Activation of the umami taste receptor (T1R1/T1R3) initiates the peristaltic reflex and pellet propulsion in the distal colon

Derek M. Kendig,1 Norman R. Hurst,1 Zachary L. Bradley,1 Sunila Mahavadi,1 John F. Kuemmerle,1,2 Vijay Lyall,1 John DeSimone,1 Karnam S. Murthy,1,2 and John R. Grider1,2

1Department of Physiology and Biophysics, Virginia Commonwealth University School of Medicine, Richmond, Virginia; and 2Department of Medicine, VCU Program in Enteric Neuromuscular Sciences, Virginia Commonwealth University School of Medicine, Richmond, Virginia

Submitted 11 July 2014; accepted in final form 9 October 2014

The ability of intraluminal nutrients to affect motility of the gastrointestinal (GI) tract has been known for decades (14). However, some of the receptors responsible for sensing intraluminal nutrients have only been discovered very recently. These receptors are the same as those responsible for the detection of taste qualities in the lingual epithelium. Multiple studies show the presence of taste receptors and the associated intracellular signaling molecules in the upper GI tract; however, there is less evidence for the existence of these receptors in the distal gut, specifically, the distal colon (13, 37, 48). While absorption of nutrients occurs almost completely in the small intestine, amino acids from the diet, dead mucosal cells, digested luminal enzymes, and amino acids generated by bacteria are present in the luminal contents of the distal colon (11, 16, 39). One receptor for luminal amino acids is the “umami” taste receptor T1R1/T1R3, which is activated by certain l-amino acids (l-AAs), including monosodium glutamate (MSG), a paradigm ligand for umami taste sensation. Other plausible luminal sensors for amino acids are the Ca2+-sensing receptor (CaSR), metabotropic glutamate receptors, and G protein-coupled receptor family C, group 6, member A (2, 7, 8, 23, 44).

While umami receptors in rodents are activated by a wide variety of l-AAs, umami taste in humans is specific to MSG, which makes MSG an excellent ligand for umami receptor activation (5, 50). Moreover, inosine 5′-monophosphate (IMP) is a potentiator of T1R1/T1R3 activation but is not a potentiator at other putative umami receptors (CaSR, metabotropic glutamate receptor, or G protein-coupled receptor family C, group 6, member A) (12, 27, 33, 49). Therefore, IMP can be used to pharmacologically determine if T1R1/T1R3 receptors are involved in changes in GI motility, as has been done in the characterization of umami taste. While much is known about the regulation of gastric emptying and small intestinal motility by luminal contents, little is known about the motility effects of nutrients in the distal GI tract. Studies have shown that activation of sweet taste receptors (T1R2/T1R3) can lead to release of glucagon-like peptide 1 (GLP-1) and regulation of glucose transporters in the small intestine (25, 31). More importantly, recent studies suggest that l-AAs activate T1R1/ T1R3 receptors in the upper small intestine and lead to release of cholecystokinin (10), although activation of the CaSR may produce the same effect (29, 44). In the present study we examined the functional effects of amino acid-sensing receptor activation on motility in the distal colon. We hypothesized that amino acid-induced changes in motility would occur in part through T1R1/T1R3 activation.

The present study shows l-AA-sensing T1R1/T1R3 receptors in the mucosal cells of human, rat, mouse, and guinea pig colon. Luminal application of the umami ligand MSG activated the peristaltic reflex and stimulated sensory pathways, as evidenced by release of the sensory neurotransmitter calcitonin gene-related peptide (CGRP) in rat colon. The effect was mediated by T1R1/T1R3 receptors, as IMP augmented the peristaltic reflex responses and CGRP release. Moreover, in T1R1−/− mice, MSG did not initiate a peristaltic reflex. MSG increased the velocity of pellet propulsion and IMP augmented this effect in guinea pig colon. Consistent with the results with MSG, the T1R1/T1R3-prefering amino acid l-cysteine in-
increased the velocity of pellet propulsion, while the CaSR-prefering amino acid l-tryptophan did not. We conclude that T1R1/T1R3 activation in distal colon results in initiation of the peristaltic reflex and enhancement of colonic motility.

**MATERIALS AND METHODS**

*Animal tissue extraction.* Male Sprague-Dawley rats (100–150 g body wt), Dunkin-Hartley guinea pigs of either sex (150–200 g body wt), and male C57BL/6 wild-type or T1R1−/− mice (Jackson Laboratory, Bar Harbor, ME) were euthanized by CO2 asphyxiation and exsanguination, as approved by Virginia Commonwealth University’s Institutional Animal Care and Use Committee. The entire distal colon was removed from guinea pigs beginning just distal of the colonic flexure through the rectum. The entire distal colon was removed from rats and mice and prepared as a flat-sheet preparation.

*Immunohistochemistry.* Colonic tissue from human, rat, mouse, or guinea pig was fixed overnight at 4°C in 4% (wt/vol) paraformaldehyde and transferred into a 30% (wt/vol) sucrose solution for 2–3 days. Human colonic tissue was obtained from histologically normal margins of segments removed at surgery from patients undergoing ileal/faloral resection for structuring Crohn’s disease, as approved by the Internal Review Board of Virginia Commonwealth University. Tissue pieces were embedded in optimal cutting temperature compound, frozen, and then sectioned in a cryostat. Sections (12 μm thick) were thaw-mounted onto gelatin-coated slides, permeabilized with 0.3% (vol/vol) Triton X, and incubated for 1 h in blocking solution [5% (vol/vol) normal donkey serum] at room temperature. Sections were incubated overnight at 4°C with primary antibody against T1R1 (rabbit polyclonal anti-T1R1, sc-50308, Santa Cruz Biotechnology; 1:200 dilution), washed multiple times with PBS, and incubated in secondary antibody (donkey anti-rabbit 488, A-21206, Life Technologies; 1:2,000 dilution) for 2 h. Antibody dilution buffers contained 5% (vol/vol) normal donkey serum in PBS. Immunofluorescence was visualized using an epifluorescence microscope (Zeiss Imager.Z1), and images were captured with AxioCam MRm.

**Measurement of the peristaltic reflex.** The peristaltic reflex was measured in a 3- to 5-cm preparation of rat or mouse distal colon that was opened to form a flat sheet and pinned mucosal-side-up in a Sylgard-lined organ bath containing Krebs buffer heated to 37°C and allowed to equilibrate for 30 min. After an initial period of 15 min, the medium was collected and treated with 100 mM MSG alone or with IMP, l-cysteine, or l-tryptophan to determine the effect on the velocity of the peristaltic reflex. The data were collected using three successive pellets inserted at 5-min intervals. Pellets were inserted into the oral end of the preparation, and movement was recorded by a camera positioned directly over the organ bath. For examination of the effects of MSG alone or with IMP, l-cysteine, or l-tryptophan on the velocity of pellet propulsion, the agents were added to the luminal Krebs buffer perfusate and the velocities were compared with the control nonperfusion condition.

**Data and statistical analysis.** CGRP released into the central compartment of the three-compartment flat-sheet preparation was measured in femtomoles per 100 mg of protein per minute. AC and DR were measured as grams of force. Velocity of artificial fecal pellet propulsion was measured as millimeters per second. Values are means ± SE from n experiments, with each experiment including a separate animal and colonic segment. Statistical significance was determined by Student’s t-test (paired) for single comparisons or repeated-measures one-way ANOVA followed by Tukey’s post hoc test for multiple comparisons. P < 0.05 was considered statistically significant.

**Materials.** CGRP antiserum RIK 6006 and 125I-labeled CGRP (125I-CGRP) were purchased from Bachem. MSG, IMP, l-cysteine, and l-tryptophan were purchased from Sigma-Aldrich (St. Louis, MO). Antibodies were purchased from Santa Cruz Biotechnology (Dallas, TX) and Life Technologies (Carlsbad, CA). All other reagents were purchased from Fisher Scientific (Pittsburgh, PA).

**RESULTS**

**Presence of T1R1 receptors in colonic mucosa of human, rat, mouse, and guinea pig.** Immunohistochemical analysis of colonic cryosections from rats, mice, guinea pigs, and humans shows the presence of T1R1 in specific cells of the mucosal layer (Figs. 1 and 2). The cells located in the crypts were triangular in appearance and extended from the basal lamina to the crypt surface, typical of open-type enteroendocrine cells (EECs). Some, but not all, cells at this location and with this morphology also costained for 5-hydroxytryptamine (5-HT) on their basolateral aspect in human colonic crypts (Fig. 2), suggesting that some of the T1R1-expressing EECs were enterochromaffin cells. Some T1R1-positive cells were located on the surface epithelium of the mucosa and possessed the elongated morphology of brush cells that have access to the same as control medium. The procedure was repeated in the same preparation by the addition of MSG + IMP. CGRP release was measured by radioimmunoassay using the antibody RIK 6006 (Bachem, Torrance, CA) (18).

**Measurement of pellet propulsion.** Velocity of artificial fecal pellet propulsion was measured in intact segments (~6 cm long) of guinea pig middle-to-distal colon, as described previously (26). Artificial pellets, similar in size to the natural fecal pellets of the guinea pigs, were made from clay. Colonic segments were pinned loosely into a Sylgard-lined organ bath containing Krebs buffer heated to 37°C and allowed 30 min to expel natural fecal pellets. Pins were placed in the oral and anal ends of the colonic segment to keep the lumen open for insertion and ejection of the fecal pellets. If all pellets were not expelled naturally, they were mechanically expelled by gentle perfusion with warmed Krebs buffer at the end of the 30-min incubation period. The control (basal) velocity of propulsion was first determined using three successive pellets inserted at 5-min intervals. Pellets were inserted into the oral end of the preparation, and movement was recorded by a camera positioned directly over the organ bath. The velocity of pellet propulsion was analyzed using software associated with the Gastrointestinal Motility Monitoring system (Catamount Research and Development, St. Albans, VT). Velocities were analyzed over a minimum distance of 2 cm of pellet movement. Each segment was perfused at a rate of 0.10 ml/min with carboxygenated (5% CO2–95% O2) Krebs buffer through PE-10 tubing inserted through the aboral end and advanced to a point just distal to the pellet. Intraluminal perfusion was initiated just prior to insertion of the artificial fecal pellet (~30 s). The pellet was allowed to move spontaneously, pushing the tubing ahead of it and out of the colon. For examination of the effects of MSG alone or with IMP, l-cysteine, or l-tryptophan on the velocity of pellet propulsion, the agents were added to the luminal Krebs buffer perfusate and the velocities were compared with the control nonperfusion condition.
intestinal lumen and its contents (not shown). We chose to immunostain for T1R1 in colonic sections, because all other components of the taste receptor canonical signaling pathway are common to umami and sweet receptors in taste cells, whereas the T1R1 component is unique to umami receptors.

**MSG induces CGRP release in rat colonic flat-sheet preparation.** The effects of MSG alone or MSG + IMP on CGRP release are presented in Fig. 3. CGRP release in a flat-sheet preparation is a marker of activation of the sensory neurons that mediate the peristaltic reflex (18, 35). MSG is a known activator of T1R1/T1R3, which we have shown to be present in the rat colonic mucosa (Fig. 1). IMP is a selective allosteric enhancer of T1R1/T1R3 activation and is useful in determining if the MSG effect is T1R1/T1R3-specific. The basal release of CGRP from rat colonic flat-sheet preparations was 7.4 ± 0.5 fmol·100 mg⁻¹·min⁻¹ (n = 6). Stimulation of the preparation with 10 mM MSG significantly increased CGRP release into the central compartment to 13.2 ± 1.3 fmol·100 mg⁻¹·min⁻¹ (n = 6, P < 0.05), whereas 10 mM MSG + 1 μM IMP significantly increased CGRP release to 17.4 ± 1.5 fmol·100 mg⁻¹·min⁻¹ (n = 6, P < 0.05) compared with basal values. IMP (1 μM) alone had no effect on CGRP release (data not shown).

![Image](http://ajpgi.physiology.org/)
MSG induces activation of the peristaltic reflex in rat colonic flat-sheet preparation. Application of MSG alone or MSG + IMP to the central compartment of a rat colonic flat-sheet preparation initiated the ascending contraction (AC) and descending relaxation (DR) limbs of the peristaltic reflex (Fig. 4A). MSG alone dose dependently increased the AC and DR components of the peristaltic reflex (Fig. 4, B and C). The AC component was increased from 0.16 ± 0.04 g (1 mM MSG, \( n = 5 \)) to 0.32 ± 0.08 g (10 mM MSG, \( n = 5 \)) and 0.58 ± 0.09 g (100 mM MSG, \( n = 5 \)). The DR component was increased from 0.08 ± 0.02 g (1 mM MSG, \( n = 5 \)) to 0.25 ± 0.04 g (10 mM MSG, \( n = 5 \)) and 0.55 ± 0.06 g (100 mM MSG, \( n = 5 \)). MSG + 1 \( \mu \)M IMP also dose dependently increased the AC and DR components of the peristaltic reflex (Fig. 4, B and C). The AC component was increased from 0.29 ± 0.03 g (1 mM MSG + 1 \( \mu \)M IMP, \( n = 5 \)) to 0.61 ± 0.07 g (10 mM MSG + 1 \( \mu \)M IMP, \( n = 5 \)) and 0.79 ± 0.06 g (100 mM MSG + 1 \( \mu \)M IMP, \( n = 5 \)). The DR component was increased from 0.21 ± 0.03 g (1 mM MSG + 1 \( \mu \)M IMP, \( n = 5 \)) to 0.52 ± 0.05 g (10 mM MSG + 1 \( \mu \)M IMP, \( n = 5 \)) and 0.70 ± 0.05 g (100 mM MSG + 1 \( \mu \)M IMP, \( n = 5 \)). The force of AC and DR in response to MSG + IMP was augmented at all concentrations compared with the same concentration of MSG alone. Moreover, the augmentation was statistically significant at 10 mM MSG for AC and DR. This enhancement of both components of the peristaltic reflex by MSG + IMP compared with MSG alone indicates that the effect is mediated by T1R1 activation. This notion was also supported by studies in T1R1−/− mice. MSG did not induce AC or DR in T1R1−/− mice (Fig. 4D), whereas mucosal mechanical stimulation induced by six mucosal strokes elicited normal AC and DR in the T1R1−/− mice. In control (T1R1+/+) mice, MSG and mucosal stroking initiated a peristaltic reflex.

Fig. 4. Activation of the peristaltic reflex in rat and mouse distal colon by MSG and augmentation by IMP in rat colon. Ascending contraction (AC) was measured in the oral compartment and descending relaxation (DR) was measured in the caudad compartment in response to MSG and IMP application into the central sensory compartment. A: representative force traces of the effect of 10 mM MSG alone or with 1 \( \mu \)M IMP on AC and DR in rat colon. B: 1 \( \mu \)M IMP (●) enhanced AC at all concentrations of MSG (○) and significantly enhanced the effect at 10 mM MSG. C: 1 \( \mu \)M IMP (●) enhanced DR at all concentrations of MSG (○) and significantly enhanced the effect at 10 mM MSG. D: representative force traces of the effect of 10 mM MSG alone on AC and DR in wild-type C57BL/6 and T1R1−/− (T1R1 knockout [KO]) mice. MSG induced a peristaltic reflex in C57BL/6 mice. MSG did not induce a peristaltic reflex in distal colon of T1R1−/− mice, but mucosal stroking still induced a normal reflex response. For B and C, *P < 0.05 vs. MSG alone at the same concentration (by paired t-test). Values are means ± SE of 5 values, with each value representing a separate animal.
MSG enhances the velocity of pellet propulsion in intact guinea pig distal colon. The velocity of artificial fecal pellet propulsion was measured in intact preparations of guinea pig distal colon. MSG (1 mM; Fig. 5) increased the velocity of pellet propulsion by 63 ± 22% (1.6 ± 0.1 mm/s, n = 6, P < 0.05) above control velocity (1.1 ± 0.1 mm/s, n = 6). The increased velocity of propulsion elicited by 1 mM MSG was further enhanced by the addition of 100 μM IMP (Fig. 5) to 95 ± 39% above control velocity (1.9 ± 0.2 mm/s, n = 6, P < 0.05). This suggests that MSG enhances propulsion by T1R1 activation. IMP alone did not change the velocity of pellet propulsion compared with control values (data not shown). This indicates that, like the taste effect of IMP, enhancement of pellet propulsion is only observed in the presence of MSG.

Perfusion of Krebs buffer (1.4 ± 0.2 mm/s, n = 6) did not change velocity compared with control values (1.4 ± 0.1 mm/s, n = 6), consistent with our previous findings (26). Also, intraluminal perfusion of 10 mM NaCl had no effect on velocity of pellet propulsion (data not shown). These control studies indicate that the effects of MSG on propulsion were mediated by glutamate and that neither additional sodium nor increased osmolarity had an effect on pellet velocity.

In addition to testing L-glutamate in the form of MSG, we tested other physiological L-AAs to confirm that the effect was not specific to L-glutamate. We tested this notion with L-cysteine, which is reported to activate T1R1 receptors, and L-tryptophan, which is reported to activate the CaSR in other systems (4, 8, 10, 33, 46). The effects were compared with the velocity of pellet propulsion during intraluminal perfusion of an equimolar concentration of the prototypical T1R1/T1R3 ligand. MSG (10 mM) increased the velocity of pellet propulsion by 47 ± 5% above control (1.8 ± 0.1 mm/s with 10 mM MSG vs. 1.3 ± 0.1 mm/s for control; Fig. 6A). Intraluminal perfusion of L-cysteine (Fig. 6B) increased pellet velocity 38 ± 7% (1.3 ± 0.1 mm/s, n = 4, P < 0.05) above control velocity (0.9 ± 0.0 mm/s, n = 4), similar to the augmentation induced by MSG. In contrast, L-tryptophan (Fig. 6C) did not affect (3 ± 8% below control) pellet velocity (1.2 ± 0.1 mm/s, n = 5) compared with control (1.2 ± 0.1 mm/s, n = 5).

Fig. 5. Velocities of artificial fecal pellet propulsion in response to MSG and MSG + IMP. Velocity of pellet propulsion was significantly increased by intraluminal perfusion of 1 mM MSG aboral to the pellet. This effect was further enhanced by addition of 100 μM IMP. *P < 0.05 vs. control (by repeated-measures 1-way ANOVA with Tukey’s post hoc test). Values are means ± SE of 6 values, with each value representing a separate animal.

Fig. 6. Velocities of artificial fecal pellet propulsion in response to 10 mM MSG, L-cysteine, or L-tryptophan. A: intraluminal perfusion of 10 mM MSG significantly increased the rate of pellet propulsion above control. B: velocity of pellet propulsion was significantly increased by intraluminal perfusion of 10 mM L-cysteine aboral to the pellet. C: velocity of pellet propulsion was unchanged by intraluminal perfusion of 10 mM L-tryptophan aboral to the pellet. This pattern of effect suggests that activation of T1R1/T1R3 receptors by MSG and L-cysteine leads to an increase in pellet velocity. In contrast, L-tryptophan, which does not activate the T1R1/T1R3 receptors, failed to enhance pellet propulsion. *P < 0.05 vs. control (by paired Student’s t-test). Values are means ± SE of 4–10 values, with each value representing a separate animal.
DISCUSSION

The discovery of taste receptors in the epithelial layer of the GI tract has expanded the field of intraluminal nutrient sensation. Several studies have shown that activation of sweet taste receptors (T1R2/T1R3) can regulate GLP-1 release, glucose transporter expression, and glucose homeostasis (25, 31). Moreover, umami taste receptors (T1R1/T1R3) can regulate cholecystokinin release in EECs of the small intestine (10), although similar effects can be mediated by CaSR (29, 44) or indirectly through peptide transporter 1 (28). Most studies have focused on the role of taste receptors in regulating gastric and intestinal hormone/paracrine release and function, while little is known of the presence of taste receptors in the colon and their role in regulation of colonic motility. We hypothesized that T1R1/T1R3 activation affects local motility in the colon. Therefore, we examined 1) T1R1 expression in cross sections of human, rat, mouse, and guinea pig colon, 2) CGRP release induced by MSG and MSG + IMP, 3) activation of the AC and DR components of the peristaltic reflex by MSG or MSG + IMP, and 4) changes in pellet propulsion induced by MSG, MSG + IMP, and L-AAs with differing selectivity for putative L-AAA (umami) receptors.

We have demonstrated the presence of the T1R1 component of the umami taste receptor in the mucosal layer of human, rat, mouse, and guinea pig colon (Figs. 1 and 2). The T1R1 protein is specific to the umami taste receptor, which is activated by L-AAs, including MSG. Our group and others have also demonstrated the presence of T1R3, α-gustducin, and transient receptor potential cation channel, subfamily M, member 5 (TRPM5) in gut tissues, as well as in the STC-1 mouse EEC line (3, 13, 38, 48). Although these signaling components are necessary for the complete functional receptor, they are not specific to the umami receptor and do not specifically identify the capacity for chemosensation of L-AAs. The location of the cells containing T1R1 suggest that T1R1/T1R3 has access to the intraluminal contents of the colon and could act as a nutrient sensor, as it does in more proximal regions of the gut.

Some of the T1R1-expressing EECs also immunostained for 5-HT, while others did not contain for 5-HT (Fig. 2). Our group and others have shown that 5-HT is involved in initiating CGRP release and the peristaltic reflex in rat, guinea pig, human, and mouse (17, 20, 24, 32, 35). Studies in the STC-1 cell line also show that T1R1/T1R3 activation is able to release several hormone/paracrine agents such as cholecystokinin, peptide YY, neurotensin, and GLP-1 (3, 9, 10, 15, 45). It seems possible that activation of these receptors and release of one or more of these agents may regulate gut motility, as well as other physiological responses to luminal L-AAs. The extent to which each of these paracrine agents is released, alone or in combination, in response to activation of the T1R1/T1R3 receptors on various EECs and the degree to which their release is involved in initiating or mediating peristalsis in the distal colon remain to be determined.

To examine the coupling of T1R1 activation to a physiological function, we next demonstrated that activation of T1R1/T1R3 receptors could initiate the peristaltic reflex. In this study we used the paradigmatic umami ligand MSG to activate T1R1/T1R3 receptors. Since MSG may also activate other amino acid-sensing receptors, we also employed the 5'-ribonucleotide IMP, an allosteric activator specific for T1R1 (27, 49). Thus the combination of MSG and IMP shows that the effects are specific to T1R1/T1R3 activation. First, we demonstrated that MSG causes release of CGRP from sensory neurons innervating the colon, which is a required initial step in activation of the peristaltic reflex (20, 36, 42). The enhanced CGRP release due to IMP suggests that CGRP release is downstream of T1R1/T1R3 activation. Although not examined in the present study, it is likely that activation of T1R1/T1R3 on an EEC causes the release of a paracrine agent that then activates the subepithelial CGRP-containing sensory neuron coupled to the circuitry of the peristaltic reflex. The nature of the intermediary paracrine agent was not investigated; however, 5-HT is a plausible candidate, since T1R receptors and α-gustducin are located on some 5-HT-containing EECs (36, 42; J. R. Grider, unpublished observations). Also we previously showed a link between paracrine 5-HT release and sensory neuronal CGRP release (20). As discussed above, potential mediators in EECs may also mediate CGRP release and peristalsis in response to activation of T1R1/T1R3 receptors. We also found that MSG initiated AC and DR components of the peristaltic reflex. AC occurred in the compartment oral to the MSG stimulus in a three-chamber flat-sheet preparation, while DR occurred in the chamber caudad to the stimulus. This pattern of activation in the flat-sheet preparation is analogous to the peristaltic reflex initiated by muscle stretch or mucosal stroking. This pattern is also similar to the motility of the intact colon, which pushes the nutrient stimulus in the aboral direction, such as in the movement of fecal pellets. Again, addition of IMP enhanced the MSG effects on AC and DR, suggesting that the changes in force are the result of T1R1/T1R3 activation. This notion was confirmed in segments of mouse colon from T1R1−/− mice. While MSG elicited a normal peristaltic reflex in wild-type mice, in the T1R1−/− mice, addition of MSG to the central compartment failed to elicit either component of the peristaltic reflex. In contrast, in the same preparations, mucosal stroking elicited a normal peristaltic reflex, indicating that the motor limbs of the reflex were intact and that the sensory pathway activated by mucosal mechanical stimulation was intact.

In the present study, we did not identify a difference in the number or size of fecal pellets expelled in T1R1−/− mice (J. R. Grider, unpublished observations). In recent studies by others, loss of tryptophan hydroxylase 1 (TPH1), which generates mucosal 5-HT, reduced, but did not abolish, fecal pellet output in TPH1−/− mice although fecal pellet size was increased (22). Also, depletion of 5-HT in the enteric neurons and removal of the mucosa only partially reduce colonic motility and initiation of the peristaltic reflex (19, 41). Thus multiple pathways initiate peristalsis, and loss of one does not preclude initiation of peristalsis and fecal pellet expulsion by the others.

Since MSG induced activation of the peristaltic reflex, we measured the effects of MSG on movement of artificial fecal pellets in guinea pig distal colon. Guinea pig distal colon was used, because rat distal colon has been found to be a poor model of pellet propulsion in in vitro preparations. We found that intraluminal perfusion of MSG aboral to the fecal pellet increased the velocity of pellet movement (Figs. 5 and 6). This effect was further enhanced by the addition of IMP, again suggesting that the change in velocity was the result of activation of T1R1/T1R3 receptors. Intraluminal perfusion of Krebs buffer or 1 μM IMP alone had no effect on pellet...
velocity. Moreover, 10 mM NaCl did not affect pellet velocity, suggesting that neither osmolarity nor sodium levels caused the increase in velocity produced by MSG. Thus the effect is due to glutamate.

MSG (1 mM) alone enhanced pellet velocity above control velocity by 63%, which was then enhanced even further to 95% above control by addition of 100 μM IMP (Fig. 5). This increase in pellet velocity by MSG and further enhancement by IMP strongly suggest that the effects of MSG are mediated by activation of T1R1/T1R3 receptors and confirm studies on the peristaltic reflex in rat and mouse described above. While we did not pursue a full concentration-response curve to MSG in pellet velocity studies, we found that 10 mM MSG also significantly augmented pellet propulsion, although to a lesser degree (47% above control). There are no studies of the level of MSG achievable in the colon of human or other species with which to compare our in vitro results; however, concentrations of amino acids in biopsies of human colon and rectum have been reported to range from 0.15 to 14.8 mol/kg (1). While T1R1/T1R3 activation by MSG was useful in showing the ability of receptor activation to affect local motility, we further explored the effects of other L-AAs, L-cysteine and L-tryptophan. L-Tryptophan has been shown to activate CaSR, but not T1R1/T1R3 receptors (4, 8, 33). Therefore, the lack of effect of L-tryptophan on velocity of pellet propulsion in the present study suggests that CaSR is not a significant mediator of the effect of L-AAs on colonic motility. In contrast, L-cysteine is one of the L-AA activators of T1R1/T1R3 receptors (33, 46), and in the present study L-cysteine also increased pellet velocity, similar to the effect of MSG. This suggests that modification of colonic motility can be achieved by physiological amino acids in the gut. Previous studies in the proximal gut show that certain L-AAs can cause activation of T1R1/T1R3 specifically, while other L-AAs may affect different amino acid-sensing receptors (10, 29, 43, 44). We have shown the effect of activation of the L-AA-sensing T1R1/T1R3 on motility of the distal colon. Considering that a large and diverse set of L-AAs are present in the colonic lumen, it is likely that the overall motility response to L-AAs is the result of activation of a combination of L-AA receptors.

Possible clinical implications. Our study suggests that activation of T1R1/T1R3 stimulates colonic motility. The effects of nutrients on motility, especially in the colon, have implications on possible causes of and/or treatments for motility disorders such as diarrhea and constipation. The search for possible dietary causes and treatments for a variety of inflammatory and functional GI disorders is a topic of great recent interest (6). A diet low in fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAPs) has potential application in treatment of a variety of gut disorders (21, 34, 40). In addition, animal-based diets, which are higher in protein and amino acids, have a greater impact on the colonic microbiome than do plant-based diets (11). Increased T1R1 activation in the colon, due to higher protein diet, may affect the microbiome through alteration of colonic motility. While many of these dietary interventions are aimed at changing nutrient fermentation by gut bacteria or intestinal permeability, the effects of activation of nutrient-sensing receptors by food may also be an important nutritional strategy. It is possible that some of the causes of or treatments for motility symptoms may involve nutrient activation of receptors within the gut wall and the subsequent effects of nutrient receptor activation. The findings of the present study raise important questions about the importance of L-AAs in changes to colonic motility. While large quantities of amino acid may not reach the colon in a normally functioning gut, it seems that, in patients with inflammatory gut disorders and impaired absorption, larger quantities of amino acid may reach the colon (30). This could affect the rate of movement of feces through the colon, and our study would suggest that increased amino acid content in the colonic lumen would increase the rate of movement of feces, perhaps producing a diarrhea-like symptom. The increased rate of colonic motility would remove the high luminal concentrations of amino acids more quickly. This would be beneficial, as the by-products of amino acid fermentation by colonic bacteria (NH₃, H₂S, and phenolic compounds) are detrimental to gut health (47).

GRANTS

This study was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-34153 (J. R. Grider). D. M. Kendig was supported by funds from National Institute of General Medical Sciences Institutional Research and Academic Career Development Award K12 GM-093857 to Virginia Commonwealth University.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES


