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Sensing of amino acids by the gut-expressed taste receptor T1R1-T1R3 stimulates CCK secretion

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Daly K, Al-Rammahi M, Moran A, Marcello M, Ninomiya Y, Shirazi-Beechey SP. Sensing of amino acids by the gut-expressed taste receptor T1R1-T1R3 stimulates CCK secretion. *Am J Physiol Gastrointest Liver Physiol* 304: G271–G282, 2013. First published November 29, 2012; doi:10.1152/ajpgi.00074.2012.—CCK is secreted by endocrine cells of the proximal intestine in response to dietary components, including amino acids. CCK plays a variety of roles in digestive processes, including inhibition of food intake, consistent with a role in satiety. In the lingual epithelium, the sensing of a broad spectrum of L-amino acids is accomplished by the heteromeric amino acid (umami) taste receptor (T1R1-T1R3). T1R1 and T1R3 subunits are also expressed in the intestine. A defining characteristic of umami sensing by T1R1-T1R3 is its potentiation by IMP or GMP. Furthermore, T1R1-T1R3 is not activated by Trp. We show here that, in response to L-amino acids (Phe, Leu, Glu, and Trp), but not D-amino acids, STC-1 enteroendocrine cells and mouse proximal small intestinal tissue explants secrete CCK and that IMP enhances Phe-, Leu-, and Glu-induced, but not Trp-induced, CCK secretion. Furthermore, small interfering RNA inhibition of T1R1 expression in STC-1 cells results in significant diminution of Phe-, Leu-, and Glu-stimulated, but not Trp-stimulated, CCK release. In STC-1 cells and mouse intestine, gurmarin inhibits Phe-, Leu-, and Glu-induced, but not Trp-stimulated, CCK secretion. In contrast, the Ca^{2+}-sensing receptor antagonist NPS2143 inhibits Phe-stimulated CCK release partially and Trp-induced CCK secretion totally in mouse intestine. However, NPS2143 has no effect on Leu- or Glu-induced CCK secretion. Collectively, our data demonstrate that functional characteristics and cellular location of the gut-expressed T1R1-T1R3 support its role as a luminal sensor for Phe-, Leu-, and Glu-induced CCK secretion.

amino acid; sensing; intestine; cholecystokinin; T1R1-T1R3

CHOLECYSTOKININ (CCK), a gastrointestinal peptide, is secreted by endocrine cells in the proximal portion of the small intestine upon ingestion of a meal (36, 57). CCK plays a variety of roles in digestive processes, such as slowing gastric emptying, mediating intestinal motility, and stimulating pancreatic and gallbladder secretions (15, 23, 30, 62). It also inhibits food intake in a manner consistent with a role in satiety (47). The major nutrients that stimulate CCK release are ingested fats and proteins, in particular protein hydrolysates, peptides, and amino acids (36). Amino acids, in particular L-Phe, at physiological concentrations (10–50 mmol/l) (26, 36) are known to increase plasma CCK levels and reduce food intake in humans, monkeys, dogs, and rodents (1, 34, 36, 44, 56). L-Leu, a branched-chain amino acid, has been shown to induce CCK release in cats (2).

In recent years, there has been increasing evidence that luminal nutrients are directly detected by enteroendocrine cells (17, 27, 32, 38, 42, 45). In 1991, Fujita (22), noting anatomic similarities between endocrine cells in the gut and taste cells in the tongue, proposed a commonality of function. More recently, taste receptors, expressed in the taste cells of the lingual epithelium that detect tastants, have been shown to be expressed in intestinal endocrine cells (18, 42, 45, 63).

In the lingual epithelium, the taste receptor 1 (T1R) family [type 1 taste G protein-coupled receptors (GPCRs)] comprises three members, T1R1, T1R2, and T1R3 (31, 45). They are distantly related to metabotropic Glu receptors (mGluRs), extracellular Ca^{2+}-sensing receptor (CaSR), and GABA type B receptor (6). On the basis of electrophysiological studies, heterologous expression of taste receptor subunits, and behavioral assays of knockout mice, the heteromeric combination of T1R2-T1R3 was shown to function as a broad-specificity sweet sensor for natural sugars, sweet proteins, and artificial sweeteners, whereas the T1R1-T1R3 combination was identified as a broad-spectrum L-amino acid sensor, responsible for mediating perception of the savory "umami" taste of monosodium glutamate (35, 49). The T1R2-T1R3 and T1R1-T1R3 heteromers are coupled to the heterotrimeric G protein gustducin to transmit intracellular signals (43). In rodents and many other mammalian species, T1R1-T1R3 responds to a wide variety of L-amino acids in the millimolar range (49). However, T1R1-T1R3 is not activated by L-Trp (49).

The human T1R1-T1R3 complex functions as a much more specific receptor, responding selectively to monosodium glutamate and Asp (as well as to the Glu analog L-AP4) (6, 28, 35).

A salient feature of amino acid taste in animals and umami taste in humans is the synergistic enhancement of potency when Glu or other amino acids are combined with the monophosphate esters of inosine or guanosine nucleotides (64, 65, 67, 69). The synergistic enhancement of umami taste by IMP or GMP is an exclusive property of T1R1-T1R3 (13, 49, 70, 72). Glu and IMP/GMP bind to adjacent domains on the NH_2-terminal Venus flytrap (VFT) module of T1R1 (70), while potentiation of intracellular signal transmission is mediated through α-gustducin (24).

With respect to the intestinal epithelium, α-gustducin was initially shown to be expressed in the mouse intestine and in a murine enteroendocrine cell line, STC-1 (63). Subsequently, we demonstrated that the T1R family members T1R1, T1R2,

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and T1R3 are also expressed in the rodent gut and STC-1 cell line (18), suggesting that taste-sensing mechanisms exist in the gastrointestinal tract. More recent work has determined that T1R2, T1R3, and α-gustducin are coexpressed in enteroendocrine L and K cells in a range of species (3, 32, 42, 45) and act as the intestinal sweet sensor (17, 19, 42). Activation of the sensor directly by natural sugars and artificial sweeteners leads to secretion of gut hormones such as glucagon-like peptide (GLP)-1, GLP-2, and the gastric inhibitory peptide (GIP) (32, 33, 42). GLP-1 and GIP act as incretins to enhance insulin secretion. The binding of GLP-2 to its receptor, expressed on enteric neurons, stimulates a reflex increase in the functional expression of intestinal Na^+-glucose cotransporter 1 in absorptive enterocytes and, hence, the capacity of the gut to absorb dietary monosaccharides (42, 45, 59). The responsiveness of the intestinal sugar/sweetener sensor to various sweeteners is remarkably similar to that of T1R2-T1R3 in taste cells of the tongue, indicating that the intestinal sweet sensor has sugar selectivity similar to that in the lingual epithelium (42).

Using the STC-1 cell line and native mouse intestinal tissue, we tested the proposition that the gut-expressed T1R1-T1R3 heteromer serves as an intestinal L-amino acid sensor modulating amino acid-induced CCK release.

To this end, we selected a number of L-amino acids, Phe, Leu, Glu, and Trp, having diverse properties. Phe, Leu, and Glu stimulation of T1R1-T1R3 is enhanced in the presence of IMP, while Trp does not activate this receptor (49). Phe, Leu, and Trp are also known to evoke CCK release (2, 26, 61). We also assessed the effect of D-isomers of these amino acids to show potential specificity.

Collectively, our data demonstrate that the functional properties and cellular location of the gut-expressed T1R1-T1R3 support its role as a luminal sensor for L-amino acid-induced CCK secretion.

MATERIALS AND METHODS

Removal of mouse intestinal tissue. Male and female C57BL/6 mice, aged 8 wk, with ad libitum access to standard chow and water were housed in standard tube cages with automatically controlled temperature and humidity and a 12:12-h light-dark cycle. They were killed by cervical dislocation (UK Home Office Schedule 1 regulations), and a portion (~4 cm) of the proximal small intestine was promptly removed. Sections (1 cm) were fixed for immunohistochemical analysis, the remainder of the proximal intestine was opened longitudinally, and the serosa was removed by gentle scrapping of the serosal side of the small intestine with a scalpel. Hematoxylin-eosin staining confirmed removal of the serosa with no damage to the circular muscle layer.

Immunohistochemistry. Immunohistochemistry was performed as described previously (16). Tissue sections from mouse proximal small intestine were fixed for 4 h in 4% (wt/vol) paraformaldehyde-PBS and then placed in 20% (wt/vol) sucrose in PBS overnight. Subsequently, tissue samples were embedded in gelatin and frozen in liquid N_2-cooled isopentane before they were sectioned on a cryostat (Bright Instrument, Huntingdon, UK). Sections (8 μm thick), thaw-mounted onto polylysine-coated slides, were washed five times for 5 min each in PBS and then incubated for 1 h in blocking solution [10% (vol/vol) donkey serum in PBS] at room temperature in a humidified chamber. Sections were then incubated overnight at 4°C with primary polyclonal antibodies to T1R1 (TR11-A, raised in rabbit; Alpha Diagnostic International, San Antonio, TX; 1:200 dilution), T1R2 (T-20, raised in goat; Santa Cruz Biotechnology, Santa Cruz, CA; 1:250 dilution), T1R3 (N-20, raised in goat; H-145, raised in rabbit; Santa Cruz Biotechnology; 1:750 dilution), α-gustducin (raised in rabbit; a gift from Prof. R. F. Margolskee, Monell Chemical Senses Center, Philadelphia, PA; 1:300 dilution), or CCK-8 (raised in rabbit; a gift from Prof. G. J. Dockray, University of Liverpool; 1:200 dilution). Antibodies to secretin, GLP-1, and GIP (S-21, C-17, and Y-20, respectively, Santa Cruz Biotechnology) were raised in goat and used at a dilution of 1:100. For double- or triple-immunofluorescent labeling, tissue sections were incubated at 4°C overnight with two or three primary antibodies (raised in different species) as appropriate with no change in the final dilutions. To demonstrate antibody specificity, primary antibodies to T1R1 and CCK-8 were also preincubated with respective peptide antigens (0.5 μg/ml). After incubation of sections with primary antibodies, slides were washed five times for 5 min each in PBS and subsequently stained for 1 h at room temperature using a 1:500 dilution of Cy3- or FITC-conjugated anti-rabbit/anti-goat IgG secondary antibodies (Stratech Scientific, Newmarket, UK). The composition of the buffer containing antibodies (primary or secondary) was 2.5% (vol/vol) donkey serum, 0.25% (wt/vol) NaN_3, and 0.2% (vol/vol) Triton X-100 in PBS. Finally, slides were washed five times for 5 min each in PBS and then mounted with Vectashield Hard Set Mounting Medium with 4,6′-diaminido-2-phenylindole (Vector Laboratories, Peterborough, UK). Immunofluorescent labeling of T1R1, T1R3, α-gustducin, and CCK proteins was visualized using an epifluorescence microscope (Nikon, Kingston-Upon-Thames, UK), and images were captured with a digital camera (model C4742-96-12G04, Hamamatsu Photonics, Welwyn Garden City, UK). Images were merged using Imaging Products Laboratory imaging software (BioVision Technologies, Golden, CO).

Confocal microscopy. Mouse intestinal tissue sections, prepared as described above, were imaged using a confocal microscope (model LSM510, Zeiss, Jena, Germany) on a Zeiss Observer Z1 with a ×63/1.4 differential interference contrast oil-immersion objective lens and a Plan Apochromat ×20/0.8 M27. An argon ion laser at 488 nm, a DPSS laser at 561 nm, and a combined photo-diode pump laser and mode-locked titanium-sapphire laser (Mai-Tai, Newport Spectra-Physics) at 810 nm were used as excitation sources. Images were captured using a Zeiss LSM510 META detector and analyzed using Zeiss AIM software.

Cell culture. STC-1 mouse enteroendocrine cells (58) are derived from an intestinal endocrine tumor that developed in a double-transgenic mouse expressing the rat insulin promoter linked to the SV40 large-T antigen and the polyoma small-t antigen. These cells express CCK mRNA and secrete the biologically active form of the peptide, CCK-8. They also express T1R1, T1R3, and α-gustducin (18). STC-1 cells were maintained by serial passage in Dulbecco's modified Eagle's medium (Sigma Aldrich) containing 10% (vol/vol) fetal bovine serum, 2 mmol/l L-Glu, 100 μg/ml streptomycin, and 100 U/ml penicillin.

CCK secretion studies. STC-1 cells were grown to 70–80% confluence in 24-well plates before incubation at 37°C in HBSS (containing 1.26 mmol/l Ca^{2+})-20 mmol/l HEPES (pH 7.4) (Life Technologies, Paisley, UK) supplemented with appropriate concentrations of test agents [protein hydrolysates, peptide, amino acids, IMP, gurmarin (Gur), and NPS2143] for 1 h. Control cells were maintained simultaneously in HBSS (containing 1.26 mmol/l Ca^{2+})-20 mmol/l HEPES (pH 7.4) containing vehicle only. After 1 h, incubation buffer was collected, centrifuged to remove cell debris, and stored at −80°C until it was used to assess CCK concentrations.

Sections (~1 cm) of mouse proximal small intestine were incubated at 37°C in HBSS (containing 1.26 mmol/l Ca^{2+})-20 mmol/l HEPES (pH 7.4) supplemented with appropriate concentrations of test agents (amino acids, IMP, Gur, and NPS2143) for 1 h immediately following removal. Control tissue was maintained simultaneously in HBSS (containing 1.26 mmol/l Ca^{2+})-20 mmol/l HEPES (pH 7.4). After 1 h, incubation buffer was collected, centrifuged to remove cell debris, and stored at −80°C until it was used for determination of CCK concentrations.

Histological studies and the absence of the cytoplasmic marker lactate dehydrogenase in the incubation buffer at the termination of the 1-h incubation period confirmed cellular and tissue integrity.

CCK concentrations were measured using a commercially available enzyme immunoassay kit (Phoenix Pharmaceuticals, Burlingame, CA) according to the manufacturer's instructions. Standard curves were constructed using GraphPad Prism 5 (GraphPad Software, La Jolla, CA).

RNA interference. The cationic lipid reagent Lipofectamine 2000 (Life Technologies, Paisley, UK) was used to transfect small interfering RNA (siRNA) duplexes, designed specifically for mouse T1R1 (5′-CTGCCGGAGAACTATAACGAA-3′; GeneSolution siRNA, Qiagen, Crawley, UK), into STC-1 cells (10). Cells were grown to 40–50% confluence and incubated for 4 h in serum-free medium containing siRNA duplex (100 nmol/l final concentration) plus Lipofectamine 2000 (1 μl/50 pmol siRNA duplex). After 4 h, the medium was resupplemented with fetal bovine serum. Inhibition of T1R1 expression, at mRNA and protein levels, was assessed by quantitative real-time RT-PCR and Western blot analysis, respectively, at 48 h posttransfection. Transfections using unrelated nonsilencing siRNA (Qiagen) were used as controls. Parallel transfection of STC-1 cells with a fluorescent oligonucleotide (Life Technologies) confirmed efficiency of transfection to be >80% (data not shown).

Quantitative real-time RT-PCR. Relative expression of T1R1 mRNA in control and siRNA "knockdown" STC-1 cells and T1R1, T1R3, and CCK mRNA in mouse small intestinal tissue was determined by quantitative PCR. RNA, isolated from STC-1 cells and mouse small intestine, was used as template for first-strand cDNA synthesis. Purified cDNA was quantified by UV spectrophotometry (with the assumption of an optical density at 260 nm of 1 = 33 μg/ml) and diluted to a final concentration of 5 μg/ml. Real-time PCR assays were then performed using 25 ng of cDNA as template to assess relative mRNA abundance of T1R1, T1R3, and CCK. Real-time amplification of β-actin (ACTB) and RNA polymerase II (POLR2A) was carried out simultaneously as control references. Primer sequences for mouse T1R1 [5′-ACTCTGAGTGGCGGCTTCA-3′ (sense) and 5′-GAAAGTGTTCTGTGTTGTTGAGTTCTG-3′ (antisense)], T1R3 [5′- AGTTCTGCTTTGGCCTGATCT-3′ (sense) and 5′-AGGGAGGTGAGCCATTGGT-3′ (antisense)], and CCK [5′-CTGCTAGCGCGATACATCCA-3′ (sense) and 5′-CCAGGCTCTGCAGGTTCTTAA-3′ (antisense)] were designed from the corresponding mRNA sequences and diluted into a 20× stock (18 μmol/l each primer). Each reaction consisted of 12.5 μl of 2× SYBR Green JumpStart *Taq* ReadyMix for quantitative PCR (Sigma Aldrich), 1.25 μl of 20× target gene stock (final concentration 900 nmol/l each primer), 6.25 μl of double-distilled H_2O, and 5 μl of cDNA (5 μg/ml). PCR cycling was performed as follows: initial denaturation at 95°C for 2 min followed by 30–40 cycles of 95°C for 15 s and 60°C for 60 s. Assays were performed in triplicate using a Rotorgene 3000 (Qiagen), and relative abundance was calculated using RG-3000 comparative quantification software.

Membrane isolation. The procedure for the isolation of postnuclear membranes (PNMs) is described elsewhere (46). Accordingly, STC-1 cells, suspended in hypotonic buffer [100 mmol/l mannitol, 2 mmol/l HEPES-Tris (pH 7.1), 0.5 mmol/l DTT, 0.2 mmol/l benzamidine, and 0.2 mmol/l PMSF] were homogenized for 20 s using a Polytron (Ystral). The homogenate was centrifuged for 10 min at 500 *g* (SS 34 rotor, Sorvall). The supernatant was subsequently decanted and centrifuged for 30 min at 30,000 *g* to pellet PNMs, which were resuspended in isotonic buffer [300 mmol/l mannitol, 20 mmol HEPES-Tris (pH 7.4), 0.2 mmol/l $MgSO_4$, and 0.02% (wt/vol) NaN_3] and further homogenized by passage 10 times through a Hamilton syringe (Scientific Glass Engineering, Ringwood, Australia). All steps were carried out at 4°C. Protein concentration in the PNM suspension was calculated by its ability to bind Coomassie blue according to the Bio-Rad assay technique (Bio-Rad, Hemel Hempstead, UK), with porcine γ-globulin as standard. PNMs were then diluted in sample buffer [62.5 mmol/l Tris·HCl (pH 6.8), 10% (vol/vol) glycerol, 2% (wt/vol) SDS, 0.05% (vol/vol) β-mercaptoethanol, and 0.05% (wt/vol) bromophenol blue] and stored at −20°C until they were used for Western blotting.

Western blot analysis for assessing T1R1 protein abundance in control and siRNA knockdown STC-1 cells. Protein components of PNMs were separated by SDS-PAGE using 8% (wt/vol) polyacrylamide gels containing 0.1% (wt/vol) SDS and electrotransferred to a polyvinylidene difluoride (PVDF) membrane (Immun-Blot, Bio-Rad). Nonspecific binding sites were blocked by incubation of PVDF membranes for 1 h at room temperature in TTBS buffer [Tris-buffered saline + 0.05% (vol/vol) Tween 20] containing 5% (wt/vol) nonfat dry milk. PVDF membranes were then incubated with T1R1 antibody (TR11-A, raised in rabbit; Alpha Diagnostic International) using a concentration of 3.5 μg/ml in TTBS with 1% (wt/vol) nonfat dry milk for 18 h at 4°C. Immunoreactive bands were detected by incubation for 1 h at room temperature with affinity-purified horseradish peroxidase-linked anti-mouse secondary antibody (Dako, Cambridge, UK) diluted 1:2,000 in TTBS containing 1% (wt/vol) nonfat dry milk and visualized using WEST-one Western blot detection system (Chembio, Hertfordshire, UK) according to the manufacturer's instructions. The intensity of the immunoreactive bands was quantified using scanning densitometry (Phoretix 1D quantifier, Non-Linear Dynamics, Newcastle-Upon-Tyne, UK). PVDF membranes were subsequently stripped: they were washed three times for 10 min each in 137 mmol/l NaCl and 20 mmol/l glycine-HCl (pH 2.5) and then probed with a monoclonal antibody to β-actin (clone AC-15, Sigma Aldrich; 1:10,000 dilution), which was used as a loading control.

Statistical analysis. Values are means ± SD. Significance of differences was determined using one-way ANOVA with Bonferroni's multiple comparison test (GraphPad Prism 5). Results were considered significant if $P < 0.05$.

RESULTS

STC-1 cells release CCK in response to protein hydrolysates, peptides, and L-amino acids. A number of previous studies have shown that protein hydrolysates, peptides, and L-amino acids stimulate STC-1 cells to secrete CCK (7, 21, 25, 37, 48, 52, 60). In Figs. 1 and 2, we show levels of CCK secretion by STC-1 cells in response to the following test agents: meat protein hydrolysate (MH; Primatone, Sigma Aldrich), albumin egg hydrolysate (AEH; Sigma Aldrich), soy

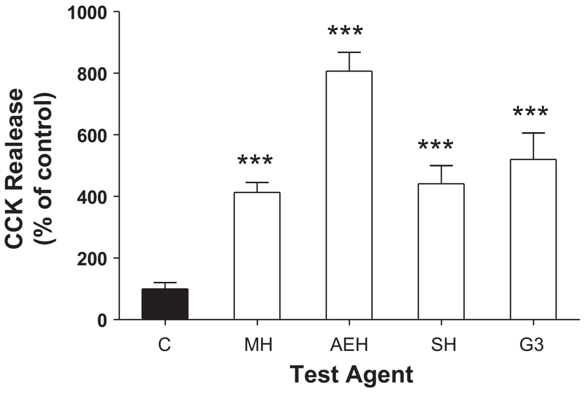

Fig. 1. CCK secretion by STC-1 cells in response to protein hydrolysates and a tripeptide. Confluent STC-1 cells were incubated for 1 h at 37°C in HBSS (containing 1.26 mmol/l Ca^{2+})-20 mmol/l HEPES (pH 7.4) supplemented with protein hydrolysates or with a glutamate tripeptide (G3) or were untreated. Values are means ± SD; $n = 15$ control (C), $n = 8$ meat hydrolysate [MH, 1% (wt/vol)], albumin egg hydrolysate [AEH, 0.2% (wt/vol)], and soy protein hydrolysate [SH, 1% (wt/vol)], and $n = 10$ G3 (10 mmol/l). $***P < 0.001$.

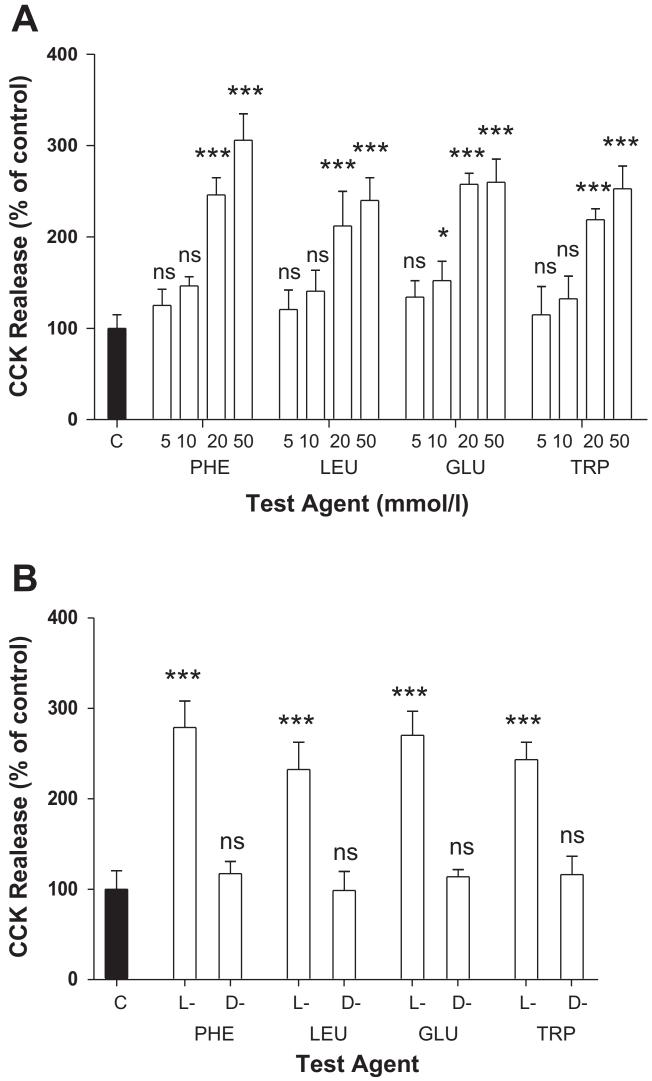

Fig. 2. CCK secretion by STC-1 cells in response to L- and D-amino acids. Confluent STC-1 cells were incubated for 1 h at 37°C in HBSS (containing 1.26 mmol/l Ca^{2+})-20 mmol/l HEPES (pH 7.4) supplemented with L-amino acids at 5, 10, 20, and 50 mmol/l (*A*) or L- and D-amino acids at 20 mmol/l (*B*). Values are means ± SD; $n = 3$ untreated control (C) in *A* and 15 in *B*, $n = 3$ Phe in *A* and 18 in *B*, $n = 3$ Leu in *A* and 18 in *B*, $n = 3$ Glu in *A* and 18 in *B*, $n = 3$ Trp in *A* and 12 in *B*. $^*P < 0.05$; $^{***}P < 0.001$; ns, not significant.

protein hydrolysate (SH), glutamate tripeptide (G3; Bachem, Bubendorf, Switzerland), and L- and D-amino acids (Phe, Leu, Glu, and Trp; Sigma Aldrich). All test agents, except D-amino acids, significantly increased CCK secretion from STC-1 cells compared with untreated controls. Protein hydrolysates [1% (wt/vol) MH, 0.2% (wt/vol) AEH, and 1% (wt/vol) SH] increased CCK secretion by 413 ± 32% ($n = 8$), 807 ± 62% ($n = 8$), and 441 ± 59% ($n = 8$), respectively, compared with untreated control cells ($n = 15$, $P < 0.001$ for all; Fig. 1). CCK secretion in response to 10 mmol/l G3 was increased by 520 ± 85% ($n = 10$, $P < 0.001$; Fig. 1). The optimal concentration of L-amino acids required to induce CCK release (20 mmol/l) was determined by measuring CCK secretion in the presence of a range (5–50 mmol/l) of amino acid concentrations (Fig. 2*A*). L-Amino acids, Phe, Leu, Glu, and Trp at 20 mmol/l increased CCK secretion by 279 ± 29% ($n = 18$), 232 ± 30% ($n = 18$), 270 ± 27% ($n = 18$), and 243 ± 19% ($n = 12$), respectively ($P < 0.001$ for all; Fig. 2*B*). To assess if CCK release in response to amino acids is specific, we also determined the effect of D-isomers of these amino acids on CCK secretion. None of the D-isomers induced CCK release from STC-1 cells ($n = 3$; Fig. 2*B*).

Inhibition of T1R1 mRNA and protein expression in STC-1 cells by siRNA. To determine the potential contribution of T1R1-T1R3 to CCK secretion by STC-1 cells, we inhibited the expression of T1R1 by siRNA and determined its effect on the ability of the receptor to initiate pathways resulting in CCK release. The effect of siRNA inhibition on T1R1 mRNA and protein expression was assessed by quantitative real-time RT-PCR and Western blot analysis, respectively. Results demonstrated a significant decline in T1R1 mRNA and protein abundance that was evident at 48 h post-siRNA transfection; relative T1R1 mRNA abundance in siRNA knockdown cells was 0.36 ± 0.06 compared with control cells, a reduction of >60% ($n = 4$, $P < 0.001$; Fig. 3*A*), whereas T1R1 protein abundance was 0.56 ± 0.12 compared with control cells, a reduction of ~45% ($n = 3$, $P < 0.05$; Fig. 3*B*).

Inhibition of T1R1 expression has no effect on protein hydrolysate- or peptide-induced CCK release by STC-1 cells. Control and T1R1 knockdown STC-1 cells were treated with 1% (wt/vol) MH, 0.2% (wt/vol) AEH, 1% (wt/vol) SH, and 10 mmol/l G3, as described above. Inhibition of T1R1 expression had no effect on CCK release by STC-1 cells in response to protein hydrolysates and G3 ($n = 3$ for all; Fig. 3*C*). The data indicate that T1R1 does not play a role in the sensing of proteins or peptides by STC-1 cells leading to CCK secretion.

Inhibition of T1R1 expression diminishes L-amino acid-induced CCK release by STC-1 cells. Control and T1R1 knockdown STC-1 cells were treated with 20 mmol/l L-amino acids Phe, Leu, Glu, and Trp, as described above. There was a significant decline in CCK secretion in response to amino acids in T1R1 knockdown cells (Fig. 3*D*). CCK release by control STC-1 cells in response to 20 mmol/l Phe, Leu, Glu, and Trp was 289 ± 20%, 253 ± 16%, 258 ± 21%, and 247 ± 17%, respectively, compared with untreated control cells ($n = 3$ for all). In T1R1 knockdown cells, however, the amount of CCK released declined to 233 ± 17% in response to Phe ($n = 3$, $P < 0.05$), 199 ± 25% in response to Leu ($n = 3$, $P < 0.05$), and 192 ± 27% in response to Glu ($n = 3$, $P < 0.05$). Overall, there was a diminution in CCK secretion of 30–40% when expression of T1R1 was inhibited by siRNA. CCK secretion in response to Trp, however, was unaffected by inhibition of T1R1 ($n = 3$). We therefore conclude that T1R1 participates in the sensing of L-amino acids Phe, Leu, and Glu by STC-1 cells leading to CCK secretion.

Effect of IMP on L-amino acid-induced CCK release by STC-1 cells. The taste of umami via T1R1-T1R3 is specifically enhanced with the addition of IMP (13, 49, 64, 65, 67, 69–71). To provide further evidence for the role of T1R1-T1R3 in the sensing of L-amino acids leading to CCK release by STC-1 cells, we supplemented each amino acid with 2.5 mmol/l IMP (Sigma Aldrich). The addition of IMP to Phe, Leu, and Glu resulted in a significant increase in CCK secretion by STC-1 cells (Fig. 4*A*). In response to Phe, CCK secretion increased from 275 ± 42% to 348 ± 31% when IMP was added ($P < 0.01$, $n = 6$). In response to Leu, CCK release increased from 238 ± 30% to 294 ± 33% with addition of IMP ($P <$

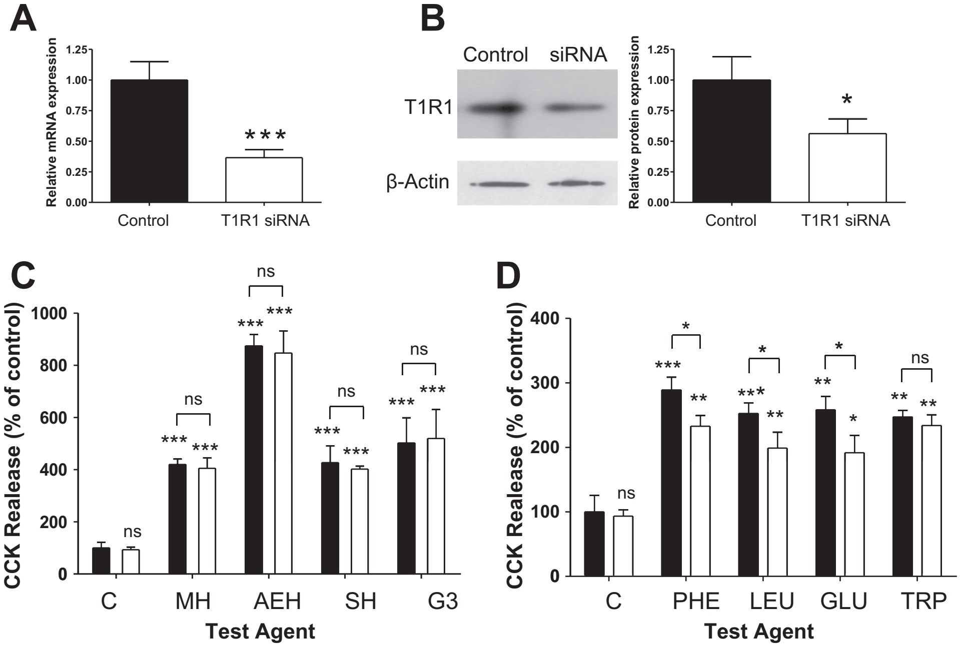

Fig. 3. Inhibition of taste 1 receptor type 1 (T1R1) expression by small interfering RNA (siRNA) and effect on protein hydrolysate-, peptide-, and L-amino acid-induced CCK release. siRNA duplexes, designed specifically for mouse T1R1, were transfected into STC-1 cells. Transfections using unrelated nonsilencing siRNA (Qiagen) were used as controls. *A* and *B*: T1R1 mRNA expression assessed by quantitative real-time PCR ($n = 4$) and protein abundance by Western blot analysis ($n = 3$) 48 h posttransfection. Expression of β-actin was used as loading control. *C* and *D*: control (solid bars) and T1R1 "knockdown" (open bars) STC-1 cells were incubated for 1 h at 37°C in HBSS (containing 1.26 mmol/l Ca^{2+})-20 mmol/l HEPES (pH 7.4) supplemented with protein hydrolysates, G3, or L-amino acids or were untreated [control (C)]. Values are means ± SD; $n = 3$. See Fig. 1 and 2 legends for abbreviations and concentrations of protein hydrolysates, G3, and amino acids. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

0.05, $n = 6$); in response to Glu, IMP addition increased CCK secretion from 276 ± 30% to 368 ± 35% ($P < 0.001$, $n = 6$) compared with untreated control cells ($n = 6$). However, Trp-induced CCK secretion was not enhanced by inclusion of IMP ($n = 6$), and IMP alone had no effect on CCK secretion in STC-1 cells ($n = 6$; Fig. 4*A*). As IMP enhances amino acid sensing exclusively via T1R1-T1R3, these data strongly support the proposition that T1R1-T1R3

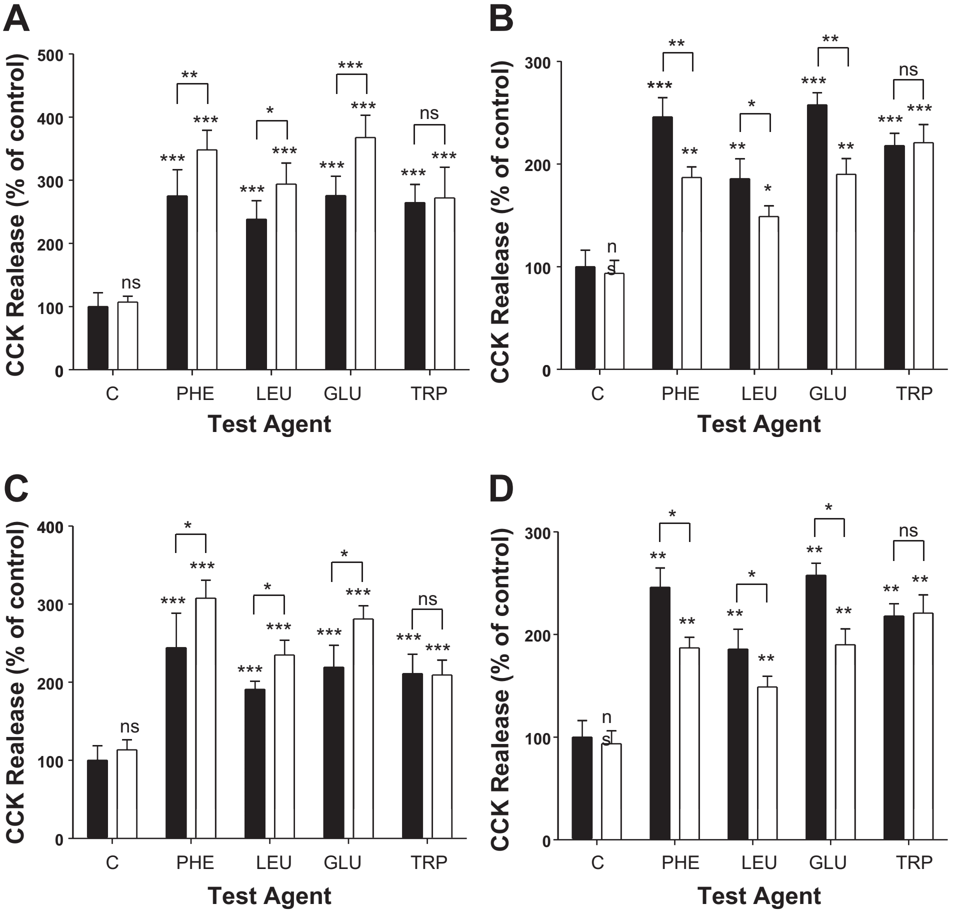

Fig. 4. Effects of IMP or gurmarin (Gur) on L-amino acid-induced CCK release by STC-1 cells and mouse proximal intestine. STC-1 cells (*A* and B) and mouse proximal intestinal tissues (*C* and *D*) were incubated for 1 h at 37°C in HBSS (containing 1.26 mmol/l Ca^{2+})-20 mmol/l HEPES (pH 7.4) supplemented with L-amino acids or were untreated in the absence (solid bars) or presence (open bars) of 2.5 mmol/l IMP (*A* and *C*) or 30 μg/ml Gur (*B* and *D*). Values are means ± SD; $n = 6$ untreated in *A* and 3 in *B–D*, $n = 6$ Phe (20 mmol/l) in *A*, 3 in *B* and *D*, and 12 in *C*, $n = 6$ Leu (20 mmol/l) in *A*, 3 in *B* and *D*, and 12 in *C*, $n = 6$ Glu (20 mmol/l) in *A*, 3 in *B* and *D*, and 12 in *C*, $n = 6$ Trp (20 mmol/l) in *A*, 3 in *B* and *D*, and 9 in *C*. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

is a sensor for Phe, Leu, and Glu in STC-1 cells leading to CCK secretion.

Effect of gurmarin on L-amino acid-induced CCK release by STC-1 cells. Gur is an inhibitor of the sweet taste receptor T1R2-T1R3 (29, 53, 68) through binding to the extracellular VFT module of T1R3 (41). Thus, binding of Gur to T1R3 has the potential to also inhibit sensing by T1R1-T1R3. Indeed, a number of studies have shown that Gur administration inhibits umami signaling (54, 66). To determine the effect of Gur on CCK release by STC-1 cells in response to L-amino acids, cells were preincubated with 30 μg/ml Gur for 30 min before addition of amino acids. As shown in Fig. 4*B*, preincubation of STC-1 cells with Gur reduced CCK secretion in response to Phe, Leu, and Glu from 246 ± 19% to 187 ± 10% ($n = 3$, $P < 0.01$), from 186 ± 23% to 140 ± 17% ($n = 3$, $P < 0.05$), and from 258 ± 12% to 190 ± 15% ($n = 3$, $P < 0.01$), respectively, compared with untreated control cells ($n = 3$). These values represent a decline in CCK secretion of 40–45% when STC-1 cells are preincubated with 30 μg/ml Gur. The lowest concentration of Gur required to inhibit amino acid-induced CCK release was assessed using 5–50 μg/ml Gur (data not shown). Preincubation with Gur had no effect on Trp-induced CCK secretion ($n = 3$). Gur alone had no effect on CCK secretion in STC-1 cells ($n = 3$). The data further support the role of T1R1-T1R3 in L-amino acid sensing leading to CCK secretion.

Assessments of the role of T1R1-T1R3 as an amino acid sensor in native intestinal tissue. Next, we assessed if T1R1-T1R3, shown to be expressed in the mouse intestine (16), acts as an L-amino acid sensor in mouse intestinal tissue. Using mouse small intestinal tissue and quantitative PCR, we demonstrated that CCK expression was 85% higher in the proximal than distal intestine and that the relative expression of T1R1 and T1R3 in proximal and distal intestine remained constant ($n = 3$, $P < 0.05$; Fig. 5). Subsequently, using triple immunohistochemistry, we demonstrated that CCK, T1R1, T1R3, and the α-subunit of gustducin are expressed in mouse proximal intestine. Moreover, CCK-expressing cells also possessed T1R1 and T1R3 (Fig. 6*A*). Furthermore, T1R1, T1R3, and α-gustducin were also coexpressed (Fig. 6*B*). Using confocal microscopy, we demonstrated that T1R1 and CCK proteins are expressed in distinct cellular domains of the same endocrine cell (Fig. 7): T1R1 is localized at the apical region, and CCK resides in the basal domain. A similar pattern of expression was also observed for coexpression of T1R3 and CCK (data not shown).

Preincubation of T1R1 and CCK primary antibodies with the corresponding peptide antigen led to the blocking of immunoreactive signals, indicating the specificity of the labeling (Fig. 6*A*, Control). Specificity of T1R3 primary antibody labeling has been previously validated (3, 12, 45).

We also assessed the frequency of T1R1, T1R3, and CCK coexpression in sections of mouse proximal intestine by immunohistochemistry. Repeated observations indicated that endocrine cells that possessed T1R1 consistently contained CCK (Fig. 8*A*); however, only ~50% of T1R3-containing cells possessed CCK (Fig. 8*B*). In proximal intestinal tissues from 6 mice, in a total of 19 enteroendocrine cells expressing T1R3, 9 coexpressed T1R1 and CCK. T1R2, the sweet receptor subunit, was never coexpressed with CCK (Fig. 8*C*), nor was T1R2 coexpressed with T1R1 (Fig. 8*D*).

The potential location of T1R1 in enteroendocrine cells other than CCK-containing I cells was investigated. When sections of mouse proximal intestine were probed with antibodies to gut hormone markers of S cells (secretin), L cells (GLP-1), and K cells (GIP), the merged images demonstrated that T1R1 is not expressed in S (Fig. 9*A*), L (Fig. 9*B*), or K (Fig. 9*C*) enteroendocrine cells.

Properties of CCK secretion by mouse proximal small intestine. Having shown that mouse proximal intestine expresses T1R1 and T1R3 in CCK-containing endocrine cells and that T1R1-T1R3 is expressed at the apical region, we aimed to determine whether *1*) mouse intestinal tissue secretes CCK in response to the range of L-amino acids tested, *2*) the level of CCK secretion is affected by IMP, the activator of T1R1-T1R3, and *3*) there are similarities in the pattern of CCK release between STC-1 cells and native mouse intestinal tissue. Sections of mouse proximal small intestine were exposed to the L-amino acids described above in the presence or absence of 2.5 mmol/l IMP. Exposure of intestinal tissue to 20 mmol/l Phe, Leu, Glu, or Trp evoked significant CCK release. CCK secretion increased by 244 ± 44% in response to Phe ($n = 12$, $P < 0.001$), by 191 ± 11% in response to Leu ($n = 6$, $P < 0.001$), by 219 ± 28% in response to Glu ($n = 12$, $P < 0.001$), and by 211 ± 25% in response to Trp ($n = 9$, $P < 0.001$) compared with untreated control tissue ($n = 12$; Fig. 4*C*). Phe-, Leu-, and Glu-induced CCK secretion was enhanced by addition of IMP. CCK release in response to Phe increased to 308 ±

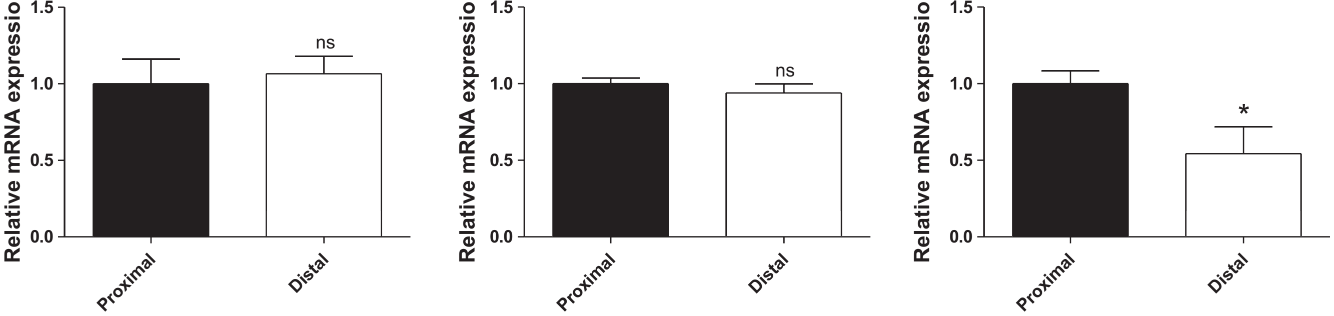

Fig. 5. Relative mRNA expression of T1R1, T1R3, and CCK in proximal and distal regions of mouse small intestine as determined by quantitative real-time RT-PCR. Values are means ± SD; $n = 3$. *$P < 0.05$.

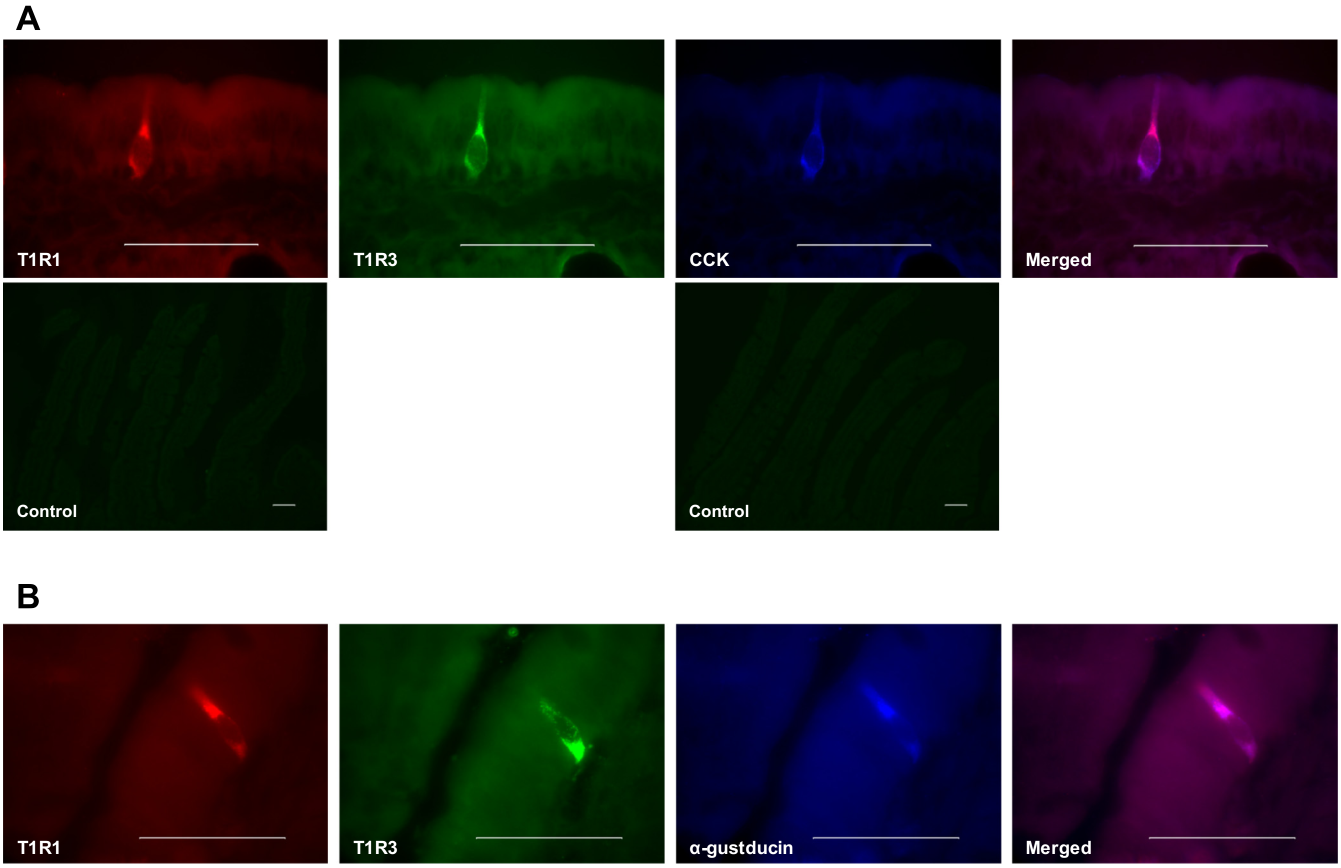

Fig. 6. Coexpression of T1R1, T1R3, CCK, and α-gustducin in mouse proximal intestine. Representative micrographs show expression of T1R1, T1R3, CCK, and α-gustducin in serial sections of mouse proximal intestine as determined by triple immunohistochemistry. Merged images (purple) show colocalization of T1R1, T1R3, and CCK (*A*) and T1R1, T1R3, and α-gustducin (*B*) in the same enteroendocrine cells. Preincubation of primary antibodies to T1R1 and CCK with the corresponding immunizing peptide blocked specific staining in mouse proximal intestine (*A*, Control). Scale bars, 10 μm.

23% ($n = 3$, $P < 0.05$) when IMP was added. In response to Leu, CCK secretion increased to 235 ± 19% ($n = 3$, $P < 0.05$) with inclusion of IMP; in response to Glu, IMP addition enhanced CCK release to 281 ± 17% ($n = 3$, $P < 0.05$; Fig. 4*C*). In contrast, Trp-induced CCK secretion was not enhanced by inclusion of IMP ($n = 3$), and IMP alone had no effect on CCK secretion ($n = 3$; Fig. 4*C*). The data support the notion that T1R1-T1R3 acts as a sensor for Phe-, Leu-, and Glu-induced CCK secretion in mouse proximal intestine and that the properties of L-amino acid sensing in STC-1 cells resemble those of the native intestinal tissue.

Gurmarin inhibits L-amino acid-induced CCK release by mouse proximal small intestine. Sections of mouse proximal small intestine were preincubated with 30 μg/ml Gur for 30 min before addition of L-amino acids. As shown in Fig. 4*D*, preincubation of intestinal tissue with Gur reduced CCK se-

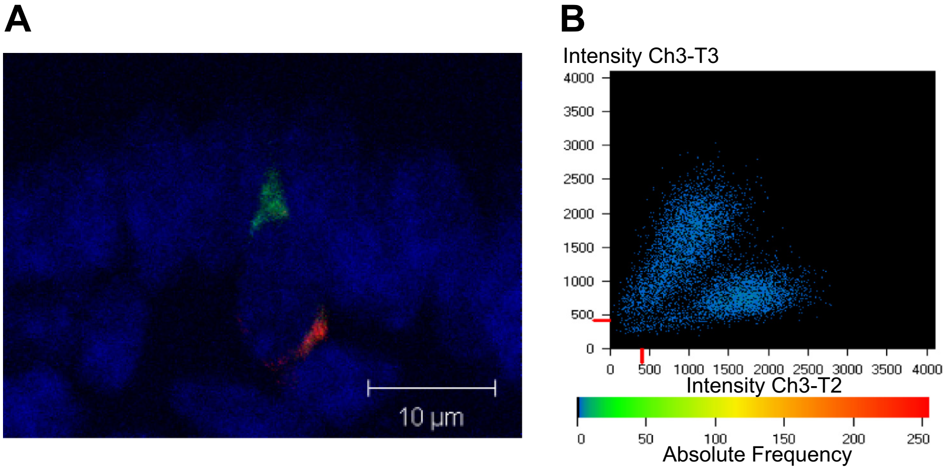

Fig. 7. T1R1 and CCK are expressed in distinct cellular domains. *A*: typical double-immunofluorescent confocal microscopy image showing expression of T1R1 (green) at the apical region and CCK (red) in the basal domain of the same endocrine cell in mouse proximal intestine. Nuclei are stained with 4′,6-diaminido-2-phenylindole (blue). *B*: scatter plot demonstrating the presence of 2 distinct antibody-labeling sites [Ch3-T3 (green) and Ch3-T2 (red)].

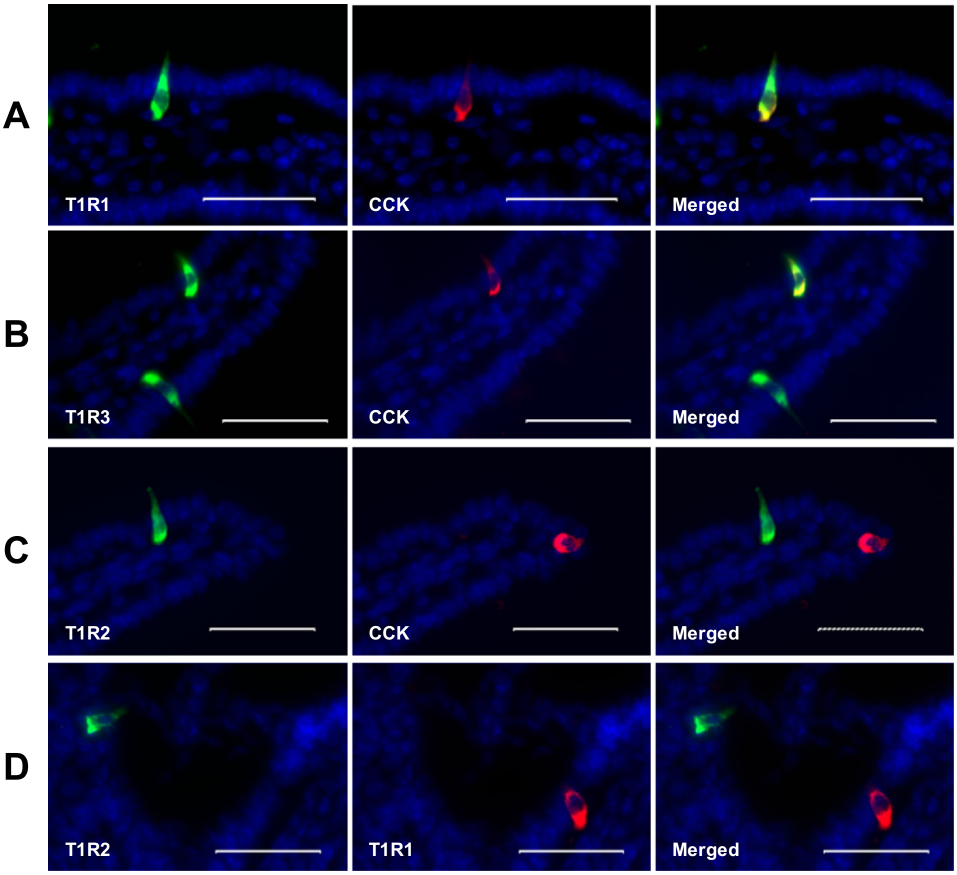

Fig. 8. Immunofluorescent images demonstrating expression of T1R subunits and CCK. Typical double-immunofluorescent images show that enteroendocrine cells that possess T1R1 consistently contain CCK (*A*), but T1R3-containing cells do not always express CCK (*B*). T1R2, the sweet receptor subunit, is not coexpressed with CCK (*C*) or with T1R1 (*D*). Scale bars, 10 μm.

cretion in response to Phe from 225 ± 30% to 179 ± 14% ($n = 3$, $P < 0.05$), in response to Leu from 195 ± 17% to 160 ± 20% ($n = 3$, $P < 0.05$), and in response to Glu from 239 ± 34% to 178 ± 12% ($n = 3$, $P < 0.05$) compared with untreated control tissue ($n = 3$). Preincubation with Gur had no effect on Trp-induced CCK secretion ($n = 3$). Gur alone had no effect on CCK secretion ($n = 3$). The data further support the role of T1R1-T1R3 in amino acid sensing in mouse small intestine evoking CCK secretion.

Effect of NPS2143 on L-amino acid-induced CCK release by mouse proximal small intestine. NPS2143 is a CasR antagonist (51) and should therefore inhibit L-amino acid-induced CCK release mediated by CasR. Sections of mouse proximal small intestine were exposed to Phe, Leu, Glu, and Trp in the presence or absence of 25 μmol/l NPS2143 (Santa Cruz Biotechnology). Figure 10 shows that inclusion of the CaSR antagonist results in ~55% inhibition of Phe-induced CCK secretion (from 218 ± 19% to 153 ± 19%, $n = 3$, $P < 0.05$) and total (100%) inhibition of Trp-induced CCK release, from 205 ± 13% to 118 ± 14% ($n = 3$, $P < 0.01$), compared with untreated control tissue ($n = 3$). However, Leu- or Glu-induced CCK secretion was not affected by NPS2143 ($n = 3$), and no effect on CCK secretion in mouse intestinal tissue was observed with NPS2143 alone ($n = 3$). These data suggest that CasR plays a role in Phe- and Trp-induced CCK release, as reported previously (26, 39, 61), but is not involved in Leu- or Glu-induced CCK secretion in mouse intestine. Inclusion of NPS2143 (25 μmol/l) and Gur (30 μg/ml) together totally inhibited Phe-induced CCK secretion ($n = 3$, $P < 0.01$; Fig. 10).

DISCUSSION

T1R1-T1R3 is expressed in taste cells of the lingual epithelium and is coupled to the G protein gustducin. T1R1 and T1R3 combine to function as a broad-spectrum L-amino acid sensor in the lingual epithelium. In rodents, the receptor responds to most of the 20 standard amino acids at millimolar concentrations (49). However, T1R1-T1R3 is not activated by Trp (49). A key feature of T1R1-T1R3 activation is its exclusive synergy with the 5′-monophosphate esters IMP and GMP (13, 49, 64, 65, 67, 69–71), which act as positive allosteric modulators. Ligand binding of Glu and IMP/GMP occurs through adjacent sites on the extracellular VFT module of T1R1 (70), while potentiation of signal transduction by IMP is mediated through α-gustducin (24). Mutation analysis of T1R1 has identified four amino acid residues within the VFT module that are critical for binding of IMP/GMP (70). Among the proposed lingual and/or intestinal epithelial amino acid receptors, T1R1-T1R3, mGluR4, mGluR1, and CaSR, only T1R1 contains all four critical residues required for IMP/GMP binding. Therefore, signal transduction in response to amino acids by other receptors cannot be enhanced by addition of these nucleotide monophosphate esters (67, 70).

It has previously been shown that the sweet taste receptor T1R2-T1R3, expressed in enteroendocrine L and K cells, acts as the sensor for sugars, evoking release of gut hormones such as GIP, GLP-1, and GLP-2 (32, 42). Therefore, we aimed to determine if gut-expressed T1R1-T1R3 is involved in sensing luminal amino acids, activating a pathway leading to CCK release.

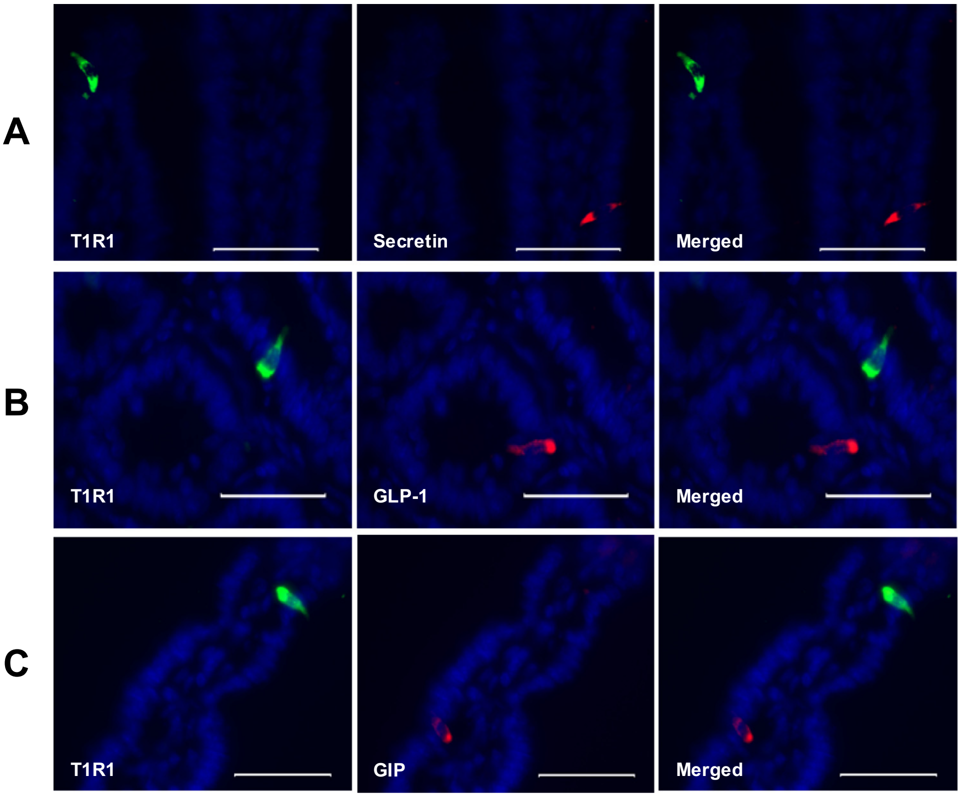

Fig. 9. Immunohistochemical localization of T1R1 and various gut hormones. Serial sections of mouse proximal intestine were immunostained with antibodies fluorescently labeled to T1R1 and the gut hormones secretin (marker for S cells), glucagon-like peptide (GLP)-1 (marker for L cells), and gastric inhibitory peptide (GIP, marker for K cells). Merged images demonstrate that T1R1 is not expressed in S, L, or K endocrine cells of mouse proximal intestine. Scale bars, 10 μm.

In light of in vivo and in vitro studies demonstrating that, in response to protein hydrolysates, peptides, and amino acids, intestinal tissue and the murine enteroendocrine cell line STC-1 secrete CCK (7, 14, 21, 25, 37, 48, 52, 55, 60), we aimed to assess the responsiveness of T1R1-T1R3 to these substrates through the secretion of CCK. We first used the CCK-secreting cell line STC-1, which expresses T1R1, T1R3, and α-gustducin (18, 63).

We demonstrated that, in response to protein hydrolysates and a tripeptide, STC-1 cells secrete CCK, as has been shown previously (7, 21, 25, 37, 48, 52, 60) (Fig. 1). Analysis of the protein hydrolysates indicated that concentrations of individual free amino acids ranged from 0.1 to 5 mmol/l (Sigma Aldrich Technical Services), below the concentration of amino acids required to evoke CCK release in STC-1 cells (10–50 mmol/l) (26; present study). Exposure of STC-1 cells to the individual L-amino acids Phe, Leu, Glu, and Trp at 20 and 50 mmol/l also provoked CCK secretion (Fig. 2). However, the D-isoforms of Phe, Leu, Glu, and Trp did not stimulate CCK release in STC-1 cells, strong evidence for the specificity of the effect of L-isoforms (Fig. 2*B*).

Inhibition of T1R1 expression in STC-1 cells by siRNA (Fig. 3, *A* and *B*) had no effect on CCK secretion in response to protein hydrolysates and G3 (Fig. 3*C*). Accordingly, we conclude that T1R1-T1R3 is not the sensor for protein hydrolysates and peptides. In contrast, inhibition of T1R1 expression led to a significant decrease in CCK secretion by STC-1 cells in response to the L-amino acids Phe, Leu, and Glu, but not Trp (Fig. 3*D*). Trp is a high-potency activator of CaSR (10) but is inactive for T1R1-T1R3 heteromers (49). These results support the notion that the gut-expressed T1R1-T1R3 combination possesses the characteristics of an L-amino acid sensor in STC-1 cells, modulating CCK secretion.

To further characterize the functional properties of T1R1-T1R3, IMP, the specific potentiator of T1R1-T1R3 activation (13, 49, 64, 65, 67, 69–71), when included in the incubation buffer, significantly enhanced the levels of CCK release by STC-1 cells in response to Phe, Leu, and Glu, but not Trp (Fig. 4*A*).

Moreover, preincubation of STC-1 cells with Gur inhibited CCK secretion significantly in response to Phe, Leu, and Glu but had no effect on Trp-induced CCK release (Fig. 4*B*). Gur has been shown to inhibit umami-sensitive chorda tympani nerve responses arising from rodent lingual epithelium on exposure to monosodium glutamate (54, 66).

We conclude that, in STC-1 cells, the functional characteristics of T1R1-T1R3 support its role as a sensor for Phe-, Leu-, and Glu-induced CCK release.

To determine if T1R1-T1R3 in native mouse intestinal tissue also acts as a sensor for L-amino acid-induced CCK release, we first determined the expression of T1R1, T1R3, and CCK in mouse small intestine. Using quantitative PCR, we demonstrated that T1R1, T1R3, and CCK mRNA is expressed in mouse intestinal tissue and that the level of CCK mRNA is significantly higher in proximal than distal small intestine (Fig. 5). By immunohistochemistry, we demonstrated that T1R1, T1R3, and CCK are coexpressed in the same endocrine cells and that T1R1, T1R3, and α-gustducin are similarly coexpressed (Fig. 6). Using confocal microscopy, we showed that T1R1 expression is confined to the apical region, while CCK resides at the basal domain (Fig. 7). This expression pattern of T1R1 and CCK is identical to that of CaSR and CCK in isolated primary I cells reported by Liou et al. (39). GPCRs, such as T1R1-T1R3 and CaSR, are known to internalize via

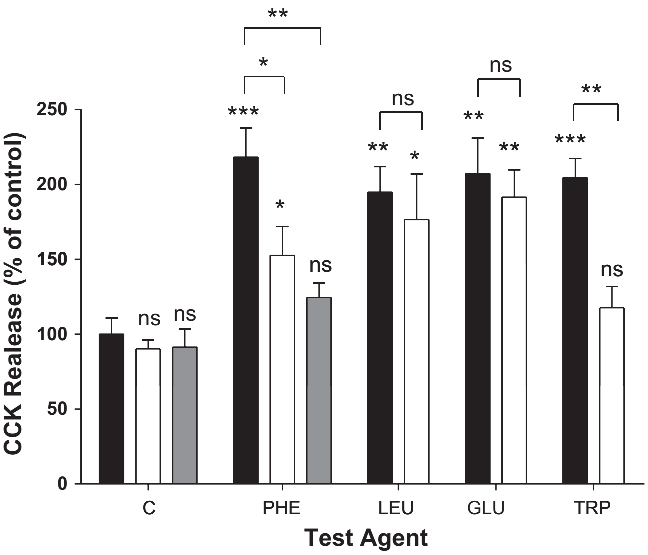

Fig. 10. Effect of NPS2143 on L-amino acid-induced CCK release by mouse proximal small intestine. Mouse proximal intestinal tissues were incubated for 1 h at 37°C in HBSS (containing 1.26 mmol/l Ca^{2+})-20 mmol/l HEPES (pH 7.4) supplemented with L-amino acids (20 mmol/l) or were untreated in the absence (solid bars) or presence of 25 μmol/l NPS2143 (open bars) or NPS2143 + 30 μg/ml Gur (shaded bars). Values are means ± SD; $n = 3$. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

endocytosis (5, 20, 31, 40), resulting in antibody labeling of the apical region, rather than just the luminal plasma membrane.

Furthermore, cells expressing T1R1 consistently contained CCK (Fig. 8*A*), but CCK was present in only 9 of 19 (~50%) T1R3-containing cells (Fig. 8*B*). This can be understood, since T1R3 also combines with T1R2 to act as the sweet receptor (17, 42). Neither CCK nor T1R1 was coexpressed with T1R2 (Fig. 8, *C* and *D*), eliminating any indirect role of T1R2 in amino acid sensing or CCK release. Immunohistochemical analysis using antibodies to secretin, GLP-1, and GIP confirmed that T1R1 is not expressed by S, L, or K endocrine cells in mouse small intestine (Fig. 9) and that T1R1 expression is confined to CCK-containing I cells (Fig. 8*A*). The coexpression of T1R subunits and α-gustducin further confirmed their association (Fig. 6*B*).

Having shown that mouse proximal intestine expresses CCK, T1R1, T1R3, and α-gustducin and that they reside in the same endocrine cells, we next examined the responsiveness of the tissue to L-amino acids via induction of CCK release. Mouse proximal intestinal explants were exposed to the L-amino acids Phe, Leu, Glu, and Trp. In response to Phe, Leu, and Glu, the gut epithelium secreted CCK, and CCK secretion was enhanced by addition of IMP (Fig. 4*C*). However, CCK secretion induced by Trp was not enhanced by IMP (Fig. 4*C*). Furthermore, CCK release in response to Phe, Leu, and Glu, but not Trp, was inhibited dramatically by preincubation of mouse intestinal tissue with Gur (Fig. 4*D*).

Therefore, the data indicate that, in native mouse proximal intestinal tissue, T1R1-T1R3, expressed in CCK-containing enteroendocrine I cells, acts as a sensor for a number of L-amino acids, leading to CCK release. It will be interesting to determine the role of T1R1-T1R3 in human intestine, with knowledge of the limited selectivity of response of the human lingual umami sensor to amino acids (6, 28, 35, 49).

CaSR is widely expressed in mammalian tissues, including enteroendocrine cells (4, 9), and has recently been recognized to act as an L-amino acid sensor implicated in mediating CCK secretion in response to aromatic amino acids (10, 26, 39, 61). Hira et al. (26) provide evidence that CaSR can function as an L-Phe receptor modulating intracellular Ca^{2+} mobilization and subsequent CCK secretion in STC-1 cells. Furthermore, they demonstrated that removal of extracellular Na^{+} does not affect the intracellular Ca^{2+} response to L-Phe, hence excluding the involvement of a plasma membrane Na^{+}-dependent amino acid transport system in the process. Recently, Wang et al. (61) and Liou et al. (39), using a transgenic mouse in which enhanced green fluorescent protein (eGFP) is expressed downstream from the mouse CCK promoter, isolated mucosal eGFP-expressing CCK (CCK-eGFP) cells and purified them by fluorescence-activated cell sorting. Using these cells, they concluded that aromatic amino acids, L-Phe and L-Trp, stimulate CCK release through activation of CaSR expressed in native intestinal I cells (CCK-expressing enteroendocrine cells). Furthermore, Liou et al. reported that deletion of CaSR in CCK-eGFP cells abolishes the ability of these cells to secrete CCK in response to L-Phe.

To determine the potential involvement of CaSR in L-amino acid-induced CCK release in mouse intestinal explants, we exposed tissue sections to Phe, Leu, Glu, and Trp in the presence or absence of NPS2143, a CaSR antagonist (51). Addition of NPS2143 inhibited Phe-stimulated CCK release partially and Trp-induced CCK secretion totally. However, it had no effect on Leu- or Glu-induced CCK secretion from mouse intestinal tissue (Fig. 10). The partial and total inhibition of CaSR-mediated Phe- and Trp-induced CCK secretion, respectively, is in agreement with the data reported by Wang et al. (61). They demonstrated similar results for Phe- and Trp-induced CCK secretion in isolated CCK-eGFP cells in the presence of a CaSR antagonist, Calhex 231. These data propose that more than one receptor is capable of sensing L-Phe. Also, in previous studies using HEK-293 cells transfected with cloned CaSR, aromatic amino acids (such as L-Phe and L-Trp) showed a specificity for CaSR, while branched-chain amino acids (such as Leu) did not activate CaSR (10). Addition of NPS2143 + Gur totally inhibited Phe-induced CCK release from mouse intestinal tissue (Fig. 10), supporting the participation of both receptors, CaSR and T1R1-T1R3, in Phe-induced CCK release.

The use of mouse intestinal explants in our studies provides a reliable method for assessment of mechanisms underlying intestinal gut hormone secretion. We previously showed that exposure of mouse intestinal explants to glucose evokes GLP-1 and GLP-2 release of a magnitude similar to in vivo studies using rats and humans given glucose orally (12). Furthermore, our data and those of others demonstrate that STC-1 cells are suitable in vitro models for identification of luminal sensors influencing gut hormone release. Characterization of the role of CaSR in STC-1 cells by Hira et al. (26) underpinned further work carried out using isolated I cells to determine the role of CaSR as a sensor for aromatic amino acids (39, 61). Our data using STC-1 cells are consistent with data obtained using intestinal tissue explants and aid in determining the involvement of T1R1-T1R3 as a sensor for a number of amino acids.

The identity of the cell surface receptor(s) involved in peptone-induced CCK release remains unknown. Recent work has shown that the peptide transporter PepT1 is not the I cell luminal membrane receptor involved in mediating peptone-induced CCK release (37), while the GPCR GPR93 has been proposed as a candidate sensor for peptones in STC-1 cells (8). Further work is required to confirm the peptone-sensing role of this GPCR in the intestine.

In summary, we have shown that T1R1-T1R3, expressed in endocrine CCK-containing cells (I cells) of mouse proximal intestine, is directly activated by a number of L-amino acids (but not their D-isomers), stimulating a pathway leading to CCK release. It has been suggested that there are multiple receptors for amino acid sensing in the lingual epithelium (67). It is likely that a similar situation may exist in the gut. CaSR may be an intestinal L-amino acid receptor specifically sensing aromatic amino acids, while T1R1-T1R3 responds to a spectrum of L-amino acids, provoking CCK secretion from intestinal endocrine cells. If we consider the complexity of the intestinal luminal environment, with massive fluctuations in the types and levels of nutrients entering the intestine, the existence of multiple modalities for sensing nutrients by enteroendocrine cells is justified.

GRANTS

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

K.D. and S.P.S.-B. are responsible for conception and design of the research; K.D., M.A.-R., A.W.M., and M.M. performed the experiments; K.D., M.A.-R., and S.P.S.-B. analyzed the data; K.D. and S.P.S.-B. interpreted the results of the experiments; Y.N. provided reagents and analyzed some data; K.D., M.A.-R., A.W.M., M.M., and S.P.S.-B. prepared the figures; K.D. and S.P.S.-B. edited and revised the manuscript; S.P.S.-B. drafted the manuscript; S.P.S.-B. approved the final version of the manuscript.

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