Radioimmunoassay of Motilin

Validation and Studies on the Relationship between Plasma Motilin and Interdigestive Myoelectric Activity of the Duodenum of Dog

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A radioimmunoassay method of motilin was developed in our laboratory and was validated in dogs with a platinum monopolar electrode in the duodenum. We confirmed that a bolus infusion of 0.3 M tris-buffer solution or 0.1 N HCl solution in the duodenum produces a significant rise in plasma immunoreactive motilin (IRM) concentrations. This coincided with a marked increase in the percentage of spike potentials on slow waves of the duodenum, similar to phase III of interdigestive myoelectric-activity (MA). A possible relationship between plasma IRM and interdigestive MA of canine duodenum was studied. It was found that cyclic changes occurred in the fasting plasma IRM concentrations in dogs. While the peak motilin concentration was always observed in phase III, the lowest concentration of motilin was found in phase I of interdigestive MA in the duodenum. In dogs with the electrodes in the duodenum and jejunum, the peak IRM concentration did not correlate with phase III of interdigestive MA in the jejunum. A dose of synthetic porcine motilin, 0.06 μg/kg/hr, which produced the plasma IRM concentration comparable to the peak fasting motilin concentration, could induce an identical phase III in the duodenum. These observations indicate that there is a relationship between cyclic changes in plasma IRM concentrations and interdigestive MA of the duodenum. It is suggested further that motilin is a hormone which may play an important role in inducing phase III of interdigestive MA in the duodenum.

Since motilin, a 22-amino acid polypeptide, was originally isolated from the hog duodenal mucosa by Brown et al (1) in 1971, both natural and synthetic porcine motilin have been known to stimulate contraction of the canine stomach (1–3), lower esophageal sphincter (2, 4, 5), and small intestine (2, 3). Synthetic porcine motilin (6) or 13-norleucine-motilin (13-nle-motilin) (7), a synthetic analogue of motilin, has also been reported to induce spike potentials of myoelectric activity in the canine antrum and small intestine (8, 9). Moreover, natural motilin or 13-nle-motilin stimulates gastric secretion of pepsin in dog (1) and in man (10), and 13-nle-motilin stimulates gastric secretion of acid and pepsin in dog (11).

In spite of available information on the biological actions of motilin or its analogue, little has been known about the release of endogenous motilin by physiological stimuli and the possible role of endogenous motilin on gastrointestinal function. Recently

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we have developed a radioimmunoassay method of motilin using antimotilin antibodies produced in New Zealand rabbits (12). In the present communication we demonstrate validation of this assay method and report our observations on the action of motilin on myoelectric activity and the relationship between plasma concentrations of endogenous motilin and myoelectric activity in the duodenum in dog.

MATERIALS AND METHODS

Radioimmunoassay Method of Motilin and Validation Experiment

A radioimmunoassay method for plasma motilin was recently developed in our laboratory (12). Antibodies were raised in randomly bred New Zealand white rabbits by repeated immunization with 0.5 mg of synthetic porcine motilin (6) coupled to 1 mg of bovine serum albumin (BSA) at monthly intervals. The immunogens were injected in the foot pads of these rabbits. Four weeks following the initial injection, two consecutive injections of 0.5 mg of conjugate were carried out at monthly intervals, and were followed by repeat booster injections of 0.05 mg of conjugate at the same intervals. Two weeks after each immunization the animals were bled by venous puncture of the ears. High titers of antibodies at a final dilution of 1:187,000 were obtained after third booster injections.

Either natural or synthetic porcine motilin was iodinated with Na[^{125}I] by a modification of Hunter and Greenwood technique (13). One μg of motilin in 50 μl of 0.5 M sodium phosphate buffer, pH 7.5, was reacted with 1 mCi of carier-free Na[^{125}I] by the addition of 25 μg of chloramine T. After 30 sec the reaction was terminated by the addition of 250 μg of sodium metabisulfite. The reaction mixture was diluted with 4 ml of H_2O, acidified to pH 6 with 1 N HCl, and immediately loaded onto a CM-Sephadex C-25 column (1 cm × 20 cm) equilibrated with 0.5% BSA in H_2O. The column was washed with approximately 20 ml of the same solution before a gradient elution consisting of 100 ml each of 0.5% BSA in H_2O in the mixing chamber and 0.5% BSA in 300 mM ammonium acetate buffer, pH 5.9, in the reservoir was carried out. Fractions of 2.2 ml were collected and assayed for radioactivity and immunoreactivity. Fractions which were consistently the major radioactive peak during gradient elution contained [^{125}I]-labeled motilin. The specific radioactivity of [^{125}I]-labeled motilin was usually in the range of 300–400 μCi/μg as determined by the self-displacement methods previously described (14). These fractions were kept frozen at −20° C. The [^{125}I]-labeled motilin possessed good immunoreactivity and gave reasonable sensitivity in radioimmunoassay within one month of storage.

The assay mixture (1.5 ml) contained 0.2 ml of known concentrations of natural porcine motilin or 0.2 ml of extracted plasma sample, 1.1 ml of diluted antiserum, and 0.2 ml of [^{125}I]-motilin (5,000 cpm). All the ingredients were prepared in the standard assay buffer, 0.1 M tris-HCl buffer, pH 7.8, containing 0.1% BSA. The incubation was continued for 48 hr at 4° C. Separation of bound from free antigen was achieved by plasma-coated charcoal as described previously (15). Both the supernatant fluid containing the antibody-bound hormone and the pellet containing the free hormone were counted by a Packard gamma-Spectrometer. The analysis of hormone concentration of each sample was carried out by a computer as previously described (15).

The antibodies showed no cross reactivities with other known gastrointestinal hormones including natural porcine secretin, cholecystokinin-pancreozymin, vasoactive intestinal polypeptide, and synthetic human gastrin I. The sensitivity of the assay was approximately 3 pg/assay tube. Unextracted plasma samples showed significant nonspecific interference in the assay as evidenced by the fact that significant variations in binding ratios were observed from one motilin-free sample to another (12). However, these interfering factors in the plasma could be removed by precipitating the plasma with four volumes of methanol at 4° C and the assay was carried out with the evaporated methanol extract. Recovery of the plasma motilin in the methanol extract was approximately 70%. The evaporated extracts from various motilin-free plasma yielded a uniform binding ratio as expected. Furthermore, parallelism of the assay could be easily demonstrated with an evaporated extract containing exogenously added motilin (12). The ratio of recovery of the added natural porcine motilin, ranging from 10 pg/tube–500 pg/ml, to methanol extract as measured by radioimmunoassay was 98%. Intraassay and interassay variations were 5.2% and 8.8% respectively. The reported plasma concentrations of motilin have been corrected for recovery during methanol extraction. A representative displacement curve of natural porcine motilin is shown in Figure 1.

To validate the radioimmunoassay, two types of experiments were performed in the three conditioned dogs with duodenal cannulae and a platinum monopolar electrode sutured on the serosal surface of the duodenum, 5 cm caudal to pyloroduodenal junction. After an 18-hr fast, 50 ml each of 0.9% NaCl, 0.1 N HCl, and 0.3 M tris-buffer solution (pH 10.2) were infused into the duodenal cannula in 3 min. Blood samples were collected in heparinized tubes at −5, 0, 3, 5, 10, 20 and 30 min after intraduodenal infusion of these three dogs (2 experiments/dog) and myoelectrical activity was recorded throughout the experimental period. Each plasma sample obtained during the experiments was coded before the assay and was decoded after the assayist determined the motilin concentration of each sample.

Experimental Dog Preparations

Twelve mongrel dogs weighing 12.0–16.5 kg were prepared with gastric cannula placed in the most dependent portion of the stomach, and a platinum monopolar electrode sutured on the serosal surface of the duodenum, 5 cm caudal to the pyloroduodenal (PD) junction. In three of these twelve dogs, two additional electrodes were also placed in the jejunum. The proximal one (J_1) was placed approximately 70 cm, and the distal one (J_2) 100 cm from the PD junction. The monopolar electrode was constructed by the method described by McCoy and Bass (16). Sterile insulated copper wires leading from

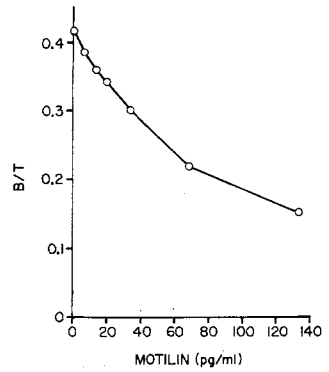

Fig 1. A motilin standard curve using a pure porcine motilin. All values represent means of triplicate determinations.

each electrode were connected to a stainless steel cannula which contained a multi-way connector. The cannula was implanted in the right anterior abdominal wall. In another three of the remaining nine dogs with the electrode in the duodenum only, a stainless steel cannula with inner diameter of 0.8 cm was placed in the duodenum, 10 cm caudal to the PD junction. The animals were allowed to recover from the surgical procedure for more than 2 wk before experiments were initiated.

Plasma Motilin Concentrations and the Myoelectric Activity of the Duodenum in Fasting State

After an 18-hr fast, the interdigestive MA in the duodenum was recorded on a Harvard Apparatus Recorder using a High-gain AC amplifier (Model No. 2171, frequency 0.2 Hz ~ 12.0 KHz, time constant 0.03 sec). The ground wire was placed in the subcutaneous tissue of a hind leg. An intravenous catheter was kept open by slow infusion of 0.9% NaCl solution. After recording one cycle of the interdigestive MA (17, 18) including phases I, II, III and IV (17), serial peripheral venous blood samples were obtained throughout the entire period of the following cycle. The time of the blood sampling was adjusted to each phase of duodenal MA and was marked on the recording paper during the entire period of recording the myoelectric activity. Five-milliliter samples of venous blood were drawn in heparinized tubes at 10–20 min intervals during phase I, at 5–10 min intervals during phase II, at 2–3 min intervals during phase III and at 2–5 min intervals during phase IV. The plasma was separated by refrigerated centrifugation at 3000 rpm for 10 min and was kept in a frozen condition below −20° C for future radioimmunoassay of motilin. Only one experiment was performed on each dog per week. Twelve experiments (2 experiments/dog) were performed while the stomach cannula was kept open to drain gastric juice. Four more experiments (2 experiments/dog) were performed in the same fashion in two of these six dogs while the gastric cannula was closed. In the three dogs with two additional electrodes in the jejunum, an identical study was carried out while the myoelectric activity was recorded from the three different parts of the small intestine. Recordings of the myoelectric activity were analyzed in 2-min intervals throughout the entire experimental period and the percent of slow waves that were accompanied by spike potentials (% spike potential) was calculated.

Effects of Synthetic Porcine Motilin on the Myoelectric Activity and Plasma Motilin Concentrations

In three of the twelve dogs, synthetic porcine motilin (6) was administered intravenously starting immediately as phase I started. Two different doses of motilin, 0.06 and 0.125 μg/kg/hr, were administered for a period of 1 hr. Six experiments were performed in these three dogs. The recordings of the myoelectric activity were analyzed and % spike potentials/2-min interval were determined.

Analysis of the Data

The results were expressed as a mean of determinations ± one standard error (SE) and a student *t* test for unpaired data (19) was used for comparison. *P* values of less than 0.05 were considered statistically significant.

RESULTS

Effect of Intraduodenal Infusion of HCl and Tris-Buffer Solution on Plasma Immunoreactive Motilin (IRM) Concentration and Myoelectric Activity of the Duodenum

Three sets of experiments were performed in three dogs (Figure 2). Each set consisted of 6 experiments (2 experiments/dog). The infusion of 50 ml of 0.9% NaCl produced no significant change in the plasma IRM concentration. The change in % spike activity on slow wave/unit time was also negligible. The infusion of 0.3 M tris-buffer solution (pH 10.2), however, resulted in a marked increase ($P < .05$–$.001$) in the plasma IRM concentration. After initiation of the infusion of tris-buffer solution, the mean IRM increased significantly in 5 min and reached its peak at 10 min. The increase lasted for as long as 20 min. This increase in the IRM coincided with a marked increase in the mean % spike activity, simi-

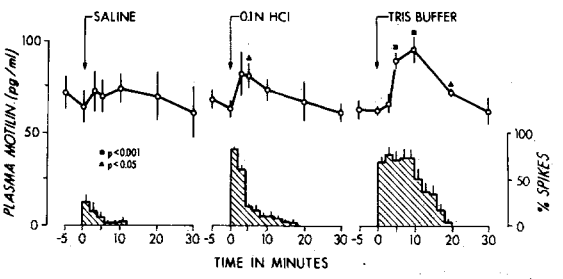

Fig 2. Mean plasma-IRM concentrations and % spike potentials on slow waves in the duodenum after intraduodenal infusions of 50 ml each of 0.9% NaCl (saline), 0.1 N HCl and 0.3 M Tris buffer (pH 10.2) in three dogs (mean ± SE of six experiments).

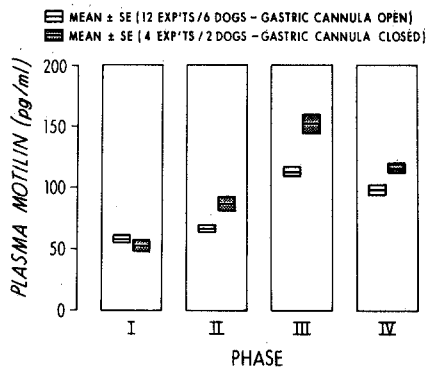

Fig 3. Mean plasma-IRM concentrations in phase I, II, III and IV of interdigestive MA in two different experimental conditions in six dogs.

lar to that seen in phase III of interdigestive MA. The infusion of 0.1 N HCl resulted in a significant increase in the IRM also. The magnitude and duration of the increase in IRM, however, was less than that observed after infusion of tris-buffer solution as shown in Figure 2. A phase III-like myoelectric activity was also produced by the infusion of HCl in the amount employed in the study.

Plasma IRM and Phasic Myoelectric Activities

Phasic cycles of the interdigestive MA were observed in the six dogs studied. The mean duration of phase I, II, III and IV was 49.00 ± 1.71 min, 32.44 ± 3.25 min, 7.00 ± 0.45 min, and 15.69 ± 1.01 min respectively. The mean plasma motilin concentrations during each phase of interdigestive MA in the duodenum are shown in Figure 3. The cyclic changes of plasma IRM concentrations occurred regardless of whether the gastric cannula was kept closed or open to drain gastric contents during the experimentation. In the experiments with an open gastric cannula in these six dogs, the mean plasma IRM concentrations were 57.66 ± 2.83 pg/ml (mean of 43 samples) in phase I, 66.91 ± 3.21 pg/ml (mean of 44 samples) in phase II, 112.65 ± 3.58 (mean of 35 samples) in phase III, and 97.75 ± 3.77 pg/ml (mean of 38 samples) in phase IV (Figure 3). The mean IRM concentrations in phase III and phase IV were significantly greater than those of phase I and II ($P < .001$). Similarly, a significant difference was observed between the means of phase I and II ($P < .05$). In the experiments with gastric cannula closed (Figure 3) a similar trend of cyclic change in the mean plasma-IRM concentrations was observed in the two dogs studied. The mean peak IRM concentration, in phase III was 151.58 ± 7.68 pg/ml (mean of 10 samples).

As shown in Figure 4, the mean IRM concentrations determined in two different periods of each phase were significantly different. The first half of each phase was arbitrarily designated as period 1 and the second half of each phase as period 2. The mean IRM concentration reached its lowest level in period 2 of phase I and gradually climbed to its peak concentration in period 2 of phase III. The differences between the two values, i.e., period 1 and 2, compared in each phase including phase 1 were all statistically significant ($P < .01 \sim .001$) except for the difference between the means observed in phase III in dogs with gastric cannula closed.

In all three dogs with additional electrodes in the jejunum, the peak plasma-IRM concentration was clearly observed in phase III of interdigestive MA in the duodenum but not in that of the jejunum (Figure 5).

Effect of Exogenous Motilin on the Plasma IRM Concentration and the Myoelectric Activity

In three dogs, six experiments, each with two different doses of motilin, were performed. The motilin was always administered starting immediately at the beginning of phase I. In each experiment, as the IRM concentration reached its peak in 30 min, a phase III-like activity occurred in the duodenum (Figure 6). During the control period with intravenous infusion of 0.9% NaCl, it took about 80 min to develop phase III. The slow wave frequently was not affected by exogenous motilin. The plasma-IRM concentration 30 min after initiation of synthetic motilin administration in a dose of 0.06 μg/kg/hr was comparable to that observed in phase III of the

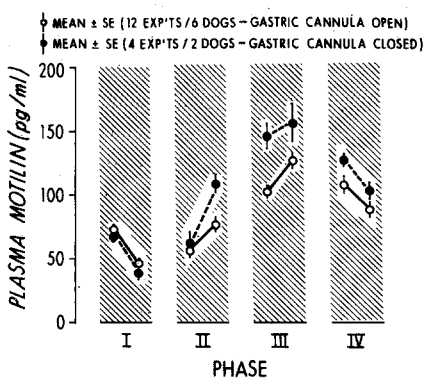

Fig 4. Mean plasma-IRM concentrations in period 1 and 2 of each phase of interdigestive MA in six dogs.

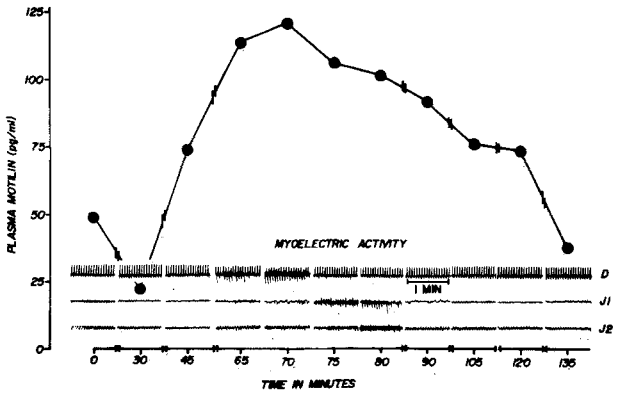

Fig 5. Plasma-IRM concentrations and myoelectric activity of the duodenum and jejunum in a dog. The electrode was sutured to serosal surface of the duodenum (5 cm from PD junction) and jejunum (J_1: 70 cm, and J_2: 100 cm from PD junction). Note that the peak concentration of motilin coincides with phase III of interdigestive MA in the duodenum but not with that in the jejunum.

interdigestive MA in the control experiment. The mean plasma-IRM concentrations and mean % spike potentials in these three dogs during intravenous infusion of synthetic porcine motilin are summarized in Figure 7. The mean plasma-IRM concentration reached the peak level in 30 min after the motilin infusion was started and remained at the plateau level until the infusion of motilin was ended. The administration of motilin in a dose of 0.06 μg/kg/hr resulted in a significant increase in the mean plasma-IRM concentrations, from the mean control concentration of 66.95 ± 13.46 pg/ml to 128.80 ± 10.75 pg/ml, 142.35 ± 15.69 pg/ml, 134.10 ± 14.54 pg/ml, and 137.07 ± 12.07 pg/ml at 30, 40, 50 and 60 min respectively. The increment in the plasma IRM concentrations was statistically significant ($P < .01$). The mean plasma IRM concentration during the motilin infusion at a rate of 0.125 μg/kg/hr increased from 81.38 ± 8.66 pg/ml to 215.33 ± 18.73 pg/ml, 230.22 ± 18.16 pg/ml, 229.40 ± 16.30 pg/ml and 206.43 ± 16.08 at 30, 40, 50 and 60 min, respectively. The magnitude of increase in the IRM concentration produced by this dose of exogenous motilin was significantly greater than that achieved by motilin in a dose of 0.06 μg/kg/hr ($P < .01 \sim .002$). During the administration of motilin in two different doses, the time interval between the end of phase IV and the beginning of phase III of the following cycle of interdigestive MA was shortened from 83.00 ± 4.88 min, to 33.60 ± 2.79 min and 28.33 ± 2.80 min, respectively (Figure 8). Although the peak values of mean % spike potentials produced by the two different dosages of motilin were similar (Figure 7), the mean % spike potentials produced during a 1-hr period by motilin in the dose of 0.125 μg/kg/hr was 52.51 ± 3.40% and was significantly greater than that, 40.58 ± 3.62, produced by 0.06 μg/kg/hr of motilin ($P < .02$). Although the IRM concentration remained elevated during the period of motilin administration, the mean % spike potentials gradually decreased after reaching their peak value as if a cyclic recurring interdigestive MA continued.

DISCUSSION

In the present study we confirm the observation reported by Dryburgh and Brown (20) that intraduodenal infusion of a tris-buffer solution with pH 10.2 produces a significant rise in the plasma-IRM concentration in dogs. In our dogs, this rise coincided with myoelectric activity of the duodenum comparable to phase III of interdigestive MA (17). Moreover, a modest elevation in the IRM concentration was observed also in dogs following a bolus infusion of 0.1 N HCl in the duodenum as observed in man by Mitznegg et al (21).

Although a bolus infusion of a strong alkaline solution with pH 10.2 in the duodenum increases plasma IRM, we have found recently that a continuous alkalinization of the duodenum by constant intraduodenal infusion of 0.1 N $NaHCO_3$ to maintain the intraduodenal pH 7.5–8.0 failed to elevate the

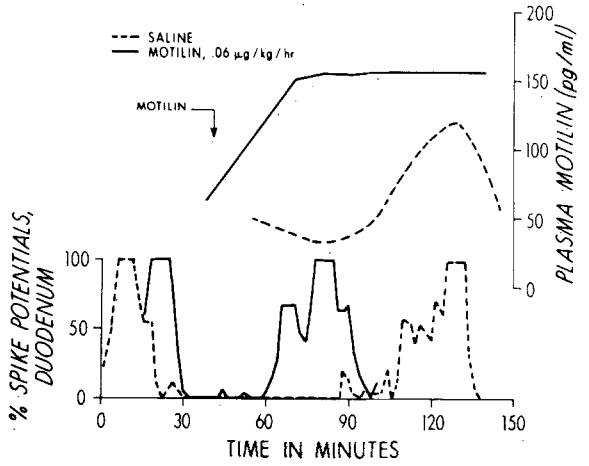

Fig 6. Plasma-IRM concentrations and % spike potentials of the duodenum in a dog during intravenous infusion of saline and synthetic porcine motilin in a dose of 0.06 μg/kg/hr.

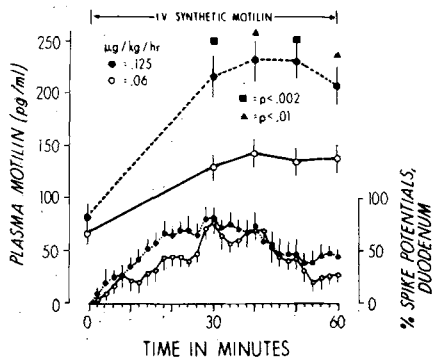

Fig 7. Effects of synthetic porcine motilin in doses of 0.06 and 0.125 μg/kg/hr on the mean plasma-IRM concentrations (± SE) and mean % spike potentials (± SE) of the duodenum in three conscious dogs (2 experiments/dog). The increments of plasma-IRM levels by exogenous motilin were dose-related ($P < 0.01 \sim 0.002$).

IRM concentration (Lee and Chey, unpublished data). In a similar fashion, continuous intraduodenal infusion of 0.1 N HCl at a rate of 2.0 ml/min failed to increase the motilin concentration, while the same amount of HCl consistently increased the plasma concentration of secretin in dog (22). Furthermore, the present study indicates clearly that the cyclic change in the plasma-IRM concentrations occurs with or without elimination of gastric juice from the stomach in fasting dogs. The observation supports the concept that an agent or agents other than gastric acid juice affect the release mechanism of endogenous motilin. Further studies are needed to uncover the release mechanism in various physiological and pathophysiological conditions.

In the present study the plasma-IRM concentrations were found to increase significantly as spike activity of interdigestive MA (17) in the duodenum increased and reach the peak during phase III. The timing of peak IRM concentration always coincided with phase III activity only in the proximal duodenum. No correlation was found between the peak IRM concentration and phase III activity of the jejunum. To investigate further a possible role of motilin on interdigestive MA, a synthetic porcine motilin was administered intravenously to produce plasma concentration of IRM comparable to that observed in fasting state. When the plasma-IRM concentration reached its peak level, which was achieved within 30 min after the initiation of motilin infusion, the myoelectrical activity identical to that of phase III occurred in the duodenum. The elapsed time before it occurs is significantly shortened. However, the motilin did not appear to shorten or prolong the normal duration of interdigestive myoelectric complex subsequent to the initial myoelectric complex induced by exogenous motilin. Wingate et al (9) also found recently that exogenous 13-nle-motilin induced phase III-like activity in the fasting dog. These observations suggest strongly that motilin may play an important role in initiating phase III of interdigestive MA of the duodenum. However, motilin does not account for the migration of interdigestive MA (18, 23, 24).

The interdigestive MA in dogs is abolished by ingestion of a meal (25). Moreover, synthetic analogues of two well-known gut hormones, pentagastrin and an octapeptide of cholecystokinin have been found to abolish the interdigestive MA (9, 25–27), suggesting strongly that they probably play an important role in interrupting interdigestive MA. The cyclic change in plasma-IRM concentrations, however, was not eliminated by these two hormones or secretin (25). Thus, some other biological clock must regulate the onset, development, and migration of interdigestive MA in dogs.

We have recently reported that the fasting plasma-IRM concentrations varied considerably in the same fasting dog (28). In 16 conscious dogs, the IRM concentrations of random plasma samples obtained in 1- or 2-hr periods ranged from 15.6–200 pg/ml with a mean of 81.3 ± 36.9 (SD). It is clear from the present study that the fasting plasma IRM concentrations vary significantly in the same dog because of its cyclic changes in the fasting state. The IRM concentration during the period of phase I is

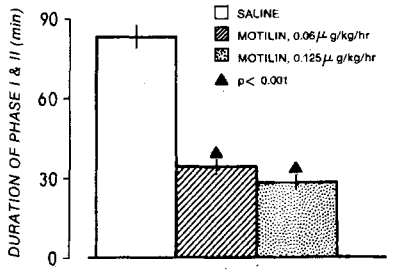

Fig 8. Mean duration of phase I and II of interdigestive MA affected by saline and synthetic porcine motilin, 0.06 and 0.125 μg/kg/hr. Mean of six experiments in three dogs (2 experiments/dog).

markedly less than that in phase III of the interdigestive MA. Moreover, there is a significant variation in the plasma IRM concentration even within the same phase of the interdigestive MA. It cannot be explained at this time why the mean IRM concentration in period 1 is significantly greater than that of period 2 of phase I. One must be careful, therefore, with interpretation of experimental data unless the cyclic change of fasting plasma IRM levels is taken into consideration.

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