Innovative Methodology

New method of manometric measurement of gastroduodenal motility in conscious mice: effects of ghrelin and Y2 depletion

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Tanaka R, Inui A, Asakawa A, Atsuchi K, Ataka K, Fujimiya M. New method of manometric measurement of gastroduodenal motility in conscious mice: effects of ghrelin and Y2 depletion. Am J Physiol Gastrointest Liver Physiol 297: G1028–G1034, 2009. First published September 3, 2009; doi:10.1152/ajpgi.90692.2008.—Since no previous studies have reported dual measurements of stomach and duodenal motility in conscious mice, we developed a manometric method to measure the gastroduodenal motility in the physiological fed and fasted states of conscious mice. By this method we measured, for the first time, the gastroduodenal motility in Y2 knockout mice and analyzed the effects of ghrelin on the gastroduodenal motility in conscious mice. To evaluate this new method, we provide the comparison on the effects of CCK-8 examined by present and previous methods. In the fasted state of mice, phase III-like contractions with frequencies of 7.8 ± 0.5 contractions/h in the antrum and 6.6 ± 0.7 contractions/h in the duodenum were observed. This fasted pattern was disrupted and replaced by the fed pattern after feeding, with an increase of the motor index (MI) immediately after feeding. Intravenous injection of ghrelin induced the fasted pattern in the duodenum when injected in the fed state and increased %MI (114.3 ± 9.8%) compared with saline-injected controls (64.4 ± 9.6%) in the antrum. Intravenous injection of CCK-8 disrupted phase III-like contractions in both antrum and duodenum, which were replaced by fast-like motor patterns accompany with the elevation of baseline pressure. In Y2 knockout mice, the frequency of phase III-like contractions was decreased in the antrum compared with wild-type mice and the immediate increase of MI after feeding seen in wild-type mice was disrupted in Y2 knockout mice. Our model provides a new method for studies of gastrointestinal motility in various mouse models, including transgenic and knockout ones.

Y2 knockout; fed and fasted motility

TO MEASURE GASTROINTESTINAL (GI) motility in experimental animals, various approaches have been reported, including in vitro preparation of isolated loops of intestinal segments and muscle strips (17), ex vivo preparation of the vascularly perfused GI tract (23), and in vivo preparation of conscious animal models (12, 16, 24). Among these methods, conscious animal experiments seem to provide more physiological information on GI motility than in vitro or ex vivo experiments because GI motility is under regulation of coordinated networks of neural and hormonal inputs from the brain and periphery. Techni
cal improvements have enabled the measurement of GI motility in conscious rats by manometric methods (4, 7, 12, 13, 19), force transducer implantation (24), and electromyographic (EMG) recordings (5), but there are very few reports of such measurement in conscious mice. Other available methods to measure the GI motility are sonometric measurement and barostat measurement. The sonomicrometric method seems to be less invasive than use of a force transducer or EMG; however, it has been employed for the measurement of stomach motility in anesthetized rats rather than in conscious animals (1, 2). Miniaturized barostat measurement of intragastro
tic pressure has been conducted for mice (20). Although barostat is a valid method to record gastric volume change, it has been used only for anesthetized mice, not conscious mice. Recent advances in transgenic and knockout technologies have provided tools to investigate the pathogenesis of disease models, and these technologies have been mostly applied to mice. Therefore, development of methods for measuring GI motility under physiological conditions in mice is important.

Because of technical difficulties, most previous studies that used conscious mice models have evaluated the upper GI motility by gastric emptying (3) and intestinal transit (11) of nutrient or nonnutrient materials. However, these parameters seem to be insufficient because these transit assays may not distinguish between the secretory and motor components. Very recently, spontaneous phase III-like contractions in conscious mice have been successfully measured by a method using transducer implantation in the mouse stomach (27). However, although this method allows single monitoring of stomach motility, it does not allow dual monitoring of stomach and duodenal motility. Since the motility in the stomach and that in the duodenum are tightly related to each other (19), dual monitoring of gastroduodenal motility seems important to evaluate the effects of various peptides. Therefore we developed manometric methods for measurement of motor activities in the stomach and the duodenum, monitoring the pressure waves by double catheters inserted into both the antrum and duodenum.

In the present study we measured fed and fasted motor activities in the antrum and duodenum of conscious mice and the effects of ghrelin and CCK-8 were examined. Ghrelin is a potent stimulant for the gastric emptying and therefore important in therapeutic strategies for GI disorders such as functional dyspepsia and gastroparesis (22). Since it is well established that ghrelin stimulates GI motility in a conscious rat model using manometric methods (13) and also in a conscious mouse model using a strain gauge transducer (27), we used ghrelin in the present study to show the reliability of manometric measurement of mouse GI motility. CCK-8, on the other hand, is a well-known peptide to inhibit the gastric emptying. In addition to the transit assays for gastric emptying in conscious mice (26), the effects of CCK-8 on the motility of specific regions of the stomach have been examined by sonometric methods in
urethane-anesthetized rats (2). We compared the data obtained with our model and those in previous studies on the effects of CCK-8 in the stomach motility. Evidence has accumulated that the NPY Y2 receptor is important in regulation of GI motility (6, 12, 15, 18). This has been shown mostly by exogenous administration of Y2 receptor agonists or antagonists. For more direct investigation, Y2 receptor knockout mice seem appropriate (21), since no previous studies have investigated their GI motility. Therefore, we examined fed and fasted motor activities in both the antrum and duodenum in Y2 knockout mice.

MATERIALS AND METHODS

Animal preparation. Male C57BL/6Jc mice (CLEA Japan, Tokyo, Japan), Y2 knockout mice, and their wild-type littermates (21) weighing 20–25 g were used. Mice were housed individually under controlled temperature (22–24°C), humidity (44–46%), and light (lights on 7:00–19:00) conditions with free access to laboratory chow pellets (CE-2; CLEA Japan) and water. Care of animals was conducted in accordance with the International Guiding Principles for Biomedical Research Involving Animals and was approved by the Office of Laboratory Animal Welfare (OLAW, no. A5808-01).

Mice were anesthetized with intraperitoneal injection of pentobarbital sodium (50 mg/kg body wt, Nembutal; Abbott Laboratories) and implanted with catheters for manometric recordings in the antrum and duodenum. A polyurethane tube (ID 0.30 × OD 0.84 mm, Eicom, Kyoto, Japan) was inserted into the stomach through a small incision to the gastric body, and the tip was placed at the gastric antrum (Fig. 1C). At the same time, a polyurethane tube was inserted through the duodenal wall and the tip was placed 7 mm from the pylorus (Fig. 1C). The tubes were fixed on the gastric wall and duodenal wall by a purse-string suture, run subcutaneously to emerge at the top of the neck, and then secured on the neck skin (Fig. 1B). In all animals, a polyurethane tube (ID 0.36 × OD 0.84 mm, Eicom, Kyoto, Japan) was placed in the right jugular vein for intravenous (IV) administration of peptides. The tube was filled with heparinized saline to prevent blood coagulation. Mice were allowed to recover for 1 wk before the experiments.

Measurement of gastroduodenal motility and experimental protocols. Mice were deprived of food but not water for 16 h before the experiment (Fig. 1). On the day of the experiment the manometric catheters from the stomach and duodenum were connected to the infusion swivel (375/D/20, Instech Laboratories, Plymouth Meeting, PA) on a single-axis counter-weighted swivel mount (TSB-23, Eicom, Kyoto, Japan) to allow free movement, and then joined to a pressure transducer (DX-100, Nihon Koden Kogyo, Tokyo, Japan) (Fig. 1, A–C). The manometric catheters were continuously infused with bubble-free distilled water at the rate of 0.15 ml/h by an infusion pump (NE-1600, New ERA Pump System, Wantagh, NY), so that the system used infused manometry, not solid-state manometry. The infusion speed was estimated on the basis of that used in rats (12). The data were recorded and stored in a PowerLab (AD Instruments, Colorado Springs, CO). The movement of animals or respiratory rhythm did not affect manometric tracing of gastroduodenal motility. The mice were placed in a black box (150 × 200 × 300 mm) with the top open (Fig. 1, A and B). At the end of the experiments measuring gut motility, animals were euthanized by intraperitoneal injection of excess doses of pentobarbital.

The following experiments were performed between 10:00 and 14:00. In the first experiment, we tried to measure fed and fasted motility in the antrum and duodenum in normal, wild-type, and Y2 knockout mice. Basal motor patterns in the antrum and duodenum were monitored for 60 min under the fasted state, and then mice were given one pellet of laboratory chow and deprived of the remainder after 20 min, during which time it was estimated that they ate 0.3 ± 0.02 g (n = 12) of chow. The motility in the antrum and duodenum was ascertained to have changed into the fed pattern and then returned

Fig. 1. Method for manometric measurements of gastrointestinal motility in conscious mice. Catheters for motility recordings are inserted into the antrum and duodenum (C), run subcutaneously to emerge at the top of the neck and connected to a pressure transducer (B). The manometric catheter is continuously infused with bubble-free distilled water by an infusion pump. The data are recorded and stored in a PowerLab (A).
to the fasted pattern at more than 150 min after feeding. In the second experiment, basal motor patterns in the antrum and duodenum were monitored for 60 min under the fasted state. Then one piece of chow was provided and the remainder removed after 20 min. Vehicle (50 μl of saline) or ghrelin (rat octanoyl ghrelin, Yanaihara, Shizuoka, Japan; 0.3 nmol in 50 μl of saline; dissolved immediately before use) was injected IV at 30 min after feeding, and motility was monitored for 150 min after IV injection. In the third experiment, basal motor patterns in the antrum and duodenum were monitored for 60 min under the fasted state, and then vehicle (50 μl of saline) or CCK-8 (sulfated form, Peptide Institute, Osaka, Japan; 100 pmol in 50 μl of saline) was injected IV and motility was monitored for 150 min after IV injection.

Statistical analysis. Pressure waves obtained by manometric methods were analyzed by using the motor index (MI: area under the manometric trace for 10 min) calculated by use of the PowerLab, as well as the frequency of phase III-like contractions. The effects of peptides were evaluated by changes in the frequency of the phase III-like contractions in the fasted motor activity and percentage motor index (%MI) of the fed motor activity in both the antrum and duodenum. The frequency of phase III-like contractions was obtained from the average of the onset of the contractions per hour as shown by arrowheads in the figures. Values of the %MI for a 10-min period in the antrum were calculated by 100 × (area under the manometric trace for each 10-min period after ghrelin or vehicle injection)/(area under the manometric trace for the 10-min period immediately before ghrelin or vehicle injection). Sequence changes in the mean value of %MI between the periods 0–20, 20–40, and 40–60 min after administration of ghrelin were compared. Data are expressed as means ± SE.

For statistical analyses of the data, Student’s t-test was used for comparison of the frequency of phase II-like contractions between fed and Y2 knockout mice, and comparison of time for initiation of phase III-like contractions in the duodenum and ghrelin injection. Two-way ANOVA for repeated measures by Bonferroni’s post hoc test was used for comparison of %MI in the fed motor activity in both the antrum between vehicle and ghrelin injection (Table 1) and comparison of MI in both antrum and duodenum between wild-type and Y2 knockout mice (Fig. 4B). Values of P < 0.05 were considered statistically significant.

RESULTS

In fasted mice, cyclic changes of pressure waves were detected in the antrum and duodenum, including a quiescent period (phase I-like contractions) followed by strong contractions (phase II-like contractions) (Fig. 3A). Phase III-like contractions were defined as clusters of pressure waves with minimum peak heights of 10 cmH2O in both the antrum and duodenum, with minimum periods of 180 s in the antrum and 190 s in the duodenum, trigger = maximum, measured using the PowerLab software (Fig. 2). Additionally, changes from the fed to the fasted pattern were detected by the appearance of at least three sequential phase III-like contractions in the antrum and duodenum. Arrowheads in all figures show phase III-like contractions. The frequencies of phase III-like contractions were 7.8 ± 0.5 contractions/h (n = 6) in the antrum and 6.6 ± 0.7 contractions/h (n = 6) in the duodenum. When mice ate 0.3 g of chow, the fasted pattern was disrupted and replaced by the fed motor pattern (Fig. 3B), which consisted of irregular contractions and continued for more than 150 min (n = 6) in the antrum and 106.5 ± 8.5 min (n = 6) in the duodenum.

When ghrelin was injected intravenously at 30 min after feeding, in the duodenum the fed pattern was replaced by the fasted pattern at 42.7 ± 4.2 min (n = 4) after injection. This time period was significantly shorter (P < 0.05) than for saline-injected controls (74.9 ± 9.4 min, n = 5) (Fig. 3C). In the antrum, on the other hand, the fed motor pattern remained but %MI was significantly increased (P < 0.05) during the period 20–40 min after intravenous injection of ghrelin (114.3 ± 9.8%, n = 4) compared with saline-injected controls (64.4 ± 9.6%, n = 8) (Fig. 3C, Table 1). However, no change was found in %MI from 0–20 and 40–60 min after injection of ghrelin (Table 1).

When CCK-8 was injected intravenously at the fasted state, phase II-like contractions were disrupted for 39.8 ± 5.6 min (n = 5) in the antrum and 29.1 ± 2.5 min (n = 5) in the duodenum, which were replaced by fed-like motor patterns (Fig. 3D). These fed-like motor patterns induced by CCK-8 were accompanied with the elevation of baseline pressure in both antrum and duodenum (Fig. 3D).

In Y2 knockout mice, in the fasted state the frequency of phase III-like contractions in the antrum (4.5 ± 0.6 contractions/h, n = 6) was significantly decreased compared with that in wild-type mice (7.8 ± 0.5 contractions/h, n = 6) (Fig. 4A). However, the frequency of phase III-like contractions in the duodenum in these mice (5.7 ± 1.0 contractions/h, n = 6) was not changed from that in wild-type mice (6.6 ± 0.7 contractions/h, n = 6) (Fig. 4A). In the fed state of animals, the sequential changes of MI were compared between wild-type (n = 10) and Y2 knockout mice (n = 6) (Fig. 4B). In wild-type mice, in the antrum MIs during the periods from 0–10 min and 10–20 min after feeding were significantly increased compared with the MI from −10 to 0 min before feeding; however, no change was found in the MI between before and after feeding in the duodenum (Fig. 4B). We compared the sequential changes of MI between wild-type and Y2 knockout mice. Significant differences in the MI were found in the antrum

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Table 1. Comparison of %MI in the antrum

<table>
<thead>
<tr>
<th>Time After Injection, min</th>
<th>0–20</th>
<th>20–40</th>
<th>40–60</th>
</tr>
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<tbody>
<tr>
<td>Vehicle (n = 8)</td>
<td>87.8±9.5</td>
<td>64.4±9.6</td>
<td>57.0±10.1</td>
</tr>
<tr>
<td>Ghrelin (n = 4)</td>
<td>104.5±4.3</td>
<td>114.3±9.8*</td>
<td>93.6±29.7</td>
</tr>
</tbody>
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Values are means ± SE. %MI, percentage motor index. *Statistically significant, P < 0.05.
during the period from 0 to 30 min after feeding and in the duodenum from 10 to 20 min after feeding (Fig. 4B).

DISCUSSION

We established a manometric method to measure the motor activities of the stomach and duodenum in the physiological fed and fasted states of conscious mice. Most previous studies that used conscious mice models evaluated GI motility by gastric emptying, measuring gastric contents after a meal (3), Tc⁹⁹m-labeled test meals (26), the [¹⁴C]octanoic acid breath test (8), or intestinal transit of dye (9). However, these transit assays may not allow measuring specifically motor events,
because they do not distinguish between the secretory and motor components. Recently direct measurements of fed and fasted motor activities were successfully performed in conscious mice by using a miniature strain gauge transducer implanted in the stomach wall (27). However, it seems difficult to monitor the duodenal motility in addition to the stomach motility because of the thinness of the duodenal wall compared with stomach wall. We overcame this problem by developing a manometric method with catheters inserted in the antrum and duodenum and connected to a pressure transducer. By this method dual monitoring of stomach and duodenal motility in conscious mice could be achieved.

We analyzed fed and fasted motor patterns in the antrum and duodenum measured by a manometric method. The fed and fasted motor activities in the antrum and duodenum in a conscious Y2 knockout mouse. The frequency of phase III-like contractions (indicated by arrowheads) in the antrum is decreased. B: sequential changes of motor index (MI) in the fed state of mice. In wild-type mice, a significant increase was detected in the MI in the antrum during the periods from 0–10 and 10–20 min after feeding compared with the MI during the period 10 min before feeding ($P < 0.01, n = 10$). In Y2 knockout mice, significant differences were found in the MI during the periods from 0–10, 10–20, and 20–30 min after feeding in the antrum, but 10–20 min in the duodenum, compared with wild type ($**P < 0.01, *P < 0.05, n = 6$).

**Fig. 4.** A: fasted and fed motor activities in the antrum and duodenum in a conscious Y2 knockout mouse. The frequency of phase III-like contractions (indicated by arrowheads) in the antrum is decreased. B: sequential changes of motor index (MI) in the fed state of mice. In wild-type mice, a significant increase was detected in the MI in the antrum during the periods from 0–10 and 10–20 min after feeding compared with the MI during the period 10 min before feeding ($P < 0.01, n = 10$). In Y2 knockout mice, significant differences were found in the MI during the periods from 0–10, 10–20, and 20–30 min after feeding in the antrum, but 10–20 min in the duodenum, compared with wild type ($**P < 0.01, *P < 0.05, n = 6$).
fasted patterns measured in the antrum and duodenum in conscious mice were similar to those obtained in conscious rats measured by manometry, except that the frequencies of phase III-like contractions in the fasted motility in the antrum and duodenum (7.8 ± 0.5 contractions/h and 6.6 ± 0.7 contractions/h, respectively) were significantly (P < 0.05) higher than those in rats (5.3 ± 0.5 contractions/h, 5.6 ± 0.8 contractions/h, respectively) (13). The frequency of phase III-like contractions in the antrum of conscious mice measured with a strain gauge transducer in a previous study (27) was relatively low (2.09 ± 0.09 contractions/30 min) compared with our model. For the fed motor activity, on the other hand, a significant increase in the MI immediately after feeding was detected in the antrum in our model; however, such an increase was not clear in the antrum measured with the strain gauge transducer (27).

Intravenous injection of ghrelin increases motor activity in the antrum and induces fasted motor activity in the duodenum when given in the fed state at a dose that stimulates gastric emptying (3). Increase in the % MI in the antrum after injection of ghrelin was consistent with previous data obtained with a conscious mouse model using a strain gauge transducer implanted in the antrum (27). On the other hand, the effects of ghrelin on the duodenal motility were compared with those obtained with manometric methods in conscious rats (13). The time intervals between injection of ghrelin and initiation of phase III-like contractions in the duodenum were 42.7 ± 4.2 min in mice and 29.6 ± 4.9 in rats (13). The delayed response is likely due to the suppression of ghrelin receptor sensitivity by low intragastric pH (13).

The effects of CCK-8 on the gastric motility have been widely examined by various models using conscious mice (26) or anesthetized rats (1, 2). The results obtained by urethane-anesthetized rats with water-filled stomach showed that CCK-8 caused a significant drop in intragastric pressure, probably due to proximal stomach relaxation (2). However, the present results showed that CCK-8 induced an increase in the luminal pressure selectively measured in the antrum, accompanied by the disappearance of phase III-like contractions. Therefore our model may provide the measurements of the GI motility more specifically compared with previous methods.

It is well known that NPY/PYY receptors in the brain and peripheral organs are involved in the regulation of GI motility (6, 10, 12, 15, 18). Although Y2 receptors primarily mediate upper GI motility, previous studies have not measured the GI motility in Y2 knockout mice but have only examined the phenotype of Y2 knockout mice. In our model, the dorsal vagal complex did not affect the basal antral motility from the fed to the fasted pattern (12) in conscious rats. On the other hand, microinjection of an Y2 agonist into the dorsal vagal complex did not affect the basal antral motility in urethane-anesthetized rats (6). The present results showed that in Y2 knockout mice motor activity in the antrum was more affected than that in the duodenum, and both fed and fasted motor activities were affected in the antrum. Because the phenotype of Y2 knockout mice might be affected by Y2 receptors located in both central and peripheral organs (21), it seems difficult to compare the present and previous studies. Nevertheless, such data obtained from knockout animals can help us to understand the involvement of Y2 receptors in the regulation of upper GI motility.

In conclusion, we developed a method to measure the physiological fed and fasted motor activities in the GI tract in conscious mice. This will open the way for new studies of GI motility in various mouse models, including overexpressing and knockout ones.

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REFERENCES


