ($0.01 \mu g/kg$) significantly stimulated endogenous release of PP, whereas PP did not stimulate endogenous release of motilin, as others have reported (8). Motilin was found to stimulate PP release through the vagal cholinergic muscarinic pathways, and there was no direct stimulatory action of motilin on the endocrine pancreas. This means that the stimulatory action of motilin is not directly transmitted to PP cells, as with humoral factors, but is indirectly mediated through cholinergic pathways (24).
In the present study, we carried out a similar experiment on insulin release and found that the effect of motilin on endogenous insulin release was similar to its effect on PP release. Moreover, during the interdigestive state, insulin was found to increase in association with the natural cyclic increase in motilin, but the inhibition of insulin release was not significant. The greatest problem concerning the conclusion of this study (that motilin causes the release of insulin) is the finding that plasma insulin reaches its peak significantly earlier than plasma motilin, and that, while the motilin concentration is still increasing, the plasma insulin level returns to the control value under natural conditions. In the present study, therefore, we carried out experiments to verify whether or not an intravenous continuous infusion of motilin at a dose reproducing the spontaneously occurring phase III contractions in the stomach really mimics the insulin release observed under natural conditions. It was found that, even when motilin was given as an intravenous continuous infusion to mimic natural conditions, the insulin peak was always observed at early phase III and quickly declined to the basal level while the motilin concentration was still increasing. Although this phenomenon appears to be at odds with general physiological knowledge, a similar situation can be seen in the interdigestive contraction of the gallbladder, as we have reported previously (13). The peaks of interdigestive gallbladder tonic contraction coincide with the border between phase II and phase III in the stomach, and the tonic contraction returns to the original tonicity at the end of phase III. The relationship between contractile activity in the stomach, the plasma motilin concentration, and gallbladder contraction is comparable with that observed in the release of insulin in response to endogenous and exogenous motilin. As...
discussed later, the action of motilin on the gallbladder is also completely dependent on the vagal cholinergic pathway, and, accordingly, it can be assumed that a common mechanism underlies both the insulin release and gallbladder contraction in response to motilin.

As with PP, insulin release in response to exogenous motilin was dose dependent, and the minimum effective dose of motilin required to stimulate endogenous insulin release was 0.01 µg/kg. This dose of motilin did not stimulate contractile activity in the gastric antrum. Motilin-induced insulin release was completely eliminated by atropine and hexamethonium and suppressed by truncal vagotomy. These findings support our hypothesis that the action of motilin is mediated to β-cells through vagal, cholinergic muscarinic pathways. The pancreatic islets appear to be densely innervated by cholinergic neurons, since there is 10-fold greater choline acetyltransferase and acetylcholinesterase activity in the islets than in the exocrine tissues (6). Stimulation of insulin release by acetylcholine has also been demonstrated in the perfused dog pancreas (16), and insulin release in response to electrical stimulation of the vagus is markedly inhibited by atropine and completely abolished during nicotinic blockade by hexamethonium (1).

The complete elimination of the endogenous insulin response to motilin by the 5-HT3 receptor antagonist strongly suggests a common pathway in the stimulatory effect of motilin on muscle contraction in the stomach (12) and on PP (24) and insulin release in the endocrine pancreas. However, the exact site of the 5-HT3 receptors in the pathway along which the action of motilin is mediated has not yet been elucidated. 5-HT3 receptors are known to be widely distributed in the central and enteric nervous systems, but, as yet, there are no reports indicating the existence of 5-HT3 receptors in the pancreas, particularly in the parasympathetic ganglion.

On the other hand, it is certain that the action of motilin on the stomach and endocrine pancreas is transmitted through the vagus, but the sites or receptors through which motilin might act are entirely unknown, particularly in the dog. For instance, Hashmonai et al. (9) compared the effects of intrathecal, intracerebroventricular, and intravenous motilin administration in conscious dogs and clearly demonstrated that the initiation of phase III could be induced only by intravenous administration. These findings (9) indicate that there is no route by which motilin can be transmitted from the intrathecal or intracerebroventricular space to the dorsal nucleus of vagus nerve but do not necessarily rule out the possibility of motilin receptors in the central nervous system, which may directly or indirectly affect the function of the dorsal nucleus of vagus nerve. The area postrema, for instance, might be a candidate because of its rich vascularity and fenestrated capillary walls surrounded by a variety of monoaminergic and peptidergic neurons in the perivascular space (25), which involve 5-HT3 receptors and are closely connected to the nucleus tractus solitarii and the parabrachial nuclei. In addition to a number of monoamines and regulatory peptides (4), the action of peripheral motilin might be transmitted to the dorsal nucleus of vagus nerve through these structures. On the other hand, because recent studies have pointed to a new mechanism underlying gastrointestinal function in the rat, involving the direct humoral action of gut hormones such as PP (23) and peptide YY (10) on the dorsal vagal complex, motilin may act directly on the dorsal vagal complex and trigger insulin release through the vagus.

The endogenous release of insulin in response to motilin was greatly enhanced by pretreatment with phentolamine, with the peak insulin response after phentolamine being about threefold that with the saline control. Because this enhancement was completely abolished by atropine, motilin-induced cholinergic excitation might be strongly inhibited by norepinephrine through postganglionic α-adrenergic receptors under normal conditions. Therefore, it seems likely that the enhanced insulin response seen after phentolamine administration was due to a relative predominance of cholinergic excitation. Indeed, adrenergic inhibition occurred soon after the start of intravenous phentolamine infusion, since the plasma insulin concentrations at 5 and 10 min were significantly higher than the prephentolamine level and the saline control level. The plasma insulin concentration in conscious baboons has also been reported to decrease after propranolol administration (7), and thus sympathetic tone seems to be one determinant of basal insulin secretion. A number of studies have also suggested that, in dogs, adrenergic agonists inhibit insulin release through α-adrenergic receptors and stimulate it via β-adrenergic receptors (15).

In the present study, there was no change in blood sugar levels, whereas the level of insulin was elevated. Although the reason for this is not fully clear, it has been shown that stimulation of the vagus nerve increases the secretion of both glucagon and insulin in a variety of species (1). Because our previous study and the present one showed that motilin stimulates the endocrine pancreas (PP, insulin) via the vagus, it is likely that endogenous pancreatic glucagon is also stimulated by motilin.

The physiological significance of periodic insulin release in association with motilin remains unclear, considering the fact that the periodic release of insulin is an event occurring in the interdigestive state when no nutrients are present in the gastrointestinal tract. As to the functions of these cyclic motor events, it has been postulated that motilin-induced phase III in the stomach plays a physiological role as an interdigestive housekeeper, in preparation for the next meal (31). The reason for the association between insulin and motilin release is not known. Bueno and Ruckebusch (3) studied the relationship between insulin and intestinal motility in conscious dogs but did not report whether insulin release increased in association with phase III in the intestine. Rayner (26) reported close relation-
ships between the migrating motor complex, glucose absorption, and insulin secretion in fed pigs and described the point at which maximum values of insulin were observed.

Many endocrine organs secrete hormones episodically or in pulses. Examples of hormones with rhythmic or pulsatile secretion are cortisol, growth hormone, prolactin, and pancreatic islet hormones. Recent studies on insulin have demonstrated oscillation of fasting blood glucose and insulin levels with periodicities ranging from 10 to 14 min to as long as several hours (28). However, it is difficult to compare these individual data with the present findings because of the different sampling times and methods of periodicity analysis that were employed and the fact that none of the previous studies assessed motor activity in the stomach or duodenum. Other studies in humans (20) and dogs (18), however, appear to describe phenomena similar to the present findings, although the details are difficult to confirm. Because pulsatile secretion of insulin with a periodicity of 10–14 min was observed in the isolated perfused pancreas (30), it is generally agreed that most components of the system that regulates islet secretory pulsatility do not lie in the central nervous system but must reside within the pancreas itself, probably in the pancreatic ganglia (29). Therefore, it can be concluded that the motilin-associated, transient release of endogenous insulin demonstrated in the present study is quite different from the extensively studied pulsatile secretion of insulin.

In conclusion, a transient but significant insulin release was observed in close association with endogenous release of motilin during the interdigestive state in conscious dogs. This endogenous insulin release was dose dependently reproduced by administration of exogenous motilin at doses of 0.01–0.3 µg/kg. Motilin-induced insulin release was mediated through vagal cholinergic muscarinic pathways and by 5-HT3 receptors. The physiological roles of the cyclic release of insulin and PP require further elucidation.

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