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

Key words: Calcium-sensing receptor: Nutrient-sensing: Amino acids: Gastrointestinal tract: Taste modulation: Taste receptors

The Ca-sensing receptor (CaSR) is a class C G-protein-coupled receptor that was originally identified as the molecular ion sensor for free ionised extracellular Ca (Ca_o^{2+}) homeostasis[1]. Although CaSR's role in divalent cation metabolism has been well defined (reviewed by Brown & MacLeod[2] and Hofer & Brown[3]), the CaSR is expressed in a number of tissues and cell types not typically associated with Ca_o^{2+} homeostasis. Over the past few years, much work has been undertaken 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.

Although 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+} and Sr^{2+}), polycations (spermine and spermidine)[6], aminoglycoside antibiotics (neomycin and gentamicin)[7–9] and cationic polypeptides (poly-L-arginine)[10] (reviewed by Brown & MacLeod[2]). Changes in ionic strength[11] and pH[12] also affect CaSR's activity, as low ionic strength and high pH enhance CaSR's sensitivity to Ca_o^{2+}.

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

The CaSR is allosterically activated by L-amino acids, being able to respond to aromatic, aliphatic and polar amino acids, but not to branched or positively charged amino acids[13]. In contrast, taste receptors can be activated by aliphatic, polar, branched-chain and, to a lesser extent, charged amino acids, but not by 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 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 specialised cells including the oesophagus, 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 Conigrave & Brown[19] and Hebert[20]) and are listed in Table 1. In this review article, we examine the emerging physiological functions of the CaSR in sensing dietary nutrients in two separate roles: (1) 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.
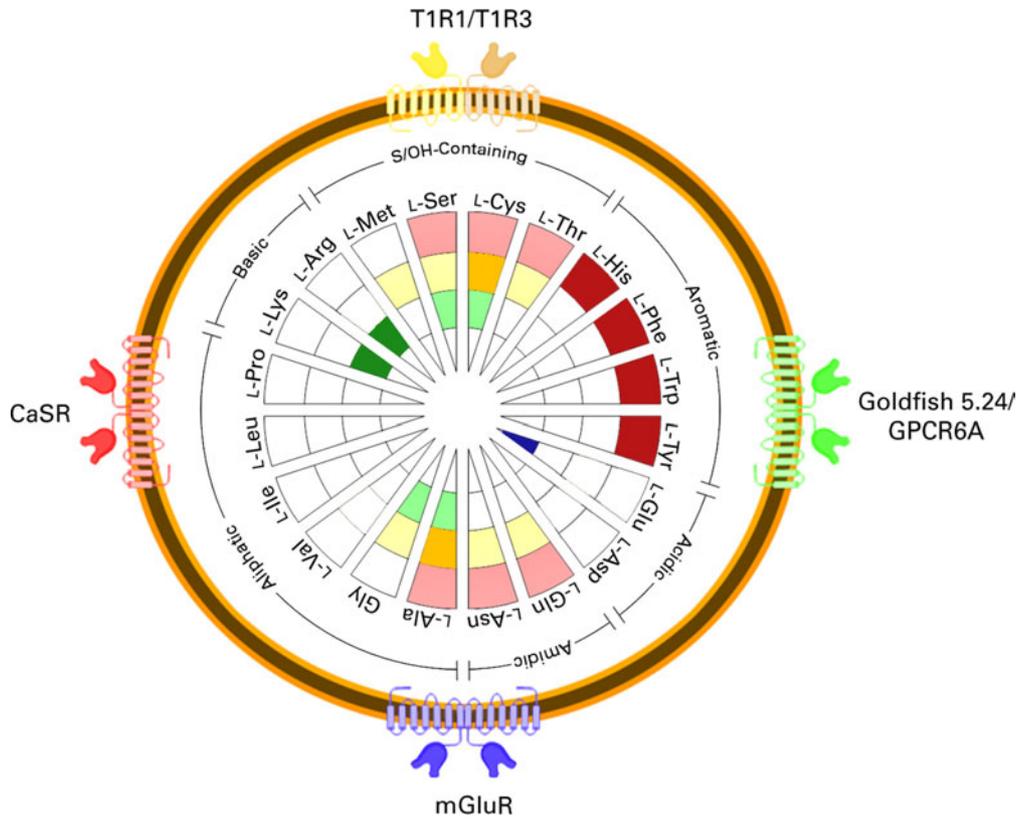
Fig. 1. Amino acid profiles of the calcium-sensing receptor (CaSR), goldfish 5.24/ GPRC6A receptors, metabotropic glutamate receptor 5 (mGluR) and type 1 taste receptor (T1R)1/T1R3 heterodimer. Effective amino acid agonists of the CaSR, goldfish 5.24/GPRC6a receptors, mGluR and T1R1/T1R3 heterodimer are shown. Data for T1R1/T1R3 efficacy were obtained from the percentage of responding cells in the HEK-293 cell population expressing the mouse T1R1/T1R3 heterodimer in the absence of 2·5 mM-inosine monophosphate and normalised to 50 mM-L-Cys response[53]. For the CaSR, amino acid efficacy was determined from the percentage of maximum reduction in half maximal effective concentration (EC$_{50}$) for extracellular Ca$_o^{2+}$ based on the method of Conigrave et al.[13] and normalised to the 10 mM-L-His response. Normalised data were obtained from Conigrave & Hampson[14]. We considered an effective amino acid agonist for the T1R1/T1R3 and CaSR to be one with ≥50% of their respective normalised responses. GPRC6A data are based on data obtained from mouse GPRC6A in the presence of 1 mM-Ca^{2+} and 1 mM-Mg$^{2[54,55]}$. Darker colours are used to indicate the top two to four amino acid activators for each receptor (where applicable) based on published results[13,14,53–55]. The receptor response to each amino acid is as follows: outer circle/red – CaSR; 2nd circle/yellow – T1R1/T1R3 heterodimer; 3rd circle/green – GPRC6A; and inner circle/blue – mGluR. (A colour version of this figure can be found online at http://www.journals.cambridge.org/bjn)

Calcium-sensing receptor as a taste receptor

There is now emerging evidence suggesting that the CaSR may play a role in the regulation of 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]. Expression of the CaSR in rat and mouse taste cells, namely in the circumvallate, foliate and, to a lesser extent, the fungiform papillae, has recently been reported[22,23].

Taste buds are generally composed of approximately 50–100 elongated taste cells, which belong to three different

Table 1. Known functions of the calcium-sensing receptor in the gastrointestinal tract

Organ	Cell	Membrane localisation	Effect	Reference
Stomach	G cells	Basolateral and apical	Gastrin secretion	
			Cell growth	Buchan et al.[36] and Feng et al.[56]
	Parietal cells	Basolateral	Acid secretion (H$^+$–K$^+$ ATPase)	Cheng et al.[18] and Dufner et al.[57]
Intestine				
Duodenum	Enteric nervous system cells	Myenteric and submucosal plexus, neurites and nerve fibres	Gut motility and inhibition of fluid secretion	Chattopadhyay et al.[17] and Cheng[58]
	I cells	Basolateral and apical	CCK secretion	Liou et al.[48]
	K cells	Unknown	GIP secretion	Mace et al.[50]
	L cells	Unknown	GLP-1 and PYY secretion	Mace et al.[50]
Colon	Colonocytes	Basolateral and apical	Inhibition of cell proliferation	Rey et al.[59,60]
			Stimulation of cell differentiation	Geibel & Hebert[35]
			Inhibition of ion/fluid secretion	Cheng[58]

CCK, cholecystokinin; GIP, gluco-indulinotropic peptide; GLP-1, glucagon-like peptide 1; PYY, peptide tyrosine tyrosine.

classes[24,25]: type I (glial-like) cells, which seem to be involved in the clearance of neurotransmitters through absorption/degradation; type II (receptor) cells, which express G-protein-coupled receptors (including the taste receptors T1R and T2R), which bind to bitter, sweet and umami compounds, the downstream signalling components (e.g. phospholipase C β2 (PLCβ2)) that transduce these taste qualities and the G-protein gustducin[26]; type III (presynaptic) 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 levels[22,23], where the CaSR appears to be distributed throughout the plasma membrane; however, there have been conflicting reports on whether the CaSR is also expressed in type I and type II cells. Bystrova *et al.*[23] showed *CaSR* mRNA expression at the single-cell level in a number of type I cells, but not in type II cells, using serial multistandard-assisted reverse transcriptase - polymerase chain reaction (SMART-PCR); however, they were unable to demonstrate functional coupling to the PLC-dependent Ca^{2+} signalling pathways in type I cells.

The possibility remains that the CaSR signals in a PLC-independent manner in type I taste cells. Type I taste cells express the renal outer medullary K^+ channel on their apical membrane, where this channel might play a role in the recycling of 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 the activation of the basolateral CaSR has been shown to inhibit apical renal outer medullary K^+ through signalling pathways involving arachidonic acid and its metabolites[28], and the type I taste cell CaSR may signal in a similar manner (Fig. 2).

Conversely, San Gabriel *et al.*[22] and Maruyama *et al.*[29] have demonstrated expression of the CaSR in a subset of taste cells that express either neural cell adhesion molecule (NCAM) (a marker of type III cells) or PLCβ2 (a marker of type II cells) using immunofluorescence. CaSR-positive, PLCβ2-expressing type II cells did not express one of the subunits required for the sweet/umami taste receptors, T1R3[29]; however, whether other receptors, such as the bitter T2R receptor, are co-expressed in CaSR-positive type II taste cells is currently unknown.

Interestingly, recent work[30] has demonstrated that in CaSR-expressing HEK-293 cells this receptor may be stimulated by the bitter compound denatonium in the millimolar range. Similar to other small peptides, such as glutathione and γ-glutamyl peptides[15,16], it seems to have a positive allosteric effect on Ca_o^{2+} concentration−response curves, although whether denatonium stimulates the CaSR in taste cells is unknown[30].

The exposure of CaSR-expressing type III taste cells to L-amino acids (L-Phe and Arg), γ-glutamyl peptides (such as glutathione and γ-glutamyl-valine-glycine) and calcimimetics (cinacalcet) has been shown to evoke intracellular $Ca^{2+}(Ca_i^{2+})$ transients, which are ablated by the non-specific PLC inhibitor U73122[23,29]. High concentrations (3 μM) of the negative CaSR allosteric modulator, the 'calcilytic' NPS 2143, have also been shown to inhibit γ-glutamyl-valine-glycine-mediated Ca_i^{2+} responses, suggesting that these
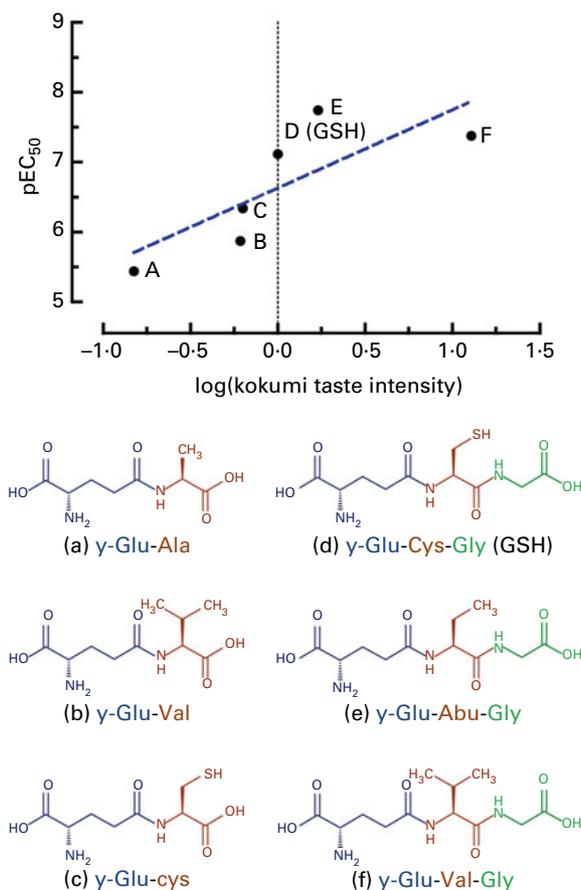

Fig. 2. Relationship between calcium-sensing receptor (CaSR) half maximal effective concentration (EC_{50}) values and kokumi taste intensity. A total of six γ-glutamyl peptides were tested for kokumi taste intensity by a panel of assessors. The intensity of kokumi taste was quantified in reference to the glutathione (GSH) concentration required to achieve an equivalent intensity of taste sensation. EC_{50} values for these substances were determined by measuring agonist-evoked increase in intracellular Ca^{2+} concentrations 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 obtained from Ohsu *et al.*[31] and redrawn. (A colour version of this figure can be found online at http://www.journals.cambridge.org/bjn)

responses might be mediated through the activation of the CaSR (Fig. 2)[29].

Although the majority of this work has been completed in rodent taste cells, there is evidence that the CaSR plays a role in human taste transduction. Human sensory analysis has demonstrated that a number of CaSR activators, including glutathione and γ-glutamyl-valine-glycine, 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 the half maximal effective concentration (EC_{50}) values in CaSR-expressing HEK-293 cells (Fig. 3, modified from that of Ohsu *et al.*[31]). In addition, high concentrations of the calcilytic NPS 2143 have been found to significantly suppress the kokumi taste effects of CaSR agonists[31].

There may also be an additional role for the CaSR in the oral detection of Ca_o^{2+}, which has a distinctive taste quality,
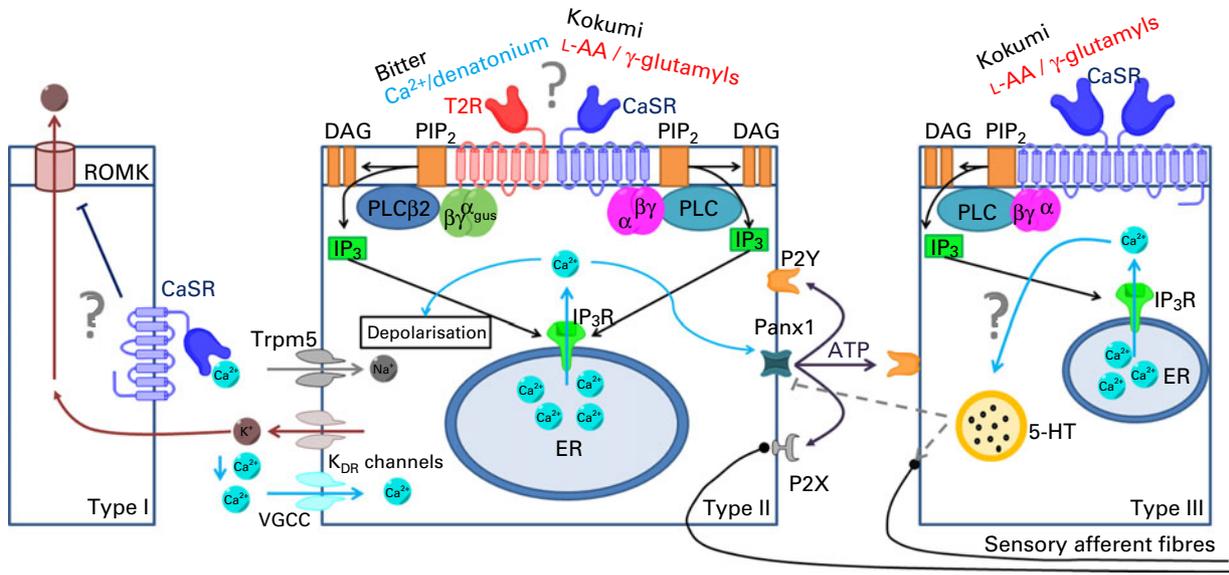
Fig. 3. Proposed roles of the calcium-sensing receptor (CaSR) within taste cells. This diagram depicts the proposed roles and signalling pathways for the CaSR in type I, II and III taste cells. In this figure, the CaSR is positioned on the membrane that is most appropriate for its postulated role. (1) In type I (glial-like) cells, activation of the basolateral CaSR may be linked to the regulation of potassium recycling by the apical renal outer medullary potassium (ROMK) channel, similar to its role in the kidney. (2) The CaSR is most probably co-expressed in type II (receptor) cells with T2R receptors on the luminal membrane, where it may play a role in the transduction of both bitter taste (oral Ca^{2+}/denatonium) and kokumi taste (L-amino acids (L-AA)/γ-glutamyl). Activation of CaSR homodimers, or possible CaSR/T2R heterodimers, leads to activation of the G-protein gustducin (α_{gus}) and phospholipase C β2 (PLCβ2). PLCβ2 catalyses the formation of inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG) from phosphatidylinositol 4,5-bisphophate (PIP_2), leading to the release of Ca^{2+} from the endoplasmic reticulum (ER) through IP_3 binding to an IP_3 receptor (IP_3R). Increased intracellular Ca^{2+} concentrations lead to the depolarisation of the cell through the actions of the Na^+ channel transient receptor potential cation channel, subfamily M, member 5 (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 never fibres[25]. (3) In type III presynaptic cells, activation of the apical CaSR by γ-glutamyl peptides leads to an increase in intracellular Ca^{2+} concentrations by a PLC-dependent pathway. An increase in intracellular Ca^{2+} concentrations in type III cells is linked to the release of the serotonin 5-hydroxytryptamine (5-HT), which can inhibit type II receptor cells; however, whether this occurs in a CaSR-dependent manner is currently unknown. (A colour version of this figure can be found online at http://www.journals.cambridge.org/bjn)

with aqueous Ca-containing solutions tasting quite bitter to humans[32]. Interestingly, Ca deprivation has been shown to increase the palatability of Ca in rodents[33]. The exact mechanism by which animals detect/'taste' Ca is still yet to be fully elucidated; however, previous work[33] has implicated the involvement of the T1R3 receptor. It has been suggested that the T1R3 receptor may heterodimerise with the CaSR to form a functional Ca^{2+} sensor in type II taste cells[33]; however, there is no evidence to date that the expression of T1R3 and that of the CaSR overlap[23,29].

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

Calcium-sensing receptor in the intestine

The CaSR is present in the stomach, where its activation stimulates the secretion of gastrin (by G cells) and of H^+ (by antral cells) (reviewed by Geibel & Hebert[35]). In gastrin-secreting G cells, the CaSR is expressed on both the apical and basolateral membranes[36], suggesting that they have the ability to respond to changes in both the luminal contents and blood[19], whereas

in parietal cells, the CaSR is expressed only on the basolateral membrane[18], which could allow the stimulation of gastric acid secretion by intestinally absorbed L-amino acids[19].

Seminal studies carried out by Geibel & Hebert[35] have demonstrated that the CaSR, expressed on both the apical and basolateral membranes of colonic crypts, plays a fundamental role in the colon in NaCl and water transport and raises the possibility of using CaSR-based therapeutics to prevent toxin-induced secretory diarrhoea, one of the most debilitating conditions in underdeveloped countries. Luminal Ca^{2+} also promotes gut epithelial differentiation, while CaSR-mediated signalling suppresses gut cell proliferation while preserving epithelial integrity[37]. Dietary Ca^{2+} intake is associated with a reduced risk of colon cancer and CaSR expression is absent in colon cancer specimens while being highly abundant in normal tissue from the same patients (see Rogers et al.[38] for a review). While a definitive and direct link between loss of CaSR expression and malignant transformation in the gut remains to be elucidated, it has been hypothesised 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 in the regulation of hormone secretion in intestinal enteroendocrine cells in response to L-amino acids. Aromatic amino acids have been shown to mediate the secretion of the GI hormone cholecystokinin (CCK)[39–43]. The secretion of CCK from enteroendocrine cells in the

small intestine is a major regulator for the release of bile by the gall bladder, as well as the secretion of digestive enzymes from the pancreas[37,42]. CCK also acts as a satiety hormone, reducing food intake in various species, including humans[44].

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 had focused on the murine enteroendocrine cell line STC-1. Here, L-Phe was shown to stimulate CCK secretion in a Ca_o^{2+}-dependent manner (Fig. 4(a) and (b))[45]. L-Phe also increased Ca_i^{2+} concentrations and Ca^{2+} channel activity, while the Ca^{2+} channel blocker diltiazem inhibited CCK secretion[45]. Phe-mediated secretion is also stereoselective for the natural L-isomer (Fig. 4(a)). High concentrations of the calcilytic 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[46]. Recent work[47] has also demonstrated that the STC-1 cells
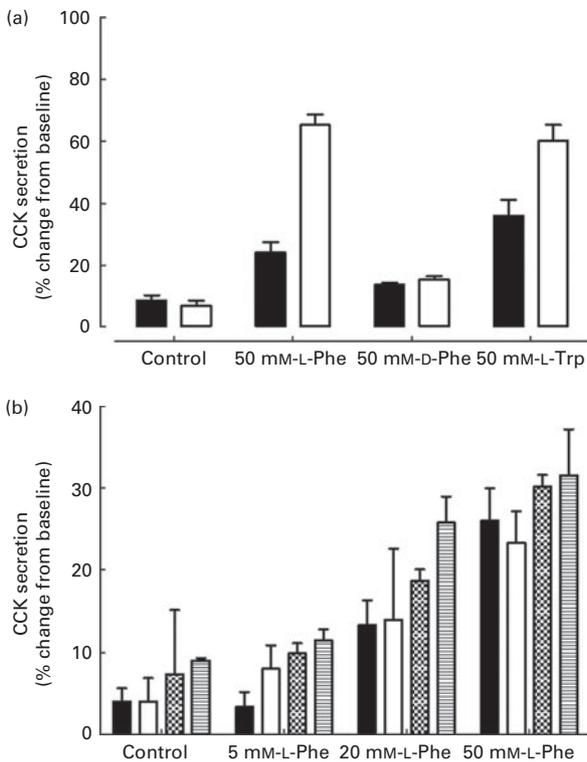

Fig. 4. L-Amino acid-induced cholecystokinin (CCK) secretion from STC-1 cells. (a) Stimulation of CCK secretion in response to L-Phe, L-Trp and D-Phe at 1·5 mm-Ca_o^{2+} (■) or 3·0 mm-Ca_o^{2+} (□) concentrations in STC-1 cells. Both the Ca-sensing receptor-active aromatic amino acids L-Phe and L-Trp stimulated increases in CCK secretion in a calcium-dependent manner. CCK secretion was stereoselective, as exposure to D-Phe had a minimal effect on CCK secretion. (b) Concentration-dependent CCK secretion in STC-1 cells. Increasing concentrations of both Ca_o^{2+} and L-Phe induced increases in CCK secretion in a concentration-dependent manner in STC-1 cells. ■, 1·0 mm-Ca^{2+}; □, 1·5 mm-Ca^{2+}; ▨, 2·0 mm-Ca^{2+}; ▤, 2·5 mm-Ca^{2+}. For (a) and (b), STC-1 cells were exposed to various agonists at different Ca_o^{2+} concentrations (1–3 mm) for 30 min at 37°C before CCK secretion was determined using a commercial CCK enzyme immunoassay kit (Phoenix Pharmaceuticals, Inc.). Values are % mean change from baseline, with their standard errors from three to four wells represented by vertical bars.

respond to protein hydrolysates (such as egg albumin, meat, potato, casein and soyabean) with an increase in CCK secretion, which is suppressed in the presence of NPS 2143, except for meat hydrolysate-induced CCK secretion. Together, these studies strongly support a role for the CaSR as an amino acid sensor in these cells.

The involvement of the CaSR in the mediation of CCK secretion has been elucidated by two groups using bacterial artificial chromosome 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 bacterial artificial chromosome transgenic mice by fluorescence-activated cell sorting[48,49].

The first such study, carried out by Liou *et al.*[48], used fluorescence-activated cell sorting to isolate native CCK-secreting duodenal I cells from CCK–eGFP bacterial artificial chromosome mice. Using quantitative RT-PCR, these isolated CCK–eGFP cells were shown to express *CaSR* mRNA transcripts at a level approximately 900-fold higher than that in non-eGFP-expressing cells, and the presence of CaSR protein was confirmed with immunofluorescence. The exposure of isolated I cells to phenylalanine induced intracellular Ca^{2+} influx, which was Ca_o^{2+} dependent and stereoselective for L-Phe. L-Phe-dependent CCK secretion in native I cells was enhanced in the presence of supraphysiological Ca_o^{2+} concentrations, indicating a synergistic effect of Ca_o^{2+} and this amino acid. Interestingly, supraphysiological Ca_o^{2+} concentrations alone were unable to evoke an increase in CCK secretion. Based on these results, the authors concluded that the CaSR acts as an amino acid sensor in this physiological setting[48].

Deletion of the CaSR from CCK–eGFP I cells did not affect basal CCK secretion; however, L-Phe-mediated Ca_i^{2+} influx was lost. Furthermore, L-Phe and supraphysiological Ca_o^{2+} concentrations surprisingly suppressed CCK secretion by approximately 20–30% in these cells, compared with basal levels, suggesting that not only is the CaSR required for L-Phe-mediated CCK secretion, but also the absence of a fully functional receptor may inhibit L-amino acid-induced CCK secretion[48].

The second study, carried out by Wang and colleagues, examined CCK-secreting intestinal mucosal cells in CCK–eGFP bacterial artificial chromosome transgenic mice. Expression of the CaSR was confirmed with quantitative RT-PCR and immunofluorescence and was found to be localised in both the apical and basolateral regions of CCK–eGFP cells, similar to that shown in the study carried out by Liou *et al*[48]. Aromatic amino acids L-Phe and L-Trp, but not the non-aromatic amino acid L-Ala, caused transient increases in Ca_i^{2+} concentrations and stimulated CCK secretion. Antagonisation of the CaSR with the calcilytic Calhex 231 blocked aromatic amino acid-mediated CCK secretion, without affecting the effect of hyperpolarising concentrations of KCl, again pointing to a role for the CaSR in the modulation of the effects of certain amino acids on CCK secretion[49].

Recently, the CaSR has also been implicated in the regulation of K- and L-cell activity in response to L-amino acids[50]. Isolated loops of the rat small intestine were used to quantify the

secretion of three anti-diabetic gut peptides (gluco-indulinotro-pic peptide, glucagon-like peptide 1 and peptide tyrosine tyro-sine) in response to a number of L-amino acids.

L-Phe, L-Trp, L-Arg, L-Asn and L-Gln induced the secretion of gluco-indulinotropic peptide, glucagon-like peptide 1 and peptide tyrosine tyrosine in the presence of physiological Ca_o^{2+} concentrations (i.e. 1·25 mM). Characteristic of a CaSR-mediated response, L-amino acid-induced secretion responses were abolished in the absence of Ca_o^{2+} for all the three peptides. High concentrations of the CaSR antagonist Calhex 231 suppressed L-amino acid secretion responses to various degrees, with the exception of L-Gln-stimulated glucagon-like peptide 1 secretion[50]. Inhibition of the CaSR by Calhex 231 was most efficient at suppressing aromatic amino acid responses[50], perhaps unsurprisingly as it is the most potent CaSR ligand[13]. Furthermore, the addition of a CaSR allosteric agonist, NPS R568, after the initial elevation of gluco-indulino-tropic peptide, glucagon-like peptide 1 or peptide tyrosine tyrosine secretion by L-amino acids further enhanced secretion to a maximal level, while an increase in Ca_o^{2+} concentrations increased the potency of L-Phe-induced L-/K-cell response.

Summary and conclusion

The role of the CaSR in nutrient sensing within the GI system continues to evolve with time. Previous studies[51,52] 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 Ca metabolism. New developments, presented in this review article, implicate the involvement of the CaSR in the modulation of appetite and control of satiety and anti-diabetic hormone secretion in response to amino acids/dietary Ca. Overall, these findings present the CaSR as a possible new therapeutic target in the ongoing fight against obesity and osteoporosis and their related disorders.

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