Biochimica et Biophysica Acta xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamcr

Calcium sensing receptor signalling in physiology and cancer☆

Sarah C. Brennan [a,b], Ursula Thiem [a,c], Susanne Roth [a,d], Abhishek Aggarwal [a,e], Irfete Sh. Fetahu [a,e], Samawansha Tennakoon [a,e], Ana Rita Gomes [a,f], Maria Luisa Brandi [a,f], Frank Bruggeman [a,d], Romuald Mentaverri [a,c], Daniela Riccardi [a,b], Enikö Kallay [a,e,*]

[a] Multifaceted CaSR Initial Training Network
[b] Cardiff School of Biosciences, Biomedical Sciences Building, Museum Avenue, Cardiff, CF10 3AX, UK
[c] INSERM U1088, University of Picardie Jules Verne, Faculty of Medicine and Pharmacy, Amiens, France
[d] Molecular Cell Physiology, Netherlands Institute for Systems Biology, Vrije Universiteit, Amsterdam, The Netherlands
[e] Department of Pathophysiology and Allergy Research, Medical University of Vienna, Austria
[f] Department of Clinical Physiopathology, University of Florence, Florence, Italy

ARTICLE INFO

Article history:
Received 28 September 2012
Received in revised form 10 December 2012
Accepted 12 December 2012
Available online xxxx

Keywords:
Parathyroid adenoma
Colorectal cancer
Breast cancer
Prostate cancer
Systems biology
Ligand-biased signalling

ABSTRACT

The calcium sensing receptor (CaSR) is a class C G-protein-coupled receptor that is crucial for the feedback regulation of extracellular free ionised calcium homeostasis. While extracellular calcium (Ca^{2+}_o) is considered the primary physiological ligand, the CaSR is activated physiologically by a plethora of molecules including polyamines and L-amino acids. Activation of the CaSR by different ligands has the ability to stabilise unique conformations of the receptor, which may lead to preferential coupling of different G proteins; a phenomenon termed 'ligand-biased signalling'. While mutations of the CaSR are currently not linked with any malignancies, altered CaSR expression and function are associated with cancer progression. Interestingly, the CaSR appears to act both as a tumour suppressor and an oncogene, depending on the pathophysiology involved. Reduced expression of the CaSR occurs in both parathyroid and colon cancers, leading to loss of the growth suppressing effect of high Ca^{2+}_o. On the other hand, activation of the CaSR might facilitate metastasis to bone in breast and prostate cancer. A deeper understanding of the mechanisms driving CaSR signalling in different tissues, aided by a systems biology approach, will be instrumental in developing novel drugs that target the CaSR or its ligands in cancer. This article is part of a Special Issue entitled: 12th European Symposium on Calcium.

1. Introduction

The calcium ion (Ca^{2+}) is crucial for the control of many important cellular functions such as proliferation, differentiation, and fluid secretion. Many organisms express cell-surface sensors for extracellular Ca^{2+} (Ca^{2+}_o). Ca^{2+}_o is the primary physiological ligand of a G protein-coupled receptor (GPCR), called the calcium sensing receptor (CaSR). The receptor is expressed primarily within the chief cells of the parathyroid glands where, when activated by an excess of Ca^{2+}_o, it decreases the release of the calcium-retaining hormone, parathyroid hormone (PTH) [1] to maintain Ca^{2+}_o within the physiological range (1.1–1.3 mM). Vice versa, in the case of hypocalcemia, the CaSR is inactive and PTH is released, an event which restores normocalcemia by acting on the kidneys, to enhance renal Ca^{2+} reabsorption; the intestine to, increase intestinal Ca^{2+} absorption and bone, to mobilise skeletal Ca^{2+} [1]. A large number of studies have shown that the CaSR is expressed in many other tissues in the body, which do not play an obvious role in Ca^{2+}_o homeostasis, such as the breast, blood vessels, liver, and placenta [1]. Altered CaSR expression and/or activity is associated not only with disorders of the parathyroid glands, but also with other conditions like osteoporosis, vascular calcification and cancer.

The CaSR is involved in the regulation of a number of diverse processes, such as hormone secretion, gene expression, ion channel activity, modulation of inflammation, proliferation, differentiation, and apoptosis, depending on cell type, and therefore represents a key molecule in physiology [1]. The CaSR is also able to respond to a variety of ligands, including polyvalent cations and amino acids.

Abbreviations: APC, adenomatous polyposis coli; bFGF, basic fibroblast growth factor; Ca^{2+}_i, intracellular calcium; Ca^{2+}_o, extracellular calcium; CaSR, calcium sensing receptor; CDX-2, caudal type homeobox 2; EGF, epidermal growth factor; ERK, extracellular-signal-regulated kinase; GEF, guanine nucleotide exchange factor; GPCR, G protein-coupled receptor; IP_3, inositol 1,4,5 tris-phosphate; MAP, mitogen-activated protein; MEK, MAP kinase kinase; pHPT, primary hyperparathyroidism; PKC, protein kinase C; PLC, phospholipase C; PTH, parathyroid hormone; PTHrP, parathyroid hormone related peptide; NFκB, Nuclear factor-kappaB; RANK, receptor activator of NFκB; RANKL, receptor activator of NFκB ligand; RGS5, regulator of G protein signalling 5; Ror2, receptor tyrosine kinase-like orphan receptor 2; SDF-1, stromal cell-derived factor 1; STAT, Signal Transducers and Activators of Transcription; SP, Specificity Protein 1/3; TGF-β, transforming growth factor beta; VDRE, vitamin D responsive element; Wnt, wingless

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* Corresponding author at: Department of Pathophysiology and Allergy Research, Medical University of Vienna, Währinger Gürtel 18-20, 1090 Vienna, Austria. Tel.: +43 1 404005123; fax: +43 1 404005130.
E-mail address: enikoe.kallay@meduniwien.ac.at (E. Kallay).

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http://dx.doi.org/10.1016/j.bbamcr.2012.12.011

Furthermore, changes of pH and ionic strength affect the activity of the receptor, making the CaSR uniquely capable of integrating several metabolic signals. Activation of the CaSR by different ligands may stabilise the CaSR in unique activation states allowing preferential stimulation of different signalling pathways — termed "ligand-biased signalling" [2].

Altered CaSR signalling is associated with a number of pathophysiological states. In cancer, CaSR expression either becomes reduced or even lost, or signalling pathways become activated that are different from those activated in the respective normal tissues. The signalling may differ dependent on the type of cancer, stage, grade, etc. Therefore, there is need for a better understanding of how individual ligands affect CaSR-mediated signalling, and how this will in turn benefit development of novel pharmaceutical therapies targeting the CaSR.

This review article aims to provide an overview of the signalling mechanism mediated by the CaSR under normal physiological conditions and in cancer.

1.1. CaSR signalling in physiology

The CaSR is a pleiotropic GPCR that is extremely sensitive to very small deviations in plasma Ca^{2+}_o (i.e. less than 10%) within the physiological range. There is a very steep, inverse sigmoid relationship between Ca^{2+}_o and PTH release, with the steepest part of the curve being centred around the physiological serum Ca^{2+}_o of 1.2 mM, at which concentration PTH secretion is already suppressed by ~25% of its maximal value [1].

Evidence gathered since the late 1980s showed that CaSR activation leads to inhibition of PTH via signalling mediated by the trimeric G protein, $G\alpha_{q/11}$. CaSR activation in the parathyroid glands and many other cellular systems promotes phosphoinositide turnover through activation of membrane-bound phospholipase C (PLC), producing inositol 1,4,5 tris-phosphate (IP_3) and diacyl glycerol. These, in turn, promote release of Ca^{2+} from intracellular, IP_3-sensitive stores [3] and protein kinase C (PKC) activation. Indeed, in mice lacking both $G\alpha_q$ and $G\alpha_{11}$ PTH levels are greatly elevated [4]. Genetic mutations of the CaSR highlight the importance of this receptor for maintaining divalent ion homeostasis [5–7].

The CaSR is also expressed in the colon where it plays an important role in nutrient sensing and intestinal fluid transport. Hebert et al., using isolated crypts from rat [8,9] and the $CaSR^{-/-}$::$GCM2^{-/-}$ knock out mouse model, demonstrated that intestinal fluid secretion involves CaSR-dependent degradation of cyclic nucleotides by phosphodiesterases [9], reversing the electrolyte secretory effect of cholera toxin and enterotoxin. Further, it has been suggested that the presence of the CaSR in enteric nerve cells, which interact with smooth muscle along the colon, could indicate involvement of the CaSR in intestinal motility [10].

Activation of the CaSR can also induce intracellular calcium (Ca^{2+}_i) oscillations, which have been linked to inhibition of proliferation in colonic epithelial cells [11]. Such CaSR-mediated Ca^{2+}_i oscillations have been observed in a number of CaSR-expressing cells [12], including parathyroid cells [13,14] and CaSR-expressing HEK293 cells (HEK-CaSR) [15]. Recent work has examined how variations in CaSR-mediated Ca^{2+}_i oscillation frequency and amplitude are physiologically significant in a variety of cell types. For example, in human colonic epithelial cells the CaSR can induce two separate oscillatory pathways: while CaSR-mediated high frequency (~3 to 4 min^{-1}) sinusoidal Ca^{2+}_i oscillations induce inhibition of proliferation, low frequency (~1.5 min^{-1}) transient Ca^{2+}_i oscillations do not [11]. Thus, the mechanisms that control Ca^{2+}_i oscillation frequency and amplitude are critical for various CaSR-dependent biological responses.

In HEK-CaSR cells, the sinusoidal oscillations (~4 min^{-1} at 37 °C) [16,17] arise from the dynamic phosphorylation and dephosphorylation of T888 [18–20], the primary PKC phosphorylation site of the CaSR [21]. Thus, receptor-induced activation of PKC leads to phosphorylation of T888 to uncouple the receptor from $G\alpha_{q/11}$-induced PLC activation and Ca^{2+}_i mobilisation [21,22]. T888 is then dephosphorylated by protein phosphatases to restore receptor coupling to PLC and Ca^{2+}_i mobilisation [20]. Interestingly, T888 phosphorylation exhibits a bi-phasic, 'bell-shaped' profile in which phosphorylation of T888 peaks at Ca^{2+}_o concentrations around 2–3 mM [20]. Further increases in Ca^{2+}_o lead to decreases in T888 phosphorylation, and Ca^{2+}_o concentrations ≥ 4.0 mM elicit sustained elevations in Ca^{2+}_i rather than Ca^{2+}_i oscillations [20]. Disruption of the T888 PKC phosphorylation site produces an increase in Ca^{2+}_o sensitivity and recently the mutation T888M was identified in a case of Autosomal Dominant Hypocalcemia (ADH) [23], demonstrating that the T888 residue and its regulation by PKC is critical for physiological CaSR function *in vivo*.

In addition to phosphoinositol turnover, the CaSR also couples to a number of other signalling pathways. For example, in thyroid C cells activation of the CaSR is linked to calcitonin-secretion through the activation of voltage-gated calcium channels [24], while in kidney cells, CaSR activation is coupled to the metabolism of arachidonic acid by cytochrome P450 (CYP450) and cyclooxygenase (COX) pathways via a G_i-dependent mechanism [25].

CaSR activation is linked also to pro-proliferative stimuli in many cell systems. The regulation of pro-proliferative CaSR signalling involves activation of the mitogen-activated protein (MAP) kinases extracellular-signal-regulated kinases (ERK) and $p38^{MAPK}$ protein kinases [26,27]. High Ca^{2+}_o induces CaSR-mediated activation of ERK1/2 in bovine parathyroid and HEK-CaSR cells, and the calcimimetic NPS-R467 markedly enhances this effect [28,29]. L-amino acids have only a relatively small effect, possibly operating as a fine-tuning mechanism [30]. Classically, ERK1/2 is activated via Ras-dependent activation of MAP kinase kinase (MEK), and specific inhibition of MEK suppresses high Ca^{2+}_o-induced ERK1/2 activation in both bovine parathyroid and HEK-CaSR cells [28].

ERK1/2 appears to be a significant site of signal convergence downstream of CaSR activation, as pertussis toxin, the PLC inhibitor U73122 and PKC inhibitor GF109203X, have all been shown to inhibit ERK1/2 activation partially implying a role for $G\alpha_i$, as well as $G\alpha_{q/11}$ and PI-PLC [28,29]. CaSR-mediated activation of ERK1/2 via $G\alpha_i$ also requires dynamin/β-arrestin-independent receptor internalisation [29]. In addition, CaSR-mediated ERK phosphorylation can also occur through "triple-pass" signalling in which CaSR activation leads to the release of an epidermal growth factor (EGF)-like peptide by matrix metalloproteinases, which in turn evokes EGF receptor-mediated cell signalling [31].

2. CaSR in cancer: tumour suppressor or oncogene?

Intracellular Ca^{2+}-dependent signalling mechanisms are frequently remodelled or deregulated in cancer cells. The current understanding is that the CaSR can either prevent, or promote tumourigenesis depending on the type of cancer [32] (see also Table 1). The mechanisms behind its impact on carcinogenesis are multiple and not well understood.

The expression of the CaSR can be decreased or even absent, as it is in parathyroid and colorectal cancer. It has been shown recently that the CaSR is expressed in differentiated neuroblastic tumours, but it is silenced in unfavourable neuroblastomas [33]. In these tumours, dearth of CaSR expression results in loss of the growth suppressing effects of high levels of Ca^{2+}_o. Activation of the receptor inhibits proliferation of these cancer cells, suggesting a tumour suppressor function for CaSR. In contrast, increased expression is observed in highly metastatic primary breast and prostate cancer cells. Furthermore, in breast cancer cells CaSR activates preferentially $G\alpha_s$ proteins and not $G\alpha_i$, as in normal breast cells [34], resulting in increased production of parathyroid hormone-related peptide (PTHrP), which is considered a primary cause of hypercalcaemia of malignancy, and a contributor to metastatic processes involving bone. In these settings, the CaSR seems to have an oncogenic role (Table 1).

Please cite this article as: S.C. Brennan, et al., Calcium sensing receptor signalling in physiology and cancer, Biochim. Biophys. Acta (2013), http://dx.doi.org/10.1016/j.bbamcr.2012.12.011