Taste Receptors in the Gastrointestinal Tract

II. L-Amino acid sensing by calcium-sensing receptors: implications for GI physiology

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Conigrave, Arthur D. and Edward M. Brown. L-Amino acid sensing by calcium-sensing receptors: implications for GI physiology. Am J Physiol Gastrointest Liver Physiol 291: G753–G761, 2006; doi:10.1152/ajpgi.00189.2006.—The extracellular calcium-sensing receptor (CaR) is a multimodal sensor for several key nutrients, notably Ca^{2+} ions and L-amino acids, and is expressed abundantly throughout the gastrointestinal tract. While its role as a Ca^{2+} ion sensor is well recognized, its physiological significance as an L-amino acid sensor and thus, in the gastrointestinal tract, as a sensor of protein ingestion is only now coming to light. This review focuses on the CaR’s amino acid sensing properties at both the molecular and cellular levels and considers new and putative physiological roles for the CaR in the amino acid-dependent regulation of gut hormone secretion, epithelial transport, and satiety.

When it was first cloned, the CaR was immediately identified by the endocrinologists. It was first cloned from a bovine parathyroid gland cDNA library (6) in an effort to identify the cell surface molecule that mediates the feedback mechanism by which parathyroid cells shut off the synthesis and secretion of parathyroid hormone once the hormone has done its job: elevating the extracellular ionized calcium concentration (Ca^{2+},o). On the basis of the available evidence, the cloned CaR does indeed mediate this critical function. Thus the CaR null (−/−) mouse exhibits uncontrolled hyperparathyroidism and attendant hypercalcemia owing to loss of feedback control of parathyroid cells. Furthermore, humans with homozygous inactivating mutations of the CaR exhibit a closely related condition, neonatal severe hyperparathyroidism (for review, see Ref. 7).

At second glance, the CaR also belongs to the nephrologists (47). Unlike homozygous inactivating mutations of the CaR, heterozygous inactivating mutations have, in general, only a limited effect on parathyroid function. Thus the CaR +/− mouse and humans heterozygous for inactivating CaR mutations exhibit only mild disturbances of parathyroid function. Instead, they exhibit a predominantly renal-based defect in calcium metabolism in which there is an inappropriate elevation in calcium reabsorption from the tubules. This is sufficient, when combined with mildly abnormal Ca^{2+}-regulated parathyroid hormone (PTH) release, to elevate Ca^{2+},o into the range commonly observed in adult primary hyperparathyroidism due to adenomatous disease (from 1.1–1.3 mM to ~1.3–1.5 mM) and to markedly lower the urinary calcium excretion below the normal range of ~2–5 mmol/day. These key clinical features of an otherwise largely benign clinical condition have led to the diagnostic name, familial hypocalciuric hypercalcemia.

Thus the CaR has key nonredundant roles in calcium homeostasis, and its most obvious roles are dependent upon its expression in the parathyroid and kidney. Interestingly, elimination of the PTH gene (35) or of the parathyroid glands themselves, via knockout of the gcm-2 gene (53), markedly limits the severity of the CaR null phenotype, indicating that its control of the parathyroid is paramount and raising questions as to its significance in other tissues.

Thus the question might be asked: “Are all other roles of the CaR, associated with its expression in a truly diverse spectrum of tissues, essentially redundant and thus of little physiological interest?” The answer to this question appears to be, in essence, “No, there are a large number of significant, nonredundant functions of the CaR that contribute to the optimal control of function in diverse physiological systems.” In the kidney, for example, local CaR plays additional roles in the regulation of water excretion by suppressing vasopressin-stimulated water reabsorption, an apparent defense against the formation of calcium-containing renal stones (for review, see Ref. 7). CaRs also contribute to the control of Na^+, K^+, and Cl^− transport in the distal nephron as revealed by the impact of severe activating mutations (56, 58).

CaRs are also expressed widely in the gastrointestinal (GI) tract. Why are they there and what are they doing? This review focuses on the roles the CaR plays, or appears to play, in the GI tract with particular attention to its recently recognized L-amino acid-sensing function and the impact this has on the control of digestion, absorption, and satiety.

The CaR as a Family C G-Protein Coupled Receptor

When it was first cloned, the CaR was immediately identified as a G-protein coupled receptor (GPCR) from hydropathy plot analysis that indicated the presence of a repetitive hydrophobic, seven-transmembrane domain (7-TMD) motif (6, 20) (Fig. 1). Subsequent experiments confirmed that the CaR couples to phosphatidylinositol (PI)-specific phospholipase C and induces the mobilization of intracellular Ca^{2+}. This neatly explained why elevated concentrations of extracellular Ca^{2+} and Mg^{2+} rapidly induce intracellular Ca^{2+} mobilization and inositol phosphate turnover in parathyroid cells via the G-
protein-dependent activation of PI-specific phospholipase C (for review, see Ref. 7).

The CaR’s atypically large NH$_2$-terminal extracellular domain (residues 22–606) and its sequence homology with the metabotropic glutamate receptors led to its placement in family C or subclass 3 of the GPCR superfamily. These receptors are, in general, composed of four main protein domains: an NH$_2$-terminal extracellular, nutrient-binding, Venus Fly Trap (VFT) domain of ~500 amino acids; a Cys-rich domain of ~90 amino acids that couples nutrient binding to receptor activation; a 7-TMD motif of ~240 amino acids; and an intracellular COOH-terminal signaling domain of ~230 amino acids that is required for the activation of intracellular signaling pathways (Fig. 1). Such a large protein potentially provides binding sites for multiple biochemical species, most obviously in the VFT domain and 7-TMD regions, but also potentially in other regions.

The CaR as a Multi-Modal, Multi-Metabolic Sensor

The CaR is activated by various multivalent cations with charge density, rather than ionic radius, being a major determinant of the potency of activators as diverse as alkaline earth metals (such as Ca$^{2+}$ and Sr$^{2+}$), lanthanides (such as Gd$^{3+}$), polycations (such as spermine and spermidine), and cationic peptides such as poly-lysine and poly-arginine (for review, see Ref. 7). All these cations might be presumed to bind on a common, spatially unrestricted, positive-charge-sensitive surface. Consistent with this idea, the CaR has also been shown recently to respond to changes in ionic strength and pH: low ionic strength (44) and high pH (43) sensitize the receptor to Ca$^{2+}$. However, the CaR also exhibits a markedly positive cooperativity for Ca$^{2+}$-induced receptor activation, pointing to the existence of multiple Ca$^{2+}$ binding sites, some of which may be truly selective for Ca$^{2+}$ ions over other multivalent cations. A recent molecular modeling analysis of the CaRs bilobed VFT domain and various mutations within this domain, for example, concluded that a Ca$^{2+}$ binding site exists in a discrete region of the crevice that lies between the lobes of each receptor subunit (49).

Because the CaR protein is most closely related to receptors for amino acids, including the metabotropic glutamate receptors (mGlus) and the fish 5,24 basic amino acid receptor, and amino acid analogs, in the case of the metabotropic GABA$_B$ receptors, it seems surprising that the protein encodes a Ca$^{2+}$-sensing receptor rather than a receptor for an amino acid or a biologically significant amino acid analog. In the spring of 1999, with our colleague Steve Quinn, we decided to systematically test whether the CaR might retain sensitivity to L-amino acids, either as coagonists or as allosteric activators such as the, then recently identified, phenylalkylamine type-II calcimimetics (41). Not knowing a priori which amino acids to test or where they might bind, we initially selected L-Phe for testing because this amino acid bears some limited structural homology with the phenylalkylamines. The choice was fortunate and, we now know, quite fortuitous because L-Phe and the phenylalkylamines bind in distinct domains (40) and, in fact, exhibit positive interactions with one another as receptor activators (62).

The CaR as an Amino Acid Receptor

For our assay system, we used HEK293 cells that stably expressed the human CaR (HEK-CaR cells). These cells exhibit a robust extracellular Ca$^{2+}$-induced intracellular Ca$^{2+}$ mobilization response that is readily detected after loading with fura 2. The CaR responded stereoselectively to L-Phe (0.3–30 mM) with acute elevations in intracellular Ca$^{2+}$ concentration provided that the cells were exposed to a submaximal Ca$^{2+}$ concentration (16) (Fig. 2). At maximally effective Ca$^{2+}$ concentrations (>5 mM), L-Phe had no effect. On the other hand, in the absence of Ca$^{2+}$ ions, or in the presence of a subthreshold Ca$^{2+}$ concentration (found to lie between 0.5 and 1.0 mM), L-Phe had no effect. Consistent with this behavior, we found that L-amino acids are not CaR agonists. Rather, like the phenylalkylamine type-II calcimimetics, L-Phe sensitized the CaR to Ca$^{2+}$, markedly shifting the concentration response to the left. We had identified a novel allosteric enhancer or, perhaps better, given that Ca$^{2+}$ is present at concentrations above threshold in normal physiological fluids, an allosteric activator (31).

There is a rather frustrating, largely old and unwieldy literature on physiological interactions between protein and calcium metabolism, plenty of evidence for links at the whole body level but very little by the way of definitive molecular or even cellular detail. So, instead, we took our next step based on observations made by GI physiologists in the 1960s and 1970s who identified a nutrient-sensing system for gut calcium receptors (CaR). The figure is an annotated map of the calcium-sensing receptor (CaR). The figure is an annotated hydropathy plot (Kyte-Doolittle) of the human CaR showing the positions of the major domains including the NH$_2$-terminal Venus Fly Trap (VFT) domain, the Cys-rich domain, the 7-transmembrane domain region, and the COOH-terminal signaling domain. Also shown are the recognized binding sites for amino acids in the VFT domain, phenylalkylamine type-II calcimimetics in the 7-transmembrane domain region, and the cytoskeletal protein filamin in the COOH-terminal tail. C-X-C denotes the sites of intermolecular disulfide bond formation.
G755

AMINO ACID SENSING BY GUT CALCIUM RECEPTORS

Themes

Location of the CaR’s Amino Acid Binding Site

On the basis of the CaR’s homology to other family C GPCRs, it might be expected that l-amino acids would bind in the cleft of the VFT domain distinct from the recognized phenylalkylamine binding site in the transmembrane domain region (22, 40). An early analysis pointing to the existence of a specific l-amino acid binding site showed that l-Phe and the phenylalkylamine R467 exerted significant positive interactions on receptor activation (62). Furthermore, mutations in the region of the key lobe I VFT domain residue S170 appeared to selectively impair amino acid responses (63). The hypothesis that amino acids bind in the CaR’s VFT domain has been recently confirmed by chimeric receptor analysis (40). In this study, replacement of the CaR’s Cys-rich, transmembrane domain, and intracellular COOH-terminal domain by the corresponding domains of the rat mGlu-1 receptor, so that only the CaR’s VFT domain remained, preserved amino acid sensing. Removal of the CaRs entire VFT domain, however, eliminated amino acid sensing, leaving Ca$^{2+}$ and phenylalkylamine-sensing intact (40). Thus l-amino acids bind in a stereoselective site in the CaR’s VFT domain demonstrating that its amino acid homology to other family C receptors is indicative of functional as well as structural relationships. A model of the dimeric VFT domains of the CaR with two bound amino acid molecules is presented in Fig. 3. It is not known whether this form is preferred or whether, instead, the dimeric CaR might more readily adopt a closed-open configuration as described for the mGlu-1 receptor (36, 51).

Amino Acid-Induced Signaling Mechanisms

The CaR couples to various G-protein linked intracellular signaling pathways. Key elements in these pathways include PI-PLC, intracellular Ca$^{2+}$ mobilization, PKC, PLA2, PLD, PI-3-kinase, and several mitogen-activated protein kinases, including ERK 1/2, p38, and JNK, all of which are activated by elevated Ca$^{2+}$, Gd$^{3+}$, or Mg$^{2+}$ concentrations, and adenylyl cyclase, which in parathyroid cells is inhibited by CaR activators (for reviews, see Refs. 7, 57). If l-amino acids were simply allosteric enhancers acting to sensitize the CaR to its cationic activators, it might be supposed that they would act with equally facility on all CaR-linked pathways. Recent analysis suggests, however, that, on a background of a suprathreshold Ca$^{2+}$o, amino acids selectively activate a discrete intracellular signaling pathway in HEK293 cells that induces intracellular Ca$^{2+}$ mobilization and has only a limited impact on PI-PLC (46).

In our initial experiments, which were conducted on large populations of fura 2-loaded cells, we observed continuous amino acid-induced elevations in cytoplasmic free Ca$^{2+}$ concentration. Digital imaging analysis of fura 2-loaded single CaR-expressing HEK293 cells, however, indicates that, on a background of a submaximal Ca$^{2+}$o, CaR-active amino acids such as l-Phe induced characteristic low-frequency oscillations (1–2 min$^{-1}$) in cytoplasmic free Ca$^{2+}$ concentration (60). Elevations in Ca$^{2+}$o above the chosen baseline level, 1.8 mM, also induced oscillations in cytoplasmic free Ca$^{2+}$ concentration, but these tended to be higher in frequency (3–5 min$^{-1}$) and took the form of sinusoidal waves rather than spikes. These differences in intracellular Ca$^{2+}$ response appear to arise from differences in the signaling pathways that are initiated by aromatic amino acids, including l-Phe and l-Trp, as well as other amino acids, that appeared to provide a molecular link between protein ingestion and gastrin release (17, 50), gastric acid secretion (27, 34, 38), pancreatic fluid secretion (39), and cholecystokinin (CCK) release (32). On the basis of these results, obvious next amino acids to test were l-TRY and others, including l-His, l-Ser, and l-Ala. In the HEK-CaR cell system, l-Trp behaved in almost identical fashion to l-Phe (Fig. 2), and all of these amino acids were effective. We then systematically worked our way through the standard 20 genetically encoded amino acids that are all readily available for intestinal absorption from dietary protein. Many, but not all, of them were effective, and l-isomers were preferred to d-isomers, although subsequent analysis indicated that l vs. d selectivity was less marked for smaller amino acids such as Ala (15). It seemed that half or even more than half of the 20 amino acids tested, including aromatics, polar, aliphatic, and acidic amino acids, were effective, with aromatic amino acids being the most potent. Two classes of amino acids were clearly ineffective: basic amino acids (a surprise for some scientists because, like Ca$^{2+}$, they are positively charged at neutral pH) and branched-chain amino acids, such as l-Leu (16). Systematic analysis of the functional group requirements for receptor activation using analogs of l-Ala led to two additional rules: the α-amino and α-carboxylate groups were absolutely required. Thus the receptor senses l-amino acids. The observations described above identified several subclasses of l-amino acids as allosteric activators of the CaR and indicated that it responds to an integrated amino acid concentration, a weighted sum of all effective amino acids.
amino acids and elevated Ca$^{2+}$ concentration. For example, clear differences in sensitivity to the PKC inhibitor RO-31-8220 were observed. Whereas RO-31-8220 converted extracellular Ca$^{2+}$-induced oscillatory responses to continuous broad transients that lasted several minutes, it completely abolished amino acid-induced responses (60). Furthermore, amino acid-induced, but not extracellular Ca$^{2+}$-induced, responses were abolished by an inhibitor of Ca$^{2+}$ release from intracellular stores, 2-aminoethoxydiphenyl-borane. Extracellular Ca$^{2+}$-induced oscillatory responses appear to require repetitive PKC-dependent phosphorylation and dephosphorylation of T888 in the CaR's COOH-terminal tail (61). The mutant T888A, which was previously shown to eliminate the CaR's extracellular Ca$^{2+}$-dependent sensitivity to PKC (3, 28), for example, exhibited continuous broad responses to elevated Ca$^{2+}$, very similar to the impact of PKC inhibitors on the wild-type CaR (61). Amino acid-induced oscillations in intracellular Ca$^{2+}$, on the other hand, were preserved in T888A. These data indicate that the CaR exhibits differential signaling in response to amino acids and extracellular Ca$^{2+}$, and a more recent analysis has provided insights into elements of the amino acid-induced intracellular pathway upstream of Ca$^{2+}$ oscillations in CaR-expressing HEK293 cells (46). These appear to include the heterotrimeric G-proteins, G$12$ or G$13$, the CaR’s distal COOH terminus, possibly corresponding to the filamin-binding domain, the small G-protein Rho, and the actin cytoskeleton. Because inhibition of none of these components disrupted extracellular Ca$^{2+}$-induced signaling, the results appear to justify the conclusion that the CaR adopts specific conformations in response to either elevated Ca$^{2+}$ or amino acids (46).

The cause and effect relationships between these various signaling elements are currently unclear. In addition, it is not yet clear how CaR-dependent amino acid-induced reorganization of the cytoskeleton regulates Ca$^{2+}$ fluxes. A recent study of cytoskeletal-plasma membrane rearrangements in CaR-expressing HEK293 cells concluded that extracellular Ca$^{2+}$, but not aromatic amino acids, induces retraction of cellular processes via the activation of Rho kinase and associated reorganization of actin stress fibers (18). Furthermore, it is not yet clear whether amino acid-dependent signaling is essentially the same in all cells in which the CaR is expressed or exhibits cell context-specific signaling as described for extracellular Ca$^{2+}$ (e.g., Ref. 24). Nevertheless, the simple concept that stabilization of a single closed form of the extracellular VFT domain by l-amino acids and/or Ca$^{2+}$ ions induces receptor activation and the activation of all associated signaling pathways is clearly inadequate.

**Modes of Operation of the CaR**

We originally envisaged that the CaR operates in several different modes dependent on specific changes in the nutrient composition of the compartment in which it is expressed (16). In one mode, it behaves as a Ca$^{2+}$ receptor under conditions in which the Ca$^{2+}$ changes either generally, requiring responses from the parathyroid, thyroid C-cells, and renal tubular cells, or locally, e.g., in response to receptor-mediated Ca$^{2+}$ efflux (26). In a second mode, it behaves as an amino acid receptor under conditions in which the Ca$^{2+}$ concentration lies between 0.5 and 1.0 mM, i.e., below the normal Ca$^{2+}$ of 1.1–1.3 mM, this mode of operation would appear to be important in all compartments exposed to significant variations in amino acid concentration, particularly in the distribution of the portal vein at the time of protein digestion and amino acid absorption but also systemically because amino acid concentrations undergo dynamic changes in systemic blood under these circumstances (for review, see Ref. 14). A third mode is also apparent in which Ca$^{2+}$ and amino acids are both elevated, e.g., in the portal circulation after a protein- and calcium-rich meal.
Although the basic concepts described above still seem reasonable, the finding that amino acids and supraphysiological concentrations of Ca\(^{2+}\) (>1.5 mM) activate distinct signaling pathways in CaR-expressing HEK293 cells indicates that a refinement is required. Thus the receptor not only responds in different modes to either amino acids or elevated Ca\(^{2+}\) concentration but, in doing so, can select specific biological responses dependent on the cell signaling apparatus available. A prominent feature of the amino acid-induced responses in CaR-expressing HEK293 cells (16, 60) and normal human parathyroid cells (15) is intracellular Ca\(^{2+}\) mobilization, known to be a potent stimulus for hormone as well as fluid and electrolyte secretion from various cell types in the GI tract.

CaR Expression in the GI Tract

Because the CaR responds to amino acids with a selectivity for aromatic and aliphatic amino acids similar to that identified for various GI processes, it seems reasonable to ask whether the CaR mediates amino acid sensing by some or many of the specialized cells and tissues of the GI tract and to consider the physiological roles that such sensing mechanisms might play.

To answer these questions, it is first necessary to consider where the CaR is expressed. Analysis of CaR expression by immunohistochemistry indicates that it is widely expressed in epithelial cells and neurons of the stomach, small intestine, and large intestine. In the stomach, for example, the CaR is expressed in acid-secreting gastric parietal cells and pepsinogen-secreting chief cells of the gastric glands (13) as well as surface mucus-secreting cells (48) (for review, see Ref. 23). Expression of these sites suggests roles in the control of gastric acid, pepsinogen, and mucus secretion, and there is now good evidence that activation of the CaR does indeed support gastric acid secretion in either the absence or presence of the potent secretagogue histamine (21). In the antrum of the stomach, the CaR is also expressed on gastrin-secreting G cells (9, 45) and that CCK-secreting small intestinal I cells require evaluation. Consistent with this latter idea, the CaR-activating amino acid L-Phe has been shown to stimulate CCK secretion (32, 37) and CCK-dependent pancreatic fluid secretion (39). The CaR is also expressed by neurons of the submucosal and myenteric plexuses throughout the stomach and intestine (12) implying roles in the control of fluid and electrolyte secretion and motility.

Regulation of Digestion and Absorption: What Are the Roles of Amino Acid-Activated CaRs?

The following discussion focuses on cells of the GI tract that 1) regulate digestion and absorption and/or contribute to the control of appetite and satiety; 2) express the CaR; and/or 3) are known to respond to aromatic amino acids, such as L-Phe and L-Trp.

Gastric phase of digestion. Upon the entry of food into the stomach, activated pepsin preferentially cleaves ingested proteins on the COOH-terminal sides of aromatic amino acids, including L-Phe, L-Trp, and L-Tyr, especially in the presence of an aromatic or other hydrophobic amino acid on the COOH side of the cleavage site (for review, see Ref. 29). Thus, as a result of pepsin action, some short hydrophobic peptides and free aromatic amino acids are released and, before exiting the stomach for absorption in the small intestine, free aromatic

![Image](https://example.com/image.png)

Fig. 4. Expression of the calcium-sensing receptor in antral G cells. Left: expression of the CaR. The longer arrow demonstrates expression of the CaR in the basolateral region of the cell. The shorter arrow demonstrates expression at the apical pole (and thus in potential contact with the gastric contents). Right: immunoreactive gastrin localized just deep to the basolateral membrane in the same cell as shown at left. The figure has been reproduced from Ref. 45 with the permission of the authors and publishers.
aa. The physiological response as demonstrated by the oral ingestion of calcium and/or peptide hydrolysates (peptones) (5) is the release of the peptide hormone gastrin followed by the further release of peptides and amino acids. Both extracellular Ca\(^{2+}\) (9, 45) and aromatic amino acids, including L-Phe and L-Trp (19, 50, 52), are recognized signals for gastrin release. Furthermore, CaRs expressed in the apical membranes of G cells are a likely target, because they mediate pronounced elevations in cytoplasmic free Ca\(^{2+}\) concentration, a potent stimulus for gastrin release (9, 45). Gastrin released into the capillary network of the stomach acts primarily on enterochromaffin-like cells in the submucosa of the gastric glands to release histamine and thus stimulate gastric acid secretion via the activation of H2 receptors on parietal cells.

It seems likely, however, that intestinally absorbed amino acids can also stimulate gastric acid release and that this process too is under CaR control, in gastric parietal cells. The demonstration that the CaR is expressed by parietal cells may explain previous observations that gastric acid secretion is activated not only by luminal amino acids, but also by intravenous infusions of amino acids including L-Phe and L-Trp (27, 33, 38). Consistent with this idea, elevated Ca\(^{2+}\) and other multivalent cation activators of the CaR mobilize intracellular Ca\(^{2+}\) in gastric parietal cells and stimulate acid secretion in a manner that is sensitive to proton pump inhibitors (21). Elevations in intracellular Ca\(^{2+}\) concentration can act either alone or in concert with histamine-induced elevations in cAMP to activate the insertion of proton pumps into the apical membrane from intracellular tubulovesicles (Fig. 6). In addition, it has been shown recently that the CaR-active amino acids, L-Phe and L-Trp, stimulate acid secretion from isolated gastric glands in a manner that is highly dependent on variations in the extracellular Ca\(^{2+}\) concentration (10). Although the system L-amino acid transporter may also contribute to amino acid-induced activation of gastric acid secretion (30), the specific inhibitor 2-amino-2-norbornane-carboxylic acid did not block these effects of L-Phe or L-Trp (10).

### Intestinal phase of digestion

In the upper small intestine, the major pancreatic protease, chymotrypsin, like pepsin, selectively cleaves proteins and peptides on the COOH side of aromatic and hydrophobic amino acid residues. This suggests that aromatic amino acids may also be preferentially released in the intestinal phase of digestion to promote, at least initially, continuing gastric acid and pepsin release after their absorption and delivery to CaRs on parietal cells in the gastric fundus while, separately, initiating key events that promote the intestinal phase of digestion and absorption. Consistent with this idea, an analysis of human jejunal contents for both free and peptide amino acids before and 3 h after the ingestion of a protein-rich meal indicated that aromatic and some other non-polar amino acids were preferentially released compared with aliphatic, polar, and acidic amino acids (2). For example, under these conditions, between 40 and 60% of the total L-Phe and L-Tyr but only ~12–15% of the total L-Ala, L-Ser, L-Thr, and L-Glu in the jejunal contents were present in their free amino acid forms. It thus seems plausible that the early entry and/or release of free aromatic amino acids into the intestinal luminal fluid provides signals for the secretion of small intestinal hormones, notably CCK, whose secretion is known to be activated by L-Phe both in vivo and in vitro (4, 32, 37). CCK
release, like gastrin release, is dependent on elevations in intracellular Ca\(^{2+}\) concentration that are blocked by an inhibitor of voltage-operated Ca\(^{2+}\) channels, diltiazem (37). Although it is tempting to hypothesize that the CaR mediates this effect of amino acids on CCK secretion, it remains to be shown that intestinal I cells express the CaR and, if so, whether it is expressed primarily on the apical membrane where it could potentially respond to intraluminal amino acids or the basolateral membrane where it would require absorbed amino acids.

The release of peptides (1) and free amino acids into the small intestinal lumen is followed shortly thereafter by their cellular absorption and, in the case of short peptides, after the action of intracellular peptidases, the release of free amino acids into the portal blood (2). CaRs expressed on hepatocytes, which are bathed by portal blood, are known to be coupled to the control of bile flow (11). It is not yet certain whether Ca\(^{2+}\), amino acids, or other putative physiological activators of the CaR regulate bile flow at the time of nutrient digestion; however, the concept that bile flow is coupled to the ingestion of nutrients is clearly attractive. Furthermore, CaRs expressed on pancreatic acinar cells may also participate in the control of pancreatic fluid and enzyme secretion (8). Whether activation of the CaR by Ca\(^{2+}\), amino acids, or other gut-derived activators such as spermine has effects on other secretory glands, e.g., on bicarbonate secretion from Brunner’s submucosal glands or pancreatic ducts, is currently uncertain.

Coincident with these effects, amino acids acting on CaRs on enteric nerves have the potential to modify enteric motor responses, which are primarily coordinated by the myenteric plexus, and/or secretory motor responses, which are coordinated by the submucous plexus, and/or communicate changes in intestinal nutrient levels to feeding and satiety centers via autonomic sensory nerves. As noted above, expression of the CaR has been detected throughout the intestinal plexuses (12). In one scenario, it seems possible that amino acid-activated CaRs on neurons of the myenteric plexus act to promote small intestinal digestion and absorption, e.g., by slowing antegrade peristalsis and by promoting static, segmenting movements. In addition, delivery of L-Phe into the small intestinal lumen is recognized as a potent satiety stimulus that may...
operate either by the release of CCK (4) or via the activation of neuronal circuits linked to central satiety centers.

As described above, the potential scope of the effects of amino acid-activated CaRs is wide on the basis of the extensive distribution of the CaR in the GI tract and the sensitivity of many GI processes to aromatic amino acids. A limited set of the effects proposed above is presented in diagrammatic form in Fig. 7.

Is There a Role for the CaR in Intestinal Calcium Absorption?

Although the ability of the CaR to sense both amino acids and Ca\(^{2+}\) ions is now well established, and this review has demonstrated how the CaR may act in the GI tract as a key switch for the control of various steps in digestion and absorption, it might be wondered whether dual regulation of the CaR also has an impact on intestinal calcium absorption. Recent analysis of this question indicates that the CaR may indeed be involved. Thus, in humans with absorptive hypercalciuria, an increased sensitivity of gastric acid secretion to the oral ingestion of calcium and protein hydrolysates has been detected (5). Elevated gastric acid production could promote calcium absorption by facilitating the release of calcium ions from insoluble precipitates. Alternatively, CaR activation might promote the activity of intestinal epithelial Ca\(^{2+}\) channels. The recent molecular cloning of the Ca\(^{2+}\) channels responsible for epithelial calcium transport in the kidney and intestine, now known as TRPV5 and TRPV6, respectively, should facilitate analysis of this question (25, 42). Consistent with a possible role for the CaR in the regulation of intestinal calcium absorption, it is expressed locally by small intestinal epithelial cells (12). In addition, variations in dietary calcium intake regulate the expression of epithelial Ca\(^{2+}\) transport channels independent of changes in 1,25 dihydroxyvitamin D levels (for review, see Ref. 55) and, interestingly, in the 1\(\alpha\)-OHase knockout mouse, high dietary calcium rescued the phenotype, at least in part, by promoting expression of TRPV6 in the duodenum (54). Although the significance of this result for the normal physiological control of intestinal calcium absorption is not yet certain, it does point to a possible regulatory role for the CaR.

Summary and Conclusions

There has been an explosion of interest in the CaR since it was first cloned (7). The realization that it has multiple ligand binding sites, is activated by multiple physiologically important biochemical species, and couples to ligand-selective signaling pathways has rather added to its charm. The story that is now evolving for amino acids in the gut, including effects on neuronal circuits linked to central satiety centers and function.

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