Calcium-sensing receptor 20 years later

Tariq I. Alfadda,1* Ahmad M. A. Saleh,1* Pascal Houillier,3 and John P. Geibel1,2

1Department of Surgery, Yale School of Medicine, New Haven, Connecticut; 2Department of Cellular and Molecular Physiology, Yale School of Medicine, New Haven, Connecticut; and 3INSERM UMR S1138, Paris, France; Paris Descartes University, Paris, France; Assistance Publique-Hopitaux de Paris, Hopital Europeen Georges Pompidou, Paris, France

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Alfadda TI, Saleh AM, Houillier P, Geibel JP. Calcium-sensing receptor 20 years later. Am J Physiol Cell Physiol 307: C221–C231, 2014. First published May 28, 2014; doi:10.1152/ajpcell.00139.2014.—The calcium-sensing receptor (CaSR) has played an important role as a target in the treatment of a variety of disease states over the past 20 plus years. In this review, we give an overview of the receptor at the cellular level and then provide details as to how this receptor has been targeted to modulate cellular ion transport mechanisms. As a member of the G protein-coupled receptor (GPCR) family, it has a high degree of homology with a variety of other members in this class, which could explain why this receptor has been identified in so many different tissues throughout the body. This diversity of locations sets it apart from other members of the family and may explain how the receptor interacts with so many different organ systems in the body to modulate the physiology and pathophysiology. The receptor is unique in that it has two large exofacial lobes that sit in the extracellular environment and sense changes in a wide variety of environmental cues including salinity, pH, amino acid concentration, and polyamines to name just a few. It is for this reason that there has been a great deal of research associated with normal receptor physiology over the past 20 years. With the ongoing research, in more recent years a focus on the pathophysiology has emerged and the effects of receptor mutations on cellular and organ physiology have been identified. We hope that this review will enhance and update the knowledge about the importance of this receptor and stimulate future potential investigations focused around this receptor in cellular, organ, and systemic physiology and pathophysiology.

G protein-coupled receptors; calcimimetics; kidney; gastrointestinal tract; divalent ions

IT IS NOW 20 YEARS since the initial review on calcium-sensing receptor (CaSR) was published (17). The initial review focused on the preliminary characterization and description of the receptor and how it related to the parathyroid and whole body calcium handling and homeostasis (17). Since that time, not only has there been a great deal of new information concerning CaSR and parathyroid disease come to light, but in addition the receptor has now been identified in many other tissues and organs in the body where it has been shown to have a diverse role in cellular and organismic pathophysiology (1, 2, 24, 28). With the further identification and functional characterization of the receptor in multiple organ systems and at the cellular level (Fig. 1), we felt it was now time to provide an updated overview of the calcium-sensing receptor and its diverse functions throughout the body.

Our review will give a brief historical review of the identification of the receptor in the parathyroid and its role as an important target for parathyroid disease. We include a discussion of the initial cloning studies and the ability of this receptor to “sense” divalent and trivalent ions in the extracellular environment. We follow this with a molecular profile of the receptor based on the many years of research into the structure and function of the receptor. In terms of the physiology of the receptor, we begin with an overview of the receptor and how modulation of the receptor can play a role in both calcium homeostasis and fluid regulation via the kidney. We then provide an overview of the receptor in the gut. We present the recent findings on the receptor related to gastric function and the recent findings on intestinal fluid and salt transport and its modulation via the receptor. In the next section, we discuss the role of the receptor in skin, and finally we give a brief overview of receptor mutations that were identified with a human phenotype, and what outcomes these activating or inactivating mutations have on the pathophysiology of the individual.

IDENTIFICATION OF A NOVEL CALCIUM SENSOR

Extracellular fluid (ECF) calcium concentration is maintained within a narrow range in normal individuals since any change in calcium entry into the ECF is rapidly matched by an identical change in urinary calcium excretion (95). Available evidence indicates that an adequate secretion of parathyroid hormone (PTH) is required for the minute-to-minute control of ECF calcium concentration (66). Parathyroid cells must appro-
priately adapt their PTH secretion to the prevailing ECF calcium concentration. Therefore, parathyroid gland cells must have the capacity to “sense” small changes in ECF calcium concentration. A very steep inverse sigmoidal relationship between PTH secretion and ECF-free calcium has been described both in vivo (10, 94, 115) and in vitro (15, 115) in humans. In addition, the response curves to agonists in the parathyroid gland cells show a Hill coefficient of about 3, suggesting a positive cooperativity that could account for the narrow range over which ECF calcium actually controls PTH secretion (13).

Upon changes in ECF calcium concentration, several intracellular signaling pathways are affected, including the cAMP-protein kinase A, phospholipase C-protein kinase C, and inositol phosphate pathways (12). This and other evidence has suggested that ECF calcium can modulate the parathyroid cell function by a receptor-like mechanism, coupled to one or more G protein-dependent pathways (26, 44).

**Cloning/Genetics**

In 1993, Brown et al. (14) isolated a 5.3 kb clone, named BoPCaR (for bovine parathyroid calcium receptor), by expression cloning in *Xenopus laevis* oocytes. BoPCaR had pharmacological properties very close to those of the calcium-sensing protein expressed in bovine parathyroid cells (14, 27). Particularly, it was confirmed that several other di- (e.g., Mg$^{2+}$, Cd$^{2+}$), tri- (e.g., Gd$^{3+}$, La$^{3+}$) or polyvalent (e.g., neomycin) cations can mimic the effect of free calcium on the parathyroid gland cells. Subsequently, the cloning of full-length CaSRs from various mammalian species, including human, rat and rabbit, was performed (1, 112).

**Primitive Receptor Found In a Variety of Animals and Fish**

The presence of CaSR was confirmed in all vertebrate classes. The strong structural conservation of CaSR throughout evolution is suggested by the high similarity in sequences across all the vertebral classes (58). Birds also express CaSR, as demonstrated by the cloning of a full-length calcium receptor from the chicken parathyroid (37).

More unexpected, perhaps, is the expression of a gene homologous to CaSR in animals that do not have parathyroid glands, such as teleost and elasmobranch fish (65, 87). The dogfish shark CaSR is expressed in several segments of the shark kidney tubule and, importantly, in many osmoregulatory segments, including rectal gland, intestine, stomach, olfactory epithelia, and gill chloride cells.

Among the major clades, both extracellular (ECD) and intracellular (ICD) domains display some differences. In the ECD, an insertion sequence is present in tetrapods, lobe-finned and elasmobranch (cartilaginous) fish, but absent in bony fish,

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**Fig. 1. Summary of some of the organ systems in which the CaSR was identified and the identified functions it carries in these locations.**
urochordates, and cephalochordates. The length of the ICD differs markedly among the various clades. Only the cephalochordates and the tetrapods CaSR contains insertion sequences (58). By contrast, the sequence of the putative calcium-binding sites within the ECD among the vertebrates remains remarkably conserved (63).

The importance of CaSR in bone development from an ontogenic standpoint was recently demonstrated (22). The role that CaSR might have played in the phylogenic development of the skeleton remains an open question for further study.

The expression of CaSR is detectable in various organs (including kidney, intestine, olfactory organ, gills) of fish. Available evidence suggests that CaSR acts as a salinity sensor in fish, to inform internal organs of changes in the salinity in surrounding water (87).

MOLECULAR PROFILE OF THE RECEPTOR

A major barrier to advancing our understanding of the role of calcium in regulating CaSR was the lack of adequate information about their calcium-binding locations, which was greatly hindered by the lack of a solved three-dimensional structure and the rapid off rates due to low calcium-binding affinities. Previously, Huang et al. (64) managed to identify three potential calcium-binding sites in a modeled CaSR structure using computational algorithms based on the geometric description and surface electrostatic potentials (64). They concluded that the calcium-binding site was on the ECD of the CaSR (64). The accurate location of the calcium-binding site on the receptor gives researchers a specific location to target when activation of the CaSR is required. CaSR, and the two type B receptors to γ-aminobutyric acid and GPRC6A, belongs to the family C of GPCRs. All these receptors have a large ECD including a “Venus flytrap” (VFT) sequence that contains the dimerization sites and the orthosteric sites for binding of endogenous agonists (Fig. 2). The ECD of the human CaSR contains 612 amino acid residues. The ECD is linked to the transmembrane domains (TMD) by a cysteine-rich domain (CRD) that contains 9 out of the 17 cysteine residues characteristic of the ECD of the receptor. These nine cysteine residues form four disulfide bridges within the CRD and one additional bridge with the VFT sequence. They are strictly required for the proper function of the receptor, indicating that the tertiary and quaternary structure is important for function.

During its intracellular biosynthesis, the CaSR is dimerized in the endoplasmic reticulum through two disulfide bonds, between the cysteine 129 and 131 of each monomer (41). However, non-covalently bound dimers might still be expressed at the cell surface when the disulfide bonds are lacking (16). The receptor’s ECD contains nine potential sites of N-linked glycosylation, which is performed in the Golgi apparatus and is required for the normal expression of the protein at the cell surface (14, 40). The fully glycosylated monomer has a molecular mass of 150–160 kDa, indicating a carbohydrate content of 35–40 kDa/monomer. While it is important for the normal membrane expression of the receptor, N-linked glycosylation is probably not critical for its biological activity (16).

The CaSR ECD is formed by two lobes (LB1 and LB2) separated by a cavity delineating the ligand-binding site representing the VFT (122). Calcium binds in the cleft between the two lobes of each VFT causing the lobes to close on one another and the VFT to rotate, leading to receptor activation (57) (Fig. 2). Recent studies have shown that the VFT contains five calcium-binding sites (63, 64, 122). These sites are located on the protein surface and contain clusters of neutral or negatively charged amino acids. Site 1, the main site, is situated in the cleft between the lobes of the VFT (63, 64, 122).

CASR AND THE KIDNEY

Location of the Receptor Throughout the Kidney

Following its initial cloning from bovine parathyroid glands (14), CaSR was cloned from the rat and human kidney (1, 112). The human kidney receptor protein was found to have more than 93% homology to bovine parathyroid and rat kidney receptor (1). The ECD of the human CaSR contains 612 amino acid residues. The ECD is linked to the transmembrane domains (TMD) by a cysteine-rich domain (CRD) that contains 9 out of the 17 cysteine residues characteristic of the ECD of the receptor. These nine cysteine residues form four disulfide bridges within the CRD and one additional bridge with the VFT sequence. They are strictly required for the proper function of the receptor, indicating that the tertiary and quaternary structure is important for function.

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Fig. 2. A schematic depicting the molecular structure of the receptor. In the inactive state shown to the left, the “Venus flytrap” is interacting with calcium ions, which leads to the activation of the receptor. Activation results in conformational changes, resulting in downstream signal transduction.
mandatory to anticipate which functions the CaSR could control.

Employing immunohistochemical studies using antibodies raised against different parts of the receptor, all authors agree that intense staining was noted on the basolateral membranes of cells of the medullary and cortical thick ascending limb (TAL) (23, 77, 110, 126). Localization of the receptor to other parts of the nephron using this technique is controversial. In addition to TAL, Riccardi et al. (110) showed that the CaSR protein is expressed in the proximal convoluted tubule (PCT), proximal straight tubule (PST), distal convoluted tubule (DCT) and cortical collecting ducts (CCD) of the rat kidney. CaSR is mainly located at the apical brush-border membrane in the proximal tubule (110) and is expressed on the apical membrane of inner medullary collecting duct (IMCD) cells (23, 118). However, other studies showed no detectable expression of the protein in the PCT, DCT, or collecting duct (77, 126).

Other studies utilizing reverse transcription-polymerase chain reaction (RT-PCR) have confirmed the presence of abundant transcription product in TAL in addition to significant product in DCT and CCD of rat kidney (111, 140). The initial study by Riccardi et al. (111) also reported transcription product in DCT and CCD of rat kidney (111, 140). Other studies showed alteration in calcium reabsorption without any change in transepithelial potential difference and the chloride transport in the microperfused rat cortical TAL (34). Other studies showed alteration in calcium reabsorption without any change in transepithelial potential or NaCl transport (77, 86).

### Role of the Receptor in the Kidney

All authors agree that CaSR is abundantly expressed in the medullary and cortical parts of the TAL of Henle’s loop (77, 110, 126). These tubular segments actively reabsorb sodium chloride. From an electrophysiological standpoint, the overall consequence of sodium chloride reabsorption in the TAL is a lumen-positive transepithelial difference that is the driving force for the passive absorption of the divalent cations Ca\(^{2+}\) and Mg\(^{2+}\) along the paracellular pathway (9, 121).

Previous experiments conducted in dogs, rats, and mice demonstrated that calcium and magnesium absorption in the TAL decrease when either peritubular calcium or magnesium concentration is increased (36, 102–105, 139). Electrophysiological studies using patch-clamp techniques demonstrated that calcium and neomycin, two nonspecific agonists of the CaSR, reduce the activity of an apical K\(^+\) channel via the generation of arachidonic acid metabolites by the cytochrome P-450 pathway (136, 137). Some investigators reported that calcium and neomycin decrease both the transepithelial potential difference and the chloride transport in the microperfused rat cortical TAL (34). Other studies showed alteration in calcium reabsorption without any change in transepithelial potential or NaCl transport (77, 86). In mice with a kidney-specific disruption of CaSR, claudin 14, a putative negative regulator of the paracellular pathway permeability in the TAL (53), was found to be downregulated, whereas claudin 16, a positive regulator, was upregulated (126).

ECF calcium is able to alter transepithelial transport and/or transporters expression or affinity in other tubular segments. For example, ECF calcium inhibits Na\(^+\)-K\(^+\)-ATPase activity in the proximal tubule, which may participate in the natriuretic effect of hypercalcemia (73). In the mouse S3 segment of the proximal tubule, luminal gadolinium, a trivalent cationic agonist of CaSR, inhibits the PTH-evoked, but not the dopamine-evoked, decrease in phosphate absorption (3). Maiti and Beckman reported that both ECF calcium and gadolinium are able to increase the expression of the vitamin D receptor in a proximal tubule-derived cell line (80), an effect mediated by the p38 MAP kinase pathway (81). Riccardi et al. (113) reported that both a chronic high-phosphate diet and an acute injection of PTH reduce the expression of both Npt2a and CaSR in the rat brush border. Recently, Capasso et al. (20) showed that both luminal calcium and the calcimimetic NPS R-568 (Table 1) increase fluid absorption and acid secretion in the PCT of both the mouse and the rat, an effect not observed in CaSR (and Gcm2) knockout mice.

The DCT and the connecting tubules are short tubular segments responsible for the fine-tuning of magnesium and calcium transport.

### Table 1. Summary of compounds targeting the CaSR

<table>
<thead>
<tr>
<th>Compound</th>
<th>Indication</th>
<th>Mechanism</th>
<th>Comments</th>
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<tbody>
<tr>
<td>Calciumimetics: stimulate CaSR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type I: direct agonists</td>
<td>Ca(^{2+}), other divalent and trivalent cations, spermine, some aminoglycosides, some amino acids, and peptides</td>
<td>Physiological or investigational</td>
<td>Binds to physiological ligand site on ECD</td>
</tr>
<tr>
<td>Cinacalcet</td>
<td>Parathyroid carcinoma</td>
<td>Binds to TMD, leading to conformational change and more sensitivity to physiological ligand</td>
<td>See Ref. 89; Reported to be effective in a patient with NSHPT (46) Different mutations of CaSR altered affinity to Cinacalcet (71)</td>
</tr>
<tr>
<td>R-467 R-568</td>
<td>Secondary hyperparathyroidism in patients with CKD on hemodialysis</td>
<td>Binds to TMD, leading to conformational change and more sensitivity to physiological ligand</td>
<td>See Ref. 90</td>
</tr>
<tr>
<td>NPS 2143; Ronacaleret; Calhex 231</td>
<td>Primary hyperparathyroidism in patients unable to undergo surgery</td>
<td>Investigational</td>
<td></td>
</tr>
<tr>
<td>Calciumimetics: negative allosteric modulators</td>
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CaSR, calcium-sensing receptor; ECD, extracellular domain; TMD, transmembrane domain; NSHPT, neonatal severe primary hyperparathyroidism.
calcium reabsorption. Both of these cations are actively transported along a transcellular route. Magnesium enters the cell through the apical Mg channel transient receptor potential melastatin member 6 (TRPM6) while calcium enters through the calcium channel transient receptor potential vanilloid member 5 (TRPV5). Both transports are activated by PTH and the active metabolite of vitamin D, 1,25(OH)2-vitamin D (61). Some groups reported the expression of CaSR in the rat and human DCT and connecting tubule cells (110, 127). In HEK-293 cells, overexpressing TRPV5 and CaSR, neomycin or phenylalanine increased the activity of TRPV5 (127), suggesting that ECF calcium might stimulate its own reabsorption in this segment.

Patients with frank hypercalcemia may have polyuria due to a decrease in the urine concentrating ability (51, 124). Using in vitro microperfused rat IMCD, Sands et al. (118) showed that the nonspecific activators of CaSR, calcium and neomycin, decrease the vasopressin-elicted, transepithelial water flux by ~30–40%. In addition, hypercalcemia and/or hypercalciuria, resulting from a treatment of rats by dihydrotachysterol, reduces the abundance of aquaporin 2 in the same segment (117). This may explain why Trpv5-null mice, which are severely hypercalciuric, also have a significant polyuria (62). Trpv5-null mice have a low urinary pH, suggesting that a high urinary calcium concentration is able to promote distal urinary acidification. Actually, luminal calcium concentration increases hydrogen ion secretion by type A intercalated cells, an effect involving the H+/K+ -ATPase while inhibition of the receptor decreases urinary calcium excretion in renal transplant recipients with secondary hyperparathyroidism (32).

**CNSR AND THE GUT**

**Stomach**

Location of the receptor in the stomach. In the stomach, the receptor was first identified in the amphibian *Necturus maculatus* using RT-PCR. It was localized to the basolateral membrane of surface epithelial cells in the antrum and to a lesser extent the acid-secreting glands in the fundus by immunohistochemical studies (31). Later, the transcript and immunohistochemical identification of the receptor was carried out on cultured human antral gastric cells (108). The presence of the receptor in gastric epithelial and glandular cells was confirmed in a model of isolated gastric glands of the rat (28). The same study demonstrated the receptor’s presence in cells of submucosal and myenteric plexuses (28). The receptor was again identified mainly in the basolateral and to a lesser extent the apical membranes of gastric mucous epithelial cells in humans (116).

Role of the receptor in the stomach. Functional studies of the CaSR in the *Necturus* stomach showed hyperpolarization of basal membranes of gastric glands when subjected to high levels of calcium or to the calcimimetic NPS-467 (31) (Table 1). In rats, activation of the CaSR by gadolinium (Gd3+) resulted in increased intracellular calcium concentrations (28). Moreover, stimulation of the receptor on the basolateral membrane of parietal cells resulted in increased acid secretion through apical H+/K+ -ATPase while inhibition of the receptor resulted in decreased acid secretion even when glands were stimulated with classic secretagogues (50). Similar results were obtained when gastric glands were subjected to different concentrations of l-amino acids, known positive allosteric modifiers of CaSR, where increasing concentrations of these amino acids resulted in increases in acid secretion independent of secretagogue presence (19). In freshly isolated human gastric
glands, activation of the receptor resulted in similar effects to that seen in rats, that is, increased acid secretion (38). Inhibition of the receptor similarly resulted in decreased acid secretion despite the presence of histamine (38).

In human antral gastrin cells in culture, stimulation of the CaSR by increasing ECF calcium levels and by the calcimimetic drug spermine (Table 1) resulted in increases in gastric secretion in a dose-dependent manner (108). In addition, increasing ECF calcium in cell cultures of gastric glandular cells induced a proliferative response (116). In 17 healthy human subjects, the administration of Cinacalcet, a CaSR allosteric agonist (Table 1), over an 11-day course caused increased gastric secretion and basal acid secretion (21). Effect of hypercalcemia on the CaSR in the stomach may explain the association of primary hyperparathyroidism with peptic ulcer disease (47).

Pharmacological target for acid secretion. Acid rebound phenomenon is a well-observed finding with the use of calcium-containing antacids (49, 108). The activation of CaSR and in turn increased acid secretion may explain this observation. In this context, modulation of gastric acid secretion by targeting CaSR on acid-secreting cells as well as neighboring G and enterochromaffin-like (ECL) cells is theoretically feasible (6, 38). In most cases the acid secretion should be decreased and this entails the inhibition of CaSR by calcilytics (38, 50). Many calcilytic compounds (Table 1) have been used in research models; some are available orally but none are approved for use in humans (68). The systemic effects of CaSR-modulating drugs should also be taken into account (21).

Small and Large Intestine

Location of the receptor in the small and large intestine. The receptor was identified along the small and large intestine (24). It was clearly localized on the basal membranes of intestinal crypt and villi epithelial cells (24). On the apical surface of epithelial cells, the receptor was only identified in the small intestinal villi (24). Similarly, a previous study had shown products of transcription across intestinal segments with major transcript in the duodenum, jejunum, and ileum (45).

The presence of the receptor in the large intestine was confirmed by RT-PCR and Northern blotting (24, 29, 45). Further immunohistochemical studies localized the receptor to both basal and apical membranes of colonic crypt cells (24, 29). Strong immunostaining was also observed in the serosa, submucosa, and nerve fibers (24).

Role of the receptor in the small and large intestine. Previous studies postulated that, in the colon, surface cells were responsible for absorption and the crypts were responsible for secretion and that these two systems worked independently. Recent studies have shown that both surface and crypt cells are capable of both secretion and absorption (75, 85, 106). These studies conclude that fluid absorption and secretion are the two main roles of the colon (75, 85, 106). The CaSR has important roles in the colon such as fluid transport and colon motility. The effect on colon motility is mainly attributed to the role CaSR plays in the smooth muscle nerve plexuses (49). The effect of the receptor on fluid transport provides a new target both for constipation and more importantly for diarrhea (49).

Early studies performed by Favus, Kathaplia, and Coe (42, 43) observed the presence of a functional calcium-sensing mechanism in the rat intestine by demonstrating that the intestines can secrete or absorb calcium in response to changes in ECF calcium concentration and modulation by vitamin D (42, 43). Recent studies have proven through immunolocalization and receptor function studies that this calcium-sensing mechanism is actually the CaSR (64). Recently, Mace, Schindler, and Patel showed that CaSR could detect amino acids in the intestine to modify gut peptide secretion (79). These findings suggest that CaSR acts as an important regulator of K- and L-cell (intestinal enteroendocrine cells) activity in response to nutrient and nonnutrient stimuli (79). These recent studies establish new functions for CaSR as a regulator of gut peptide secretion that senses nutrients and provides signaling pathways for the release of glucose-dependent insulinoctopic polypeptide (GIP), glucagon-like peptide 1 (GLP-1), and peptide YY (PYY), which are anti-diabetic gut peptides (79).

Pharmacological target for diarrhea and inflammatory bowel disease. Fluid and electrolyte loss in secretory diarrhea is cyclic nucleotide dependent. Exposure to choleran toxin (CTX) results in upregulation of cAMP, which leads to diarrhea. On the other hand, Escherichia coli heat stable toxin (STA) upregulates cGMP. This fluid and electrolyte loss occurs not only through an increase in the secretory process process via chloride loss but also in the reduced absorptive capacity of the intestines via the basolateral sodium hydrogen exchanger’s subtype 2 and 3 (52, 78). Recent studies have shown that stimulation of the CaSR in the presence of cyclic nucleotides (i.e., CTX, STA, and guanylin) will suppress secretion and increase absorption (48). This occurs mainly through cyclic nucleotide degradation via phosphodiesterases (48). The reversal of CTX and STA endotoxin-induced fluid secretion by a small-molecule CaSR agonist (R-568) suggests that these compounds may provide a unique therapy for secretory diarrhea (48).

Inflammatory bowel disease (IBD; secretory type; IBD-S) is characterized by a large amount of fluid loss due to diarrhea and has been a topic of importance for researchers. The significance of finding a relation between IBD-S and CaSR has been an area of interest for years. Researchers have tried to link the association between patients who have familial hypocalciuric hypercalcemia due to a CaSR mutation and Crohn’s disease, yet the link still remains unclear (60).

SKIN/KERATINOCYTES

Skin although not directly involved in mineral homeostasis also expresses the CaSR (7). ECF calcium is a critical factor for differentiation in epidermal keratinocytes: increasing ECF calcium concentration evokes accumulation of inositol phosphates and elevation in free cytosolic calcium concentration (7). It also stimulates the binding of E-cadherins from adjacent cells as well as the interactions with catenins to form the core structure of adherens junctions. Keratinocyte differentiation, as well as the interaction between E-cadherin and catenins, is blocked when CaSR expression is inhibited (128). The differentiated keratinocytes display a lower responsiveness to ECF calcium than nondifferentiated keratinocytes (93). The reason might be that keratinocytes do express a splice variant of CaSR, lacking exon 5, which exerts a dominant negative effect of the full-length CaSR, and that the expression of this truncated splice variant increases as differentiation progresses (93).
Pharmacological Targeting of Skin CaSR

Since CaSR was identified, its effect on keratinocyte growth and differentiation was established, but no disease states of the skin were associated with mutations of the receptor (2, 129). Deletions in the CaSR resulted in abnormal ultrastructure and differentiation of the epidermis while overexpression led to accelerated hair follicle formation and increased differentiation markers (130). How this observation can be exploited in modulation of skin conditions, e.g., wound healing, is currently unknown but is of potential clinical use and worthy of further investigation.

RECEPTOR MUTATIONS

Activating Mutations

Autosomal dominant hypocalcemia. Since CaSR was first cloned, more than 90 gain-of-function mutations were reported (99). Most of these mutations have been described in patients with autosomal dominant hypocalcemia (99). The disease state is characterized by low calcium and below normal or inappropriately low PTH levels (39). Some patients also suffer from hypercalcemia with nephrolithiasis and nephrocalcinosis (107). In addition, intracranial calcifications and seizures have been described (18, 107, 123).

The pathophysiology involves a lower set point for calcium detection by CaSR in the parathyroid glands, resulting in higher sensitivity of the receptor to circulating calcium and in turn a lower PTH production (11, 125). In most cases the responsiveness to changes in ECF calcium levels is still maintained albeit to bring calcium to the new “normal” lower level (25). Most activating mutations are found on the ECD and seem to increase the receptor’s affinity for calcium (25).

Sporadic idiopathic hyperparathyroidism. Several de novo activating mutations have been reported in patients with sporadic hyperparathyroidism (5, 35, 76). Further genetic testing of the parents in these cases failed to demonstrate the mutations (5, 35, 76). Nevertheless, not all cases of sporadic idiopathic hyperparathyroidism were associated with activating mutations in the CaSR, suggesting that other mechanisms are involved (119).

Bartter syndrome type V. Bartter syndrome is a condition of deficient reabsorption of sodium and chloride in the kidney, leading to hypokalemic metabolic alkalosis, hyperreninemia, and hyperaldosteronemia (138). Type V Bartter syndrome is caused by some of the activating mutations of the CaSR and is typically associated with autosomal dominant hypocalcemia (132, 138). The mutation is thought to inhibit sodium reabsorption in the TAL, leading to the clinical manifestations (132).

Activating autoantibodies. Activating autoantibodies against the CaSR have also been reported (54, 67). The clinical spectrum included mainly patients with sporadic hyperparathyroidism and includes patients with autoimmune polyendocrine syndrome type 1 (54, 67).

Inactivating Mutations

Familial hypocalciuric hypercalcaemia (familial benign hypercalcaemia). Familial hypocalciuric hypercalcaemia (FHH) is an autosomal dominant disease, with a high degree of penetrance. In most cases, FHH is the consequence of a heterozygous loss-of-function mutation of the CaSR (100), although recently, loss-of-function mutations of two other proteins, adaptor protein 2 σ-subunit (AP2σ) (92) and Gα11 (91), have been involved in some kindred. A lifelong, most often asymptomatic, hypercalcaemia together with a normal urinary calcium excretion are the hallmarks of the disease (82, 83). The serum PTH concentration is typically normal, inappropriate to the prevailing hypercalcaemia, though it may be high in 20% of the afflicted patients, making it difficult to confirm the diagnosis of primary hyperparathyroidism (30). A few patients suffer from pancreatitis or chondrocalcinosis.

Neonatal severe primary hyperparathyroidism. Neonatal severe primary hyperparathyroidism (NSHPT) is a rare disease characterized by the early onset of extreme hypercalcaemia, severe hyperparathyroidism related to parathyroid hyperplasia, skeletal demineralization, respiratory distress, and hypotonia (8, 114). Physicians have often observed the familial coincidence between FHH and NSHPT. In 1994, Pollak et al. (101) reported that NSHPT is the homozygous form of FHH, the afflicted patients bearing either two identical mutations (homozygous, most often issued from consanguineous parents) or two distinct mutations (compound heterozygous). However, Pearce et al. (96) reported that NSHPT can be caused by heterozygous mutations in the CaSR gene, seemingly because some mutant proteins might exert a negative dominant effect.

Signs and symptoms of patients with NSHPT are reminiscent of the phenotype of Casr-null mice (59), which have a marked elevation in blood calcium and PTH concentrations, parathyroid gland hyperplasia, bone abnormalities, failure to thrive, and premature death. In mice, the bone phenotype and lethality can be efficiently rescued by the genetic deletion of parathyroid glands, as observed in the double homozygous Casr- and Gcm2- or Pth-deficient mice (69, 131). Most of the skeletal effects of CaSR disruption can therefore be ascribed to the attendant severe hyperparathyroidism. By contrast, hypercalciuria remains in the double homozygous mice, further indicating that the renal effect of CaSR is independent of the level of PTH.

As it is the case in the mouse, NSHPT can be fatal in humans if not aggressively treated. Recent observations suggest that bisphosphonates help to improve the life-threatening hypercalcaemia and severe bone demineralization (135), while in the past, total parathyroidectomy was sometimes needed in the most severe cases. A recent report demonstrated good response to Cinacalcet in a neonate with NSHPT (46).

SUMMARY

We have tried in this review to give an overview of the receptor and its molecular profile along with the identification of the various organ systems where it has been shown, to date, to play an important role. Over the past 20 plus years since the identification of the receptor, the roles for receptor function and interactions with cellular and organ homeostasis have expanded greatly from the initial observations in the parathyroid. We hope that this review serves two functions: provide the reader with some insights into the receptor at all levels and, secondarily, induce new interest in the receptor and its potential roles in yet to be studied cellular and organ systems.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).
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Review

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