Role of Transient Receptor Potential Ankyrin 1 in Gastric Accommodation in Conscious Guinea Pigs

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ABSTRACT
We report the establishment of a new model for measuring gastric tone and liquid meal-induced accommodation in conscious guinea pigs and the role played by transient receptor potential ankyrin 1 (TRPA1). An indwelling polyethylene bag was placed in proximal stomaches of 5-week-old male Hartley guinea pigs. Gastric tone was measured by distending the bag and recording changes in intrabag pressure at various volumes. Gastric accommodation was measured by administering liquid meals and recording intrabag pressure over time. N_ϵ-nitro-L-arginine methyl ester hydrochloride (L-NAME) (a nitric-oxide synthase inhibitor), atropine sulfate (atropine) (a muscarinic receptor antagonist), allyl isothiocyanate (AITC) (a TRPA1 agonist), or theophylline-7-((N-4-isopropylphenyl) acetamide (HC-030031) (a selective TRPA1 antagonist) was administered 15 to 60 min before measurement. Gastric tone was increased by stepwise distension of the bag and was further significantly increased by L-NAME and significantly decreased by atropine. A liquid meal (15% w/v; 1.7 kcal) significantly decreased intrabag pressure 5 to 20 min after administration, indicating gastric accommodation; this was completely suppressed by L-NAME and further enhanced by atropine. AITC significantly increased gastric tone; this increase was decreased by HC-030031 and atropine. A combination of AITC and L-NAME significantly increased gastric tone compared with L-NAME alone. HC-030031 alone significantly decreased gastric tone. Liquid meal-induced gastric accommodation was significantly suppressed by pretreatment with AITC. We established a new model for measuring gastric tone and accommodation in conscious guinea pigs. TRPA1 activation suppresses gastric accommodation by increasing gastric tone through cholinergic neuronal pathways.

Introduction
Gastric accommodation functions as a reservoir for ingested food and fluid; disorders in gastric accommodation are associated with conditions such as functional dyspepsia and diabetic gastroparesis (Tack et al., 1998; Kumar et al., 2008). Elucidating the mechanisms underlying gastric accommodation is extremely important for understanding the pathophysiology of functional dyspepsia.

Gastric accommodation describes a reflex response in the proximal stomach when food enters, leading to decreased gastric tone and increased gastric compliance. Currently nonadrenergic and noncholinergic nerves are thought to be involved in gastric accommodation; of these, nitrergic nerves are strong candidates, and the response has been reported to be initiated by the vagus or intrinsic nerves (Azpiroz and Malagelada, 1986; Desai et al., 1991a,b; Tonini et al., 2000). In contrast, gastric tone is a sustained muscular contraction of the stomach wall and is maintained by the activity of cholinergic neurons innervated by the vagus nerve (Morgan et al., 1981; Azpiroz and Malagelada, 1987).

Capsaicin, a transient receptor potential vanilloid receptor 1 (TRPV1) agonist, has been reported to decrease gastric tone (Lee et al., 2004). Transient receptor potential ankyrin 1 (TRPA1) is a nonselective cation channel of the TRP family, cloned from human fibroblast cells, that is involved in nociception, neuropathic pain, and inflammatory pain (Jaquemar et al., 1999; Story et al., 2003; Obata et al., 2005; Bautista et al., 2006; Kwan et al., 2006). TRPV1 has been reported to be involved in the contractile response of the small intestine, pain-induced gastric distension, and delayed gastric emptying.

ABBREVIATIONS: TRPA1, transient receptor potential ankyrin 1; L-NAME, N_ϵ-nitro-L-arginine methyl ester hydrochloride; AITC, allyl isothiocyanate; HC-030031, theophylline-7-((N-4-isopropylphenyl) acetamide; TRPV1, transient receptor potential vanilloid receptor 1; NO, nitric oxide; LM, liquid meal.
ing (Penuelas et al., 2007; Doihara et al., 2009a,b; Kondo et al., 2009). Although functional analysis of TRPA1 in the gastrointestinal tract has progressed, the role of TRPA1 in gastric tone or accommodation remains unclear.

Noninvasive technological advances, such as the barostat, conventional and three-dimensional ultrasound imaging, and single-photon emission-computed tomography, have enabled the measurement of gastric relaxation in humans (Tack et al., 1998; Schwizer et al., 2002). Although in vivo studies in the conscious condition have generally been performed in large animals, such as dogs and cats, using the barostat with a bag or balloon, studies in small animals, such as rodents, have been performed mainly under anesthesia (Azpiroz and Malagelada, 1987; Coulie et al., 1989). Furthermore, although gastric accommodation in conscious rats has been measured (Janssen et al., 2008), rodents have a forestomach, which is histologically different from the human gastric fundus; therefore, mechanisms that regulate gastric accommodation may differ from those of humans. On the other hand, the guinea pig has a gastric fundus analogous to that of humans.

The aims of the present study were to establish a new experimental model for evaluating gastric tone and accommodation in the conscious guinea pig, and then to use the model to study the role of TRPA1 in gastric tone and accommodation induced by liquid meals.

Materials and Methods

Animals. Male, 4-week-old Hartley guinea pigs were purchased from Japan SLC, Inc. (Hamamatsu, Japan). After 1 week of acclimation, the guinea pigs were used for experiments at 5 weeks of age. During the experimental period, the guinea pigs were kept in a 12-h light/dark cycle (7:00 AM-7:00 PM) at 23°C and 50 ± 20% humidity. They were allowed free intake of food and water. All experiments were performed between 9:00 AM and 6:00 PM and were in compliance with guidelines established by the Animal Ethics Committee of Tsumura & Co.

Chemicals. The following compounds were used in experiments: a nitric-oxide (NO) synthase inhibitor, N-nitro-l-arginine methyl ester hydrochloride (L-NAME) (Sigma, St. Louis, MO); a muscarinic receptor antagonist, atropine sulfate (atropine) (Wako Pure Chemicals, Osaka, Japan); a TRPA1 agonist, allyl isothiocyanate (AITC) (Wako Pure Chemicals); and a selective TRPA1 antagonist, tephrillin-7-(N-4-isopropylphenyl) acetamide (HC-030031) (Enzo Life Sciences, Plymouth Meeting, PA).

Placement of Polyethylene Bag and Polyurethane Cannula. The guinea pigs were fasted for at least 18 h, then anesthetized with sodium pentobarbital (30 mg/kg i.p.) (Kyoritsu Seiyaku Corporation, Tokyo, Japan). A 4- to 5-cm abdominal laparotomy was performed to expose the stomach. A polyethylene bag (maximum capacity 14 ml; 0.01 mm thick) was inserted into the proximal stomach from the distal part of the stomach and left in place. A polyethylene tube (PE-60; BD Biosciences, Franklin Lakes, NJ) connected to the bag was threaded through the abdominal wall of the right flank and exteriorized in the back of the neck under the skin.

A polyurethane cannula (BC-3P; Access Technologies, Skokie, IL) was inserted into the right jugular vein for systemic drug administration. To prevent blood clots, the cannula was flushed once every 2 days with 100 units/ml heparin sodium (Mochida Pharmaceutical Co., Ltd., Tokyo, Japan).

Body weights and food intake were recorded after the operation. The location of the bag was confirmed after the measurements, when the animals were sacrificed. Extremely low intrabag pressure was detected when the bag was dislocated from the proximal stomach to the distal stomach. We have excluded animals in which dislocation occurred to secure the reliability of the data.

Measurement of Gastric Tone. After allowing at least 7 days for recovery from surgery, measurement of gastric tone was performed on conscious animals. Before each experimental session, the guinea pigs fasted for at least 18 h. The intragastric bag was connected to a pressure transducer (MLT0699; ADInstruments Pty Ltd., Castle Hill, Australia), and air was injected into the bag by using a syringe pump (KDS-200; KD Scientific, Holliston, MA) at a flow rate of 2 ml/min. Intrabag volume was increased in a stepwise fashion, without intervening deflation, to 1, 2, 4, 6, and 8 ml of air; the syringe pump was temporarily stopped at each volume step, and pressure changes were measured and recorded at intervals of 30 s by using the BP Amp (ADInstruments Pty Ltd.) and PowerLab 4/26 (ADInstruments Pty Ltd.) devices. For each experiment, three volume-pressure curves were obtained; a minimum of 15 min was allowed between the end of one distention cycle and the beginning of the next. The first distention cycle was used to unfold the intragastric bag, and the second was used to measure gastric tone induced by the vehicle. Before the third distention cycle test, drugs were administered as follows: L-NAME and atropine were dissolved in saline and administered intravenously 30 and 15 min before measurements, respectively; AITC was dissolved in an aqueous solution of 0.3% (v/v) ethanol and 1% (v/v) Tween80 and administered orally 15 min before measurements; HC-030031 was suspended in 0.5% (w/v) methylcellulose and administered intraperitoneally 60 min before measurements. The treatment conditions of L-NAME and atropine were those used by De Ponti et al. (2003). The treatment conditions of AITC and HC-030031 were determined in our preliminary examinations and by referring to the reports of Doihara et al. (2009a) and Kondo et al. (2009). The animals were used in consecutive experiments after a washout period of at least 3 days within 3 weeks after surgery.

Gastric tone was recorded twice on the same day with a 60-min interval and on 2 separate days (Supplemental Table 1). There were no significant differences in tone between intraday and interday examinations.

Measurement of Liquid Meal-Induced Accommodation. After allowing at least 7 days for recovery from surgery, measurement of gastric accommodation was performed on conscious animals. Each experiment consisted of a 5-min premeal baseline period and a 30-min postmeal period. To measure the baseline pressure, a pressure transducer was connected to the intragastric bag and 6 ml of air were injected at a flow rate of 2 ml/min by using the syringe pump. Five minutes after air injection, the intrabag pressure was recorded for 1 min by using the BP Amp and PowerLab 4/26. The air was temporarily withdrawn from the bag, and animals were allowed to rest for at least 15 min. Immediately after a liquid meal administration, 6 ml of air were again injected into the intragastric bag and maintained for 30 min. Intrabag pressure was recorded for 1 min at 5, 10, 15, 20, 25, and 30 min after the liquid meal. The liquid meal consisted of powdered feed pellets for guinea pigs (CG-7; CLEA Japan, Tokyo, Japan) [278.1 kcal, 18.1% (w/w) crude proteins, 3.4% (w/w) crude fats, and 16.8% (w/w) crude fibers per 100 g] suspended in distilled water at 15% (w/v) and then homogenized for 1 min at room temperature by using a Polytron homogenizer (Kinematica Inc., Littau-Lucerne, Switzerland). This liquid meal (1.7 kcal in 4 ml) was administered by gavage using an oral sonde (RZ-2; CLEA Japan, Inc.). L-NAME, atropine, AITC, and HC-030031 were administered 30, 15, and 60 min, respectively, before liquid meal administration.

Gastric accommodation was recorded on 2 separate days (Supplemental Table 2). There was no significant difference in accommodation between interday examinations.

Statistical Analysis. The intrabag pressure was calculated as the average pressure by using LabChart6 (ADInstruments Pty Ltd.). The mean pressure change (Δ intrabag pressure) was calculated by averaging the differences between premeal and postmeal pressures of each 5-min interval. The intrabag pressures in gastric tone experiments were analyzed with paired t tests. The premeal and postmeal
intrabag pressures in gastric accommodation experiments were analyzed with Dunnett’s test. The mean Δ intrabag pressure was determined by using the Williams test or Student’s t test. All data were expressed as mean ± S.D. and analyzed for statistical significance by using StatLight (Yukms Co., Ltd., Tokyo, Japan); p values < 0.05 were considered statistically significant.

Results

Measurement of Gastric Tone and Influences of L-NAME and Atropine. Stepwise gastric distention by air infusion increased intrabag pressure (Fig. 1A). After L-NAME (10 mg/kg) administration, intrabag pressure significantly increased compared with the vehicle (Fig. 1B). However, atropine (100 µg/kg) significantly decreased intrabag pressure compared with the vehicle (Fig. 1C).

Gastric Accommodation Induced by Liquid Meal Administration and Influences of L-NAME and Atropine. Intrabag pressures before and after water or a 7.5% (w/v) liquid meal did not differ. In contrast, a 15% (w/v) liquid meal significantly decreased intrabag pressure 5 to 20 min after administration and then recovered to previous levels after 30 min (Fig. 2A). Compared with water, a 15% (w/v) liquid meal significantly decreased the mean Δ intrabag pressure during the 30-min period after administration (Fig. 2B). When various volumes of the 15% (w/v) liquid meal were administered, intrabag pressure decreased, compared with baseline levels, at volumes ≥2 ml (Fig. 2C). When intrabag pressures 30 min after various volumes of liquid meals were compared, administration of ≥2 ml of 15% (w/v) liquid meal showed a volume-dependent decrease in intrabag pressure (Fig. 2D).

To determine whether the liquid meal-induced decrease in intrabag pressure was regulated by nitrergic or cholinergic neurons, pretreatment with L-NAME or atropine was used. L-NAME (10 mg/kg) significantly inhibited the decrease in intrabag pressure caused by 15% (w/v) liquid meal administration compared with the vehicle (Fig. 2E); however, atropine (100 µg/kg) significantly promoted this decrease (Fig. 2F).

Combined Influence of AITC and HC-030031 on Gastric Tone. Neither vehicle nor 1 mg/kg AITC administration changed intrabag pressure (Fig. 3, A and B). However, 2 mg/kg AITC significantly increased intrabag pressure compared with vehicle (Fig. 3C); this increase was inhibited by pretreatment with 100 mg/kg HC-030031 (Fig. 3D).

Involvement of Cholinergic Nerves in AITC-Induced Increase in Gastric Tone. The 2 mg/kg AITC-induced increase in intrabag pressure was suppressed by concurrent administration of atropine (100 µg/kg) (Fig. 4A). L-NAME (10 mg/kg) significantly increased intrabag pressure compared with vehicle (Fig. 4B); administration of L-NAME (10 mg/kg) and AITC (2 mg/kg) significantly increased intrabag pressure compared with L-NAME alone (Fig. 4B).

Influence of HC-030031 on Gastric Tone. HC-030031 (100 mg/kg) alone significantly suppressed the increase in intrabag pressure caused by air injection compared with vehicle (Fig. 5).

Fig. 1. Measurement of gastric tone and influences of L-NAME and atropine. A, gastric distention caused a stepwise increase in intrabag pressure. B, L-NAME (10 mg/kg) significantly increased intrabag pressure at each volume compared with vehicle. C, atropine (100 µg/kg) significantly decreased intrabag pressure at each volume compared with vehicle. Mean ± S.D. of four animals is shown. *, p < 0.05; **, p < 0.01 versus vehicle (paired t test).
Influences of AITC and HC-030031 on Liquid Meal-Induced Gastric Accommodation. AITC (2 mg/kg) significantly inhibited the decrease in intrabag pressure by 15% (w/v) liquid meal administration compared with vehicle (Fig. 6A); HC-030031 (100 mg/kg) tended to promote this decrease (Fig. 6B).

Discussion

In the present study, we placed in-dwelling polyethylene bags in the stomachs of guinea pigs and have established a small-animal model for measuring gastric tone and accommodation in the conscious condition. This model can be used to study mechanisms of gastric accommodation and the brain-gut axis in physiological or pathophysiological conditions after experimental stressors in the conscious state. Using this model, we concluded that TRPA1 may suppress gastric accommodation by increasing gastric tone via cholinergic pathways.

We first determined the effect of placing these polyethylene bags in the stomachs of guinea pigs. After placement, body weight and food intake temporarily decreased, but were restored to preoperative levels after 5 days (data not shown). This demonstrated that the influence of polyethylene bag placement on weight and appetite was minimal.

Gastric accommodation is reportedly induced by calorie-containing foods and nutrient liquids (Ahluwalia et al., 1996; McLaughlin et al., 1998). Ahluwalia et al. reported a significant decrease in intragastric pressure after a liquid meal alone when saline and a liquid meal were administered together to humans under fasting conditions.

In the present study, when water and 7.5% (0.85 kcal) or 15% (1.7 kcal) liquid meals were administered to guinea pigs a significant decrease in intrabag pressure was observed only after the 15% meal, indicating this liquid meal induced gastric accommodation. We also administered 3.4- or 6.8-kcal liquid meals in a constant volume to determine whether additional calories would decrease intrabag pressure more than the 1.7-kcal liquid meal; no differences were seen (data not shown). Although the intrabag pressure decreased in a volume-dependent manner after 15% liquid meals, the pressure induced by 8 ml was similar to that observed after 4 ml. Therefore, we chose to use the 15% liquid meal (4 ml) to study gastric accommodation.
Atropine reduces gastric tone in fasting dogs (Azpiroz and Malagelada, 1987; Moro et al., 2005). In contrast, the NO synthase inhibitor L-NMMA decreases basal fundic volume and suppresses meal-induced gastric relaxation in humans (Kuiken et al., 2002; Tack et al., 2002). In addition, in rats both choline acetyltransferase- and NO synthase-positive neurons are found in the rostral and caudal dorsal motor nucleus of the vagus nerve (in which vagal preganglionic cell bodies are located), and previous reports have indicated that stimulation of each site with L-glutamate can induce gastric contraction and relaxation (Zhou et al., 2008). Those reports suggest that gastric tone and accommodation are controlled by cholinergic and nitrergic neurons. To evaluate the validity of our experimental model, the effects of atropine and L-NAME were examined. Atropine significantly decreased gastric tone and promoted liquid meal-induced accommodation. The decreasing effect of atropine on gastric tone was consistent with results reported in dogs (Azpiroz and Malagelada, 1987; Moro et al., 2005). In humans, on the other hand, atropine has been reported to have no effect on fasting and postprandial gastric tone (Bruley des Varannes et al., 1995). However, we used atropine at a concentration of 100 μg/kg, whereas the dose used in humans in previous reports was 6 μg/kg/h (Bruley des Varannes et al., 1995). We attribute the inconsistencies in these results to differences in dose. In our model, L-NAME increased gastric tone and completely inhibited liquid meal-induced accommodation. Although NO synthase inhibitors differed among experiments, the results in the present study are consistent with those of previous reports in humans (Kuiken et al., 2002; Tack et al., 2002). On the basis of these results with atropine and L-NAME, we confirmed that cholinergic neurons inhibit gastric accommodation by increasing gastric tone, whereas nitrergic neurons promote gastric accommodation by decreasing gastric tone.

The method has a limitation for the measurement of gastric accommodation. Gastric accommodation in humans usually is quantified by isobaric changes in the volume of the proximal stomach with a barostat, because it is reported that intragastric pressure measurement in fixed-volume distention of the stomach using a balloon distorts the normal pattern of gastric motor activity (Azpiroz and Malagelada, 1985). However, it is not possible to insert a hard barostat tube into conscious animals and measure gastric accommodation by an isobaric method, and it is important to perform the experiments on conscious animals. Although it was necessary to leave the bag in the stomach of the animals before the experiments and the isobaric method could not be performed, our method has the merit of allowing experiments on conscious animals. New methods for examining gastric accommodation in rats and humans were also established by measuring intragastric pressure with a manometer (Janssen et al., 2008, 2011).

AITC is a pungent component of mustard oil and is a TRPA1 agonist (Jordt et al., 2004). In the present study, a significant increase was observed in gastric tone when AITC was orally administered at concentrations 2 mg/kg. In contrast, pretreatment with HC-030031 suppressed this AITC-induced increase. These results indicate that TRPA1 activation increases gastric tone. The AITC-induced increase in gastric tone was inhibited when atropine was coadministered. In addition, when AITC
was administered with L-NAME gastric tone further increased compared with L-NAME alone. These results suggest that the AITC-induced increase in gastric tone is not an effect from nitrergic neurons, but is rather a cholinergic response.

Because TRPA1 is expressed mainly on sensory nerves in the target organ (Story et al., 2003; Penuelas et al., 2007; Kondo et al., 2009), we hypothesize that the AITC-induced increase in gastric tone was a response mediated by TRPA1 expressed on nerves in the proximal stomach. In fact, an immunohistochemical study conducted in rat gastric tissue revealed positive TRPA1 staining in nerve fibers in the mucosa, submucosa, perivascular, and myenteric regions (Kondo et al., 2009). Because TRPA1 expression in the proximal stomach of guinea pigs has not been reported, we attempted immunofluorescent staining; however, the procedure was unsuccessful, perhaps because the antibody used might not cross-react with guinea pig TRPA1. TRPA1 is highly expressed in enterochromaffin cells in the duodenum of rats and humans, and stimulation by AITC causes serotonin release from these cells (Nozawa et al., 2009), resulting in contraction of the small intestine via the serotonin type 3 receptor. We did not examine the involvement of serotonin receptors in the AITC-induced gastric tone increase in this model. Additional studies are needed to clarify the precise mechanisms of action involved, including localization of TRPA1 expression.

AITC significantly inhibited the liquid meal-induced decrease in intrabag pressure, indicating that AITC inhibited gastric accommodation by increasing gastric tone via TRPA1 activation. HC-030031 tended to further promote the decrease in intrabag pressure after liquid meal administration and decreased gastric tone. In addition, TRPA1 has been reported to be activated by mechanical distension (Kwan et al., 2006). Therefore, TRPA1 in the proximal stomach may be activated by a liquid meal or distension, leading to an increase in gastric tone, and in turn, suppressing a decrease in excessive gastric wall tonus by gastric accommodation. On the other hand, TRPV1 is coexpressed on TRPA1 and sensory neurons (Story et al., 2003), and the TRPV1 agonist capsaicin relaxes muscle strips from rat gastric fundus (Lefebvre et al., 1991). Gastric relaxation in an isolated guinea pig stomach is suppressed by capsaicin desensitization (Uno et al., 1997). Furthermore, capsaicin has been reported to decrease gastric tone in the human proximal stomach (Lee et al., 2004). On the basis of the present study, TRPA1 and TRPV1 exert reciprocal regulation on gastric tone under physiological conditions, suggesting that they regulate gastric function.

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known that TRPA1 channels are activated by pungent ex-
tracts. Therefore, TRPA1 may mediate exacerbation of ab-
dominal symptoms by suppression of gastric accommodation
in this pathological condition.
In conclusion, we established a new model for measuring
gastric tone and accommodation in conscious guinea pigs.
TRPA1 activation may suppress gastric accommodation by
increasing gastric tone via cholinergic neuronal pathways.

**Authorship Contributions**

**Participated in research design:** Koseki, Oshima, Hattri, Kase, and Miwa.

**Conducted experiments:** Koseki, Kondo, and Tomita.

**Performed data analysis:** Fukui and Watari.

**Wrote or contributed to the writing of the manuscript:** Koseki, Oshima, and Miwa.

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