By 5 and 6 hr, none of the products elicited any significant antipyretic effect.

When the various commercial acetaminophen suppositories (A–E) were compared to each other and to the laboratory-prepared product (F), no significant differences in antipyretic response were found at 0.5, 1, 2, 4, 5, and 6 hr. At 3 hr, there were no significant differences among Products C–F; however, Products A and B showed some differences. Product B showed its maximum effect at 3 hr (Table I) and exhibited the slowest onset of action; Product A was indistinguishable from the controls at 3 hr and thereafter, thus providing the shortest duration of action.

The magnitude of the variability, as indicated by the standard error (*SE*) (Table I), differed among products, with some showing more intraproduct variability than others. Because of the large variability when compared with other products, Product A was retested; the results were not significantly different from the original results. Product A showed a marked variability *via* visual examination in consistency and coloration when quartered longitudinally. Uniform drug distribution is essential for consistent absorption; and since only one-fourth of the suppository was inserted rectally into the animal, the lack of homogeneity and uniformity among Product A suppositories may account for the large variability in effect.

All five commercial products were stated to contain polyethylene glycol vehicles, but none listed the types or percentages of polyethylene glycol. Thus, a comparison of the differences in effect among formulations was not possible. The differences in the polyethylene glycol vehicle formulations, as well as other formulation factors, could account for the slight differences in the onset of action, duration of action, and time to reach peak antipyretic effect among the products tested.

All products were obtained from commercial sources and were tested within their expiration dates, but their shelftime prior to purchase was not known. The laboratory-manufactured acetaminophen and control suppositories were prepared and used within 1 month of manufacture. Stability of the vehicles is an important consideration in efficacy. Aging can influence the physical–chemical properties of the vehicle (3, 8) and may have contributed to the slight differences in effect among the products tested.

Correlation between *in vivo* and *in vitro* results was previously reported for benzocaine suppositories (9). Similar *in vitro* testing of acetaminophen suppositories A–F did not show any significant differences in drug release. The effects of aging, the polyethylene glycol composition, and other manufacturing and formulation factors all could account for the slight differences among products in this study. Nevertheless, all products tested, including the laboratory-manufactured product, did decrease the rectal temperatures of the yeast-fevered animals significantly. This animal model was a viable method for studying the efficacy of rectally administered antipyretic agents.

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Species Difference in GI Motor Response to Somatostatin

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Received January 29, 1979, from the *Department of Physiology and Biophysics, Health Sciences Center, Temple University, Philadelphia, PA 19140.* Accepted for publication March 2, 1979.

Abstract □ Acute experiments were performed on overnight fasted chloralose–urethan anesthetized dogs, cats, rabbits, and rats. Under these conditions, somatostatin practically abolished gastric contractions and decreased GI tonus in all species examined. The canine duodenum, jejunum, and ileum exhibited only a contractile response to somatostatin, whereas motor activities of the small intestines of the cat, rabbit, and rat were inhibited. In all instances and at all dosages, both the inhibitory and excitatory effects showed suggestions of tachyphylaxis. The data also indicate that excitatory or inhibitory effects were not dependent on the presence of long arc pathways. It is concluded that somatostatin exerts a direct stimulatory effect on the canine small intestine that is mediated by the muscularis mucosa.

Keyphrases □ Somatostatin—effect on GI motor activities, species specificity, dogs, cats, rabbits, rats □ GI motility—somatostatin effect, species specificity, dogs, cats, rabbits, rats □ Growth hormone inhibitors—somatostatin, effect on GI motor activities, species specificity, dogs, cats, rabbits, rats

Intravenous somatostatin administration to the anesthetized dog is associated with anatomically defined GI motor effects: gastric antrum relaxation and a generalized augmentation of the small intestine segmenting activities (1). Similar observations have been made in conscious dogs.

Because more recent abstracts (2, 3) documented similar observations for the canine small intestine, a series of experiments in four mammalian species was designed to determine the mechanism of the excitatory effect on the small bowel as well as its species specificity.

EXPERIMENTAL

Animals and General Procedure—Acute experiments were performed on 15 mongrel dogs, four cats, four rabbits, and four adult male Sprague–Dawley rats. Following an overnight fast with tap water *ad libitum*, all animals were anesthetized by the intravenous administration of a mixture of α-chloralose (5%, dissolved in polyethylene glycol 200) and urethan (50% in 0.9% saline). Each animal was individually titrated to a surgical plane of anesthesia using this mixture. Anesthesia was maintained by individual administration of the chloralose–urethan mixture.

Surgical and Recording Procedures—In all species, a tracheal cannula was inserted to ensure airway patency or, when necessary, to maintain artificial ventilation with a positive pressure pump. The femoral artery was cannulated to record blood pressure. The ipsilateral vein was cannulated to permit either bolus or infusion administration of drugs and fluids. A midline laparotomy was performed.

Recording balloons were inserted *via* the oral route for gastric recording and retrograde *via* a stab wound in the ileum for small intestinal recording. Motor activities of the large intestine were monitored by surgi-

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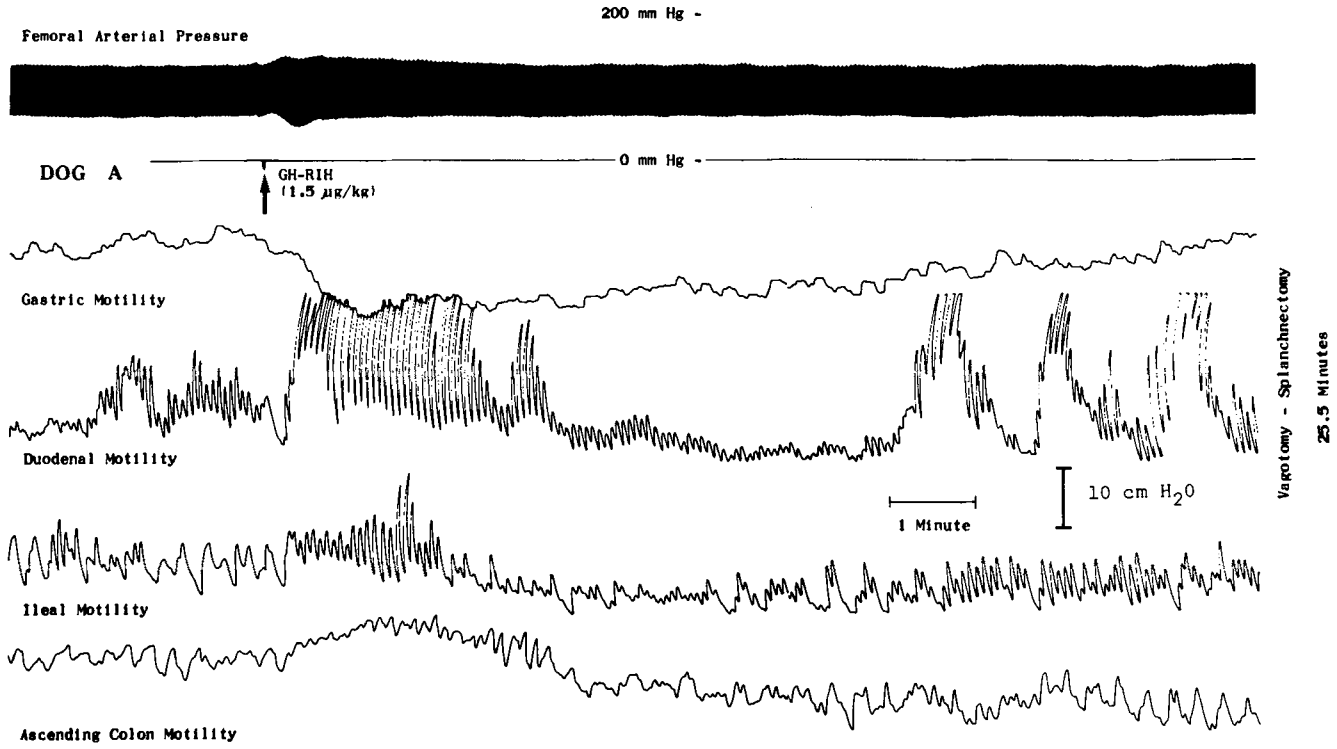

Figure 1—*Time courses of arterial blood pressure and various GI parameters showing the effects of intravenous somatostatin administered to an anesthetized dog. Stomach relaxation and increased motor activity of the intestinal tract are seen consistently. GH-RIH = growth hormone release inhibiting hormone (somatostatin).*

cally placed intraluminal water-filled balloons. Polygraphic control tracings were obtained before and at stated intervals after the physical and chemical stimulatory procedures in each group of animals. In all instances, intestinal pressure records were linked to a particular motility response on the basis of a visual observation that such a response occurred concurrently with the recorded events.

The following specific procedures were employed, depending on the particular canine experiment design. In some experiments, the vagus nerves were exposed cervically, and either the splanchnic nerves or the sympathetic chains were exposed at the T-9–T-10 level. In other experiments, the contractions of the mucosal musculature and of the longitudinal fibers of the small intestine tunica muscularis of the dog were recorded, both in excised surviving strips and in the living animal with the circulation intact according to a modified version (4) of a literature technique (5). Briefly, a small longitudinal incision was made through the entire wall of the intestinal segment opposite the mesenteric arcade attachment point. Two circular incisions were performed to create flaps such that the incisions or flap reflections did not interrupt the arcade blood vessels or innervation. The tunica muscularis was separated from the muscularis mucosa by blunt dissection and affixed by suture thread to a force transducer. It was then possible to monitor the muscularis mucosa activity by an intraluminal balloon.

Chemicals—In most experiments, drugs were administered intravenously through the femoral venous pressure cannula. Occasionally, intraarterial drug administration in the region of the denuded bowel segment was performed *via* a cannula inserted into a mesenteric artery,

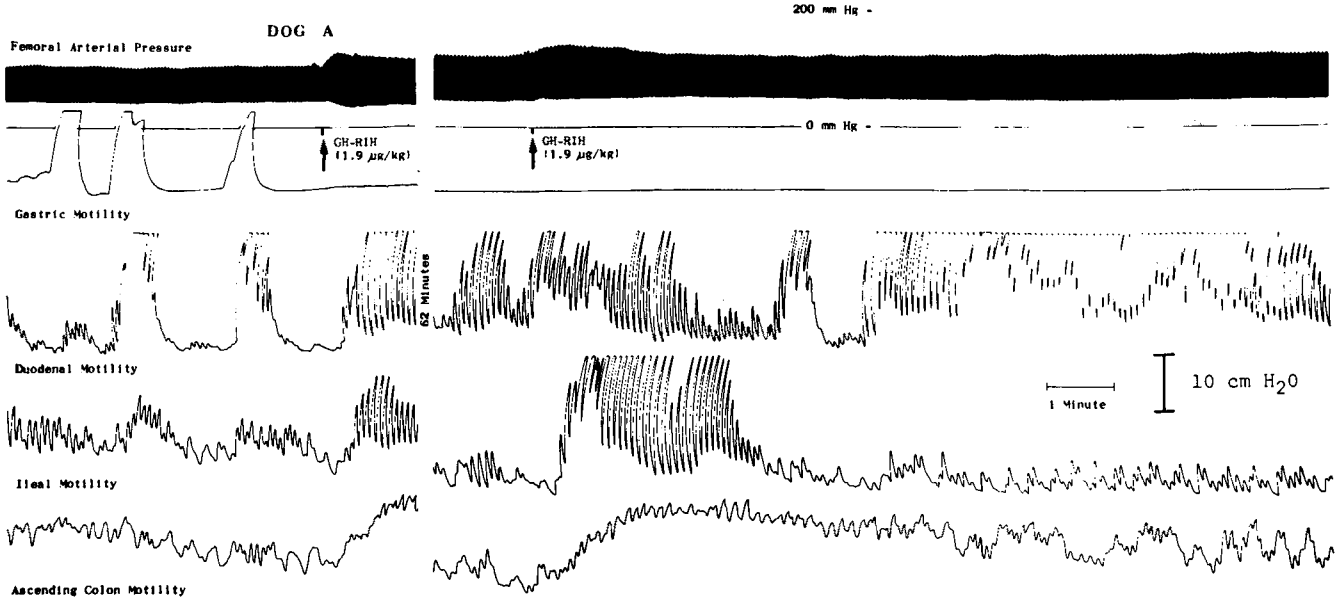

Figure 2—*Continuation of the experimental record depicted in Fig. 1 following bilateral cervical vagectomy and splanchnectomy. The same effects depicted in Fig. 1 continue with multiple subsequent intravenous somatostatin dosages.*

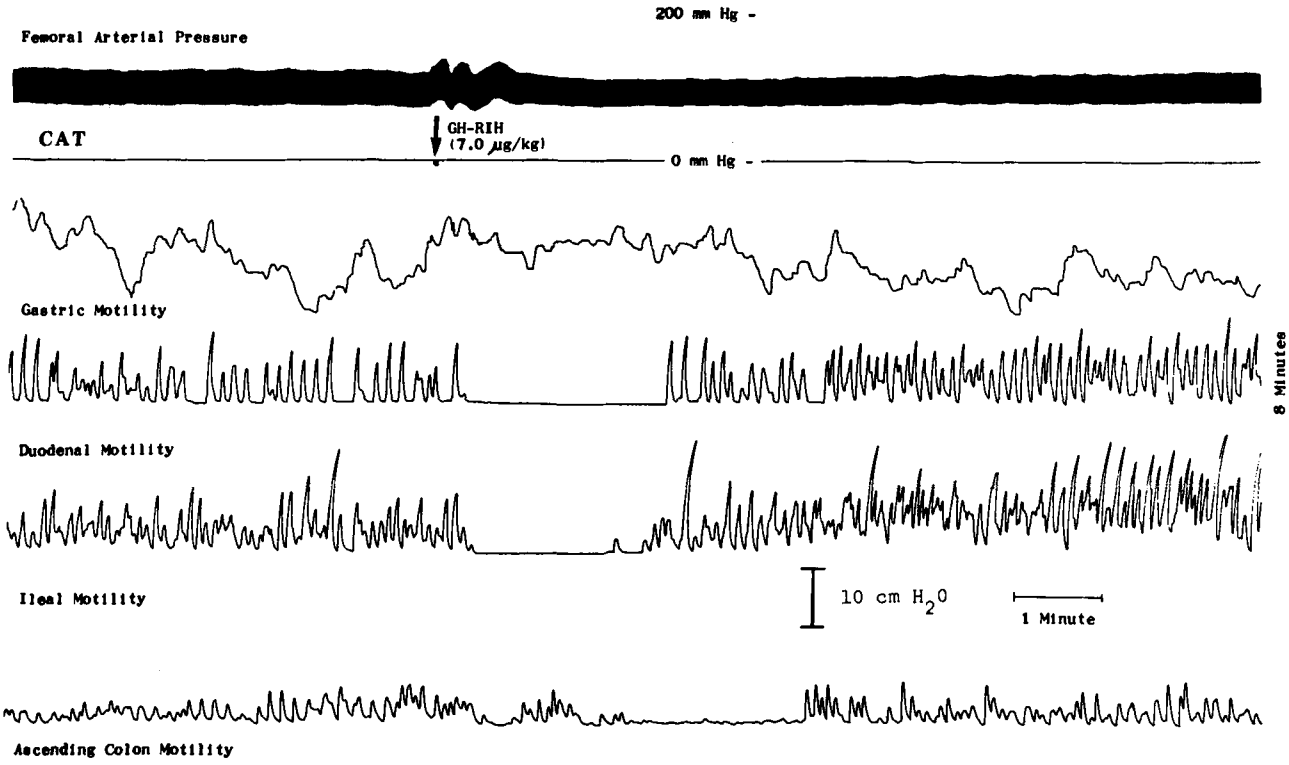

Figure 3—*Experimental record showing the effects of an intravenous bolus dosage of somatostatin administered to an anesthetized cat. In this case, there was a generalized intestinal motor activity inhibition, but spontaneous gastric motor activity was not affected.*

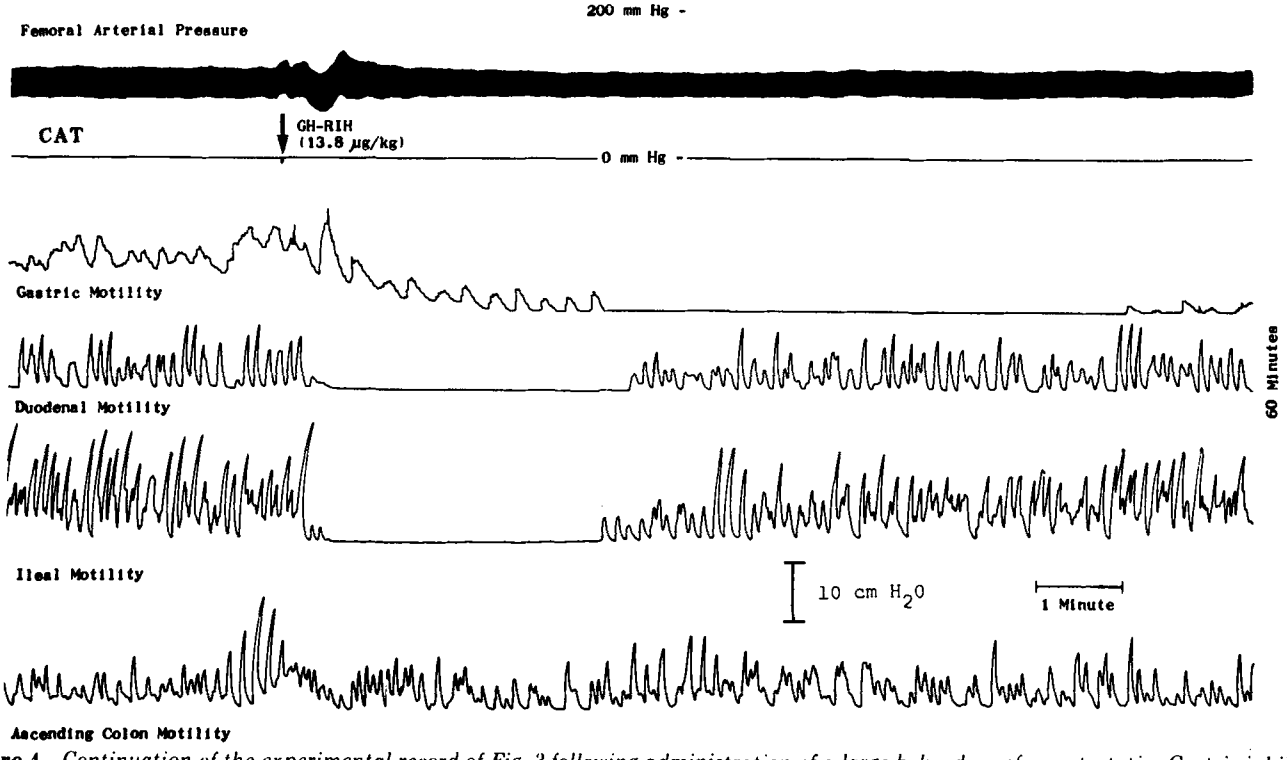

Figure 4—*Continuation of the experimental record of Fig. 3 following administration of a large bolus dose of somatostatin. Gastric inhibition accompanied small bowel inhibition, while the large intestine was no longer affected.*

which appeared to supply the immediate vicinity of the split bowel preparation. Intravenously administered agents were synthetic somatostatin (C-11873)[1], epinephrine hydrochloride[2], atropine sulfate[2], and tetraethylammonium chloride[3]. Somatostatin also was introduced into the arterial supply of the canine small intestine. The following drugs were used in the classical tissue bath: somatostatin, acetylcholine chloride[2], and epinephrine hydrochloride. The drug dose was specified as the final concentration of the active base present in the tissue bath.

[1] Wyeth Laboratories.
[2] Sigma.
[3] Etamon, Parke, Davis.

RESULTS AND DISCUSSION

As illustrated in Fig. 1, the canine stomach antrum exhibited a unan-

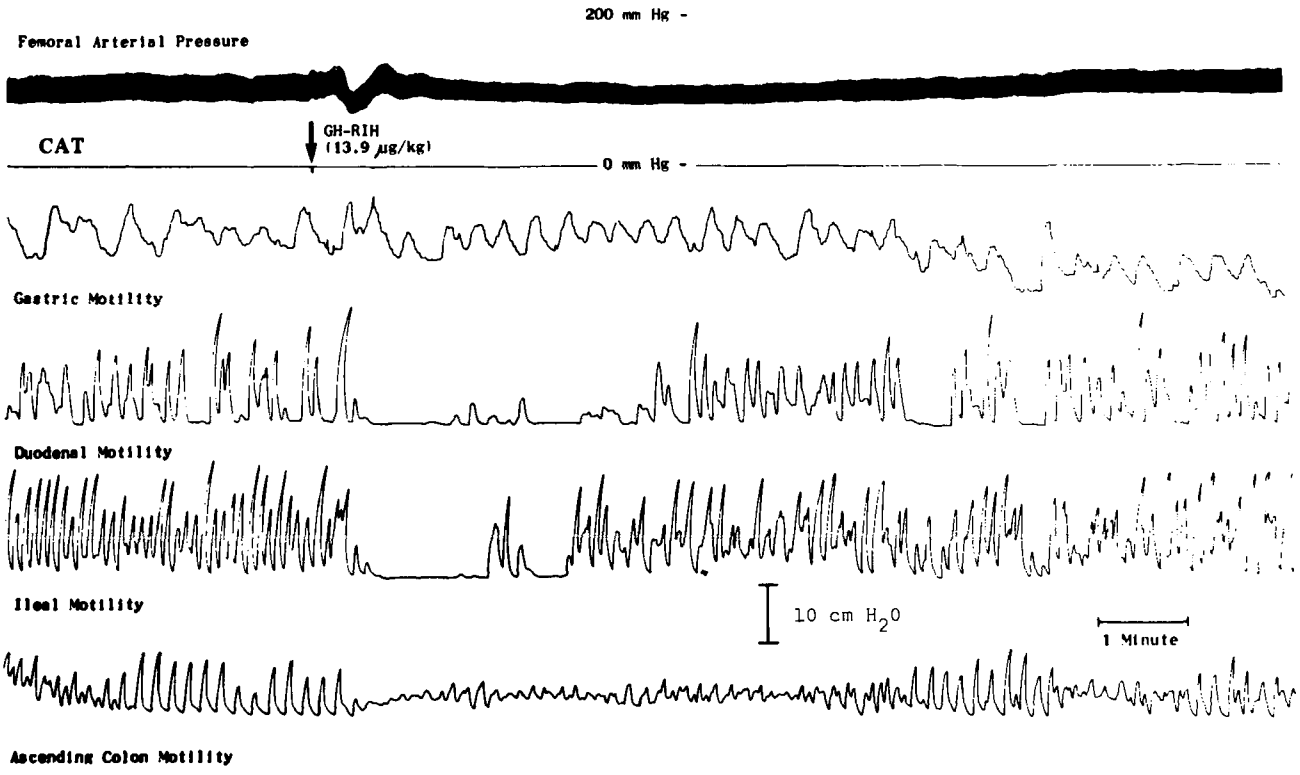

Figure 5—*Continuation of the experimental record of Fig. 4 following a 1-hr interval. A repeat administration of nearly the same bolus somatostatin dose resulted in shorter durations of inhibition of spontaneous small intestinal motor activity and no noticeable effect on gastric motor activity.*

imous relaxation response in 45 trials to a fixed bolus somatostatin dose, whereas the small bowel exhibited only a contractile response in all 15 animals, each to the same dose. Both responses showed dosage dependence and tachyphylaxis.

Gastric motor inhibition and augmented small intestinal segmenting activity always continued for a variable time (10–60 min) subsequent to the cessation of somatostatin administration. Somatostatin had minor and seemingly inconsistent excitatory effects on the ascending colon spontaneous motor activities. No effects on the descending colon were noted.

Figures 1 and 2 show that GI inhibitory and excitatory effects in response to somatostatin were not blocked in four surgically denervated

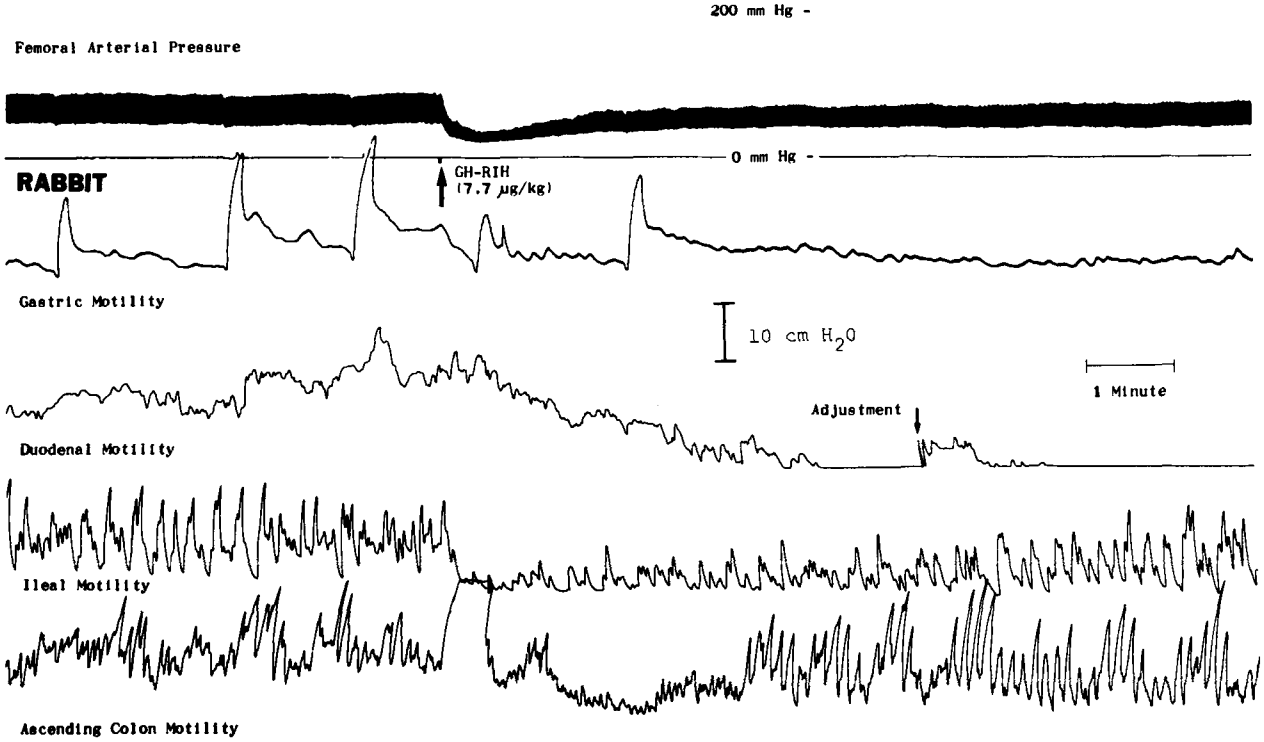

Figure 6—*Polygram depicting the time courses of arterial blood pressure and GI motor activities in an anesthetized rabbit following the intravenous somatostatin bolus dosage. Gastric and duodenal activities were noticeably inhibited, with marginal inhibition of the ileum and ascending colon.*

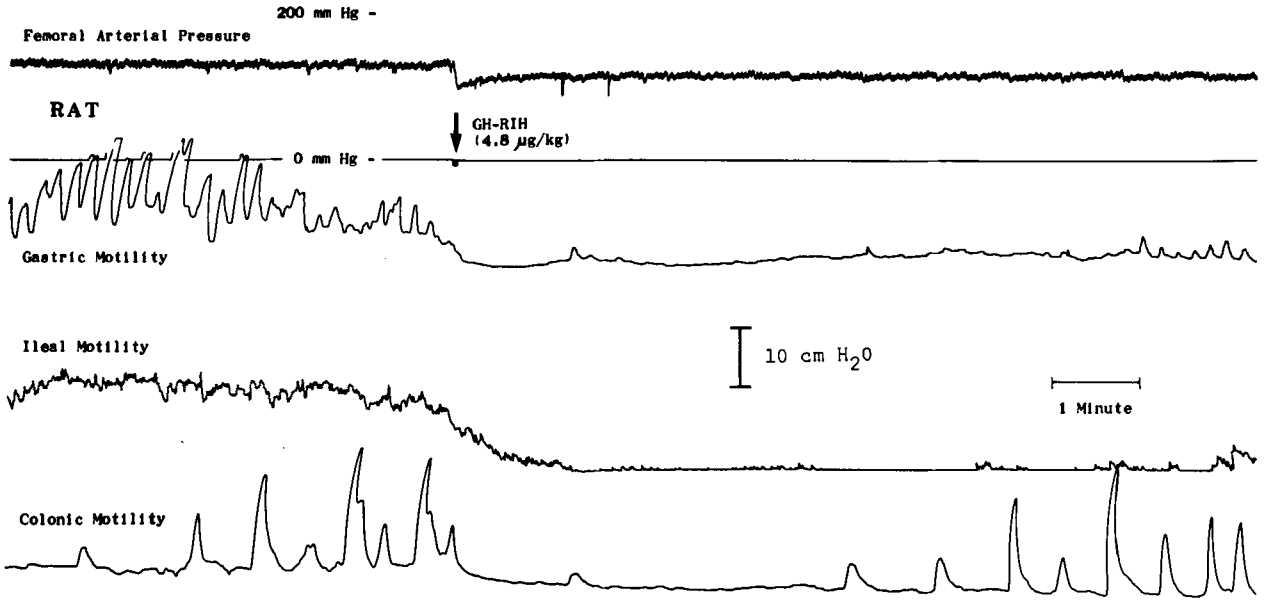

Figure 7—*Polygram from an anesthetized rat showing generalized GI motor inhibition by a single intravenous somatostatin bolus dosage.*

canine preparations. Although not depicted, the polygrams from four other dogs also revealed that the inhibitory and excitatory effects produced by somatostatin on the stomach and small intestinal tissues could not be blocked by prior intravenous administration of high doses (10 mg/kg) of the ganglionic blocking agent tetraethylammonium chloride.

Intravenous somatostatin infusions to three dogs resulted in essentially normal gastric activity following the initial depression, but small intestinal excitation (particularly in the duodenum) was maintained throughout the infusion period.

In all cases, low bolus intravenous somatostatin dosages to the cat promptly inhibited small intestinal motility but had no gastric effect (Fig. 3). Higher dosages inhibited gastric activity as well as small intestinal motor activity (Fig. 4). Repeated administration of high somatostatin dosages to the cat resulted in a loss of gastric inhibition and a reduction in the duration of small intestine inhibition (Fig. 5). In any event, stimulation of small intestinal activity was never observed in cats, regardless of the dosage or administration method.

Bolus dosages of intravenous somatostatin in four rabbits produced protracted gastric and duodenal inhibition and transient ileal inhibition (Fig. 6). There was no evidence of small intestinal stimulatory effects. Intravenous somatostatin also was effective in inhibiting GI motor activities in each instance in the rat, with even bolus dosages producing long inhibition periods (Fig. 7). No evidence of a small intestinal stimulatory effect was observed in the rat.

The excitatory effect of somatostatin on the dog small bowel was investigated in four animals by the split bowel preparation. Simultaneous recording indicated that ileal excitation to close intraarterial somatostatin was due to vigorous circular muscularis mucosa contractions while the longitudinal tunica muscularis was inhibited (Fig. 8). Results of *in vitro*

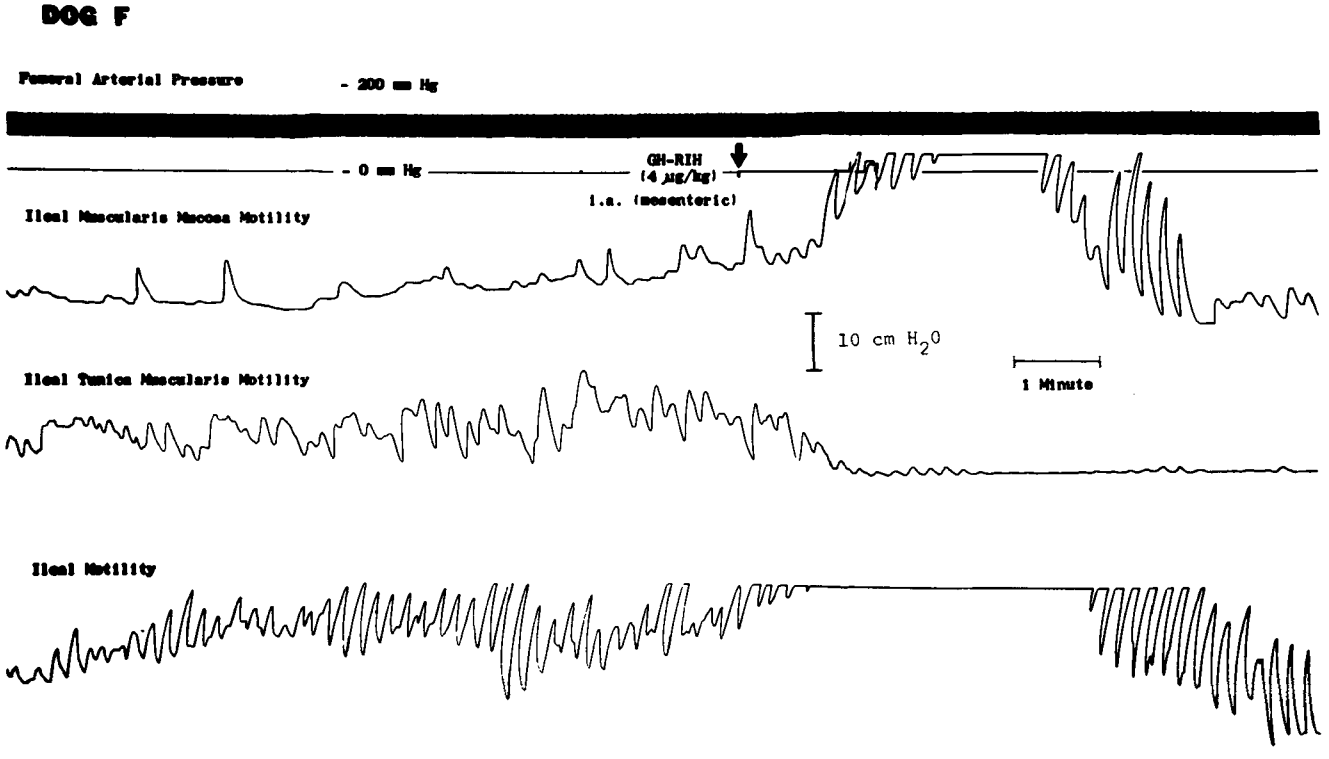

Figure 8—*Experimental record from an anesthetized dog showing mechanical responses of a small intestinal split bowel preparation to a close intraarterial bolus dosage of somatostatin. Tunica muscularis relaxation occurred in parallel with an increase in activity of the underlying muscularis mucosa. The overall effect, as recorded from an intraluminal balloon in an adjacent intact segment, was excitation.*

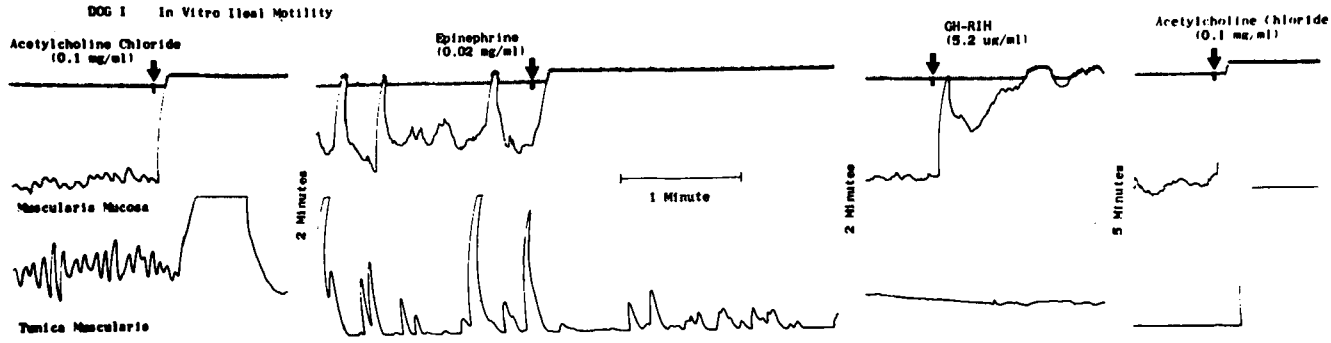

Figure 9—*Force records obtained* in vitro *from strips of canine muscularis mucosa and tunica muscularis showing the effects of somatostatin. Somatostatin produced an excitatory effect on the muscularis mucosa and an inhibitory effect on the tunica muscularis. In contrast, epinephrine excited the muscularis mucosa and inhibited the tunica muscularis; acetylcholine excited both.*

experiments on denuded canine small intestine segments supported the *in vivo* observations in the same animal. The *in vitro* pharmacological responsiveness of a circular strip of the muscularis mucosa and the outer tunica muscularis is depicted in the top and bottom traces of Fig. 9. Both ileal muscular components exhibited spontaneous motility, and they exhibited tonic contractions when challenged with acetylcholine.

As expected, epinephrine had opposite effects in different parts of the ileum of the same animal. As illustrated, the tunica muscularis portion of the ileum exhibited a relaxation response to epinephrine, whereas the muscularis mucosa portion exhibited only a tonic contractile response to the same dose of the catecholamine. Somatostatin produced a prolonged stimulation of ileal muscularis mucosa tone with no evidence of a stimulatory effect on the tunica muscularis.

This mechanistic explanation fits in well with the comparative histology of the small intestines of the species studied, viewed from the perspective of coupled mechano-effector systems. Although not depicted, only the dog small intestine has a well-developed circular component of the muscularis mucosa, and, aside from the dog, only the rabbit small intestine has any developed muscularis mucosa, albeit the orientation is longitudinal. The cat has a rudimentary longitudinal layer, and the rat has practically none.

It has been reported (6) that somatostatin acutely inhibits acetylcholine release by the guinea pig ileum myenteric plexus as in the *in vitro* preparation described previously (7). As suggested (6), the data may explain, at least in part, the ability of somatostatin to inhibit or decrease spontaneous or elicited gastric, gut, and gallbladder contractions.

In experiments to determine the effects of somatostatin on the myenteric plexus, Guillemin (6) observed tachyphylaxis, occasionally persisting for as long as 2 hr. This observation is in complete agreement with our findings on the duration of tachyphylaxis in feline preparations.

A review of the experiments by Guillemin (6) indicates that the guinea pig ileum failed to contract regardless of the somatostatin concentration in the bath. Interestingly enough, the guinea pig ileum has no circular muscularis mucosa and very little of the longitudinal components. Experiments in this laboratory have not produced an *in vitro* stimulatory effect on the rat small intestine, which is almost exclusively tunica muscularis (Fig. 10). Therefore, a mechanistic explanation for the anatomically specific canine responses probably can be advanced. The stimulatory effect is due to the action of the circular muscularis mucosa and cannot be produced in species that do not have this muscular component. The inhibitory effect probably is due to inhibition *via* the myenteric plexus of the tunica muscularis, which is the only significant muscle layer in the small intestines of the noncanine species studied.

In the dog stomach, where presumably both effector layers are present, the primary inhibitory effect may be due partly to the difference in ge-

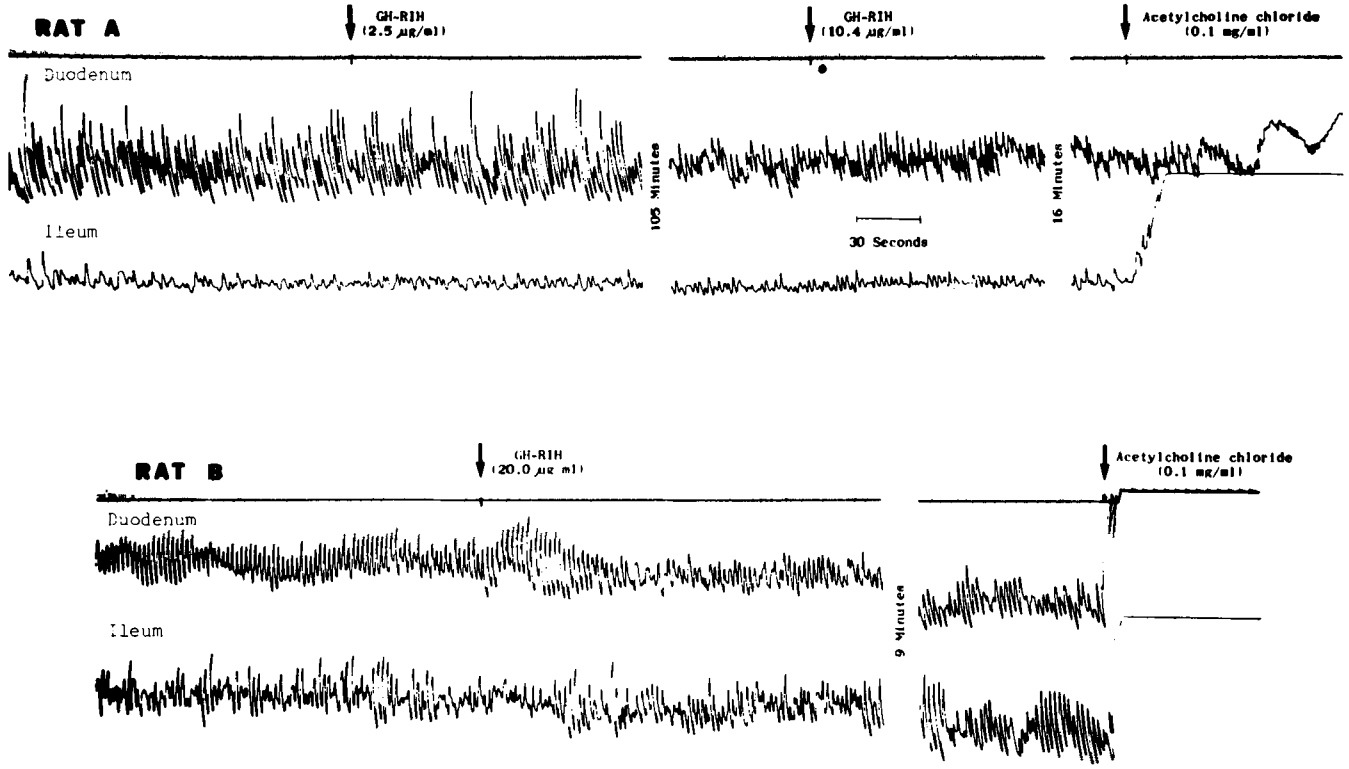

Figure 10—In vitro *force records of spontaneous muscle contractions in whole bowel segments from rats showing responses to challenges by somatostatin and acetylcholine.*

ometry. The stomach is neither circular nor does its average diameter approximate that of the small intestine. Consequently, there is no reason to expect that simultaneous excitation of the muscularis mucosa and inhibition of the tunica muscularis would increase intraluminal pressure sufficiently to register with a water-filled intraluminal recording balloon, and the contrary might well be the case if the external effector layers are indeed inhibited.

No acceptable adaptation of the split bowel preparation to the stomach appears practical, but indirect means are available to estimate at least the presence or absence of spontaneous motor activity. Such demonstrations will be necessary to determine whether the observed net inhibition of canine gastric motor activity is due to the relative geometries of coupled effector systems.

With the canine small intestine, paradoxical motor effects were observed by intraluminal pressure measurements, probably because the test agents had opposite effects on the two muscle layers. This is amply illustrated in Fig. 8, which shows the effect of somatostatin on the canine ileum. Clearly, the tunica muscularis is inhibited and the muscularis mucosa is stimulated, while an overall stimulatory effect is seen from an intraluminal balloon in an adjacent intact small bowel segment. These data confirm and explain the excitation mechanism reported by several investigators (1–3) in the somatostatin effect on the motor activity of the canine small intestine. These results also lead to the conclusion that the circular components of the canine small intestine muscularis mucosa possess physiologically significant contractile powers.

The overall significance of these findings is that both the canine and human small intestine constitute pharmacologically separate, but mechanically coupled, muscular systems, either one of which can show excitation when the coupled effects are measured by any system that does not differentiate the identity of the effector system involved. In the future, it will be necessary to evaluate motor effects of test agents on a separate basis. Those that have been reported to produce paradoxical effects should be reevaluated. Obviously, the use of the split bowel technique would not be appropriate for chronic preparations, but these results also suggest a cautious interpretation of the mechanisms from experimental data obtained from either open-ended catheters or intraluminal pressure telemetry capsules.

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High-Performance Liquid Chromatographic Microdetermination of Indoprofen in Human Milk

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Received August 30, 1978, from the **Chemical Sciences Division, Midwest Research Institute, Kansas City, MO 64110,* and ‡*Adria Laboratories, Inc., Columbus, OH 43216.* Accepted for publication March 6, 1979.

Abstract □ A previously reported high-performance liquid chromatographic (HPLC) method for indoprofen determination in physiological fluids was modified and extended to provide quantitative data on drug concentrations in human milk samples at a low nanogram per milliliter level. The reversed-phase HPLC technique was modified to give a better separation of the drug and milk components. To achieve the necessary cleanup for low level determination, the milk samples required protein precipitation, liquid–liquid drug extraction, and concentration. Excellent indoprofen recovery was obtained with this technique; the average recovery from 20 milk samples spiked with various nanogram drug levels was 95%. The analytical technique showed excellent reproducibility; the calibration solutions over 15 days had a relative standard deviation of 3.2%. Results for indoprofen levels in milk and plasma samples from seven subjects who received either a single or multiple oral drug dose are presented.

Keyphrases □ Indoprofen—analysis, high-performance liquid chromatography, human milk □ High-performance liquid chromatography—analysis, indoprofen, human milk □ Anti-inflammatory agents—indoprofen, high-performance liquid chromatographic analysis, human milk □ Milk, human—analysis, indoprofen, high-performance liquid chromatography

Indoprofen, 4-(1,3-dihydro-1-oxo-2*H*-isoindol-2-yl)-α-methylbenzeneacetic acid (I), has been reported to have analgesic activity in animals and humans (1–4). In an earlier report (5), a reversed-phase high performance liquid chromatographic (HPLC) method was presented for the quantitation of drug levels in plasma and urine samples. This method was very simple and had sufficient sensitivity for determination of microgram per milliliter levels. However, in certain cases, such as determinations in later plasma time point samples for pharmacokinetic studies or in physiological fluids such as milk where the drug level is expected to be low, a more sensitive technique is required. Therefore, a sample preparation procedure was developed that provides the necessary cleanup and sensitivity for low level analyses. The method has the necessary precision and accuracy to provide quantitative data for nanogram indoprofen levels in milk samples and may be applicable to other physiological fluids containing low drug levels.

EXPERIMENTAL

Apparatus and Reagents—The liquid chromatograph consisted of a high-pressure pump[1], a loop injector[2], a UV detector[3], and a strip-chart recorder[4]. The chromatographic column was μBondapak C_{18}[5] (300 × 4 mm i.d.).

[1] Model 6000A, Waters Associates, Milford, Mass.
[2] Model U6K, Waters Associates, Milford, Mass.
[3] Model 440, Waters Associates, Milford, Mass.
[4] Model SR-240, Heath/Schlumberger Instruments, Benton Harbor, Mich.
[5] Waters Associates, Milford, Mass.

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