Luminal Amino Acid-Sensing Cells in Gastric Mucosa

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Key Words

Glutamate · Gastric mucosa · Endocrine cell · Chemosensing · Somatostatin

Abstract

Chemosensing of nutrients in the gastrointestinal tract plays physiologically important roles in the regulation of food intake behaviors, including digestion, absorption, metabolism and other subsequently occurring body functions via brain activation. Free amino acids, liberated from ingested foods, are of course essential nutrients which compose the body proteins and sometimes determine the taste of the food. Glutamate, one of the most abundant amino acids in the foods and the liberated free form, critically contributes to the ‘umami’ taste perception. Recently, it has been revealed that dietary glutamate has many beneficial functions in the gastrointestinal tract. However, the precise mechanism of glutamate sensing still remains unclear. Using primary rat gastric mucosal cell cultures, we demonstrated that somatostatin-secreting D cells are candidate cells for glutamate sensing in the stomach through inhibition of somatostatin release. Considering that somatostatin is one of the major negative regulators of gastric functions, it is suggested that some parts of glutamate’s beneficial effects could be explained by suppression of the inhibitory somatostatin effects, i.e. stimulation, by glutamate.

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Introduction

The gastrointestinal (GI) tract is an essential organ for the digestion of dietary foods following absorption and metabolism of nutrients liberated from the digested foods such as amino acids, sugar, fatty acids, etc. These nutrients are detected by a ‘nutrient chemosensing system’ involving luminal sensors on the GI mucosa. Information from this system is transmitted from the GI tract to the brain and it has important roles in the recognition of nutrient quality and in the regulation of food intake.

Recent progress in the taste field, using molecular biology and cell isolation techniques, has revealed that certain types of cells in the GI mucosa, especially endocrine cells, express taste- and nutrient-specific sensors. These sensors contribute to the regulation of hormone secretion in response to taste substances and nutrients as shown in table 1.

Since the 1970s, it has been reported that free amino acids, liberated from protein during digestion, induce the secretion of GI hormones such as gastrin and cholecystokinin (table 1). Glutamate is one of the most abundant amino acids in foods and serves as the agonist of ‘umami’ taste receptors such as the taste receptor type 1/type 3 heterodimer (T1R1/T1R3) and metabotropic glutamate receptor subtypes (mGluRs) located on the taste buds of the tongue [1]. In the taste field, ‘umami’ taste is now recognized as one of the five basic tastes, along with sweet-

Table 1. Expression profiles of hormones and nutrient sensors in GI endocrine cells

GI hormone	Cell type	Open or closed type	Luminal stimulant	Nutrient receptor	GI expression								
					stomach		small intestine			large intestine			
					fundus corpus	antrum	duodenum	jejunum	ileum	cecum	colon	rectum	
Histamine	ECL	Closed [21]	–	?	++								
Ghrelin	P/D1 (human) [22] X/A-like (rat) [22]	Mainly closed, some open [23]	?	?	++	±							
Nesfatin-1	Mainly X/A-like ^(rat) [24, 25]	Mainly closed, some open [25]	?	?	++	±							
Somatostatin	D [26]	Corpus; closed? Antrum; open	H ⁺ [27, 28]	mGluR2, 3, 4, 7	++	++	+	±					
Gastrin	G	Open	Amino acids, peptides [29], Ca ²⁺	CaSR [30–32], T1R1 [33]		++	+						
Serotonin	EC	Stomach; closed Intestine; open	Glucose [34], odorants [35], bitter [33], AITC [36–38], etc.	SGLT1, OR1G1 [35], T2Rs [33], TRPA1 [36–38]	+	+	+	+	+	+	+	+	
Secretin	S [39, 40]	Open	H ⁺ [41]	?		±	+	+					
CCK	I [42]	Open	Fatty acids [43, 44], peptides [45], amino acids [46], bitter [47, 48], etc.	GPR40 [43, 44], 93 [45], 120 [43, 44], CaSR [46], T2Rs [47, 48]			++	+	±				
Motilin	Mo [49]	Open	Fat, H ⁺ : + sugar, amino acids; – [50, 51]	?			+	+					
GIP	K [52]	Open	Fatty acids [53], glucose [54], amino acids [55]	GPR40 [53], SGLT1 [54]			+	+	±				
Neurotensin	N [56]	Open	Fatty acids [57]	?				+	+				
GLP-1, 2 PYY Oxyntomodulin	L [58]	Open	Sugar, amino acids, fatty acids, bile acid, etc. [59]	SGLT1, GPR40,120, 131 [59]			±	+	++	+	+	+	
Amylin	β (human) D, G, L (rat) [60, 61]	Open	?	?	+	±							
					+	++	±	±	±				

ness, sourness, bitterness and saltiness [1]. Our group has been studying the physiological roles of dietary glutamate on food intake behavior, comprehensively from the digestive tract to the brain [2–7]. Through the research to clarify the mechanism for sensing umami (glutamate) and other amino acids in the GI tract, we have characterized the distribution of these possible sensors using dispersed rat gastric mucosal cells fractionized by counterflow elutriation and density-gradient centrifugation. In the present study, we bridge the sensor distribution analysis with a functional analysis and propose new roles for these amino acid sensors in rat gastric functions.

Materials and Methods

Cell Isolation, Enrichment and Real-Time RT-PCR

Animals used for this study were maintained in accordance with the guidelines of the Committee on Animals at Ajinomoto Co., Inc. Isolated cells were prepared from Sprague-Dawley rat stomachs (Charles-River, Yokohama, Japan) by density-gradient and centrifugal elutriation techniques as described previously [8]. For amino acid sensor expression analysis, we quantified the expressions of T1R1, mGluR1–8, calcium-sensing receptor (CaSR) and G-protein-coupled receptor family C group 6 subtype A (GPRC6A).

Somatostatin Release from Cultured Smaller Endocrine Cells

After characterization of each cell type, D-cell-rich smaller endocrine cell fractions were cultured for 2 days in DMEM/F12 (1:1) supplemented with heat-inactivated 10% FBS (Gibco/BRL,

Gaithersburg, Md., USA), 10 ng/ml epidermal growth factor, 100 units/ml penicillin, 100 units/ml streptomycin and 0.25 µg/ml amphotericin B. Next, the confluent cells were then incubated for 90 min with the indicated amino acid (10 mM) in standard (STD) buffer containing (in mM) 147 Na⁺, 5.0 K⁺, 131 Cl⁻, 1.3 Mg²⁺, 1.3 SO₄²⁻, 2 Ca²⁺, 25 HCO₃⁻, 15 Hepes and 20 D-glucose at pH 7.4. Somatostatin release from the cells into the culture medium was measured by commercially available ELISA kits (Phoenix Pharmaceuticals, Inc., Burlingame, Calif., USA).

Results

Expressions of Glutamate and Amino Acid Sensors in Gastric Mucosal Cell Fractions

As the result of each cell marker expression analysis by real-time RT-PCR methods, we designated five enriched fractions as surface mucous cells (Muc5ac), smaller endocrine cells (somatostatin and ghrelin), larger endocrine cells (ghrelin and histidine decarboxylase, HDC, i.e. histamine), parietal cells (H⁺,K⁺-ATPase β-subunit) and chief cells (pepsinogen C), respectively (table 2). The endocrine marker expressions indicate that somatostatin-secreting D cells are enriched mainly in the smaller endocrine cell fraction.

Using these specific cell-enriched fractions, we examined which cells express glutamate and amino acid sensors as shown in table 2. Because the level of expressions of several sensors such as T1R1, mGluR1, 2, 3, 4, 7, and GPRC6A was quite low, we quantified these sensors by nested PCR. The expression of T1R1 was moderately higher in parietal cell fraction compared with total gastric mucosa. Among group 1 metabotropic glutamate receptors (which couple with G_q protein to activate phospholipase C), the expression of mGluR1 was observed in the parietal and larger endocrine cells, while expression of mGluR5 was observed in both the smaller and larger endocrine cells. In contrast, the expression profiles were dramatically changed in group 2 and 3 metabotropic glutamate receptors (both of which couple with G_i protein to inhibit adenylate cyclase). Both mGluR2 and mGluR3 were expressed specifically to the smaller endocrine cells and parietal cells. In contrast, the expressions of mGluR4 and mGluR7 were specific to the smaller endocrine cells, while that of mGluR6 was moderately high in the chief cells and larger endocrine cells.

Concerning other amino acid sensors, CaSR was expressed specifically in the smaller endocrine cells, while the expression of GPRC6A was high in parietal cells and smaller endocrine cell and moderately higher in chief cells and larger endocrine cells.

Table 2. Expression profiles of glutamate and amino acid sensors in cell fractions isolated from rat gastric mucosa

	Surface mucous cell	Parietal cell	Chief cell	Smaller endocrine cell	Larger endocrine cell
MUC5ac	++	--	--	--	--
H ⁺ ,K ⁺ -ATPase β	-	++	--	--	--
Pepsinogen C	--	--	++	--	--
Somatostatin	--	--	--	++++	++
Ghrelin	--	--	--	++++	++++
HDC	--	--	--	+++	++++
T1R1	--	++	--	-	0
mGluR1	-	+	--	--	+
mGluR2	0	+++	++	++++	++
mGluR3	--	+++	--	++	--
mGluR4	+	+	-	+++	+
mGluR5	0	0	0	+	+
mGluR6	0	+	++	0	++
mGluR7	+	++	0	++++	++
mGluR8	--	--	--	+	0
CaSR	--	--	--	++	0
GPRC6A	-	+++	++	+++	++

Expression levels of cell-specific markers and glutamate sensors in each cell fraction were compared with those of gastric mucosa (as 100%) and indicated according to the following classification: -- = much less than gastric mucosa (<50%); - = moderately less than gastric mucosa (50% < X < 75%); 0 = similar to gastric mucosa (75% < X < 125%); + = slightly more than gastric mucosa (125% < X < 200%); ++ = moderately more than gastric mucosa (200% < X < 500%); +++ = much more than gastric mucosa (500% < X < 1,000%); ++++ = extremely more than gastric mucosa (X > 1,000%).

Bolding of the marker indicates that the cDNAs amplified prior to PCR were used due to low expression.

This list is modified from Nakamura et al. [8].

Effect of Amino Acids on Somatostatin Release from Gastric Smaller Endocrine Cell Fraction

From real-time RT-PCR results, the smaller endocrine cell fraction highly expressed CaSR, GPRC6A and Gi-coupling mGluRs such as mGluR2, 3, 4, 7. Thus, we next evaluated the effect of these receptor-favorable amino acids on somatostatin release from the cultured smaller endocrine cells. CaSR-favorable amino acids such as phenylalanine, tryptophan and histidine significantly increased somatostatin release more than two-fold compared with control. Similarly, both CaSR- and GPRC6A-favorable cysteine significantly stimulated the somatostatin release more than twofold. In contrast,

GPCR6A-favorable lysine had no effect compared with control. Interestingly, mGluR-favorable glutamate and aspartate significantly decreased somatostatin release by 20%.

Discussion

We have previously reported that luminal application of glutamate in the rat stomach caused increases in the activity of the afferent fibers of the gastric branch of vagus nerve [6]. Interestingly, other amino acids failed to show such effects, although each of the amino acids responded to luminal application in the intestine [9, 10]. Thus, among 20 dietary amino acids which compose body proteins, only glutamate can transmit nutrient information from the stomach to the brain. However, the precise mechanism for luminal glutamate sensing remains unclear.

In the present study, we characterized the distribution of glutamate and other amino acid sensors on the gastric mucosa in an attempt to elucidate the mechanism of luminal glutamate sensing. Yet, we encountered two serious experimental problems: (1) we could not locate sufficient specific antibodies for immunostaining, and (2) G-protein-coupled receptor (GPCR) expressions were very low when the samples were collected as a whole tissue. Based on these findings, we concluded that whole tissue analyses could be misleading. To overcome these problems, we utilized a modified cell isolation method that allowed us to culture the cells for several days and to evaluate cell functions directly.

As reported previously, we could successfully fractionate the rat gastric mucosa into a variety of cell types, including surface mucous cells, parietal cells, chief cells, and smaller and larger endocrine cells. Additionally we could culture some of the fractions. Most of these cells (except for complete closed-type of endocrine cells) may contact dietary glutamate and amino acids via the lumen of gastric glands, so have the potential to regulate gastric digestive functions.

Using these fractions, we quantified the expressions of amino acid sensors in family C GPCRs such as mGluRs, T1R1, CaSR and GPCR6A. Wellendorph and Brauner-Osborne [11] have recently reported that this class of GPCRs is important for nutrient, especially amino acid sensing. Their amino acid selectivity profiles for CaSR, GPCR6A, and the T1R1/T1R3 heterodimer indicated that aromatic amino acids such as phenylalanine, tryptophan and histidine were CaSR-specific. Cysteine

is both CaSR- and GPCR6A-favorable. In contrast, lysine seems to be GPCR6A-favorable. Interestingly, our results showed that the somatostatin-secreting D cell-rich smaller endocrine cell fraction expressed highly, and relatively specifically, CaSR, GPCR6A and Gi-coupling mGluRs such as mGluR 2, 3, 4, 7. The expression of CaSR in parietal cells was very low compared with a D cell-rich smaller endocrine cell fraction. Thus, we functionally evaluated the effect of the family C GPCRs-favored amino acids on somatostatin release using this fraction culture.

As somatostatin is a paracrine hormone in the gastric mucosa [12–15], it is less worthwhile to measure the systemic concentration of somatostatin in the blood. In contrast, primary D cell culture is a valuable system to quantify the hormone locally. Using our cell culture system, CaSR-specific amino acids, including phenylalanine, tryptophan and histidine, significantly stimulated somatostatin release more than twofold. Similarly, both CaSR- and GPCR6A-favorable cysteine significantly stimulated the somatostatin release more than twofold. However, GPCR6A-favorable lysine had no effect. These results indicate that CaSR, but not GPCR6A, plays a functionally important role in the stimulation of somatostatin release in D cells.

Interestingly, glutamate and aspartate, despite showing a weak CaSR-agonistic activity, significantly decreased somatostatin release. The fraction also highly expressed G_i-protein-coupled mGluRs the activation of which inhibits adenylate cyclase [12–14]. We speculate that mGluRs may be more effective receptors than CaSR for suppression of somatostatin release. This would provide a mechanism for the previously reported effects of glutamate in the stomach. Indeed, dietary glutamate reportedly stimulates gastric secretion and motility in dogs and humans [16–18]. Since somatostatin is an inhibitory regulator of gastric exocrine and endocrine secretion [12–15], it is reasonable to assume that the inhibition of somatostatin release via G_i-protein-coupled mGluRs stimulation (i.e., inhibition of inhibitory effect) results in stimulation of gastric secretion. Although D cells are the closed-type endocrine cells in the corpus of the stomach [12–15], we observed, using immunohistochemical analysis, that a portion of the D cells in the corpus seemed to be open-type [unpublished data]. In addition, D cells express neuron-specific markers and function like the paraneuronal cells [19, 20]. Thus, D cells may be directly involved in modulating the luminal glutamate signaling to vagal nerves in the gastric mucosa.

In conclusion, using D cells isolated from rat gastric mucosa we demonstrated the role of family C GPCR, mGluRs and CaSR in glutamate and amino acid sensing and in the regulation of somatostatin release. Further studies are needed to clarify the functional relationships between glutamate, G_i-protein-coupled mGluRs and somatostatin release in the stomach.

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Disclosure Statement

No conflict of interest exists.

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