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CVIII. Calcium-Sensing Receptor Nomenclature, Pharmacology, and Function

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ABBREVIATIONS: ADH, autosomal dominant hypocalcemia; AP2, adaptor-related protein complex-2; BMS, Bristol Myers Squibb; BTU, benzothiazole trisubstituted urea; Ca_i^{2+} , intracellular calcium; Ca_o^{2+} , extracellular calcium; CaSR, calcium-sensing receptor; CCK, cholecystokinin; CKD, chronic kidney disease; Col, collagen; CR, cysteine-rich; cryo-EM, cryogenic electron microscopy; DSS, dextrane sulfate sodium; EC, effective concentration; ECD, extracellular domain; ECL, extracellular loop; ENS, enteric nervous system; ERK, extracellular signal-regulated kinase; FDA, Food and Drug Administration; FHH, familial hypocalciuric hypercalcemia; GCM2, glial cell missing-2; GLP-1, glucagon-like peptide 1; GNA, guanine nucleotide-binding protein α ; GPCR, G protein-coupled receptor; GRK, GPCR kinase; HEK, human embryonic kidney; ICL, intracellular loop; IL, interleukin; IP, inositol phosphate; MAPK, mitogen-activated protein kinase; mGluR, metabotropic glutamate receptor; NAM, negative allosteric modulator; NPS, Natural Product Services; NSHPT, neonatal severe hyperparathyroidism; $1,25(\text{OH})_2\text{D}$, 1,25-dihydroxyvitamin D; OMIM, Online Mendelian Inheritance of Man; p-, phosphorylated; PAM, positive allosteric modulator; PDB, Protein Data Base; PKC, protein kinase C; PLC, phospholipase C; PTH, parathyroid hormone; PTHrP, PTH-related protein; PYY, protein YY; SNP, single-nucleotide polymorphism; TAL, thick ascending limb; 7TM, 7-transmembrane; TNCA, L-1,2,3,4-tetrahydronorharman-3-carboxylic acid; VDR, vitamin D receptor; VFT, Venus flytrap.

Abstract—The calcium-sensing receptor (CaSR) is a class C G protein-coupled receptor that responds to multiple endogenous agonists and allosteric modulators, including divalent and trivalent cations, L-amino acids, γ -glutamyl peptides, polyamines, polycationic peptides, and protons. The CaSR plays a critical role in extracellular calcium (Ca_o^{2+}) homeostasis, as demonstrated by the many naturally occurring mutations in the CaSR or its signaling partners that cause Ca_o^{2+} homeostasis disorders. However, CaSR tissue expression in mammals is broad and includes tissues unrelated to Ca_o^{2+} homeostasis, in which it, for example, regulates the secretion of digestive hormones, airway constriction, cardiovascular effects, cellular differentiation, and proliferation. Thus, although the CaSR is targeted clinically by the positive allosteric modulators (PAMs) cinacalcet, evocalcet, and etelcalcetide in hyperparathyroidism, it is also a putative therapeutic target in diabetes, asthma, cardiovascular disease, and cancer. The CaSR is somewhat unique in possessing multiple ligand binding sites, including at least five putative sites for the “orthosteric” agonist Ca_o^{2+} , an allosteric site for endogenous L-amino acids, two

further allosteric sites for small molecules and the peptide PAM, etelcalcetide, and additional sites for other cations and anions. The CaSR is promiscuous in its G protein-coupling preferences, and signals via $\text{G}_{q/11}$, $\text{G}_{i/o}$, potentially $\text{G}_{12/13}$, and even G_s in some cell types. Not surprisingly, the CaSR is subject to biased agonism, in which distinct ligands preferentially stimulate a subset of the CaSR's possible signaling responses, to the exclusion of others. The CaSR thus serves as a model receptor to study natural bias and allostery.

Significance Statement—The calcium-sensing receptor (CaSR) is a complex G protein-coupled receptor that possesses multiple orthosteric and allosteric binding sites, is subject to biased signaling via several different G proteins, and has numerous (patho)physiological roles. Understanding the complexities of CaSR structure, function, and biology will aid future drug discovery efforts seeking to target this receptor for a diversity of diseases. This review summarizes what is known to date regarding key structural, pharmacological, and physiological features of the CaSR.

I. Introduction

A. Identification and Cloning of the Calcium-Sensing Receptor

Ca^{2+} is an essential ion, both intracellularly and extracellularly, in mammals. Intracellular Ca^{2+} (Ca_i^{2+}) is maintained at approximately 100 nM but rises to low micromolar concentrations upon membrane or endoplasmic reticulum Ca^{2+} channel opening, thus serving as an important second messenger (Brini et al., 2013). Ca^{2+} also functions as a key first messenger via activation of the calcium-sensing receptor (CaSR) (Alexander, et al., 2017; Bikle et al., 2019), which plays a pivotal role in tightly regulating ionized (free) extracellular calcium (Ca_o^{2+}). In human plasma, total calcium (referred to herein as calcium to signify ionized and nonionized calcium) levels are maintained between 2.1 and 2.6 mM, of which roughly half is in an ionized form (Brini et al., 2013).

In the mid 1980s, there was significant interest in the mechanisms regulating parathyroid hormone (PTH) release from the parathyroid glands. It was consequently shown that elevated Ca_o^{2+} increased Ca_i^{2+} levels and decreased PTH release (LeBoff et al., 1985; Nemeth et al., 1986). In the following years, elevated Ca_o^{2+} was demonstrated to increase inositol phosphate (IP) and decrease cAMP levels, which led to the suggestion of a cell surface calcium-sensing G protein-coupled receptor (GPCR) (Nemeth and Scarpa, 1986, 1987; Brown et al., 1987a; Chen et al., 1989). Further evidence for the receptor was provided via activation of Ca^{2+} -sensitive Cl^- channels in *Xenopus* oocytes injected with mRNA isolated from bovine parathyroid cells (Racke et al., 1993), which subsequently led to expression cloning of the bovine CaSR (Brown et al., 1993). In isolated

parathyroid cells, the cloned bovine CaSR was activated (in rank order of potency) by gadolinium (Gd^{3+}) > neomycin > Ca_o^{2+} > magnesium (Mg^{2+}) and signaled through elevation of Ca_i^{2+} , providing strong evidence of the cloned receptor being the long-sought CaSR (Brown et al., 1993).

Analyses of the cloned receptor sequence revealed a 1085 amino acid-long protein consisting of a large amino-terminal extracellular domain (ECD) of 613 amino acids comprised of a “Venus flytrap” (VFT) domain, which closes upon activation much like the VFT plant, and a cysteine-rich domain, a 7-transmembrane (7TM) domain of 250 amino acids and an intracellular carboxy terminus of 222 amino acids (Brown et al., 1993). The analyses also revealed that the CaSR was homologous to the metabotropic glutamate receptors, which were later shown to form the class C GPCRs together with GABA_B , taste type 1; GPRC6A; and a handful of orphan receptors (Wellendorph and Bräuner-Osborne, 2009). The structurally conserved class C GPCR VFT domain is homologous to bacterial periplasmic binding proteins, and thus it has been predicted that class C GPCRs arose from fusion of the GPCR 7TM with a periplasmic binding protein (O'Hara et al., 1993). Nucleic acid hybridization techniques quickly led to cloning of the human (Garrett et al., 1995a), rat (Riccardi et al., 1995; Ruat et al., 1995), rabbit (Butters et al., 1997), chicken (Diaz et al., 1997), and shark (Nearing et al., 2002) CaSR orthologs, and genome data base mining subsequently suggested that the CaSR is evolutionarily conserved in flies and worms (Bjarnadóttir et al., 2005).

B. General Gene Structure

The human *CASR* gene has been mapped to chromosome 3q13.3-21 by fluorescence in situ hybridization

(Janicic et al., 1995) and linkage analyses (Chou et al., 1992). The human CaSR is encoded by seven exons, of which exons 2-6 encode the ECD, and exon 7 encodes the 7Tm and intracellular carboxy terminus (Pollak et al., 1993; Pearce et al., 1995). Two different 5'-untranslated promoter regions, termed exon 1A and exon 1B, have been identified in humans (Chikatsu et al., 2000), and both splice with the same site in exon 2. As recently reviewed (Hendy and Canaff, 2016), the promoters, and thus CaSR expression, are regulated by *cis*-elements responding to 1,25-dihydroxyvitamin D [$1,25(\text{OH})_2\text{D}$], proinflammatory cytokines, and the transcription factor glial cells missing-2 (GCM2s).

Tissue-specific splice variants lacking exon 3 (Bradbury et al., 1998) and exon 5 (Oda et al., 1998) have been reported, but their function (if any) remains elusive. The exon 5 splice variant is of particular interest because it is functional in growth plate chondrocytes (Rodriguez et al., 2005) despite being non-functional when recombinantly expressed in HEK293 and CHO cells. These latter findings led to an initial underestimation of the role of the CaSR in bone development because the original exon 5 knockout mouse (Ho et al., 1995) displayed a mild bone phenotype compared with a more severe phenotype in the exon 7 knockout mouse model (Chang et al., 2008).

C. Tissue Distribution

mRNA probes and antibodies have revealed that the CaSR is widely expressed both in tissues directly involved in controlling systemic Ca^{2+} homeostasis as well as in tissues with other functions. As detailed in the section, V. (Patho)physiology of the Calcium-Sensing Receptor and Its Ligands, the plasma calcium level is mainly regulated via actions on the parathyroid gland (PTH release), thyroid gland (calcitonin release, although calcitonin in humans is less important than in rodents), and kidney (production of $1,25(\text{OH})_2\text{D}_3$ and regulation of ion excretion), but other tissues, such as the bone (release of skeletal Ca^{2+}) and small intestine (Ca^{2+} absorption), also play a role both via direct CaSR activation and via PTH, calcitonin, and $1,25(\text{OH})_2\text{D}_3$ (Brown and MacLeod, 2001; Brown, 2013; Lee et al., 2019). In addition, the CaSR is expressed in a range of tissues not involved in systemic Ca^{2+} homeostasis, such as the keratinocytes of the skin (VE. *Calcium-Sensing Receptor in Keratinocytes*), colon (VF. *Calcium-Sensing Receptor in the Gastrointestinal Tract*), pancreas (VG. *Calcium-Sensing Receptor in the Pancreas*), mammary glands (VH. *Calcium-Sensing Receptor in Mammary Glands*), airway smooth muscle and epithelium (VI. *Calcium-Sensing Receptor in Airway Smooth Muscle and Epithelium*), vascular smooth muscle and endothelium (VJ. *Calcium-Sensing Receptor in the Vasculature*), and the brain (VK. *Calcium-Sensing Receptor in the Brain and Nervous System*), in which the CaSR regulates a range of (patho)physiological functions.

D. Signal Transduction Pathways

The principal CaSR signaling pathways are shown in Fig. 1. The CaSR primarily elicits its functions by coupling to the $\text{G}_{i/o}$ and $\text{G}_{q/11}$ families of heterotrimeric G proteins to activate intracellular signaling pathways that inhibit PTH synthesis and release from parathyroid cells (A. *Calcium-Sensing Receptor in the Parathyroid Glands*). CaSR activation of $\text{G}_{i/o}$ proteins leads to inhibition of the cAMP-synthesizing enzyme, adenylyl cyclase, causing a decrease in intracellular cAMP levels (Chang et al., 1998; Kifor et al., 2001). CaSR coupling to $\text{G}_{q/11}$ is usually considered the primary signaling pathway, which activates phospholipase C (PLC)- β to hydrolyze phosphatidylinositol 4,5-bisphosphate to the second messengers, IP_3 and diacylglycerol (Brown et al., 1993; Chang et al., 1998). IP_3 triggers release of Ca_i^{2+} from intracellular stores, such as the endoplasmic reticulum, and diacylglycerol alone or in combination with Ca_i^{2+} activates protein kinase C (PKC). Cytosolic phospholipase A_2 , which is the rate-limiting enzyme in arachidonic acid metabolism, is also activated by the CaSR-mediated $\text{G}_{q/11}$ pathway through calmodulin and the Ca^{2+} /calmodulin-dependent protein kinase II (Handlogten et al., 2001).

The importance of the $\text{G}_{q/11}$ pathway in CaSR physiology has been demonstrated by the similarities between selective parathyroid knockout of the genes encoding $\text{G}\alpha_q$ (*Gnaq*) and $\text{G}\alpha_{11}$ (*Gna11*) in mice, which results in a phenotype with almost all the features of *Casr* germline knockout mice (Wettschureck et al., 2007). Similarly, human *CASR* and *GNA11* loss- or gain-of-function mutations cause familial hypocalciuric hypercalcemia (FHH) types 1 (*CASR*) and 2 (*GNA11*) or autosomal dominant hypocalcemia (ADH) types 1 (*CASR*) or 2 (*GNA11*), respectively (Pollak et al., 1993, 1994; Nesbit et al., 2013a) (VI. *Calcium-Sensing Receptor-Related Genetic Diseases and Therapeutic Interventions*).

Studies of CaSR coupling to $\text{G}_{12/13}$ are limited because of a lack of inhibitors and suitable functional readouts. However, the CaSR activates phospholipase D in Madin-Darby canine kidney cells through a $\text{G}_{q/11}$ - and $\text{G}_{i/o}$ -independent pathway involving activation of the Rho family of small GTPases, most likely via $\text{G}_{12/13}$ coupling (Huang et al., 2004). The $\text{G}_{12/13}$ pathway is also likely to be the $\text{G}_{q/11}$ - and $\text{G}_{i/o}$ -independent pathway that activates the phosphatidylinositol 4-kinase responsible for the first step in inositol biosynthesis through Rho (Huang et al., 2002). However, CaSR can activate RhoA by a $\text{G}_{q/11}$ pathway in HEK293 cells (Pi et al., 2002) and phospholipase D by a PKC-dependent mechanism likely mediated by $\text{G}_{q/11}$ in HEK293 cells and parathyroid cells (Kifor et al., 1997), so it remains unclear whether CaSR also couples to $\text{G}_{12/13}$ in these cells.

CaSR coupling to G_s and the consequent increase in intracellular cAMP levels activates PKA and stimulates PTH-related protein (PTHrP) release in immortalized

and malignant breast cells and in the AtT-20 pituitary tumor-derived cell line (Mamillapalli et al., 2008; Mamillapalli and Wysolmerski, 2010) (*VH. Calcium-Sensing Receptor in Mammary Glands*). Stimulation of cAMP production is not observed in HEK293 cells recombinantly expressing the CaSR (Thomsen et al., 2012a), and the molecular mechanism for the switch in G protein preference in breast cancer and AtT-20 cells remains unknown.

The CaSR activates several mitogen-activated protein kinase (MAPK) cascades, including extracellular signal-regulated kinase (ERK) 1/2, p38 MAPK, and c-Jun N-terminal kinase to regulate PTHrP release, proliferation, and other functions (MacLeod et al., 2003; Tfelt-Hansen et al., 2003; Chattopadhyay et al., 2004). ERK1/2 is activated by phosphorylation (pERK1/2) through multiple CaSR-mediated pathways, including parallel G protein-dependent pathways involving either $G_{q/11}$ and PKC or $G_{i/o}$ and epidermal growth factor receptor transactivation (Kifor et al., 2001; MacLeod et al., 2004; Thomsen et al., 2012a). Ras and phosphatidylinositol 3-kinase are also involved in ERK1/2 activation by the CaSR (Hobson et al., 2003), but it is unclear whether this pathway overlaps with the $G_{q/11}$ - or $G_{i/o}$ -dependent pathways. The CaSR can also activate ERK1/2 through a β -arrestin-dependent and G protein-independent pathway (Thomsen et al., 2012a). Furthermore, an Arg680^{3.32}Gly [numbering shown in superscript after residue numbers throughout this manuscript is based on Ballesteros-Weinstein numbering assigned in Ballesteros and Weinstein (1995) for class A GPCRs and in Dore et al. (2014) for class C

GPCRs] CaSR mutation associated with ADH1 selectively increases β -arrestin-dependent ERK1/2 activation, in which the mutation is predicted to disrupt an extracellular salt bridge between Arg680^{3.32} and Glu767 in the second extracellular loop (ECL) (Gorvin et al., 2018a).

In some cell types, the CaSR stimulates opening of L-type voltage-gated Ca^{2+} channels (Fajtova et al., 1991; McGehee et al., 1997; Muff et al., 1988) and nonselective cation channels, including transient receptor potential cation channels (Ye et al., 1996; El Hiani et al., 2006; Meng et al., 2014), although the pathways that couple the CaSR to ion channels are poorly defined.

II. Agonists and Allosteric Modulators

A. Endogenous and Exogenous Agonists

1. Polyvalent Cations. The CaSR is now well-known for its ability to sense fluctuations in Ca_o^{2+} . CaSR radioligand-binding assays to quantify the affinity of Ca_o^{2+} and other agonists have to date not been possible because of low agonist affinity, a lack of suitable radioligands, and complexities in quantifying agonist binding to multiple binding sites. However, spectroscopic studies indicate Ca_o^{2+} binds to the VFT with an affinity in the range of 3.0–5.0 mM (Zhang et al., 2014b). These findings are supported by the use of an operational model of agonism for receptors with multiple agonist binding sites, in which Ca_o^{2+} affinity at the full-length CaSR was 1.1–1.3 mM (Gregory et al., 2020). The low millimolar Ca_o^{2+} affinity is consistent with Ca_o^{2+} potency in healthy human subjects, in which Ca_o^{2+} suppresses PTH

Fig. 1. Key CaSR-signaling pathways. The CaSR primarily couples to $G_{q/11}$ and $G_{i/o}$ proteins to mediate many of its physiological responses, including PTH release. The CaSR may also couple to $G_{12/13}$, but the physiological relevance of this is unknown; therefore, $G_{12/13}$ is semitransparent in the figure. AC, adenylate cyclase; ADIS, agonist-driven insertional signaling; Akt, protein kinase B; β -Arr, β -arrestin; CaM, calmodulin; DAG, diacylglycerol; EGFR, epidermal growth factor receptor; ER, endoplasmic reticulum; PI3K, phosphatidylinositol 3-kinase; PI4K, phosphatidylinositol 4-kinase; PLA_2 , phospholipase A2; PLD, phospholipase D.

secretion with an approximate IC_{50} of 1.2 mM (which is also the approximate free Ca_o^{2+} concentration in human serum) (Brown, 1991; Ramirez et al., 1993), whereas in cultured parathyroid cells, the Ca_o^{2+} IC_{50} for PTH release is closer to 1 mM (Brown, 1983, 1991). The Ca_o^{2+} -PTH relationship is characterized by a Hill coefficient greater than unity (Brown, 1983, 1991; Ramirez et al., 1993). This is because multiple Ca_o^{2+} ions bind to the CaSR in a positively cooperative manner, allowing the CaSR to respond to minute changes in Ca_o^{2+} concentrations that span less than 100 μ M (Brown, 1983, 1991; Ramirez et al., 1993). Thus, although Ca_o^{2+} is considered the primary endogenous and therefore orthosteric agonist of the CaSR, strictly speaking it is an allosteric modulator of its own activity.

In addition to Ca_o^{2+} , the CaSR is activated by many other polyvalent cations, including Mg^{2+} , zinc, manganese, ferrous iron, strontium (Sr^{2+}), barium, cadmium, cobalt, nickel, lead, terbium, Gd^{3+} , europium, and yttrium (Brown et al., 1990; Ruat et al., 1996; Handlogten et al., 2000). Trivalent cations are generally more potent than divalent cations, of which Ca_o^{2+} and Mg^{2+} are the most physiologically relevant. The role of non- Ca_o^{2+} cations in CaSR-mediated (patho)physiology is unknown. Agonists that mimic the actions of Ca_o^{2+} at the CaSR have traditionally been called type I calcimimetics.

Although much larger and structurally more complex than the small cations described above, polyamines are CaSR agonists. Polyamines are found in all eukaryotes, with spermine, spermidine, and their diamine precursor putrescine the most abundant in mammals. Polyamines are synthesized ubiquitously in the body and are also ingested in the diet and secreted by intestinal bacteria. Although polyamines activate the CaSR in the absence of Ca_o^{2+} , there is some evidence they also potentiate the potency of Ca_o^{2+} (Quinn et al., 1997). Spermine is the most potent CaSR agonist, followed by spermidine and then putrescine (Quinn et al., 1997). Spermine IC_{50} for suppression of PTH release from cultured bovine parathyroid cells is ~ 200 μ M (Quinn et al., 1997). Blood polyamine concentrations in healthy humans are ~ 5 – 10 μ M (Casti et al., 1982; Soda et al., 2009), concentrations that are likely sufficient to activate the CaSR in tissues where receptor density is high. In the lung, polyamines and other polycations stimulate CaSR-mediated airway contraction (Yarova et al., 2015) (described in VI. *Calcium-Sensing Receptor in Airway Smooth Muscle and Epithelium*). Intriguingly, other overlapping functions of the CaSR and polyamines exist, including promotion of osteoblast, keratinocyte, vascular smooth muscle cell, and gastrointestinal epithelial cell differentiation and proliferation (Riccardi and Kemp, 2012; Leach et al., 2014; Miller-Fleming et al., 2015). Thus, polyamines may contribute to multiple (patho)physiological processes mediated by the CaSR.

Not surprisingly, additional positively charged molecules activate the CaSR, including poly-L-arginine, protamine, and aminoglycoside antibiotics, including neomycin, tobramycin, and gentamicin (McLarnon and Riccardi, 2002). Poly-L-arginine is a mimetic of eosinophil major basic protein released to activate mast cells, neutrophils, basophils, and macrophages in asthma and other allergic diseases. Ca_i^{2+} mobilization in CaSR-HEK293 cells stimulated by the related eosinophil cationic protein was completely absent in untransfected HEK293 cells and was blocked by structurally distinct CaSR inhibitors, demonstrating a CaSR-dependent signaling mechanism (Yarova et al., 2015).

B. Endogenous and Exogenous Allosteric Modulators

Allosteric modulators bind to sites that are topographically distinct from the orthosteric binding site and act to either potentiate [positive allosteric modulators (PAMs)], inhibit [negative allosteric modulators (NAMs)], or have no effect on (neutral allosteric ligands) the binding or efficacy of the orthosteric agonist. Allosteric modulators may also be agonists (or inverse agonists) in the absence of orthosteric agonists and can simultaneously act as agonists and PAMs (PAM-agonists). CaSR PAMs have been termed type II calcimimetics and CaSR NAMs calcilytics.

1. *L-Amino Acids.* L-amino acids are endogenous CaSR activators that are generally recognized as PAMs. Thus, L-amino acids have no activity in the absence of Ca_o^{2+} or another cationic activator, such as Gd^{3+} or spermine, but potentiate CaSR-mediated responses in the presence of submaximal concentrations of cationic activators (Conigrave et al., 2000). In a Ca_i^{2+} mobilization assay performed in CaSR-HEK293 cells, the magnitude of Ca_o^{2+} potentiation mediated by 10 mM amino acids followed the rank order L-Phe, L-Trp, L-histidine > L-alanine > L-serine, L-proline, L-glutamic acid > L-aspartic acid (but not L-lysine, L-arginine, L-leucine, and L-isoleucine) (Conigrave et al., 2000). Similarly, in human parathyroid cells in culture, aromatic amino acids, such as L-Trp and L-Phe, were the most potent L-amino acid CaSR activators in Ca_i^{2+} mobilization assays (Conigrave et al., 2004). Thus, the CaSR, like a number of other class C GPCRs, is a promiscuous sensor of L-amino acids (Conigrave and Hampson, 2006, 2010; Smajilovic et al., 2014).

As would be expected for a positive binding interaction, L-amino acids and Ca_o^{2+} markedly enhance the CaSR's sensitivity to one another in a reciprocal manner (Conigrave et al., 2000). Based on observations of Ca_i^{2+} mobilization and PTH secretion assays in vitro, amino acids support normal physiological Ca_o^{2+} sensitivity and thus underpin the physiological Ca_o^{2+} concentration set point for the parathyroid at around 1.1–1.2 mM (Conigrave et al., 2004).

Recent crystal structures of the CaSR's VFT (Zhang et al., 2016) and entire extracellular (Geng et al., 2016)

domains as well as mutational studies suggest that L-amino acids and analogs might be better viewed as coagonists of the receptor rather than PAMs (see *III. Receptor Structure*). As detailed later, L-amino acids display pronounced biased signaling properties (*IIC. Biased Agonism and Biased Allosteric Modulation*), and L-amino acid signaling appears to be attenuated by PKC-mediated phosphorylation of Thr888 in the C-terminal tail of CaSR (*IVA. Phosphorylation and Dephosphorylation*).

2. *γ -Glutamyl Peptides*. Wang et al. (2006) demonstrated that the γ -glutamyl peptide, glutathione, is a potent activator of the CaSR and of another class C GPCR, the fish 5.24 receptor. Subsequently, various natural and synthetic analogs of glutathione were found to activate the CaSR in the presence of threshold Ca_o^{2+} concentrations in a similar manner to L-amino acids. A receptor double mutant (Thr145Ala + Ser170Thr) exhibits similar impairments of function when exposed to either L-amino acids (Mun et al., 2005) or the glutathione analog, S-methylglutathione (Broadhead et al., 2011), suggesting overlapping binding sites. Interestingly, γ -glutamyl peptides active at the CaSR are also potent activators of kokumi taste (Ohsu et al., 2010; Amino et al., 2016).

3. *pH*. Large supraphysiological changes in buffer pH alter the potencies of Ca_o^{2+} and Mg^{2+} at the CaSR (Quinn et al., 2004). In the blood, pH rarely varies by more than 0.2 U; however, this represents a change in H^+ concentration of $\sim 58\%$. Such acidosis can occur in advanced chronic kidney disease (CKD), which has relevance to the CaSR (see *V. (Patho)physiology of the Calcium-Sensing Receptor and Its Ligands*). Interestingly, altering buffer pH from 7.4 to just 7.2 or 7.6 elicits significant attenuation or enhancement of CaSR signaling, respectively, as observed in both HEK293 cells and bovine parathyroid cells (Campion et al., 2015). The site of H^+ action is unknown, although it is not apparently mediated via the CaSR's extracellular histidine residues (Campion et al., 2015). Crucially, pathophysiological changes in pH elicit significant changes in PTH secretion from isolated human parathyroid cells (Campion et al., 2015). This indicates the potential clinical relevance of altered acid or base balance in CaSR-modulated mineral metabolism.

4. *Phosphate*. Crystallization of the CaSR ECD has revealed up to four anion binding sites (Geng et al., 2016) (see *III. Receptor Structure*), and a recent study has revealed that phosphate inhibits the CaSR directly and in a noncompetitive manner (Centeno et al., 2019). This phosphate effect is more substantial than can be explained by buffering of free Ca_o^{2+} ions, and mutation of Arg62 inhibits the phosphate action. Exposure of human and murine parathyroid cells to pathophysiological phosphate concentrations induces rapid and reversible PTH secretion indicative of a receptor-mediated action (Centeno et al., 2019). Similarly, other

anions, such as sulfate (SO_4^{2-}), act as inhibitors of the CaSR (Geng et al., 2016) potentially also acting via Arg62 (Centeno et al., 2019).

5. *Osmolarity*. High sodium chloride (NaCl) concentrations are inhibitory for the CaSR, such that concomitant Ca_o^{2+} concentration-response curves are right-shifted, whereas lowering the NaCl concentration raises the potency of Ca_o^{2+} for the CaSR (Quinn et al., 1998). Accordingly, in dispersed bovine parathyroid cells, raising extracellular osmolarity with either NaCl or sucrose elicits rapid (within minutes) and substantial PTH secretion, an effect that cannot be suppressed by raising Ca_o^{2+} concentrations (Chen et al., 1987). Although this means that the CaSR could represent an ionic strength sensor where it is expressed in, for example, the renal tubules or the subfornical organ of the brain, there is little evidence to date that the CaSR is a substantive contributor to mammalian osmoregulation. Indeed, Na^+ is a well-known negative allosteric modulator of multiple class A GPCRs, in which it binds in a conserved 7TM domain pocket. Therefore, allosteric modulation of GPCRs, at least by Na^+ , is likely a general phenomenon. Nonetheless, some severe gain-of-function clinical CaSR mutations (see *VI. Calcium-Sensing Receptor-Related Genetic Diseases and Therapeutic Interventions*) can elicit a Bartter-like salt-wasting syndrome, whereas loss-of-function CaSR mutations can enhance the natriuretic response to loop diuretics indicative of mild Na^+ retention (Huang and Miller, 2010; Tyler Miller, 2013).

6. *Small-Molecule Allosteric Modulators*. A detailed review on the discovery and development of CaSR small-molecule drugs has recently been published (Nemeth et al., 2018). Therefore, for the purposes of this review, the focus will be on small molecules for which detailed pharmacological or clinical data are available. To date, all CaSR small-molecule binding sites have been localized to the 7TM domain and/or ECLs (Petrel et al., 2003, 2004; Miedlich et al., 2004; Bu et al., 2008; Leach et al., 2016). These sites are distinct from the predominant Ca_o^{2+} -, L-amino acid, or γ -glutamyl binding sites in the ECD (see *III. Receptor Structure*), and thus all small-molecule CaSR drugs identified so far are allosteric.

For the majority of small-molecule PAMs and NAMs, pharmacological characterization has been based on their ability to potentiate or inhibit a single concentration of Ca_o^{2+} , usually in a Ca_i^{2+} mobilization or IP accumulation assay (see Table 1). This approach provides a measure of modulator potency, which is a composite value of affinity, cooperativity (the magnitude and direction of modulator potentiation or inhibition of the orthosteric agonist), and efficacy (i.e., agonism or inverse agonism). Although potency measurements facilitate drug comparisons in a series when in vitro assays are performed under identical conditions, they can be misleading when different assay conditions are

employed (e.g., different orthosteric agonist concentrations, different signaling outputs) (Gregory et al., 2018). Therefore, more recent work has quantified PAM and NAM affinity, cooperativity, and efficacy values as separate parameters using an operational model of allostery or an allosteric ternary complex model (Davey et al., 2012; Leach et al., 2013, 2016; Cook et al., 2015; Diepenhorst et al., 2018; Gregory et al., 2018, 2020).

7. Small-Molecule Positive Allosteric Modulators. The structural and chemical diversity of small-molecule CaSR PAMs is relatively limited, with few distinct series discovered. Two chemically and structurally related small-molecule PAMs, cinacalcet and evocalcet (Table 1), are clinically approved. Cinacalcet is FDA-approved for the treatment of primary hyperparathyroidism in patients who cannot undergo parathyroidectomy, and for hypercalcemia in adults with parathyroid carcinoma. Cinacalcet is also FDA-approved for secondary hyperparathyroidism in patients on renal replacement therapy, and has been used off-label to treat naturally occurring loss-of-function mutations in the CaSR or its signaling partners that cause disorders of Ca_0^{2+} and PTH homeostasis (described in VI. *Calcium-Sensing Receptor-Related Genetic Diseases and Therapeutic Interventions*). Cinacalcet was the first GPCR allosteric modulator to be approved for clinical use in 2004. Evocalcet was approved in Japan in 2018 for the treatment of secondary hyperparathyroidism in patients on dialysis. Cinacalcet and evocalcet potentiate Ca_0^{2+} activity at the CaSR, thus left-shifting the Ca_0^{2+} -PTH concentration-response relationship in the body. This means lower Ca_0^{2+} concentrations are required to suppress PTH release, thus normalizing elevated serum PTH levels. However, both cinacalcet and evocalcet carry a risk of hypocalcemia in patients that limits their clinical utility (Fukagawa et al., 2018), presumably in part from potentiation of the CaSR in the kidney and enhanced CaSR-mediated calcitonin secretion from thyroid parafollicular C cells (see V. *(Patho) physiology of the Calcium-Sensing Receptor and Its Ligands*). Furthermore, cinacalcet and evocalcet are associated with adverse gastrointestinal side effects, including nausea and vomiting, which may occur via the CaSR expressed in the gastrointestinal tract. In rats and humans, however, evocalcet appears to have reduced actions in the gastrointestinal tract in comparison with cinacalcet (Fukagawa et al., 2018; Kawata et al., 2018).

Cinacalcet and evocalcet belong to the arylalkylamine family of PAMs derived from the nonselective calcium channel blocker, fendiline. A number of structurally related arylalkylamine PAMs have been identified, including NPS R-467 and NPS R-568 (the precursors to the discovery of cinacalcet), calindol, and calcimimetic B (Table 1). The activity of these PAMs is highly dependent upon their stereoselectivity, in which the

R-configuration of the methyl between the aromatic and secondary nitrogen is more active than the *S*-configuration (Nemeth et al., 2018). Although NPS R-568, cinacalcet, and calindol exhibit similar affinity and cooperativity values when measured in a Ca_i^{2+} mobilization assay (Davey et al., 2012; Cook et al., 2015; Leach et al., 2016; Diepenhorst et al., 2018; Keller et al., 2018), *R,R*-calcimimetic B has a roughly 10-fold higher affinity but comparable cooperativity (Cook et al., 2015). Although concentrations of cinacalcet that exceed 1 μM weakly activate the CaSR in the absence of divalent cations (Nemeth et al., 2018), suggesting it is a “PAM agonist,” arylalkylamine PAMs demonstrate negligible agonism at concentrations that robustly potentiate CaSR activity (Cook et al., 2015; Keller et al., 2018). In contrast, *R,R*-calcimimetic B is a PAM and a partial agonist at micromolar concentrations (Cook et al., 2015). Arylalkylamine PAMs also exhibit pronounced positive interactions with L-amino acids (Zhang et al., 2002a) and glutathione (Broadhead et al., 2011).

A benzothiazole series of CaSR PAMs that is structurally and chemically distinct from the arylalkylamines has been discovered. These PAMs include the small benzothiazole, AC265347 (Table 1), which has been characterized in detail. AC265347 has comparable affinity and cooperativity to cinacalcet when measured in a Ca_i^{2+} mobilization assay (Cook et al., 2015; Leach et al., 2016; Diepenhorst et al., 2018), and similar to the arylalkylamine PAMs, AC265347 is a PAM agonist, although AC265347 is more potent and efficacious as an agonist than the arylalkylamines (Cook et al., 2015). Although AC265347 has not been tested in humans, in healthy rats, AC265347 suppressed serum PTH levels with greater potency than cinacalcet and demonstrated a lower propensity to cause hypocalcemia (Ma et al., 2011).

Trisubstituted urea compounds have been identified as another potent class of CaSR PAMs (Temal et al., 2013) (Table 1). Benzothiazole trisubstituted urea (BTU) compound 13 (Deprez et al., 2013) is the best characterized of this series. BTU compound 13 has similar affinity and cooperativity to cinacalcet at the CaSR in a Ca_i^{2+} mobilization assay (Cook et al., 2015; Diepenhorst et al., 2018). Much like AC265347, BTU compound 13 suppressed PTH levels in a rat model of CKD while avoiding significant hypocalcemia (Deprez et al., 2013).

8. Peptide Positive Allosteric Modulator, Etelcalcetide. In 2017, a novel CaSR PAM, etelcalcetide (chemical name *N*-acetyl-D-cysteiny-D-alanyl-D-arginyl-D-arginyl-D-arginyl-D-alanyl-D-argininamide disulfide with L-cysteine hydrochloride), was FDA-approved for the treatment of secondary hyperparathyroidism in patients with CKD on dialysis. Etelcalcetide is administered intravenously at the end of dialysis. Similar to cinacalcet and evocalcet, etelcalcetide is associated with adverse gastrointestinal side effects and hypocalcemia (Hamano et al., 2017).

TABLE 1
Representative CaSR agonists or endogenous and small-molecule allosteric modulators and their pharmacological properties

Ligand	Structure	(Cell Type or Model, Assay)	Potency ^a or Affinity ^b	Cooperativity ^c with Ca _v ²⁺	References
Agonists					
Ca _v ²⁺	Ca ²⁺	Human, PTH release Parathyroid cell, PTH release HEK293, Ca _i ²⁺ HEK293, Ca _i ²⁺	pEC ₅₀ 2.9 pEC ₅₀ 3.0 pEC ₅₀ 2.5–3.5 pK _B 3.0	NA NA NA NA	Brown, 1983, 1991; Ramirez et al., 1993; Quinn et al., 1997; Gregory et al., 2018, 2020
Spermine		HEK293, Ca _i ²⁺	pEC ₅₀ 3.3–4.4	NA	Quinn et al., 1997; Gregory et al., 2018
Neomycin		HEK293, Ca _i ²⁺	pEC ₅₀ 4.4	NA	McLarnon et al., 2002
PAMs					
L-Trp		HEK293, Ca _i ²⁺	pEC ₅₀ 2.6	ND	Conigrave et al., 2000
Cinacalcet		HEK293, Ca _i ²⁺ HEK293, IP ₁ HEK293, pERK1/2 HEK293, membrane ruffling HEK293, SRF-RE luc ^d HEK293, Ca _i ²⁺	pK _B 5.9–6.7 pK _B 6.1 pK _B 5.9–6.5 pK _B 8.1 pK _B 7.1 pK _B 6.0–6.6	2.6–4.7 2.6–4.8 1.3–2.9 2.6 4.5 3.0–3.9	Davey et al., 2012; Leach et al., 2013, 2016; Cook et al., 2015; Diepenhorst et al., 2018
NPS R-568		CHO, aequorin CHO/HEK293, IP ₁ HEK293, pERK1/2 HEK293, membrane ruffling	pK _B 6.2 pK _B 6.2–6.8 pK _B 5.6–6.6 pK _B 9.4	2.7 4.3–4.5 2.0–5.1 1.7	Lu et al., 2009; Davey et al., 2012; Cook et al., 2015; Gregory et al., 2018; Keller et al., 2018
Calindol		HEK293, Ca _i ²⁺ HEK293, IP ₁ HEK293, pERK1/2	pK _B 6.3 pK _B 6.4 pK _B 5.2	5.4 4.7 8.1	Cook et al., 2015
Evocalcet		HEK293, Ca _i ²⁺	pEC ₅₀ 7.0	ND	Kawata et al., 2018
R,R-calcimimetic B		HEK293, Ca _i ²⁺ HEK293, IP ₁ HEK293, pERK1/2	pK _B 7.2 pK _B 7.0 pK _B 7.1	1.9 3.2 3.0	Cook et al., 2015
AC265347		HEK293, Ca _i ²⁺ HEK293, IP ₁ HEK293, pERK1/2 HEK293, SRF-RE luc	pK _B 6.2–6.4 pK _B 7.3–8.0 pK _B 6.1–6.7 pK _B 6.2	2.5–4.3 4.0–4.7 4.5–10 13	Cook et al., 2015; Leach et al., 2016; Diepenhorst et al., 2018

(continued)

TABLE 1—Continued

Ligand	Structure	(Cell Type or Model, Assay)	Potency ^a or Affinity ^b	Cooperativity ^c with Ca _v ²⁺	References
BTU compound 13		HEK293, Ca _v ²⁺ HEK293, IP ₁ HEK293, pERK1/2 HEK293, SRF-RE luc	pK _B 6.7 pK _B 7.2 pK _B 6.2 pK _B 6.5	3.2 2.9 1.2 17	Diepenhorst et al., 2018
Etelcalcetide		HEK293, IP ₁	pEC ₅₀ 4.6	ND	Walter et al., 2013
NAMs					
NPS 2143		HEK293, Ca _v ²⁺ HEK293, pERK1/2 HEK293, membrane ruffling	pK _B 6.2–6.7 pK _B 6.2–6.6 pK _B 7.8	0.3–0.5 0.3–0.6 0.3	Davey et al., 2012; Leach et al., 2016
NPSP795		HEK293, assay not disclosed	pIC ₅₀ 7.1	ND	Kumar et al., 2010
Ronacaleret		HEK293, Ca _v ²⁺	pK _B 6.4	0.03	Josephs et al., 2019
JTT-305/MK-5442		PC12h, zif luc ^e	pIC ₅₀ 7.9	ND	Shinagawa et al., 2011
ATF936		HEK293, Ca _v ²⁺	pK _B 7.6	0.005	Josephs et al., 2019
BMS compound 1		HEK293, Ca _v ²⁺	pK _B 7.0	0.03	Josephs et al., 2019
3H-pyrimidine-4-one compound (R)-2h		HEK293, Ca _v ²⁺	pK _B 7.8	0.009	Josephs et al., 2019

(continued)

TABLE 1—Continued

Ligand	Structure	(Cell Type or Model, Assay)	Potency ^a or Affinity ^b	Cooperativity ^c with Ca _v ²⁺	References
Benzimidazole compound 40		Hamster fibroblasts, Ca _v ²⁺	pIC ₅₀ 8.4	ND	Gerspacher et al., 2010
Mixed PAM/NAM Calhex 231		HEK293, Ca _v ²⁺	pK _B 6.5	ND	Gregory et al., 2018

luc, luciferase; NA, not applicable; ND, not determined; SRF-RE, serum response factor–response element; zif, zinc finger.

^apEC₅₀ or pIC₅₀ is the negative logarithm of ligand concentration that mediates a 50% response determined in a functional assay. For a PAM or NAM, the pEC₅₀ or pIC₅₀ is the modulator's ability to half maximally potentiate or inhibit a single Ca_v²⁺ concentration (usually EC₈₀ or EC₂₀, respectively).

^bpK_B is the negative logarithm of the ligand concentration that achieves 50% receptor occupancy (the equilibrium dissociation constant) determined in a functional assay in which the interaction between an agonist (usually Ca_v²⁺) and the PAM or NAM was fitted to an operational model of allosterism or an allosteric ternary complex model.

^cαβ values between the allosteric modulator and Ca_v²⁺, in which α is the binding cooperativity, and β is a scaling factor that describes the effect of the modulator on agonist efficacy. Because of a lack of commercially available CaSR radioligands, cooperativity was estimated as a composite αβ value in functional assays.

^dSerum response factor–response element luciferase reporter gene assay.

^eZif promoter luciferase reporter gene assay.

Etelcalcetide is comprised of seven D-amino acids linked via a disulfide bond to L-cysteine. Not surprisingly, given it is the only peptide CaSR PAM identified, etelcalcetide has a unique mode of PAM action in comparison to small-molecule PAMs that involves binding to the CaSR via disulfide bond formation (Alexander et al., 2015) (see *III. Receptor Structure*). Although etelcalcetide has been classified as a PAM agonist, assays used to discern agonism contained 0.5 mM MgCl₂; therefore, it is currently uncertain whether observed etelcalcetide efficacy for stimulation of IP₁ accumulation in the absence of Ca_v²⁺ is true agonism or potentiation of Mg²⁺ (Walter et al., 2013). The affinity and cooperativity of etelcalcetide at the CaSR has not been quantified, but its potency for potentiation of 1.2 mM Ca_v²⁺ in an HEK293 IP₁ accumulation assay was 25 μM (Walter et al., 2013).

9. Small-Molecule Negative Allosteric Modulators. Due to the role of the CaSR in regulation of PTH secretion, there was significant interest in the development of CaSR NAMs that could stimulate PTH release. Intermittent and transient increases in serum PTH levels enhance the formation of new bone via the differentiation and proliferation of bone-forming osteoblasts. This is evidenced by clinical use of recombinant PTH1-34 injections to promote bone formation in osteoporosis. However, if PTH levels remain elevated, PTH stimulates the differentiation and proliferation of bone-resorbing osteoclasts, resulting in bone breakdown (Dobnig and Turner, 1997).

Although several pharmaceutical companies have embarked on CaSR NAM discovery programs, similar to CaSR PAMs, there is fairly limited structural and chemical diversity in the NAM scaffolds identified to date. NPS 2143 (Table 1) was one of the first CaSR NAMs to be discovered (Gowen et al., 2000) and is structurally and chemically related to cinacalcet and

other arylalkylamines. Like CaSR PAMs, NAMs have generally been evaluated for their potency to inhibit a single Ca_v²⁺ (usually EC₈₀) concentration. Nevertheless, more recent studies have employed an operational model of allosterism to quantify NPS 2143 activity and have indicated that NPS 2143 binds at the CaSR with micromolar to submicromolar affinity depending on the assay (Table 1) (Davey et al., 2012; Leach et al., 2013, 2016; Gregory et al., 2020). Importantly, NPS 2143 is a partial NAM at the CaSR, meaning that it does not fully inhibit Ca_v²⁺-mediated signaling (Cook et al., 2015; Leach et al., 2016; Gregory et al., 2018).

In rats, NPS 2143 stimulated the release of PTH, resulting in an increase in bone turnover markers, but it did not promote the formation of new bone (Gowen et al., 2000). The lack of new bone formation was hypothesized to be due to the prolonged, rather than transient, PTH release in response to NPS 2143, resulting in both bone formation and resorption. Efforts to develop shorter-acting CaSR NAMs based on the structure of NPS 2143 led to the discovery of ronacaleret (Fitzpatrick et al., 2011a,b, 2012) and JTT-305/MK-5442 (Shinagawa et al., 2011) (Table 1). However, in rats, JTT-305/MK-5442 did not increase bone mass and density (Fisher et al., 2012), whereas in human clinical trials, both ronacaleret and JTT-305/MK-5442 lacked efficacy in treating postmenopausal osteoporosis (Fitzpatrick et al., 2011a,b, 2012; Halse et al., 2014).

Further efforts to identify additional CaSR NAMs that may prove successful in treating osteoporosis led to the discovery of four chemically distinct NAM series exemplified by the quinazolinones ATF936 and AXT914 (Gerspacher et al., 2010), the pyridine Bristol Myers Squibb (BMS) compound 1 (Arey et al., 2005), a series of 3H-quinazoline-4-ones and 3H-pyrimidine-4-ones (Shcherbakova et al., 2005; Didiuk et al., 2009), and benzimidazoles (Gerspacher et al., 2010). A recent study

revealed that the affinity of ATF936 was 17-fold higher than that of ronacaleret, and ATF936 also demonstrated higher negative cooperativity (Josephs et al., 2019) (Table 1). However, despite findings that the quinazolinone NAMs may be superior to ronacaleret in terms of desirable drug properties, when AXT914 was evaluated for its effects on bone turnover in humans, the trial was terminated early because of a lack of effect on bone turnover markers and a propensity to cause hypercalcemia (John et al., 2014).

After the failure of three different NAMs in human clinical trials of osteoporosis, the development of CaSR NAMs diminished. However, there has been recent interest in repurposing these NAMs for the treatment of Ca_o^{2+} homeostasis disorders caused by gain-of-function mutations in the CaSR or its interactors (described in VI. *Calcium-Sensing Receptor-Related Genetic Diseases and Therapeutic Interventions*). Indeed, the NAM, NPSP795 (SHP635), has recently undergone clinical testing for its therapeutic potential in the treatment of ADH1 (Roberts et al., 2019).

10. Calhex 231: A Mixed Positive Allosteric Modulator and Negative Allosteric Modulator. Although the arylalkylamine, calhex 231, was originally classified as a NAM based on its ability to inhibit an EC_{100} Ca_o^{2+} concentration (Kessler et al., 2006), a recent study has revealed that calhex 231 is both a PAM and a NAM (Gregory et al., 2018). This novel mode-switching mechanism is due to allostery across the CaSR dimer, wherein calhex 231 acts as a PAM when it occupies a single protomer in the dimer and a NAM when bound to both protomers. Mixed PAM and NAM activity was observed in HEK293 cells stably expressing the CaSR and in primary cultures of human parathyroid cells, demonstrating that mode-switching may occur under physiological conditions (Gregory et al., 2018). Because several CaSR NAMs have been characterized based on their ability to modulate only a single Ca_o^{2+} concentration, it is unclear at present whether other CaSR allosteric modulators also exhibit mixed PAM and NAM activity. However, whereas other CaSR NAMs were identified from high throughput screens of large compound libraries, calhex 231 originated from a PAM scaffold (Kessler et al., 2004, 2006), which likely contributes to its mixed PAM and NAM activity.

C. Biased Agonism and Biased Allosteric Modulation

Given that the CaSR responds to a diverse array of different ligands, it is unsurprising that the CaSR is subject to biased agonism and biased modulation. Biased agonism is the phenomenon by which distinct ligands stabilize preferred GPCR signaling states, with each state having the potential to stimulate or inhibit discrete subsets of the full repertoire of intracellular signaling pathways that couple to a given receptor (Kenakin and Christopoulos, 2013). This is in contrast to the earlier dogma that all agonists activate the same

subsets of GPCR signaling pathways to greater (e.g., full agonists) or lesser (e.g., partial agonists) extents. Similarly, biased modulation arises when an allosteric ligand differentially modulates different agonist-mediated signaling pathways.

For instance, in CaSR-HEK293 cells, Ca_o^{2+} preferentially mediates stimulation of Ca_i^{2+} mobilization over pERK1/2, whereas spermine preferentially activates pERK1/2 (Thomsen et al., 2012a). Similarly, L-amino acids activate Ca_i^{2+} mobilization and ERK phosphorylation (Lee et al., 2007) and also inhibit cAMP synthesis. However, they are inactive in stimulating phosphatidylinositol-PLC and various other signaling events, including Rho-dependent actin stress fiber formation (Davies et al., 2006) and cAMP responsive element-binding protein phosphorylation (Avlani et al., 2013), and appear to promote Ca_i^{2+} mobilization via a $\text{G}_{12/13}$ /transient receptor potential cation 1-dependent Ca_o^{2+} influx pathway (Rey et al., 2005, 2006).

Evidence from patients with FHH suggests CaSR bias may arise in part from spatial and temporal CaSR-signaling patterns. Loss-of-function germline mutations of the adaptor-related protein complex-2 (AP2)-S1 gene, which encodes the sigma subunit of the heterotetrameric $\text{AP2}\sigma$, cause FHH3 (Nesbit et al., 2013b; Hannan et al., 2015a). $\text{AP2}\sigma$ forms part of the heterotetrameric AP2 that plays a critical role in clathrin-mediated endocytosis. $\text{AP2}\sigma$ mutations increase CaSR cell surface expression yet reduce CaSR signaling because CaSR residency time in clathrin-coated pits is increased, consequently impairing CaSR $\text{G}_{q/11}$ signaling from endosomes (Gorvin et al., 2018c). In contrast, $\text{G}_{i/o}$ -mediated signaling is less sensitive to $\text{AP2}\sigma$ mutations. Thus, whereas the plasma membrane localized CaSR signals via $\text{G}_{q/11}$ and $\text{G}_{i/o}$, endosomal CaSRs signal predominantly via $\text{G}_{q/11}$ (Gorvin et al., 2018c).

It must be noted that many of the studies reporting differential CaSR-mediated pathway activation have not been performed in a systematic manner using identical conditions across assays (e.g., buffers, duration of agonist stimulation, etc.) or the same cellular background. Furthermore, bias has not been quantified in these studies. Therefore, it remains to be definitively proven whether biased agonism is truly operative at the CaSR or whether previous observations were due to observational bias (e.g., different assay conditions, different cell types) or system bias (e.g., the relative efficiency with which the receptor couples to different pathways).

Nonetheless, small-molecule allosteric modulators do appear to exhibit true biased modulation at the CaSR. Evidence of biased modulation comes from reversals in the magnitude of cooperativity in different pathways between distinct PAMs or NAMs or from differences in PAM or NAM affinity for receptor states that couple to different signal transducers. For instance, although cinacalcet and NPS 2143 preferentially potentiate or

inhibit, respectively, Ca_o^{2+} -mediated Ca_i^{2+} mobilization over pERK1/2, AC265347 and *R,R*-calcimimetic B show reversed bias for CaSR-mediated pERK1/2 over Ca_i^{2+} mobilization (Cook et al., 2015; Leach et al., 2016; Diepenhorst et al., 2018). Similarly, AC265347, NPS R-568, and calindol, but not cinacalcet or *R,R*-calcimimetic B, have a higher functional affinity (i.e., an affinity quantified in a functional assay using an operational model of allostereism (Leach et al., 2007)) for the CaSR state that signals to IP_1 accumulation versus Ca_i^{2+} mobilization (Cook et al., 2015; Diepenhorst et al., 2018), whereas cinacalcet, NPS R-568, and NPS 2143 all have a higher functional affinity for the CaSR state that couples to membrane ruffling (Davey et al., 2012).

Evidence for small-molecule PAM and NAM bias also comes from pharmacochaperone studies, which reveal that although cinacalcet, AC265347, and BTU compound 13 are all PAMs in multiple CaSR-mediated signaling assays, only cinacalcet positively modulates the trafficking of an endosomally-trapped, naturally occurring mutant CaSR, rescuing its cell surface expression back to levels comparable to wild-type CaSR (Leach et al., 2013; Cook et al., 2015; Diepenhorst et al., 2018). In contrast, although NPS 2143 is a NAM of CaSR signaling, it is a PAM of loss-of-expression mutant receptor trafficking (Leach et al., 2013). This is in contrast to the actions of NPS 2143 at the wild-type CaSR, wherein it reduces CaSR surface expression (Huang and Breitwieser, 2007), suggesting naturally occurring mutations (which cause Ca_o^{2+} homeostasis disorders; see VI. *Calcium-Sensing Receptor-Related Genetic Diseases and Therapeutic Interventions*) may engender bias in CaSR function. Indeed, Ca_o^{2+} -mediated bias toward Ca_i^{2+} mobilization is abolished by some naturally occurring mutations (Leach et al., 2012).

Although the physiological relevance of biased agonism and biased modulation at the CaSR is not at present known, differences in the propensity of CaSR PAMs to cause hypocalcemia could be linked to this phenomenon. For instance, as already mentioned, *R,R*-calcimimetic B and AC265347 are effective suppressors of PTH release. However, in comparison with cinacalcet, *R,R*-calcimimetic B and AC265347 demonstrate reduced propensity to cause hypocalcemia in rats successfully treated for severe hyperparathyroidism induced by CKD (*R,R*-calcimimetic B) or in normal rats (AC265347). The reduced incidence of hypocalcemia with *R,R*-calcimimetic B and AC265347 is presumably linked, in part, to their lower potency and efficacy for the stimulation of calcitonin secretion versus suppression of PTH release (Henley et al., 2011; Ma et al., 2011). Importantly, although suppression of PTH release has been associated with pERK1/2, calcitonin release is independent of pERK1/2 in rat medullary thyroid carcinoma cells (Thomsen et al., 2012b). This highlights

differences in the coupling specificity of the CaSR in distinct tissues and is consistent with observations that when compared with cinacalcet, AC265347 and *R,R*-calcimimetic B show reversed bias for CaSR-mediated pERK1/2 over Ca_i^{2+} mobilization.

Another apparent difference between CaSR PAMs points toward putative clinical advantages for cinacalcet. The CaSR agonist Sr^{2+} reduces the differentiation of bone-resorbing osteoclasts (Bonnellye et al., 2008) and stimulates osteoclast apoptosis (Hurtel-Lemaire et al., 2009) (described in V. *(Patho)physiology of the Calcium-Sensing Receptor and Its Ligands*). In cultured osteoclasts differentiated from human CD14⁺ monocytes, although cinacalcet potentiated Sr^{2+} -mediated tartrate-resistant acid phosphatase expression (a marker of osteoclast activity) and robustly inhibited osteoclast-mediated hydroxyapatite artificial bone resorption, AC265347 and BTU compound 13 were without effect in these two assays (Diepenhorst et al., 2018). Although it is not clear whether differences in the biased profile of AC265347 and BTU compound 13 versus cinacalcet are responsible for their distinct PAM activities in osteoclasts, it is interesting that only cinacalcet, and not AC265347 or BTU compound 13, can pharmacochaperone loss-of-function mutant CaSRs potentially via differential stabilization of different conformations of the CaSR. A more detailed understanding of the signaling and trafficking pathways that couple the CaSR to its many physiological responses will aid our understanding of why the CaSR responds to so many endogenous activators and may facilitate the development of biased compounds with improved, tissue-specific effects.

In addition to bias engendered by small-molecule allosteric modulators, CaSR autoantibodies that cause acquired hypocalciuric hypercalcemia can act as biased allosteric modulators. Biased autoantibodies directed against the CaSR VFT can potentiate IP accumulation while inhibiting pERK1/2 generation (Makita et al., 2007; Makita and Iiri, 2014), whereas others inhibit pERK1/2 generation but have no effect on IP accumulation (Pallais et al., 2011). Importantly, cinacalcet corrected the severe hypercalcemia associated with acquired hypocalciuric hypercalcemia caused by a biased autoantibody (Makita et al., 2019). Taken together, these findings once again highlight how bias and allostery are key features of CaSR (patho)physiology and drug actions.

III. Receptor Structure

To date, the complete structure of the CaSR has not been determined. Current CaSR structural knowledge comes from the inactive (Geng et al., 2016) and active (Geng et al., 2016; Zhang et al., 2016) crystal structures of the CaSR ECD in isolation, from mutagenesis studies and homology modeling of the 7TM based on the crystal structures of the metabotropic glutamate receptors

(mGluRs) 1 and 5 7TMs (Dore et al., 2014; Christopher et al., 2015, 2019), and from comparisons with the low resolution cryogenic electron microscopy (cryo-EM) structure of mGlu₅ (Koehl et al., 2019).

The CaSR is an obligate homodimer (Romano et al., 1996; Bai et al., 1998a; Ward et al., 1998; Ray et al., 1999; Zhang et al., 2001; Pidasheva et al., 2006), with each protomer comprised of an extracellular VFT domain (amino acids 20–542) and a cysteine-rich (CR) domain (9 Cys residues within amino acids 542–612) that links the VFT to the prototypical GPCR 7TM domain (amino acids 613–862) (Fig. 2). The 7TM domain is followed by a long intracellular tail (amino acids 863–1078), which is predicted to be largely unstructured but is important for trafficking and phosphorylation (Bai et al., 1998b; Chang et al., 2001; Stepanchick et al., 2010; Zhuang et al., 2012).

A. Calcium-Sensing Receptor Extracellular Domain

1. Structural Overview of the Calcium-Sensing Receptor Extracellular Domain. The VFT extends outside the cell and is comprised of two lobe subdomains (lobe 1 and 2; Fig. 2), with each lobe forming part of a ligand binding cleft. In other class C GPCRs, this cleft forms the orthosteric binding pocket (Kunishima et al., 2000; Tsuchiya et al., 2002; Muto et al., 2007). However, in the CaSR, it is an allosteric or coagonist binding site for L-amino acids, with Ca²⁺ and other cations binding elsewhere.

Two recent VFT crystal structures confirm that the CaSR VFT forms a dimer, with each CaSR protomer orientated next to each other as mirror images (Fig. 2). The dimer orientation of the extracellular domain is similar to that reported for other class C GPCRs,

including mGluRs (Kunishima et al., 2000; Tsuchiya et al., 2002; Muto et al., 2007) and the GABA_B receptor (Geng et al., 2012, 2013). In the inactive state, the two VFT lobes adopt an open conformation [buried surface area of 740 Å², calculated using methods described in Krissinel and Henrick (2007)], and the interdomain cleft is empty. In contrast, the active-state structures adopt a closed conformation and a resulting increase in the buried surface area to just over 1000 Å² between the VFT lobes (Fig. 2). Upon VFT closure, the interdomain cleft interface rotates 29°, mediated by interactions between the two lobes of the VFT (Geng et al., 2016).

The crystal structure of the CaSR VFT plus the CR domains shows an 83-Å distance between the CR domains when the CaSR VFT is in the open (inactive) conformation, which is reduced to 23 Å once the VFT is closed (active; Fig. 2D) (Geng et al., 2016). This change is consistent with other X-ray structures of class C ECDs (Muto et al., 2007; Chappell et al., 2016), likely driving a similar reorientation of the 7TM domains as seen in the mGlu₅ cryo-EM structure a "transition-state" that is partially active but not coupled to G proteins (Koehl et al., 2019). This reorientation is sustained by the rigid CR domain and its nine Cys residues, which form five covalent disulfide bonds: four within the CR domain and one that anchors the CR domain to lobe 2 of the VFT. Consequently, mutation of the Cys residues compromises this rigidity, impacting significantly on receptor function (Fan et al., 1998).

2. Amino Acid and γ -Glutamyl Peptide Binding Site. Although Ca²⁺ has long been considered the orthosteric agonist for the CaSR, Ca²⁺ does not occupy the conserved cleft that forms the orthosteric binding site in other class C GPCRs. Both mutagenesis (Zhang et al.,

Fig. 2. Structural conformation of the CaSR. (A) Model of the CaSR based on homology with full-length mGlu₅ (PDB 6N51). The CaSR (cartoon ribbon) comprises an extracellular VFT domain composed of lobe 1 (LB1, dark blue) and lobe 2 (LB2, teal) and a CR domain (yellow) anchored to the 7TM (orange). (B) Inactive ECD monomer (PDB 5K5T). The bilobed VFT adopts an open conformation revealing a conserved binding cleft between the two lobes. (C) Inactive ECD dimer (left, front view; right, side view). The CR domains of the inactive ECDs are separated. (D) Active ECD monomer (PDB 5K5S). Upon activation, the bilobed VFT closes the amino acid-binding site, narrowing the cleft. (E) Active ECD dimer (left, front view; right, side view). Upon activation, each protomer (orange and yellow) is drawn closer together.

2002b, 2014a; Mun et al., 2004, 2005) and, more recently, the crystal structures of the VFT have revealed that L-amino acids bind the conserved cleft (between lobe 1 and 2), similar to L-Glu binding in the mGluRs (Wellendorph and Bräuner-Osborne, 2009). Thus, L-amino acids and analogs might be better viewed as coagonists rather than PAMs.

The binding of L-Trp (Geng et al., 2016) or the tryptophan derivative, L-1,2,3,4-tetrahydronorharman-3-carboxylic acid (TNCA) (Zhang et al., 2016), stabilizes closing of the bilobed domains through hydrogen bonding and hydrophobic interactions with the receptor. Mutational analysis of residues in the conserved interdomain cleft support binding of L-Trp here (Zhang et al., 2002b, 2014a; Mun et al., 2004, 2005). Interestingly, residues in the conserved cleft are also important for Ca_o^{2+} activation of the CaSR (Bräuner-Osborne et al., 1999; Kunishima et al., 2000; Tsuchiya et al., 2002; Muto et al., 2007; Geng et al., 2013; Jacobsen et al., 2017), suggesting that L-amino acids are required for Ca_o^{2+} activation in line with a classification as coagonists. However, these mutational studies have not accounted for mutation-induced changes in receptor expression, therefore the mutation-induced signaling impairments may be due to reduced receptor expression and consequent reductions in apparent agonist efficacy.

Receptor contacts with L-Trp or TNCA are predominantly through backbone interactions, and the fact that these interactions are largely not L-Trp- or TNCA-specific means other amino acids could be accommodated within this pocket, explaining the L-amino acid promiscuity of the CaSR (see *IIB. Endogenous and Exogenous Allosteric Modulators*). Interestingly, TNCA was not included as a constituent of the crystallization conditions. This highlights not only the diversity of ligands that can bind and activate the CaSR but also suggests that TNCA has such high affinity for the CaSR that it is difficult to remove during the purification process.

γ -Glutamyl peptides are also potent CaSR PAMs that can promote Ca_o^{2+} -dependent Ca_i^{2+} mobilization, suppress intracellular cAMP levels, and inhibit PTH secretion from normal parathyroid cells (see *IIB. Endogenous and Exogenous Allosteric Modulators*) (Broadhead et al., 2011). This activity is lost when Thr145 and Ser170 located in the interdomain cleft are mutated to Ala, indicating that the γ -glutamyl peptides likely share the same binding site as the amino acids (Mun et al., 2005; Broadhead et al., 2011).

3. Cation Binding Sites. In both crystal structures of the CaSR VFT domain, cation binding sites were identified, but these sites differed in their number, location (with the exception of cation binding site 1), and the cation that was bound to each site (Fig. 3).

Anomalous difference mapping indicated four Ca_o^{2+} binding sites in the VFT structure solved by

Geng et al. (2016) (Fig. 3). In lobe 1 of the active (L-Trp bound and closed) VFT conformation, backbone carbonyl oxygen atoms of Ile81, Ser84, Leu87, and Leu88 coordinate Ca_o^{2+} binding at cation binding site 1 (PDB: 5K5S). There was no Ca_o^{2+} coordinated at cation binding site 1 in the inactive structure (PDB: 5K5T), even though this site is not significantly different in the active versus inactive structures (Geng et al., 2016). As such, it is possible that Ca_o^{2+} , which was used at a lower concentration in the crystallization conditions for the inactive structure, could bind to this site without the need for the VFT domain to be closed.

Cation binding site 2 is located adjacent to the L-Trp binding site above the interdomain cleft in lobe 1 of the VFT. Cation binding site 2 is occupied by Ca_o^{2+} in both the inactive and active structures, in which Ca_o^{2+} is coordinated by the hydroxyl group of Thr100 in both states and by the carbonyl of Asn102 via a water molecule in the active structure. Thr145 also lines cation binding site 2 and forms part of the L-Trp binding cleft in the active state (Geng et al., 2016).

The hydroxyl groups of Ser302 and Ser303 coordinate cation binding site 3, either directly or indirectly through water molecules, at the edge of the interdomain cleft of lobe 2. The closing of lobe 1 and lobe 2 of the VFT is facilitated by Ca_o^{2+} stabilization of a conformation that permits an interdomain hydrogen bond interaction between lobe 1 residue Arg66 and lobe 2 residue Ser301 (Geng et al., 2016).

Finally, upon agonist binding, cation binding site 4 forms part of the homodimer interface bridging the lobe 2 domain of one subunit and the CR domain of the second subunit. Three interfacial residues, the carboxylate group of Asp234, and carbonyl oxygen of Glu231 and Gly557, coordinate Ca_o^{2+} binding to site 4 (Geng et al., 2016).

The anomalous difference map intensities varied at each of the Ca_o^{2+} binding sites, where intensity was ranked as Ca_o^{2+} binding site 1 = 2 > 3 > 4. The lower anomalous signal for Ca_o^{2+} in sites 3 and 4 indicates incomplete occupancy or higher flexibility at these positions in the crystal lattice. The authors suggested the lower signal reflects a lower Ca_o^{2+} affinity at these sites. In support of a lower Ca_o^{2+} binding affinity for cation binding site 4, the authors proposed that Ca_o^{2+} binding at site 4 stabilizes the active homodimer conformation, and thus the site is occupied only at elevated concentrations required for receptor activation (Geng et al., 2016).

In contrast to the structures by Geng et al. (2016), Zhang et al. (2016) identified two cation binding sites in their active VFT structures. Electron density and geometric restraints were used to identify Mg^{2+} occupying these cation binding sites, one of which overlapped with cation binding site 1 in the structure by Geng et al. (2016). However, in contrast to the unoccupied cation binding site 1 in the inactive structure by Geng et al.

(2016), cation binding site 1 was occupied by Mg^{2+} in the inactive structure by Zhang et al. (2016). The Mg^{2+} is coordinated by Ser84 and backbone interactions with Ile81, Ile87, and Leu88, in addition to two water molecules. This site is similarly occupied by a Mg^{2+} cation in the rat mGlu₁ VFT structure (Kunishima et al., 2000).

The second Mg^{2+} binding site (cation-binding site 5) is located at the dimerization interface of lobe 2 and is coordinated through Ser240 and four water molecules (Zhang et al., 2016). The highly conserved residues Glu228 and Glu231 from one protomer and Glu241 from the other protomer surround this site.

Anomalous difference maps identified a Gd^{3+} binding site (cation binding site 6) coordinated by Glu232, Glu228, and Glu229 adjacent to cation binding site 5 on the lobe 2 dimerization interface (PDB: 5FBN) (Zhang et al., 2016). The Glu228Ile and the double mutant Glu228Ile/Glu229Ile have previously been shown to reduce Mg^{2+} -induced Ca_i^{2+} mobilization; therefore, other cations could bind here (Huang et al., 2009).

The crystal structures of the ECD suggest that Ca_o^{2+} and other cations play a role in: 1) local stabilization of the CaSR ECD; and 2) activation of the receptor via stabilization of the homodimer through cation binding at sites

4-6 (Jensen et al., 2002; Geng et al., 2016; Zhang et al., 2016). It is unknown whether Ca_o^{2+} alone can activate the receptor or whether it requires the presence of the cleft-binding ligands. Although Geng et al. (2016) obtained an active (closed) structure in the absence of amino acids, an unidentified continuous stretch of density in the conserved interdomain cleft was observed, which could be attributed to an endogenous ligand or a ligand acquired during the crystallization process. If ligands that bind the conserved interdomain cleft are difficult to remove during crystallography studies, it is likely that these same ligands are present during in vitro assays that measure CaSR activation. Furthermore, cations identified in the crystal structures could be artifacts of the crystallization conditions and merely stabilize the crystal contacts required for structure determination. Although mutagenesis was used to corroborate the observed cation binding sites (Geng et al., 2016; Zhang et al., 2016), these mutational studies neither accounted for mutation-induced changes in receptor expression nor quantified changes in cation affinity and efficacy. Therefore, a reduction in cation binding upon mutation of these sites has not been validated. Furthermore, analysis of Ca_o^{2+} -binding proteins to predict the CaSR's Ca_o^{2+} sites, coupled with

Fig. 3. Binding sites within the CaSR crystal structures. ECD conformations of the (A) active (PDB 5K5S) and (B) inactive (PDB 5K5S) calcium-bound structures and the VFT conformations of the (C) active Mg^{2+} -bound (PDB 5FBK) and (D) active Mg^{2+} - and Gd^{3+} -bound (PDB 5FBH) structures. Crystal structures are shown as cartoon ribbon within the transparent molecular surface and colored as in Fig. 2. Hydrogen bond interactions (dashed lines) of calcium (red spheres), Mg^{2+} (green spheres), and Gd^{3+} (yellow spheres) with key residues or water molecules (black spheres) are shown for each proposed binding site.

mutagenesis and spectroscopic techniques to validate the predictions, confirmed multiple VFT Ca_0^{2+} binding sites, but they differed to those identified in the VFT crystal structures (Huang et al., 2007, 2009; Kirberger et al., 2008; Wang et al., 2009, 2010; Zhao et al., 2012). Moreover, analyses of a “headless” CaSR, in which the ECD has been removed, has shown that Ca_0^{2+} can also activate the CaSR via binding sites in the 7TM domain (see *IIIB. Calcium-Sensing Receptor 7-Transmembrane Domain*) (Ray and Northup, 2002; Leach et al., 2016). Accordingly, the cooperative binding of Ca_0^{2+} at multiple binding sites likely maximizes the CaSR’s ability to respond to Ca_0^{2+} over a narrow physiological range. Additional active-state structures, biophysical studies, and mutagenesis work are required to fully understand how these sites interact.

Site-directed mutagenesis and functional studies show that Ca_0^{2+} and L-amino acids potentiate each other’s activity in a positively cooperative manner (Conigrave et al., 2000; Zhang et al., 2002b, 2014a,b; Mun et al., 2005). Under physiological conditions, L-amino acids potentiate Ca_0^{2+} potency for evoking intracellular responses (Conigrave et al., 2007), and mutating residues important for L-amino acid binding eliminated L-Phe potentiation of Ca_i^{2+} mobilization (Conigrave et al., 2000; Zhang et al., 2002a). The ability of Ca_0^{2+} and L-amino acids to cooperatively activate the CaSR was further demonstrated using saturation transfer difference NMR (Zhang et al., 2014b). Using saturation transfer difference NMR, L-Phe was estimated to bind to the CaSR with an affinity of ~ 10 mM in the absence of Ca_0^{2+} , whereas in the presence of Ca_0^{2+} , L-Phe affinity was increased. Similarly, and as expected for reciprocal cooperativity, the binding affinity of Ca_0^{2+} in the presence of 10 mM L-Phe was increased. Therefore, dual binding of Ca_0^{2+} and amino acids enhances the sensitivity of the CaSR to changes in concentrations of these ligands.

4. Anion Binding Sites. A total of four anion binding sites in the inactive and active extracellular domain structures were identified based on electron density and crystallization conditions (Geng et al., 2016). Anion binding sites 1-3 are located above the interdomain cleft in lobe 1, and anion site 4 is located in lobe 2. Although SO_4^{2-} and PO_4^{3-} anions were modeled into these structures, it is possible other anions may be present. These anions act to stabilize the local conformation of the receptor in the crystal structure because, in the absence of PO_4^{3-} in the inactive crystal structure, several binding site residue side chains are disordered. In the inactive structure, anions were bound at sites 1-3, whereas in the active structure, only sites 2 and 4 were occupied. In the crystal structures, anions may have stabilized the CaSR to aid crystallization. However, like all GPCRs, the CaSR can sample multiple conformations not captured in these crystal structures. Thus, under physiological conditions, anions may act to stabilize intermediate CaSR states.

5. Etelcalcetide Binding Site. The polypeptide allosteric modulator etelcalcetide binds to a distinct site in the CaSR’s VFT domain and requires a covalent S-S bond formed directly with the CaSR VFT to retain activity (Alexander et al., 2015). This interaction occurs when the free Cys482, which is located at the back of VFT lobe 1 near the hinge loops, exchanges with a L-Cys disulphide bound to a D-Cys in the etelcalcetide D-amino acid peptide sequence. Despite this covalent linkage, the interaction appears transient, and the effect of etelcalcetide on plasma PTH levels rapidly diminishes immediately after withdrawal of intravenous injection (Alexander et al., 2015). It is not known how etelcalcetide binding potentiates CaSR activity at a structural level; therefore, further structural and mutagenesis studies are needed to determine the conformational changes stabilized by etelcalcetide that mediate its PAM activity.

B. Calcium-Sensing Receptor 7-Transmembrane Domain

1. Structural Basis of Calcium-Sensing Receptor 7-Transmembrane Activation. The only full-length class C GPCR structure is of mGlu₅, which was determined using cryo-EM. The full-length mGlu₅ structure shows how the inactive (or open) VFT receptor complex disrupts the interface between the 7TM domains, whereas the activated (closed) complex forces a reorientation of the 7TM domains, fostering an interface between the top of TM6 and TM7 (Koehl et al., 2019). Without a comparable structure available for the CaSR, similar conformational changes driving CaSR activation can only be hypothesized. Nevertheless, there is significant structural and functional data that are available for the CaSR 7TM that is important for understanding its activity.

Like all GPCRs, the CaSR’s 7TM helices are joined by intracellular loops (ICLs) 1-3, which are important for effector coupling, and ECLs 1-3, in which ECL2 and ECL3 contain a number of residues important for receptor activation (Leach et al., 2012; Goolam et al., 2014). Structural and biochemical data for other GPCR classes show that receptor activation involves an outward movement of TM5 and TM6 to permit G protein coupling and signal transduction. 7TM movements are driven by a number of conserved amino acid sequences important for receptor activation, which are known as switch motifs. How this process may happen in the CaSR is discussed in this section.

Although the CaSR responds to a diverse array of stimuli through its VFT, the VFT is not required for the receptor to respond to Ca_0^{2+} . The CaSR 7TM domain alone signals in response to Ca_0^{2+} , albeit with lower potency and a significant reduction in the Ca_0^{2+} Hill coefficient (Ray and Northup, 2002; Leach et al., 2016). This indicates that the CaSR 7TM also contains one or more orthosteric binding sites. Regrettably, no

structures of the CaSR 7TM have been determined experimentally. However, sequence comparisons between the CaSR and mGlu₁ or mGlu₅ reveal that putative switch motifs important for receptor activation are shared throughout the 7TMs of class C GPCRs, guiding our understanding of CaSR activation.

With the lack of a CaSR 7TM domain structure, the CaSR 7TM has been the subject of extensive mutagenesis and structure-function studies in an attempt to understand this domain. Guided by naturally occurring and engineered mutations and sequence homology with other GPCRs, residues important for Ca_o²⁺ activity, allosteric modulation, biased agonism, and biased modulation have been identified (Leach et al., 2013, 2014; Goolam et al., 2014; Cook et al., 2015). Indeed, the putative Ca_o²⁺ binding site within the 7TM has been predicted using this approach, in which Ca_o²⁺ is hypothesized to mediate an interaction network between Glu767^{ECL2} and Glu837^{7.32} (Leach et al., 2016).

The mGlu₁ and mGlu₅ X-ray structures revealed an ionic lock formed between Lys^{3.50} (Lys698^{3.50} in the CaSR) and Glu^{6.35} (Glu803^{6.35} in the CaSR) (Dore et al., 2014; Christopher et al., 2015, 2019). These ionic lock residues are conserved across class C GPCRs and this “switch motif” is believed to stabilize the inactive conformation of the class C 7TM domain in the absence of agonist (Dore et al., 2014). Furthermore, a conserved sequence in class A GPCRs important for their activation called the “toggle” switch motif (protein sequence: FxxCWxP^{6.50}) is replaced by a “wl switch motif” (protein sequence: W^{6.50}L^{6.51}) in class C GPCRs (Trzaskowski et al., 2012). Although the wl switch motif differs markedly in sequence from the class A toggle switch motif, most notably by its lack of Pro^{6.50} to induce a characteristic kink in TM6 (Lagerström and Schiöth, 2008), Trp^{6.50} in the class C GPCR wl motif (Trp818^{6.50} in the CaSR) is in an identical position to Trp^{6.48} in the class A GPCR FxxCWxP^{6.50} motif (Trzaskowski et al., 2012; Dore et al., 2014). Rotation of the Trp^{6.48} side chain is a central feature of the toggle switch motif during class A GPCR activation. Molecular dynamic simulations suggest a similar rotation of Trp^{6.50} may occur in mGluR₂ upon activation (Perez-Benito et al., 2017), whereas the mGlu₅ crystal structures demonstrate that Trp^{6.50} can alternate between two distinct rotomers when bound to different NAMs, indicating it differentially orientates upon binding of different ligands (Dore et al., 2014; Christopher et al., 2015, 2019). Thus, it is hypothesized that Trp^{6.50} in class C GPCRs fulfills an equivalent toggle switch function to Trp^{6.48} in class A GPCRs (Trzaskowski et al., 2012; Dore et al., 2014). Finally, the CaSR and other class C GPCRs contain a P^{7.56}KxY motif, which is believed to perform an analogous role to the NP^{7.50}xxY(x)_{5/6}F motif (wherein F sits five or six residues away from the Y) in class A GPCRs. The NP^{7.50}xxY(x)_{5/6}F motif undergoes significant rearrangement during activation (Fritze et al.,

2003; Katritch et al., 2013; Dore et al., 2014). Nevertheless, without high resolution structures of the CaSR and with only inactive mGlu₁ and mGlu₅ 7TM structures available, it is difficult to confidently determine any importance of these motifs to CaSR activation and effector coupling.

2. Small-Molecule Allosteric Modulator Binding Sites. The CaSR 7TM contains allosteric binding sites for small-molecule allosteric modulators (Ray and Northup, 2002; Petrel et al., 2003, 2004; Miedlich et al., 2004; Hu et al., 2006; Bu et al., 2008; Gerspacher et al., 2010; Leach et al., 2016; Gregory et al., 2018; Keller et al., 2018; Josephs et al., 2019). These sites have been established by mutagenesis studies that examined changes in modulator potency or affinity coupled with homology modeling to understand the context of this mutagenesis data. Initial homology modeling was based on the solved X-ray crystallography structures of class A GPCRs (Miedlich et al., 2004; Hu et al., 2006; Bu et al., 2008; Gerspacher et al., 2010), but this was later extended to modeling based on the NAM-bound 7TM structures of mGlu₁ and mGlu₅ (Leach et al., 2016; Gregory et al., 2018; Keller et al., 2018; Josephs et al., 2019).

Mutagenesis and homology modeling has established that the CaSR 7TM domain contains an extended allosteric binding pocket formed by Phe668^{2.56}, Arg680^{3.32}, Phe684^{3.36}, Phe688^{3.40}, Glu767^{ECL2}, Leu776^{5.43}, Trp818^{6.50}, Phe821^{6.53}, Tyr825^{6.56}, Val833^{ECL3}, Ser834^{ECL3}, Glu837^{7.32}, Ala840^{7.35}, Ile841^{7.36}, and Ala844^{7.39} (Leach et al., 2016). This extended pocket overlaps with the allosteric and orthosteric binding sites in biogenic amine class A GPCRs (Kruse et al., 2013) and contains multiple binding sites. For instance, arylalkylamine PAMs and NAMs, such as cinacalcet and NPS 2143, are predicted to form direct salt-bridge interactions with Glu837^{7.32} at the top of the extended binding pocket supported by substitutions of Glu837^{7.32} with uncharged or positively charged amino acids, which abolish or significantly reduce arylalkylamine activity (Miedlich et al., 2004; Bu et al., 2008; Leach et al., 2016; Jacobsen et al., 2017; Gregory et al., 2018; Keller et al., 2018; Josephs et al., 2019). AC265347 is believed to bind lower in the allosteric pocket because it lacks the capacity to interact with Glu837^{7.32} (Leach et al., 2016). Although ATF936 is predicted to bind in a comparable position to the arylalkylamines, mutation of Glu837^{7.32} has no effect on ATF936 potency or affinity; therefore, some of its binding interactions with the CaSR differ to the arylalkylamines (Gerspacher et al., 2010; Josephs et al., 2019).

Excitingly, the established 7TM allosteric pocket is unlikely to be the only binding site for small-molecule allosteric modulators. The CaSR NAM, BMS compound 1, does not appear to use this binding site because it interacts in a noncompetitive manner with NPS 2143 and is largely unaffected by many of the 7TM mutations that reduce the affinity of other CaSR NAMs (Arey

et al., 2005; Josephs et al., 2019). Thus, there remains scope for allosteric modulator binding to multiple sites in the CaSR 7TM.

3. Structural Basis of Small-Molecule Allosteric Modulator Cooperativity, Efficacy, and Bias. Fitting an operational model of agonism or allosterism to functional CaSR data has revealed structural features important for allosteric cooperativity, agonism, and bias. For the PAMs cinacalcet, NPS R-568, and AC265347, mutations Glu767^{ECL2}Ala, Val817^{6.49}Ala, or Ala844^{7.37}Val all reduced the cooperativity of these PAMs (Leach et al., 2016; Keller et al., 2018). However, substantial differences between PAMs have also been described. For instance, although mutation of Phe688^{3.40}Ala, Tyr825^{6.57}Ala, or Leu848^{7.43}Ala reduced the cooperativity of the two arylalkylamine PAMs, cinacalcet and NPS R-568, mutation of Ala615^{1.42}Val or Lys831^{ECL3}Ala only reduced the cooperativity of cinacalcet. Furthermore, mutation of Trp818^{6.50}Ala, which is part of the wl motif discussed above, increased cooperativity of cinacalcet but had no significant effect on NPS R-568 cooperativity. Although structurally and pharmacologically similar, the divergent residues mediating cinacalcet or NPS R-568 cooperativity demonstrate how subtle differences in chemical scaffolds can stabilize distinct structural conformations of the CaSR 7TM domain (Leach et al., 2016; Keller et al., 2018).

The PAM agonist, AC265347, demonstrated further differences from cinacalcet and NPS R-568. For instance, unlike cinacalcet and NPS R-568, mutations Tyr825^{6.57}Ala or Leu848^{7.43}Ala had no effect on AC265347 cooperativity, whereas mutation of Phe688^{3.40}Ala altered AC265347 cooperativity (Leach et al., 2016; Keller et al., 2018). Interestingly, AC265347 biased modulation of pERK1/2 versus Ca_i^{2+} mobilization was altered by the mutations Leu776^{5.43}Ala or Trp818^{6.50}Ala. Here, these two mutations increased or decreased AC265347 cooperativity in pERK1/2 assays without altering cooperativity in Ca_i^{2+} mobilization assays, providing some insight into 7TM residues that specifically mediated CaSR signaling toward a specific signaling pathway (Cook et al., 2015; Leach et al., 2016). Furthermore, allosteric agonism mediated by AC265347 has different requirements to Ca_o^{2+} agonism. Although mutation of Leu776^{5.43}Ala or V817^{6.49}Ala reduced efficacy of both AC265347 and Ca_o^{2+} , mutations Phe684^{3.36}Ala or Phe688^{3.40}Ala decreased AC265347 efficacy without altering the efficacy or affinity of Ca_o^{2+} (Leach et al., 2016; Keller et al., 2018).

Similar to residues that transmit cooperativity mediated by PAMs, distinct amino acids transmit negative cooperativity mediated by different NAMs. For instance, of the residues analyzed to date, only the mutation Leu776^{5.43}Ala significantly altered NPS 2143 cooperativity (Leach et al., 2016). In contrast, a number of mutations that had no effect on NPS 2143 cooperativity increased or decreased ATF936 cooperativity, including Glu767^{ECL2}Ala, Trp818^{6.50}Ala, and

Ile841^{7.36}Ala (Josephs et al., 2019). Other NAMs were sensitive to different mutations (Josephs et al., 2019). Further analysis of additional 7TM mutations will help to unravel cooperativity networks that drive global and ligand-specific allosteric effects.

C. Calcium-Sensing Receptor Dimerization

Like all class C GPCRs, CaSR dimerization is a key feature governing receptor function. The dominant interaction underpinning the CaSR dimer is two covalent disulfide bonds formed at the top of lobe 1 of the VFT domains between Cys129 and Cys131 (Ray et al., 1999). However, the CaSR is not dependent on the disulfide links for activity, as is evidenced by mutation of these residues to Ser, which does not alter surface expression or Ca_o^{2+} potency in vitro (Fan et al., 1998; Zhang et al., 2001).

Dimerization influences allosteric modulation at the CaSR. For instance, negative allosteric modulators must bind both protomers to block signaling, whereas PAMs only need occupy one protomer to exert their full modulatory effect (Hauache et al., 2000; Jacobsen et al., 2017; Gregory et al., 2018). This feature likely reflects agonist-mediated signal transmission through the CaSR, which occurs across the dimer rather than propagating through a single protomer (Hauache et al., 2000). Consequently, transactivation across the dimer can result in unique pharmacology for CaSR allosteric modulators. An example is calhex 231, which shows positive allosteric activity when bound to the allosteric site in only one protomer but shows negative allosteric activity when occupying both the allosteric sites of the dimer (Gregory et al., 2018).

Immunoprecipitation data have demonstrated that the CaSR forms heterodimers in vitro with mGlu_{1/5} or the GABA_B receptor, with heterodimers detected in bovine and mouse brain lysates, respectively (Gama et al., 2001; Chang et al., 2007). On the other hand, fluorescence resonance energy transfer studies have revealed that the CaSR does not heterodimerize with its closest receptor homolog, the GPRC6A receptor (Jacobsen et al., 2017). Heterodimerization may facilitate the varied functional roles of the CaSR in different tissues, particularly in the brain, wherein the expression of the GABA_B receptor regulates CaSR expression and vice versa (discussed in VK. *Calcium-Sensing Receptor in the Brain and Nervous System*).

D. Calcium-Sensing Receptor Glycosylation

The CaSR VFT domain contains 11 potential N-linked glycosylation sites; however, not all of these sites have been experimentally verified. The CaSR is glycosylated in the endoplasmic reticulum with mannose (immature) carbohydrate prior to mature complex glycosylation processing in the Golgi. Disruption of at least three glycosylation sites can impair receptor processing and cell surface expression (Ray et al., 1998). Eight

glycosylation sites (Asn90, Asn130, Asn261, Asn287, Asn446, Asn468, Asn488, and Asn541) have been experimentally validated, whereas questions remain over the three remaining sites (Asn386, Asn400, and Asn594). Notably, Asn594 was glycosylated in the solved X-ray crystal structure, whereas Asn386 was mutated to Gln to prevent glycosylation and aid crystallization. However, it is unclear whether this observation at a truncated CaSR sample reflects the glycosylation arrangement of the full-length CaSR. Importantly, the functional role of glycosylation beyond controlling surface expression needs further investigation.

IV. CaSR Regulation

A. Phosphorylation and Dephosphorylation

PKC-mediated phosphorylation of the CaSR provides a rapid and quickly reversible mechanism for inhibiting receptor activity. Indeed, treatment of parathyroid cells with PKC-activating phorbol esters overcomes the inhibitory effect of Ca_o^{2+} on PTH release (Brown et al., 1984; Nemeth et al., 1986). When first cloned, the CaSR was predicted to contain five PKC consensus motifs, although 54 serine and threonine residues reside in the receptor's C-terminal tail and ICLs (Garrett et al., 1995a). However, the key inhibitory phosphorylation site is Thr888 in the C-terminal tail (Bai et al., 1998b). Thr888 is most likely phosphorylated by PKC α (Young et al., 2014) and dephosphorylated by a calyculin A-sensitive protein phosphatase (McCormick et al., 2010).

The functional importance of inhibitory Thr888 phosphorylation is most apparent with the clinical mutant, Thr888Met, which cannot be phosphorylated. In vitro Thr888Met is a gain-of-function mutant, whereas it suppresses PTH secretion in vivo, resulting clinically in ADH (see *VIC. Autosomal Dominant Hypocalcemia and Bartter Syndrome Type V*) (Lazarus et al., 2011). Therefore, CaSR phosphorylation contributes significantly to CaSR activity in vivo and thus to the overall control of PTH secretion and Ca_o^{2+} homeostasis [reviewed in Conigrave and Ward (2013)].

The nonphosphorylatable mutant, Thr888Val, also produced a significant gain of function, which was not further enhanced by comutating the other four predicted PKC sites (Bai et al., 1998b). However, PKC inhibition at the wild-type CaSR resulted in a greater gain of function than produced at the Thr888Val mutant, thus it appeared likely that another unknown site may be phosphorylated in tandem with Thr888. However, the identity of this site has remained elusive. In mGlu₅, the key PKC phosphorylation site, Ser839 (Kim et al., 2005), aligns not with Thr888 in the CaSR but with Ser875, a residue not originally predicted to be phosphorylated by PKC (Garrett et al., 1995a). Intriguingly, current data indicate removal of this putative phosphorylation site from the CaSR (Ser875Ala) also produces a gain of function, similar to that of Thr888Ala, whereas

a phosphomimetic mutation at this site (Ser875Asp) produces a loss of function (Binmahfouz et al., 2019). The double Ser875Ala plus Thr888Ala mutant exhibits a greater gain of function than Ser875Ala alone, and concomitant PKC inhibition exerts no further signal enhancement. Thus, Ser875 is most likely the second major inhibitory PKC site in the CaSR (Binmahfouz et al., 2019).

Ca_o^{2+} induces biphasic concentration-dependent phosphorylation of Thr888 in CaSR-HEK cells, with 0.5–2.5 mM Ca_o^{2+} eliciting increased Thr888 phosphorylation after 10 minutes, whereas 2.5–5 mM Ca_o^{2+} decreases phosphorylation apparently by activating a calyculin A-sensitive protein phosphatase (McCormick et al., 2010). The decrease in Thr888 phosphorylation mediated by 2.5–5 mM Ca_o^{2+} occurs at the same Ca_o^{2+} concentrations that elicit sustained, as opposed to oscillatory, Ca_i^{2+} mobilization. Consistent with this, the Thr888Ala mutant is not only gain-of-function but also exhibits less oscillatory and more sustained Ca_i^{2+} mobilization, as does the wild-type CaSR when cotreated with a PKC inhibitor (Davies et al., 2007). Furthermore, PKC-dependent phosphorylation of Thr888 attenuates L-amino acid-dependent signaling in a manner similar to its effect on Ca_o^{2+} (Bai et al., 1998b; McCormick et al., 2010). Since PKC-mediated Thr888 phosphorylation is thus a critical regulator of CaSR function, differential CaSR phosphorylation could provide a mechanism to permit biased signaling in different cells or in response to various agonists.

CaSR signaling is also modulated by the GPCR kinases (GRKs). Specifically, overexpression of GRK2 and GRK3 decreases CaSR-induced IP formation in a HEK-derived cell line by >70% (Lorenz et al., 2007). Mutating GRK2 so that it could no longer bind G_q overcame the inhibitory effect of GRK2 on CaSR signaling, indicating that GRK2 inhibition of CaSR signaling might be caused by sequestering of G_q rather than by phosphorylation of the CaSR. Overexpression of either β -arrestin 1 or β -arrestin 2 partly inhibits CaSR-induced IP production, and this effect was abolished by deleting all five of the predicted PKC sites as identified by Bai et al. (1998b) (Lorenz et al., 2007).

B. Internalization and Agonist-Driven Insertional Signaling

In heterologous expression systems, the CaSR undergoes constitutive internalization (Reyes-Ibarra et al., 2007; Gorvin et al., 2018c; Mos et al., 2019) and agonist-induced internalization (Lorenz et al., 2007; Zhuang et al., 2012; Nesbit et al., 2013b). Furthermore, CaSR internalization is increased by the CaSR PAM, NPS R-568, and agonist-induced but not constitutive internalization is inhibited by the NAM, NPS 2143 (Mos et al., 2019). GPCR internalization usually involves desensitization by kinase phosphorylation and subsequent β -arrestin binding followed by recruitment to clathrin-

coated pits by β -arrestin and the clathrin-binding AP2 heterotetramer (Hanyaloglu and von Zastrow, 2008). As described above, phosphorylation by PKC and GRKs, or β -arrestin recruitment, is involved in CaSR desensitization (Pi et al., 2005b; Lorenz et al., 2007). Similarly, agonist-induced CaSR internalization requires β -arrestin, which is in contrast with the GABA_B and mGluRs, which function independently of β -arrestin (Pin and Bettler, 2016). However, constitutive and agonist-induced CaSR internalization is largely independent of G_{q/11} and G_{i/o} in HEK293 cells (Mos et al., 2019), thus indicating that G protein-mediated activation of PKC is not involved in this cell line. Studies of the internalization mechanisms of endogenously expressed CaSRs in nonrecombinant cells are still lacking because of the technical difficulty of performing such experiments.

The CaSR is also predicted to couple directly to AP2 σ through a dileucine motif in the CaSR C-terminal tail (Nesbit et al., 2013b). Similar to loss-of-function mutations in the *CASR* or *GNA11* genes (Pollak et al., 1993; Nesbit et al., 2013a), mutations that disrupt the CaSR interaction with AP2 σ reduce the sensitivity of CaSR-expressing cells to Ca_o²⁺ (Nesbit et al., 2013b). Similarly, germline mutations of the *AP2S1* gene that lead to alteration of Arg15 in AP2 σ cause FHH3 (Nesbit et al., 2013b), which is clinically the most severe of the three FHH types (Hannan et al., 2015a) (VI. *Calcium-Sensing Receptor-Related Genetic Diseases and Therapeutic Interventions*). AP2 σ Arg-15 mutations inhibit CaSR internalization (Nesbit et al., 2013b; Gorvin et al., 2018c), and the functional similarity between loss-of-function mutations in the *CASR*, *GNA11*, and *AP2S1* genes shows a close relationship between internalization and CaSR signaling. This relationship could be explained by reduced resensitization and/or intracellular signaling when internalization is inhibited (Reyes-Ibarra et al., 2007; Zhuang et al., 2012; Gorvin et al., 2018c).

After internalization, GPCRs are either resensitized and recycled to the cell surface or degraded (Hanyaloglu and von Zastrow, 2008). Cell surface expression of CaSR is constant under basal conditions (Reyes-Ibarra et al., 2007; Zhuang et al., 2012), which means constitutively internalized receptors are replaced. In heterologous cells, internalized CaSR is recycled through Rab11a-dependent slow-recycling endosomes (Reyes-Ibarra et al., 2007) to be sorted to lysosomes for degradation (Grant et al., 2011; Zhuang et al., 2012). The CaSR's C-terminal tail is involved in postendocytic sorting, as deletion of residues 920–970 increased cell surface expression and reduced colocalization with a lysosomal marker (Zhuang et al., 2012). Similarly, overexpression of associated molecule with the SH3 domain of signal-transducing adapter molecule, which interacts with the CaSR C-terminal tail, downregulated cell surface CaSR (Herrera-Vigener et al., 2006; Reyes-Ibarra et al., 2007).

The interaction of 14-3-3 proteins with an arginine-rich motif in the CaSR C-terminal tail partly retains an intracellular CaSR pool (regulated by Ser899 phosphorylation) (Stepanchick et al., 2010; Grant et al., 2011, 2015), but the CaSR is upregulated at the cell surface upon agonist stimulation via a G_{q/11}-dependent mechanism called agonist-driven insertional signaling (Grant et al., 2011; Gorvin et al., 2018c). This process involves rapid mobilization of the intracellular pool of receptors to the cell surface and initiation of receptor synthesis to support prolonged upregulation (Grant et al., 2011, 2015). The rapid increase in cell surface receptors is proposed to support the high sensitivity of the CaSR to increases in Ca_o²⁺.

V. (Patho)physiology of the CaSR and Its Ligands

A. Calcium-Sensing Receptor in the Parathyroid Glands

CaSR expression appears during parathyroid development in response to key parathyroid-determining genes, including *GCM2* (encoding GcmB) and *SHH* (encoding the inhibitory controller, Sonic Hedgehog) (Grevellec et al., 2011). Consistent with a direct connection between GcmB and CaSR expression, *GCM2* control elements have been identified in the *CASR* promoters (Canaff et al., 2009), and short hairpin RNA directed against *GCM2* in parathyroid cell cultures suppressed the protein levels of GcmB and the CaSR (Mizobuchi et al., 2009).

The CaSR's nonredundant roles in Ca_o²⁺ metabolism have been clearly established by the hypercalcemic disorders, neonatal severe hyperparathyroidism (NSHPT) and FHH, and the hypocalcemic disorders, ADH and Bartter syndrome type V. These are discussed together with animal models of loss- and gain-of-function mutations of the CaSR in VI. *Calcium-Sensing Receptor-Related Genetic Diseases and Therapeutic Interventions*.

The CaSR negatively controls parathyroid function by suppressing acute PTH secretion primarily from chief cells [review: (Conigrave, 2016)], inhibiting cell proliferation and thus cell number and gland size (Fan et al., 2018), and reducing PTH gene transcription [review: (Chen and Goodman, 2004)]. It also activates the local synthesis, particularly in parathyroid oxyphil cells, of 1,25(OH)₂D₃ (Ritter et al., 2012), a recognized inhibitor of PTH synthesis. The CaSR's effects on cell proliferation are particularly noticeable in the context of primary hyperparathyroidism (e.g., due to adenomatous disease) or hyperplasia in the context of CKD. Interestingly, in parathyroid adenoma and CKD, cellular CaSR expression is reduced (Kifor et al., 1996). Nonetheless, in patients with CKD and in rat models of secondary hyperparathyroidism, sustained treatment with cinacalcet suppresses parathyroid gland size as well as serum PTH levels (Colloton et al., 2005; Yamada et al., 2015). Similarly, exposure of parathyroid cells to

cinacalcet in vitro suppresses proliferation and promotes apoptosis (Tatsumi et al., 2013).

The parathyroid CaSR continuously monitors the Ca_o^{2+} concentration as well as various other stimuli that affect CaSR function, including the plasma levels of L-amino acids (Conigrave et al., 2004), pH (Campion et al., 2015), ionic strength (Quinn et al., 1998), and, perhaps, locally generated polyamines (Quinn et al., 1997) (see *II. Agonists and Allosteric Modulators*). CaSR activity in the parathyroid glands is resistant to desensitization, in part because of efficient receptor recycling as well as a large intracellular receptor pool that undergoes a high rate of trafficking from the endoplasmic reticulum and Golgi to the plasma membrane [reviews: (Breitwieser, 2013; Ray, 2015)]. Whether agonist-driven insertional signaling operates in parathyroid glands is unknown, but the CaSR interacts with a signaling assembly dependent on caveolin-1 (Kifor et al., 1998) and undergoes AP2 σ -regulated endocytosis (Nesbit et al., 2013b; Gorvin et al., 2018c).

1. Parathyroid Hormone Secretion Control. The set point for the CaSR's half-maximal inhibitory effect on PTH secretion lies at the lower limit of the normal free Ca_o^{2+} concentration range (1.1–1.3 mM). In this way, the disinhibited parathyroid provides the body's primary defense against hypocalcemia ($\text{Ca}_o^{2+} < 1.1$ mM). However, the parathyroid CaSR does not provide the primary defense against hypercalcemia, which is mediated by CaSRs in the renal cortical thick ascending limbs (TALs) of Henle's loop, which accelerate Ca_o^{2+} excretion (Kantham et al., 2009; Loupy et al., 2012). Furthermore, as Ca_o^{2+} increases, its inhibitory effect on PTH secretion suppresses bone resorption.

Two distinct paradigms for CaSR-mediated inhibition of PTH secretion have been identified at the cellular level: 1) stimulation of $\text{G}_{i/o}$ proteins, which oppose cAMP-dependent increases in PTH secretion mediated by G_s -coupled receptors, for example, adrenaline (β -adrenergic receptors 1 and 2), dopamine, histamine, and prostanoid receptors [review: (Conigrave, 2016)]; and 2) inhibition of endogenous PTH secretion mechanisms that occur in the absence of exogenous activators, at least in part via stimulation of $\text{G}_{q/11}$ proteins. Endogenous PTH secretion mechanisms may depend upon the intrinsic production of activators for parathyroid secretion [review: (Conigrave, 2016)] or may represent true constitutive secretion (Muresan and MacGregor, 1994).

2. Calcium-Sensing Receptor Structure and Function in the Parathyroid. The primary protein form adopted by the CaSR in parathyroid cells is a disulphide-linked homodimer (Kifor et al., 2003) similar to that observed when the CaSR is expressed in HEK293 cells (Bai et al., 1998a). However, it may also form heterodimers with some other receptors, for example, GABA_B receptors, as reported for growth plate chondrocytes (Cheng et al.,

2007), with unknown consequences for parathyroid cell signaling and function (see *IIIC. Calcium-Sensing Receptor Dimerization*).

The CaSR in the parathyroid couples to various signaling pathways as it does when expressed heterologously in HEK293 cells and in other cell types [review: (Conigrave and Ward, 2013)]. As described above, in parathyroid cells the CaSR couples to multiple heterotrimeric G proteins, including, most notably, $\text{G}_{i/o}$ and $\text{G}_{q/11}$. Of these, $\text{G}_{i/o}$ supports Ca_o^{2+} -mediated suppression of PTH secretion stimulated by G_s -coupled receptors [e.g., for dopamine (Brown et al., 1990)] but not intrinsic PTH secretion (Brown et al., 1992). Of apparently greater importance, $\text{G}_{q/11}$ signaling is absolutely required for CaSR-mediated control of PTH secretion. Thus, mice that are global null for $\text{G}_{\alpha_{11}}$ or have conditional deletion of G_{α_q} in the parathyroid exhibit mild-moderate hyperparathyroidism. Interestingly, however, crossbreeding to generate mice that are *both* global null for $\text{G}_{\alpha_{11}}$ and parathyroid null for G_{α_q} results in severe neonatal hyperparathyroidism (Wettschureck et al., 2007) that is comparable to that seen in human neonates with homozygous or compound heterozygous loss-of-function CaSR mutations (Pollak et al., 1993). Consistent with these observations, loss-of-function mutations of $\text{G}_{\alpha_{11}}$, which only partially impair signaling, have been linked to a variant form of FHH in humans, now known as FHH2, and gain-of-function mutations of $\text{G}_{\alpha_{11}}$ have been linked to a variant form of ADH, now known as ADH2 (Nesbit et al., 2013a). The Arg60Cys and Ile62Val gain-of-function mutations in $\text{G}_{\alpha_{11}}$ also induce ADH2 in mice, in which treatment with the NAM, NPS 2143, or the specific $\text{G}_{q/11}$ inhibitor, YM-254890, increases PTH and Ca_o^{2+} concentrations (Gorvin et al., 2017; Roszko et al., 2017). These findings demonstrate the critical importance of $\text{G}_{q/11}$ in control of PTH synthesis and/or secretion. CaSR signaling in the parathyroid is also negatively regulated by the GTPase activator RGS5, and overexpression of RGS5 in the parathyroid induces hyperparathyroidism in mice (Koh et al., 2011). Whether RGS5 has a preference for either G_q or G_{11} in parathyroid cells is unknown. Interestingly, studies in other tissues suggest that RGS5 preferentially suppresses the function of G_q with little or no effect on G_{11} (Ladds et al., 2007). Whether this might support a parathyroid-based preference for CaSR-mediated activation of G_{11} rather than G_q is unknown.

The mechanism by which the CaSR controls PTH secretion downstream of $\text{G}_{q/11}$ is surprisingly ill-defined. Contributing factors appear to include phosphatidylinositol-PLC, which is robustly activated by Ca_o^{2+} stimulation in parathyroid cells (Brown et al., 1987a; Shoback et al., 1988); Ca_i^{2+} signals whose frequency and amplitude are dependent on the phosphorylation status of Thr888 (McCormick et al., 2010; Lazarus et al., 2011); and the MAPK, ERK1/2 (Corbetta et al., 2002). Evidence has also been presented for

a convergent signaling pathway mediated by α -klotho and the CaSR on PTH synthesis and parathyroid hyperplasia downstream of FGF receptors (Fan et al., 2018). In other work, parathyroid Na^+/K^+ -ATPase activity has been implicated in the control mechanism (Brown et al., 1987b; Imura et al., 2007). Whether this might operate via changes in cell volume or intracellular ion concentrations is unclear; changes in Ca_i^{2+} concentration appear to have been excluded (Brown et al., 1987b).

B. Calcium-Sensing Receptor in the Thyroid Gland

In the thyroid the CaSR is expressed at high levels in a relatively small subpopulation of cells, the parafollicular C cells (Garrett et al., 1995b). In C cells, the CaSR acts to promote secretion of the peptide hormone calcitonin. Evidence that the CaSR stimulates calcitonin secretion is supported by studies in CaSR knockout mice, in which plasma calcitonin levels were suppressed (Fudge and Kovacs, 2004; Kantham et al., 2009). Thus, elevated Ca_o^{2+} stimulates calcitonin release, which, in turn, lowers the plasma calcium level, primarily by suppressing bone resorption. Both the CaSR and calcitonin (or calcitonin gene-related peptide) genes are under inhibitory regulation by thyroid transcription factor-1 in C cells, and CaSR activation promotes calcitonin synthesis, at least in part by suppressing the levels of thyroid transcription factor-1 (Suzuki et al., 1998). CaSR coupling to G proteins in C cells is similar to its coupling in parathyroid cells and various other cell types, and the C-cell CaSR thereby activates plasma membrane phospholipases and cellular protein kinases (McGehee et al., 1997). Activation of the CaSR also stimulates acute elevations in Ca_i^{2+} in various C-cell models. In some cell types, this occurs via Ca_o^{2+} entry through plasma membrane L-type voltage-gated Ca^{2+} channels (Muff et al., 1988; Fajtova et al., 1991; McGehee et al., 1997) and, in others, via Ca_i^{2+} mobilization (Freichel et al., 1996). Recently, the amino acid-activated CaSR was shown to stimulate calcitonin release from human C cells (Mun et al., 2019). Despite these insights, the molecular mechanisms by which the CaSR stimulates calcitonin secretion are largely unknown.

C. Calcium-Sensing Receptor in the Kidney

CaSR expression in the kidney is one of the highest in the body, and the renal CaSR plays a major role in the regulation of renal function in both a hormone-dependent and independent fashion [see Riccardi and Valenti (2016) and references therein]. A large body of functional, molecular, and genetic evidence indicates that the kidney CaSR plays a crucial role in mineral ion homeostasis. Indeed, the CaSR is widely expressed along the nephron at both the apical and basolateral sides of kidney cells, and thereby it is uniquely poised to monitor both urine and plasma and alter the final

ultrafiltrate composition accordingly (Riccardi et al., 1998; Graca et al., 2016). Urinary calcium excretion mirrors serum calcium levels and is directly proportional to the filtered calcium load (Brown, 1991). Within the kidney, the TAL of Henle's loop is the main site for active divalent cation movement, mostly via the paracellular route, and is coupled to NaCl reabsorption (Friedman, 1998). The latter occurs through a concerted action of an apical $\text{Na}^+:\text{K}^+:2\text{Cl}^-$ cotransporter, NKCC2, and this is followed by basolateral exit via the voltage-gated Cl^- channel, chloride channel Kb, and the $\text{Na}^+:\text{K}^+:\text{ATPase}$. Overall, NaCl movement generates a favorable transepithelial electrochemical gradient for positively charged ions to move from the urine toward the basolateral side. In concert, the tight junctional proteins, claudins 14, 16, and 19, establish a divalent cation-selective permeable route, thereby allowing Ca_o^{2+} (and Mg^{2+}) reabsorption (Gong and Hou, 2014). The TAL has the highest CaSR expression, and here the CaSR is expressed basolaterally (Riccardi et al., 1998). In the event of hypercalcemia, CaSR activation dampens Ca_o^{2+} reabsorption in two ways: firstly, it inhibits NaCl reabsorption, hence the driving force for divalent cation movement; secondly, it directly reduces Ca_o^{2+} and Mg^{2+} junctional permeability through its actions on claudin 14 by activating microRNA-9 and -374 (Gong and Hou, 2014). If the hypercalcemic stimulus persists, hypercalciuria can occur with excess urinary calcium excretion in the terminal collecting duct.

In the presence of hypovolemia, the antidiuretic hormone, vasopressin, promotes water reabsorption through the insertion of aquaporin-2 water channels into the lumen of inner medullary collecting duct cells. However, excessive water reabsorption could lead to suprasaturating urinary calcium concentrations and attendant pathologic kidney stone formation, which could severely impair renal function. The CaSR is expressed at the luminal side of inner medullary collecting duct cells, where it monitors Ca_o^{2+} concentration in the urine (Sands et al., 1997). Thus, CaSR activation inhibits the tubular response to vasopressin by limiting the number of apical aquaporin-2 water-channel insertions (Procino et al., 2012). In addition, CaSR activation stimulates the activity of the proton pump, V-ATPase, thereby evoking urine acidification and reducing the risk of precipitation (Renkema et al., 2009).

Further, the kidney proximal tubule is a major site of PTH action that promotes a phosphaturia by inhibiting the activity of the $\text{Na}^+:\text{P}_i$ cotransporters, Npt2a, and Npt2c (Murer et al., 2001). Excess phosphate in the urine could also exacerbate the risk of calcium-phosphorus stone formation by the distal nephron. In the proximal tubule, a luminal CaSR blunts the phosphaturic action of PTH and promotes acid secretion via stimulation of the $\text{Na}^+:\text{H}^+$ exchanger, $\text{Na}^+:\text{H}^+$ exchanger 3 (Capasso et al., 2013). Thus, by monitoring both urine

and plasma composition, together with the integration of inputs deriving from urinary phosphate content, concentration, and acidification, the renal CaSR accomplishes divalent cation homeostasis while minimizing the risk of developing nephrolithiasis and nephrocalcinosis, which could arise as a consequence of enhanced urinary calcium excretion by the TAL (Hebert et al., 1997). The corollary is that altered CaSR expression or function due to CaSR mutations leads to FHH1, ADH1, or Bartter syndrome type V (see VI. *Calcium-Sensing Receptor-Related Genetic Diseases and Therapeutic Interventions*). In all circumstances, the aberrant calciuria is not the consequence of an impairment of renal function but rather the result of altered Ca_0^{2+} sensing by the CaSR in the parathyroid glands and the kidney. In the context of CKD, hyperphosphatemia caused by decreased renal phosphate excretion and acidosis may both elicit CaSR underactivation, leading to secondary hyperparathyroidism. Therefore, similarly to the parathyroid CaSR, the kidney CaSR is a drug target and, indeed, pharmacological CaSR PAMs are employed to rectify abnormal Ca_0^{2+} sensing by the kidney (Riccardi and Valenti, 2016). Furthermore, the use of NAMs for the treatment of nephrolithiasis and nephrocalcinosis could also be postulated (Riccardi and Valenti, 2016). Finally, it should be noted that CaSR PAMs increase urinary calcium excretion by means of their actions on both the parathyroid and kidney CaSR, and indeed, cinacalcet promotes calciuria in patients with secondary hyperparathyroidism, but this occurs in the absence of an increase in urine output (Courbebaisse et al., 2012). Given the clinical use of PAMs, the impact of their long-term use on urine production, acidification, and concentration, particularly in the context of kidney stone formation, remains to be fully understood (Riccardi and Valenti, 2016).

D. Calcium-Sensing Receptor in the Bone

The CaSR is expressed by several types of bone cells, including osteoblasts, osteocytes, osteoclasts, and some chondrocytes (Santa Maria et al., 2016). Although some controversies exist, there is good evidence that Ca_0^{2+} and the CaSR contribute to skeletal development and maintenance (Chang et al., 2008; Goltzman and Hendy, 2015; Hannan et al., 2018a) and that bone CaSRs may even contribute to overall Ca_0^{2+} homeostasis (Al-Dujaili et al., 2016). Elucidation of the CaSR's roles in skeletal tissue was historically complicated by models that examined global deletion of the *Casr* gene (Kos et al., 2003). Global *Casr* deletion has numerous effects, partly through large alterations in PTH secretion and changes in serum calcium and phosphate concentrations (Hannan et al., 2018a), thus it is difficult to elucidate tissue-specific CaSR effects. To further complicate matters, early *Casr* knockouts involved deletion of *Casr* exon 5, which results in mice encoding a nonfunctional CaSR lacking a portion of its extracellular domain (Kos

et al., 2003). When *Casr* exon 5-deleted mice were crossed with mice that had a deletion of *Gcm2* (which results in no parathyroid gland development) or the *Pth* gene, the skeletal abnormalities seen in the global *Casr* knockout mice were largely abolished (Kos et al., 2003; Tu et al., 2003). Furthermore, studies of the *Casr* exon 5-deleted mice revealed an alternatively spliced *Casr* transcript in the growth plate and other organs, such as skin, that could compensate for the absence of full-length CaSR in cartilage and bone (Rodriguez et al., 2005). Nonetheless, alternative *Casr* knockout models and bone-specific *Casr* deletion has confirmed that the CaSR is critical to bone development and maintenance, as described below.

1. *Osteoblast Calcium-Sensing Receptors.* Perhaps the clearest evidence for a role of the CaSR in skeletal development and maintenance comes from studies in which exon 7 of the *Casr* gene was deleted during different stages of osteoblast differentiation. *Casr* exon 7 deletion removes most of the 7TM and C-terminal tail, resulting in a nonfunctional receptor (Chang et al., 2008; Dvorak-Ewell et al., 2011). *Casr* exon 7 deletion was achieved by Cre-recombinase in osteoblasts under the control of the 2.3-kb *Col(I) α1* subunit promoter [2.3Col(I)-Cre], which is expressed in early- and late-stage cells of the osteoblast lineage (Chang et al., 2008); an *α1(I) collagen* promoter [Col 3.6-Cre], which is expressed throughout cells of the osteoblastic lineage (Dvorak-Ewell et al., 2011); or the *osterix* promoter, which is expressed in early osteoblasts (Chang et al., 2008). In studies using the collagen-Cre promoters, heterozygous *Casr* knockout mice grew relatively normally (Chang et al., 2008; Dvorak-Ewell et al., 2011). In contrast, homozygous *Casr* knockout using any of the Cre promoters resulted in severe bone defects (Chang et al., 2008; Dvorak-Ewell et al., 2011). There was marked reduction in the size of the knockout mice and their skeletons evident as early as 3 days after birth, and at day 20 the weight of the *Casr* knockout mice was only 30% that of controls (Chang et al., 2008; Dvorak-Ewell et al., 2011). Their skeletons were severely undermineralized, even in the skull, as well as in the vertebrae and long bones (Chang et al., 2008; Dvorak-Ewell et al., 2011). There was a marked reduction in bone volume in both the trabecular and cortical bones (Chang et al., 2008). Most of these mice died with multiple fractures by 3 to 4 weeks after birth (Chang et al., 2008; Dvorak-Ewell et al., 2011). Osteoblasts from *Casr* knockout mice were poorly differentiated, with both early and late differentiation markers markedly reduced, compared with controls (Chang et al., 2008; Dvorak-Ewell et al., 2011). mRNA levels of the local growth factor, insulin-like growth factor-1, were also substantially decreased, as were those of factors supporting cell survival, such as B-cell lymphoma 2 (*Bcl-2*) and *Bcl-2L1* (Chang et al., 2008). In contrast, mRNA for IL-10, an inducer of apoptosis in

many cell types, was increased, along with evidence of augmented apoptotic osteoblast and osteocyte numbers in sections from bone (Chang et al., 2008; Dvorak-Ewell et al., 2011). mRNA-encoding genes that inhibit mineralization, such as osteopontin, ankylosis protein, and nucleotide pyrophosphatase/phosphodiesterase 1 (NPP1), showed increased expression in the knockouts (Dvorak-Ewell et al., 2011). In addition to impaired osteoblastic differentiation and activity, deletion of *Casr* in early and late osteoblasts led to increased expression of mRNA for the bone resorption-promoting protein, receptor activator of nuclear factor- κ B ligand, together with a doubling of osteoclast numbers and activity, with bone loss in trabecular and cortical bone (Chang et al., 2008; Dvorak-Ewell et al., 2011). Whether these effects of osteoblast *Casr* knockout are entirely specific is not clear, since transplantation of vertebrae from 10-day-old wild-type or homozygous *Casr* knockout mice into athymic mice resulted in no differences in the volume or composition of transplanted bones, as assessed by microCT or histomorphometry after 4 weeks (Al-Dujaili et al., 2016). Furthermore, bones of transgenic mice that expressed a constitutively active mutant CaSR in late osteoblasts under the control of the osteocalcin promoter (Dvorak et al., 2007) also showed increased receptor activator of nuclear factor- κ B ligand expression and increased osteoclast activity, with resultant bone loss over the lifespan of the mice, but only in trabecular and not cortical bone (Dvorak et al., 2007). Other osteoblastic markers and function were largely unaffected, except for a slight decrease in bone-forming activity indicated by a small drop in mineral apposition rate (Dvorak et al., 2007). It is difficult to explain these observations in a comprehensive model of the CaSR's role in bone, despite attempts to propose age-related differences in the interactions between the CaSR and PTH/PTH1R in bone or the presence of mild hyperparathyroidism in the CaSR knockout models, but not in the mutant constitutively active CaSR mice (Dvorak et al., 2007; Goltzman and Hendy, 2015). Under conditions of expression of a constitutively active CaSR, however, normal feedback mechanisms would not function. The existing evidence indicates that Ca_o^{2+} and the CaSR, together with PTH/PTHrP and PTH1R, interact with one another in whole animals in ways that cannot easily be predicted (Goltzman and Hendy, 2015; Santa Maria et al., 2016; Yang and Wang, 2018).

2. Osteoclast Calcium-Sensing Receptors. Local regulatory pathways relevant to Ca_o^{2+} and the CaSR are likely to involve osteoclasts, which, along with bone marrow monocytes and macrophages, express the CaSR (House et al., 1997; Kameda et al., 1998; Diepenhorst et al., 2018). Activation of the CaSR in these cells with high concentrations of Ca_o^{2+} or Sr^{2+} inhibited osteoclast maturation and secretion of acid phosphatase, which is critical for bone resorption, and increased apoptosis of mature osteoclasts, all of which would suppress

bone resorption (Zaidi et al., 1991; Kameda et al., 1998; Kanatani et al., 1999; Mentaverri et al., 2006; Diepenhorst et al., 2018). Although high Ca_o^{2+} concentrations were required to activate these osteoclast responses, this might be relevant in vivo, since an acid environment, as present in resorption pits, increases Ca_o^{2+} potency at the CaSR (Quinn et al., 2004), and the Ca_o^{2+} concentration in bone resorption pits can be as high as 40 mM (Silver et al., 1988). There is some recent evidence that cinacalcet can inhibit the actions of osteoclasts (Diepenhorst et al., 2018), raising the possibility of CaSR activation in osteoclasts as a potential antiresorptive strategy in osteoporosis. However, another study found no effect of cinacalcet on osteoclast-mediated resorption (Shalhoub et al., 2003).

3. Osteoblast and Osteoclast Calcium-Sensing Receptors as Therapeutic Targets. Given the negative effects of CaSR deletion on bone mass and bone cell survival (Chang et al., 2008; Dvorak-Ewell et al., 2011; Santa Maria et al., 2016), it follows that there would be interest in targeting the CaSR in osteoblasts/osteocytes for a bone anabolic effect (Marie, 2010; Goltzman and Hendy, 2015; Diepenhorst et al., 2018). Indeed, there is evidence that Sr^{2+} , which displays higher potency than Ca_o^{2+} in osteoblasts (Brennan et al., 2009), increased bone mineral density and reduced fractures in the clinic (Reginster et al., 2005). Other receptors, including GPRC6A, may also mediate the effects of Sr^{2+} (Pi et al., 2005a; Rybchyn et al., 2009). Unfortunately, reported cardiovascular side effects of Sr^{2+} ranelate (marketed as Protelos/Osseor) narrowed the potential patient population so that this agent was withdrawn from the market. Nevertheless, preclinical studies showed Sr^{2+} reduced bone-resorbing signals and increased bone cell anabolism and survival under stress (Bonnelye et al., 2008; Brennan et al., 2009; Rybchyn et al., 2011). They also reported that Sr^{2+} stimulated the important bone anabolic Wnt pathway downstream of the CaSR and Akt phosphorylation in osteoblasts (Rybchyn et al., 2011). CaSR-dependent activation of the Wnt pathway in bone cells was in turn dependent on the formation of a complex involving CaSR, Homer1 (a long isoform of this scaffold protein), and mechanistic target of rapamycin complex-2, which phosphorylates Akt on Serine 475 (Rybchyn et al., 2019). These observations provide proof of principle that selective activation of the CaSR in osteoblasts might be a suitable strategy for osteoporosis therapies, either alone or in combination with other anabolic agents, such as intermittent PTH. Intermittent PTH has anabolic effects on bone but also stimulates osteoclast activity. Given that CaSR activation in osteoclasts suppresses bone resorption, as discussed above, and has anabolic effects on bone, the use of CaSR PAMs in conjunction with intermittent PTH may reduce the likelihood of hypercalcemia and enhance the bone anabolic effects of intermittent PTH. Indeed, administration of the PAM, NPS R-568, in combination with intermittent PTH in

mice reduced blood Ca_0^{2+} concentrations, increased trabecular bone, and increased cortical bone strength compared with intermittent PTH alone (Santa Maria et al., 2016). The effect of intermittent PTH on trabecular bone volume as a fraction of total bone volume was slightly but significantly blunted in mice in which the *Casr* gene was deleted in early and late osteoblasts (Al-Dujaili et al., 2016).

4. Chondrocyte Calcium-Sensing Receptors. High levels of CaSR protein are present in hypertrophic chondrocytes in the growth plate of long bones (Santa Maria et al., 2016). When mice with the loxP sites flanking exon 7 of the *Casr* were crossed with mice expressing the *Cre* transgene under the control of the *type II collagen $\alpha 1$ subunit* [Col(II)] promoter [Col(II)-Cre], which targets growth plate chondrocytes and other types of chondrocytes, they all died in utero at around E13 (Chang et al., 2008). Whether this was due to interference in heart valve development is unclear. When the Col(II)-Cre promoter was modified to a tamoxifen-inducible variant and 4-hydroxytamoxifen was given at E18-19, the resultant growth plate chondrocyte targeted *Casr* knockout and produced small, undermineralized skeletons, with expansion and reduced mineralization of the hypertrophic zone of the growth plate (Chang et al., 2008). Gene expression analysis confirmed reduced expression of terminal differentiation markers and reduced expression of insulin-like growth factor-1 and its receptor (Chang et al., 2008).

The CaSR is also expressed in articular cartilage chondrocytes, with increased expression reported in chondrocytes from osteoarthritic joints (Burton et al., 2005). Increased expression of the CaSR was also reported in cartilage endplate chondrocytes adjacent to degenerated intervertebral discs from human subjects along with high total calcium concentrations and low water content (Grant et al., 2016). Treatment of cartilage endplate chondrocytes in vitro with increasing Ca_0^{2+} resulted in lower accumulation of collagens I and II and aggrecan, whereas catabolic enzymes were increased, an effect that was abrogated by knockdown of the *CASR* (Grant et al., 2016). The authors proposed a role for increased Ca_0^{2+} and the CaSR in intervertebral disc degeneration (Grant et al., 2016). In a dental malocclusion model affecting the temporomandibular joint in rats, increased expression of CaSR in articular chondrocytes and in the endoplasmic reticulum of these cells was also observed (Zhang et al., 2019). These provided some evidence to support a role for endoplasmic reticulum expressed CaSR, as opposed to cell membrane CaSR in chondrocytes under stress (Zhang et al., 2019). Increased whole-cell CaSR and increased endoplasmic reticulum CaSR were also observed in vitro using articular cartilage chondrocytes exposed to shear stress (Zhang et al., 2019). Shear stress resulted in increased expression of chondrocyte terminal differentiation markers, such as alkaline

phosphatase, osteocalcin, and matrix metalloproteinase-13, which contributes to cartilage degradation. Critically, local CaSR knockdown or the use of the NAM, NPS 2143, reduced the shear stress-induced increases in terminal differentiation markers in chondrocytes in culture and reduced the severity of osteoarthritis in the temporomandibular joint of a rat model of dental malocclusion (Zhang et al., 2019). In contrast, injection of the PAM, cinacalcet, into the temporomandibular joint of these rats promoted thinning and loss of articular cartilage (Zhang et al., 2019). These studies in chondrocytes raise the possibility that the chondrocyte CaSR is a potential therapeutic target for prevention or management of joint degeneration.

E. Calcium-Sensing Receptor in Keratinocytes

The CaSR is highly expressed in keratinocytes, the main epidermal cell type. Moreover, an ionic calcium gradient exists in the epidermis, which increases from the basal proliferative layer to reach a maximum in the stratum granulosum, wherein the keratinocytes are well-differentiated, decreasing again in the relatively water-deficient lipid-containing cells of the stratum corneum (Menon et al., 1985; Celli et al., 2010). The epidermal calcium gradient and the CaSR are critically important for various epidermal functions, including keratinocyte differentiation, water and xenobiotic barrier function, and wound healing (Tu and Bikle, 2013; Hannan et al., 2018a). Interestingly, the epidermal calcium gradient is predominantly present in intracellular organelles of keratinocytes, such as the endoplasmic reticulum and Golgi, although an extracellular gradient makes some contribution to the gradient (Celli et al., 2010).

Keratinocytes cultured in low-calcium media (<0.07 mM) proliferate well. Raising the Ca_0^{2+} concentration above 0.1 mM promotes differentiation, as indicated by the appearance of E-cadherin/catenin complexes (adherens junctions) and desmosomes, upregulation of keratins 1 and 10, stratification of cells, and then formation of cornified envelope precursors (Braga et al., 1995). Disruption of the permeability barrier of the skin by tape stripping disrupts the epidermal calcium gradient, resulting in disorganization of the normally differentiated cell layers (Menon et al., 1994). When the calcium gradient is re-established over the next day or so, the permeability barrier also recovers. Skin diseases, such as psoriasis, characterized by abnormal barrier function also exhibit a loss of the calcium gradient (Menon and Elias, 1991).

CaSR expression increases in upper layers of the epidermis with the increase in differentiation, with high expression in the stratum granulosum but weak expression in the corneocytes (Komuves et al., 2002). There is some expression of the CaSR on the plasma membrane of keratinocytes, but its predominant localization in these cells is intracellular and in the

cytoplasm around the nucleus (Komuves et al., 2002). This perinuclear localization is also seen, although not to the same extent, in rodent osteoblasts and chondrocytes (Chang et al., 1999). It is likely that Ca_o^{2+} signals to the keratinocyte via the plasma membrane CaSR in a manner similar to that of more classic calcium targets, such as the parathyroid or kidney. The function of the intracellular CaSR is unclear at this time. Knockdown or inactivation of the CaSR in keratinocytes abrogates calcium-induced inhibition of proliferation and stimulation of differentiation of these cells (Tu et al., 2008). Not surprisingly, in mice in which there had been knockdown of the CaSR in the epidermis, skin barrier function was disrupted with impaired differentiation of keratinocytes, and these problems were exacerbated by a low-calcium diet (Tu et al., 2012). Keratinocytes from these epidermal *Casr*^{-/-} mice had blunted Ca_i^{2+} mobilization in response to Ca_o^{2+} , decreased Ca_i^{2+} pools, defective cell-cell adhesion, and reduced expression of differentiation markers (Tu et al., 2012).

In contrast, mice engineered to constitutively over-express the CaSR in basal keratinocytes displayed enhanced keratinocyte differentiation and barrier formation during development as well as accelerated hair growth at birth (Turksen and Troy, 2003). There was hypertrophy of the suprabasal keratinocyte layers with increased expression of early and late differentiation markers together with upregulation of epidermal growth factor and noncanonical Wnt-signaling pathways (Turksen and Troy, 2003).

In the epidermis, there are interactions between the CaSR and the vitamin D system in skin. The active vitamin D hormone, $1,25(\text{OH})_2\text{D}$, increases the calcium response in keratinocytes (Ratnam et al., 1999). Deletion of the epidermal CaSR reduces expression of both the vitamin D receptor (VDR) and CYP27B1, the enzyme that produces $1,25(\text{OH})_2\text{D}$ from 25-hydroxyvitamin D (Tu et al., 2012). It is likely that these effects on the vitamin D system contribute to impaired differentiation of the epidermis in these mice and reduced function of the innate immune system (Schauber et al., 2007). Moreover, $1,25(\text{OH})_2\text{D}$ increases transcription of the *CASR* (Canaff and Hendy, 2002).

It has previously been reported that wound healing is impaired in mice with epidermal deletion of the VDR (Oda et al., 2017). Very low dietary calcium or deletion of the *Casr* gene exacerbates this impairment in wound healing in epidermal VDR-deficient mice (Oda et al., 2017). There is a robust increase in Ca^{2+} in the bed of wounds within minutes of injury (Jungman et al., 2012), rapid increase in Ca_i^{2+} in cells near the site of the wound, and spreading to surrounding cells (Tsutsumi et al., 2013), with all of these indicating that the CaSR may play an important role in wound healing. The CaSR is coexpressed with E-cadherin at the cell membranes of migratory keratinocytes. Blockade of either the CaSR or E-cadherin inhibited keratinocyte proliferation and

migration after wound induction (Tu et al., 2019). Accordingly, the PAM, NPS R-568, accelerated wound healing in normal mice, potentially pointing to the epidermal CaSR as a therapeutic target to enhance repair of skin wound (Tu et al., 2019).

Mice with epidermal knockout of the VDR are more susceptible to UV- or chemically induced skin tumor formation (Zinser et al., 2002; Ellison et al., 2008). Neither the epidermal VDR knockout mice nor mice with epidermal *Casr* knockout develop skin tumors spontaneously, but mice null for both epidermal *Vdr* and *Casr* are reported to spontaneously develop squamous cell carcinomas (Bikle et al., 2015). In keratinocytes, stimulation of Wnt signaling results in β -catenin translocation to the nucleus and subsequent transcriptional activity, which may be important in skin tumorigenesis (Wei et al., 2007; Youssef et al., 2012). The VDR appears to suppress this β -catenin transcriptional activity in skin (Wei et al., 2007), in part by helping to keep β -catenin at the cell membrane as part of the E-cadherin/catenin complex (adherens junctions). As noted earlier, the CaSR is also important for the development of the E-cadherin/catenin complex, which helps to retain β -catenin at the cell membrane by promoting wound healing and differentiation of the skin cells (Oda et al., 2017) and inhibiting nuclear translocation and associated protumorigenic activities of β -catenin (Wei et al., 2007). This is in direct contrast with osteoblasts, wherein activation of the CaSR predominantly promotes β -catenin stabilization, subsequent β -catenin translocation to the nucleus, and increased transcriptional activity (Rybchyn et al., 2011). Some preliminary data indicate that both CaSR PAMs and NAMs enhance DNA repair after UV damage in cultured keratinocytes (Yang et al., 2016), although the mechanism and why PAMs and NAMs have a similar effect are unclear. How this observation fits with observed effects of CaSR knockdown in mice remains to be examined.

F. Calcium-Sensing Receptor in the Gastrointestinal Tract

The CaSR is expressed along the entire gastrointestinal tract in parietal and G cells of stomach gastric glands (Ray et al., 1997; Busque et al., 2005; Feng et al., 2010; Engelstoft et al., 2013), epithelial and enteroendocrine cells of the small and large intestine (Liou et al., 2011; Wang et al., 2011; Cheng et al., 2014; Alamshah et al., 2017), and neurons of the submucosal and myenteric plexuses of the enteric nervous system (ENS) (Geibel et al., 2006; Cheng, 2012; Tang et al., 2018). In the gastrointestinal tract, the CaSR functions as a nutrient sensor, binding not only Ca^{2+} , Mg^{2+} , and other cations but also L-amino acids and dipeptides and polypeptides (e.g., glutamyl dipeptides, poly-L-lysine). The CaSR is involved in regulation of gastric acid and hormone secretion, nutrient absorption, intestinal fluid

homeostasis, energy metabolism, cellular differentiation and proliferation, motility and enteric nerve activity, maintenance of gut microbiota, immune homeostasis, and intestinal inflammation (Dufner et al., 2005; Ceglia et al., 2009; Geibel and Hebert, 2009; Feng et al., 2010; Brennan et al., 2014; Cheng et al., 2014; Tang et al., 2016b, 2018; Alamshah et al., 2017; Sun et al., 2018).

The CaSR responds to alterations in nutrient levels by regulating hormone secretion from enteroendocrine cells (Geibel and Hebert, 2009; Liou et al., 2011; Wang et al., 2011; Alamshah et al., 2017; Liu et al., 2018). In global *Casr* knockout mice, gastric G cell number was significantly reduced, suggesting the CaSR regulates G cell growth. Further, in wild-type but not knockout mice, NPS 2143 inhibited gastrin secretion after gavage of Ca_0^{2+} , L-Phe, or cinacalcet (Feng et al., 2010). In rat whole-stomach preparations, ex vivo exposure to Ca_0^{2+} increased acid production in parietal cells by enhancing H^+ - K^+ -ATPase activity. These effects were potentiated by L- but not D-amino acids, implicating the CaSR (Busque et al., 2005). The function of the recently identified acid secretory protein, vacuolar H^+ -ATPase, in parietal cells is also dependent on CaSR activity (Kitay et al., 2018).

The amino acid-stimulated CaSR may influence appetite and satiety via stimulatory effects on satiety hormones, cholecystokinin (CCK), glucagon-like peptide 1 (GLP-1) and protein YY (PYY) (Alamshah et al., 2017), and/or inhibitory effects on the release of the appetite-stimulating hormone ghrelin (Engelstoft et al., 2013). In the mouse enteroendocrine cell line, STC-1, L-Phe increased PYY and GLP-1 secretion, an effect inhibited by NPS 2143, suggesting involvement of the CaSR (Alamshah et al., 2017). In a transgenic mouse model expressing a CCK promoter-driven enhanced GFP, the CaSR was enriched in CCK-producing duodenal I cells, in which L-Phe and cinacalcet induced Ca_i^{2+} changes and stimulated CCK in the presence of Ca_0^{2+} (Liou et al., 2011). L-Phe- and Trp-stimulated CCK secretion was inhibited by calhex 231 (Wang et al., 2011). In intestinal L-cells, the CaSR was involved in peptone-stimulated GLP-1 release (Pais et al., 2016), whereas in a ghrelinoma cell line, the CaSR partially mediated the L-Phe, L-Ala, and peptone-induced secretion of octanoyl ghrelin (Vancleef et al., 2015). In swine duodenum, ex vivo L-Trp perfusion induced secretion of CCK and glucose-dependent insulinotropic peptide and upregulated CaSR expression. This effect was inhibited by NPS 2143 (Zhao et al., 2018). To conclude that the Trp-induced gut-hormone secretion is mediated by the CaSR, further proof is needed. In mice and rats, L-Phe reduced short-time food intake and plasma ghrelin release, affecting the appetite of the animals (Alamshah et al., 2017). In minks, CaSR-mediated secretion of CCK and PYY led to emesis (Wu et al., 2017). These findings might explain why cinacalcet

and other CaSR PAMs cause gastrointestinal side effects (Block et al., 2017).

Specific knockout of intestinal epithelial cell *Casr* leads to epithelial cell hyperproliferation and changes in intestinal crypt structure driven by β -catenin signaling (Rey et al., 2012). Mice additionally have decreased intestinal transepithelial resistance and reduced levels of colonic tight-junction proteins, suggesting that the epithelial CaSR maintains intestinal barrier function (Cheng et al., 2014). The inadequate epithelial barrier function was associated with lower amounts of beneficial *Lactobacilli* bacteria and more *Deferribacteraceae* bacteria, which are linked to colitis (Cheng et al., 2014). This intestinal dysbiosis has been associated with more severe proinflammatory responses in the intestinal epithelium-specific *Casr* null mice compared with wild-type controls (Owen et al., 2016).

The amino acid-stimulated CaSR has recently been found to suppress intestinal inflammation in inflammatory bowel disease and other settings [reviews: (Owen et al., 2016; Sun et al., 2018)]. Inflammatory cytokine expression, including IL-1R, was higher in the distal colons of the CaSR knockout mice, in addition to a marked increase in nuclear factor- κ B-dependent genes (Cheng et al., 2014). These mice developed more severe colitis with delayed recovery than the CaSR-expressing littermates when challenged with dextrane sulfate sodium (DSS) (Cheng et al., 2014). In a mouse model of colitis, poly-L-lysine (commonly used as a food preservative) and glutamyl dipeptides reduced DSS-induced inflammation, whereas intravenous administration of NPS 2143 inhibited this effect (Mine and Zhang, 2015; Zhang et al., 2015). Dietary supplementation of Trp, L-Phe, and Tyr also reduced the expression of intestinal inflammatory markers in piglets after short-term induction of inflammation by lipopolysaccharides (Liu et al., 2018). However, a recent study found that the CaSR PAMs, cinacalcet and GSK3004774 (an intestine-specific modulator), did not reduce the inflammatory effects of DSS, whereas NPS 2143 ameliorated the DSS-induced symptoms and reduced immune cell infiltration (Elajnaf et al., 2019).

The anti-inflammatory effect of the CaSR was also shown in vitro in cell lines. In a colonic myofibroblast cell line, activation of the CaSR inhibited tumor necrosis factor- α secretion (Kelly et al., 2011) and increased expression of bone morphogenetic protein-2, which is a promoter of colonic epithelial barrier maturation (Peiris et al., 2007). In colon cancer cell lines, amino acids and dipeptides inhibited proinflammatory cytokine secretion, and the effect was reversed by NPS 2143 (Mine and Zhang, 2015; Zhang et al., 2015). Inflammatory cytokines, such as tumor necrosis factor- α , IL-1 β , and IL-6, increased the expression of the CaSR at the mRNA and protein level in some colon cancer cell lines (Fetahu et al., 2014), which could be a defense mechanism against inflammation in the intestines.

Bicarbonate (HCO_3^-) secretion in the colon is fine-tuned by the CaSR (Tang et al., 2015). However, it seems that the neurogenic secretory responses in the intestinal epithelium are mediated mainly by the CaSR expressed in the ENS and not in the epithelium (Geibel et al., 2006; Cheng, 2012; Tang et al., 2018). Because abnormalities of the ENS affect the severity of intestinal inflammation and contribute to the pathogenesis of inflammatory bowel disease (Margolis et al., 2011), the CaSR could be a potential therapeutic target.

Increased dietary intake of calcium reduces the risk of several cancers. The inverse correlation between calcium intake and risk of colorectal cancer has been known for decades, although the mechanisms driving the protective effect of calcium were not clear. There is some evidence that the CaSR is one of the central mediators of the antitumorigenic effects of calcium and acts as a tumor suppressor (Kállay et al., 1997, 2003; Yang et al., 2018). In colon cancer cells, activation of the CaSR increased differentiation and reduced proliferation, epithelial-to-mesenchymal transition, and expression of stem cell markers (Aggarwal et al., 2015, 2017). The signaling pathways involved in these processes still need to be determined. Interestingly, in the upper intestinal tract, it seems that the CaSR functions as an oncogene, as it promoted gastric cancer cell proliferation (Xie et al., 2017).

G. Calcium-Sensing Receptor in the Pancreas

The CaSR is expressed in pancreatic acinar cells (Bruce et al., 1999), which promote digestion via nutrient-stimulated release of digestive enzymes and fluid. The CaSR is also expressed in the pancreatic islets on glucagon-secreting α cells and insulin-secreting β cells (Babinsky et al., 2017). Thus, the CaSR may influence not only protein metabolism but also carbohydrate and fat metabolism.

Ca_o^{2+} is critical for pancreatic islet function and acts via voltage-gated Ca^{2+} channels to trigger the exocytosis of insulin- and glucagon-containing secretory granules from β - and α -cells, respectively (Rorsman et al., 2012). Ca_o^{2+} also activates the pancreatic islet CaSR, with ex vivo and in vitro studies demonstrating a role for the CaSR in mediating islet hormone secretion. Thus, stimulation of isolated human islets and an insulin-secreting mouse cell line (MIN6) with the PAM, NPS R-568, potentiated Ca_o^{2+} -mediated insulin secretion (Gray et al., 2006), whereas knockdown of the CaSR through RNA interference diminished glucose-induced insulin secretion in MIN6 cells that were cultured as pseudoislets (Kitsou-Mylona et al., 2008). Studies involving MIN6 pseudoislets have also revealed CaSR-stimulated insulin secretion to be mediated by PLC and the MAPK pathway (Gray et al., 2006). Furthermore, CaSR-mediated MAPK activation in MIN6 cells induces β -cell proliferation, thus highlighting a potential role for the CaSR in the regulation of β -cell mass (Kitsou-

Mylona et al., 2008). The CaSR also upregulates the expression of E-cadherin in MIN6 cells, which is associated with increased adherence between neighboring β -cells (Hills et al., 2012). Thus, the CaSR may facilitate cell-to-cell communication within an individual pancreatic islet to coordinate insulin secretion from β -cells (Hodgkin et al., 2008). In addition, the level of islet CaSR expression correlates with insulin secretion from isolated wild-type mouse pancreatic islets (Oh et al., 2016). Studies in wild-type mice have shown that islet CaSR expression increases with age, which may compensate for the insulin resistance in aged mice by increasing insulin secretion (Oh et al., 2016). Transient stimulation of isolated human islets with Ca_o^{2+} and NPS R-568 also promoted glucagon secretion, thereby indicating a role for the CaSR in α -cells (Gray et al., 2006). The intestinal CaSR, which is activated by dietary amino acids and peptides, may also influence pancreatic islet function by regulating the secretion of incretin hormones. In support of this, studies involving isolated mouse intestine have shown that the CaSR is expressed in GLP-1-secreting L-cells and also that oligopeptides enhance GLP-1 secretion by activation of the CaSR (Diakogiannaki et al., 2013).

The role of the CaSR in systemic glucose homeostasis has been investigated in studies involving human subjects. An association study reported a common coding-region *CASR* gene variant to be an independent determinant of plasma glucose concentrations in renal transplant recipients (Babinsky et al., 2015). However, another study involving patients with FHH1 (caused by germline loss-of-function *CASR* mutations) did not reveal any alterations in insulin secretion or glucose tolerance (Wolf et al., 2014). The effect of altered CaSR function on glucose tolerance has also been evaluated in an ADH1 mouse model, which is referred to as *Nuclear flecks* (*Nuf*) because the mutant mouse was initially identified to have nuclear cataracts (Babinsky et al., 2017). *Nuf* mice, which harbor a germline gain-of-function CaSR mutation (Leu723^{ICL2}Gln) causing hypocalcemia, have impaired glucose tolerance and hypoinsulinemia in association with reductions in pancreatic islet mass and β -cell proliferation (Babinsky et al., 2017). *Nuf* mice also lack glucose-mediated suppression of glucagon secretion, which was associated with an increase in α -cell proliferation and an impairment of α -cell membrane depolarization (Babinsky et al., 2017). Administration of the NAM, ronacalcet, ameliorated the hypocalcemia and glucose intolerance of *Nuf* mice, and these findings highlight the potential utility of targeted CaSR compounds for modulating glucose metabolism (Babinsky et al., 2017).

H. Calcium-Sensing Receptor in Mammary Glands

The CaSR is expressed in breast epithelial cells, in which its main role is to fine tune maternal Ca^{2+} metabolism by balancing Ca_o^{2+} mobilization and usage: It ensures the supply of Ca^{2+} for milk while protecting

against maternal hypocalcemia (Cheng et al., 1998; VanHouten et al., 2004, 2007; Kim and Wysolmerski, 2016). The expression of the CaSR is increased during lactation when it regulates Ca_o^{2+} transport into milk. In parallel, it inhibits synthesis of PTHrP by coupling with G_i to inhibit adenylyl cyclase activity and cAMP production (VanHouten et al., 2004). During milk production, the CaSR enables the lactating breast to participate in the regulation of systemic Ca_o^{2+} and bone metabolism. VanHouten and Wysolmerski (2013) suggested a negative feedback between systemic Ca_o^{2+} delivered to the lactating breast and PTHrP synthesis and secretion by mammary epithelial cells. When the mother's serum calcium level is adequate, the CaSR in breast epithelial cells stimulates calcium secretion into milk, but reduces Ca_o^{2+} usage when the mother's calcium supply becomes limited (VanHouten and Wysolmerski, 2013). In mammary epithelial cells, the CaSR regulates Ca_o^{2+} transport by altering the activity of the plasma membrane Ca^{2+} ATPase 2; however, the detailed molecular mechanism is not yet known (VanHouten et al., 2007; VanHouten and Wysolmerski, 2007).

The CaSR is expressed also in the neoplastic mammary gland. In contrast with normal breast cells, in breast cancer cells the CaSR stimulates PTHrP secretion. This is possible because the CaSR switches coupling from $G_{i/o}$ to G_s , leading to stimulation of cAMP and PTHrP synthesis (Mamillapalli et al., 2008). The higher PTHrP levels, secreted because of activation of the CaSR, inhibit the cell cycle inhibitor $p27^{\text{kip1}}$ and the apoptosis-inducing factor, stimulating cell proliferation and reducing apoptosis (Kim et al., 2016). Moreover, PTHrP is an activator of osteoclasts and often stimulates osteolytic bone destruction when secreted from cancer cells that metastasize to bone (Wysolmerski, 2012).

The CaSR is highly expressed by metastatic breast cancer cells and potentiates their osteolytic ability, promoting a more aggressive behavior. In vitro, the CaSR promoted breast cancer cell migration only in cells capable of forming bone metastases (e.g., MDA-MB-231, MCF-7) but not in BT474 cells that have no bone-metastatic potential, even though CaSR levels were similar in all cell types (Saidak et al., 2009). It has been shown recently that breast cancer cells overexpressing the wild-type CaSR injected intratibially into BALB/c-Nude mice led to osteolytic lesions through an epiregulin-mediated mechanism (Boudot et al., 2017). The oncogenic potential of the CaSR in breast cancer cells was also suggested by the fact that activation of the CaSR by NPS R-568 or Ca_o^{2+} increased secretion of proangiogenic and chemotactic cytokines and growth factors from the highly invasive MDA-MB-231 breast cancer cells (Hernández-Bedolla et al., 2015). Another group, however, found that activating the CaSR with Ca_o^{2+} induced sensitivity of MCF-7 and MDA-MB-435 cells to the chemotherapeutic drug

paclitaxel and reduced malignant behavior. The paclitaxel-resistant cells expressed no CaSR (Liu et al., 2009). This group suggested a positive link between the tumor suppressive functions of BRCA1 and the CaSR (Promkan et al., 2011).

I. Calcium-Sensing Receptor in Airway Smooth Muscle and Epithelium

Asthma is characterized by airway hyperresponsiveness, inflammation, and remodeling of the conducting airways. A number of mechanisms, many driven by inflammation, have been hypothesized to contribute to airway hyperresponsiveness and/or remodeling. Among these, local increases of polycations are seen in the airways of patients who are asthmatic (Kurosawa et al., 1992) and, vice versa, increased inflammation increases the local concentration of polycations. Furthermore, the polycations, eosinophil cationic protein and major basic protein, are markers for asthma severity and stability. Elevated arginase activity increases the consumption of L-arginine to enhance production of the polycations, spermine, spermidine, and putrescine (North et al., 2013). Indeed, arginase inhibitors have been proposed to have therapeutic potential for allergic asthma (van den Berg et al., 2018). Recent evidence suggests that the CaSR is expressed in the airway epithelium, smooth muscle, and inflammatory cells and that polycations act at the CaSR and are directly implicated in the pathogenesis of asthma (Yarova et al., 2015). Yarova et al. (2015) have also shown that inhaled CaSR NAMs delivered topically reverse airway hyperresponsiveness, inflammation, and remodeling in in vivo models of allergic asthma and other inflammatory lung diseases, such as chronic obstructive pulmonary disease. Inhaled NAMs also show efficacy in nonallergic asthma, which is often associated with poor response to steroids, and for which currently there is no treatment (Riccardi unpublished observations). Four CaSR NAMs have been studied in humans; NPSP795, ronacaleret, AXT914, and JTT-305 (*IIB. Endogenous and Exogenous Allosteric Modulators*, Table 1), which could be repurposed, via the inhaled route, as novel asthma treatments. Crucially, delivery of CaSR NAMs directly to the lung does not significantly affect serum calcium levels up to 24 hours post-treatment, suggesting absence of any significant systemic overspill and possible effects on whole-body mineral ion homeostasis in vivo. Thus, CaSR NAMs could provide a new therapeutic approach to treating inflammatory lung disease in humans.

J. Calcium-Sensing Receptor in the Vasculature

The CaSR is expressed in the intima, media, and adventitia of the blood vessels in endothelial, smooth muscle cells and in the perivascular neurons. Although consumption of dietary calcium reduces blood pressure (Nakamura et al., 2019; Rietsema et al., 2019), direct actions of Ca_o^{2+} on isolated blood vessels have yielded

contrasting effects, with both relaxation and constriction reported (Bohr, 1963). Furthermore, the molecular mechanisms underlying these actions are elusive. Studies carried out over the last two decades indicate that the CaSR could mediate at least some of the effects of Ca_o^{2+} on vascular function, with opposing effects in the endothelium and smooth muscle cell layers of the blood vessels. Specifically, CaSR activation by the CaSR PAM, cinacalcet, in the vascular endothelium leads to hyperpolarization and attendant nitric oxide release and vasodilatation (Smajilovic et al., 2007). In contrast, studies of *Casr* gene ablation in the vascular smooth muscle cells show that activation of the CaSR in these cells leads to contraction, as evidenced by the fact that *Casr* knockout mice exhibit impaired vascular reactivity, hypotension, and reduced contractile response to Ca_o^{2+} (Schepelmann et al., 2016). Thus, the CaSR sets blood vessel tone by integrating prorelaxing (endothelium-mediated) actions with procontractile (smooth muscle-mediated) effects (Schepelmann et al., 2016). Therefore, altered CaSR expression within either the endothelium or smooth muscle could account for the abnormal vascular reactivity seen in advanced CKD or in type 2 diabetes. Indeed, systemic administration of the CaSR PAM, NPS R-568, initially evokes an increase in blood pressure in control and in uremic rats (a model for advanced CKD), which is followed by a reduction in blood pressure, but only in uremic animals (Odenwald et al., 2006), suggesting partial loss of the CaSR-dependent contractile component of the vasculature.

However, there is some controversy regarding the role of the CaSR in regulating blood pressure. In ex vivo studies in rat mesenteric arteries, relaxant responses to cinacalcet and calindol were not blocked by calhex 231 (Thakore and Ho, 2011), which at the time was believed to be a CaSR NAM (but has now been shown to have mixed PAM and NAM activity, see *II.B. Endogenous and Exogenous Allosteric Modulators*). Nonetheless, Ca_o^{2+} influx into these vessels stimulated by the $\alpha 1$ adrenergic receptor agonist, methoxamine, was inhibited, not potentiated, by cinacalcet and calindol, as were contractions in response to an L-type calcium channel activator (Thakore and Ho, 2011). Given that arylalkylamine PAMs are structurally related to the nonselective calcium channel blocker, fendiline, and have low affinity for calcium channels, the relaxing effects of CaSR PAMs in arteries may in part arise from off-target calcium channel effects. Further support for a non-CaSR-mediated effect of PAMs in the vasculature comes from findings that, although the *S*-enantiomers of arylalkylamine PAMs have little activity at the CaSR, the effects of NPS R-568 on vascular tone, blood pressure, and heart rate are not stereoselective and only occur at concentrations in excess of those required to inhibit PTH secretion (Nakagawa et al., 2009).

End-stage CKD is associated with impaired mineral ion metabolism, which can lead to pathologic vascular calcification of the medial layer of the blood vessel, left ventricular hypertrophy, and increased cardiovascular mortality (Locatelli et al., 2002). CaSR expression is significantly reduced in the medial layer of calcifying blood vessels and is completely absent in areas of extensive medial calcification, suggesting an involvement of the CaSR in the vascular calcification process (Alam et al., 2009). Human and bovine vascular smooth muscle cells exposed to Ca^{2+} and phosphate concentrations mimicking those seen during pathologic CKD exhibit marked calcification in vitro, an effect that is exacerbated by CaSR downregulation and that is reversed by the CaSR PAM, NPS R-568 (Alam et al., 2009). In addition, NPS R-568 reduces blood pressure and ameliorates cardiac remodeling in animal models of advanced CKD in vivo (Ogata et al., 2003). Taken together, these results suggest that loss of CaSR expression by the medial layer of the blood vessels in advanced CKD leads to vascular calcification and that CaSR PAMs might be vasculo-protective by directly restoring normal CaSR expression levels within the vasculature. However, CaSR PAMs reduce systemic levels of serum P_i and PTH through their actions on the parathyroid CaSR, and parathyroidectomy suppresses vascular calcification (Kawata et al., 2008); therefore, PAM-mediated reduction of vascular calcification may be dependent on activation of parathyroid CaSRs. Although in vitro and in vivo studies support a direct role for the vascular CaSR in protecting vascular function, human observational studies of clinical evaluation of the CaSR PAM, cinacalcet, assessed by the Evaluation of Cinacalcet Hydrochloride Therapy to Lower Cardiovascular Events randomized controlled trial failed to reach its endpoints (reduction of all-cause and cardiovascular mortality in patients with advanced CKD) (Chertow et al., 2012). However, a recent Bayesian meta-analysis combined with a systematic literature review concluded that once subject ages and high drop-out rates throughout the trial are accounted for, cinacalcet treatment does reduce mortality rates in patients with secondary hyperparathyroidism on hemodialysis (Lozano-Ortega et al., 2018). Therefore, further clinical studies are needed to fully evaluate the effects of CaSR PAMs on cardiovascular and all-cause mortality in patients with advanced CKD.

Finally, it should be pointed out that the CaSR is also expressed in arterial smooth muscle cells of the pulmonary vasculature, wherein receptor activation leads to pulmonary vasoconstriction and proliferation. Here, CaSR NAMs prevent the development and progression of pulmonary hypertension in mouse and rat models in vivo (Tang et al., 2016a). Thus, targeting the CaSR in the pulmonary arteries with inhaled NAMs might provide a novel treatment of patients with idiopathic pulmonary hypertension.

K. Calcium-Sensing Receptor in the Brain and Nervous System

For a comprehensive review of all evidence for CaSR function in the brain, readers are directed to a recent review (Giudice et al., 2019).

Although the role of the CaSR in human brain requires validation, the CaSR is expressed throughout the rat brain, with particular abundance in the hippocampus, striatum, cerebellum, pituitary, and olfactory bulb (Ruat et al., 1995). However, CaSR expression can change with developmental age (Vizard et al., 2008), which supports a role for the CaSR in brain development. For instance, rat CaSR expression increases in fetal oligodendrocyte precursor cells and postnatal immature oligodendrocytes during myelination of nerve axons, but expression declines in mature oligodendrocytes (Chattopadhyay et al., 1998, 2008; Ferry et al., 2000).

Although hyperparathyroidism and consequent early lethality resulting from global *Casr* ablation preclude determination of the role of the CaSR in brain development, concomitant *Casr* and *PTH* ablation prevents hyperparathyroidism, and mice survive to adulthood (Kos et al., 2003). In brains of *Casr*^{-/-}/*Pth*^{-/-} mice, neuron and glial cell differentiation markers were reduced after birth, whereas differentiation of neural stem cells from *Casr*^{-/-} mice was delayed (Liu et al., 2013). These mice also had reduced numbers of gonadotropin-releasing hormone-positive neurons in the hypothalamus. These findings suggest a role for the CaSR in neuron and glial cell differentiation.

To elucidate region-specific CaSR brain functions, hippocampus-specific *Casr* ablation 3 weeks postbirth has been undertaken (Kim et al., 2014). Although mice did not display an obvious phenotype under normal conditions, they were protected from hippocampal neuronal damage in response to ischemia-induced injury, which mimics injury sustained during cardiac arrest or stroke. In line with these findings, hypoxia increases CaSR expression in rat hippocampal neurons in vivo and in vitro (Bai et al., 2015), but neuroprotection from ischemia is blocked when the related class C GPCR, GABA_BR1, is also ablated (Kim et al., 2014). This may be explained by the discovery of an increase in CaSR expression in hippocampal neurons in culture upon suppression of GABA_BR1 levels (Chang et al., 2007), which is also observed in cortical neurons of mice who have experienced controlled cortical impact as a model for traumatic brain injury (Kim et al., 2013). In support of a role for the CaSR in the hippocampus, in rat hippocampal neurons from wild-type but not *Casr*^{-/-} mice, CaSR activation opens nonselective cation channels (Ye et al., 1997b). Similarly, transfection of a dominant negative Arg185Gln mutant CaSR into pyramidal neurons of hippocampal brain slice cultures resulted in significantly shorter and less

complex dendritic branching (Vizard et al., 2008). Taken together, these studies suggest that CaSR NAMs could be neuroprotective.

In addition to neuroprotective effects upon brain injury, inhibition of brain CaSRs may afford neuroprotection in Alzheimer disease. The first evidence for a possible role of the CaSR in Alzheimer disease came from a study that demonstrated activation of nonselective cation channels in cultured hippocampal pyramidal neurons from wild-type rats and mice but not from *Casr*^{-/-} mice (Ye et al., 1997a). Although additional studies have since suggested β -amyloid proteins activate the CaSR (Conley et al., 2009; Dal Pra et al., 2014), these findings warrant further validation. Nonetheless, CaSR expression is increased in the hippocampus of an Alzheimer disease mouse model (Gardenal et al., 2017), and there is a positive association between CaSR SNPs and Alzheimer disease, although this is only in patients who do not harbor the Alzheimer risk allele encoding apolipoprotein E4 (Conley et al., 2009). Furthermore, in human cortical astrocytes and neurons in culture, neurotoxic β -amyloid_{25–35} stimulates full-length β -amyloid₄₂ secretion, an effect that is blocked by the CaSR NAM, NPS 2143 (Armato et al., 2013; Chiarini et al., 2017b). NPS 2143 also blocked β -amyloid_{25–35}-mediated GSK-3 β activation and subsequent phosphorylation of τ in cultured human astrocytes (Chiarini et al., 2017a).

The CaSR has also been implicated in the etiology of neuroblastomas, tumors originating from precursor nerve cells of the sympathetic nervous system. Approximately 98% of neuroblastomas are associated with spontaneous mutations in a variety of genes (Aygun, 2018). Analysis of mRNA from neuroblastoma tumors indicates that although the CaSR is expressed in benign differentiated tumors, epigenetic hypermethylation of the *CASR* P2 promoter region silences *CASR* transcription in some aggressive neuroblastomas (de Torres et al., 2009; Casalà et al., 2013). Similarly, two non-coding *CASR* SNPs (rs7652579 and rs1501899) that reduce CaSR expression are present in homozygous or heterozygous form in 58% of neuroblastoma tumors but in only 47% of the general population (Masvidal et al., 2017). In a subset of ganglioneuromas, *CASR* expression was absent in four out of six tumors harboring rs7652579 and rs1501899. However, neuroblastoma patients with rs7652579 and rs1501899 SNPs did not have poorer outcomes or survival (Masvidal et al., 2017). In contrast, neuroblastomas with a haplotype SNP in the *CASR* gene-coding region were associated with poorer outcomes, including increased risk of death (Masvidal et al., 2013). Importantly, cinacalcet reduced neuroblastoma tumor growth in immunocompromised mice carrying neuroblastoma xenografts by inducing endoplasmic reticulum stress, tumor differentiation, and fibrosis as well as upregulation of cancer-testis antigens (Rodríguez-Hernández et al., 2016).

Finally, approximately 40% of patients harboring ADH1 gain-of-function *CASR* mutations present with

seizures (Gorvin, 2019). Although this could be associated with the consequent reduction in serum calcium concentrations, a gain-of-expression CaSR mutation, Arg898Gln, was identified in a patient with idiopathic epilepsy who did not have low serum concentrations of calcium or PTH (Kapoor et al., 2008). These findings suggest a possible role for CaSR in neurotransmission, which is supported by numerous in vitro studies suggesting the CaSR regulates synaptic transmission and neuronal activity via activation of nonselective cation channels on presynaptic terminals [reviewed in Jones and Smith (2016)].

VI. Calcium-Sensing Receptor-Related Genetic Diseases and Therapeutic Interventions

A. Loss- and Gain-of-Function Mutations in the Calcium-Sensing Receptor and Its Signaling Partners

Alterations in CaSR signaling, which lead to derangements of mineral homeostasis, can result from loss-of-function germline mutations of the *CASR* gene on chromosome 3q21.1, which cause FHH1 and NSHPT, or gain-of-function germline *CASR* mutations, which lead to ADH1 and Bartter syndrome type V (Fig. 4; Table 2) (Hannan et al., 2012; Hannan and Thakker, 2013). In addition, loss- and gain-of-function germline mutations of the *GNA11* gene on chromosome 19p13.3, which encodes $G\alpha_{11}$, are associated with FHH2 and ADH2, respectively (Table 2) (Nesbit et al., 2013a; Hannan et al., 2016). Furthermore, loss-of-function germline mutations of the *AP2S1* gene on chromosome 19q13.3, which encodes AP2 σ , cause FHH3 (Table 2) (Nesbit et al., 2013b; Hannan et al., 2015a).

B. Familial Hypocalciuric Hypercalcemia and Neonatal Severe Hyperparathyroidism

FHH is a genetically heterogeneous autosomal dominant disorder characterized by lifelong nonprogressive elevations of serum calcium concentrations, mild hypermagnesemia, normal or mildly raised serum PTH concentrations, and low urinary calcium excretion (Table 2) (Hannan and Thakker, 2013). FHH1 (OMIM 145980) accounts for ~65% of all FHH cases and is usually an asymptomatic disorder. It has been associated with >150 different *CASR* mutations (Hannan et al., 2018a). The majority (>85%) of these loss-of-function *CASR* mutations are heterozygous missense substitutions, which are predominantly located in the VFT of the CaSR ECD and also in the 7TM (Hannan et al., 2012). These FHH1-associated missense mutations cause a loss of function by diminishing the signaling responses of CaSR-expressing cells (Leach et al., 2012) or by reducing CaSR anterograde trafficking and cell surface expression (Huang and Breitwieser, 2007; White et al., 2009). In addition, FHH1-causing missense mutations may induce biased agonism by switching from a wild-type CaSR that preferentially

increases Ca_i^{2+} mobilization to mutant receptors that demonstrate equal preference for Ca_i^{2+} and MAPK pathways or that preferentially act via MAPK (Leach et al., 2012, 2013; Leach and Gregory, 2017). Between 10% and 15% of FHH1 cases are caused by heterozygous deletion, insertion, nonsense, and splice-site mutations that lead to nonsense-mediated decay of mRNA and CaSR haploinsufficiency or truncate the CaSR protein (Hannan et al., 2012). The offspring of two parents with FHH1 can harbor biallelic loss-of-function *CASR* mutations that cause NSHPT (OMIM 239200), which is associated with marked hyperparathyroidism that leads to hypercalcemia and bone demineralization causing fractures and respiratory distress (Hannan and Thakker, 2013). Occasionally, biallelic loss-of-function *CASR* mutations can lead to primary hyperparathyroidism, which presents in adulthood (Table 2) (Hannan et al., 2010). Furthermore, some heterozygous mutations (e.g., Arg185Gln) can cause NSHPT due to dominant negative effects on the wild-type CaSR (Bai et al., 1997).

FHH2 (OMIM 145981) is the least common form of FHH and has been reported in four probands to date (Nesbit et al., 2013a; Gorvin et al., 2016, 2018b). FHH2 appears to have a mild clinical presentation with serum-adjusted total calcium concentrations usually between 2.55 and 2.80 mM (normal range 2.10–2.55 mM). Urinary calcium excretion may be normal or low (Table 2) (Nesbit et al., 2013a; Gorvin et al., 2016, 2018b). The *GNA11* mutations reported in FHH2 probands consist of three missense substitutions (Thr54Met, Leu135Gln, Phe220Ser) and an in-frame isoleucine deletion (Ile200del) (Nesbit et al., 2013a; Gorvin et al., 2016, 2018b). All of these mutations impair CaSR-signaling responses and are located within key domains of the $G\alpha_{11}$ protein (Nesbit et al., 2013a; Gorvin et al., 2016, 2018b). Thus, the Ile200del and Phe220Ser mutations are located within the $G\alpha_{11}$ GTPase domain and are predicted to diminish the interaction of $G\alpha_{11}$ with the CaSR or PLC, respectively (Nesbit et al., 2013a; Gorvin et al., 2018b). In contrast, the Leu135Gln mutation is situated within the PLC-interacting portion of the $G\alpha_{11}$ helical domain, and the Thr54Met mutation is located at the interface between the helical and GTPase domains and may potentially affect GTP binding (Nesbit et al., 2013a; Gorvin et al., 2016).

FHH3 (OMIM 600740) has been reported in >60 FHH probands and has a more marked clinical phenotype than FHH1. Thus, FHH3 is associated with significant elevations of serum calcium and magnesium and also a significantly reduced urinary calcium excretion compared with FHH1 (Table 2) (Hannan et al., 2015a; Vargas-Poussou et al., 2016). In addition, hypercalcemic symptoms, low bone mineral density, and alterations in cognitive function have been described in some patients with FHH3 (McMurtry et al., 1992;

Fig. 4. CaSR snakeplot with residues linked to loss- and gain-of-function germline mutations. Snakeplot of the CaSR showing the location of the ECD, 7TM, ICLs, ECLs, and carboxy terminus. Sites of loss- and gain-of-function germline mutations causing FHH1/NSHPT (red), ADH1/Bartter syndrome type V (green), or both FHH1/NSHPT and ADH1/Bartter syndrome type V (yellow), respectively. Snakeplot generated by GPCRdb (Munk et al., 2016) with data from the Human Gene Mutation Database (Stenson et al., 2012). C-term, C terminal; N-term, N terminal.

TABLE 2
Calcitropic disorders caused by germline *CASR*, *GNA11*, and *AP2S1* mutations

Gene Mutation and Disease	Genotype	Serum Calcium	Serum PTH	Urine Calcium
<i>CASR</i> mutations				
Loss-of-function				
FHH1	Heterozygous ^a	High	Normal or high	Low
NSHPT	Heterozygous, compound heterozygous, or homozygous	High	High	Normal, low or high
Primary hyperparathyroidism (PHPT) ^b	Heterozygous or homozygous	High	High	Normal, low or high
Gain-of-function				
ADH1	Heterozygous ^a	Low	Normal or low	Normal, low or high
Bartter syndrome type V	Heterozygous	Low	Low	High
<i>GNA11</i> mutations				
Loss-of-function				
FHH2	Heterozygous	High	Normal or high	Normal or low
Gain-of-function				
ADH2	Heterozygous	Low	Normal or low	Normal or low
<i>AP2S1</i> mutations				
Loss-of-function				
FHH3	Heterozygous	High	Normal or high	Low

^aMay occasionally be caused by homozygous *CASR* mutations (Lietman et al., 2009; Cavaco et al., 2018).

^b*CASR* mutations are a rare cause of primary hyperparathyroidism.

Hannan et al., 2015a). Nearly all FHH3 cases are caused by a missense mutation of the AP2 σ Arg15 residue (Arg15Cys, Arg15His, or Arg15Leu) (Fujisawa et al., 2013; Nesbit et al., 2013b; Hendy et al., 2014; Hannan et al., 2015a; Howles et al., 2016; Vargas-Poussou et al., 2016; Hovden et al., 2017). In addition, a genotype-phenotype correlation has been observed at the AP2 σ Arg15 residue with the Arg15Leu mutation being associated with significant increases in serum calcium and an earlier age of presentation compared with patients harboring the Arg15Cys or Arg15His AP2 σ mutations (Hannan et al., 2015a; Hovden et al., 2017). The AP2 σ subunit forms part of the heterotetrameric AP2 complex, which is involved in clathrin-mediated endocytosis (Kelly et al., 2008), and the FHH3-causing AP2 σ Arg15 mutations have been shown to reduce CaSR endocytosis and impair endosomal signaling from the internalized CaSR (Gorvin et al., 2018c). However, given the role of AP2 in clathrin-mediated endocytosis, it remains to be established whether phenotypic observations, such as cognitive deficits in FHH3, are attributable to CaSR dysregulation or potentially due to alterations in the endocytosis of other plasma membrane proteins.

C. Autosomal Dominant Hypocalcemia and Bartter Syndrome Type V

ADH is comprised of two genetically distinct variants, designated ADH1 and 2, which are caused by germline gain-of-function mutations of the CaSR and G α_{11} , respectively (Table 2) (Hannan et al., 2016). ADH1 (OMIM 601198) is characterized by mild-to-moderate hypocalcemia in association with mild hypomagnesemia, hyperphosphatemia, and serum PTH concentrations that are

usually detectable but within the lower half of the reference range (Nesbit et al., 2013a). Patients with ADH1 have significantly increased urinary calcium excretion compared with patients with hypoparathyroid (Yamamoto et al., 2000), and ~10% of patients with ADH1 have an absolute hypercalciuria (Nesbit et al., 2013a). Some patients with ADH1 may have ectopic calcifications and/or elevations in bone mineral density (Pearce et al., 1996), and patients with a severe gain-of-function CaSR mutation may also develop a Bartter syndrome (referred to as Bartter syndrome type V) (Table 2), which is characterized by renal salt wasting leading to volume depletion, hyper-reninemic hyperaldosteronism, and hypokalemic alkalosis (Watanabe et al., 2002). Over 90 different ADH1-causing CaSR mutations have been reported (Hannan and Thakker, 2013; Hannan et al., 2016), and around 95% of these are heterozygous missense substitutions, whereas ~5% are in-frame or frameshift insertion or deletion mutations (Hannan et al., 2012). ADH1 mutations cluster within the second loop of the VFT domain (residues 116–136), which contributes to the dimeric CaSR interface (Geng et al., 2016) (Fig. 4). A second ADH1 mutational hotspot is located in a region that encompasses transmembrane helices 6 and 7 and the intervening third ECL of the CaSR (residues 819–837) (Hannan et al., 2016). This transmembrane region may participate in a network of interactions with other transmembrane helices (Dore et al., 2014), thereby causing the CaSR to adopt an inactive conformational state.

ADH2 (OMIM 615361) (Table 2) has been reported in seven probands (Mannstadt et al., 2013; Nesbit et al., 2013a; Li et al., 2014a; Piret et al., 2016; Tenhola et al., 2016). Patients with ADH2 generally have mild-to-moderate hypocalcemia, in keeping with the serum

biochemical phenotype of ADH1 (Hannan et al., 2016). However, ADH2 is associated with a milder urinary phenotype, with significantly reduced urinary calcium excretion compared with ADH1 (Li et al., 2014a). Moreover, short stature caused by postnatal growth insufficiency has been reported in two ADH2 kindreds (Li et al., 2014a; Tenhola et al., 2016). ADH2-causing mutations all comprise missense substitutions (Arg60Cys, Arg60Leu, Arg181Gln, Ser211Trp, Val340Met, and Phe341Leu), which enhance CaSR-mediated signaling responses, consistent with a gain of function (Nesbit et al., 2013a; Li et al., 2014a; Piret et al., 2016). ADH2-causing mutations cluster at the interface between the $G\alpha_{11}$ helical and GTPase domains (Piret et al., 2016) and may enhance the exchange of GDP and GTP, thereby leading to G protein activation. ADH2 mutations also affect the C-terminal portion of the $G\alpha_{11}$ protein, which facilitates G protein–GPCR coupling (Piret et al., 2016).

D. Animal Models of Genetic Diseases

Mouse models for FHH, NSHPT, and ADH have been generated using gene knockout and knock-in techniques and also by using chemical mutagenesis (Piret and Thakker, 2011).

1. Familial Hypocalciuric Hypercalcemia/Neonatal Severe Hyperparathyroidism Mouse Models. A mouse model lacking the CaSR was generated by replacing part of exon 5 with a neomycin resistance gene (Ho et al., 1995). Mice harboring this germline heterozygous CaSR deletion ($CaSR^{+/-}$) had mild hypercalcemia and hypocalciuria, similar to patients with FHH1, whereas mice with a homozygous CaSR deletion ($CaSR^{-/-}$) had a phenotype resembling NSHPT with parathyroid hyperplasia, severe hypercalcemia, bone abnormalities, and retarded growth (Ho et al., 1995). The $CaSR^{-/-}$ mice died within the first 30 days of life (Ho et al., 1995), which was attributed to severe hyperparathyroidism. In support of this, correction of the hyperparathyroidism by the additional germline ablation of the *Pth* or *Gcm2* genes rescued the early lethality and bone demineralization in $CaSR^{-/-}$ mice (Kos et al., 2003; Tu et al., 2003). The importance of the parathyroid CaSR in the pathogenesis of NSHPT has been further highlighted by mice harboring a parathyroid-specific ablation of the CaSR, which developed severe hypercalcemia and hyperparathyroidism (Chang et al., 2008; Fan et al., 2018). In contrast, mice with a kidney-specific ablation of the CaSR do not have alterations in serum calcium or PTH but are hypocalciuric, and these findings support an independent role of the kidney CaSR in the regulation of urinary calcium excretion (Toka et al., 2012).

A mouse model for FHH2 has been generated by chemical mutagenesis using the *N*-ethyl-*N*-nitrosourea-alkylating agent (Howles et al., 2017). The mutant mice harbor a germline loss-of-function *Gna11* mutation, Asp195Gly (D195G) (Howles et al., 2017). Heterozygous ($Gna11^{+/195G}$)

mice have mild hypercalcemia and normal plasma PTH concentrations (Howles et al., 2017). Homozygous ($Gna11^{195G/195G}$) mice have significantly increased plasma calcium and PTH concentrations compared with $Gna11^{+/195G}$ and wild-type mice (Howles et al., 2017). However, $Gna11^{195G/195G}$ mice do not have growth retardation, bone demineralization, or early lethality to suggest an NSHPT phenotype (Howles et al., 2017). Thus, these studies indicate that the loss-of-function D195G $G\alpha_{11}$ mutation is associated with a mild calcitropic phenotype. Furthermore, the $Gna11^{+/195G}$ and $Gna11^{195G/195G}$ mice have no alterations in urinary calcium excretion (Howles et al., 2017), which suggests that $G\alpha_{11}$ may not play a major role in the renal handling of calcium.

2. Autosomal Dominant Hypocalcemia Mouse Models. Three different ADH1 mouse models have been reported (Hough et al., 2004; Dong et al., 2015). *Nuf* mice (described in *V.G. Calcium-Sensing Receptor in the Pancreas*) were generated by chemical mutagenesis using the isopropyl methane sulfonate-alkylating agent (Hough et al., 2004). Heterozygous and homozygous *Nuf* mice have hypocalcemia, hyperphosphatemia, reduced plasma PTH concentrations, and ectopic calcifications caused by a germline gain-of-function CaSR mutation, Leu723Gln (Hough et al., 2004). Two knock-in mouse models, which harbor ADH1-causing germline Cys129Ser or Ala843Glu gain-of-function CaSR mutations, have also been generated (Dong et al., 2015). Homozygous mutant knock-in mice exhibited embryonic or perinatal lethality, whereas heterozygous knock-in mice have hypocalcemia, hyperphosphatemia, reduced plasma PTH, hypercalciuria, and renal calcifications, consistent with the phenotype of patients with ADH1 (Dong et al., 2015).

Two mouse models for ADH2 have been described (Gorvin et al., 2017; Roszko et al., 2017). One mouse model, which is known as *Dark skin 7*, was generated by *N*-ethyl-*N*-nitrosourea chemical mutagenesis (Gorvin et al., 2017) and harbors a germline gain-of-function $G\alpha_{11}$ mutation, Ile62Val, whereas the other ADH2 mouse model was generated by CRISPR-Cas9 gene editing and harbors a human ADH2-causing germline $G\alpha_{11}$ mutation, Arg60Cys (Roszko et al., 2017). Both of these ADH2 mouse models have hypocalcemia, hyperphosphatemia, reduced plasma PTH, and normocalciuria in association with increased skin pigmentation (Gorvin et al., 2017; Roszko et al., 2017).

E. Therapeutic Interventions—Successes and Failures

CaSR PAMs represent a targeted therapy for symptomatic forms of FHH (Hannan et al., 2018b) and potentiate the signaling responses of cells expressing FHH-associated CaSR, $G\alpha_{11}$, or AP2 σ mutant proteins in vitro (Table 3) (Rus et al., 2008; Leach et al., 2013; Babinsky et al., 2016; Howles et al., 2016; Gorvin et al., 2018b). Furthermore, cinacalcet treatment is effective

TABLE 3

Summary of key studies assessing effectiveness of PAMs and NAMs for FHH, NSHPT, and ADH

Adapted from Hannan FM, Olesen MK, Thakker RV. Calcimimetic and calcilytic therapies for inherited disorders of the calcium-sensing receptor–signaling pathway. *Br J Pharmacol* (2018) 175:4083–4094.

Disorder	In Vitro Studies	In Vivo Studies
Hypercalcemic disorders		
FHH1/NSHPT	NPS R-568 and cinacalcet enhance the signaling responses and cell surface expression of loss-of-function FHH1/NSHPT-causing CaSR mutants (Rus et al., 2008; Leach et al., 2013)	Cinacalcet lowers serum calcium and PTH concentrations and improves hypercalcemic symptoms in patients with FHH1 (Alon and VandeVoorde, 2010; Rasmussen et al., 2011; Sethi et al., 2017) Cinacalcet lowers serum calcium and PTH concentrations in patients with NSHPT harboring a heterozygous Arg185Gln <i>CASR</i> mutation (Reh et al., 2011; Gannon et al., 2014; Fisher et al., 2015) but is less effective for NSHPT caused by biallelic truncating <i>CASR</i> mutations (García Soblechero et al., 2013; Atay et al., 2014)
FHH2	Cinacalcet enhances the signaling responses of cells expressing loss-of-function FHH2-causing $G\alpha_{11}$ mutants (Babinsky et al., 2016)	Cinacalcet lowers serum calcium and PTH concentrations in a mouse model for FHH2 (Howles et al., 2017) and also normalizes serum calcium concentrations in a patient with FHH2 (Gorvin et al., 2018b)
FHH3	Cinacalcet enhances the signaling responses of cells expressing loss-of-function FHH3-causing Arg15Cys, Arg15His, or Arg15Leu <i>AP2σ</i> mutants (Howles et al., 2016)	Cinacalcet lowers serum calcium and PTH concentrations and improves hypercalcemic symptoms in patients with FHH3 with Arg15Cys, Arg15His, or Arg15Leu <i>AP2S1</i> mutations (Howles et al., 2016)
Hypocalcemic disorders		
ADH1	NPS 2143 reduces the signaling responses of cells expressing gain-of-function ADH1-causing CaSR mutants but has limited efficacy for constitutively active CaSR mutants (Letz et al., 2010; Leach et al., 2013) ATF936 and AXT914 rectify the gain of function caused by constitutively active CaSR mutants (Letz et al., 2014)	Acute administration of NPS 2143 and JTT-305/MK-5442 increases serum calcium and PTH concentrations in mouse models for ADH1 (Dong et al., 2015; Hannan et al., 2015b) Administration of JTT-305/MK-5442 over 12 wk reduces urinary calcium excretion and prevents nephrocalcinosis in mouse models for ADH1 (Dong et al., 2015) Intravenous infusion of NPSP795 increases serum PTH concentrations and reduces urinary calcium excretion in patients with ADH1 (Roberts et al., 2019)
ADH2	NPS 2143 reduces the signaling responses of cells expressing gain-of-function ADH2-causing $G\alpha_{11}$ mutants (Babinsky et al., 2016; Gorvin et al., 2017; Roszko et al., 2017)	NPS 2143 increases serum calcium and PTH concentrations in mouse models for ADH2 (Gorvin et al., 2017; Roszko et al., 2017)

at decreasing serum calcium concentrations in patients with FHH1 and has been reported to improve hypercalcemic symptoms occasionally associated with FHH1, such as anorexia, polydipsia, and constipation (Table 3) (Alon and VandeVoorde, 2010; Rasmussen et al., 2011; Sethi et al., 2017). However, the response of NSHPT to cinacalcet is variable and appears to depend on the underlying *CASR* mutation. Indeed, cinacalcet rectifies the hypercalcemia and hyperparathyroidism in patients with NSHPT harboring a heterozygous Arg185Gln CaSR mutation (Reh et al., 2011; Gannon et al., 2014; Fisher et al., 2015) but is less effective for patients with NSHPT with biallelic truncating *CASR* mutations (Table 3) (García Soblechero et al., 2013; Atay et al., 2014), which would be a consequence of the truncated mutant receptor being unable to bind cinacalcet or couple with intracellular signaling proteins (Hannan et al., 2018b). Cinacalcet has also rectified the hypercalcemia in a mouse model for FHH2 (Howles et al., 2017) and ameliorated the hypercalcemia in a patient with symptomatic FHH2 (Table 3) (Gorvin et al., 2018b). Furthermore, cinacalcet is an effective therapy for symptomatic hypercalcemia caused by all three types of FHH3-causing Arg15 *AP2 σ* mutations (Table 3) (Howles et al., 2016). However, hypocalcemic symptoms have occurred in a cinacalcet-treated child

affected by FHH3 and the chromosome 22q11.2 deletion syndrome (Tenhola et al., 2015). Thus, long-term surveillance is required to detect hypocalcemia in cinacalcet-treated patients with FHH (Howles et al., 2016).
CaSR NAMs have been evaluated as a potential targeted therapy for ADH. In vitro studies have demonstrated that NPS 2143 normalizes the signaling responses associated with gain-of-function *CASR* and *GNA11* mutations, which cause ADH1 and ADH2, respectively (Table 3) (Letz et al., 2010; Leach et al., 2013; Hannan et al., 2015b; Babinsky et al., 2016). However, NPS 2143 is less effective for gain-of-function mutations causing Bartter syndrome type V (Letz et al., 2010; Leach et al., 2013). In contrast, the quinazolinone-derived NAMs rectify gain-of-function *CASR* mutations that cause Bartter syndrome V (Table 3) (Letz et al., 2014). CaSR NAMs have also been characterized in vivo, and single-dose studies have demonstrated that NPS 2143 significantly increases circulating concentrations of calcium and PTH in ADH1 and ADH2 mouse models (Table 3) (Hannan et al., 2015b; Gorvin et al., 2017; Roszko et al., 2017). In addition, repetitive dosing studies have shown that the NAM, JTT-305/MK-5442, prevents the occurrence of nephrocalcinosis in mouse models of ADH1 (Dong et al., 2015). Furthermore, the

NAM, NPSP795, has been administered to five ADH1 patients in a phase IIa clinical trial and increased plasma PTH concentrations and reduced urinary calcium excretion (Table 3) (Roberts et al., 2019). However, circulating calcium concentrations were not altered in these patients, and the optimal dosing regimen of NPSP795 for ADH remains to be established.

VII. Conclusions and Perspective

The CaSR is a highly complex GPCR, as evidenced by its widespread tissue expression and varied physiological roles, its capacity to respond to multiple stimuli that act via numerous binding sites, and the ability of different stimuli to bias CaSR signaling toward distinct subsets of G protein-dependent and independent signaling pathways. The existence of multiple allosterically linked binding sites for endogenous CaSR ligands demonstrates how allostery is fundamental to CaSR activity. It is therefore unsurprising that the CaSR was the first GPCR for which an allosteric therapeutic, cinacalcet, was FDA-approved. The clinical success of cinacalcet in treating various forms of hyperparathyroidism highlights the potential of targeting the CaSR with allosteric drugs. Given the many fundamental roles of the CaSR, the CaSR is a putative therapeutic target for numerous diseases beyond Ca_o^{2+} homeostasis, including asthma, diabetes, and cancer. Thus, drug discovery efforts at the CaSR will no doubt continue.

In addition to the aforementioned CaSR (patho) physiology, ongoing research is expanding the known roles of this receptor. Analysis of *CASR* SNPs supports associations between CaSR expression or activity and the risk of kidney stones (Vezzoli et al., 2011), vascular calcification (Babinsky et al., 2015), breast cancer (Li et al., 2014b; Wang et al., 2017), psoriasis (Zuo et al., 2015), and serum glucose concentrations (Babinsky et al., 2015). Furthermore, the sensitivity of the CaSR to amino acids and other stimuli raises the possibility that Ca_o^{2+} is not solely responsible for CaSR-mediated Ca_o^{2+} homeostasis. Indeed, high dietary protein intake modestly increases bone density at some sites and reduces hospital stay after fracture (Dawson-Hughes, 2003; Shams-White et al., 2017). In contrast, low-protein diets induce secondary hyperparathyroidism (Kerstetter et al., 2000; Dubois-Ferrière et al., 2011) and acute increases in L-amino acids suppress PTH secretion and potentiate Ca_o^{2+} -mediated Ca_i^{2+} mobilization in human parathyroid cells (Conigrave et al., 2004). Thus, the CaSR could couple protein metabolism to changes in Ca_o^{2+} homeostasis (Conigrave et al., 2002, 2008).

Although novel analytical methods, such as the operational model of allostery, have facilitated quantification of CaSR drug actions, CaSR drug discovery still suffers from limited tools to directly probe drug binding (e.g., commercially available radioligands or

fluorescently labeled ligands) and from the lack of 7TM and full-length CaSR structures for structure-based drug discovery. Furthermore, given the critical importance of Ca_o^{2+} homeostasis to human health, novel drugs that target the CaSR outside the parathyroid glands and kidney must have limited on-target effects in these tissues (e.g., by delivery of the drug regiospecifically to the targeted tissue). Alternatively, biased signaling has the potential to revolutionize our ability to target GPCRs in a tissue-specific manner by directing receptor signaling toward desirable pathways that mediate therapeutic effects at the expense of pathways linked to unwanted effects. Furthermore, major advances in GPCR structural biology resulting in 7TM and full-length structures of the class C GPCRs, mGlu_1 , and mGlu_5 (Dore et al., 2014; Wu et al., 2014; Koehl et al., 2019) provide confidence for forthcoming CaSR structural biology efforts. Thus, the future holds much promise for the design of novel drugs that target the CaSR.

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Wrote or contributed to the writing of the manuscript: Leach, Hannan, Josephs, Keller, Möller, Ward, Kallay, Mason, Thakker, Riccardi, Conigrave, Bräuner-Osborne.

References

- Aggarwal A, Prinz-Wohlgenannt M, Gröschel C, Tennakoon S, Meshcheryakova A, Chang W, Brown EM, Mechtcheriakova D, and Kállay E (2015) The calcium-sensing receptor suppresses epithelial-to-mesenchymal transition and stem cell-like phenotype in the colon. *Mol Cancer* **14**:61.
- Aggarwal A, Schulz H, Manhardt T, Bilban M, Thakker RV, and Kallay E (2017) Expression profiling of colorectal cancer cells reveals inhibition of DNA replication licensing by extracellular calcium. *Biochim Biophys Acta Mol Cell Res* **1864**: 987–996.
- Alam MU, Kirton JP, Wilkinson FL, Towers E, Sinha S, Rouhi M, Vizard TN, Sage AP, Martin D, Ward DT, et al. (2009) Calcification is associated with loss of functional calcium-sensing receptor in vascular smooth muscle cells. *Cardiovasc Res* **81**:260–268.
- Alamshah A, Spreckley E, Norton M, Kinsey-Jones JS, Amin A, Ramgulum A, Cao Y, Johnson R, Saleh K, Akalestou E, et al. (2017) l-phenylalanine modulates gut hormone release and glucose tolerance, and suppresses food intake through the calcium-sensing receptor in rodents. *Int J Obes* **41**:1693–1701.
- Al-Dujaili SA, Koh AJ, Dang M, Mi X, Chang W, Ma PX, and McCauley LK (2016) Calcium sensing receptor function supports osteoblast survival and acts as a co-factor in PTH anabolic actions in bone. *J Cell Biochem* **117**:1556–1567.
- Alexander SPH, Christopoulos A, Davenport AP, Kelly E, Marrion NV, Peters JA, Faccenda E, Harding SD, Pawson AJ, Sharman JL, et al.; CGTP Collaborators (2017) The concise guide to pharmacology 2017/18: G protein-coupled receptors. *Br J Pharmacol* **174** (Suppl 1):S17–S129.
- Alexander ST, Hunter T, Walter S, Dong J, Maclean D, Baruch A, Subramanian R, and Tomlinson JE (2015) Critical cysteine residues in both the calcium-sensing receptor and the allosteric activator AMG 416 underlie the mechanism of action. *Mol Pharmacol* **88**:853–865.
- Alon US and VandeVoorde RG (2010) Beneficial effect of cinacalcet in a child with familial hypocalciuric hypercalcemia. *Pediatr Nephrol* **25**:1747–1750.
- Amino Y, Nakazawa M, Kaneko M, Miyaki T, Miyamura N, Maruyama Y, and Eto Y (2016) Structure-CaSR-activity relation of kokumi γ -glutamyl peptides. *Chem Pharm Bull (Tokyo)* **64**:1181–1189.
- Areay BJ, Seethala R, Ma Z, Fura A, Morin J, Swartz J, Vyas V, Yang W, Dickson JK Jr, and Feyen JH (2005) A novel calcium-sensing receptor antagonist transiently stimulates parathyroid hormone secretion in vivo. *Endocrinology* **146**:2015–2022.
- Armato U, Chiarini A, Chakravarthy B, Chioffi F, Pacchiana R, Colarusso E, Whitfield JF, and Dal Prà I (2013) Calcium-sensing receptor antagonist (calcilytic) NPS 2143 specifically blocks the increased secretion of endogenous Aβ42 prompted by exogenous

- fibrillary or soluble A β 25-35 in human cortical astrocytes and neurons-therapeutic relevance to Alzheimer's disease. *Biochim Biophys Acta* **1832**:1634–1652.
- Atay Z, Bereket A, Haliloglu B, Abali S, Ozdogan T, Altuncu E, Canaff L, Vilaça T, Wong BY, Cole DE, et al. (2014) Novel homozygous inactivating mutation of the calcium-sensing receptor gene (CASR) in neonatal severe hyperparathyroidism-lack of effect of cinacalcet. *Bone* **64**:102–107.
- Avlani VA, Ma W, Mun H-C, Leach K, Delbridge L, Christopoulos A, and Conigrave AD (2013) Calcium-sensing receptor-dependent activation of CREB phosphorylation in HEK293 cells and human parathyroid cells. *Am J Physiol Endocrinol Metab* **304**:E1097–E1104.
- Aygun N (2018) Biological and genetic features of neuroblastoma and their clinical importance. *Curr Pediatr Rev* **14**:73–90.
- Babinsky VN, Hannan FM, Gorvin CM, Howles SA, Nesbit MA, Rust N, Hanyaloglu AC, Hu J, Spiegel AM, and Thakker RV (2016) Allosteric modulation of the calcium-sensing receptor rectifies signaling abnormalities associated with G-protein α -11 mutations causing hypercalcemic and hypocalcemic disorders. *J Biol Chem* **291**:10876–10885.
- Babinsky VN, Hannan FM, Ramracheya RD, Zhang Q, Nesbit MA, Huggill A, Bentley L, Hough TA, Joynton E, Stewart M, et al. (2017) Mutant mice with calcium-sensing receptor activation have hyperglycemia that is rectified by calcilytic therapy. *Endocrinology* **158**:2486–2502.
- Babinsky VN, Hannan FM, Youhanna SC, Maréchal C, Jadoul M, Devuyt O, and Thakker RV (2015) Association studies of calcium-sensing receptor (CaSR) polymorphisms with serum concentrations of glucose and phosphate, and vascular calcification in renal transplant recipients. *PLoS One* **10**:e0119459.
- Bai M, Pearce SH, Kifor O, Trivedi S, Stauffer UG, Thakker RV, Brown EM, and Steinmann B (1997) In vivo and in vitro characterization of neonatal hyperparathyroidism resulting from a de novo, heterozygous mutation in the Ca $^{2+}$ -sensing receptor gene: normal maternal calcium homeostasis as a cause of secondary hyperparathyroidism in familial benign hypocalciuric hypercalcemia. *J Clin Invest* **99**:88–96.
- Bai M, Trivedi S, and Brown EM (1998a) Dimerization of the extracellular calcium-sensing receptor (CaR) on the cell surface of CaR-transfected HEK293 cells. *J Biol Chem* **273**:23605–23610.
- Bai M, Trivedi S, Lane CR, Yang Y, Quinn SJ, and Brown EM (1998b) Protein kinase C phosphorylation of threonine at position 888 in Ca $^{2+}$ -sensing receptor (CaR) inhibits coupling to Ca $^{2+}$ store release. *J Biol Chem* **273**:21267–21275.
- Bai S, Mao M, Tian L, Yu Y, Zeng J, Ouyang K, Yu L, Li L, Wang D, Deng X, et al. (2015) Calcium sensing receptor mediated the excessive generation of β -amyloid peptide induced by hypoxia in vivo and in vitro. *Biochem Biophys Res Commun* **459**:568–573.
- Ballesteros JA and Weinstein H (1995) Integrated methods for the construction of three-dimensional models and computational probing of structure-function relations in G protein-coupled receptors, in *Methods in Neurosciences* (Stuart CS ed) pp 366–428, Academic Press, San Diego, CA.
- Bikle D, Bräuner-Osborne H, Brown EM, Chang W, Conigrave A, Hannan F, Leach K, Riccardi D, Shoback D, Ward DT, et al. (2019) Calcium-sensing receptor (version 2019.4) in the IUPHAR/BPS Guide to Pharmacology Database. *IUPHAR/BPS Guide to Pharmacology CITE* **2019** DOI: 10.2218/gtopdb/F12/2019.4.
- Bikle DD, Oda Y, Tu CL, and Jiang Y (2015) Novel mechanisms for the vitamin D receptor (VDR) in the skin and in skin cancer. *J Steroid Biochem Mol Biol* **148**: 47–61.
- Binnahfouz LS, Centeno PP, Conigrave AD, and Ward DT (2019) Identification of serine-875 as an inhibitory phosphorylation site in the calcium-sensing receptor. *Mol Pharmacol* **96**:204–211.
- Bjarnadóttir TK, Fredriksson R, and Schiöth HB (2005) The gene repertoire and the common evolutionary history of glutamate, pheromone (V2R), taste(1) and other related G protein-coupled receptors. *Gene* **362**:70–84.
- Block GA, Bushinsky DA, Cheng S, Cunningham J, Dehmel B, Drueke TB, Ketteler M, Kewalramani R, Martin KJ, Moe SM, et al. (2017) Effect of etelcalcetide vs cinacalcet on serum parathyroid hormone in patients receiving hemodialysis with secondary hyperparathyroidism: a randomized clinical trial. *JAMA* **317**:156–164.
- Bohr DF (1963) Vascular smooth muscle: dual effect of calcium. *Science* **139**:597–599.
- Bonnelye E, Chabadel A, Saltel F, and Jurdic P (2008) Dual effect of strontium ranelate: stimulation of osteoblast differentiation and inhibition of osteoclast formation and resorption in vitro. *Bone* **42**:129–138.
- Boudot C, Hénaut L, Thiem U, Geraci S, Galante M, Saldanha P, Saidak Z, Six I, Clézardin P, Kamel S, et al. (2017) Overexpression of a functional calcium-sensing receptor dramatically increases osteolytic potential of MDA-MB-231 cells in a mouse model of bone metastasis through epiregulin-mediated osteoprotegerin downregulation. *Oncotarget* **8**:56460–56472.
- Bradbury RA, Sunn KL, Crossley M, Bai M, Brown EM, Delbridge L, and Conigrave AD (1998) Expression of the parathyroid Ca $^{2+}$ -sensing receptor in cytotrophoblasts from human term placenta. *J Endocrinol* **156**:425–430.
- Braga VM, Hodiava KJ, and Watt FM (1995) Calcium-induced changes in distribution and solubility of cadherins, integrins and their associated cytoplasmic proteins in human keratinocytes. *Cell Adhes Commun* **3**:201–215.
- Bräuner-Osborne H, Jensen AA, Sheppard PO, O'Hara P, and Krogsgaard-Larsen P (1999) The agonist-binding domain of the calcium-sensing receptor is located at the amino-terminal domain. *J Biol Chem* **274**:18382–18386.
- Breitwieser GE (2013) The calcium sensing receptor life cycle: trafficking, cell surface expression, and degradation. *Best Pract Res Clin Endocrinol Metab* **27**:303–313.
- Brennan SC, Davies TS, Schepelmann M, and Riccardi D (2014) Emerging roles of the extracellular calcium-sensing receptor in nutrient sensing: control of taste modulation and intestinal hormone secretion. *Br J Nutr* **111** (Suppl 1):S16–S22.
- Brennan TC, Rybchyn MS, Green W, Atwa S, Conigrave AD, and Mason RS (2009) Osteoblasts play key roles in the mechanisms of action of strontium ranelate. *Br J Pharmacol* **157**:1291–1300.
- Brini M, Ottoloni D, Cali T, and Carafoli E (2013) Calcium in health and disease, in *Interrelations between Essential Metal Ions and Human Diseases Metal Ions in Life Sciences* (Sigel A, Sigel H, and Sigel RKO eds) pp 81–137, Springer, Dordrecht, Netherlands.
- Broadhead GK, Mun HC, Avlani VA, Jourdon O, Church WB, Christopoulos A, Delbridge L, and Conigrave AD (2011) Allosteric modulation of the calcium-sensing receptor by γ -glutamyl peptides: inhibition of PTH secretion, suppression of intracellular cAMP levels, and a common mechanism of action with L-amino acids. *J Biol Chem* **286**:8786–8797.
- Brown E, Enyedi P, LeBoff M, Rotberg J, Preston J, and Chen C (1987a) High extracellular Ca $^{2+}$ and Mg $^{2+}$ stimulate accumulation of inositol phosphates in bovine parathyroid cells. *FEBS Lett* **218**:113–118.
- Brown EM (1983) Four-parameter model of the sigmoidal relationship between parathyroid hormone release and extracellular calcium concentration in normal and abnormal parathyroid tissue. *J Clin Endocrinol Metab* **56**:572–581.
- Brown EM (1991) Extracellular Ca $^{2+}$ sensing, regulation of parathyroid cell function, and role of Ca $^{2+}$ and other ions as extracellular (first) messengers. *Physiol Rev* **71**: 371–411.
- Brown EM (2013) Role of the calcium-sensing receptor in extracellular calcium homeostasis. *Best Pract Res Clin Endocrinol Metab* **27**:333–343.
- Brown EM, Butters R, Katz C, Kifor O, and Fuleihan GE (1992) A comparison of the effects of concanavalin-A and tetradecanoylphorbol acetate on the modulation of parathyroid function by extracellular calcium and neomycin in dispersed bovine parathyroid cells. *Endocrinology* **130**:3143–3151.
- Brown EM, Chen CJ, Fuleihan GE, and Kifor O (1990) A comparison of the effects of divalent and trivalent cations on parathyroid hormone release, 3',5'-cyclic-adenosine monophosphate accumulation, and the levels of inositol phosphates in bovine parathyroid cells [published correction appears in *Endocrinology* 1992 131: 862]. *Endocrinology* **127**:1064–1071.
- Brown EM, Gamba G, Riccardi D, Lombardi M, Butters R, Kifor O, Sun A, Hediger MA, Lyttton J, and Hebert SC (1993) Cloning and characterization of an extracellular Ca $^{2+}$ -sensing receptor from bovine parathyroid. *Nature* **366**:575–580.
- Brown EM and MacLeod RJ (2001) Extracellular calcium sensing and extracellular calcium signaling. *Physiol Rev* **81**:239–297.
- Brown EM, Redgrave J, and Thatcher J (1984) Effect of the phorbol ester TPA on PTH secretion. Evidence for a role for protein kinase C in the control of PTH release. *FEBS Lett* **175**:72–75.
- Brown EM, Watson EJ, Thatcher JG, Koletsky R, Dawson-Hughes BF, Posillico JT, and Shoback DM (1987b) Ouabain and low extracellular potassium inhibit PTH secretion from bovine parathyroid cells by a mechanism that does not involve increases in the cytosolic calcium concentration. *Metabolism* **36**:36–42.
- Bruce JJ, Yang X, Ferguson CJ, Elliott AC, Steward MC, Case RM, and Riccardi D (1999) Molecular and functional identification of a Ca $^{2+}$ (polyvalent cation)-sensing receptor in rat pancreas. *J Biol Chem* **274**:20561–20568.
- Bu L, Michino M, Wolf RM, and Brooks CL III (2008) Improved model building and assessment of the Calcium-sensing receptor transmembrane domain. *Proteins* **71**: 215–226.
- Burton DW, Foster M, Johnson KA, Hiramoto M, Deftos LJ, and Terkeltaub R (2005) Chondrocyte calcium-sensing receptor expression is up-regulated in early Guinea pig knee osteoarthritis and modulates PTHrP, MMP-13, and TIMP-3 expression. *Osteoarthritis Cartilage* **13**:395–404.
- Busque SM, Kerstetter JE, Geibel JP, and Insogna K (2005) L-type amino acids stimulate gastric acid secretion by activation of the calcium-sensing receptor in parietal cells. *Am J Physiol Gastrointest Liver Physiol* **289**:G664–G669.
- Butters RR Jr, Chattopadhyay N, Nielsen P, Smith CP, Mithal A, Kifor O, Bai M, Quinn S, Goldsmith P, Hurwitz S, et al. (1997) Cloning and characterization of a calcium-sensing receptor from the hypercalcemic New Zealand white rabbit reveals unaltered responsiveness to extracellular calcium. *J Bone Miner Res* **12**: 568–579.
- Campion KL, McCormick WD, Warwicker J, Khayat ME, Atkinson-Dell R, Steward MC, Delbridge LW, Mun HC, Conigrave AD, and Ward DT (2015) Pathophysiologic changes in extracellular pH modulate parathyroid calcium-sensing receptor activity and secretion via a histidine-independent mechanism. *J Am Soc Nephrol* **26**: 2163–2171.
- Canaff L and Hundy GN (2002) Human calcium-sensing receptor gene. Vitamin D response elements in promoters P1 and P2 confer transcriptional responsiveness to 1,25-dihydroxyvitamin D. *J Biol Chem* **277**:30337–30350.
- Canaff L, Zhou X, Mosesova I, Cole DE, and Hundy GN (2009) Glial cells missing-2 (GCM2) transactivates the calcium-sensing receptor gene: effect of a dominant-negative GCM2 mutant associated with autosomal dominant hypoparathyroidism. *Hum Mutat* **30**:85–92.
- Capasso G, Geibel PJ, Damiano S, Jaeger P, Richards WG, and Geibel JP (2013) The calcium sensing receptor modulates fluid reabsorption and acid secretion in the proximal tubule. *Kidney Int* **84**:277–284.
- Casalà C, Gil-Guñón E, Ordóñez JL, Miguel-Queralt S, Rodríguez E, Galván P, Lavarino C, Munell F, de Alava E, Mora J, et al. (2013) The calcium-sensing receptor is silenced by genetic and epigenetic mechanisms in unfavorable neuroblastomas and its reactivation induces ERK1/2-dependent apoptosis. *Carcinogenesis* **34**:268–276.
- Casti A, Orlandini G, Reali N, Bacciotini F, Vanelli M, and Bernasconi S (1982) Pattern of blood polyamines in healthy subjects from infancy to the adult age. *J Endocrinol Invest* **5**:263–266.
- Cavaco BM, Canaff L, Nolin-Lapalme A, Vieira M, Silva TN, Saramago A, Domingues R, Rutter MM, Hudon J, Gleason JL, et al. (2018) Homozygous calcium-sensing receptor polymorphism R544Q presents as hypocalcemic hypoparathyroidism. *J Clin Endocrinol Metab* **103**:2879–2888.
- Ceglia L, Harris SS, Rasmussen HM, and Dawson-Hughes B (2009) Activation of the calcium sensing receptor stimulates gastrin and gastric acid secretion in healthy participants. *Osteoporos Int* **20**:71–78.
- Celli A, Sanchez S, Behne M, Hazlett T, Gratton E, and Mauro T (2010) The epidermal Ca $^{2+}$ gradient: measurement using the phasor representation of fluorescent lifetime imaging. *Biophys J* **98**:911–921.

- Centeno PP, Herberger A, Mun H-C, Tu C, Nemeth EF, Chang W, Conigrave AD, and Ward DT (2019) Phosphate acts directly on the calcium-sensing receptor to stimulate parathyroid hormone secretion. *Nat Commun* **10**:4693.
- Chang W, Pratt S, Chen TH, Bourguignon L, and Shoback D (2001) Amino acids in the cytoplasmic C terminus of the parathyroid Ca^{2+} -sensing receptor mediate efficient cell-surface expression and phospholipase C activation. *J Biol Chem* **276**: 44129–44136.
- Chang W, Tu C, Chen TH, Bikle D, and Shoback D (2008) The extracellular calcium-sensing receptor (CaSR) is a critical modulator of skeletal development. *Sci Signal* **1**:ra1.
- Chang W, Tu C, Chen TH, Komuves L, Oda Y, Pratt SA, Miller S, and Shoback D (1999) Expression and signal transduction of calcium-sensing receptors in cartilage and bone. *Endocrinology* **140**:5883–5893.
- Chang W, Tu C, Cheng Z, Rodriguez L, Chen TH, Gassmann M, Bettler B, Margeta M, Jan LY, and Shoback D (2007) Complex formation with the Type B gamma-aminobutyric acid receptor affects the expression and signal transduction of the extracellular calcium-sensing receptor. Studies with HEK-293 cells and neurons. *J Biol Chem* **282**:25030–25040.
- Chang W, Pratt S, Chen TH, Nemeth E, Huang Z, and Shoback D (1998) Coupling of calcium receptors to inositol phosphate and cyclic AMP generation in mammalian cells and *Xenopus laevis* oocytes and immunodetection of receptor protein by region-specific antipeptide antisera. *J Bone Miner Res* **13**:570–580.
- Chappell MD, Li R, Smith SC, Dressman BA, Tromiczak EG, Tripp AE, Blanco MJ, Vetman T, Quimby SJ, Matt J, et al. (2016) Discovery of (1S,2R,3S,4S,5R,6R)-2-amino-3-[(3,4-difluorophenyl)sulfanylmethyl]-4-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid hydrochloride (LY3020371-HCl): a potent, metabotropic glutamate 2/3 receptor antagonist with antidepressant-like activity. *J Med Chem* **59**: 10974–10993.
- Chattopadhyay N, Espinosa-Jeffrey A, Tfelt-Hansen J, Yano S, Bandyopadhyay S, Brown EM, and de Vellis J (2008) Calcium receptor expression and function in oligodendrocyte commitment and lineage progression: potential impact on reduced myelin basic protein in CaR-null mice. *J Neurosci Res* **86**:2159–2167.
- Chattopadhyay N, Yano S, Tfelt-Hansen J, Rooney P, Kanuparthi D, Bandyopadhyay S, Ren X, Terwilliger E, and Brown EM (2004) Mitogenic action of calcium-sensing receptor on rat calvarial osteoblasts. *Endocrinology* **145**:3451–3462.
- Chattopadhyay N, Ye CP, Yamaguchi T, Kifor O, Vassilev PM, Nishimura R, and Brown EM (1998) Extracellular calcium-sensing receptor in rat oligodendrocytes: expression and potential role in regulation of cellular proliferation and an outward K^+ channel. *Glia* **24**:449–458.
- Chen CJ, Anast CS, and Brown EM (1987) High osmolality: a potent parathyroid hormone secretagogue in dispersed parathyroid cells. *Endocrinology* **121**:958–964.
- Chen CJ, Barnett JV, Congo DA, and Brown EM (1989) Divalent cations suppress 3',5'-adenosine monophosphate accumulation by stimulating a pertussis toxin-sensitive guanine nucleotide-binding protein in cultured bovine parathyroid cells. *Endocrinology* **124**:233–239.
- Chen RA and Goodman WG (2004) Role of the calcium-sensing receptor in parathyroid gland physiology. *Am J Physiol Renal Physiol* **286**:F1005–F1011.
- Cheng I, Klingensmith ME, Chattopadhyay N, Kifor O, Butters RR, Soybel DI, and Brown EM (1998) Identification and localization of the extracellular calcium-sensing receptor in human breast. *J Clin Endocrinol Metab* **83**:703–707.
- Cheng SX (2012) Calcium-sensing receptor inhibits secretagogue-induced electrolyte secretion by intestine via the enteric nervous system. *Am J Physiol Gastrointest Liver Physiol* **303**:G60–G70.
- Cheng SX, Lightfoot YL, Yang T, Zadeh M, Tang L, Sahay B, Wang GP, Owen JL, and Mohamadzadeh M (2014) Epithelial CaSR deficiency alters intestinal integrity and promotes proinflammatory immune responses. *FEBS Lett* **588**:4158–4166.
- Cheng Z, Tu C, Rodriguez L, Chen TH, Dvorak MM, Margeta M, Gassmann M, Bettler B, Shoback D, and Chang W (2007) Type B γ -aminobutyric acid receptors modulate the function of the extracellular Ca^{2+} -sensing receptor and cell differentiation in murine growth plate chondrocytes. *Endocrinology* **148**:4984–4992.
- Chertow GM, Block GA, Correa-Rotter R, Drüeke TB, Floege J, Goodman WG, Herzog CA, Kubo Y, London GM, Mahaffey KW, et al.; EVOLVE Trial Investigators (2012) Effect of cinacalcet on cardiovascular disease in patients undergoing dialysis. *N Engl J Med* **367**:2482–2494.
- Chiarini A, Armato U, Gardenal E, Gui L, and Dal Prà I (2017a) Amyloid β -exposed human astrocytes overproduce phospho-tau and overrelease it within exosomes, effects suppressed by calcilytic NPS 2143-further implications for Alzheimer's therapy. *Front Neurosci* **11**:217.
- Chiarini A, Armato U, Liu D, and Dal Prà I (2017b) Calcium-sensing receptor antagonist NPS 2143 restores amyloid precursor protein physiological non-amyloidogenic processing in $\text{A}\beta$ -exposed adult human astrocytes. *Sci Rep* **7**:1277.
- Chikatsu N, Fukumoto S, Takeuchi Y, Suzawa M, Obara T, Matsumoto T, and Fujita T (2000) Cloning and characterization of two promoters for the human calcium-sensing receptor (CaSR) and changes of CaSR expression in parathyroid adenomas. *J Biol Chem* **275**:7553–7557.
- Chou YH, Brown EM, Levi T, Crowe G, Atkinson AB, Arnqvist HJ, Toss G, Fuleihan GE, Seidman JG, and Seidman CE (1992) The gene responsible for familial hypocalcemic hypercalcemia maps to chromosome 3q in four unrelated families. *Nat Genet* **1**:295–300.
- Christopher JA, Aves SJ, Bennett KA, Doré AS, Errey JC, Jazayeri A, Marshall FH, Okrasa K, Serrano-Vega MJ, Tehan BG, et al. (2015) Fragment and structure-based drug discovery for a class C GPCR: discovery of the mGlu5 negative allosteric modulator HTL14242 (3-chloro-5-[6-(5-fluoropyridin-2-yl)pyrimidin-4-yl]benzotriazole). *J Med Chem* **58**:6653–6664.
- Christopher JA, Orgován Z, Congreve M, Doré AS, Errey JC, Marshall FH, Mason JS, Okrasa K, Rucktooa P, Serrano-Vega MJ, et al. (2019) Structure-based optimization strategies for G protein-coupled receptor (GPCR) allosteric modulators: a case study from analyses of new metabotropic glutamate receptor 5 (mGlu5) X-ray structures. *J Med Chem* **62**:207–222.
- Colloton M, Shatz E, Miller G, Stehman-Breen C, Wada M, Lacey D, and Martin D (2005) Cinacalcet HCl attenuates parathyroid hyperplasia in a rat model of secondary hyperparathyroidism. *Kidney Int* **67**:467–476.
- Conigrave AD (2016) The calcium-sensing receptor and the parathyroid: past, present, future. *Front Physiol* **7**:563.
- Conigrave AD, Brown EM, and Rizzoli R (2008) Dietary protein and bone health: roles of amino acid-sensing receptors in the control of calcium metabolism and bone homeostasis. *Annu Rev Nutr* **28**:131–155.
- Conigrave AD, Franks AH, Brown EM, and Quinn SJ (2002) L-amino acid sensing by the calcium-sensing receptor: a general mechanism for coupling protein and calcium metabolism? *Eur J Clin Nutr* **56**:1072–1080.
- Conigrave AD and Hampson DR (2006) Broad-spectrum L-amino acid sensing by class 3 G-protein-coupled receptors. *Trends Endocrinol Metab* **17**:398–407.
- Conigrave AD and Hampson DR (2010) Broad-spectrum amino acid-sensing class C G-protein coupled receptors: molecular mechanisms, physiological significance and options for drug development. *Pharmacol Ther* **127**:252–260.
- Conigrave AD, Mun H-C, Delbridge L, Quinn SJ, Wilkinson M, and Brown EM (2004) L-amino acids regulate parathyroid hormone secretion. *J Biol Chem* **279**: 38151–38159.
- Conigrave AD, Mun HC, and Lok HC (2007) Aromatic L-amino acids activate the calcium-sensing receptor. *J Nutr* **137**(6 Suppl 1):1524S–1527S, discussion 1548S.
- Conigrave AD, Quinn SJ, and Brown EM (2000) L-amino acid sensing by the extracellular Ca^{2+} -sensing receptor. *Proc Natl Acad Sci USA* **97**:4814–4819.
- Conigrave AD and Ward DT (2013) Calcium-sensing receptor (CaSR): pharmacological properties and signaling pathways. *Best Pract Res Clin Endocrinol Metab* **27**:315–331.
- Conley YP, Mukherjee A, Kammerer C, DeKosky ST, Kambh MI, Finegold DN, and Ferrell RE (2009) Evidence supporting a role for the calcium-sensing receptor in Alzheimer disease. *Am J Med Genet B Neuropsychiatr Genet* **150B**:703–709.
- Cook AE, Mistry SN, Gregory KJ, Furness SG, Sexton PM, Scammells PJ, Conigrave AD, Christopoulos A, and Leach K (2015) Biased allosteric modulation at the CaS receptor engendered by structurally diverse calcimimetics. *Br J Pharmacol* **172**: 185–200.
- Corbetta S, Lania A, Filipanti M, Vicentini L, Ballaré E, and Spada A (2002) Mitogen-activated protein kinase cascade in human normal and tumoral parathyroid cells. *J Clin Endocrinol Metab* **87**:2201–2205.
- Courbebaisse M, Diet C, Timsit MO, Mamzer MF, Thervet E, Noel LH, Legendre C, Friedlander G, Martinez F, and Prié D (2012) Effects of cinacalcet in renal transplant patients with hyperparathyroidism. *Am J Nephrol* **35**:341–348.
- Dal Prà I, Armato U, Chioffi F, Pacchiana R, Whitfield JF, Chakravarthy B, Gui L, and Chiarini A (2014) The $\text{A}\beta$ peptides-activated calcium-sensing receptor stimulates the production and secretion of vascular endothelial growth factor-A by normoxic adult human cortical astrocytes. *Neuromolecular Med* **16**:645–657.
- Davey AE, Leach K, Valant C, Conigrave AD, Sexton PM, and Christopoulos A (2012) Positive and negative allosteric modulators promote biased signaling at the calcium-sensing receptor. *Endocrinology* **153**:1232–1241.
- Davies SL, Gibbons CE, Vizard T, and Ward DT (2006) Ca^{2+} -sensing receptor induces Rho kinase-mediated actin stress fiber assembly and altered cell morphology, but not in response to aromatic amino acids. *Am J Physiol Cell Physiol* **290**:C1543–C1551.
- Davies SL, Ozawa A, McCormick WD, Dvorak MM, and Ward DT (2007) Protein kinase C-mediated phosphorylation of the calcium-sensing receptor is stimulated by receptor activation and attenuated by calyculin-sensitive phosphatase activity. *J Biol Chem* **282**:15048–15056.
- Dawson-Hughes B (2003) Calcium and protein in bone health. *Proc Nutr Soc* **62**: 505–509.
- Deprez P, Temal T, Jary H, Auberval M, Lively S, Guédin D, and Vevert JP (2013) New potent calcimimetics. II. Discovery of benzothiazole trisubstituted ureas. *Bioorg Med Chem Lett* **23**:2455–2459.
- de Torres C, Beleta H, Diaz R, Toran N, Rodríguez E, Lavarino C, García I, Acosta S, Suñol M, and Mora J (2009) The calcium-sensing receptor and parathyroid hormone-related protein are expressed in differentiated, favorable neuroblastic tumors. *Cancer* **115**:2792–2803.
- Diakogiannaki E, Pais R, Tolhurst G, Parker HE, Horscroft J, Rauscher B, Zietek T, Daniel H, Gribble FM, and Reimann F (2013) Oligopeptides stimulate glucagon-like peptide-1 secretion in mice through proton-coupled uptake and the calcium-sensing receptor. *Diabetologia* **56**:2688–2696.
- Diaz R, Hurwitz S, Chattopadhyay N, Pines M, Yang Y, Kifor O, Einat MS, Butters R, Hebert SC, and Brown EM (1997) Cloning, expression, and tissue localization of the calcium-sensing receptor in chicken (*Gallus domesticus*). *Am J Physiol* **273**: R1008–R1016.
- Didiuk MT, Griffith DA, Benbow JW, Liu KK, Walker DP, Bi FC, Morris J, Guzman-Perez A, Gao H, Bechle BM, et al. (2009) Short-acting 5-(trifluoromethyl)pyrido [4,3-d]pyrimidin-4(3H)-one derivatives as orally-active calcium-sensing receptor antagonists. *Bioorg Med Chem Lett* **19**:4555–4559.
- Diepenhorst NA, Leach K, Keller AN, Rueda P, Cook AE, Pierce TL, Nowell C, Pastoureau P, Sabatini M, Summers RJ, et al. (2018) Divergent effects of strontium and calcium-sensing receptor positive allosteric modulators (calcimimetics) on human osteoclast activity. *Br J Pharmacol* **175**:4095–4108.
- Dobnig H and Turner RT (1997) The effects of programmed administration of human parathyroid hormone fragment (1-34) on bone histomorphometry and serum chemistry in rats. *Endocrinology* **138**:4607–4612.
- Dong B, Endo I, Ohnishi Y, Kondo T, Hasegawa T, Amizuka N, Kiyonari H, Shioi G, Abe M, Fukumoto S, et al. (2015) Calcilytic ameliorates abnormalities of mutant calcium-sensing receptor (CaSR) knock-in mice mimicking autosomal dominant hypocalcemia (ADH). *J Bone Miner Res* **30**:1980–1993.
- Doré AS, Okrasa K, Patel JC, Serrano-Vega M, Bennett K, Cooke RM, Errey JC, Jazayeri A, Khan S, Tehan B, et al. (2014) Structure of class C GPCR metabotropic glutamate receptor 5 transmembrane domain. *Nature* **511**:557–562.

- Dubois-Ferrière V, Brennan TC, Dayer R, Rizzoli R, and Ammann P (2011) Calcitropic hormones and IGF-I are influenced by dietary protein. *Endocrinology* **152**: 1839–1847.
- Dufner MM, Kirchhoff P, Remy C, Hafner P, Müller MK, Cheng SX, Tang LQ, Hebert SC, Geibel JP, and Wagner C (2005) The calcium-sensing receptor acts as a modulator of gastric acid secretion in freshly isolated human gastric glands. *Am J Physiol Gastrointest Liver Physiol* **289**:G1084–G1090.
- Dvorak MM, Chen TH, Orwoll B, Garvey C, Chang W, Bikle DD, and Shoback DM (2007) Constitutive activity of the osteoblast Ca^{2+} -sensing receptor promotes loss of cancellous bone. *Endocrinology* **148**:3156–3163.
- Dvorak-Ewell MM, Chen TH, Liang N, Garvey C, Liu B, Tu C, Chang W, Bikle DD, and Shoback DM (2011) Osteoblast extracellular Ca^{2+} -sensing receptor regulates bone development, mineralization, and turnover. *J Bone Miner Res* **26**:2935–2947.
- Elajnaif T, Iamartino L, Mesteri I, Müller C, Bassetto M, Manhardt T, Baumgartner-Parzer S, Kallay E, and Schepelmann M (2019) Nutritional and pharmacological targeting of the calcium-sensing receptor influences chemically induced colitis in mice. *Nutrients* **11**:E3072.
- El Hiani Y, Ahidouch A, Roudbaraki M, Guenin S, Brûlé G, and Ouadid-Ahidouch H (2006) Calcium-sensing receptor stimulation induces nonselective cation channel activation in breast cancer cells. *J Membr Biol* **211**:127–137.
- Ellison TI, Smith MK, Gilliam AC, and MacDonald PN (2008) Inactivation of the vitamin D receptor enhances susceptibility of murine skin to UV-induced tumorigenesis. *J Invest Dermatol* **128**:2508–2517.
- Engelstoft MS, Park WM, Sakata I, Kristensen LV, Husted AS, Osborne-Lawrence S, Piper PK, Walker AK, Pedersen MH, Nøhr MK, et al. (2013) Seven transmembrane G protein-coupled receptor repertoire of gastric ghrelin cells. *Mol Metab* **2**:376–392.
- Fajtova VT, Quinn SJ, and Brown EM (1991) Cytosolic calcium responses of single rMTC 44-2 cells to stimulation with external calcium and potassium. *Am J Physiol* **261**:E151–E158.
- Fan GF, Ray K, Zhao XM, Goldsmith PK, and Spiegel AM (1998) Mutational analysis of the cysteines in the extracellular domain of the human Ca^{2+} receptor: effects on cell surface expression, dimerization and signal transduction. *FEBS Lett* **436**: 353–356.
- Fan Y, Liu W, Bi R, Densmore MJ, Sato T, Mannstadt M, Yuan Q, Zhou X, Olsson H, Larsson TE, et al. (2018) Interrelated role of Klotho and calcium-sensing receptor in parathyroid hormone synthesis and parathyroid hyperplasia. *Proc Natl Acad Sci USA* **115**:E3749–E3758.
- Feng J, Petersen CD, Coy DH, Jiang JK, Thomas CJ, Pollak MR, and Wank SA (2010) Calcium-sensing receptor is a physiologic multimodal chemosensor regulating gastric G-cell growth and gastrin secretion. *Proc Natl Acad Sci USA* **107**: 17791–17796.
- Ferry S, Traiffort E, Stinnakre J, and Ruat M (2000) Developmental and adult expression of rat calcium-sensing receptor transcripts in neurons and oligodendrocytes. *Eur J Neurosci* **12**:872–884.
- Fetahu IS, Hummel DM, Manhardt T, Aggarwal A, Baumgartner-Parzer S, and Kallay E (2014) Regulation of the calcium-sensing receptor expression by 1,25-dihydroxyvitamin D₃, interleukin-6, and tumor necrosis factor alpha in colon cancer cells. *J Steroid Biochem Mol Biol* **144**:228–231.
- Fisher JE, Scott K, Wei N, Zhao JZ, Cusick T, Tijerina M, Karanam B, Duong L, and Glantschnig H (2012) Pharmacodynamic responses to combined treatment regimens with the calcium sensing receptor antagonist JTT-305/MK-5442 and alendronate in osteopenic ovariectomized rats. *Bone* **50**:1332–1342.
- Fisher MM, Cabrera SM, and Imel EA (2015) Successful treatment of neonatal severe hyperparathyroidism with cinacalcet in two patients. *Endocrinol Diabetes Metab Case Rep* **2015**:150040.
- Fitzpatrick LA, Dabrowski CE, Cicconetti G, Gordon DN, Fuerst T, Engelke K, and Genant HK (2012) Ronacaleret, a calcium-sensing receptor antagonist, increases trabecular but not cortical bone in postmenopausal women. *J Bone Miner Res* **27**:255–262.
- Fitzpatrick LA, Dabrowski CE, Cicconetti G, Gordon DN, Papapoulos S, Bone HG III, and Bilezikian JP (2011a) The effects of ronacaleret, a calcium-sensing receptor antagonist, on bone mineral density and biochemical markers of bone turnover in postmenopausal women with low bone mineral density. *J Clin Endocrinol Metab* **96**:2441–2449.
- Fitzpatrick LA, Smith PL, McBride TA, Fries MA, Hossain M, Dabrowski CE, and Gordon DN (2011b) Ronacaleret, a calcium-sensing receptor antagonist, has no significant effect on radial fracture healing time: results of a randomized, double-blinded, placebo-controlled Phase II clinical trial. *Bone* **49**:845–852.
- Freichel M, Zink-Lorenz A, Holloschi A, Hafner M, Flockerzi V, and Raue F (1996) Expression of a calcium-sensing receptor in a human medullary thyroid carcinoma cell line and its contribution to calcitonin secretion. *Endocrinology* **137**:3842–3848.
- Friedman PA (1998) Codependence of renal calcium and sodium transport. *Annu Rev Physiol* **60**:179–197.
- Fritze O, Filipek S, Kukus V, Palczewski K, Hofmann KP, and Ernst OP (2003) Role of the conserved NPxxY(x)5,6F motif in the rhodopsin ground state and during activation. *Proc Natl Acad Sci USA* **100**:2290–2295.
- Fudge NJ and Kovacs CS (2004) Physiological studies in heterozygous calcium sensing receptor (CaSR) gene-ablated mice confirm that the CaSR regulates calcitonin release in vivo. *BMC Physiol* **4**:5.
- Fujisawa Y, Yamaguchi R, Satake E, Ohtaka K, Nakanishi T, Ozono K, and Ogata T (2013) Identification of AP2S1 mutation and effects of low calcium formula in an infant with hypercalcemia and hypercalciuria. *J Clin Endocrinol Metab* **98**: E2022–E2027.
- Fukagawa M, Shimazaki R, and Akizawa T; Evocalcet Study Group (2018) Head-to-head comparison of the new calcimimetic agent evocalcet with cinacalcet in Japanese hemodialysis patients with secondary hyperparathyroidism. *Kidney Int* **94**: 818–825.
- Gama L, Wilt SG, and Breitwieser GE (2001) Heterodimerization of calcium sensing receptors with metabotropic glutamate receptors in neurons. *J Biol Chem* **276**: 39053–39059.
- Gannon AW, Monk HM, and Levine MA (2014) Cinacalcet monotherapy in neonatal severe hyperparathyroidism: a case study and review. *J Clin Endocrinol Metab* **99**: 7–11.
- García Soblechero E, Ferrer Castillo MT, Jiménez Crespo B, Domínguez Quintero ML, and González Fuentes C (2013) Neonatal hypercalcemia due to a homozygous mutation in the calcium-sensing receptor: failure of cinacalcet. *Neonatology* **104**: 104–108.
- Gardenal E, Chiariari A, Armato U, Dal Prà I, Verkhatsky A, and Rodríguez JJ (2017) Increased calcium-sensing receptor immunoreactivity in the hippocampus of a triple transgenic mouse model of Alzheimer's disease. *Front Neurosci* **11**:81.
- Garrett JE, Capuano IV, Hammerland LG, Hung BC, Brown EM, Hebert SC, Nemeth EF, and Fuller F (1995a) Molecular cloning and functional expression of human parathyroid calcium receptor cDNAs. *J Biol Chem* **270**:12919–12925.
- Garrett JE, Tamir H, Kifor O, Simin RT, Rogers KV, Mithal A, Gagel RF, and Brown EM (1995b) Calcitonin-secreting cells of the thyroid express an extracellular calcium receptor gene. *Endocrinology* **136**:5202–5211.
- Geibel J, Sritharan K, Geibel R, Geibel P, Persing JS, Seeger A, Roepke TK, Deichstetter M, Prinz C, Cheng SX, et al. (2006) Calcium-sensing receptor abrogates secretagogue-induced increases in intestinal net fluid secretion by enhancing cyclic nucleotide destruction. *Proc Natl Acad Sci USA* **103**:9390–9397.
- Geibel JP and Hebert SC (2009) The functions and roles of the extracellular Ca^{2+} -sensing receptor along the gastrointestinal tract. *Annu Rev Physiol* **71**:205–217.
- Geng Y, Bush M, Mosyak L, Wang F, and Fan QR (2013) Structural mechanism of ligand activation in human GABA(B) receptor. *Nature* **504**:254–259.
- Geng Y, Mosyak L, Kurinov I, Zuo H, Sturchler E, Cheng TC, Subramanyam P, Brown AP, Brennan SC, Mun HC, et al. (2016) Structural mechanism of ligand activation in human calcium-sensing receptor. *eLife* **5**:e13662.
- Geng Y, Xiong D, Mosyak L, Malito DL, Kniazef J, Chen Y, Burmakina S, Quick M, Bush M, Javitch JA, et al. (2012) Structure and functional interaction of the extracellular domain of human GABA(B) receptor GBR2. *Nat Neurosci* **15**:970–978.
- Gerspacher M, Altmann E, Beerli R, Buhl T, Endres R, Gamse R, Kamenit-Tcheudji J, Kneissel M, Krawinkler KH, Missbach M, et al. (2010) Penta-substituted benzimidazoles as potent antagonists of the calcium-sensing receptor (CaSR-antagonists). *Bioorg Med Chem Lett* **20**:5161–5164.
- Giudice ML, Mihalik B, Dinnyés A, and Kobolák J (2019) The nervous system relevance of the calcium sensing receptor in health and disease. *Molecules* **24**:E2546.
- Goltzman D and Hendy GN (2015) The calcium-sensing receptor in bone—mechanistic and therapeutic insights. *Nat Rev Endocrinol* **11**:298–307.
- Gong Y and Hou J (2014) Claudin-14 underlies Ca^{++} -sensing receptor-mediated Ca^{++} metabolism via NFAT-microRNA-based mechanisms. *J Am Soc Nephrol* **25**: 745–760.
- Goolam MA, Ward JH, Avlani VA, Leach K, Christopoulos A, and Conigrave AD (2014) Roles of intraloops-2 and -3 and the proximal C-terminus in signalling pathway selection from the human calcium-sensing receptor. *FEBS Lett* **588**: 3340–3346.
- Gorvin CM (2019) Molecular and clinical insights from studies of calcium-sensing receptor mutations. *J Mol Endocrinol* **63**:R1–R16.
- Gorvin CM, Babinsky VN, Malinauskas T, Nissen PH, Schou AJ, Hanyaloglu AC, Siebold C, Jones EY, Hannan FM, and Thakker RV (2018a) A calcium-sensing receptor mutation causing hypocalcemia disrupts a transmembrane salt bridge to activate β -arrestin-biased signaling. *Sci Signal* **11**:ean3714.
- Gorvin CM, Cranston T, Hannan FM, Rust N, Qureshi A, Nesbit MA, and Thakker RV (2016) A G-protein subunit- α 11 loss-of-function mutation, Thr54Met, causes familial hypocalciuric hypercalcemia type 2 (FHH2). *J Bone Miner Res* **31**:1200–1206.
- Gorvin CM, Hannan FM, Cranston T, Valtia H, Makitie O, Schalin-Jantti C, and Thakker RV (2018b) Cinacalcet rectifies hypercalcemia in a patient with familial hypocalciuric hypercalcemia type 2 (FHH2) caused by a germline loss-of-function $\text{G}\alpha_{11}$ mutation. *J Bone Miner Res* **33**:32–41.
- Gorvin CM, Hannan FM, Howles SA, Babinsky VN, Piret SE, Rogers A, Freidin AJ, Stewart M, Paudyal A, Hough TA, et al. (2017) $\text{G}\alpha_{11}$ mutation in mice causes hypocalcemia rectifiable by calcilytic therapy. *JCI Insight* **2**:e91103.
- Gorvin CM, Rogers A, Hastoy B, Tarasov AI, Frost M, Sposini S, Inoue A, Whyte MP, Rorsman P, Hanyaloglu AC, et al. (2018c) AP2 α mutations impair calcium-sensing receptor trafficking and signaling, and show an endosomal pathway to spatially direct G-protein selectivity. *Cell Rep* **22**:1054–1066.
- Gowen M, Stroup GB, Dodds RA, James IE, Votta BJ, Smith BR, Bhatnagar PK, Lago AM, Callahan JF, DelMar EG, et al. (2000) Antagonizing the parathyroid calcium receptor stimulates parathyroid hormone secretion and bone formation in osteopenic rats. *J Clin Invest* **105**:1595–1604.
- Graca JA, Schepelmann M, Brennan SC, Reens J, Chang W, Yan P, Toka H, Riccardi D, and Price SA (2016) Comparative expression of the extracellular calcium-sensing receptor in the mouse, rat, and human kidney. *Am J Physiol Renal Physiol* **310**:F518–F533.
- Grant MP, Cavanaugh A, and Breitwieser GE (2015) 14-3-3 proteins buffer intracellular calcium sensing receptors to constrain signaling. *PLoS One* **10**: e0136702.
- Grant MP, Epure LM, Bokhari R, Roughley P, Antoniou J, and Mwale F (2016) Human cartilaginous endplate degeneration is induced by calcium and the extracellular calcium-sensing receptor in the intervertebral disc. *Eur Cell Mater* **32**: 137–151.
- Grant MP, Stepanchick A, Cavanaugh A, and Breitwieser GE (2011) Agonist-driven maturation and plasma membrane insertion of calcium-sensing receptors dynamically control signal amplitude. *Sci Signal* **4**:ra78.
- Gray E, Muller D, Squires PE, Asare-Anane H, Huang GC, Amiel S, Persaud SJ, and Jones PM (2006) Activation of the extracellular calcium-sensing receptor initiates insulin secretion from human islets of Langerhans: involvement of protein kinases. *J Endocrinol* **190**:703–710.
- Gregory KJ, Giraldo J, Diao J, Christopoulos A, and Leach K (2020) Evaluation of operational models of agonism and allosterism at receptors with multiple orthosteric binding sites. *Mol Pharmacol* **97**:35–45.

- Gregory KJ, Kufareva I, Keller AN, Khajehali E, Mun HC, Goolam MA, Mason RS, Capuano B, Conigrave AD, Christopoulos A, et al. (2018) Dual action calcium-sensing receptor modulator unmasks novel mode-switching mechanism. *Pharm Trans Sci* 1:96–109.
- Grevellec A, Graham A, and Tucker AS (2011) Shh signalling restricts the expression of Gcm2 and controls the position of the developing parathyroids. *Dev Biol* 353: 194–205.
- Halse J, Greenspan S, Cosman F, Ellis G, Santora A, Leung A, Heyden N, Samanta S, Doleckij S, Rosenberg E, et al. (2014) A phase 2, randomized, placebo-controlled, dose-ranging study of the calcium-sensing receptor antagonist MK-5442 in the treatment of postmenopausal women with osteoporosis. *J Clin Endocrinol Metab* 99:E2207–E2215.
- Hamano N, Komaba H, and Fukagawa M (2017) Etelcalcetide for the treatment of secondary hyperparathyroidism. *Expert Opin Pharmacother* 18:529–534.
- Handlogten ME, Huang C, Shiraishi N, Awata H, and Miller RT (2001) The Ca²⁺-sensing receptor activates cytosolic phospholipase A2 via a Gq α -dependent ERK-independent pathway. *J Biol Chem* 276:13941–13948.
- Handlogten ME, Shiraishi N, Awata H, Huang C, and Miller RT (2000) Extracellular Ca(2+)-sensing receptor is a promiscuous divalent cation sensor that responds to lead. *Am J Physiol Renal Physiol* 279:F1083–F1091.
- Hannan FM, Babinsky VN, and Thakker RV (2016) Disorders of the calcium-sensing receptor and partner proteins: insights into the molecular basis of calcium homeostasis. *J Mol Endocrinol* 57:R127–R142.
- Hannan FM, Howles SA, Rogers A, Cranston T, Gorvin CM, Babinsky VN, Reed AA, Thakker CE, Bockenhauer D, Brown RS, et al. (2015a) Adaptor protein-2 sigma subunit mutations causing familial hypocalcemic hypercalcaemia type 3 (FHH3) demonstrate genotype-phenotype correlations, codon bias and dominant-negative effects. *Hum Mol Genet* 24:5079–5092.
- Hannan FM, Kallay E, Chang W, Brandt ML, and Thakker RV (2018a) The calcium-sensing receptor in physiology and in calcitropic and noncalcitropic diseases. *Nat Rev Endocrinol* 15:33–51.
- Hannan FM, Nesbit MA, Christie PT, Lissens W, Van der Schueren B, Bex M, Bouillon R, and Thakker RV (2010) A homozygous inactivating calcium-sensing receptor mutation, Pro339Thr, is associated with isolated primary hyperparathyroidism: correlation between location of mutations and severity of hypercalcaemia. *Clin Endocrinol (Oxf)* 73:715–722.
- Hannan FM, Nesbit MA, Zhang C, Cranston T, Curley AJ, Harding B, Fratter C, Rust N, Christie PT, Turner JJ, et al. (2012) Identification of 70 calcium-sensing receptor mutations in hyper- and hypo-calcaemic patients: evidence for clustering of extracellular domain mutations at calcium-binding sites. *Hum Mol Genet* 21: 2768–2778.
- Hannan FM, Olesen MK, and Thakker RV (2018b) Calcimimetic and calcilytic therapies for inherited disorders of the calcium-sensing receptor signalling pathway. *Br J Pharmacol* 175:4083–4094.
- Hannan FM and Thakker RV (2013) Calcium-sensing receptor (CaSR) mutations and disorders of calcium, electrolyte and water metabolism. *Best Pract Res Clin Endocrinol Metab* 27:359–371.
- Hannan FM, Walls GV, Babinsky VN, Nesbit MA, Kallay E, Hough TA, Fraser WD, Cox RD, Hu J, Spiegel AM, et al. (2015b) The calcilytic agent NPS 2143 rectifies hypocalcaemia in a mouse model with an activating calcium-sensing receptor (CaSR) mutation: relevance to autosomal dominant hypocalcaemia type 1 (ADH1). *Endocrinology* 156:3114–3121.
- Hanyaloglu AC and von Zastrow M (2008) Regulation of GPCRs by endocytic membrane trafficking and its potential implications. *Annu Rev Pharmacol Toxicol* 48:537–568.
- Hauache OM, Hu J, Ray K, and Spiegel AM (2000) Functional interactions between the extracellular domain and the seven-transmembrane domain in Ca²⁺ receptor activation. *Endocrine* 13:63–70.
- Hebert SC, Brown EM, and Harris HW (1997) Role of the Ca(2+)-sensing receptor in divalent mineral ion homeostasis. *J Exp Biol* 200:295–302.
- Hendy GN and Canaff L (2016) Calcium-sensing receptor gene: regulation of expression. *Front Physiol* 7:394.
- Hendy GN, Canaff L, Newfield RS, Tripto-Shkolnik L, Wong BY, Lee BS, and Cole DE (2014) Codon Arg15 mutations of the AP2S1 gene: common occurrence in familial hypocalcemic hypercalcaemia cases negative for calcium-sensing receptor (CaSR) mutations. *J Clin Endocrinol Metab* 99:E1311–E1315.
- Henley C III, Yang Y, Davis J, Lu JY, Morony S, Fan W, Florio M, Sun B, Shatzken E, Pretorius JK, et al. (2011) Discovery of a calcimimetic with differential effects on parathyroid hormone and calcitonin secretion. *J Pharmacol Exp Ther* 337: 681–691.
- Hernández-Bedolla MA, Carretero-Ortega J, Valadez-Sánchez M, Vázquez-Prado J, and Reyes-Cruz G (2015) Chemotactic and proangiogenic role of calcium sensing receptor is linked to secretion of multiple cytokines and growth factors in breast cancer MDA-MB-231 cells. *Biochim Biophys Acta* 1853:166–182.
- Herrera-Vigorin F, Hernández-García R, Valadez-Sánchez M, Vázquez-Prado J, and Reyes-Cruz G (2006) AMSh regulates calcium-sensing receptor signaling through direct interactions. *Biochem Biophys Res Commun* 347:924–930.
- Hills CE, Younis MY, Bennett J, Siamantouras E, Liu KK, and Squires PE (2012) Calcium-sensing receptor activation increases cell-cell adhesion and β -cell function. *Cell Physiol Biochem* 30:575–586.
- Ho C, Conner DA, Pollak MR, Ladd DJ, Kifor O, Warren HB, Brown EM, Seidman JG, and Seidman CE (1995) A mouse model of human familial hypocalcemic hypercalcaemia and neonatal severe hyperparathyroidism. *Nat Genet* 11:389–394.
- Hobson SA, Wright J, Lee F, McNeil SE, Bilderback T, and Rodland KD (2003) Activation of the MAP kinase cascade by exogenous calcium-sensing receptor. *Mol Cell Endocrinol* 200:189–198.
- Hodgkin MN, Hills CE, and Squires PE (2008) The calcium-sensing receptor and insulin secretion: a role outside systemic control 15 years on. *J Endocrinol* 199:1–4.
- Hough TA, Bogani D, Cheeseman MT, FAVOR J, Nesbit MA, Thakker RV, and Lyon MF (2004) Activating calcium-sensing receptor mutation in the mouse is associated with cataracts and ectopic calcification. *Proc Natl Acad Sci USA* 101:13566–13571.
- House MG, Kohlmeier L, Chattopadhyay N, Kifor O, Yamaguchi T, Leboff MS, Glowacki J, and Brown EM (1997) Expression of an extracellular calcium-sensing receptor in human and mouse bone marrow cells. *J Bone Miner Res* 12:1959–1970.
- Hovden S, Rejnmark L, Ladefoged SA, and Nissen PH (2017) AP2S1 and GNA11 mutations - not a common cause of familial hypocalcemic hypercalcaemia. *Eur J Endocrinol* 176:177–185.
- Howles SA, Hannan FM, Babinsky VN, Rogers A, Gorvin CM, Rust N, Richardson T, McKenna MJ, Nesbit MA, and Thakker RV (2016) Cinacalcet for symptomatic hypercalcaemia caused by AP2S1 mutations. *N Engl J Med* 374:1396–1398.
- Howles SA, Hannan FM, Gorvin CM, Piret SE, Paudyal A, Stewart M, Hough TA, Nesbit MA, Wells S, Brown SD, et al. (2017) Cinacalcet corrects hypercalcaemia in mice with an inactivating G α 11 mutation. *JCI Insight* 2:96540.
- Hu J, Jiang J, Costanzi S, Thomas C, Yang W, Feyen JH, Jacobson KA, and Spiegel AM (2006) A missense mutation in the seven-transmembrane domain of the human Ca²⁺ receptor converts a negative allosteric modulator into a positive allosteric modulator. *J Biol Chem* 281:21558–21565.
- Huang C and Miller RT (2010) Novel Ca receptor signaling pathways for control of renal ion transport. *Curr Opin Nephrol Hypertens* 19:106–112.
- Huang C, Handlogten ME, and Miller RT (2002) Parallel activation of phosphatidylinositol 4-kinase and phospholipase C by the extracellular calcium-sensing receptor. *J Biol Chem* 277:20293–20300.
- Huang C, Hujer KM, Wu Z, and Miller RT (2004) The Ca²⁺-sensing receptor couples to G α 12/13 to activate phospholipase D in Madin-Darby canine kidney cells. *Am J Physiol Cell Physiol* 286:C22–C30.
- Huang Y and Breitwieser GE (2007) Rescue of calcium-sensing receptor mutants by allosteric modulators reveals a conformational checkpoint in receptor biogenesis. *J Biol Chem* 282:9517–9525.
- Huang Y, Zhou Y, Castiblanco A, Yang W, Brown EM, and Yang JJ (2009) Multiple Ca(2+)-binding sites in the extracellular domain of the Ca(2+)-sensing receptor corresponding to cooperative Ca(2+) response. *Biochemistry* 48:388–398.
- Huang Y, Zhou Y, Yang W, Butters R, Lee HW, Li S, Castiblanco A, Brown EM, and Yang JJ (2007) Identification and dissection of Ca(2+)-binding sites in the extracellular domain of Ca(2+)-sensing receptor. *J Biol Chem* 282:19000–19010.
- Hurtel-Lemaire AS, Mentaverri R, Caudrillier A, Courmarie F, Wattel A, Kamel S, Terwilliger EF, Brown EM, and Brazier M (2009) The calcium-sensing receptor is involved in strontium ranelate-induced osteoclast apoptosis. New insights into the associated signaling pathways. *J Biol Chem* 284:575–584.
- Imura A, Tsuji Y, Murata M, Maeda R, Kubota K, Iwano A, Obuse C, Togashi K, Tominaga M, Kita N, et al. (2007) alpha-Klotho as a regulator of calcium homeostasis. *Science* 316:1615–1618.
- Jacobsen SE, Gether U, and Bräuner-Osborne H (2017) Investigating the molecular mechanism of positive and negative allosteric modulators in the calcium-sensing receptor dimer. *Sci Rep* 7:46355.
- Janicic N, Soliman E, Pausova Z, Seldin MF, Riviere M, Szpirer J, Szpirer C, and Hendy GN (1995) Mapping of the calcium-sensing receptor gene (CaSR) to human chromosome 3q13.3-21 by fluorescence in situ hybridization, and localization to rat chromosome 11 and mouse chromosome 16. *Mamm Genome* 6:798–801.
- Jensen AA, Greenwood JR, and Bräuner-Osborne H (2002) The dance of the clams: twists and turns in the family C GPCR homodimer. *Trends Pharmacol Sci* 23: 491–493.
- John MR, Harfst E, Loeffler J, Belleli R, Mason J, Bruin GJ, Seuwen K, Klickstein LB, Mindeholm L, Widler L, et al. (2014) AXT914 a novel, orally-active parathyroid hormone-releasing drug in two early studies of healthy volunteers and postmenopausal women. *Bone* 64:204–210.
- Jones BL and Smith SM (2016) Calcium-sensing receptor: a key target for extracellular calcium signaling in neurons. *Front Physiol* 7:116.
- Josephs TM, Keller AN, Khajehali E, DeBono A, Langmead CJ, Conigrave AD, Capuano B, Kufareva I, Gregory KJ, and Leach K (2019) Negative allosteric modulators of the human calcium-sensing receptor bind to overlapping and distinct sites within the 7-transmembrane domain. *Br J Pharmacol* DOI: 10.1111/bph. 14961 [published ahead of print].
- Jungman E, Pirot F, and Maibach H (2012) Ex vivo calcium percutaneous egression in normal and tape-stripped human skin. *Cutan Ocul Toxicol* 31:1–6.
- Kállay E, Bonner E, Wrba F, Thakker RV, Peterlik M, and Cross HS (2003) Molecular and functional characterization of the extracellular calcium-sensing receptor in human colon cancer cells. *Oncol Res* 13:551–559.
- Kállay E, Kifor O, Chattopadhyay N, Brown EM, Bischof MG, Peterlik M, and Cross HS (1997) Calcium-dependent c-myc proto-oncogene expression and proliferation of Caco-2 cells: a role for a luminal extracellular calcium-sensing receptor. *Biochem Biophys Res Commun* 232:80–83.
- Kameda T, Mano H, Yamada Y, Takai H, Amizuka N, Kobori M, Izumi N, Kawashima H, Ozawa H, Ikeda K, et al. (1998) Calcium-sensing receptor in mature osteoclasts, which are bone resorbing cells. *Biochem Biophys Res Commun* 245: 419–422.
- Kanatani M, Sugimoto T, Kanzawa M, Yano S, and Chihara K (1999) High extracellular calcium inhibits osteoclast-like cell formation by directly acting on the calcium-sensing receptor existing in osteoclast precursor cells. *Biochem Biophys Res Commun* 261:144–148.
- Kantham L, Quinn SJ, Egbuna OI, Baxi K, Butters R, Pang JL, Pollak MR, Goltzman D, and Brown EM (2009) The calcium-sensing receptor (CaSR) defends against hypercalcaemia independently of its regulation of parathyroid hormone secretion. *Am J Physiol Endocrinol Metab* 297:E915–E923.
- Kapoor A, Satishchandra P, Ratnapriya R, Reddy R, Kadandale J, Shankar SK, and Anand A (2008) An idiopathic epilepsy syndrome linked to 3q13.3-q21 and missense mutations in the extracellular calcium sensing receptor gene. *Ann Neurol* 64:158–167.
- Katritch V, Cherezov V, and Stevens RC (2013) Structure-function of the G protein-coupled receptor superfamily. *Annu Rev Pharmacol Toxicol* 53:531–556.
- Kawata T, Nagano N, Obi M, Miyata S, Koyama C, Kobayashi N, Wakita S, and Wada M (2008) Cinacalcet suppresses calcification of the aorta and heart in uremic rats. *Kidney Int* 74:1270–1277.

- Kawata T, Tokunaga S, Murai M, Masuda N, Haruyama W, Shoukei Y, Hisada Y, Yanagida T, Miyazaki H, Wada M, et al. (2018) A novel calcimimetic agent, evocalcet (MT-4580/KHK7580), suppresses the parathyroid cell function with little effect on the gastrointestinal tract or CYP isozymes in vivo and in vitro. *PLoS One* **13**:e0195316.
- Keller AN, Kufareva I, Josephs TM, Diao J, Mai VT, Conigrave AD, Christopoulos A, Gregory KJ, and Leach K (2018) Identification of global and ligand-specific calcium sensing receptor activation mechanisms. *Mol Pharmacol* **93**:619–630.
- Kelly BT, McCoy AJ, Späte K, Miller SE, Evans PR, Höning S, and Owen DJ (2008) A structural explanation for the binding of endocytic dileucine motifs by the AP2 complex. *Nature* **456**:976–979.
- Kelly JC, Lungchukiet P, and Macleod RJ (2011) Extracellular calcium-sensing receptor inhibition of intestinal epithelial TNF signaling requires CaSR-mediated Wnt5a/Ror2 interaction. *Front Physiol* **2**:17.
- Kenakin T and Christopoulos A (2013) Signalling bias in new drug discovery: detection, quantification and therapeutic impact. *Nat Rev Drug Discov* **12**:205–216.
- Kerstetter JE, Svatisalee CM, Caseria DM, Mitnick ME, and Insogna KL (2000) A threshold for low-protein-diet-induced elevations in parathyroid hormone. *Am J Clin Nutr* **72**:168–173.
- Kessler A, Faure H, Petrel C, Rognan D, Césario M, Ruat M, Dauban P, and Dodd RH (2006) N1-Benzoyl-N2-[1-(1-naphthyl)ethyl]-trans-1,2-diaminocyclohexanes: development of 4-chlorophenylcarboxamide (calhex 231) as a new calcium sensing receptor ligand demonstrating potent calcilytic activity. *J Med Chem* **49**:5119–5128.
- Kessler A, Faure H, Roussanne MC, Ferry S, Ruat M, Dauban P, and Dodd RH (2004) N(1-Arylsulfonyl-N(2)-(1-(1-naphthyl)ethyl)-1,2-diaminocyclohexanes: a new class of calcilytic agents acting at the calcium-sensing receptor. *ChemBioChem* **5**:1131–1136.
- Kifor O, Diaz R, Butters R, and Brown EM (1997) The Ca²⁺-sensing receptor (CaR) activates phospholipases C, A₂, and D in bovine parathyroid and CaR-transfected, human embryonic kidney (HEK293) cells. *J Bone Miner Res* **12**:715–725.
- Kifor O, Diaz R, Butters R, Kifor I, and Brown EM (1998) The calcium-sensing receptor is localized in caveolin-rich plasma membrane domains of bovine parathyroid cells. *J Biol Chem* **273**:21708–21713.
- Kifor O, Kifor I, Moore FD Jr, Butters RR Jr, Cantor T, Gao P, and Brown EM (2003) Decreased expression of caveolin-1 and altered regulation of mitogen-activated protein kinase in cultured bovine parathyroid cells and human parathyroid adenomas. *J Clin Endocrinol Metab* **88**:4455–4464.
- Kifor O, MacLeod RJ, Diaz R, Bai M, Yamaguchi T, Yao T, Kifor I, and Brown EM (2001) Regulation of MAP kinase by calcium-sensing receptor in bovine parathyroid and CaR-transfected HEK293 cells. *Am J Physiol Renal Physiol* **280**:F291–F302.
- Kifor O, Moore FD Jr, Wang P, Goldstein M, Vassilev P, Kifor I, Hebert SC, and Brown EM (1996) Reduced immunostaining for the extracellular Ca²⁺-sensing receptor in primary and uremic secondary hyperparathyroidism. *J Clin Endocrinol Metab* **81**:1598–1606.
- Kim CH, Braud S, Isaac JT, and Roche KW (2005) Protein kinase C phosphorylation of the metabotropic glutamate receptor mGluR5 on Serine 839 regulates Ca²⁺ oscillations. *J Biol Chem* **280**:25409–25415.
- Kim JY, Ho H, Kim N, Liu J, Tu CL, Yenari MA, and Chang W (2014) Calcium-sensing receptor (CaSR) as a novel target for ischemic neuroprotection. *Ann Clin Transl Neurol* **1**:851–866.
- Kim JY, Kim N, Yenari MA, and Chang W (2013) Hypothermia and pharmacological regimens that prevent overexpression and overactivity of the extracellular calcium-sensing receptor protect neurons against traumatic brain injury. *J Neurotrauma* **30**:1170–1176.
- Kim W, Takyar FM, Swan K, Jeong J, VanHouten J, Sullivan C, Dann P, Yu H, Fiaschi-Taesch N, Chang W, et al. (2016) Calcium-sensing receptor promotes breast cancer by stimulating intracellular actions of parathyroid hormone-related protein. *Cancer Res* **76**:5348–5360.
- Kim W and Wysolmerski JJ (2016) Calcium-sensing receptor in breast physiology and cancer. *Front Physiol* **7**:440.
- Kirberger M, Wang X, Deng H, Yang W, Chen G, and Yang JJ (2008) Statistical analysis of structural characteristics of protein Ca²⁺-binding sites. *J Biol Inorg Chem* **13**:1169–1181.
- Kitay AM, Schneebacher MT, Schmitt A, Heschl K, Kopic S, Alfadda T, Alsaihati A, Link A, and Geibel JP (2018) Modulations in extracellular calcium lead to H⁺-ATPase-dependent acid secretion: a clarification of PPI failure. *Am J Physiol Gastrointest Liver Physiol* **315**:G36–G42.
- Kitsou-Mylonas I, Burns CJ, Squires PE, Persaud SJ, and Jones PM (2008) A role for the extracellular calcium-sensing receptor in cell-cell communication in pancreatic islets of langerhans. *Cell Physiol Biochem* **22**:557–566.
- Koehl A, Hu H, Feng D, Sun B, Zhang Y, Robertson MJ, Chu M, Kobilka TS, Laeremans T, Steyaert J, et al. (2019) Structural insights into the activation of metabotropic glutamate receptors. *Nature* **566**:79–84.
- Koh J, Dar M, Untch BR, Dixit D, Shi Y, Yang Z, Adam MA, Dressman H, Wang X, Gesty-Palmer D, et al. (2011) Regulator of G protein signaling 5 is highly expressed in parathyroid tumors and inhibits signaling by the calcium-sensing receptor. *Mol Endocrinol* **25**:867–876.
- Komuves L, Oda Y, Tu CL, Chang WH, Ho-Pao CL, Mauro T, and Bikle DD (2002) Epidermal expression of the full-length extracellular calcium-sensing receptor is required for normal keratinocyte differentiation. *J Cell Physiol* **192**:45–54.
- Kos CH, Karaplis AC, Peng JB, Hediger MA, Goltzman D, Mohammad KS, Guise TA, and Pollak MR (2003) The calcium-sensing receptor is required for normal calcium homeostasis independent of parathyroid hormone. *J Clin Invest* **111**:1021–1028.
- Krissinel E and Henrick K (2007) Inference of macromolecular assemblies from crystalline state. *J Mol Biol* **372**:774–797.
- Kruse AC, Ring AM, Manglik A, Hu J, Hu K, Eitel K, Hübner H, Pardon E, Valant C, Sexton PM, et al. (2013) Activation and allosteric modulation of a muscarinic acetylcholine receptor. *Nature* **504**:101–106.
- Kumar S, Matheny CJ, Hoffman SJ, Marquis RW, Schultz M, Liang X, Vasko JA, Stroup GB, Vaden VR, Haley H, et al. (2010) An orally active calcium-sensing receptor antagonist that transiently increases plasma concentrations of PTH and stimulates bone formation. *Bone* **46**:534–542.
- Kunishima N, Shimada Y, Tsuji Y, Sato T, Yamamoto M, Kumasaka T, Nakanishi S, Jingami H, and Morikawa K (2000) Structural basis of glutamate recognition by a dimeric metabotropic glutamate receptor. *Nature* **407**:971–977.
- Kurosawa M, Shimizu Y, Tsukagoshi H, and Ueki M (1992) Elevated levels of peripheral-blood, naturally occurring aliphatic polyamines in bronchial asthmatic patients with active symptoms. *Allergy* **47**:638–643.
- Ladds G, Goddard A, Hill C, Thornton S, and Davey J (2007) Differential effects of RGS proteins on G alpha(q) and G alpha(11) activity. *Cell Signal* **19**:103–113.
- Lagerström MC and Schiöth HB (2008) Structural diversity of G protein-coupled receptors and significance for drug discovery. *Nat Rev Drug Discov* **7**:339–357.
- Lazarus S, Pretorius CJ, Khafagi F, Campion KL, Brennan SC, Conigrave AD, Brown EM, and Ward DT (2011) A novel mutation of the primary protein kinase C phosphorylation site in the calcium-sensing receptor causes autosomal dominant hypocalcemia. *Eur J Endocrinol* **164**:429–435.
- Leach K and Gregory KJ (2017) Molecular insights into allosteric modulation of class C G protein-coupled receptors. *Pharmacol Res* **116**:105–118.
- Leach K, Gregory KJ, Kufareva I, Khajehali E, Cook AE, Abagyan R, Conigrave AD, Sexton PM, and Christopoulos A (2016) Towards a structural understanding of allosteric drugs at the human calcium-sensing receptor. *Cell Res* **26**:574–592.
- Leach K, Sexton PM, and Christopoulos A (2007) Allosteric GPCR modulators: taking advantage of permissive receptor pharmacology. *Trends Pharmacol Sci* **28**:382–389.
- Leach K, Sexton PM, Christopoulos A, and Conigrave AD (2014) Engendering biased signalling from the calcium-sensing receptor for the pharmacotherapy of diverse disorders. *Br J Pharmacol* **171**:1142–1155.
- Leach K, Wen A, Cook AE, Sexton PM, Conigrave AD, and Christopoulos A (2013) Impact of clinically relevant mutations on the pharmacoregulation and signaling bias of the calcium-sensing receptor by positive and negative allosteric modulators. *Endocrinology* **154**:1105–1116.
- Leach K, Wen A, Davey AE, Sexton PM, Conigrave AD, and Christopoulos A (2012) Identification of molecular phenotypes and biased signaling induced by naturally occurring mutations of the human calcium-sensing receptor. *Endocrinology* **153**:4304–4316.
- LeBoff MS, Shoback D, Brown EM, Thatcher J, Leombruno R, Beaudoin D, Henry M, Wilson R, Pallotta J, Marynick S, et al. (1985) Regulation of parathyroid hormone release and cytosolic calcium by extracellular calcium in dispersed and cultured bovine and pathological human parathyroid cells. *J Clin Invest* **75**:49–57.
- Lee HJ, Mun H-C, Lewis NC, Crouch MF, Culverston EL, Mason RS, and Conigrave AD (2007) Allosteric activation of the extracellular Ca²⁺-sensing receptor by L-amino acids enhances ERK1/2 phosphorylation. *Biochem J* **404**:141–149.
- Lee JJ, Liu X, O'Neill D, Beggs MR, Weissgerber P, Flockner V, Chen XZ, Dimke H, and Alexander RT (2019) Activation of the calcium sensing receptor attenuates TRPV6-dependent intestinal calcium absorption. *JCI Insight* **5**:e128013.
- Letz S, Haag C, Schulze E, Frank-Raue K, Raue F, Hofner B, Mayr B, and Schöfl C (2014) Amino alcohol- (NPS-2143) and quinazolinone-derived calcilytics (ATF936 and AXT914) differentially mitigate excessive signalling of calcium-sensing receptor mutants causing Bartter syndrome Type 5 and autosomal dominant hypocalcemia. *PLoS One* **9**:e115178.
- Letz S, Rus R, Haag C, Dörr HG, Schnabel D, Möhlig M, Schulze E, Frank-Raue K, Raue F, Mayr B, et al. (2010) Novel activating mutations of the calcium-sensing receptor: the calcilytic NPS-2143 mitigates excessive signal transduction of mutant receptors. *J Clin Endocrinol Metab* **95**:E229–E233.
- Li D, Opas EE, Tuluc F, Metzger DL, Hou C, Hakonarson H, and Levine MA (2014a) Autosomal dominant hypoparathyroidism caused by germline mutation in GNA11: phenotypic and molecular characterization. *J Clin Endocrinol Metab* **99**:E1774–E1783.
- Li X, Kong X, Jiang L, Ma T, Yan S, Yuan C, and Yang Q (2014b) A genetic polymorphism (rs17251221) in the calcium-sensing receptor is associated with breast cancer susceptibility and prognosis. *Cell Physiol Biochem* **33**:165–172.
- Lietman SA, Tenenbaum-Rakover Y, Jap TS, Yi-Chi W, De-Ming Y, Ding C, Kussiny N, and Levine MA (2009) A novel loss-of-function mutation, Gln459Arg, of the calcium-sensing receptor gene associated with apparent autosomal recessive inheritance of familial hypocalciuric hypercalcemia. *J Clin Endocrinol Metab* **94**:4372–4379.
- Liou AP, Sei Y, Zhao X, Feng J, Lu X, Thomas C, Pechhold S, Raybould HE, and Wank SA (2011) The extracellular calcium-sensing receptor is required for cholecystokinin secretion in response to L-phenylalanine in acutely isolated intestinal I cells. *Am J Physiol Gastrointest Liver Physiol* **300**:G538–G546.
- Liu G, Cao W, Jia G, Zhao H, Chen X, and Wang J (2018) Calcium-sensing receptor in nutrient sensing: an insight into the modulation of intestinal homeostasis. *Br J Nutr* **120**:881–890.
- Liu G, Hu X, and Chakrabarty S (2009) Calcium sensing receptor down-regulates malignant cell behavior and promotes chemosensitivity in human breast cancer cells. *Cell Calcium* **45**:216–225.
- Liu XL, Lu YS, Gao JY, Marshall C, Xiao M, Miao DS, Karaplis A, Goltzman D, and Ding J (2013) Calcium sensing receptor absence delays postnatal brain development via direct and indirect mechanisms. *Mol Neurobiol* **48**:590–600.
- Locatelli F, Cannata-Andia JB, Drüeke TB, Hörl WH, Fouque D, Heimbürger O, and Ritz E (2002) Management of disturbances of calcium and phosphate metabolism in chronic renal insufficiency, with emphasis on the control of hyperphosphataemia. *Nephrol Dial Transplant* **17**:723–731.
- Lorenz S, Frenzel R, Paschke R, Breitwieser GE, and Miedlich SU (2007) Functional desensitization of the extracellular calcium-sensing receptor is regulated via distinct mechanisms: role of G protein-coupled receptor kinases, protein kinase C and β -arrestins. *Endocrinology* **148**:2398–2404.
- Loupy A, Ramakrishnan SK, Wootla B, Chabmrey R, de la Faille R, Bourgeois S, Bruneval P, Mandet C, Christensen EI, Faure H, et al. (2012) PTH-independent

- regulation of blood calcium concentration by the calcium-sensing receptor. *J Clin Invest* **122**:3355–3367.
- Lozano-Ortega G, Waser N, Bensink ME, Goring S, Bennett H, Block GA, Chertow GM, Trotman ML, Cooper K, Levy AR, et al. (2018) Effects of calcimimetics on long-term outcomes in dialysis patients: literature review and Bayesian meta-analysis. *J Comp Eff Res* **7**:693–707.
- Lu JY, Yang Y, Gnacadja G, Christopoulos A, and Reagan JD (2009) Effect of the calcimimetic R-568 [3-(2-chlorophenyl)-N-((1R)-1-(3-methoxyphenyl)ethyl)-1-propylamine] on correcting inactivating mutations in the human calcium-sensing receptor. *J Pharmacol Exp Ther* **331**:775–786.
- Ma JN, Owens M, Gustafsson M, Jensen J, Tabatabaei A, Schmelzer K, Olsson R, and Burstein ES (2011) Characterization of highly efficacious allosteric agonists of the human calcium-sensing receptor. *J Pharmacol Exp Ther* **337**:275–284.
- MacLeod RJ, Chattopadhyay N, and Brown EM (2003) PTHrP stimulated by the calcium-sensing receptor requires MAP kinase activation. *Am J Physiol Endocrinol Metab* **284**:E435–E442.
- MacLeod RJ, Yano S, Chattopadhyay N, and Brown EM (2004) Extracellular calcium-sensing receptor transactivates the epidermal growth factor receptor by a triple-membrane-spanning signaling mechanism. *Biochem Biophys Res Commun* **320**:455–460.
- Makita N, Ando T, Sato J, Manaka K, Mitani K, Kikuchi Y, Niwa T, Ootaki M, Takeba Y, Matsumoto N, et al. (2019) Cincalcet corrects biased allosteric modulation of CaSR by AHH autoantibody. *JCI Insight* **4**.
- Makita N and Iiri T (2014) Biased agonism: a novel paradigm in G protein-coupled receptor signaling observed in acquired hypocalciuric hypercalcemia. *Endocr J* **61**:303–309.
- Makita N, Sato J, Manaka K, Shoji Y, Oishi A, Hashimoto M, Fujita T, and Iiri T (2007) An acquired hypocalciuric hypercalcemia autoantibody induces allosteric transition among active human Ca-sensing receptor conformations. *Proc Natl Acad Sci USA* **104**:5443–5448.
- Mamillapalli R, VanHouten J, Zawlich W, and Wysolmerski J (2008) Switching of G-protein usage by the calcium-sensing receptor reverses its effect on parathyroid hormone-related protein secretion in normal versus malignant breast cells. *J Biol Chem* **283**:24435–24447.
- Mamillapalli R and Wysolmerski J (2010) The calcium-sensing receptor couples to Galphas and regulates PTHrP and ACTH secretion in pituitary cells. *J Endocrinol* **204**:287–297.
- Mannstadt M, Harris M, Bravenboer B, Chitturi S, Dreijerink KM, Lambright DG, Lim ET, Daly MJ, Gabriel S, and Juppner H (2013) Germline mutations affecting Gc11 in hypoparathyroidism. *N Engl J Med* **368**:2532–2534.
- Margolis KG, Stevanovic K, Karamooz N, Li ZS, Ahuja A, D'Autreaux F, Saurman V, Chalazontis A, and Gershon MD (2011) Enteric neuronal density contributes to the severity of intestinal inflammation. *Gastroenterology* **141**:588–598, 598.e1–598.e2.
- Marie PJ (2010) The calcium-sensing receptor in bone cells: a potential therapeutic target in osteoporosis. *Bone* **46**:571–576.
- Masvidal L, Iniesta R, Casalá C, Galván P, Rodríguez E, Lavarino C, Mora J, and de Torres C (2013) Polymorphisms in the calcium-sensing receptor gene are associated with clinical outcome of neuroblastoma. *PLoS One* **8**:e59762.
- Masvidal L, Iniesta R, García M, Casalá C, Lavarino C, Mora J, and de Torres C (2017) Genetic variants in the promoter region of the calcium-sensing receptor gene are associated with its down-regulation in neuroblastic tumors. *Mol Carcinog* **56**:1281–1289.
- McCormick WD, Atkinson-Dell R, Campion KL, Mun HC, Conigrave AD, and Ward DT (2010) Increased receptor stimulation elicits differential calcium-sensing receptor (T888) dephosphorylation. *J Biol Chem* **285**:14170–14177.
- McGehee DS, Aldersberg M, Liu KP, Hsuing S, Heath MJ, and Tamir H (1997) Mechanism of extracellular Ca²⁺ receptor-stimulated hormone release from sheep thyroid parafollicular cells. *J Physiol* **502**:31–44.
- McLarnon S, Holden D, Ward D, Jones M, Elliott A, and Riccardi D (2002) Aminoglycoside antibiotics induce pH-sensitive activation of the calcium-sensing receptor. *Biochem Biophys Res Commun* **297**:71–77.
- McLarnon SJ and Riccardi D (2002) Physiological and pharmacological agonists of the extracellular Ca²⁺-sensing receptor. *Eur J Pharmacol* **447**:271–278.
- McMurtry CT, Schranck FW, Walkenhorst DA, Murphy WA, Kocher DB, Teitelbaum SL, Rupich RC, and Whyte MP (1992) Significant developmental elevation in serum parathyroid hormone levels in a large kindred with familial benign (hypocalciuric) hypercalcemia. *Am J Med* **93**:247–258.
- Meng K, Xu J, Zhang C, Zhang R, Yang H, Liao C, and Jiao J (2014) Calcium sensing receptor modulates extracellular calcium entry and proliferation via TRPC3/6 channels in cultured human mesangial cells. *PLoS One* **9**:e98777.
- Menon GK and Elias PM (1991) Ultrastructural localization of calcium in psoriatic and normal human epidermis. *Arch Dermatol* **127**:57–63.
- Menon GK, Elias PM, and Feingold KR (1994) Integrity of the permeability barrier is crucial for maintenance of the epidermal calcium gradient. *Br J Dermatol* **130**:139–147.
- Menon GK, Grayson S, and Elias PM (1985) Ionic calcium reservoirs in mammalian epidermis: ultrastructural localization by ion-capture cytochemistry. *J Invest Dermatol* **84**:508–512.
- Mentaverri R, Yano S, Chattopadhyay N, Petit L, Kifor O, Kamel S, Terwilliger EF, Brazier M, and Brown EM (2006) The calcium sensing receptor is directly involved in both osteoclast differentiation and apoptosis. *FASEB J* **20**:2562–2564.
- Miedlich SU, Gama L, Seuwen K, Wolf RM, and Breitwieser GE (2004) Homology modeling of the transmembrane domain of the human calcium sensing receptor and localization of an allosteric binding site. *J Biol Chem* **279**:7254–7263.
- Miller-Fleming L, Olin-Sandoval V, Campbell K, and Ralser M (2015) Remaining mysteries of molecular biology: the role of polyamines in the cell. *J Mol Biol* **427**:3389–3406.
- Mine Y and Zhang H (2015) Anti-inflammatory effects of poly-L-lysine in intestinal mucosal system mediated by calcium-sensing receptor activation. *J Agric Food Chem* **63**:10437–10447.
- Mizobuchi M, Ritter CS, Krits I, Slatopolsky E, Sicard G, and Brown AJ (2009) Calcium-sensing receptor expression is regulated by glial cells missing-2 in human parathyroid cells. *J Bone Miner Res* **24**:1173–1179.
- Mos I, Jacobsen SE, Foster SR, and Bräuner-Osborne H (2019) Calcium-sensing receptor internalization is β -arrestin-dependent and modulated by allosteric ligands. *Mol Pharmacol* **96**:463–474.
- Muff R, Nemeth EF, Haller-Brem S, and Fischer JA (1988) Regulation of hormone secretion and cytosolic Ca²⁺ by extracellular Ca²⁺ in parathyroid cells and C-cells: role of voltage-sensitive Ca²⁺ channels. *Arch Biochem Biophys* **265**:128–135.
- Mun HC, Culverston EL, Franks AH, Collyer CA, Clifton-Bligh RJ, and Conigrave AD (2005) A double mutation in the extracellular Ca²⁺-sensing receptor's venus flytrap domain that selectively disables L-amino acid sensing. *J Biol Chem* **280**:29067–29072.
- Mun HC, Franks AH, Culverston EL, Krapcho K, Nemeth EF, and Conigrave AD (2004) The venus fly trap domain of the extracellular Ca²⁺-sensing receptor is required for L-amino acid sensing. *J Biol Chem* **279**:51739–51744.
- Mun HC, Leach KM, and Conigrave AD (2019) L-amino acids promote calcitonin release via a calcium-sensing receptor: Gq/11-mediated pathway in human C-cells. *Endocrinology* **160**:1590–1599.
- Munk C, Isberg V, Mordalski S, Harpsøe K, Rataj K, Hauser AS, Kolb P, Bojarski AJ, Vriend G, and Gloriam DE (2016) GPCRdb: the G protein-coupled receptor database - an introduction. *Br J Pharmacol* **173**:2195–2207.
- Murer H, Hernandez N, Forster I, and Biber J (2001) Molecular aspects in the regulation of renal inorganic phosphate reabsorption: the type IIa sodium/inorganic phosphate co-transporter as the key player. *Curr Opin Nephrol Hypertens* **10**:555–561.
- Muresan Z and MacGregor RR (1994) The release of parathyroid hormone and the exocytosis of a proteoglycan are modulated by extracellular Ca²⁺ in a similar manner. *Mol Biol Cell* **5**:725–737.
- Muto T, Tsuchiya D, Morikawa K, and Jingami H (2007) Structures of the extracellular regions of the group II/III metabotropic glutamate receptors. *Proc Natl Acad Sci USA* **104**:3759–3764.
- Nakagawa K, Parekh N, Koleganova N, Ritz E, Schaefer F, and Schmitt CP (2009) Acute cardiovascular effects of the calcimimetic R-568 and its enantiomer S-568 in rats. *Pediatr Nephrol* **24**:1385–1389.
- Nakamura H, Tsujiguchi H, Hara A, Kambayashi Y, Miyagi S, Thu Nguyen TT, Suzuki K, Tao Y, Sakamoto Y, Shimizu Y, et al. (2019) Dietary calcium intake and hypertension: importance of serum concentrations of 25-hydroxyvitamin D. *Nutrients* **11**:E911.
- Nearing J, Betka M, Quinn S, Hentschel H, Elger M, Baum M, Bai M, Chattopadhyay N, Brown EM, Hebert SC, et al. (2002) Polyvalent cation receptor proteins (CaRs) are salinity sensors in fish. *Proc Natl Acad Sci USA* **99**:9231–9236.
- Nemeth EF and Scarpa A (1986) Cytosolic Ca²⁺ and the regulation of secretion in parathyroid cells. *FEBS Lett* **203**:15–19.
- Nemeth EF and Scarpa A (1987) Rapid mobilization of cellular Ca²⁺ in bovine parathyroid cells evoked by extracellular divalent cations. Evidence for a cell surface calcium receptor. *J Biol Chem* **262**:5188–5196.
- Nemeth EF, Van Wagenen BC, and Balandrin MF (2018) Discovery and development of calcimimetic and calcilytic compounds. *Prog Med Chem* **57**:1–86.
- Nemeth EF, Wallace J, and Scarpa A (1986) Stimulus-secretion coupling in bovine parathyroid cells. Dissociation between secretion and net changes in cytosolic Ca²⁺. *J Biol Chem* **261**:2668–2674.
- Nesbit MA, Hannan FM, Howles SA, Babinsky VN, Head RA, Cranston T, Rust N, Hobbs MR, Heath H III, and Thakker RV (2013a) Mutations affecting G-protein subunit $\alpha 11$ in hypercalcemia and hypocalcemia. *N Engl J Med* **368**:2476–2486.
- Nesbit MA, Hannan FM, Howles SA, Reed AA, Cranston T, Thakker CE, Gregory L, Rimmer AJ, Rust N, Graham U, et al. (2013b) Mutations in AP2S1 cause familial hypocalciuric hypercalcemia type 3. *Nat Genet* **45**:93–97.
- North ML, Grasemann H, Khanna N, Inman MD, Gauvreau GM, and Scott JA (2013) Increased ornithine-derived polyamines cause airway hyperresponsiveness in a mouse model of asthma. *Am J Respir Cell Mol Biol* **48**:694–702.
- Oda Y, Hu L, Nguyen T, Pong C, Tu CL, and Bikle DD (2017) Combined deletion of the vitamin D receptor and calcium-sensing receptor delays wound re-epithelialization. *Endocrinology* **158**:1929–1938.
- Oda Y, Tu CL, Pillai S, and Bikle DD (1998) The calcium sensing receptor and its alternatively spliced form in keratinocyte differentiation. *J Biol Chem* **273**:23344–23352.
- Odenwald T, Nakagawa K, Hadtstein C, Roesch F, Gohlke P, Ritz E, Schaefer F, and Schmitt CP (2006) Acute blood pressure effects and chronic hypotensive action of calcimimetics in uremic rats. *J Am Soc Nephrol* **17**:655–662.
- Ogata H, Ritz E, Odoni G, Amann K, and Orth SR (2003) Beneficial effects of calcimimetics on progression of renal failure and cardiovascular risk factors. *J Am Soc Nephrol* **14**:959–967.
- Oh YS, Seo EH, Lee YS, Cho SC, Jung HS, Park SC, and Jun HS (2016) Increase of calcium sensing receptor expression is related to compensatory insulin secretion during aging in mice. *PLoS One* **11**:e0159689.
- O'Hara PJ, Sheppard PO, Thøgersen H, Venezia D, Haldeman BA, McGrane V, Houamed KM, Thomsen C, Gilbert TL, and Mulvihill ER (1993) The ligand-binding domain in metabotropic glutamate receptors is related to bacterial periplasmic binding proteins. *Neuron* **11**:41–52.
- Ohsu T, Amino Y, Nagasaki H, Yamanaka T, Takeshita S, Hatanaka T, Maruyama Y, Miyamura N, and Eto Y (2010) Involvement of the calcium-sensing receptor in human taste perception. *J Biol Chem* **285**:1016–1022.
- Owen JL, Cheng SX, Ge Y, Sahay B, and Mohammadzadeh M (2016) The role of the calcium-sensing receptor in gastrointestinal inflammation. *Semin Cell Dev Biol* **49**:44–51.
- Pais R, Gribble FM, and Reimann F (2016) Signalling pathways involved in the detection of peptones by murine small intestinal enteroendocrine L-cells. *Peptides* **77**:9–15.
- Pallais JC, Kemp EH, Bergwitz C, Kanthan L, Slovik DM, Weetman AP, and Brown EM (2011) Autoimmune hypocalciuric hypercalcemia unresponsive to

- glucocorticoid therapy in a patient with blocking autoantibodies against the calcium-sensing receptor. *J Clin Endocrinol Metab* **96**:672–680.
- Pearce SH, Trump D, Wooding C, Besser GM, Chew SL, Grant DB, Heath DA, Hughes IA, Paterson CR, Whyte MP, et al. (1995) Calcium-sensing receptor mutations in familial benign hypercalcemia and neonatal hyperparathyroidism. *J Clin Invest* **96**:2683–2692.
- Pearce SH, Williamson C, Kifor O, Bai M, Coulthard MG, Davies M, Lewis-Barned N, McCredie D, Powell H, Kendall-Taylor P, et al. (1996) A familial syndrome of hypocalcemia with hypercalciuria due to mutations in the calcium-sensing receptor. *N Engl J Med* **335**:1115–1122.
- Peiris D, Pacheco I, Spencer C, and MacLeod RJ (2007) The extracellular calcium-sensing receptor reciprocally regulates the secretion of BMP-2 and the BMP antagonist Noggin in colonic myofibroblasts. *Am J Physiol Gastrointest Liver Physiol* **292**:G753–G766.
- Perez-Benito L, Doornbos MLJ, Cordomi A, Peeters L, Lavreysen H, Pardo L, and Tresadern G (2017) Molecular switches of allosteric modulation of the metabotropic glutamate 2 receptor. *Structure* **25**:1153–1162.e4.
- Petrel C, Kessler A, Dauban P, Dodd RH, Rognan D, and Ruat M (2004) Positive and negative allosteric modulators of the Ca²⁺-sensing receptor interact within overlapping but not identical binding sites in the transmembrane domain. *J Biol Chem* **279**:18990–18997.
- Petrel C, Kessler A, Maslah F, Dauban P, Dodd RH, Rognan D, and Ruat M (2003) Modeling and mutagenesis of the binding site of Calhex 231, a novel negative allosteric modulator of the extracellular Ca(2+)-sensing receptor. *J Biol Chem* **278**:49487–49494.
- Pi M, Faber P, Ekema G, Jackson PD, Ting A, Wang N, Fontilla-Poole M, Mays RW, Brunden KR, Harrington JJ, et al. (2005a) Identification of a novel extracellular calcium-sensing G-protein-coupled receptor. *J Biol Chem* **280**:40201–40209.
- Pi M, Oakley RH, Gesty-Palmer D, Cruickshank RD, Spurney RF, Luttrell LM, and Quarles LD (2005b) β -arrestin- and G protein receptor kinase-mediated calcium-sensing receptor desensitization. *Mol Endocrinol* **19**:1078–1087.
- Pi M, Spurney RF, Tu Q, Hinson T, and Quarles LD (2002) Calcium-sensing receptor activation of rho involves flamin and rho-guanine nucleotide exchange factor. *Endocrinology* **143**:3830–3838.
- Pidasheva S, Grant M, Canaff L, Ercan O, Kumar U, and Hendy GN (2006) Calcium-sensing receptor dimerizes in the endoplasmic reticulum: biochemical and biophysical characterization of CASR mutants retained intracellularly. *Hum Mol Genet* **15**:2200–2209.
- Pin JP and Bettler B (2016) Organization and functions of mGlu and GABA_B receptor complexes. *Nature* **540**:60–68.
- Piret SE, Gorvin CM, Pagnamenta AT, Howles SA, Cranston T, Rust N, Nesbit AM, Glaser B, Taylor JC, Buchs AE, et al. (2016) Identification of a G-Protein subunit- α 11 gain-of-function mutation, Val340Met, in a family with autosomal dominant hypocalcemia type 2 (ADH2). *J Bone Miner Res* **31**:1207–1214.
- Piret SE and Thakker RV (2011) Mouse models for inherited endocrine and metabolic disorders. *J Endocrinol* **211**:211–230.
- Pollak MR, Brown EM, Chou YH, Hebert SC, Marx SJ, Steinmann B, Levi T, Seidman CE, and Seidman JG (1993) Mutations in the human Ca²⁺-sensing receptor gene cause familial hypocalcemic hypercalcemia and neonatal severe hyperparathyroidism. *Cell* **75**:1297–1303.
- Pollak MR, Brown EM, Estep HL, McLaine PN, Kifor O, Park J, Hebert SC, Seidman CE, and Seidman JG (1994) Autosomal dominant hypocalcemia caused by a Ca²⁺-sensing receptor gene mutation. *Nat Genet* **8**:303–307.
- Procino G, Mastrofrancesco L, Tammaro G, Lasorsa DR, Ranieri M, Stringini G, Emma F, Svelto M, and Valenti G (2012) Calcium-sensing receptor and aquaporin 2 interplay in hypercalciuria-associated renal concentrating defect in humans. An in vivo and in vitro study. *PLoS One* **7**:e33145.
- Promkan M, Liu G, Patmasiriwat P, and Chakrabarty S (2011) BRCA1 suppresses the expression of survivin and promotes sensitivity to paclitaxel through the calcium sensing receptor (CaSR) in human breast cancer cells. *Cell Calcium* **49**:79–88.
- Quinn SJ, Bai M, and Brown EM (2004) pH Sensing by the calcium-sensing receptor. *J Biol Chem* **279**:37241–37249.
- Quinn SJ, Kifor O, Trivedi S, Diaz R, Vassilev P, and Brown E (1998) Sodium and ionic strength sensing by the calcium receptor. *J Biol Chem* **273**:19579–19586.
- Quinn SJ, Ye CP, Diaz R, Kifor O, Bai M, Vassilev P, and Brown E (1997) The Ca²⁺-sensing receptor: a target for polyamines. *Am J Physiol* **273**:C1315–C1323.
- Racke FK, Hammerland LG, Dubyak GR, and Nemeth EF (1993) Functional expression of the parathyroid cell calcium receptor in *Xenopus* oocytes. *FEBS Lett* **333**:132–136.
- Ramirez JA, Goodman WG, Gornbein J, Menezes C, Moulton L, Segre GV, and Salusky IB (1993) Direct in vivo comparison of calcium-regulated parathyroid hormone secretion in normal volunteers and patients with secondary hyperparathyroidism. *J Clin Endocrinol Metab* **76**:1489–1494.
- Rasmussen AQ, Jørgensen NR, and Schwarz P (2011) Clinical and biochemical outcomes of cinacalcet treatment of familial hypocalcemic hypercalcemia: a case series. *J Med Case Rep* **5**:564.
- Ratnam AV, Bikle DD, and Cho JK (1999) 1,25 dihydroxyvitamin D3 enhances the calcium response of keratinocytes. *J Cell Physiol* **178**:188–196.
- Ray JM, Squires PE, Curtis SB, Meloche MR, and Buchan AM (1997) Expression of the calcium-sensing receptor on human antral gastrin cells in culture. *J Clin Invest* **99**:2328–2333.
- Ray K (2015) Calcium-sensing receptor: trafficking, endocytosis, recycling, and importance of interacting proteins. *Prog Mol Biol Transl Sci* **132**:127–150.
- Ray K, Clapp P, Goldsmith PK, and Spiegel AM (1998) Identification of the sites of N-linked glycosylation on the human calcium receptor and assessment of their role in cell surface expression and signal transduction. *J Biol Chem* **273**:34558–34567.
- Ray K, Hauschild BC, Steinbach PJ, Goldsmith PK, Hauache O, and Spiegel AM (1999) Identification of the cysteine residues in the amino-terminal extracellular domain of the human Ca(2+) receptor critical for dimerization. Implications for function of monomeric Ca(2+) receptor. *J Biol Chem* **274**:27642–27650.
- Ray K and Northup J (2002) Evidence for distinct cation and calcimimetic compound (NPS 568) recognition domains in the transmembrane regions of the human Ca²⁺-receptor. *J Biol Chem* **277**:18908–18913.
- Reginster JY, Seeman E, De Vernejoul MC, Adams S, Compston J, Phenekos C, Devogelaer JP, Curiel MD, Sawicki A, Goemaere S, et al. (2005) Strontium ranelate reduces the risk of nonvertebral fractures in postmenopausal women with osteoporosis: Treatment of Peripheral Osteoporosis (TROPOS) study. *J Clin Endocrinol Metab* **90**:2816–2822.
- Reh CM, Hendy GN, Cole DE, and Jeandron DD (2011) Neonatal hyperparathyroidism with a heterozygous calcium-sensing receptor (CASR) R185Q mutation: clinical benefit from cinacalcet. *J Clin Endocrinol Metab* **96**:E707–E712.
- Renkema KY, Velic A, Dijkman HB, Verkaar S, van der Kemp AW, Nowik M, Timmermans K, Doucet A, Wagner CA, Bindels RJ, et al. (2009) The calcium-sensing receptor promotes urinary acidification to prevent nephrolithiasis. *J Am Soc Nephrol* **20**:1705–1713.
- Rey O, Chang W, Bikle D, Rozengurt N, Young SH, and Rozengurt E (2012) Negative cross-talk between calcium-sensing receptor and β -catenin signaling systems in colonic epithelium. *J Biol Chem* **287**:1158–1167.
- Rey O, Young SH, Papazyan R, Shapiro MS, and Rozengurt E (2006) Requirement of the TRPC1 cation channel in the generation of transient Ca²⁺ oscillations by the calcium-sensing receptor. *J Biol Chem* **281**:38730–38737.
- Rey O, Young SH, Yuan J, Slice L, and Rozengurt E (2005) Amino acid-stimulated Ca²⁺ oscillations produced by the Ca²⁺-sensing receptor are mediated by a phospholipase C/inositol 1,4,5-trisphosphate-independent pathway that requires G12, Rho, filamin-A, and the actin cytoskeleton. *J Biol Chem* **280**:22875–22882.
- Reyes-Ibarra AP, García-Regalado A, Ramírez-Rangel I, Esparza-Silva AL, Valadez-Sánchez M, Vázquez-Prado J, and Reyes-Cruz G (2007) Calcium-sensing receptor endocytosis links extracellular calcium signaling to parathyroid hormone-related peptide secretion via a Rab11a-dependent and AMSH-sensitive mechanism. *Mol Endocrinol* **21**:1394–1407.
- Riccardi D, Hall AE, Chattopadhyay N, Xu JZ, Brown EM, and Hebert SC (1998) Localization of the extracellular Ca²⁺/polyvalent cation-sensing protein in rat kidney. *Am J Physiol* **274**:F611–F622.
- Riccardi D and Kemp PJ (2012) The calcium-sensing receptor beyond extracellular calcium homeostasis: conception, development, adult physiology, and disease. *Annu Rev Physiol* **74**:271–297.
- Riccardi D, Park J, Lee WS, Gamba G, Brown EM, and Hebert SC (1995) Cloning and functional expression of a rat kidney extracellular calcium/polyvalent cation-sensing receptor. *Proc Natl Acad Sci USA* **92**:131–135.
- Riccardi D and Valenti G (2016) Localization and function of the renal calcium-sensing receptor. *Nat Rev Nephrol* **12**:414–425.
- Rietsema S, Eelderink C, Joutstra ML, van Vliet IMY, van Londen M, Corpeleijn E, Singh-Povel CM, Geurts JMW, Kootstra-Ros JE, Westerhuis R, et al. (2019) Effect of high compared with low dairy intake on blood pressure in overweight middle-aged adults: results of a randomized crossover intervention study. *Am J Clin Nutr* **110**:340–348.
- Ritter CS, Haughey BH, Armbricht HJ, and Brown AJ (2012) Distribution and regulation of the 25-hydroxyvitamin D3 1 α -hydroxylase in human parathyroid glands. *J Steroid Biochem Mol Biol* **130**:73–80.
- Roberts MS, Gafni RI, Brillante B, Guthrie LC, Streit J, Gash D, Gelb J, Krusinska E, Brennan SC, Schepelmann M, et al. (2019) Treatment of autosomal dominant hypocalcemia type 1 with the calcilytic NPS795 (SHP635). *J Bone Miner Res* **34**:1609–1618.
- Rodriguez L, Tu C, Cheng Z, Chen TH, Bikle D, Shoback D, and Chang W (2005) Expression and functional assessment of an alternatively spliced extracellular Ca²⁺-sensing receptor in growth plate chondrocytes. *Endocrinology* **146**:5294–5303.
- Rodríguez-Hernández CJ, Mateo-Lozano S, García M, Casala C, Briansó F, Castrejón N, Rodríguez E, Suñol M, Carcaboso AM, Lavarino C, et al. (2016) Cinacalcet inhibits neuroblastoma tumor growth and upregulates cancer-testis antigens. *Oncotarget* **7**:16112–16129.
- Romano C, Yang WL, and O'Malley KL (1996) Metabotropic glutamate receptor 5 is a disulfide-linked dimer. *J Biol Chem* **271**:28612–28616.
- Rorsman P, Braun M, and Zhang Q (2012) Regulation of calcium in pancreatic α - and β -cells in health and disease. *Cell Calcium* **51**:300–308.
- Roszkó KL, Bi R, Gorvin CM, Bräuner-Osborne H, Xiong XF, Inoue A, Thakker RV, Strömgaard K, Gardella T, and Mannstadt M (2017) Knockin mouse with mutant G α ₁₁ mimics human inherited hypocalcemia and is rescued by pharmacologic inhibitors. *JCI Insight* **2**:e91079.
- Ruat M, Molliver ME, Snowman AM, and Snyder SH (1995) Calcium sensing receptor: molecular cloning in rat and localization to nerve terminals. *Proc Natl Acad Sci USA* **92**:3161–3165.
- Ruat M, Snowman AM, Hester LD, and Snyder SH (1996) Cloned and expressed rat Ca²⁺-sensing receptor. *J Biol Chem* **271**:5972–5975.
- Rus R, Haag C, Bumke-Vogt C, Bähr V, Mayr B, Möhlig M, Schulze E, Frank-Raue K, Raue F, and Schöfl C (2008) Novel inactivating mutations of the calcium-sensing receptor: the calcimimetic NPS R-568 improves signal transduction of mutant receptors. *J Clin Endocrinol Metab* **93**:4797–4803.
- Rybchyn MS, Green WL, Conigrave AD, and Mason RS (2009) Involvement of both GPRC6A and the calcium-sensing receptor in strontium ranelate-induced osteoclastogenic signal expression and replication in primary human osteoblasts. *Bone* **44**:S317–S317.
- Rybchyn MS, Islam KS, Brennan-Speranza TC, Cheng Z, Brennan SC, Chang W, Mason RS, and Conigrave AD (2019) Homer1 mediates CaSR-dependent activation of mTOR complex 2 and initiates a novel pathway for AKT-dependent β -catenin stabilization in osteoblasts. *J Biol Chem* **294**:16337–16350.
- Rybchyn MS, Slater M, Conigrave AD, and Mason RS (2011) An Akt-dependent increase in canonical Wnt signaling and a decrease in sclerostin protein levels are involved in strontium ranelate-induced osteogenic effects in human osteoblasts. *J Biol Chem* **286**:23771–23779.

- Saidak Z, Boudot C, Abdoune R, Petit L, Brazier M, Mentaverri R, and Kamel S (2009) Extracellular calcium promotes the migration of breast cancer cells through the activation of the calcium sensing receptor. *Exp Cell Res* **315**:2072–2080.
- Sands JM, Naruse M, Baum M, Jo I, Hebert SC, Brown EM, and Harris HW (1997) Apical extracellular calcium/polyvalent cation-sensing receptor regulates vasopressin-elicited water permeability in rat kidney inner medullary collecting duct. *J Clin Invest* **99**:1399–1405.
- Santa Maria C, Cheng Z, Li A, Wang J, Shoback D, Tu CL, and Chang W (2016) Interplay between CaSR and PTHrP signaling in skeletal development and osteoanabolism. *Semin Cell Dev Biol* **49**:11–23.
- Schauber J, Dorschner RA, Coda AB, Büchau AS, Liu PT, Kiken D, Helfrich YR, Kang S, Elalieh HZ, Steinmeyer A, et al. (2007) Injury enhances TLR2 function and antimicrobial peptide expression through a vitamin D-dependent mechanism. *J Clin Invest* **117**:803–811.
- Schepelmann M, Yarova PL, Lopez-Fernandez I, Davies TS, Brennan SC, Edwards PJ, Aggarwal A, Graça J, Rietdorf K, Matchkov V, et al. (2016) The vascular Ca²⁺-sensing receptor regulates blood vessel tone and blood pressure. *Am J Physiol Cell Physiol* **310**:C193–C204.
- Sethi BK, Nagesh VS, Kelwade J, Parekh H, and Dukle V (2017) Utility of cinacalcet in familial hypocalciuric hypercalcaemia. *Indian J Endocrinol Metab* **21**:362–363.
- Shalhoub V, Grisanti M, Padagas J, Scully S, Rattan A, Qi M, Varnum B, Vezina C, Lacey D, and Martin D (2003) In vitro studies with the calcimimetic, cinacalcet HCl, on normal human adult osteoblastic and osteoclastic cells. *Crit Rev Eukaryot Gene Expr* **13**:89–106.
- Shams-White MM, Chung M, Du M, Fu Z, Insogna KL, Karlens MC, LeBoff MS, Shapses SA, Sackey J, Wallace TC, et al. (2017) Dietary protein and bone health: a systematic review and meta-analysis from the National Osteoporosis Foundation. *Am J Clin Nutr* **105**:1528–1543.
- Shcherbakova I, Huang G, Geoffroy OJ, Nair SK, Swierczek K, Balandrin MF, Fox J, Heaton WL, and Conklin RL (2005) Design, new synthesis, and calcilytic activity of substituted 3H-pyrimidin-4-ones. *Bioorg Med Chem Lett* **15**:2537–2540.
- Shinagawa Y, Inoue T, Katsushima T, Kiguchi T, Ikenogami T, Ogawa N, Fukuda K, Hirata K, Harada K, Takagi M, et al. (2011) Discovery of a potent and short-acting oral calcilytic with a pulsatile secretion of parathyroid hormone. *ACS Med Chem Lett* **2**:238–242.
- Shoback DM, Membreno LA, and McGhee JG (1988) High calcium and other divalent cations increase inositol trisphosphate in bovine parathyroid cells. *Endocrinology* **123**:382–389.
- Silver IA, Murrills RJ, and Etherington DJ (1988) Microelectrode studies on the acid microenvironment beneath adherent macrophages and osteoclasts. *Exp Cell Res* **175**:266–276.
- Smajilovic S, Sheykhdade M, Holmgard HN, Haunso S, and Tfelt-Hansen J (2007) Calcimimetic, AMG 073, induces relaxation on isolated rat aorta. *Vasc Pharmacol* **47**:222–228.
- Smajilovic S, Wellendorph P, and Bräuner-Osborne H (2014) Promiscuous seven transmembrane receptors sensing L-α-amino acids. *Curr Pharm Des* **20**:2693–2702.
- Soda K, Kano Y, Sakuragi M, Takao K, Lefor A, and Konishi F (2009) Long-term oral polyamine intake increases blood polyamine concentrations. *J Nutr Sci Vitaminol (Tokyo)* **55**:361–366.
- Stenson PD, Ball EV, Mort M, Phillips AD, Shaw K, and Cooper DN (2012) The Human Gene Mutation Database (HGMD) and its exploitation in the fields of personalized genomics and molecular evolution. *Curr Protoc Bioinformatics Chapter 1*:Unit1.13.
- Stepanchick A, McKenna J, McGovern O, Huang Y, and Breitwieser GE (2010) Calcium sensing receptor mutations implicated in pancreatitis and idiopathic epilepsy syndrome disrupt an arginine-rich retention motif. *Cell Physiol Biochem* **26**:363–374.
- Sun X, Tang L, Winesett S, Chang W, and Cheng SX (2018) Calcimimetic R568 inhibits tetrodotoxin-sensitive colonic electrolyte secretion and reduces c-fos expression in myenteric neurons. *Life Sci* **194**:49–58.
- Suzuki K, Lavaroni S, Mori A, Okajima F, Kimura S, Katoh R, Kawaoi A, and Kohn LD (1998) Thyroid transcription factor 1 is calcium modulated and coordinately regulates genes involved in calcium homeostasis in C cells. *Mol Cell Biol* **18**:7410–7422.
- Tang H, Yamamura A, Yamamura H, Song S, Fraidenburg DR, Chen J, Gu Y, Pohl NM, Zhou T, Jiménez-Pérez L, et al. (2016a) Pathogenic role of calcium-sensing receptors in the development and progression of pulmonary hypertension. *Am J Physiol Lung Cell Mol Physiol* **310**:L846–L859.
- Tang L, Cheng CY, Sun X, Pedicone AJ, Mohammadzadeh M, and Cheng SX (2016b) The extracellular calcium-sensing receptor in the intestine: evidence for regulation of colonic absorption, secretion, motility, and immunity. *Front Physiol* **7**:245.
- Tang L, Jiang L, McIntyre ME, Petrova E, and Cheng SX (2018) Calcimimetic acts on enteric neuronal CaSR to reverse cholera toxin-induced intestinal electrolyte secretion. *Sci Rep* **8**:7851.
- Tang L, Peng M, Liu L, Chang W, Binder HJ, and Cheng SX (2015) Calcium-sensing receptor stimulates Cl⁻- and SCFA-dependent but inhibits cAMP-dependent HCO₃⁻ secretion in colon. *Am J Physiol Gastrointest Liver Physiol* **308**:G874–G883.
- Tatsumi R, Komaba H, Kanai G, Miyakogawa T, Sawada K, Kakuta T, and Fukagawa M (2013) Cinacalcet induces apoptosis in parathyroid cells in patients with secondary hyperparathyroidism: histological and cytological analyses. *Nephron Clin Pract* **124**:224–231.
- Temal T, Jary H, Auberval M, Lively S, Guédin D, Vevert JP, and Deprez P (2013) New potent calcimimetics: I. Discovery of a series of novel trisubstituted ureas. *Bioorg Med Chem Lett* **23**:2451–2454.
- Tenhola S, Hendy GN, Valta H, Canaff L, Lee BS, Wong BY, Välimäki MJ, Cole DE, and Mäkitie O (2015) Cinacalcet treatment in an adolescent with concurrent 22q11.2 deletion syndrome and familial hypocalciuric hypercalcaemia type 3 caused by AP2S1 mutation. *J Clin Endocrinol Metab* **100**:2515–2518.
- Tenhola S, Voutilainen R, Reyes M, Toiviainen-Salo S, Jüppner H, and Mäkitie O (2016) Impaired growth and intracranial calcifications in autosomal dominant hypocalcaemia caused by a GNA11 mutation. *Eur J Endocrinol* **175**:211–218.
- Tfelt-Hansen J, MacLeod RJ, Chattopadhyay N, Yano S, Quinn S, Ren X, Terwilliger EF, Schwarz P, and Brown EM (2003) Calcium-sensing receptor stimulates PTHrP release by pathways dependent on PKC, p38 MAPK, JNK, and ERK1/2 in H-500 cells. *Am J Physiol Endocrinol Metab* **285**:E329–E337.
- Thakore P and Ho WS (2011) Vascular actions of calcimimetics: role of Ca²⁺(+)-sensing receptors versus Ca²⁺(+) influx through L-type Ca²⁺(+) channels. *Br J Pharmacol* **162**:749–762.
- Thomsen AR, Hvidtfeldt M, and Bräuner-Osborne H (2012a) Biased agonism of the calcium-sensing receptor. *Cell Calcium* **51**:107–116.
- Thomsen AR, Worm J, Jacobsen SE, Stahlhut M, Latta M, and Bräuner-Osborne H (2012b) Strontium is a biased agonist of the calcium-sensing receptor in rat medullary thyroid carcinoma 6-23 cells. *J Pharmacol Exp Ther* **343**:638–649.
- Toka HR, Al-Romaih K, Koshy JM, DiBartolo S III, Kos CH, Quinn SJ, Curhan GC, Mount DB, Brown EM, and Pollak MR (2012) Deficiency of the calcium-sensing receptor in the kidney causes parathyroid hormone-independent hypocalcaemia. *J Am Soc Nephrol* **23**:1879–1890.
- Trzaskowski B, Latek D, Yuan S, Ghoshdastider U, Debinski A, and Filipek S (2012) Action of molecular switches in GPCRs—theoretical and experimental studies. *Curr Med Chem* **19**:1090–1109.
- Tsuchiya D, Kunishima N, Kamiya N, Jingami H, and Morikawa K (2002) Structural views of the ligand-binding cores of a metabotropic glutamate receptor complexed with an antagonist and both glutamate and Gd3+. *Proc Natl Acad Sci USA* **99**:2660–2665.
- Tsutsumi M, Goto M, and Denda M (2013) Dynamics of intracellular calcium in cultured human keratinocytes after localized cell damage. *Exp Dermatol* **22**:367–369.
- Tu CL and Bikle DD (2013) Role of the calcium-sensing receptor in calcium regulation of epidermal differentiation and function. *Best Pract Res Clin Endocrinol Metab* **27**:415–427.
- Tu CL, Celli A, Mauro T, and Chang W (2019) Calcium-sensing receptor regulates epidermal intracellular Ca²⁺ signaling and re-epithelialization after wounding. *J Invest Dermatol* **139**:919–929.
- Tu CL, Chang W, Xie Z, and Bikle DD (2008) Inactivation of the calcium sensing receptor inhibits E-cadherin-mediated cell-cell adhesion and calcium-induced differentiation in human epidermal keratinocytes. *J Biol Chem* **283**:3519–3528.
- Tu CL, Crumrine DA, Man MQ, Chang W, Elalieh H, You M, Elias PM, and Bikle DD (2012) Ablation of the calcium-sensing receptor in keratinocytes impairs epidermal differentiation and barrier function. *J Invest Dermatol* **132**:2350–2359.
- Tu Q, Pi M, Karsenty G, Simpson L, Liu S, and Quarles LD (2003) Rescue of the skeletal phenotype in CasR-deficient mice by transfer onto the Gcm2 null background. *J Clin Invest* **111**:1029–1037.
- Turksen K and Troy TC (2003) Overexpression of the calcium sensing receptor accelerates epidermal differentiation and permeability barrier formation in vivo. *Mech Dev* **120**:733–744.
- Tyler Miller R (2013) Control of renal calcium, phosphate, electrolyte, and water excretion by the calcium-sensing receptor. *Best Pract Res Clin Endocrinol Metab* **27**:345–358.
- Vancleef L, Van Den Broeck T, Thijs T, Steensels S, Briand L, Tack J, and Depoortere I (2015) Chemosensory signalling pathways involved in sensing of amino acids by the ghrelin cell. *Sci Rep* **5**:15725.
- van den Berg MP, Meurs H, and Gosens R (2018) Targeting arginase and nitric oxide metabolism in chronic airway diseases and their co-morbidities. *Curr Opin Pharmacol* **40**:126–133.
- VanHouten J, Dann P, McGeoch G, Brown EM, Krapcho K, Neville M, and Wysolmerski JJ (2004) The calcium-sensing receptor regulates mammary gland parathyroid hormone-related protein production and calcium transport. *J Clin Invest* **113**:598–608.
- VanHouten JN, Neville MC, and Wysolmerski JJ (2007) The calcium-sensing receptor regulates plasma membrane calcium adenosine triphosphatase isoform 2 activity in mammary epithelial cells: a mechanism for calcium-regulated calcium transport into milk. *Endocrinology* **148**:5943–5954.
- VanHouten JN and Wysolmerski JJ (2007) Transcellular calcium transport in mammary epithelial cells. *J Mammary Gland Biol Neoplasia* **12**:223–235.
- Vanhouten JN and Wysolmerski JJ (2013) The calcium-sensing receptor in the breast. *Best Pract Res Clin Endocrinol Metab* **27**:403–414.
- Vargas-Poussou R, Mansour-Hendili L, Baron S, Bertocchio JP, Travers C, Simian C, Treard C, Baudouin V, Beltran S, Broux F, et al. (2016) Familial hypocalciuric hypercalcaemia types 1 and 3 and primary hyperparathyroidism: similarities and differences. *J Clin Endocrinol Metab* **101**:2185–2195.
- Vezzoli G, Scillitani A, Corbetta S, Terranegra A, Dogliotti E, Guarnieri V, Arcidiacono T, Paloschi V, Rainone F, Eller-Vainicher C, et al. (2011) Polymorphisms at the regulatory regions of the CASR gene influence stone risk in primary hyperparathyroidism. *Eur J Endocrinol* **164**:421–427.
- Vizard TN, O'Keeffe GW, Gutierrez H, Kos CH, Riccardi D, and Davies AM (2008) Regulation of axonal and dendritic growth by the extracellular calcium-sensing receptor. *Nat Neurosci* **11**:285–291.
- Walter S, Baruch A, Dong J, Tomlinson JE, Alexander ST, Janes J, Hunter T, Yin Q, Maclean D, Bell G, et al. (2013) Pharmacology of AMG 416 (Velcalcetide), a novel peptide agonist of the calcium-sensing receptor, for the treatment of secondary hyperparathyroidism in hemodialysis patients. *J Pharmacol Exp Ther* **346**:229–240.
- Wang L, Widatalla SE, Whalen DS, Ochieng J, and Sakwe AM (2017) Association of calcium sensing receptor polymorphisms at rs1801725 with circulating calcium in breast cancer patients. *BMC Cancer* **17**:511.
- Wang M, Yao Y, Kuang D, and Hampson DR (2006) Activation of family C G-protein-coupled receptors by the tripeptide glutathione. *J Biol Chem* **281**:8864–8870.
- Wang X, Kirberger M, Qiu F, Chen G, and Yang JJ (2009) Towards predicting Ca²⁺-binding sites with different coordination numbers in proteins with atomic resolution. *Proteins* **75**:787–798.

- Wang X, Zhao K, Kirberger M, Wong H, Chen G, and Yang JJ (2010) Analysis and prediction of calcium-binding pockets from apo-protein structures exhibiting calcium-induced localized conformational changes. *Protein Sci* **19**:1180–1190.
- Wang Y, Chandra R, Samsa LA, Gooch B, Fee BE, Cook JM, Vigna SR, Grant AO, and Liddle RA (2011) Amino acids stimulate cholecystokinin release through the Ca²⁺-sensing receptor. *Am J Physiol Gastrointest Liver Physiol* **300**:G528–G537.
- Ward DT, Brown EM, and Harris HW (1998) Disulfide bonds in the extracellular calcium-polyvalent cation-sensing receptor correlate with dimer formation and its response to divalent cations in vitro. *J Biol Chem* **273**:14476–14483.
- Watanabe S, Fukumoto S, Chang H, Takeuchi Y, Hasegawa Y, Okazaki R, Chikatsu N, and Fujita T (2002) Association between activating mutations of calcium-sensing receptor and Bartter's syndrome. *Lancet* **360**:692–694.
- Wei G, Ku S, Ma GK, Saito S, Tang AA, Zhang J, Mao JH, Appella E, Balmain A, and Huang EJ (2007) HIPK2 represses beta-catenin-mediated transcription, epidermal stem cell expansion, and skin tumorigenesis. *Proc Natl Acad Sci USA* **104**:13040–13045.
- Wellendorph P and Bräuner-Osborne H (2009) Molecular basis for amino acid sensing by family C G-protein-coupled receptors. *Br J Pharmacol* **156**:869–884.
- Wettschurek N, Lee E, Libutti SK, Offermanns S, Robey PG, and Spiegel AM (2007) Parathyroid-specific double knockout of Gq and G11 alpha-subunits leads to a phenotype resembling germline knockout of the extracellular Ca²⁺-sensing receptor. *Mol Endocrinol* **21**:274–280.
- White E, McKenna J, Cavanaugh A, and Breitwieser GE (2009) Pharmacochaperone-mediated rescue of calcium-sensing receptor loss-of-function mutants. *Mol Endocrinol* **23**:1115–1123.
- Wolf P, Krššák M, Winhofer Y, Anderwald CH, Zwettler E, Just Kukurová I, Gessl A, Trattng S, Luger A, Baumgartner-Parzer S, et al. (2014) Cardiometabolic phenotyping of patients with familial hypocalcemic hypercalcemia. *J Clin Endocrinol Metab* **99**:E1721–E1726.
- Wu H, Wang C, Gregory KJ, Han GW, Cho HP, Xia Y, Niswender CM, Katritch V, Meiler J, Cherezov V, et al. (2014) Structure of a class C GPCR metabotropic glutamate receptor 1 bound to an allosteric modulator. *Science* **344**:58–64.
- Wu W, Zhou HR, Bursian SJ, Link JE, and Pestka JJ (2017) Calcium-sensing receptor and transient receptor ankyrin-1 mediate emesis induction by deoxynivalenol (Vomitoxin). *Toxicol Sci* **155**:32–42.
- Wysolmerski JJ (2012) Parathyroid hormone-related protein: an update. *J Clin Endocrinol Metab* **97**:2947–2956.
- Xie R, Xu J, Xiao Y, Wu J, Wan H, Tang B, Liu J, Fan Y, Wang S, Wu Y, et al. (2017) Calcium promotes human gastric cancer via a novel coupling of calcium-sensing receptor and TRPV4 channel. *Cancer Res* **77**:6499–6512.
- Yamada S, Tokumoto M, Taniguchi M, Toyonaga J, Suehiro T, Eriguchi R, Fujimi S, Ooboshi H, Kitazono T, and Tsuruya K (2015) Two years of cinacalcet hydrochloride treatment decreased parathyroid gland volume and serum parathyroid hormone level in hemodialysis patients with advanced secondary hyperparathyroidism. *Ther Apher Dial* **19**:367–377.
- Yamamoto M, Akatsu T, Nagase T, and Ogata E (2000) Comparison of hypocalcemic hypercalciuria between patients with idiopathic hypoparathyroidism and those with gain-of-function mutations in the calcium-sensing receptor: is it possible to differentiate the two disorders? *J Clin Endocrinol Metab* **85**:4583–4591.
- Yang C, Rybchyn MS, Conigrave AD, and Mason RS (2016) Role of calcium sensing receptor (CaSR) modifiers in photoprotection. *Wound Repair Regen* **24**:A16.
- Yang W, Liu L, Masugi Y, Qian ZR, Nishihara R, Keum N, Wu K, Smith-Warner S, Ma Y, Nowak JA, et al. (2018) Calcium intake and risk of colorectal cancer according to expression status of calcium-sensing receptor (CASR). *Gut* **67**:1475–1483.
- Yang Y and Wang B (2018) PTH1R-CaSR cross talk: new treatment options for breast cancer osteolytic bone metastases. *Int J Endocrinol* **2018**:7120979.
- Yarova PL, Stewart AL, Sathish V, Britt RD Jr, Thompson MA, P Lowe AP, Freeman M, Aravamudan B, Kita H, Brennan SC, et al. (2015) Calcium-sensing receptor antagonists abrogate airway hyperresponsiveness and inflammation in allergic asthma. *Sci Transl Med* **7**:284ra60.
- Ye C, Ho-Pao CL, Kanazirska M, Quinn S, Rogers K, Seidman CE, Seidman JG, Brown EM, and Vassilev PM (1997a) Amyloid-beta proteins activate Ca(2+)-permeable channels through calcium-sensing receptors. *J Neurosci Res* **47**:547–554.
- Ye C, Ho-Pao CL, Kanazirska M, Quinn S, Seidman CE, Seidman JG, Brown EM, and Vassilev PM (1997b) Deficient cation channel regulation in neurons from mice with targeted disruption of the extracellular Ca²⁺-sensing receptor gene. *Brain Res Bull* **44**:75–84.
- Ye C, Rogers K, Bai M, Quinn SJ, Brown EM, and Vassilev PM (1996) Agonists of the Ca(2+)-sensing receptor (CaR) activate nonselective cation channels in HEK293 cells stably transfected with the human CaR. *Biochem Biophys Res Commun* **226**:572–579.
- Young SH, Rey O, Sinnett-Smith J, and Rozengurt E (2014) Intracellular Ca²⁺ oscillations generated via the Ca²⁺-sensing receptor are mediated by negative feedback by PKCα at Thr888. *Am J Physiol Cell Physiol* **306**:C298–C306.
- Youssef KK, Lapouge G, Bouvrée K, Rorive S, Brohée S, Appelstein O, Larsimont JC, Sukumaran V, Van de Sande B, Pucci D, et al. (2012) Adult interfollicular tumour-initiating cells are reprogrammed into an embryonic hair follicle progenitor-like fate during basal cell carcinoma initiation. *Nat Cell Biol* **14**:1282–1294.
- Zaidi M, Kerby J, Huang CL, Alam T, Rathod H, Chambers TJ, and Moonga BS (1991) Divalent cations mimic the inhibitory effect of extracellular ionised calcium on bone resorption by isolated rat osteoclasts: further evidence for a “calcium receptor”. *J Cell Physiol* **149**:422–427.
- Zhang C, Huang Y, Jiang Y, Mulpuri N, Wei L, Hamelberg D, Brown EM, and Yang JJ (2014a) Identification of an L-phenylalanine binding site enhancing the co-operative responses of the calcium-sensing receptor to calcium. *J Biol Chem* **289**:5296–5309.
- Zhang C, Zhang T, Zou J, Miller CL, Gorkhali R, Yang JY, Schillmiller A, Wang S, Huang K, Brown EM, et al. (2016) Structural basis for regulation of human calcium-sensing receptor by magnesium ions and an unexpected tryptophan derivative co-agonist. *Sci Adv* **2**:e1600241.
- Zhang C, Zhuo Y, Moniz HA, Wang S, Moremen KW, Prestegard JH, Brown EM, and Yang JJ (2014b) Direct determination of multiple ligand interactions with the extracellular domain of the calcium-sensing receptor. *J Biol Chem* **289**:33529–33542.
- Zhang H, Kovacs-Nolan J, Koder T, Eto Y, and Mine Y (2015) γ-Glutamyl cysteine and γ-glutamyl valine inhibit TNF-α signaling in intestinal epithelial cells and reduce inflammation in a mouse model of colitis via allosteric activation of the calcium-sensing receptor. *Biochim Biophys Acta* **1852**:792–804.
- Zhang M, Yang H, Wan X, Lu L, Zhang J, Zhang H, Ye T, Liu Q, Xie M, Liu X, et al. (2019) Prevention of injury-induced osteoarthritis in rodent temporomandibular joint by targeting chondrocyte CaSR. *J Bone Miner Res* **34**:726–738.
- Zhang Z, Jiang Y, Quinn SJ, Krapcho K, Nemeth EF, and Bai M (2002a) L-phenylalanine and NPS R-467 synergistically potentiate the function of the extracellular calcium-sensing receptor through distinct sites. *J Biol Chem* **277**:33736–33741.
- Zhang Z, Qiu W, Quinn SJ, Conigrave AD, Brown EM, and Bai M (2002b) Three adjacent serines in the extracellular domains of the CaR are required for L-amino acid-mediated potentiation of receptor function. *J Biol Chem* **277**:33727–33735.
- Zhang Z, Sun S, Quinn SJ, Brown EM, and Bai M (2001) The extracellular calcium-sensing receptor dimerizes through multiple types of intermolecular interactions. *J Biol Chem* **276**:5316–5322.
- Zhao K, Wang X, Wong HC, Wohlhueter R, Kirberger MP, Chen G, and Yang JJ (2012) Predicting Ca²⁺-binding sites using refined carbon clusters. *Proteins* **80**:2666–2679.
- Zhao X, Xian Y, Wang C, Ding L, Meng X, Zhu W, and Hang S (2018) Calcium-sensing receptor-mediated L-tryptophan-induced secretion of cholecystokinin and glucose-dependent insulinotropic peptide in swine duodenum. *J Vet Sci* **19**:179–187.
- Zhuang X, Northup JK, and Ray K (2012) Large putative PEST-like sequence motif at the carboxyl tail of human calcium receptor directs lysosomal degradation and regulates cell surface receptor level. *J Biol Chem* **287**:4165–4176.
- Zinser GM, Sundberg JP, and Welsh J (2002) Vitamin D(3) receptor ablation sensitizes skin to chemically induced tumorigenesis. *Carcinogenesis* **23**:2103–2109.
- Zuo X, Sun L, Yin X, Gao J, Sheng Y, Xu J, Zhang J, He C, Qiu Y, Wen G, et al. (2015) Whole-exome SNP array identifies 15 new susceptibility loci for psoriasis. *Nat Commun* **6**:6793.