Ghrelin/motilin-related peptide is a potent prokinetic to reverse gastric postoperative ileus in rat

L. TRUDEL,1 C. TOMASETTO,2 M. C. RIO,2 M. BOUIN,1 V. PLOURDE,3 P. EBERLING,2 AND P. POITRAS1
1Department of Gastroenterology, Centre Hospitalier de l’Université de Montréal, Montréal, Québec, Canada; and 2Institut de Génétique et de Biologie Moléculaire et Cellulaire, Institut National de la Santé et de la Recherche Médicale/Centre National de la Recherche Scientifique/Université Louis Pasteur, Strasbourg, France

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A novel peptide called ghrelin or motilin-related-peptide (MTLRP) was found in the stomach of various mammals. We studied its effect on the motor function of the rat gastrointestinal tract. In normal, conscious unoperated animals, ghrelin/MTLRP (5 or 20 μg/kg iv) significantly accelerated the gastric emptying of a methylcellulose liquid solution (gastric residue after 15 min: 57 ± 7, 42 ± 11, 17 ± 4, and 9 ± 3% of the ingested meal with doses of 0, 1, 5, and 20 μg/kg iv, respectively) Transit of the methylcellulose liquid solution was also accelerated by ghrelin/MTLRP in the small intestine but not in the colon. Des-[Gln14]ghrelin, also found in the mammalian stomach, was as potent as ghrelin in emptying the stomach (gastric residue after 15 min: 12 ± 3% at a dose of 20 μg/kg iv). In rats in which postoperative gastrointestinal ileus had been experimentally induced, ghrelin/MTLRP (5 or 20 μg/kg iv) reversed the delayed gastric evacuation (gastric residue after 15 min: 28 ± 7% of the ingested meal vs. 82 ± 9% with saline). In comparison, the gastric ileus was not modified by high doses of motilin (77 ± 7%) or erythromycin (82 ± 6%) and was only partially improved by calcitonin gene-related peptide (CGRP) 8–37 antagonist (59 ± 7%). Ghrelin/MTLRP, therefore, accelerates the gastric emptying and small intestinal transit of a liquid meal and is a strong prokinetic agent capable of reversing the postoperative gastric ileus in rat.

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A NOVEL PEPTIDE WAS IDENTIFIED recently in endocrine cells of the gastric mucosa of mouse, rat, dog, and human (9, 20, 22). It was discovered simultaneously by two research teams who named it, respectively, motilin-related peptide (MTLRP) and ghrelin. Tomasetto et al. (21) used molecular biology to look for new proteins of the gastric epithelium that could provide insight to the differentiation and function of the gastric unit. They discovered a protein expressed in secretory granules of the mouse stomach endocrine cells in which chromogranin A, serotonin, and somatostatin also localized. The protein has sequence similarity (47%) with preprotinin, and the secreted peptide was, therefore, tentatively named motilin-related peptide. At the same time, Japanese researchers (9), looking for an endogenous ligand for growth hormone secretagogue receptor, purified and identified from rat stomach a peptide of 28 amino acids they called ghrelin (ghre is the proto-Indo-European root of the word grow). Ghrelin is now most often used to identify the peptide.

Because it interacts with the growth hormone secretagogue receptor of the pituitary, ghrelin has definite actions on the release of growth hormone (9, 18, 20, 24), but also on appetite stimulation (counterbalancing the effect of leptin) (1, 12, 24) and on body weight evolution (by a mechanism independent of growth hormone or hyperphagia) (23, 24). In the digestive tract, ghrelin appears to have stimulatory potential for gastric acid secretion (5, 10). It could also stimulate gastric contractions in urethane-anesthetized rats (10), and it seemed to accelerate gastric emptying of food in mice (1).

Two molecular forms of ghrelin were found in the rat stomach, where the 28-amino acid ghrelin peptide is accompanied by des-[Gln14]ghrelin, a 27-amino acid peptide whose sequence is identical to ghrelin except for one glutamine in position 14. Created by alternative splicing of the ghrelin gene, des-[Gln14]ghrelin, which constitutes one-fifth of ghrelin immunoreactivity of the rat stomach, is as potent as ghrelin-28 to stimulate growth hormone secretion (8).

These studies were undertaken to characterize the effect of ghrelin on the motor function of the gastrointestinal tract in the rat. Therefore, we verified the effect of ghrelin on emptying of the stomach and transit through the small intestine or the colon. We compared the effect of ghrelin-28 and des-[Gln14]ghrelin-27 on...
gastric motor activity. We tested the gastrokinetic capacity of ghrelin in a model of postoperative ileus.

MATERIALS AND METHODS

These studies were approved by the Animal Care Committee of the Centre Hospitalier de l’Université de Montréal. Male Sprague-Dawley rats weighing 200–250 g (Charles River, Montreal, PQ, Canada) were used. Animals were deprived of food for 18 h before the beginning of the experiments.

Preliminary experiments were done in anesthetized animals to have a simple and comfortable experimental model to test ghrelin motor activity. In rats under anesthesia with urethane (1.25 μg/kg iv) or with inhaled isoflurane, a jugular catheter was installed immediately before gavage of the methylcellulose solution and death of the animal later on by CO2 inhalation as described below. Because of the poor results obtained with this model (see RESULTS), experiments were then performed in conscious animals as discussed below.

Gastrointestinal Transit Studies

The animals were first submitted to a brief anesthesia with isoflurane to insert a catheter in the right jugular vein 2 h before start of the experiments.

Gastric emptying in normal unoperated animals. Gastric emptying was measured by the following technique. A distilled water solution (1.5 ml) containing 1.5% methylcellulose and technetium-99m (99mTc) (∼100,000 counts per minute) was given intragastrically through a stainless steel tube in conscious rats. Animals were killed by CO2 inhalation at the desired time points (0, 15, 30 min, etc.) for the various experimental protocols, and the abdomen was opened. Stomachs, clamped at the pylorus and cardia, were removed and placed in test tubes for counting with the use of a gamma counter the amount of radioactivity left in the stomach.

Small intestine and colon transit. Twenty-four hours before the experiments, the animals were anesthetized with isoflurane and a laparotomy was performed to insert a small Silastic catheter into the duodenum (1 cm postpylorus) or into the cecum (1 cm postileum). The catheter was fixed to the organ with glue and exteriorized to the back of the animal via a subcutaneous tunnel. Laparotomy was closed with stitches, and the animals were allowed to recover in their individual cages.

Twenty-four hours after the intestinal tube was installed, the animals were briefly anesthetized with isoflurane to insert a catheter in the right jugular vein. Two hours later, 0.5 ml of the methylcellulose solution was administered via the Silastic tube in the duodenum or in the cecum. Experimental drugs were then injected via the jugular catheter. Animals were killed 15 min later by CO2 inhalation. The abdomen was opened, and the gastrointestinal tract was separated into multiple sections by silk ligations. The following organs or anatomic sections were identified: stomach, duodenum, ileum-1 (upper one-third), ileum-2 (middle one-third), ileum-3 (lower one-third), cecum, colon-1 (proximal one-half), colon-2 (distal one-half). All sections of the gut were then individually removed and placed in separate test tubes for counting in a gamma counter the amount of radioactivity present in each organ or section. Colon transit was also evaluated by looking at the fecal output during the 15-min observation period after drug administration.

Gastric ileus studies. Gastric ileus was induced as previously described (15). Briefly, rats were anesthetized with isoflurane and submitted to laparotomy. The cecum was exteriorized and gently manipulated (patted between hands for 1 min in saline-soaked gauze). Abdominal muscles and skin were then closed with silk sutures and the animals were allowed to recover for 5 min before gavage for gastric emptying studies. Methylcellulose was then administered as described earlier.

Substances Tested

The substance to be tested was injected intravenously at the end of the methylcellulose administration at time 0.

The human ghrelin-28 and des-(Gln14)ghrelin were synthesized at the Institut de Génétique et de Biologie Moléculaire et Cellulaire on solid phase using Nc-9-fluorenylmethoxycarbonyl chemistry on a peptide synthesizer (model 431A; Applied Biosystems). Acetylation with n-octanoic acid on Ser5 was carried out as previously described by Kojima et al. (9). Human motilin 1–22 and calcitonin gene-related peptide (CGRP) 8–37 were synthesized in the laboratory of Dr. S. St-Pierre (Université du Québec, Montreal, PQ, Canada). Erythromycin lactobionate was purchased from Abbott Laboratories (Montreal, PQ, Canada). Anesthesia was induced by isoflurane (Abbott Laboratories) or urethane (Sigma). Hepatate II tin colloid (99mTc) was purchased from Amersham Canada (Oakville, ON, Canada).

Data Analysis

Gastric residue at different times postingestion of the methylcellulose solution was measured by counting the amount of 99mTc left in the removed organ. Data expressed in percentage of the administered dose are shown as means ± SE. Statistical analysis was done by ANOVA (with Tukey-Kramer posttests with one-to-one comparisons) or unpaired t-test.

RESULTS

In preliminary experiments with urethane- and isoflurane-anesthetized animals, the spontaneous gastric emptying was slow (gastric residue 83 ± 9 and 94 ± 4%, respectively, after 15 min). Ghrelin (20 μg/kg iv) failed to modify the gastric emptying rate in these urethane- or isoflurane-anesthetized animals (residue after 15 min: 69 ± 12 and 91 ± 4%, respectively; n = 3 rats per group). Because of these poor results with

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**Fig. 1.** Gastric emptying curves in conscious rats receiving saline or ghrelin (20 μg/kg) given at time 0 after the ingestion of a methylcellulose liquid meal containing technetium-99m (99mTc) as a radioactive marker.
Ghrelin, experiments were then performed in conscious animals as described in MATERIALS AND METHODS.

**Gastric Emptying in Normal Unoperated Animals**

Gastric emptying over a 1-h period of the methylcellulose meal was first compared in rats receiving saline or ghrelin (20 μg/kg iv) at time 0 (*n* = 6 rats per time point in each group). As shown on Fig. 1, gastric emptying was progressive in control saline animals (gastric residue was 95.1% of the administered dose at time 0 and decreased to 57 ± 7, 30 ± 8, 23 ± 8, and 19 ± 4% after 15, 30, 45, and 60 min, respectively). In ghrelin-treated animals, gastric emptying was dramatically accelerated 15 min after drug administration (gastric residue = 9 ± 3%; *P* < 0.0001).

The dose-response curve to ghrelin was tested at 15 min. As shown on Fig. 2, the effect of ghrelin on the gastric residue at 15 min after meal ingestion was dose related (57 ± 7, 42 ± 11, 17 ± 4, and 9 ± 3% with ghrelin doses of 0, 1, 5, and 20 μg/kg, respectively; *n* = 6 animals per dose).

Des-[Gln14]ghrelin (20 g/kg iv) had the same stimulatory effect on the gastric motor function as ghrelin (gastric residue at 15 min = 12 ± 3%; *n* = 12; *P* not significant vs. 20 g/kg ghrelin).

**Small Intestine Transit**

Fifteen minutes after the administration of the methylcellulose meal into the duodenum, 46 ± 13 and 49 ± 13% of the 99mTc marker was found in ileum-1 and ileum-2 in the saline control animals (*n* = 8). In ghrelin-treated rats (*n* = 8), most of the marker was found in ileum-2 (83 ± 6%; *P* = 0.03 vs. saline), and only 14 ± 7% (*P* = 0.05 vs. saline) was found in ileum-1, suggesting that the intestinal transit was faster in ghrelin-treated animals. These data are shown on Fig. 3.

**Colon Transit**

Distribution of the radioactive tracer was similar in ghrelin- or saline-treated animals (*n* = 4 rats per group). After 15 min, most of the 99mTc marker stayed in the cecum (93 ± 3 vs. 86 ± 5%; *P* not significant; data not shown). None of the animals expelled feces during the 15-min observation period.

**Gastric Emptying in the Postoperative Ileus Model**

Laparotomy and manipulation of the cecum had, as expected, a definite effect on the gastric emptying func-
tion. After 15 min, the gastric residue was 54 ± 4% in a control group of 25 unoperated animals and decreased to 81 ± 3% in 29 operated rats.

As shown on Fig. 4, the gastric residue after 15 min was dramatically decreased (P = 0.0003) in rats treated with ghrelin (20 μg/kg; 28 ± 7%; n = 7) compared with those receiving saline. The effect of ghrelin appeared to be dose related and maximal at a dose of 20 μg/kg (not different from 50 μg/kg).

In comparison, motilin (given iv at time 0) was unable to accelerate the gastric emptying of the postoperative ileus; 84 ± 6% (n = 5) and 77 ± 7% (n = 9) of the ingested material was left in the stomach 15 min postprandially in rats treated with 10 or 100 ng/kg motilin. Erythromycin (5 mg/kg iv) was also without effect (82 ± 6%; n = 5). These pharmacological doses of motilin and erythromycin were selected on the basis of the information accumulated in other species with these compounds (3, 16, 19). As expected from previous studies (15), CGRP 8–37 improved the gastric ileus [50 μg/kg (88 ± 4%, n = 13) and 150 μg (59 ± 7%, n = 8) of residue left over 15 min], but the effect was less marked than with ghrelin (20 μg/kg; 28 ± 7%).

DISCUSSION

Ghrelin appears, therefore, as a strong gastrokinetic agent. It could dramatically accelerate the normal emptying process in conscious unoperated rats, and it was the most potent drug to reverse postoperative gastric ileus. Ghrelin could also accelerate the transit of the small intestine but had no effect on the colon.

Masuda et al. (10) described the contractile response of the stomach to the intravenous administration of ghrelin. Atropine or vagotomy blocked the contractions induced by ghrelin in these urethane-anesthetized rats. Asakawa et al. (1) observed decreased gastric food residue 1 and 2 h after meal ingestion in mice treated by ghrelin administered intraperitoneally or intracerebroventricularly, suggesting that ghrelin could accelerate gastric emptying. Our data clearly indicate that ghrelin can accelerate gastric emptying in rats. Whether this propulsive effect is solely due to the gastric contractions recorded in the anesthetized animals remains unclear, because the capacity of the peptide to stimulate gastric emptying appeared to be seriously compromised in our anesthetized animals. The doses of ghrelin we needed to stimulate gastric emptying in our animals appeared to be comparable to the dose of peptide required in other studies (18, 20) to stimulate the release of growth hormone in the blood. Our study also demonstrates that both forms of ghrelin present in the stomach, the 28-amino acid and the des(Gln14)-27 amino acid octanoylated peptides, are equally bioactive.

On the basis of sequence similarity with prepropomotilin, the protein had been called motilin-related peptide by Tomasetto et al. (21). Our results, showing a propulsive action of ghrelin/MTLRP in an animal ileus model in which the stimulation of motilin receptors by motilin or by the motilin receptor agonist erythromycin (14) was without biological effect, clearly suggest that ghrelin is acting on receptors different from those identified for motilin. These results are in agreement with structure-activity studies showing that bioactivity of motilin or ghrelin relied on the first four or seven amino acids of each molecule, respectively (2, 11). The poor similitude in the NH2-terminal segments of these two peptides, indeed, was not in favor of a reciprocal influence or cross reaction on a common receptor.

The effect of ghrelin on postoperative ileus is most interesting. Abdominal surgery inhibits gastric emptying and digestive motor activity in various mammalian species and in humans. In humans, abdominal surgery with manipulation of visera postoperatively induces a state of digestive motor inactivity that may occasionally be overly prolonged while associated with significant morbidity, and these patients could certainly benefit from a specific and effective treatment. Moreover, shortening of the normal postoperative ileus could possibly have a serious economic impact by reducing hospital stay and facilitating ambulatory surgery. The cause of postoperative ileus remains only partly understood, and its treatment remains often empirical and suboptimal. Attempts by various prokinetics (such as acetylcholine, cisapride, or even motilides) to stimulate smooth muscle cells directly or via the efferent neural pathways frequently gave disappointing results (4, 6, 13, 17, 25). More recent studies suggested, however, that an inhibitory influence by afferent nerves could be involved in the genesis of postoperative ileus. In the same rat model used in the current manuscript, the defunctionalization of afferent nerves by systemic capsaicin helped to prevent postoperative gastric ileus (7), and antagonists to CGRP were among the few agents capable of reversing, at least partly, the inhibition of gastric emptying after surgery (15). In support of this theory on abdominal afferent nerves, Asakawa et al. (1) observed that ghrelin decreased gastric vagal afferent discharge. Our results in animals with postoperative ileus indicate that ghrelin is more active than CGRP 8–37 and is the most potent tested drug to accelerate gastric emptying.

Ghrelin/MTLRP is, therefore, a very powerful gastrokinetic agent. Future studies are needed to elucidate its physiological contribution in mammals and to identify its pharmacological potential in human medicine.

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