

Emerging roles of the extracellular calcium-sensing receptor in nutrient sensing: control of taste modulation and intestinal hormone secretion.

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ABSTRACT

The extracellular calcium-sensing receptor (CaSR) is a sensor for a number of key nutrients within the body, including calcium ions (Ca^{2+}) and L-amino acids. The CaSR is expressed in a number of specialized cells within the gastrointestinal (GI) tract, and much work has been done to examine the CaSR's role as a nutrient sensor in this system. This review article examines two emerging roles for the CaSR within the GI tract – CaSR-mediated modulation of *kokumi* taste in taste cells and its role as a regulator of dietary hormone release in response L-amino acids in the intestine.

INTRODUCTION

The calcium-sensing receptor (CaSR) is a class C G protein-coupled receptor (GPCR) which was originally identified as the molecular ion sensor for free ionised extracellular calcium (Ca^{2+}_o) homeostasis [1]. While the CaSR's role in divalent cation metabolism has been well defined (reviewed in references [2,3]), the CaSR is expressed in a number of tissues and cell types not typically associated with Ca^{2+}_o homeostasis. Over the past few years much work has taken place to elucidate the functional significance of CaSR expression in a wide number of other tissues including the brain and central nervous system [4], the vasculature [5] and the gastrointestinal (GI) tract.

While the main physiological agonist of the CaSR is Ca^{2+} , this receptor can be activated by a diverse array of other multivalent cations including alkaline metals (Mg^{2+} , Sr^{2+}), lanthanides (Gd^{3+}), polycations (spermine, spermidine) [6], aminoglycoside antibiotics (neomycin, gentamycin) [7-9], and cationic polypeptides (poly-L-arginine) [10] (reviewed in ref [2]). Changes in ionic strength [11] and pH [12] also affect the CaSR's activity, as low ionic strength and high pH enhance the CaSR sensitivity to Ca^{2+}_o .

Furthermore, as a class C GPCR, the CaSR sits within a family of extracellular amino acid sensors including the metabotropic glutamate receptors. The CaSR, along with the heterodimeric taste receptors (T1R1 and T1R3), the goldfish 5.24 receptor and its mammalian ortholog GPRC6A form a distinct subgroup of broad spectrum amino acid sensing receptors, which have distinct yet overlapping sensitivities to different amino acids (Figure 1).

The CaSR is allosterically activated by L-amino acids, being able to respond to aromatic, aliphatic and polar amino acids but not branched or positively charged amino acids [13]. In contrast, taste can be activated by aliphatic, polar, branched-chain and, to a lesser extent, charged amino acids, but not aromatic amino acids. Lastly, the goldfish 5.24/GPRC6A receptors respond to basic, aliphatic and polar amino acids [14]. The CaSR has also been shown to respond to small peptides, including glutathione (GSH) and other γ -glutamyl peptides [15,16].

This variety in ligands enables the CaSR to act physiologically as a multi-modal sensor for several key nutrients throughout the body, including the GI tract. Within the GI tract the CaSR is widely expressed in a number of specialized cells including the esophagus, stomach, small intestine and colon [17-19] and has roles in gastrin secretion, colonic fluid transport and intestinal epithelial cell growth, all of which have been reviewed in depth previously (see [20] and [19]) and are listed in Table 1. In this review article, we examine emerging physiological functions of the CaSR in sensing dietary nutrients in two separate roles; 1) the role of the CaSR as a taste receptor for both protein and oral Ca^{2+} , and 2) as an amino acid sensor for the release of dietary hormones within the intestine.

CALCIUM-SENSING RECEPTOR AS A TASTE RECEPTOR

There is now emerging evidence suggesting that the CaSR may play a role in regulating appetite for nutrients by modulating taste perception. The first demonstration of the possible involvement of the CaSR in taste perception was given in bullfrogs, where a positive allosteric modulator of the CaSR, the “calcimimetic” NPS R-467, stimulated taste cells with accompanying neuronal responses [21]. CaSR expression has now been reported in rat and mouse taste cells, namely in the circumvallate, foliate and, to a lesser extent, the fungiform papillae [22,23].

Taste buds are generally composed of ~ 50 – 100 elongated taste cells, which belong to three different classes [24,25]: Type I (glial-like) cells seem to be involved in clearing neurotransmitters through absorption/degradation; Type II (receptor) cells express G protein-coupled receptors (including the taste receptors T1Rs and T2Rs), which bind bitter, sweet and umami compounds, the downstream signalling components (e.g. phospholipase $\beta 2$ (PLC $\beta 2$)) which transduce these taste qualities and the G protein gustducin [26]; and Type III (pre-synaptic) cells which form synaptic contacts with nerve terminals and are known to receive and integrate signals from Type II cells.

Expression of the CaSR has been observed in Type III taste cells at the mRNA and protein level [22,23], however there have been conflicting reports on whether the CaSR is also expressed in Type I and Type II cells. Bystrova et al. showed CaSR mRNA expression at the single cell level in a number of Type I, but not Type II cells, using SMART-PCR, however they were unable to demonstrate functional coupling to the PLC-dependent Ca^{2+} signalling pathways in Type I cells [23].

The possibility does remain that the CaSR signals in a PLC-independent manner in Type I taste cells. Type I taste cells express the renal outer medullary potassium K^+ (ROMK) channel on their apical membrane, where this channel might play a role in recycling the K^+ that accumulates in the restricted spaces between Type II and Type III cells [27]. This scenario would be reminiscent of the thick ascending limb of the kidney, where activation of the basolateral CaSR has been

shown to inhibit apical ROMK through signalling pathways involving arachidonic acid and its metabolites [28] and the Type I taste cell CaSR may signal in a similar manner (Figure 2).

Conversely, San Gabriel et al. and Maruyama et al. [22,29] have both demonstrated expression of the CaSR in subset of taste cells that express either NCAM (a marker of Type III cells) or PLC β 2 (Type II cell marker) using immunofluorescence. CaSR positive, PLC β 2 expressing Type II cells did not express the sweet/umami taste receptors, T1R3 [29], however whether other receptors, such as the bitter T2R receptors, are co-expressed in CaSR-positive Type II taste cells is currently unknown.

Interestingly, recent work demonstrated in CaSR-expressing HEK293 cells that this receptor may be stimulated by the bitter compound denatonium the mM range [30]. Similar to other small peptides, like glutathione (GSH) and γ -glutamyls [15,16], it seems to have a positive allosteric effect on Ca²⁺_o concentration response curve, although whether denatonium stimulates the CaSR in taste cells is unknown [30].

Exposure of CaSR-expressing Type III taste cells to L-amino acids (L-Phe and Arg), γ -glutamyl peptides (like GSH and γ -glutamyl-valine-glycine [γ EVG]) and calcimimetics (cinacalcet) has been shown to evoke intracellular Ca²⁺ (Ca²⁺_i) transients, which are ablated by the non-specific PLC inhibitor U73122 [23,29]. High concentrations (3 μ M) of the negative CaSR allosteric modulator, the “calcilytic” NPS 2143, were also shown to inhibit the γ EVG-mediated Ca²⁺_i response, suggesting these responses might be mediated through CaSR activation (Figure 2) [29].

Whilst the majority of this work has been completed in rodent taste cells, there does seem to be a role for the CaSR in human taste transduction. Human sensory analysis has demonstrated that a number of CaSR agonists, including GSH and γ EVG, act as *kokumi* taste substances [31], enhancing sweet, salty and umami tastes without producing a taste of their own. There seems to be a positive correlation between *kokumi* taste intensity and CaSR agonist activity, as determined by EC₅₀ values in CaSR-expressing HEK293 cells (Figure 3, modified from [31]). In addition, high concentrations of the calcilytic NPS 2143 significantly suppressed the *kokumi* taste effects of CaSR agonists [31].

There may also be an additional role of the CaSR in the oral detection of Ca²⁺_o, which has a distinctive taste quality, with aqueous calcium-containing solutions tasting quite bitter to humans [32]. Interestingly, calcium deprivation has been shown to increase the palatability of calcium in rodents [33]. The exact mechanism by which animals detect / ‘taste’ calcium is still yet to be fully elucidated, however previous work has implicated the involvement of the T1R3 receptor [33]. It has been suggested the T1R3 receptor may heterodimerise with the CaSR to form a functional Ca²⁺ sensor in Type II taste cells [33], however there is no evidence to date that T1R3 and CaSR expression overlap [23,29].

As the activation of the CaSR-positive taste cells has already been implicated in *kokumi* taste modulation, it is perhaps not such a great leap to imagine the CaSR could play a similar role in the detection of oral calcium. In support of this argument is the finding that a substantial proportion of variation in calcium taste preference among mice is due to CaSR polymorphisms [34].

CALCIUM-SENSING RECEPTOR IN THE INTESTINE

CaSR is present in the stomach, where its activation stimulates secretion of gastrin (by G cells) and of H^+ (by antral cells) (reviewed by [35]). Seminal studies by Hebert, Geibel et al have demonstrated that the CaSR plays a fundamental role in the colon in NaCl and water transport and raised the possibility of using CaSR-based therapeutics to prevent toxin-induced secretory diarrhoea, one of the most debilitating conditions in underdeveloped countries (reviewed by [35]). Luminal Ca^{2+} also promotes gut epithelial differentiation while CaSR-mediated signalling suppresses gut cell proliferation while preserving epithelial integrity [36]. Dietary Ca^{2+} intake is associated with a reduced risk of colon cancer and CaSR expression is absent in colon cancer specimens while is highly abundant in normal tissue from the same patients (see review [37]). While a definitive and direct link between loss of CaSR expression and malignant transformation in the gut remains to be elucidated, it has been hypothesized that the CaSR might be a drug target for the treatment of colon cancer. Recent evidence has demonstrated that the CaSR also plays an important role regulating hormone secretion in intestinal enteroendocrine cells in response to L-amino acids.

Aromatic amino acids have been shown to mediate secretion of the gastrointestinal hormone cholecystokinin (CCK) [38-42]. Secretion of CCK from enteroendocrine cells in the small intestine is a major regulator for the release of bile by the gallbladder, as well as the secretion of digestive enzymes from the pancreas [36,41]. CCK also acts as a satiety hormone, reducing food intake in a number of different species, including humans [43].

Due to the difficulties in obtaining sufficient amounts of homogeneous I cells from intestinal tissue, initial experiments examining the cellular mechanism by which aromatic amino acids mediate CCK secretion focused on the murine enteroendocrine cell line, STC-1. Here, L-Phe was shown to stimulate CCK secretion in a Ca^{2+}_o -dependent manner (Figure 4A & B) [44]. L-Phe also increased Ca^{2+}_i levels and Ca^{2+} channel activity, while the Ca^{2+} channel blocker diltiazem inhibited CCK secretion [44]. Phe-mediated secretion is also stereoselective for the natural L-isomer (Figure 4A). High concentrations of the calcilytics NPS 2143 abolished L-Phe stimulated CCK secretion, suggesting that the CaSR may play a role in L-Phe mediated CCK secretion in STC-1 cells. Consistent with this hypothesis, CaSR mRNA expression was detected in STC-1 cells using RT-PCR [45]. Recent work has also demonstrated that the STC-1 cells respond to protein hydrolysates (such as egg albumin, meat, potato, , casein, and soy bean) with an increase in CCK

secretion, which is suppressed in the presence of NPS 2143, except for meat hydrolysate-induced CCK secretion [46]. Together, these studies strongly support a role for the CaSR as an amino acid and dietary peptide sensor in these cells.

The involvement of the CaSR in mediating CCK secretion has been elucidated by two groups who have used bacterial artificial chromosome (BAC) transgenic mice, which permitted the identification of specific cell types using enhanced green fluorescent protein (eGFP). This elegant approach has permitted the isolation of CCK-secreting cells from CCK-eGFP BAC transgenic mice by fluorescence-activated cell sorting (FACS) [47,48].

The first such study, by Liou *et al.*, used FACS to isolate native CCK-secreting duodenal I cells from CCK-eGFP BAC mice [47]. Using quantitative RT-PCR these isolated CCK-eGFP cells were shown to express CaSR mRNA transcripts at a level approximately 900-fold higher than non-eGFP expressing cells, and the presence of CaSR protein was confirmed with immunofluorescence. Exposure of isolated I cells to phenylalanine induced intracellular Ca^{2+} influx which was Ca^{2+}_o -dependent and stereoselective for L-Phe. L-Phe dependent CCK secretion in native I cells was enhanced in the presence of supraphysiological Ca^{2+}_o concentrations, indicating a synergistic effect of Ca^{2+}_o and this amino acid. Interestingly, supraphysiological Ca^{2+}_o concentrations alone were unable to evoke an increase in CCK secretion. Based on these results, the authors concluded that CaSR acts as an amino acids sensor in this physiological setting [47].

Deletion of the CaSR from CCK-eGFP I cells did not effect basal CCK secretion, however L-Phe mediated Ca^{2+}_i influx was lost. Furthermore, L-Phe and supraphysiological Ca^{2+}_o surprisingly suppressed CCK secretion by ~ 20 – 30% in these cells, compared to basal levels, suggesting that not only is the CaSR required for L-Phe mediated CCK secretion, but that the absence of a fully functional receptor may inhibit L-amino acid-induced CCK secretion [47].

The second study, by Wang and colleagues, examined CCK-secreting intestinal mucosal cells from CCK-eGFP BAC transgenic mice. Expression of the CaSR was confirmed with quantitative RT-PCR and immunofluorescence, where CaSR expression was localized on both the apical and basolateral regions of CCK-eGFP cells. Aromatic amino acids, L-Phe and L-Trp, but not the non-aromatic amino acid L-Ala, caused transient increases in Ca^{2+}_i and stimulated CCK secretion. Antagonising the CaSR with the calcilytic Calhex 231 blocked aromatic amino acid mediated CCK secretion, without affecting the effect of hyperpolarizing concentrations of KCl, again pointing to a role for the CaSR in the modulation of the effects of certain amino acids on CCK secretion [48].

Recently, the CaSR has also been implicated in regulating K- and L-cell activity in response L-amino acids [49]. Isolated loops of the rat small intestine were used to quantify secretion of

three anti-diabetic gut peptides (gluco-indulinotropic peptide (GIP), glucagon-like peptide-1 (GLP-1) and peptide tyrosine tyrosin (PYY)) in response to a number of L-amino acids.

L-Phe, L-Trp, L-Arg, L-Asn, and L-Gln all induced secretion of GIP, GLP-1 and PYY in the presence of physiological (i.e., 1.25 mM) Ca^{2+}_o . Characteristic of a CaSR-mediated response, L-amino acid-induced secretion responses were abolished in the absence of Ca^{2+}_o for all three peptides. High concentrations of the CaSR antagonist, Calhex 231, suppressed L-amino acid secretion responses to various extent, with the exception of L-Gln stimulated GLP-1 secretion [49]. Inhibition of the CaSR by Calhex 231 was most efficient in suppressing aromatic amino acid responses [49], perhaps unsurprisingly as they are the more potent CaSR ligand [13]. Furthermore, the addition of a CaSR allosteric agonist, NPS R568, after the initial elevation of GIP, GLP-1 or PYY secretion by L-amino acids, further enhanced secretion to a maximal level, while increasing in Ca^{2+}_o increased the potency of L-Phe-induced L-/K-cell response.

SUMMARY & CONCLUSION

The role of the CaSR in nutrient sensing within the GI system continues to evolve as time carries on. Previous studies have demonstrated that a protein-rich diet improves bone health and is associated with a reduced risk of fracture and an improved post-fracture recovery, underlying a link between dietary protein intake and calcium metabolism [50,51]. New developments, presented in this review, implicate the CaSR in modulation of appetite and control of satiety and anti-diabetic hormone secretion in response to amino acids/dietary calcium. Overall, these finding present the CaSR as a possible new therapeutic target in ongoing fight against obesity and bone health, and their related disorders.

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FIGURE LEGENDS

Figure 1: Amino acid profiles of CaSR, goldfish 5.24/GPRC6A, mGluR and the T1R1/T1R3 heterodimer.

The figure shows effective amino acids agonists of CaSR, goldfish 5.24/GPRC6a, mGluR and T1R1/T1R3 heterodimer. Data for T1R1/T1R3 efficacy were determined by the percentage of response cells in the presence of 2.5 mM inosine monophosphate (IMP) and normalized to 50 mM L-Cys response [52]. For CaSR, amino acid efficacy was determined by the % maximum reduction in EC_{50} for extracellular Ca^{2+}_o based on [13] and normalized to the 10 mM L-His response. Normalized data were taken from [14]. We considered an effective amino acid agonist for T1R1/T1R3 and CaSR to be one $\geq 40\%$ of their respective normalized responses. GPCR6a data are based on data obtained from mouse GPCR6a in the presence of 1 mM Ca^{2+} and 1 mM Mg^{2+} [53,54]. The receptor response to each amino acid is as follows: *red* – CaSR; *yellow* – T1R1/T1R3 heterodimer; *green* – GPCR6a and *blue* – mGluR.

Figure 2: The relationship between CaSR EC_{50} values and *kokumi* taste intensity

Six γ -glutamyl peptides were tested for *kokumi* taste intensity by a panel of assessors. Intensity of *kokumi* taste was quantified in reference to the glutathione (GSH) concentration required to achieve an equivalent intensity of taste sensation. Half maximal effective concentration (EC_{50}) values for these substances were determined by measuring agonist-evoked increase in intracellular Ca^{2+} concentration in HEK-293 cells transiently expressing human CaSR. Substances with stronger *kokumi* taste intensity exhibited a higher potency for CaSR activation than substances with lower *kokumi* taste intensity. Data were taken and redrawn from Ohsu *et al.* [31].

Figure 3: Proposed roles of the CaSR within taste cells.

This diagram depicts the proposed roles and signalling pathways for the CaSR in Type I, Type II and Type III taste cells. **(1)** In Type I (glial-like) cells, CaSR activation may be linked to regulation of potassium recycling by the apical ROMK channel, similar to its role in the kidney. **(2)** The CaSR is most likely co-expressed in Type II (receptor) cells with T2R receptors, where it may play a role in transducing both bitter (oral Ca^{2+} /denatonium) and *kokumi* (L-amino acids/ γ -glutamyl) taste. Activation of CaSR homodimers, or possible CaSR/T2R heterodimers, leads to activation of the G-protein gustducin (α gust) and phospholipase $\beta 2$ (PLC $\beta 2$). PLC $\beta 2$ catalyses the formation of inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG) from phosphatidylinositol 4,5-bisphosphate (PIP_2), leading to release of Ca^{2+} from the endoplasmic reticulum through IP_3 binding to an IP_3 receptor (IP_3R). Increased intracellular Ca^{2+} leads to depolarization of the cell through the actions of the Na^+ channel Trpm5, delayed rectifying potassium channels (K_{DR}

channels) and voltage-gated calcium channels (VGCC). Furthermore, the cells release ATP through pannexin 1 (panx1), exciting the ATP receptors P2Y and P2X on sensory nerve fibres [25]. **(3)** In Type III pre-synaptic cells, activation of the CaR by γ -glutamyl peptides leads to an increase in intracellular Ca^{2+} by a PLC-dependent pathway. An increase in intracellular Ca^{2+} in Type III cells is linked to the release of the neurotransmitter serotonin (5-HT), which can inhibit Type II receptor cells, however whether this occurs in a CaSR-dependent manner is currently unknown.

Figure 4: CaSR-mediated L-amino acid-induced CCK secretion from STC-1 cells.

A, Stimulation of CCK secretion in response to L-Phe, L-Trp and D-Phe at 1.5 or 3.0 mM Ca^{2+}_o in STC-1 cells. The CaSR-active aromatic amino acids, L-Phe and L-Trp, both stimulated increases in CCK secretion in a calcium-dependent manner. CCK secretion was stereoselective, as exposure to D-Phe had minimal effect on CCK secretion. **B**, Concentration dependent CCK secretion in STC-1 cells. Increasing Ca^{2+}_o and L-Phe concentration both induced increases in CCK secretion in a concentration dependent manner in STC-1 cells. For **A & B** STC-1 cells were exposed to various agonists at different Ca^{2+}_o concentrations (1 – 3 mM) for 30 min at 37 °C before CCK secretion was determined by a commercial CCK enzyme immunoassay kit ((Phoenix Pharmaceuticals Inc., Belmont, CA). Values are presented as % mean change from baseline \pm SEM from 3 – 4 wells.

Table 1: Known functions of the CaSR in the gastrointestinal tract

Organ	Cell	Effect	Reference	
Stomach	G cells	Gastrin secretion Cell growth	[55]	
	Parietal cells	Acid secretion (H ⁺ -K ⁺ ATPase)	[56]	
Intestine	Duodenum	Enteric nervous system cells	Gut motility	[35]
		I cells	CCK secretion	[47]
		K cells	GIP secretion	[49]
		L cells	GLP-1 and PYY secretion	[49]
		Enteric nervous system cells	Inhibition of fluid secretion	[57]
	Colon	Colonocytes	Inhibition of cell proliferation Stimulation of cell differentiation Inhibition of ion/fluid secretion	[58,59] [35] [35]

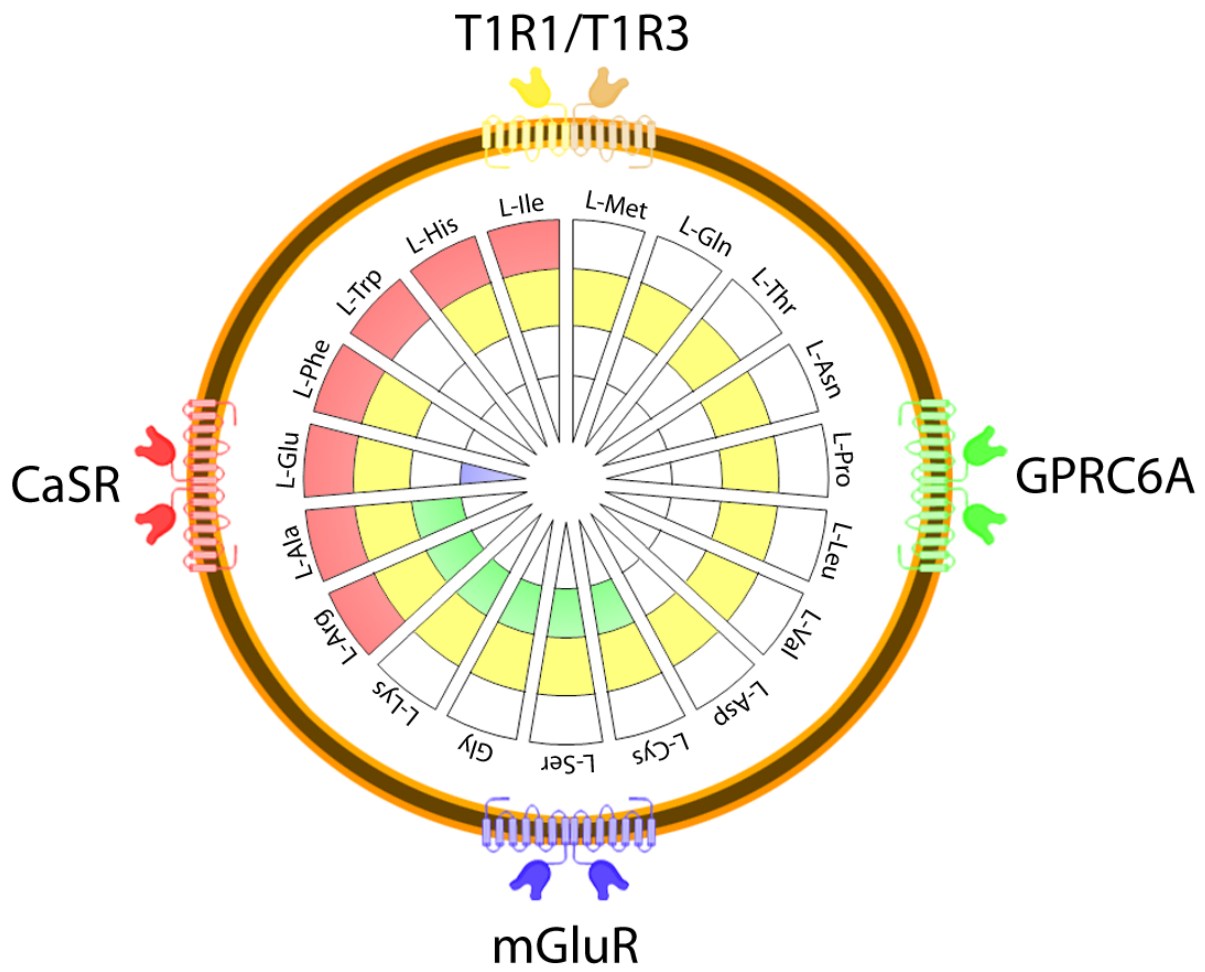
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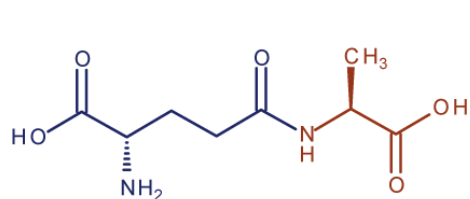
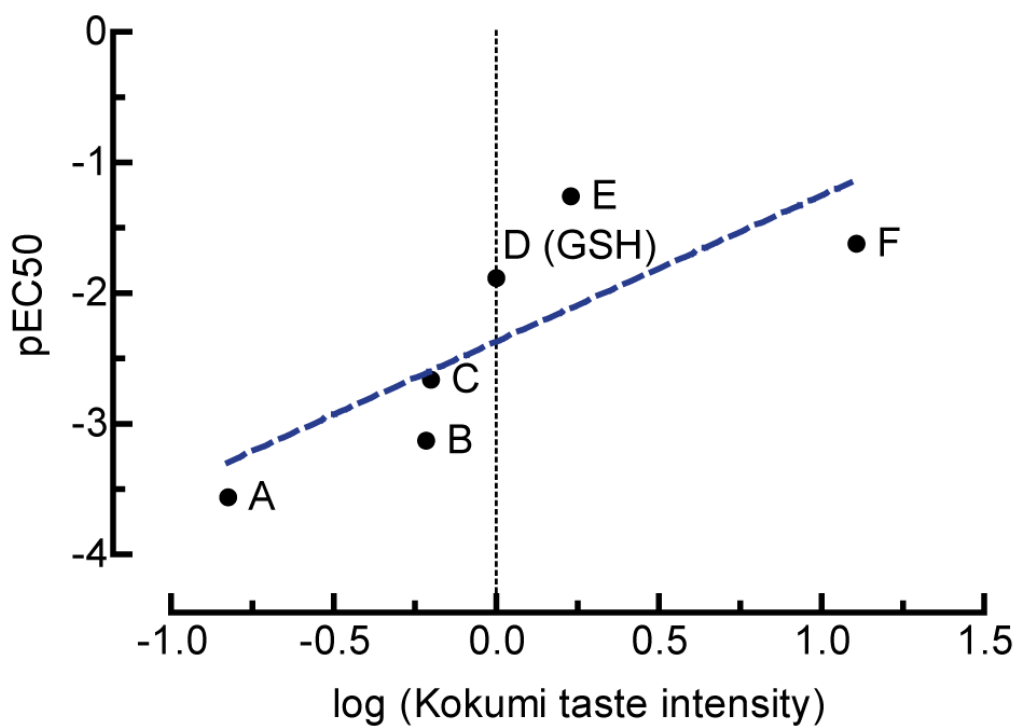
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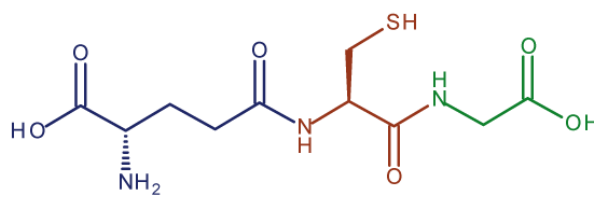
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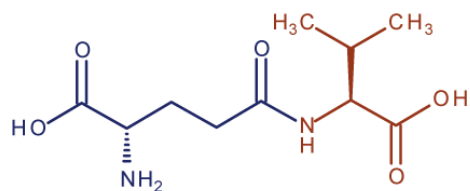




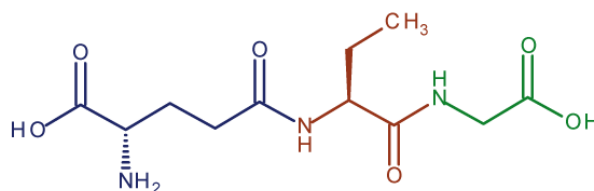
A: γ -Glu-Ala



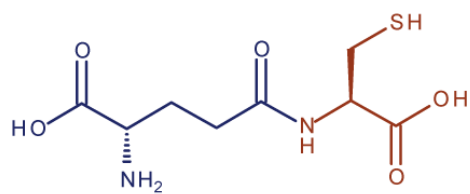
D: γ -Glu-Cys-Gly (GSH)



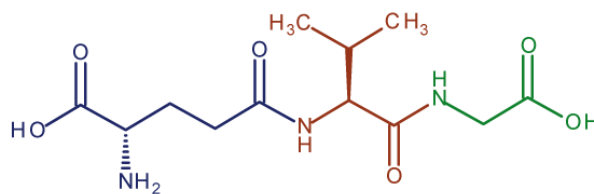
B: γ -Glu-Val



E: γ -Glu-Abu-Gly



C: γ -Glu-Cys



F: γ -Glu-Val-Gly

