Physiological characteristics of gastric contractions and circadian gastric motility in the free-moving conscious house musk shrew (Suncus murinus)

Satoshi Sakahara,1 Zuoyun Xie,1 Kanako Koike,1 Satoya Hoshino,1 Ichiro Sakata,2 Sen ichi Oda,3 Toku Takahashi,4 and Taka fumi Saki1,5

1 Area of Regulatory Biology, Division of Life Science, Graduate School of Science and Engineering, Saitama Univ., 255 Shimo-ohkubo, Sakuraku Saitama 338-8570, Japan; 2 Laboratory of Animal Management & Resources, School of Bio-Agricultural Sciences, Nagoya University, Nagoya, Japan; 3 Department of Internal Medicine, The University of Texas Southwestern Medical Center, Dallas, Texas; and 4 Department of Surgery, Medical College of Wisconsin and Zablocki Veterans Affairs Medical Center, Milwaukee, Wisconsin

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Sakahara S, Xie Z, Koike K, Hoshino S, Sakata I, Oda S, Takahashi T, Sakai T. Physiological characteristics of gastric contractions and circadian gastric motility in the free-moving conscious house musk shrew (Suncus murinus). Am J Physiol Regul Integr Comp Physiol 299: R1106–R1113, 2010. First published August 4, 2010; doi:10.1152/ajpregu.00278.2010.—Although many studies have demonstrated the physiological action of motilin on the migrating motor complex, the precise mechanisms remain obscure. To obtain new insights into the mechanisms, we focused on the house musk shrew (Suncus murinus, suncus used as a laboratory name) as a small model animal for in vivo motilin study, and we studied the physiological characteristics of suncus gastrointestinal motility. Strain gauge transducers were implanted on the serosa of the gastric body and duodenum, and we recorded gastrointestinal contractions in the free-moving conscious suncus and also examined the effects of intravenous infusion of various agents on gastrointestinal motility. During the fasted state, the suncus stomach and duodenum showed clear migrating phase III contractions (intervals of 80–150 min) as found in humans and dogs. Motilin (bolus injection, 100–300 ng/kg; continuous infusion, 10–100 ng·kg⁻¹·min⁻¹) and erythromycin (80 μg·kg⁻¹·min⁻¹) induced gastric phase III contractions, and motilin injection also increased the gastric motility index in a dose-dependent manner (P < 0.05, vs. saline). Pretreatment with atropine completely abolished the motilin-induced gastric phase III contractions. On the other hand, in the free-feeding condition, the suncus showed a relatively long fasting period in the light phase followed by spontaneous gastric phase III contractions. The results suggest that the suncus has almost the same gastrointestinal motility and motilin response as those found in humans and dogs, and we propose the suncus as a new small model animal for studying gastrointestinal motility and motilin in vivo.

DURING A FASTED STATE, THE STOMACH AND SMALL INTESTINE UNDERGO A TEMPORARILY COORDINATED CYCLIC MOTOR PATTERN KNOWN AS MIGRATING MOTOR COMPLEX (MMC) IN DOGS (38) AND HUMANS (44). IT HAS BEEN ESTABLISHED THAT THESE COORDINATED CONTRACTIONS CONSIST OF THREE PHASES, PHASE I (PERIOD OF MOTOR QUIESCENCE), PHASE II (PERIOD OF PRECEDING IRREGULAR CONTRACTIONS), AND PHASE III (PERIOD OF CLUSTERED POTENT CONTRACTIONS), AND THE MMC IS STIMULATED BY ENDGENOUS MOTILIN THAT IS RELEASED IN THE FASTED STATE.

Motilin was originally purified from porcine intestinal mucosa in the 1970s, and its molecular structure was determined to be a 22-amino-acid polypeptide. It has been demonstrated that plasma motilin is released at ~100-min intervals at the interdigestive state (20, 21). Physiological study of motilin in vivo has been mainly performed by using dogs and humans, and endogenous motilin and exogenous motilin have been shown to induce phase III contractions through the cholinergic pathway because atropine pretreatment completely abolished the motilin-induced contractions (11).

However, in addition to a lack of information on the precise physiological mechanism of motilin-induced MMC, there is a lack of basic information for motilin study such as information on the detailed distribution of motilin and motilin receptor in the body and changes in motilin release under some physiological states. One of the reasons for the difficulty in motilin study is that rodents such as rats and mice cannot be used for motilin study because the motilin gene is inactivated in the common ancestor of mice and rats (12).

Ghrelin, which is involved in stimulation of growth hormone (GH) secretion (23) and food intake (31), forms a peptide family with motilin because of their similarities in not only peptides but also their receptors (34). Ghrelin also stimulates gastric contractions in rats (26) and mice (48), suggesting that ghrelin serves as an alternative to motilin with regard to gastrointestinal motility in motilin-lacking rodents (34). It has also been shown that ghrelin induces premature phase III contractions in the human stomach (39). Therefore, it is reasonable to assume that there are some additional or synergic effects of ghrelin and motilin on induction of gastric contraction and gastric emptying. However, because of a lack of suitable model animals, few findings regarding the physiological relationships between motilin and ghrelin have been reported.

To find a small mammal that can be used as a new model animal for study of the mechanism of motilin-induced gastric contraction and motilin/ghrelin family peptide, we focused on the house musk shrew (Suncus murinus, suncus used as a laboratory name). The suncus is a small mammal that belongs to the order Insectivora, family Soricidae, genus Suncus (3). We previously reported the complete cDNA sequences and tissue distribution of motilin and ghrelin in the suncus (15, 43), and reproducible contractile responses of the suncus stomach to suncus or human motilin were found in an in vitro organ bath experiment (43).
In this study, to determine the characteristics of suncus gastrointestinal motility and the usefulness of the suncus for physiological study of motilin in vivo, we established a system for recording gastrointestinal contractions in the conscious and free-moving suncus and then conducted a series of fundamental observations, i.e., MMC in the fasted suncus, effects of motilin and erythromycin on gastrointestinal motility, and gastric contractions and behaviors for 24 h under a free-feeding condition.

**MATERIALS AND METHODS**

**Animals and experimental protocol.** Experiments were carried out using adult male suncus (10–30 wk of age) of an outbred KAT strain established from a wild population in Kathmandu, Nepal (32), weighing between 70 and 100 g. The animals were housed individually in plastic cages equipped with an empty can for a nest box under conditions (23 ± 2°C, lights on from 0800 to 2000) with free access to water and commercial feeding pellets (no. 5P; Nippon Fostera Feed Manufacturing, Yokohama, Japan). The metabolizable energy content of the pellets was 344 kcal/100 g, and the pellets consisted of 54.1% protein, 30.1% carbohydrate, and 15.8% fat. Because there is no cecum in the suncus, it is difficult to distinguish the small intestine and colon. Therefore, in this study, we divided the intestine into six equal segments (intestines 1–6) as previously reported (15, 43). All procedures were approved and performed in accordance with the Saitama University Committee on Animal Research. All efforts were made to minimize animal suffering and to reduce the number of animals in experiments. We repeated the recording experiments at least individually three times and obtained similar results. We also showed the numbers of animals used for statistical analysis in legends for Figs. 1–4 and in Tables 1 and 2.

**Animal preparation for gastrointestinal motility recording.** After 3 h fasting, each suncus was anesthetized with intraperitoneal injection (100 mg/kg) of pentobarbital sodium (50 mg/kg). Through a midline laparotomy, strain gauge force transducers were implanted on the serosal surface of the gastric body and intestine 2 (equivalent to the duodenum or jejunum) for recording circular muscle contractions. The wires from the transducer were exteriorized through the abdominal wall and ran under the skin toward the back of the neck. An intravenous catheter was inserted in the right jugular vein and also exteriorized to the back. The catheter was filled with heparinized saline (100 U/ml) to prevent coagulation. The wires and the catheter were protected by a protective jacket. Suncus started to eat food 1 day after surgery, the food intake was similar to that in the nonoperated group (data not shown), and motilin or chemical compounds were administered on day 3 after surgery.

**Assessment of fix feeding.** The animals were divided into two groups on the 2nd day after the operation. Animals in the fixed-feeding group were deprived of food from 1000 to 2000 (10 h fasting) and received food from 2000 to 1000 (14 h feeding), whereas animals in the free-feeding group had free access to food for the whole day. To assess the influence of fixed feeding on body weight, the animals were maintained on a feeding schedule that alternated weekly between free feeding (24 h ad libitum feeding) and fixed feeding (14 h ad libitum feeding) for 3 wk, and all animals were weighed daily at 1000.

**Monitoring of gastrointestinal motility.** The strain gauge force transducers used in this study were made in our laboratory with appropriate modification of the previously reported method (18). Waterproofing and response property were checked in all transducers before implantation. The amplified analog signals were converted with an analog-digital converter (ADC-20; Pico Technology, St. Neots, UK), and then the digital signals were recorded with a computer. Spontaneous gastrointestinal motility was recorded for 8–10 h in a fasted state or 24 h in a free-feeding state. The definition of phase III contractions of the MMC in the conscious suncus was based on that in dogs and humans, i.e., clustered contractions with an amplitude of >8 g and lasting for >5 min were counted as phase III contractions.

**Effect of motilin and erythromycin administration on gastrointestinal motility in the fasted state.** Administration of motilin and erythromycin was initiated 15–25 min after completion of spontaneous phase III contractions in the fasted suncus. Synthetic suncus motilin was given as a single bolus intravenous injection at doses of 100 and 300 ng/kg or as a continuous infusion at doses of 10, 17.5, and 100 ng·kg⁻¹·min⁻¹ for 10 min, and erythromycin (80 µg·kg⁻¹·min⁻¹) was continuously intravenously infused for 10 min. A single bolus intravenous injection volume was 100 µl/suncus, and a continuous intravenous infusion volume was 50 µl·100 g body wt⁻¹·min⁻¹. Quantified motilin-induced gastric motility was represented by the motility index (MI). In this study, MI during 10 min motilin infusion was defined as the percentage of the area under the curve for the 10-min duration of adjacent phase III contractions.

**Effect of atropine pretreatment on motilin-induced gastric contractions.** To study whether the cholinergic pathway is involved in mediating phase III contractions, atropine was intravenously administered by single-bolus injection (100 µl/100 g body wt) and subsequent continuous 20-min infusion (50 µg/kg + 0.83 µg·kg⁻¹·min⁻¹). After the atropine pretreatment initiation (5 min), motilin was continuously infused (100 ng·kg⁻¹·min⁻¹) for 10 min. As a control, saline was used instead of atropine.

**Monitoring of behaviors.** To observe behaviors (movement, feeding, and drinking) of the suncus under a free-feeding condition, we installed a web camera (CAM 130 Night Vision 2; Timely, Tokyo, Japan) and monitored behaviors with the gastric contractions for 24 h. The behaviors in the dark period were observed with the night-vision mode of the camera. In this study, we defined the active stage of the suncus as the duration of moving and grooming.

**Data and statistical analysis.** Values were given as means ± SE. Statistical analysis was performed using ANOVA followed by Student’s t-test and Scheffé’s test with Stat View statistics software (SAS Institute, Cary, NC). A P value <0.05 was considered to be statistically significant.

**Materials.** Suncus motilin (Scrum, Tokyo, Japan), erythromycin lactobionate [500 mg (potency)/vial; Abbott Japan, Tokyo, Japan], and atropine (Mylan, Tokyo, Japan) were dissolved in each solvent and diluted with saline immediately before use.

**RESULTS**

**Daily body weight in the free-fed and fixed-fed animals.** Body weight was not changed significantly under the conditions of 24 h free feeding and 14 h fixed feeding (data not shown), suggesting that the suncus under the fixed-feeding condition in this study consumes its essential daily food within 14 h and that this 14-h fixed-feeding condition does not cause any severe nutritional problems.

**Gastrointestinal contractions in the fasted state and postprandial state in the conscious suncus.** In the fasted state, the same clear interdigestive contractions as those that occur in humans and dogs were observed in the suncus stomach (gastric body). Phase I (period of motor quiescence), phase II (period of preceding irregular contractions), and phase III (period of clustered potent contractions) were clearly recognized at regular intervals (Fig. 1A). Spontaneous phase III contractions of the stomach were observed every 80–150 min (Table 1). The interdigestive patterns in the stomach were disrupted immediately after feeding and were replaced by postprandial patterns (Fig. 1B). Although interdigestive phase I and phase II contractions of the duodenum were not synchronized with those of the stomach, spontaneous gastric phase III contractions migrated from the gastric body to intestinal tract (intestine 2)
Mean migrating speed of phase III contractions from the stomach to upper small intestine was calculated to be ~1 cm/min. The frequencies of contractions in the suncus stomach and duodenum during phase III are 14 times/min and 35 times/min, respectively.

**Effect of motilin, erythromycin, and atropine administration on gastrointestinal motility in the fasted state.** At 15–25 min after completion of spontaneous phase III contractions, saline, motilin, or erythromycin was intravenously injected. Figure 2A shows typical contractions evoked by single bolus injection of synthetic suncus motilin at doses of 100 and 300 ng/kg, and Fig. 2B shows typical contractions evoked by the infusion of synthetic suncus motilin at doses of 17, 50, and 100 ng·kg⁻¹·min⁻¹. Although infusion of motilin at 50–100 ng·kg⁻¹·min⁻¹ always induced strong phase III-like gastric contractions, contractile reactivity for 17 ng·kg⁻¹·min⁻¹ administration depended on the individual. Percentages of area under the curve for 10 min in the motilin-induced contractions to that of spontaneous phase III contractions were calculated and represented as MI. MI significantly increased in a dose-dependent manner (Fig. 2C, Scheffé’s test, P < 0.05). Motilin-induced gastric phase III-like contractions also migrated from the gastric body to intestinal tract (duodenum) (*spontaneous phase III contractions). Arrows indicate the movement of contractions.

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**Fig. 1.** Spontaneous gastrointestinal contractions in the fasted state and postprandial state in the conscious free-moving suncus. A: two examples of gastric contraction in the fasted suncus. Clear interdigestive contractions that are the same as those occurring in humans and dogs were observed in the suncus stomach (gastric body). Contractions of phase I (period of motor quiescence), phase II (period of preceding irregular contractions), and phase III (period of clustered potent contractions) were clearly recognized at regular intervals (magnified). B: the interdigestive patterns in the stomach were disrupted immediately after feeding and were replaced by postprandial patterns. C: interdigestive contractions of the duodenum were not like those of the stomach, but spontaneous gastric phase III contractions migrated from the gastric body to intestinal tract (duodenum) (*spontaneous phase III contractions).
intestine (intestine 2) as was found for spontaneous phase III contractions (Fig. 2D).

Ten minutes of erythromycin infusion at a dose of 80 μg·kg⁻¹·min⁻¹ also induced typical gastric contractions as found in motilin treatment (Fig. 3A). On the other hand, bolus and continuous administration of atropine (50 μg/kg + 0.83 μg·kg⁻¹·min⁻¹) completely abolished motilin (100 ng·kg⁻¹·min⁻¹)-induced gastric contractions (Fig. 3B).

Twenty-four-hour monitoring of gastric contractions and behaviors of the free-fed suncus. The gastric contractions and behaviors were recorded from 1000 for 24 h. Figure 4 shows a typical chart of 24-h monitoring of gastric contractions and behaviors (movement, feeding, and drinking) in the free-fed suncus. A clear circadian rhythm was found in the gastric contraction and behavior. Relatively long fasting (almost 6 h in the case of Fig. 4) was found in the light phase, and typical gastric phase III contractions were found in this fasting period, suggesting that spontaneous MMC occurs in the natural state of this species. The suncus was found to be more active during the dark phase, and frequency of feeding was increased signifi-
It has been demonstrated that suncus motilin is expressed in the upper small intestine (43). In the present study, we found that the stomach and small intestine of the conscious suncus showed spontaneous gastric phase III contractions every 80–150 min, almost the same intervals as those found in humans and dogs. Moreover, suncus stomachs responded to motilin treatment in vivo, and phase III-like strong clustered gastric contractions were found. Furthermore, it has been reported that bolus injection of motilin to dogs (100–300 ng/kg) and continuous intravenous infusion in humans (4–69 ng·kg\(^{-1}\)·min\(^{-1}\) for 30 min) or dogs (5–45 ng·kg\(^{-1}\)·min\(^{-1}\) for 20 min) induced gastric contraction in vivo (17, 25, 37). In the present study, we clearly demonstrated that almost the same dose of motilin (bolus injection of 100–300 ng/kg, continuous infusion of 10–100 ng·kg\(^{-1}\)·min\(^{-1}\) for 10 min) also stimulated suncus gastric contraction. Taken together, the results suggest that the suncus stomach has almost the same threshold and reactivity to motilin as those of humans and dogs. From these results, we concluded that the suncus has the unique property of gastrointestinal motility and motilin reactivity, and, to our knowledge, this is the first small laboratory animal showing human- and dog-like MMC in the fasted state and motilin reactivity in vivo and in vitro.

It has been well established that erythromycin, a nonpeptide motilin receptor agonist (33, 41), mimics the effect of motilin on gastrointestinal contractile activity in humans and dogs in vivo (19, 42). In this study, erythromycin also induced gastric contractions in the conscious suncus (Fig. 3), suggesting the existence of physiological active motilin receptor in the suncus. In fact, we have determined the sequence of suncus motilin receptor mRNA by using the PCR cloning method and found that suncus GPR38 mRNA is mainly expressed in the suncus stomach (unpublished observations). Because the suncus is a small animal, it is easy to study the expression and distribution of these receptors by using molecular-biological and morphological techniques. Therefore, by using the suncus, the mechanisms of motilin- and/or ghrelin-induced gastrointestinal contraction can be easily studied from the receptor level to whole body physiology, and results of such studies will shed light on the mechanism of MMC in the fasted state.

Many efforts have been made to find a small laboratory animal that produces motilin. Xu et al. (46) reported the guinea pig motilin amino acid sequence predicted by cDNA cloning. Although results of several electrophysiological studies on motilin using the guinea pig have been reported (22, 47), to our knowledge, few morphological and molecular biological results have been reported. Moreover, Furness et al. (8) showed that erythromycin-induced muscle contraction in the guinea pig is not caused through the motilin receptor, and many studies have demonstrated that guinea pig muscle preparations did not respond to motilin treatment in vitro (27, 36). Taken together, the guinea pig may not always be a suitable model animal for motilin study.

It has been reported that motilin stimulates gastrointestinal smooth muscle contraction through a direct or indirect pathway, i.e., myenteric neurons (28), autonomic nervous system (16), or smooth muscles (36), and various reports have pointed out the importance of vagal nerves (2, 10, 14, 40). Several studies have suggested that the main pathway of motilin-induced gastrointestinal contractions is different in each spe-

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**DISCUSSION**

It has been demonstrated that plasma motilin is released at ~100-min intervals during the interdigestive state and induces phase III contractions of MMC in humans (45) and dogs (20). However, in rodents, although specific phase III-like contractions were observed every 15 min in the fasted state (7, 48), many reports claimed that administration of motilin or a motilin agonist failed to stimulate contraction of rat intestinal muscle strips and gastric emptying. For example, Depoortere et al. (4, 5) found that motilin did not accelerate rat gastric emptying and transit, and they suggested that motilin and its receptor do not exist or are nonfunctional in these species. Moreover, although several research groups, including our group, have tried to determine the sequence of rat motilin and motilin receptor knockouts. These results strongly suggest that rats and mice do not produce motilin and that rodents are therefore not suitable animals for motilin study. On the other hand, we previously confirmed the production of physiological active motilin in the suncus by using molecular cloning and an in vitro organ bath experiment, and we also

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**Fig. 3. Effect of erythromycin and atropine on gastric contraction.**

* A: 10 min erythromycin infusion at a dose of 80 µg·kg\(^{-1}\)·min\(^{-1}\) induced typical gastric contractions as found in motilin treatment. B: bolus and continuous administration of atropine (50 µg/kg + 0.83 µg·kg\(^{-1}\)·min\(^{-1}\)) completely abolished motilin (100 ng·kg\(^{-1}\)·min\(^{-1}\))-induced gastric contractions (*spontaneous phase III contractions). Arrows indicate the timing of the bolus injection of saline or atropine.

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*Significantly (Student’s t-test, *P < 0.05), whereas frequency of phase IIIB contractions and total duration of phase I were decreased in the dark phase (Table 2, Student’s t-test, *P < 0.05) (n = 5).*

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cies. For example, in vitro contractility studies with smooth muscle strips of the rabbit upper small intestine demonstrated that contractile effects of motilin were mediated through a direct action on smooth muscle cells (36), and isolated smooth muscle cells from the rabbit antrum were contracted by superfusion with motilin (29). In contrast, in the dog, no contractile effects could be obtained with porcine motilin treatment in gastric smooth muscle preparations (6), and endogenous and exogenous motilin induced phase III contractions through a myenteric plexus and vagal pathway (28). We demonstrated that motilin-induced suncus gastric contractions are completely abolished by atropine pretreatment. Moreover, in our previous study, we showed that the whole suncus stomach in an organ bath responded to motilin treatment in a dose-dependent manner through a tetrodotoxin- and atropine-sensitive neural pathway, suggesting that motilin-induced suncus gastric contractions are involved in the myenteric cholinergic neural network as found in dogs. However, the importance of the vagal pathway and the relationship between the vagus and myenteric plexus in motilin-induced suncus gastric contractions remains obscure. For further understanding of the mechanisms of motilin-induced gastric contraction, these points should be determined by in vitro and in vivo studies.

Table 2. Characteristics of behavior and gastric motility of the free-fed suncus in light and dark phases

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<tr>
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<th>Light Phase</th>
<th>Dark Phase</th>
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<tr>
<td>Total duration of movement, min/12 h</td>
<td>94 ± 27</td>
<td>168 ± 28</td>
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<tr>
<td>Frequency of feeding, times/12 h</td>
<td>6.0 ± 0.4</td>
<td>9.8 ± 1.2*</td>
</tr>
<tr>
<td>Frequency of drinking, times/12 h</td>
<td>8.4 ± 0.8</td>
<td>16.6 ± 4.3</td>
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<tr>
<td>Frequency of phase III contractions, times/12 h</td>
<td>3.8 ± 0.7</td>
<td>1.0 ± 0.4*</td>
</tr>
<tr>
<td>Total duration of phase I, min/12 h</td>
<td>232 ± 39</td>
<td>44 ± 19†</td>
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</table>

Values are means ± SE; n = 5 experiments. Frequency of phase III contractions and total duration of phase I were significantly increased in the light phase, and frequency of feeding was decreased in the dark phase. *P < 0.05 and †P < 0.01 by Student’s t-test.

The suncus is among the smallest mammals and has a high relative resting metabolic rate (35). As a consequence, the suncus has a high energetic demand met by multiple feeding periods during the daily cycle (3). However, in the suncus, even in the free-feeding condition, long spontaneous fastings were found in the light period, and the suncus usually stayed in the nest and was immobilized, suggesting that spontaneous fasting and subsequent gastric phase III contractions occurred in the sleeping state of the suncus. It has been demonstrated that phase III contractions also occur in humans during sleep (9). Taken together, the suncus may be a good model animal for study of the properties and mechanisms of MMC during sleep. In the light phase, gastric phase III induced by long-lasting spontaneous fasting and subsequently phase I were observed, and, interestingly, the suncus always fed at the end of phase I and then postprandial contractions appeared. Considering the physiological effect of motilin, it is important to elucidate the changes in plasma concentration of motilin for understanding these spontaneous phase III contractions.

It has been reported that ghrelin regulates not only food intake (31) and GH release (23) but also gastrointestinal motility in many species, including rats (26), mice (48), guinea pigs (30), and humans (24), and that ghrelin may be a major prokinetic hormone in motilin-lacking animals. Given the prokinetic effects of motilin and ghrelin, additive or synergistic effect on gastrointestinal contractions by these peptides is very interesting. We previously demonstrated that physiological active ghrelin is produced in the suncus, and we recently found that ghrelin also stimulated suncus gastric motility in specific physiological states (unpublished observations). Taken together, the results indicate that the suncus may be a useful animal for study of not only the action of motilin but also that of ghrelin on gastrointestinal motility as an alternative to humans and dogs for study of gastrointestinal motility.
Perspectives and Significance

We have successfully established a system for recording suncus gastrointestinal motility in vivo, and we demonstrated motilin-induced gastric contraction and spontaneous MMC in the free-moving conscious suncus. Suncus, a motilin- and ghrelin-producing laboratory animal, will be useful for studying not only physiological mechanisms of motilin but also effects of the motilin/ghrelin family on gastrointestinal motility.

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