Role of bitter taste receptors in regulating gastric accommodation in guinea pigs

Yumi Harada\textsuperscript{1*}, Junichi Koseki\textsuperscript{1}, Hitomi Sekine\textsuperscript{1}, Naoki Fujitsuka\textsuperscript{1}, Hiroyuki Kobayashi\textsuperscript{2}

1. Tsumura Kampo Research Laboratories, Tsumura & Co. (Y.H, J.K, H.S, N.F)
2. Center for Advanced Kampo Medicine and Clinical Research, Juntendo Graduate School of Medicine, Tokyo (H.K.)
Running title: Bitter taste receptors regulate gastric accommodation

*Corresponding author: Yumi Harada Ph.D.

Tsumura Kampo Research Laboratories, Tsumura & Co.

3586 Yoshiwara, Ami-machi, Inashiki-Gun, Ibaraki 300-1192, Japan

Tel.: 81-29- 889- 3855, Fax: 81-29- 889- 2158

E-mail: harada_yumi@mail.tsumura.co.jp

Number of text pages: 36

Number of figures: 5

Number of references: 53

Number of words in the abstract: 193

Number of words in the introduction: 392

Number of words in the discussion: 1233

Abbreviations:

CPA: Change of membrane potential caused by absorption, DB: Denatonium benzoate,
DW: Distilled water, FD: Functional dyspepsia, GA: Gastric accommodation, GI:
Gastrointestinal, IG: Intragastrically, IO: Intra-orally, RKT: Rikkunshito, SEM: Standard
error of the mean, TAS2R: Bitter taste receptor

Recommended section assignment: Gastrointestinal, Hepatic, Pulmonary, and Renal
Abstract

Taste stimulants play important roles in triggering digestion and absorption of nutrients and in toxin detection, under the control of the gut-brain axis. Bitter compounds regulate gut hormone secretion and gastrointestinal motility through bitter taste receptors (TAS2Rs), which are located in the taste buds on the tongue and in the enteroendocrine cells. Gastric accommodation (GA) is an important physiological function. However, the role of TAS2R agonists in regulating GA remains unclear. To clarify whether GA is influenced by bitter stimulants, we examined the effect of TAS2R agonist denatonium benzoate (DB) administered intra-orally and intragastrically, by measuring the consequent intrabag pressure in the proximal stomach of guinea pigs. Effects of the Kampo medicine Rikkunshito (RKT) and its bitter components liquiritigenin and naringenin on GA were also examined. Intra-oral DB (0.2 nmol/mL) administration enhanced GA. Intragastric DB administration (0.1 and 1 nmol/kg) promoted GA, whereas higher DB doses (30 μmol/kg) inhibited it. Similar changes in GA were observed with intragastric (1000 mg/kg) and intra-oral (200 mg/mL) RKT administration. Liquiritigenin and naringenin also promoted GA. These findings suggest that GA is affected by the stimulation of TAS2Rs in the oral cavity or gut in guinea pigs.
Introduction

Human beings can distinguish between five basic taste qualities – sweet, sour, bitter, salty, and umami (Barretto et al., 2015). Each taste is detected by taste receptor cells assembled into taste buds on the tongue. Hence, taste receptors play a prominent role in taste discrimination. Taste stimulants increase gastric acid and gastrin secretion through the oropharyngeal taste receptors by cephalic phase responses and induce the activation of digestive functions (Katschinski, 2000). In addition, taste receptors are also expressed in the gut and regulate appetite and gastrointestinal (GI) functions (Depoortere, 2014). Sensing the luminal content though taste receptors is important to initiate appropriate responses to digestion and absorption of nutrients, which are regulated via the gut-brain axis (Daly et al., 2012; Janssen and Depoortere, 2013). Among the taste receptors, the taste 2 receptor family (TAS2Rs) is involved in the perception of bitter compounds (Chandrashekar et al., 2000). Twenty-five TAS2R subtypes have been identified in humans and are considered to play a role in the detection of toxins. These receptors also affect the secretion of gut hormone and regulate GI motility (Wu et al., 2002; Shi et al., 2003; Chen et al., 2006; Rozengurt and Sternini, 2007; Hao et al., 2008; Jeon et al., 2008; Janssen et al., 2011; Daly et al., 2013).

Gastric accommodation (GA) is an essential physiological function, which allows the
stomach to accommodate large amounts of food by reflex relaxation of the proximal stomach during food intake (Tack et al., 1998; Tack et al., 2002; Kindt and Tack, 2006). However, the association between GA and TAS2Rs expressed in the taste buds or GI tract remains poorly understood. In this study, we determined the effects of intra-oral (i.o.) and intragastric (i.g.) administration of the bitter agonist denatonium benzoate (DB) on GA in guinea pigs. Impaired GAs have been reported to be associated with the relaxation of the fundus and are considered a therapeutic target of functional dyspepsia (FD) (van den Elzen and Boeckxstaens, 2006). The Japanese traditional medicine Rikkunshito (RKT), is used to treat gastric symptoms in FD patients (Tominaga et al., 2018) with GA impairment (Kusunoki et al., 2010; Shiratori et al., 2011; Miwa et al., 2016). It has been reported that some flavonoids in RKT have agonistic activity towards TAS2Rs (Roland et al., 2013). Therefore, the effects of RKT and these flavonoids on GA in guinea pigs were also examined.
Materials and methods

Animals

Four-week-old male Hartley guinea pigs were purchased from Japan SLC, Inc. (Hamamatsu, Japan). During the experimental period following a week of acclimation, they were housed in a regulated environment, with controlled conditions of room temperature (23 ± 3 °C), humidity (50 ± 20%), and lighting (12-h light-dark cycle). Animals were provided with standard laboratory chow and water ad libitum. All experiments were approved by and conducted according to the guidelines of the Experimental Animal Ethics Committee of Tsumura & Co. (Ibaraki, Japan, approved protocol No.: 10-020, 12-008, 13-042)

Test samples

The following test samples were used – TAS2Rs agonist DB (Sigma-Aldrich Co. LLC, St. Louis, MO), Japanese traditional medicine RKT (Tsumura and Co., Tokyo, Japan), liquiritigenin (Wako Pure Chemical Industries, Ltd., Osaka, Japan), and naringenin (Sigma-Aldrich Co. LLC). RKT is a powder extract, which is prepared by extracting a mixture of Glycyrrhiza radix, Zingiberis rhizoma, Atractylodis lancea rhizoma, Zizyphi fructus, Citri unshiu pericarpium, Ginseng radix, Pinelliae tuber and Poria in hot water, followed by spray drying. The quality, efficacy, and safety of the drugs were evaluated by
the Pharmaceuticals and Medical Devices Agency (PMDA) (available at http://www.pmda.go.jp).

**Animal preparation**

Guinea pigs are widely used for studying gastric contraction and motility (Ji et al., 2003; Cellini et al., 2011; Tsai et al., 2018). The methods to evaluate GA in the conscious guinea pig have been previously established (Koseki et al., 2012; Youn et al., 2015; Miwa et al., 2016; Ikeo et al., 2017). Briefly, a balloon-like apparatus, comprising a polyethylene bag (maximum volume 14 mL, thickness 0.01 mm) connected to a polyethylene tube (PE 60; Becton, Dickinson and Company, Franklin Lakes, NJ) was prepared before the surgery. Guinea pigs food-deprived for over 16 h were anesthetized with sodium pentobarbital (30 mg/kg, intraperitoneally, Kyoritsu Seiyaku Corporation, Tokyo, Japan) and were maintained under anesthesia by isoflurane inhalation for about 30 min. After abdominal laparotomy for exposing the stomach, a small incision was made near the distal part of the stomach, and the residual content in the stomach was removed. The polyethylene bag of the balloon-like apparatus was deflated and inserted into the proximal stomach from the distal part and was left in the fundus part. The polyethylene tube was then withdrawn from the inside of the stomach through the incision at the distal part and sutured and fixed together with the incision. The polyethylene tube was then subcutaneously threaded...
through the abdominal wall of the right flank and emerged from the back of the neck. All incisions were closed surgically with sutures. This is illustrated in the report by Y. H et al (Youn et al., 2015). The measurements were performed under conscious conditions for 5 to 28 days after polyethylene bag placement.

**Measurement of liquid meal-induced GA**

GA was measured in conscious animals as described in a previous report (Koseki et al., 2012). During measurement, conscious guinea pigs were placed in a small transparent plastic box. The polyethylene tube emerging from the back of the neck was connected to a pressure transducer (MLT0699; AD Instruments Pty. Ltd., Bella Vista, NSW, Australia) and a syringe pump (KDS-200; KD Scientific Inc., Holliston, MA) to inject 6 mL of air into the bag at a flow rate of 2 mL/min. The intrabag pressure was recorded using a pressure amplifier (BP Amp; AD Instruments Pty. Ltd.) and a data acquisition device (PowerLab 4/26; AD Instruments Pty. Ltd.). The baseline intrabag pressure for 1 min was measured 5 min after the start of air injection. Then, the air was withdrawn from the bag and the animals were allowed to rest for about 10 min. After that, a liquid meal (4 mL, 1.7 kcal) was orally administered using a gavage needle (RZ-2; CLEA Japan, Inc.). The liquid meal consisted of powdered standard laboratory chow (CG-7; CLEA Japan, Inc., Tokyo, Japan [278.1 kcal, 18.1% (w/w) crude protein, 3.4% (w/w) crude fat, and 16.8%
(w/w) crude fiber per 100 g]) suspended in DW at 15% (w/v) using a Polytron homogenizer. Immediately after administration of the liquid meal, 6 mL air was again injected into the bag, and the intrabag pressure for 1 min was recorded every 5 min until 30 min after the start of air injection. The mean change in intrabag pressure (mean Δ intrabag pressure), which is the average of changes in intrabag pressure from the basal level at 5-min intervals, was calculated using LabChart 6 (AD Instruments Pty. Ltd.). In this study, each guinea pig was used for up to three times. When used for multiple measurements, the animals were rested for a minimum of three days between measurements. The baseline intrabag pressure was determined before every measurement and if it was low (<4.0 mmHg/min), the animal was not used. The animals were sacrificed at the end of the study and the integrity and location of the bags were evaluated. Extremely low intrabag pressures were detected when the bag was damaged or dislocated to the distal stomach. The animals with dislocated or damaged bags were excluded from the study.

**Administration of test samples**

DB (0.02 nmol/mL to 60 μmol/mL) was dissolved in DW. RKT (100 or 200 mg/mL: no toxicologically meaningful change was observed in single dose toxicity studies in rats) and its ingredients liquiritigenin (0.2 to 0.4 mg/mL) and naringenin (0.4 to 0.8 mg/mL)
were suspended in DW. DB (0.02 nmol/mL to 2 μmol/mL), RKT, liquiritigenin, or naringenin was administered into the oral cavity (0.5 mL/kg). Fifteen minutes later, the liquid meal was orally administered as described above, in order to induce physiological GA. In addition, DB (0.2 nmol/mL to 60 μmol/mL) or RKT (200 mg/mL) was administered into the stomach (0.5 or 5 mL/kg, respectively) and the liquid meal was orally administered 30 min later.

**Taste measurement**

DB (0.1 nmol/mL to 100 nmol/mL) was dissolved in DW; KCl was added to the solution to a final concentration of 10 mM. RKT (200 mg/mL) and its ingredients liquiritigenin (0.4 mg/mL) and naringenin (0.8 mg/mL) were suspended in DW; KCl was added to the solution to a final concentration of 10 mM. After centrifugation, the supernatant was collected and used as the test samples. The test samples were analyzed for their prominent taste using the taste-sensing system SA402B (Intelligent Sensor Technology, Inc.). This system is equipped with seven artificial lipid membrane sensor probes that can detect the taste factors of anionic bitterness, aftertaste of anionic bitterness, aftertaste of catatonic bitterness 1, aftertaste of catatonic bitterness 2, astringency, aftertaste of astringency, umami, aftertaste of umami, saltiness, and acidity (Table 1). Taste intensity was estimated from the outputs of the artificial lipid membrane sensor probes, which is based on Weber–
Fechner’s law that the intensity of perception is proportional to the logarithm of stimulus intensity (Uchida et al., 2003; Kobayashi et al., 2010; Tahara et al., 2011).

**Statistical analysis**

All data are expressed as mean ± standard error. To assess the differences in intrabag pressure among groups, a Student’s t-test, Dunnett’s test was performed using StatLight (Yukms Co., Ltd., Tokyo, Japan). Values of $p < 0.05$ were considered statistically significant.
Results

Effects of DB administration on GA

The time course of intrabag pressure after liquid meal administration in guinea pigs is shown in Figure 1A. The intrabag pressure in the distilled water (DW)-treated group was temporally decreased 5 or 10 min after liquid meal administration and then gradually increased. In guinea pigs, intra-oral (i.o.) administration of DW or DB decreased the intrabag pressure 5 min after liquid meal administration in all groups (Figure 1A). As shown in Figure 1B, the decrease in the mean change in intrabag pressure was significantly promoted by 0.2 nmol/mL i.o. DB treatment, but not by other doses of DB (0.02 or 2 nmol/mL, i.o.), compared to the DW-treatment group (DW: -1.34 ± 0.20 mmHg, DB 0.2 nmol/mL: -2.62 ± 0.29; p<0.01 by Dunnett’s test).

A decrease in intrabag pressure was also observed in the guinea pigs that received intragastric DB (Figure 2A). As shown in Figure 2B, the mean change in intrabag pressure, which is calculated as the change in intrabag pressure from the basal level, significantly decreased in the intragastric (i.g.) DB (0.1 and 1 nmol/kg)-treated group, compared to the DW-treated group (DW: -1.85 ± 0.15 mmHg, DB 0.1 nmol/kg: -2.66 ± 0.18 mmHg, p<0.05, DB 1 nmol/kg: -2.85 ± 0.23 mmHg, p<0.01 by Dunnett’s test). These results indicate that GA was enhanced by intragastric administration of the potent TAS2Rs
agonist, DB in guinea pigs. However, the effect was attenuated at a higher DB dose (10 nmol/kg, i.g.). The treatment with DB at higher doses (30 μmol/kg, i.g.) significantly suppressed the decrease in the mean change in intrabag pressure (DW: -1.80 ± 0.18 mmHg, DB: -1.17 ± 0.21, p<0.05 by Student’s t-test) (Figure 2C).

**Effects of RKT and its flavonoids on GA**

Intra-oral administration of RKT (200 mg/mL) in guinea pigs significantly enhanced the mean change in intrabag pressure, compared to DW administration (DW: -1.26 ± 0.13 mmHg, RKT: -2.46 ± 0.32, p<0.01 by Dunnett’s test) (Figure 3A). Additionally, a promoting effect was also observed by i.g. administration of RKT at a dose of 1000 mg/kg (DW: -1.08 ± 0.16 mmHg, RKT: -2.01 ± 0.29, p<0.05 by Student’s t-test) (Figure 3B).

The effects of liquiritigenin and naringenin, which are components of RKT and have TAS2R-agonistic activity, on the mean change in intrabag pressure after liquid meal administration were assessed. Intra-oral administration of 0.4 mg/mL liquiritigenin significantly promoted the decrease in the mean change in intrabag pressure (DW: -1.23 ± 0.12 mmHg, liquiritigenin: -1.89 ± 0.13; p<0.01 by Dunnett’s test) (Figure 4A). Intra-oral administration of 0.8 mg/mL naringenin significantly promoted the decrease in the mean change in intrabag pressure (DW: -1.29 ± 0.22 mmHg, naringenin: -2.34 ± 0.28, p<0.05 by Dunnett’s test) (Figure 4B).
Taste patterns of test samples

Ten taste factors of the test samples were measured using a taste-sensing system. As shown in Figure 5, the aftertastes of cationic bitterness 1 and cationic bitterness 2 were detected in DB solution (10 and 100 nmol/mL). The taste intensity increased in a dose-dependent manner. Other taste factors were not detectable. As shown in Table 2, the taste intensity of anionic bitterness was the highest of the ten taste factors in liquiritigenin (0.4 mg/mL) and naringenin (0.8 mg/mL) solutions. In contrast, almost all taste factors were detected in the RKT (200 mg/mL) solution. Among them, a remarkable increase in the aftertaste of cationic bitterness 2 was observed.
Discussion

We found that the i.o. or i.g. administration of the bitter compound DB promoted GA in guinea pigs. DB is indicated to bind to eight of the TAS2R subtypes and has TAS2Rs agonist activities in human (Meyerhof et al., 2010). TAS2Rs are expressed in taste receptor cells assembled into taste buds on the tongue, enteroendocrine cells, and smooth muscle cells of the GI tract. Several studies have demonstrated that TAS2Rs are co-expressed with the signal element taste-specific G-protein gustducin, and involved in regulating gut hormone release and GI motility in humans and animals (Wu et al., 2002; Shi et al., 2003; Chen et al., 2006; Rozengurt and Sternini, 2007; Hao et al., 2008; Jeon et al., 2008; Janssen et al., 2011; Daly et al., 2013). Therefore, the GA-promoting effect of DB could be mediated by a gut hormone or a direct action on smooth muscle cells in guinea pigs.

The present study showed that i.o. administration of DB-induced GA-promotion in guinea pigs. Taste stimulation of taste buds on the tongue simultaneously activates GI motility and stimulate the secretion of gastric acids, gastrin, and pancreatic polypeptide 2. Recent evidence suggests that bitter-tasting plants elicit cephalic responses through the oropharyngeal TAS2Rs and facilitate digestive activity (McMullen et al., 2015). Moreover, it has been demonstrated that G-protein gustducin, which is an important first
signal transmitter of taste signals to the brain (Behrens and Meyerhof, 2011), is
extensively co-expressed with TAS2Rs in the soft palate and fungiform papillae on the
tongue (Tomonari et al., 2012), and that motilin secreted from the thyroid into the
peripheral plasma by paraventricular nucleus stimulation affects gastric motility (Guo et
al., 2011). Motilin is a gut hormone released from the endocrine cells of the duodenal and
jejunal mucosa as a regulator of gastrointestinal motility (Guo et al., 2011). Motilin
infusion caused a contraction of the proximal stomach in humans, resulting in the
inhibition of GA induced by satiety drinking (Cuomo et al., 2006). It has also been
reported that taste stimulation accelerates the efferent activity of the gastric branch of the
vagus nerve (Niijima, 1991; Niot and Besnard, 2017). Therefore, the promoting effect of
DB on GA in guinea pigs could probably be involved in regulating motilin secretion or a
direct action on smooth muscles through the cephalic phase responses.

In healthy female volunteers, i.g. administration of DB inhibited the increase in plasma
motilin levels after an overnight fast and reduced the fluctuation of antral motility
(Deloose et al., 2017; Deloose et al., 2018). Therefore, it was suggested that the promoting
effect of DB on GA was also mediated by the decreased release of motilin through
TAS2Rs in the gut. In addition, cellular studies indicated that the activation of TAS2Rs
with DB induced the secretion of glucagon-like peptide-1 (GLP-1) from enteroendocrine
cells (Kim et al., 2014). The increase in GLP-1 secretion by DB was also observed in db/db mice (van Avesaat et al., 2015). GLP-1 causes a reduction in cholinergic contractions in the GI smooth muscles by acting on the enteric nervous system in the mouse intestine (Amato et al., 2010; Rotondo et al., 2011). These findings suggest that the enhancement of GA by i.g. administration of DB in guinea pigs might be mediated by the regulation of GLP-1 release from the enteroendocrine cells.

On the contrary, i.o. and i.g. treatment with DB at a higher dose did not promote GA. Furthermore, i.g. treatment with DB at a higher dose (30 μmol/kg) significantly suppressed GA in guinea pigs in this study. It was reported that thresholds for the bitter taste in foods can be greater than, equal to, or less than those for toxicity and that the bitter rejection response is maladaptive for omnivores or herbivores because it would cause them to limit drastically the range of potential foods (Glendinning, 1994). Therefore, the bitter substance can be rejected at high concentrations above the threshold. It was also demonstrated that DB-induced contraction in mouse gastric fundic smooth muscle strips by an increase in intracellular calcium concentration in smooth muscle cells (Avau et al., 2015). Furthermore, i.g. DB (1 μmol/kg) administration impaired the relaxation of the fundus in response to nutrient infusion and increased the satiation in healthy volunteers (Avau et al., 2015). These reports are similar to the effect of i.g. treatment with DB at a
higher dose (30 μmol/kg) on GA in guinea pigs, although the sensitivity to bitter stimulants seems to be different between humans and guinea pigs. TAS2R subtypes TAS2R5, 10, and 14 reportedly have a predominant role in inducing relaxation of smooth muscle cells (Grassin-Delyle et al., 2013). DB has binding activities against TAS2R10 (Meyerhof et al., 2010). These findings suggest that the optimal dose of DB for binding TAS2Rs might differ between TAS2R subtypes. Hence, low dose DB-induced stimulation of TAS2Rs may have enhanced GA in guinea pigs, which is different from the effect of high dose DB administration. Therefore, it may be considered that DB regulated GA by several mechanisms associated with TAS2Rs. In addition, it may be important to evaluate gastric emptying following GA. Bert Avau et al. has demonstrated, that intragastric administration of DB (60 μmol/kg, PO) inhibited gastric emptying rate (GE) in mice (Avau et al., 2015). TAS2R stimulation is also thought to affect GE. However, it is unclear whether the regulation of GA by DB is involved in the suppression of GE.

Several studies have demonstrated that RKT enhances GA in humans and animals. In a study of GA reflex in FD patients using extracorporeal ultrasonography, the expansion rate of the proximal stomach after ingestion of a liquid meal was increased by RKT treatment (Kusunoki et al., 2010). The proximal gastric volume increased immediately after liquid infusion in conscious dogs, and the effect of RKT was significantly greater
than that of water (Furukawa et al., 2013). In this study, i.o. and i.g. RKT-treated guinea pigs exhibited a potentiated physiological GA. Furthermore, the enhancement of GA in guinea pigs was observed after i.o. administration of the components of RKT, liquiritigenin, and naringenin. These components reportedly have agonistic effects on TAS2R14 and TAS2R39 (Rouzade et al., 1998), suggesting that RKT could regulate GA by stimulating TAS2R signaling in guinea pigs.

Recent evidence has suggested that regulation of gastrointestinal function may also be affected by the taste sensory system (Loper et al., 2015). Taste stimulants except for the bitter taste also regulate gut hormone release and GI motility (Lavin et al., 2002; Kendig et al., 2014). The assessment of taste function using a taste-sensing system demonstrated that liquiritigenin and naringenin showed the highest intensity to bitterness among all tastes. However, the type of bitterness of these components was different from that of DB, suggesting that different TAS2R subtypes were involved. These results indicated that liquiritigenin and naringenin specifically stimulated the TAS2Rs and that other taste receptors may not be involved in these effects on GA in guinea pigs. Our results suggest that TAS2R may be involved in the regulation of gastrointestinal motility. We speculate that the modulation of TAS2R activity could be a novel therapeutic approach for functional gastrointestinal disorders such as FD, gastroesophageal reflux disease, and
non-erosive reflux disease.

In conclusion, the bitter stimulants regulated GA in guinea pigs, which could be mediated by TAS2R activation in the oral cavity and gut. However, the mechanisms of GA activation mediated through the cephalic phase responses or gut hormone are not well understood and need further investigation.
Acknowledgments

We appreciate Dr. H Miwa for the guidance on the gastric accommodation evaluation system using guinea pigs and for his helpful technical advice.
Authorship contributions

Participated in research design: Y Harada, N Fujitsuka

Conducted experiments: Y Harada, J Koseki, H Sekine

Performed data analysis: Y Harada, N Fujitsuka

Wrote or contributed to the writing of the manuscript: Y Harada, N Fujitsuka, H Kobayashi
References

Amato A, Cinci L, Rotondo A, Serio R, Faussone-Pellegrini MS, Vannucchi MG and Mule F (2010) Peripheral motor action of glucagon-like peptide-1 through enteric neuronal receptors. Neurogastroenterol Motil 22:664-e203.

Avau B, Rotondo A, Thijs T, Andrews CN, Janssen P, Tack J and Depoortere I (2015) Targeting extra-oral bitter taste receptors modulates gastrointestinal motility with effects on satiation. Sci Rep 5:15985.

Barretto RP, Gillis-Smith S, Chandrashekar J, Yarmolinsky DA, Schnitzer MJ, Ryba NJ and Zuker CS (2015) The neural representation of taste quality at the periphery. Nature 517:373-376.

Behrens M and Meyerhof W (2011) Gustatory and extragustatory functions of mammalian taste receptors. Physiol Behav 105:4-13.

Cellini J, DiNovo K, Harlow J and LePard KJ (2011) Regional differences in neostigmine-induced contraction and relaxation of stomach from diabetic guinea pig. Auton Neurosci 160:69-81.

Chandrashekar J, Mueller KL, Hoon MA, Adler E, Feng L, Guo W, Zuker CS and Ryba NJ (2000) T2Rs function as bitter taste receptors. Cell 100:703-711.

Chen MC, Wu SV, Reeve JR, Jr. and Rozengurt E (2006) Bitter stimuli induce Ca2+
signaling and CCK release in enteroendocrine STC-1 cells: role of L-type voltage-sensitive Ca2+ channels. *Am J Physiol Cell Physiol* **291**:C726-739.

Cuomo R, Vandaele P, Coulie B, Peeters T, Depoortere I, Janssens J and Tack J (2006) Influence of motilin on gastric fundus tone and on meal-induced satiety in man: role of cholinergic pathways. *Am J Gastroenterol* **101**:804-811.

Daly K, Al-Rammahi M, Arora DK, Moran AW, Proudman CJ, Ninomiya Y and Shirazi-Beechey SP (2012) Expression of sweet receptor components in equine small intestine: relevance to intestinal glucose transport. *Am J Physiol Regul Integr Comp Physiol* **303**:R199-208.

Daly K, Al-Rammahi M, Moran A, Marcello M, Ninomiya Y and Shirazi-Beechey SP (2013) Sensing of amino acids by the gut-expressed taste receptor T1R1-T1R3 stimulates CCK secretion. *Am J Physiol Gastrointest Liver Physiol* **304**:G271-282.

Deloose E, Corsetti M, Van Oudenhove L, Depoortere I and Tack J (2018) Intragastric infusion of the bitter tastant quinine suppresses hormone release and antral motility during the fasting state in healthy female volunteers. *Neurogastroenterol Motil* **30**.

Deloose E, Janssen P, Corsetti M, Biesiekierski J, Masuy I, Rotondo A, Van Oudenhove L, Depoortere I and Tack J (2017) Intragastric infusion of denatonium benzoate
attenuates interdigestive gastric motility and hunger scores in healthy female volunteers. *Am J Clin Nutr* **105**:580-588.

Depoortere I (2014) Taste receptors of the gut: emerging roles in health and disease. *Gut* **63**:179-190.

Furukawa N, Manabe N, Kase Y, Hattori T, Imamura H, Kusunoki H and Haruma K (2013) Intragastric infusion of rikkunshito (kampo) induces proximal stomach relaxation in conscious dogs. *Auton Neurosci* **179**:14-22.

Glendinning JI (1994) Is the bitter rejection response always adaptive? *Physiol Behav* **56**:1217-1227.

Grassin-Delyle S, Abrial C, Fayad-Kobeissi S, Brollo M, Faisy C, Alvarez JC, Naline E and Devillier P (2013) The expression and relaxant effect of bitter taste receptors in human bronchi. *Respir Res* **14**:134.

Guo F, Xu L, Sun X, Gao S and Zhu H (2011) The paraventricular nucleus modulates thyroidal motilin release and rat gastric motility. *J Neuroendocrinol* **23**:767-777.

Hao S, Sternini C and Raybould HE (2008) Role of CCK1 and Y2 receptors in activation of hindbrain neurons induced by intragastric administration of bitter taste receptor ligands. *Am J Physiol Regul Integr Comp Physiol* **294**:R33-38.

Ikeo K, Oshima T, Sei H, Kondo T, Fukui H, Watari J and Miwa H (2017) Acotiamide
improves stress-induced impaired gastric accommodation. *Neurogastroenterol Motil* **29**.

Janssen S and Depoortere I (2013) Nutrient sensing in the gut: new roads to therapeutics? *Trends Endocrinol Metab* **24**:92-100.

Janssen S, Laermans J, Verhulst PJ, Thijs T, Tack J and Depoortere I (2011) Bitter taste receptors and alpha-gustducin regulate the secretion of ghrelin with functional effects on food intake and gastric emptying. *Proc Natl Acad Sci U S A* **108**:2094-2099.

Jeon TI, Zhu B, Larson JL and Osborne TF (2008) SREBP-2 regulates gut peptide secretion through intestinal bitter taste receptor signaling in mice. *J Clin Invest* **118**:3693-3700.

Ji SW, Park HJ, Cho JS, Lim JH and Lee SI (2003) Investigation into the effects of mosapride on motility of Guinea pig stomach, ileum, and colon. *Yonsei Med J* **44**:653-664.

Katschinski M (2000) Nutritional implications of cephalic phase gastrointestinal responses. *Appetite* **34**:189-196.

Kendig DM, Hurst NR, Bradley ZL, Mahavadi S, Kuemmerle JF, Lyall V, DeSimone J, Murthy KS and Grider JR (2014) Activation of the umami taste receptor
(T1R1/T1R3) initiates the peristaltic reflex and pellet propulsion in the distal colon. *Am J Physiol Gastrointest Liver Physiol* **307**:G1100-1107.

Kim KS, Egan JM and Jang HJ (2014) Denatonium induces secretion of glucagon-like peptide-1 through activation of bitter taste receptor pathways. *Diabetologia* **57**:2117-2125.

Kindt S and Tack J (2006) Impaired gastric accommodation and its role in dyspepsia. *Gut* **55**:1685-1691.

Kobayashi Y, Habara M, Ikezazki H, Chen R, Naito Y and Toko K (2010) Advanced taste sensors based on artificial lipids with global selectivity to basic taste qualities and high correlation to sensory scores. *Sensors (Basel)* **10**:3411-3443.

Koseki J, Oshima T, Kondo T, Tomita T, Fukui H, Watari J, Hattori T, Kase Y and Miwa H (2012) Role of transient receptor potential ankyrin 1 in gastric accommodation in conscious guinea pigs. *J Pharmacol Exp Ther* **341**:205-212.

Kusunoki H, Haruma K, Hata J, Ishii M, Kamada T, Yamashita N, Honda K, Inoue K, Imamura H, Manabe N, Shiotani A and Tsunoda T (2010) Efficacy of Rikkunshito, a traditional Japanese medicine (Kampo), in treating functional dyspepsia. *Intern Med* **49**:2195-2202.

Lavin JH, French SJ and Read NW (2002) Comparison of oral and gastric administration
of sucrose and maltose on gastric emptying rate and appetite. *Int J Obes Relat Metab Disord* **26**:80-86.

Loper HB, La Sala M, Dotson C and Steinle N (2015) Taste perception, associated hormonal modulation, and nutrient intake. *Nutr Rev* **73**:83-91.

McMullen MK, Whitehouse JM and Towell A (2015) Bitters: Time for a New Paradigm. *Evid Based Complement Alternat Med* **2015**:670504.

Meyerhof W, Batram C, Kuhn C, Brockhoff A, Chudoba E, Bufe B, Appendino G and Behrens M (2010) The molecular receptive ranges of human TAS2R bitter taste receptors. *Chem Senses* **35**:157-170.

Miwa H, Koseki J, Oshima T, Hattori T, Kase Y, Kondo T, Fukui H, Tomita T, Ohda Y and Watari J (2016) Impairment of gastric accommodation induced by water-avoidance stress is mediated by 5-HT2B receptors. *Neurogastroenterol Motil* **28**:765-778.

Niijima A (1991) Effects of oral and intestinal stimulation with umami substance on gastric vagus activity. *Physiol Behav* **49**:1025-1028.

Niot I and Besnard P (2017) Appetite control by the tongue-gut axis and evaluation of the role of CD36/SR-B2. *Biochimie* **136**:27-32.
drug review form. Update November 2014, available at: https://www.pmda.go.jp

Roland WS, van Buren L, Gruppen H, Driesse M, Gouka RJ, Smit G and Vincken JP (2013) Bitter taste receptor activation by flavonoids and isoflavonoids: modeled structural requirements for activation of hTAS2R14 and hTAS2R39. *J Agric Food Chem* 61:10454-10466.

Rotondo A, Amato A, Lentini L, Baldassano S and Mule F (2011) Glucagon-like peptide-1 relaxes gastric antrum through nitric oxide in mice. *Peptides* 32:60-64.

Rouzade ML, Fioramonti J and Bueno L (1998) Decrease in gastric sensitivity to distension by 5-HT1A receptor agonists in rats. *Dig Dis Sci* 43:2048-2054.

Rozengurt E and Sternini C (2007) Taste receptor signaling in the mammalian gut. *Curr Opin Pharmacol* 7:557-562.

Shi P, Zhang J, Yang H and Zhang YP (2003) Adaptive diversification of bitter taste receptor genes in Mammalian evolution. *Mol Biol Evol* 20:805-814.

Shiratori M, Shoji T, Kanazawa M, Hongo M and Fukudo S (2011) Effect of rikkunshito on gastric sensorimotor function under distention. *Neurogastroenterol Motil* 23:323-329, e155-326.

Tack J, Demedts I, Meulemans A, Schuurkes J and Janssens J (2002) Role of nitric oxide in the gastric accommodation reflex and in meal induced satiety in humans. *Gut*
Tack J, Piessevaux H, Coulie B, Caenepeel P and Janssens J (1998) Role of impaired gastric accommodation to a meal in functional dyspepsia. *Gastroenterology* **115**:1346-1352.

Tahara Y, Ikeda A, Maehara Y, Habara M and Toko K (2011) Development and evaluation of a miniaturized taste sensor chip. *Sensors (Basel)* **11**:9878-9886.

Tominaga K, Sakata Y, Kusunoki H, Odaka T, Sakurai K, Kawamura O, Nagahara A, Takeuchi T, Fujikawa Y, Oshima T, Kato M, Furuta T, Murakami K, Chiba T, Miwa H, Kinoshita Y, Higuchi K, Kusano M, Iwakiri R, Fujimoto K, Tack JF and Arakawa T (2018) Rikkunshito simultaneously improves dyspepsia correlated with anxiety in patients with functional dyspepsia: A randomized clinical trial (the DREAM study). *Neurogastroenterol Motil* **30**:e13319.

Tomonari H, Miura H, Nakayama A, Matsumura E, Ooki M, Ninomiya Y and Harada S (2012) Galpha-gustducin is extensively coexpressed with sweet and bitter taste receptors in both the soft palate and fungiform papillae but has a different functional significance. *Chem Senses* **37**:241-251.

Tsai CC, Tey SL, Lee MC, Liu CW, Su YT and Huang SC (2018) Mechanism of resveratrol-induced relaxation of the guinea pig fundus. *Phytomedicine* **43**:55-59.
Uchida T, Tanigake A, Miyanaga Y, Matsuyama K, Kunitomo M, Kobayashi Y, Ikezaki H and Taniguchi A (2003) Evaluation of the bitterness of antibiotics using a taste sensor. *J Pharm Pharmacol* **55**:1479-1485.

van Avesaat M, Troost FJ, Ripken D, Peters J, Hendriks HF and Mascreel AA (2015) Intraduodenal infusion of a combination of tastants decreases food intake in humans. *Am J Clin Nutr* **102**:729-735.

van den Elzen BD and Boeckxstaens GE (2006) Review article: a critical view on impaired accommodation as therapeutic target for functional dyspepsia. *Aliment Pharmacol Ther* **23**:1499-1510.

Wu SV, Rozengurt N, Yang M, Young SH, Sinnett-Smith J and Rozengurt E (2002) Expression of bitter taste receptors of the T2R family in the gastrointestinal tract and enteroendocrine STC-1 cells. *Proc Natl Acad Sci U S A* **99**:2392-2397.

Youn YH, Choi EJ, Lee YH, Oshima T, Miwa H and Park H (2015) The effects of 5-hydroxytryptamine1a receptor agonist, buspirone on the gastric fundus accommodation in an animal model using guinea pigs. *Neurogastroenterol Motil* **27**:532-541.
Footnotes

H.K received a research grant from Tsumura & Co.

Y.H, J.K, H.S, and N.F are employed by Tsumura & Co.

All authors declare that they have no conflict of interests.
Figure legends

**Figure 1.** Influence of intra-oral administration of denatonium benzoate (DB) on gastric accommodation in guinea pigs. (A) Time course of intrabag pressure after liquid meal administration in guinea pigs. (B) The mean Δ intrabag pressure was decreased by DB (0.2 nmol/mL) administration, compared to that in the DW-treated group. Data are expressed as mean ± standard error (n = 5-7). **p<0.01 (B: Dunnett’s test).

**Figure 2.** Influence of intragastric administration of denatonium benzoate (DB) on gastric accommodation in guinea pigs. (A) Time course of intrabag pressure after liquid meal administration in guinea pigs. (B) The mean Δ intrabag pressure (the changes in intrabag pressure from basal level) was decreased by DB (0.1 and 1 nmol/kg) administration, compared to that in the DW-treated group. (C) The treatment with DB (30 μmol/kg) suppressed the decrease in the mean Δ intrabag pressure. Data are expressed as mean ± standard error (n = 5). *p<0.05, **p<0.01 (B: Dunnett’s test, C: Student’s t-test).

**Figure 3.** Influence of Rikkunshito (RKT) on gastric accommodation in guinea pigs. Intra-oral (A) and intragastric (B) administration of RKT in guinea pigs enhanced the decrease in the mean Δ intrabag pressure. Data are expressed as mean ± standard error (n
Figure 4. Influence of the ingredients of Rikkunshito (RKT) on gastric accommodation in guinea pigs. Intra-oral administration of liquiritigenin (A) and naringenin (B) in guinea pigs enhanced the decrease in the mean Δ intrabag pressure. Data are expressed as mean ± standard error (n =6-7). *p<0.05, **p<0.01 (Dunnett’s test).

Figure 5. Effect of denatonium benzoate on several taste factors in the taste-sensing system. The aftertastes of cationic bitterness 1 and cationic bitterness were detected in DB solution (10 and 100 nmol/mL). Other taste factors were not detected.
### Table 1. Characteristics of taste information on taste sensors

| Sensor probes | Taste factor                                      |
|---------------|--------------------------------------------------|
| C00           | Anionic bitterness                               |
|               | Aftertaste of anionic bitterness<sup>a</sup>     |
| AC0           | Aftertaste of cationic bitterness 1<sup>a</sup>  |
| AN0           | Aftertaste of cationic bitterness 2 (mineral)<sup>a</sup> |
| AE1           | Astringency                                      |
|               | Aftertaste of astringency<sup>a</sup>            |
| AAE           | Umami                                            |
|               | Aftertaste of umami<sup>a</sup>                  |
| CT0           | Saltiness                                        |
| CA0           | Acidity                                          |

<sup>a</sup> Converted from change of membrane potential caused by absorption (CPA) values of each sensor probe; others converted from relative potentials.
| Taste factors | Taste intensities of each taste factor (mean ± SEM) |
|---------------|---------------------------------------------------|
|               | RKT (200 mg/mL) | Liquiritigenin (0.4 mg/mL) | Naringenin (0.8 mg/mL) |
| Anionic bitterness | 5.77 ± 0.05 | 1.57 ± 0.07 | 1.41 ± 0.15 |
| Aftertaste of anionic bitterness | 5.30 ± 0.06 | 0.33 ± 0.00 | 0.82 ± 0.03 |
| Aftertaste of cationic bitterness 1 | 7.32 ± 0.18 | -0.16 ± 0.06 | -0.27 ± 0.13 |
| Aftertaste of cationic bitterness (Mineral) | 64.41 ± 0.57 | -0.41 ± 0.12 | -0.61 ± 0.41 |
| Astringency | 5.96 ± 0.15 | 0.44 ± 0.01 | 0.25 ± 0.01 |
| Aftertaste of astringency | 3.28 ± 0.07 | 0.33 ± 0.01 | 0.22 ± 0.01 |
| Umami | 2.71 ± 0.15 | 0.12 ± 0.01 | 0.13 ± 0.04 |
| Aftertaste of umami | 5.19 ± 0.16 | 0.14 ± 0.01 | 0.16 ± 0.01 |
| Saltiness | 10.78 ± 0.24 | 0.07 ± 0.01 | -0.01 ± 0.01 |
| Acidity | 0.20 ± 0.24 | -0.27 ± 0.01 | -0.48 ± 0.09 |
Figure 1
Figure 2
Figure 3

**Mean ∆ intrabag pressure (mmHg)**

A

- DW 100 200

RKT (mg/mL, i.o.)

B

- DW 1000

RKT (mg/kg, i.g.)

- Mean ∆ intrabag pressure (mmHg)
- Mean ∆ intrabag pressure (mmHg)

**Note:** This article has not been copyedited and formatted. The final version may differ from this version.
Figure 4

Panel A
Mean Δintrabag pressure (mmHg)

Panel B
Mean Δintrabag pressure (mmHg)

-3.0
-2.5
-2.0
-1.5
-1.0
-0.5
0.0

Liquiritigenin (mg/mL, i.o.)
0.2
0.4

Naringenin (mg/mL, i.o.)
0.4
0.8

* **
Figure 5