Regular Article
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Denatonium and 6-n-Propyl-2-thiouracil, Agonists of Bitter Taste Receptors, Inhibit Contraction of Various Types of Smooth Muscles in the Rat and Mouse

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Received May 19, 2015; accepted November 4, 2015; advance publication released online November 14, 2015

Recently the global expression of taste 2 receptors (TAS2Rs) on smooth muscle cells in human airways was demonstrated. Here, the effects of agonists of taste receptor, type 2, denatonium and 6-n-propyl-2-thiouracil, on smooth-muscle contraction were examined in the rat and mouse. Contractions induced by carbachol (CCh), high K+, and sodium fluoride, but not calcycin-A, were inhibited significantly in the presence of a TAS2R agonist in the bronchial smooth muscle of mice. The contraction induced by CCh was inhibited by TAS2R agonists in ileal smooth muscle. Phenylephrine-induced contraction was also inhibited by TAS2R agonists in aortic smooth muscle. Gastrointestinal motility and blood pressure were attenuated by administration of TAS2R agonists in vivo. These findings suggest that TAS2R may be a receptor for endogenous biologically active substances as well as for bitter tastes on the tongue. TAS2R signaling could be employed in the development of anti-asthmatic, anti-spasmodic, and anti-hypertensive drugs.

Key words  bitter-taste type-2 receptor; bronchus; ileum; aorta; smooth muscle contraction

Bitter-taste type-2 receptors (TAS2Rs) are G protein-coupled receptors (GPCRs) on cell surfaces that mediate perception of gustatory taste on the tongue. For a long time, TAS2Rs were thought to be expressed only in the specialized epithelial cells in the taste buds of the tongue that allow organisms to avoid harmful toxins and noxious substances characterized by bitterness. Recently, TAS2Rs were found in the smooth muscle of human airways, and found to mediate tracheal relaxation in mice. Stimulation of TAS2Rs increases signaling of intracellular calcium ions (Ca2+), which has been suggested to activate large-conductance potassium channels and result in hyperpolarization of cell membranes. However, how TAS2Rs cause airway relaxation is not known. Nevertheless, TAS2R agonists have shown greater relaxation and inhibition of airway hyperresponsiveness than a β2-adrenoceptor agonist in mice. Given the large selection of known natural and synthetic agonists recognized by 25 TAS2Rs, studies have suggested that bitter-taste receptors could be new targets for asthma pharmacotherapy. Recent studies have revealed that smooth-muscle contraction is mediated by an increase in intracellular Ca2+, i.e., [Ca2+]i, that initiates a cascade of mechanisms using calmodulin, myosin light chain (MLC) kinase (MLCK), and phosphorylation of regulatory MLC to induce contractions. For agonist-induced contractions, this action is followed by a decrease in the activity of myosin light chain phosphatase (MLCP) and a resultant further increase in MLC phosphorylation, which induces further contractions. Reagents that induce the relaxation of smooth muscle are used as anti-asthmatic, anti-spasmodic, and anti-hypertensive drugs.

Of the 35 known mouse TAS2Rs, ligands have been identified for taste receptor, type 2, member 108 (TAS2R108): denatonium and 6-n-propyl-2-thiouracil (PTU). Here, we investigated the gene expression of TAS2R108 and gustducin (Ggust) in multiple tissues, as well as the effect of exogenous bitter-taste receptor ligands for TAS2R on various agonist-induced contractions in rat bronchial smooth muscle and various types of smooth muscle in mice. We also examined the effect of administration of denatonium and PTU on the gastrointestinal motility and blood pressure of mice in vivo.

MATERIALS AND METHODS

Animals  Male Wistar rats (6 weeks; specific pathogen-free) and C57BL/6J mice (7–8 weeks; specific pathogen-free) were used. All experiments were done according to theGuiding Principles for the Care and Use of Laboratory Animals approved by the Animal Care Committee of Hoshi University (Tokyo, Japan).

Functional Studies of Various Types of Smooth Muscles  Animals were killed by exsanguination from the abdominal aorta under anesthesia. The main left bronchus, ileum and thoracic aorta were isolated. Resultant tissue preparations (rings) were suspended in a 5-mL organ bath at a resting tension (in g force) of rat bronchi of 1.0, murine bronchi of 0.4, murine ileum of 0.4, and murine thoracic aorta of 0.8. Isometric contraction of circular smooth muscle was measured with a Force–Displacement Transducer (TB-612T; Nihon Kohden, Tokyo, Japan). The organ bath contained modified Krebs–Henseleit solution with the following composition (mM): NaCl, 118.0; KCl, 4.7; CaCl2, 2.5; MgCl2, 1.2; NaHCO3, 25.0; KH2PO4, 1.2; glucose, 10.0 (pH 7.4). The buffer solution was maintained at 37°C and oxygenated with a mixture of 95% O2 and 5% CO2. During an equilibration period in the organ bath, tissues were washed four times at 15-min intervals. In bronchial smooth muscle, tissues were treated with a TAS2R agonist (denatonium benzoate or PTU) for 20 min before application.
tion of carbachol (CCh; 3 nM to 100 µM), calyculin-A (1 µM), high K+ (10–60 mM) or sodium fluoride (NaF; 20 mM). Development of force by constrictors (except CCh) was observed in the presence of atropine (1 µM) and indomethacin (1 µM). In other experiments, a TAS2R agonist was applied cumulatively to strips after the contraction induced by 10 µM CCh reached a plateau. In the case of a combination of a TAS2R agonist and isoprenaline, the TAS2R agonist (10 µM) was applied to bronchial strips after the contraction induced by 0.3 µM CCh reached a plateau, and then isoprenaline (10 nM to 1 µM) was applied cumulatively to strips. In ileal smooth muscle, tissues were treated with a TAS2R agonist (denatonium benzoate or PTU) for 20 min before application of CCh (1 µM). Levels of tension obtained with normal Krebs–Henseleit solution (basal tension) and the contraction induced by 1 µM CCh was assigned to be 0 and 100%, respectively. In aortic smooth muscle, a TAS2R agonist was applied cumulatively to strips after the contraction induced by 1 µM phenylephrine reached a plateau. In the case of pre-treatment with a TAS2R agonist, tissues were treated with a TAS2R agonist (denatonium benzoate or PTU) for 20 min before application of 1 µM phenylephrine. Levels of tension obtained with normal Krebs–Henseleit solution (basal tension) and the contraction induced by 1 µM phenylephrine was assigned to be 0% and 100%, respectively.

Protein Extraction Homogenates of tissue were prepared by the method described previously with minor modifications. In brief, the murine bronchi, ileum and thoracic aorta tissue was equilibrated in oxygenated Krebs–Henseleit solution (37°C) for 60 min with 15-min washout intervals. After the equilibration period, the bronchial, ileal and aortae segments were stimulated by an indicated concentration of CCh (37°C) for 60 min with 15-min washout intervals. After the contraction induced by 10 µM CCh or PTU) for 20 min before application of CCh (1 µM). Levels of tension obtained with normal Krebs–Henseleit solution (basal tension) and the contraction induced by 1 µM CCh as assigned to be 0 and 100%, respectively. In aortic smooth muscle, a TAS2R agonist was applied cumulatively to strips after the contraction induced by 1 µM phenylephrine reached a plateau. In the case of pre-treatment with a TAS2R agonist, tissues were treated with a TAS2R agonist (denatonium benzoate or PTU) for 20 min before application of 1 µM phenylephrine. Levels of tension obtained with normal Krebs–Henseleit solution (basal tension) and the contraction induced by 1 µM phenylephrine was assigned to be 0% and 100%, respectively.

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Immunoblotting To quantify the phosphorylated MLC, Western blot was performed. In brief, the samples (10 µg of total protein per lane) were subjected to 5–15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The proteins were then electrophoretically transferred to a polyvinylidene difluoride (PVDF) membrane. Transferred PVDF membranes were incubated in primary antibodies, either rabbit anti-pSer19-MLC (1:1000 dilution; Cell Signaling Technology, Beverly, MA, U.S.A.). Then the membranes were incubated with horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin (IgG) (1:3000 dilution; GE Healthcare, Buckinghamshire, U.K.); the detection was done by an enhanced chemiluminescence (ECL) system. To normalize the p-MLC contents to MLC, immunoblotting was also performed after stripping antibodies on the same gel by using rabbit anti-MLC and then incubated horseradish peroxidase-conjugated goat anti-rabbit IgG (GE Healthcare). The ratio of corresponding p-MLC was calculated as an index of p-MLC.

Analyses by Reverse Transcription-Polymerase Chain Reaction (RT-PCR) Tissues (tongue, bronchi, ileum, colon, thoracic aorta) were removed from mice, washed with cold physiologic (0.9%) saline, and stored in TRI Reagent™ (Sigma-Aldrich, Saint Louis, MO, U.S.A.) at −80°C. Total RNA was removed from various tissues with a one-step guanidinium–phenol–chloroform extraction procedure using TRI Reagent. cDNAs were prepared from total RNA (1.0 µg) using QuantiTect Reverse Transcriptase (Qiagen, Hilden, Germany) after incubation with gDNA Wipeout Buffer at 42°C for 3 min to remove contaminating genomic DNA. To the RT reaction mixture (10 µL) were added 1 µL of 10 µM forward primer, 1 µL of 10 µM reverse primer, and 5 µL of 2X PCR Master Mix (0.05 U/mL Taq DNA polymerase, 4 mM MgCl2, and 0.4 mM of each deoxyribonucleotide triphosphate (dNTP); Fermentas Life Science, Waltham, MA, U.S.A.) in a total volume of 20 µL. RT-generated cDNAs encoding gene sequences were amplified by PCR using specific primers (Table 1), which were designed from published sequences. The thermal cycle was denaturing at 95°C for 3 min; annealing primers at 55°C for 15 s; extending primers for 1 min at 72°C. PCR amplifications were undertaken for 35 cycles. PCR mixtures were subjected to electrophoresis on 1% agarose gel with a DNA molecular weight standard marker (100-bp DNA ladder; TaKaRa Bio, Shiga, Japan) and visualized by staining with ethidium bromide.

Measurement of Gastrointestinal Motility Twenty minutes after administration of denatonium (3 and 10 mg/kg, intraperitoneally [i.p.]), PTU (10 and 30 mg/kg, i.p.) and their vehicle (saline), 0.1 mL/kg Fountain Pen Ink (INK-30; Pilot Pen, Jacksonville, FL, U.S.A.) was administered into the stomach by orogastric gavage. Twenty-minutes later, mice were killed, the abdomen opened, and the small intestine dissected. Total length of the small intestine (pylorus–cecum) and the distance traveled by carbon ink were measured. Results were expressed as the percentage of the distance traveled by carbon ink to the total length of the small intestine.

Measurement of Blood Pressure Twenty minutes after

Table 1. Primers Used in This Study

| Accession number | Primer deoxyribonucleotide | Product size (base pairs) |
|------------------|---------------------------|--------------------------|
| Taste receptor, type 2, member 108 (T2R108) | NM_020502.1 | Forward 5′-GTGTTTGCTGCTGGTTTT-3′ Reverse 5′-TCTATGCGGAGGCGAATT-3′ 115 |
| Gustducin (Gnat3) | NM_001081143.1 | Forward 5′-GCAGCGTACTAGGCTTAGC-3′ Reverse 5′-AATGGTAGGTGGTTGCAAATG-3′ 120 |
| Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) | NM_017008 | Forward 5′-CCATCACTGCCAAGGAGAC-3′ Reverse 5′-TACTCTTGGAGGCCATGAG-3′ 469 |
administration of denatonium (3 and 10 mg/kg, i.p.) and its vehicle (saline), the tail-cuff method (Visitech Systems BP-2000) was used to measure systolic, diastolic, and mean blood pressure in mice.

**Statistical Analyses** One- or two-way ANOVA and Bonferroni/Dunn’s post hoc test (two-tailed test) were carried out using GraphPad Prism (San Diego, CA, U.S.A.). p < 0.05 was considered significant.

**RESULTS**

To examine the effect of TAS2R on contractions of the bronchial smooth muscles in rats, the effects of TAS2R agonists were investigated. Contractions induced by CCh were inhibited significantly by pretreatment with denatonium in a concentration-dependent manner (Fig. 1A). Basal tension was not affected by denatonium treatment. Contractions induced by high K⁺ depolarization were inhibited markedly by pretreatment with 10⁻³ M denatonium (Fig. 1B). To investigate effect of denatonium in other agonist, the effect of denatonium on endothelin (ET)-1 (which activates GPCRs as well as contractions induced by CCh in bronchial smooth muscle) was examined. ET-1-induced contractions were also inhibited by denatonium in rat bronchial smooth muscle (Fig. 1C). NaF is a known activator of G-proteins. NaF-induced contractions were also significantly decreased by denatonium pretreatment (Fig. 1C). However, the contractions induced by 1 μM calyculin-A (potent inhibitor of MLCP) were not affected by denatonium in rat bronchial smooth muscle. The sustained contractions induced by 1 μM CCh were also inhibited by denatonium application in a concentration-dependent manner (Figs. 2A, B). β-Agonists are bronchodilators used for the rapid relief of asthma symptoms, so we examined co-treatment of β-agonists and denatonium. Post-treatment of isoprenaline attenuated CCh-induced contractions in a concentration-dependent manner. Isoprenaline-induced relaxation was augmented by denatonium pre-treatment in rat bronchial smooth muscle (Fig. 2C).

We examined species differences in the effects of denatonium. As well as rat bronchial smooth muscle, denatonium relaxed the sustained contractions induced by CCh, high K⁺- and NaF in murine bronchial smooth muscle (Figs. 3A–C). We examined the effects of another TAS2R agonist, PTU, on the contractions induced by CCh, high K⁺- and NaF in murine
bronchial smooth muscle. Not only denatonium but also PTU attenuated the contractions induced by various stimuli (Figs. 3D–F). As well as the contraction, CCh-induced phosphorylation of MLC was inhibited by denatonium and PTU treatment (Fig. 3G). Furthermore, isoprenaline-induced relaxation of CCh-induced contraction was augmented by co-treatment with denatonium or PTU (Figs. 3H, I).

We examined the gene expression of Ggust and TAS2R108 in the murine smooth muscles. mRNAs for Ggust and TAS2R108 were detected in the tongue, bronchus, ileum, colon, and thoracic aorta after 35 cycles of amplification. Gel staining for PCR products from the total RNA of the tissues of mice revealed amplified cDNA fragments that corresponded to the predicted sizes of glyceraldehyde 3-phosphate dehydrogenase (469 bp; Fig. 4A), Ggust (120 bp; Fig. 4B), and TAS2R108 (115 bp; Fig. 4C).

To ascertain if the ileal contractions was mediated by TAS2R108/Ggust pathway, we examined the effects of TAS2R agonists on CCh-induced contractions in the ileal tissue of mice. Denatonium and PTU caused slight force development, but these TAS2R agonists attenuated CCh-induced contractions (Figs. 5A, B). CCh-induced the phosphorylation of MLC was also inhibited by denatonium and PTU, although denatonium and PTU caused slight phosphorylation of MLC (Fig. 5C). Therefore, we examined the effect of TAS2R agonists on gastrointestinal motility in mice: it was inhibited by pretreatment with denatonium and PTU (Figs. 5D, E).

Subsequently, we examined the effects of TAS2R agonists on phenylephrine-induced contractions in the aortic tissue of mice. Phenylephrine-induced contractions were inhibited by post-treatment of denatonium and PTU (Figs. 6A, B). Next, we examined the effect of pretreatment of TAS2R agonists on phenylephrine-induced contractions. Denatonium treatment caused slight force development. Contractions induced by phenylephrine were inhibited by pre-treatment with denatonium or PTU (Figs. 7A, B). Phenylephrine-induced the phosphorylation of MLC was also inhibited by pretreatment of denatonium and PTU, although denatonium and PTU caused slight phosphorylation of MLC (Fig. 7C). Furthermore, systemic, diastolic, and mean blood pressures were lowered by treatment with denatonium (30 mg/kg, i.p.) in mice in vivo (Figs. 7D–F).

DISCUSSION

We showed that denatonium and PTU attenuate the contraction of bronchi, ileum, and thoracic aorta, and that these TAS2R agonists relax the smooth muscle of these tissues. Furthermore, treatment of TAS2R agonists in vivo inhibited gastrointestinal motility and lowered blood pressure.

Recently, widespread expression of TAS2Rs on smooth muscle cells in human airways was demonstrated using a GPCR DNA array. It has also been reported that bitter tastants cause dilation of tracheal smooth muscle in mice, suggesting that TAS2Rs can be classified not only as taste receptors but also as receptors for endogenous chemical mediators.5)

First, we examined the effect of TAS2R agonists on the contraction of bronchial smooth muscle in the rat and mouse. Contractions induced by CCh, high K\textsuperscript{+}, and NaF (but not calyculin-A) were inhibited significantly in the presence of 10\textsuperscript{−5} M denatonium in rat bronchial smooth muscle. Columns are the mean±S.E.M. from four independent experiments, respectively. *p<0.05 and ***p<0.001 vs. vehicle (saline).
Fig. 3. Effects of Denatonium and 6-α-Propyl-2-thiouracil (PTU) on Contractions in the Bronchial Smooth Muscles of Mice

Effects of post-treatment with denatonium (10⁻⁶–10⁻³M)/PTU (10⁻⁶–10⁻³M) on CCh (10⁻⁵M)-induced contractions in murine bronchial smooth muscle (A/D). Effect of 10⁻³M denatonium/PTU on the contraction induced by K⁺ depolarization (B/E) and 20mM NaF (C/F). Typical immunoblots of phosphorylated MLC (upper; p-MLC) by CCh (10μM) with or without 1mM denatonium and 0.3mM PTU (G left). The bands were scanned, and the level of phosphorylated MLC was expressed as density ratio of the phosphorylated MLC to the corresponding total MLC bands. The data are summarized in (G right). Each point represents the mean ± S.E.M. from three independent experiments. *p<0.05 vs. vehicle, #p<0.05 vs. CCh stimulation. Effect of isoprenaline (Iso)-induced relaxation on 3×10⁻⁷M CCh-induced contractions in the presence of 10⁻⁴M denatonium (H) or PTU (I) in murine bronchial smooth muscle. *p<0.05, **p<0.01, and ***p<0.001 vs. vehicle (saline or dimethyl sulfoxide).
Fig. 4. mRNA Expressions of TAS2R108 and Gustducin in the Tongue (Lane 1), Bronchus (Lane 2), Ileum (Lane 3), Colon (Lane 4), and Aorta (Lane 5) of Mice Were Determined by RT-PCR Analyses

PCR amplifications were undertaken for 35 cycles. Predicted size (see Table 1) of the bands for TAS2R108, gustducin, and glyceraldehyde 3-phosphate dehydrogenase was detected. Photographs are representative of four independent experiments. Lane M, molecular-weight marker (100-bp DNA ladder).

Fig. 5. Effects of Denatonium and 6-n-Propyl-2-thiouracil (PTU) on Contraction of Ileal Smooth Muscle in the Mouse

Effect of pre-treatment with denatonium ($10^{-3}$M)/PTU ($3 \times 10^{-4}$M) on CCh ($10^{-6}$M)-induced contractions on ileal smooth muscle in the mouse (A and B). Effect of administration of denatonium and PTU in vivo on gastrointestinal motility. Typical immunoblots of phosphorylated MLC (upper; p-MLC) by CCh ($1 \mu$M) with or without 1 mM denatonium and 0.3 mM PTU (C left). The bands were scanned, and the level of phosphorylated MLC was expressed as density ratio of the phosphorylated MLC to the corresponding total MLC bands. The data are summarized in (C right). Each point represents the mean±S.E.M. from four independent experiments. **p<0.01 vs. vehicle, #p<0.05 vs. CCh stimulation. D shows representative images from the stomach to the cecum isolated from mice after administration of vehicle and denatonium. Results are summarized in E. Columns are the mean±S.E.M. from four independent experiments. *p<0.05 and ***p<0.001 vs. vehicle.
The potassium equilibrium potential to activate L-type Ca\(^{2+}\) channels are regulated solely by augmentation of cytosolic Ca\(^{2+}\) concentration.\(^{14}\) However, it was reported that K\(^+\) depolarization-induced entry of Ca\(^{2+}\) activates the RhoA/Rho kinase, indicating in augmentation of myosin phosphatase, target subunit 1 (MYPT1) phosphorylation, MLCP inhibition and MLC phosphorylation.\(^{15}\) It is known that NaF, an activator of G-proteins,\(^{10,11,13,16}\) activates the trimeric G protein and RhoA pathways, which results in the development of contractions.\(^{17}\)

Conversely, the potent inhibitor of protein phosphatases calyculin-A augments MLCK activity by inhibiting MLCP activity, thereby increasing MLC phosphorylation and the development of tension. Contractions induced by calyculin-A were not affected by denatonium treatment. Thus, it seems unlikely that denatonium treatment affects the contractile apparatus of smooth muscle (e.g., activity of actomyosin ATPase). Taken together, the TAS2R pathway may inhibit not only the Ca\(^{2+}\)/CaM/MLCK pathway but also the RhoA/Rho kinase/MLCP pathway, although we need to investigate that effect of TAS2R agonists on contractile agonist-induced RhoA activation and MLCP inhibition (phosphorylation of MYPT 1, myosin-binding subunit of myosin phosphatase) and the contraction-induced by contractile agonists and Ca\(^{2+}\) alone in permeabilized smooth muscle. Furthermore, we showed that TAS2R agonists augmented \(\beta\)-agonist-induced relaxation. Therefore, a medication combining TAS2R and a \(\beta\)-agonist could be used to treat asthma and chronic obstructive pulmonary disease.

The ligands have been identified for taste receptor, type 2, member 108 (TAS2R108): denatonium and 6-n-propyl-2-thiouracil (PTU).\(^{3,10}\) TAS2R108 expression was investigated further in various smooth-muscle tissues of mice. Gene expression of TAS2R108 was found not only in the tongue, but also in the bronchus, ileum, and aorta. Effects of the TAS2R agonist denatonium on smooth-muscle contractions of these tissues in mice were also examined. Interestingly, denatonium caused a slight force development in the ileum. Previous studies showed that bitter tastants increase global [Ca\(^{2+}\)], and that these Ca\(^{2+}\) changes in cells at rest are mediated via activation of the canonical bitter-taste signaling cascade (i.e., the TAS2R-gustducin-phospholipase C/\(\beta\)-inositol-1,4,5-triphosphate receptor).\(^{18}\) Therefore, slight force development might be caused by the canonical bitter-taste signaling cascade in aortal and ileal smooth muscle, although the detail mechanism is not unclear in this study. On the other hand, Deshpande et al.\(^{3}\) discussed that the relaxation induced by TAS2Rs is associated with a localized [Ca\(^{2+}\)]\(_i\) response at the cell membrane, which opens large-conductance Ca\(^{2+}\) -activated K\(^+\) channels, leading to tracheal smooth muscle membrane hyperpolarization.

Contractions induced by CCh in the ileum were also inhibited by treatment with TAS2R agonists. Furthermore, gastrointestinal motility was inhibited by administration of TAS2R agonists in vivo. In the aorta, denatonium also caused slight force development but the contractions induced by phenylephrine were also inhibited by denatonium treatment. Moreover, denatonium administration in vivo lowered systolic, diastolic, and mean blood pressure. Foster et al. recently demonstrated that agonists for TAS2R108 led to decrease in left ventricular developed pressure in the murine hearts.\(^{19}\) These findings suggest that TAS2R may be the receptor for endogenous biologically active substances as well as a receptor for bitter tastants on the tongue.

Not only TAS2R108 but also some TAS2R subtypes are activation by denatonium and PTU.\(^{7}\) Therefore, it is hard to suggest that smooth muscle relaxation activity was mediated by TAS2R108 alone. Moreover, it is possible that localization of TAS2R subtypes having different action exists in various smooth muscle. In the present study, potency of relaxation induced by denatonium and PTU was different among bronchi, aortal and ileal smooth muscle. Also, as mentioned above, high K\(^+\)-induced contractions were inhibited markedly in the presence with TAS2R agonists in the present study. However, Manson et al. recently showed that TAS2R agonists (chloroquine, denatonium, dextromethorphan, noscapine and quinine) caused only weak relaxations of depolarization-induced contractions evoked by KCl.\(^{20}\) Although it is not possible to explain these contradiction in the present, the relaxation effect of each TAS2R agonist therefore may be different in various smooth muscles.

Specific antagonists for TAS2R- or TAS2R-deficient animals are lacking. Hence, whether the reported activities of TAS2R agonists in the present study and on smooth muscles\(^{5,21,22}\) are mediated through TAS2R has yet to be elucidated. The observed broad range of activities by the TAS2R agonists studied may have important implications because these agonists are used commonly to define TAS2R functions. Therefore, future research is required to determine the contribution of TAS2R to the mechanism(s) underlying these
Fig. 7. Effects of Pre-treatment of Denatonium and PTU on Contraction of Aortic Smooth Muscle in the Mouse

Traces of phenylephrine (Phe)-induced contraction are representative of four independent experiments (A). Effects of denatonium on Phe-induced contractions of aortic rings isolated from mice. $10^{-3}$M denatonium or $10^{-4}$ and $3\times10^{-4}$M PTU were added 20 min before Phe treatment. Data are summarized in B. Typical immunoblots of phosphorylated MLC (upper; p-MLC) by Phe (1μM) with or without 1mM denatonium and 0.3 mM PTU (C upper). The bands were scanned, and the level of phosphorylated MLC was expressed as density ratio of the phosphorylated MLC to the corresponding total MLC bands. The data are summarized in (C lower). Each point represents the mean±S.E.M. from four independent experiments. *$p<0.05$ vs. vehicle, **$p<0.01$ and ***$p<0.005$ vs. Phe stimulation. Effect of administration of denatonium in vivo on systolic, diastolic, and mean blood pressure of mice (D–F). Columns are the mean±S.E.M. from four independent experiments. **$p<0.01$ and *$p<0.05$ vs. vehicle.
responses in various types of smooth muscle. Activation of TAS2R signaling may apply to the development of anti-asthma, anti-spasmodic, and anti-hypertensive drugs.

Acknowledgments We thank Mr. Atsunobu Sagara and Ms. Nanoka Sasaki for technical assistance. This work was supported by a Lotte Research Promotion Grant and MEXT-Supported Program for the Strategic Research Foundation at Private Universities (S1411019).

Conflict of Interest The authors declare no conflict of interest.

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