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Review

Pharmacoperones and the calcium sensing receptor: Exogenous and endogenous regulators

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ABSTRACT

Calcium sensing receptor (CaSR) mutations or altered expression cause disorders of calcium handling. Recent studies suggest that reduced targeting to the plasma membrane is a feature common to many CaSR loss-of-function mutations. Allosteric agonists (calcimimetics) can rescue signaling of a subset of CaSR mutants. This review evaluates our current understanding of the subcellular site(s) for allosteric modulator rescue of CaSR mutants. Studies to date make a strong case for calcimimetic potentiation of signaling not only at plasma membrane-localized CaSR, but at the endoplasmic reticulum, acting as pharmacoperones to assist in navigation of multiple quality control checkpoints. The possible role of endogenous pharmacoperones, calcium and glutathione, in folding and stabilization of the CaSR extracellular and transmembrane domains are considered. Finally, the possibility that dihydropyridines act as unintended pharmacoperones of CaSR is proposed. While our understanding of pharmacoperone rescue of CaSR requires refinement, promising results to date argue that this may be a fruitful avenue for drug discovery.

Contents

Abbreviations: ADH, autosomal dominant hypocalcemia; CaSR, calcium sensing receptor; xCT, cystine-glutamate transporter; DHPs, dihydropyridines; EAAT, excitatory amino acid transporter; EGFR, endothelial growth factor receptor; ER, endoplasmic reticulum; ERK1/2, extracellular signal activated kinase 1 and 2; ER QC, endoplasmic reticulum quality control; FHH, familial hypocalciuric hypercalcemia; FIH, familial isolated hypoparathyroidism; GABA, gamma amino isobutyric acid; GCM2, glial cells missing homolog 2; GPCR, G protein-coupled receptor; IPAH, idiopathic pulmonary arterial hypertension; mGlu5, metabotropic glutamate receptor type 5; N-linked, asparagine-linked; IP3R, inositol 1,4,5-trisphosphate receptor; NSHPT, neonatal severe primary hyperparathyroidism; p24A, TMED2, transmembrane emp24 domain trafficking protein 2; PLCβ, phospholipase C beta; PTH, parathyroid hormone; SERCA pump, sarco/endoplasmic reticulum Ca^{2+}-ATPase.

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1. Introduction

The calcium sensing receptor (CaSR) is a member of Family C of the G protein-coupled receptor (GPCR) superfamily that is activated by extracellular calcium over the concentration range found in serum. CaSR activation by extracellular calcium is potentiated by endogenous amino acids and/or peptides acting at allosteric sites within the extracellular domain [1,2]. Pharmacologic modulation of CaSR function targets overlapping allosteric sites within the heptahelical transmembrane domain, and include both allosteric agonists (calcimimetics) and antagonists (calcilytics) [3–7]. Cinacalcet (Sensipar™/Mimpara™, Amgen) is the first FDA-approved allosteric agonist for a GPCR, and is used to treat secondary hyperparathyroidism resulting from chronic kidney disease or parathyroid adenomas [8]. Both conditions lead to a reduction in CaSR function and/or expression. Cinacalcet potentiates CaSR signaling, and may also up-regulate CaSR expression in some contexts.

1.1. Importance of CaSR in physiology

CaSR is integral to establishing and maintaining systemic calcium homeostasis [reviewed in 2], acting at the parathyroid gland to link serum calcium levels to PTH secretion, and at the intestine, kidney and bone to regulate calcium uptake, reuptake, and deposition/resorption, respectively. Mutations of CaSR identified in patients attest to the critical role of CaSR in calcium homeostasis [9,10]. Loss-of-function mutations with reduced sensitivity to extracellular calcium cause familial hypocalciuric hypercalcemia (FHH, OMIM145980) when present on one allele. A severe autosomal recessive disorder, neonatal severe primary hyperparathyroidism (NSHPT, OMIM239200), results from loss-of-function mutations in both alleles. Rare gain-of-function mutations of CaSR leading to increased sensitivity to calcium or constitutive activity, cause familial isolated hypoparathyroidism (FIH, OMIM146200), also called autosomal dominant hypoparathyroidism or autosomal dominant hypocalcemia. Some highly active gain-of-function mutations also lead to type 5 Bartter Syndrome (OMIM601198). Beyond the role of CaSR in regulation of serum calcium, CaSR is also expressed in the vasculature, nervous system, and many endocrine and epithelial cell types, where it contributes to regulation of cellular calcium homeostasis and/or secretion, but may have additional tissue-specific roles as yet undefined. Altered expression of CaSR, either up-regulation or epigenetic silencing, may contribute to cancer progression and/or metastasis [2,10].

1.2. CaSR signaling

CaSR is a promiscuous GPCR, capable of activating G_q, G_i, $G_{12/13}$, and G_s-mediated signaling pathways in the appropriate cell context [11,12]. The lack of high affinity agonists or antagonists which can be used to identify CaSR by specific binding assays has led to reliance on CaSR signaling outputs as an inferred measure of CaSR activation. Most commonly used measures of CaSR activation are (1) increases in cellular inositol phosphates or intracellular calcium, resulting from G_q-mediated activation of PLCβ, or (2) increased ERK1/2 phosphorylation, resulting from activation of the MAPK cascade. Using these assays, mutants of CaSR identified in patients with FHH/NSHPT or FIH can be assessed for functional differences. Many mutations, however, lead to decreased plasma membrane targeting of CaSR, causing reductions in signaling which do not necessarily reflect an inability of these mutants to activate the appropriate signaling pathways, but rather to access the appropriate cellular compartment, i.e., the plasma membrane, where signaling is initiated [13–19]. Recent careful examination of loss-of-function mutants highlights another complication in assessing the impact on CaSR function, i.e., mutation-dependent alterations in CaSR signaling bias [19,20]. Signaling bias connotes the relative ability of a ligand to activate all possible signaling outputs. Alterations in signaling bias can be driven by ligand structure, which may favor a subset of receptor conformations, but can also be altered by mutation. Reliance on a single assay may therefore not provide a nuanced view of the impact of mutations on CaSR function. Careful dissection of mutation-dependent alterations in signaling bias may, however, provide insight into the minimal signaling required for efficient trafficking to the plasma membrane.

2. Biosynthesis and regulation of CaSR expression

CaSR expression is regulated at both the transcriptional and translational levels. Here our focus is on the regulatory steps initiated when CaSR mRNA is translated at the ER. CaSR synthesis and membrane insertion at rough ER requires recognition of the CaSR signal sequence; mutations which disrupt the CaSR signal sequence have been identified in patients with FHH [13]. As with other membrane proteins, CaSR undergoes cotranslational glycosylation at up to 11 N-linked sites at the extracellular domain [21,22]. At this point, newly synthesized CaSR is interrogated by successive checkpoints, including general quality control, and those required for ER release [15,18,23].

2.1. ER QC checkpoints

All GPCRs must pass a series of quality control checkpoints during biosynthesis/folding in the ER. For Family C GPCRs, which have large (>600 residues) and autonomously folding extracellular domains [24,25] stabilized by hydrophobic helical interactions and disulfide bonds [26], initiation of proper folding likely begins prior to full synthesis of the transmembrane domain. Although glycosylation is required for ER exit (see Section 2.2), it is not required for proper folding of the extracellular domain, since CaSR having asparagine (N) to glutamine (Q) mutations at all glycosylation sites (CaSR(N11Q)) fold and dimerize at the ER [22]. Carboxyl terminal truncations with a single transmembrane helix or expressing only the non-membrane anchored extracellular domain dimerize, are secreted, and bind neomycin and calcium [24,25,27]. Folding and proper helical packing of the transmembrane domain of CaSR requires appropriate alignment of interacting residues to stabilize particular helix–helix interactions, and proper assembly of the helical bundle. Thus there are two autonomous domains of CaSR which must be properly folded for monomeric CaSR to pass initial quality control criteria. Aspects of the initial folding of CaSR likely

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