Calcium-Sensing Receptor in Cardiac Physiology

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Abstract: Calcium is a crucial signal molecule in the cardiovascular system. Calcium (Ca^{2+}) acts as a second messenger *via* changes in intracellular Ca^{2+} levels through the actions of calcium channels and pumps. However, it is now well known that calcium may also be an extracellular first messenger through a G-protein-coupled receptor that senses extracellular Ca^{2+} concentration, the calcium-sensing receptor (CaR). The CaR is one of the key players in extracellular calcium homeostasis, but besides being expressed in the major organs involved in calcium homeostasis, the parathyroid gland, kidney and intestine, the CaR has also been found to be functionally expressed in other tissues. Although several studies demonstrated the CaR in heart and blood vessels, exact roles of the receptor in the cardiovascular system still remain to be elucidated. This review will summarize the current knowledge on the expression and possible functions of the CaR in the cardiac tissue.

Keywords: CaSR, cardiomyocytes, smooth muscle cells, hypertension.

1. INTRODUCTION

Calcium ion (Ca^{2+}) is a crucial signal molecule in the heart. For example, it is well-accepted that intracellular calcium release from the sarcoplasmic reticulum (SR) is required for cardiac muscle contraction. Indeed, with each heartbeat the calcium concentration in the cytosol of cardiac myocytes is elevated approximately 10-fold from a resting level. Thus, Ca^{2+} acts as the most important second messenger *via* changes in its intracellular levels, through the actions of calcium channels and pumps. However, it is now commonly believed that calcium is also an extracellular first messenger through a G-protein-coupled receptor that senses extracellular Ca^{2+} concentration, the calcium-sensing receptor (CaR)**.** The binding of extracellular Ca^{2+} to the CaR elicits complex intracellular signals through modulation of a wide range of proteins, including G proteins and phospholipase C (PLC), which in turn stimulate inositol triphosphate production, thereby increasing intracellular Ca^{2+} release. Downstream of or in parallel with PLC, the CaR also activates mitogen-activated protein kinases (MAPKs).

The most important function of the CaR is to regulate calcium homeostasis throughout the body [1]. Activation of the CaR in parathyroid glands inhibits secretion of parathyroid hormone (PTH), the key calcium-regulating hormone. The CaR is also expressed in other organs involved in calcium homeostasis, namely bone, kidney, and intestine. However, the receptor is also widely expressed in tissues uninvolved in calcium homeostasis and modulates various cellular functions, including secretion of peptides, ion-channel/transporter activity, gene expression, proliferation, differentiation, apoptosis, and chemotaxis. There is growing evidence that the CaR is functionally expressed in cardiac tissue [2-4]. This review aims to provide a brief background of CaR structure, signaling, and function, followed by a more detailed discussion of the expression and potential role of the CaR in the heart.

2. CALCIUM-SENSING RECEPTOR STRUCTURE AND SIGNALING

The CaR belongs to the family C of the G protein-coupled receptors (GPCRs), which are also called seven-transmembrane receptors (7TMs) [1]. The 7TMs constitute the largest group of cell surface membrane receptors, and they have become one of the most important drug targets. The human CaR consists of 1078 amino-acid residues and, like all 7TMs, has three structural domains: an amino (N)-terminal domain of 612 amino acid residues, which is an unusually large extracellular domain (ECD) characteristic of the family C receptors of the 7TMs; a seven-transmembrane domain (TMD) of 250 amino acid residues; and a 216-amino-acid intracellular carboxyl (C) -terminal domain (ICD). The receptor is modified by N-linked glycosylation, which is important for cell-surface expression [5]. The cell-surface CaR is present in a homodimeric configuration, which is crucial for its normal function [6]. The ECD is the main extracellular Ca^{2+} ($Ca^{2+}{}_o$) binding site, but a mutated CaR that lacks the ECD also responds to $Ca^{2+}{}_o$, implying that the TMD also participates in calcium sensing [7].

Although the main ligand of the CaR is $Ca^{2+}{}_o$, the CaR is a promiscuous receptor with a variety of ligands, which can

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Table 1. A List of Known Agonists and Antagonists of the CaR

Direct Agonists	Positive Allosteric Modulators	Negative Allosteric Modulators
Ca^{2+}	L-type amino acids	Calcilytics
Other cations *(e.g. Gd^{3+}, Mg^{2+})*	Calcimimetics	
Polyamines *(e.g. spermine)*		
Antibiotics *(e.g. neomycin)*		
pH		
Ionic strength		

be divided into type I and type II [1, 8]. Type I ligands are direct agonists (orthosteric agonists), whereas type II are allosteric modulators that change the affinity of the receptor to Ca^{2+}_o and other direct agonists. The type I ligands are all poly-charged cations, both organic and inorganic (listed in Table **1**). In general, CaR agonists with a high positive charge density tend to have higher potency. Furthermore, the CaR has also been shown to be sensitive to changes in ionic strength and pH [9, 10]. Type II agonists compromise two groups: small pharmacological drugs, termed calcimimetics; and L-amino acids [8, 11]. The calcimimetics bind to the TMD of the CaR and increase its sensitivity to Ca^{2+}_o [12]. The calcimimetic drug AMG 073, called Mimpara in Europe or Sensipara in the US, is used in the treatment of uremic secondary hyperparathyroidism [13, 14]. Earlier drugs as NPS 567 and NPS 467 are not used clinically; AMG 073 is currently the drug of choice due to pharmacokinetic considerations. Drugs that negatively modulate the CaR in an allosteric fashion are termed calcilytics.

The CaR is a low-affinity receptor: Ca^{2+}_o produces half-maximal activation of the CaR at about 3.5 mM in CaR-transfected human embryonic kidney (HEK-CaR) cells *in vitro* [15]. However, the Hill coefficient, a measure of how well the receptor responds to small changes in agonists, is 3-4 in HEK-CaR and bovine parathyroid cells [this will mean more if you provide a little explanation of the Hill coefficient]. This allows the CaR to detect very small fluctuations in extracellular Ca^{2+} levels.

The intracellular signalling apparatus of the CaR is very complex (for an overview, see Fig. **1**) and depends markedly on the cell type in which the receptor is expressed. In most cells, CaR stimulation elicits phospholipase C (PLC)-mediated inositol triphosphate (IP_3) formation with intracellular Ca^{2+} (Ca^{2+}_i) mobilization, indicative of $G\alpha_q$ activation [2, 16]. This interaction also induces activation of protein kinase C (PKC), which in turns modulates the activity of the receptor by a negative feedback system [17, 18]. In parallel, the CaR has been shown to activate phosphatidylinositol 4-kinase (PI4K), an enzyme that facilitates the first step in inositol lipid biosynthesis [18]. The CaR interacts directly not only with $G\alpha_q$, but also with pertussis toxin-sensitive $G\alpha_i$, which results in the inhibition of adenylate cyclase and therefore a reduction in cellular cyclic adenosine monophosphate (cAMP) levels [19].

The CaR has also been linked by several signalling pathways to various mitogen-activated protein kinases (MAPKs) such as MAPK kinase 1 (MEK1), extracellular signal-regulated kinases (ERKs), p38 MAPK, and Jun amino-terminal kinase (JNK), which account for many distal effects of the CaR, such as proliferation, differentiation, regulation of peptide secretion, and ion channel activity [2, 20-23]. As is the case with many other cell-surface receptors, it is at present poorly understood how the activation of a single receptor type, in this case the CaR, can result in such varied biological endpoints depending on the cellular context in which the receptor is expressed.

3. THE CALCIUM-SENSING RECEPTOR IN CALCIUM HOMEOSTASIS

The CaR is one of the key players in calcium homeostasis, its major function being to inhibit parathyroid hormone (PTH) release from the parathyroid glands. A decrease in plasma concentration of Ca^{2+} results in a CaR-mediated increase in PTH secretion from the parathyroid cells. The augmented PTH promotes distal renal tubular Ca^{2+}_o reabsorption and bone resorption by the lining cells, both leading to an increase in Ca^{2+}_o [24]. Furthermore, the relative hypocalcemia also results in decreased secretion of calcitonin from thyroid C cells mediated by the CaR, preventing inhibition of bone resorption by calcitonin [25]. Both PTH and low Ca^{2+} induce synthesis of $1.25(OH)_2$ vitamin D_3 in the proximal tubuli cells of the kidney. The vitamin D metabolite stimulates intestinal Ca^{2+}_o absorption.

In addition to parathyroid glands, the CaR is also expressed in other main organs involved in ion homeostasis, namely kidney, bone, and intestine. The CaR is expressed in many segments of the nephron; the cellular localization depends on the cell type [26]. The expression of the CaR is highest in the thick ascending limb (TAL) of the nephron, where it impairs NaCl reabsorption, creating a lumen-positive gradient that limits Ca^{2+} and Mg^{2+} reabsorption [27]. In the proximal tubule, activation of the CaR appears to attenuate PTH-induced, but not dopamine-induced, inhibition of phosphate resorption [28]. Therefore, the CaR in the proximal tubule may reduce the renal phosphate loss in PTH-dependent hypercalcemia, perhaps maintaining the phosphate-calcium product at a correct level for processes such as bone mineralization. Activation of the CaR in the collecting duct reduces vasopressin-elicited water resorption, resulting in more dilute urine, and thereby possibly decreasing the risk of stone formation [29].

CaR expression has also been demonstrated in several cell types of bone and intestine [30-32]. However, the exact roles of the CaR in these tissues are now only beginning to be elucidated, and much work remains.

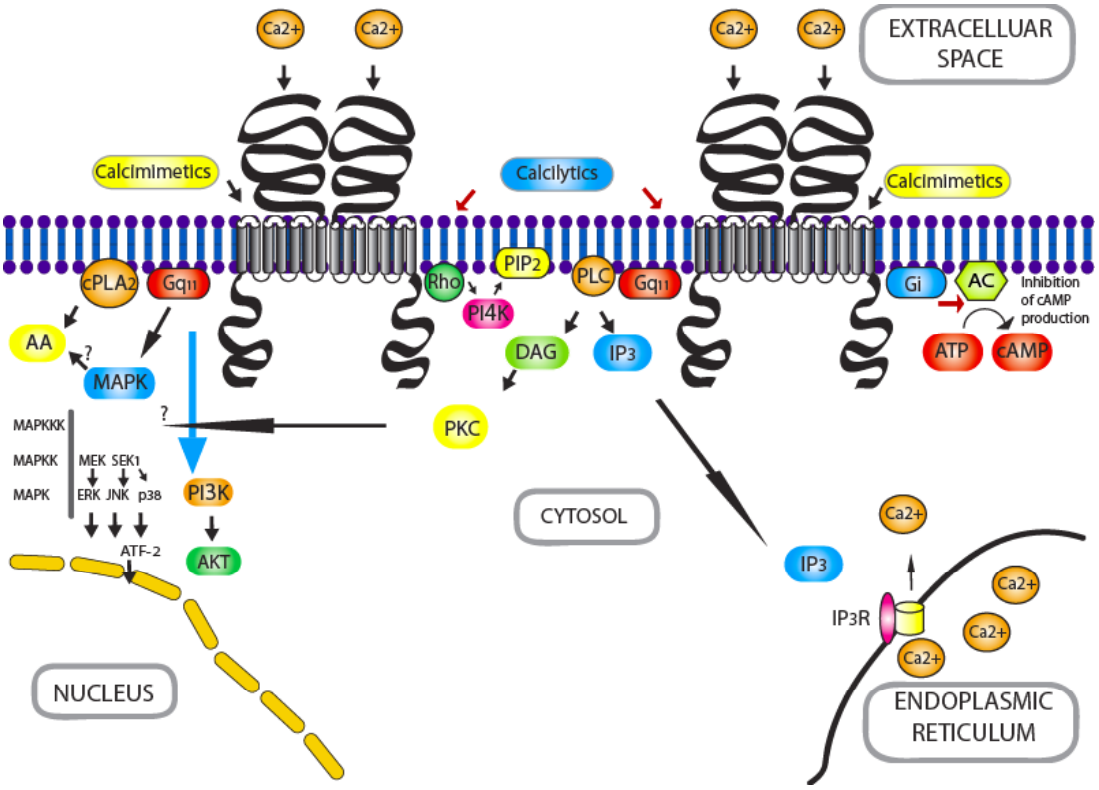

Fig. (1). Signaling pathways activated by CaR. CaR is activated by Ca^{2+}_o, calcimimetics and numerous other agents. Please refer to text for detailed information. Black and blue arrows indicate stimulation and red arrow indicates inhibition. Abbreviations: Arachidonic acid (AA), Adenylyl cyclase (AC), protein kinase B (AKT), activating transcription factor-2 (ATF-2), adenosine triphosphate (ATP), cyclic adenosine monophosphate (cAMP), extracellular regulated kinase (ERK), alpha subunit of i and q subtype of the heterotrimeric G proteins (Gi and Gq_{11}), inositol-1,4,5-triphosphate (IP_3), Jun amino terminal kinase (JNK), mitogen-activated protein kinase (MAPK), MAPK kinase (MEK), p38 MAPK (p38) phosphatidylinositol 4-kinase (PI4K), phosphatidylinositol 3-kinase (PI3K), protein kinase C (PKC), phosphatidylinositol-4,5-biphosphate (PIP_2), and stress-activated protein kinase ERK kinase 1 (SEK1).

4. EXPRESSION AND FUNCTION OF THE CAR IN THE HEART

Besides being expressed in all four organs involved in calcium homeostasis, the CaR has also been found to be functionally expressed in tissues uninvolved in calcium homeostasis, including cardiac tissue [33, 34]. The first evidence of the presence of the CaR in heart came in 2003 when Wang *et al.* detected CaR messenger RNA (mRNA) and protein in rat adult atrial and ventricular cardiomyocytes [3]. Challenging isolated ventricular myocytes with Ca^{2+}_o and other type 1 CaR agonists (Gd^{3+} and spermine) induced concentration-dependent increases in Ca^{2+}_i concentration as well as in intracellular inositol phosphate (IP) levels, suggesting that the CaR is linked to the PLC/IP pathway. We established that the CaR is also present in rat neonatal ventricular cardiomyocytes and that it is coupled to the PLC/IP and MEK1/ERK pathways [2].

We used two approaches to verify that Ca^{2+}_o-induced effects in cardiomyocytes are CaR-mediated. First, we infected the cells with adeno-associated virus containing the dominant negative CaR (R186Q [35]) and compared the effects of Ca^{2+}_o on IP accumulation with those in cells infected with the control adeno-associated virus expressing β-galactosidase, a protein approximately the same size as the CaR. Expressing the dominant negative CaR produced a downward and rightward shift in the concentration-response curve for Ca^{2+}_o-induced IP accumulation in cardiomyocytes. Similar inhibitory effects of this mutant dominant negative CaR on the response of the wild-type CaR to Ca^{2+}_o were also produced in other CaR-expressing cells [22, 35-37]. We also utilized the calcimimetic AMG 073 to further test the hypothesis that the CaR is the mediator of the effects of Ca^{2+}_o. As expected, AMG 073 augmented the effects of Ca^{2+}_o on IP accumulation. Furthermore, ERK1/2 activation was more rapid in response to AMG 073 than with Ca^{2+}_o alone. Different kinetics of ERK1/2 activation with Ca^{2+}_o and calcimimetics have been noted in other systems [19]. Stimulating the CaR with AMG 073 induced a decrease in DNA synthesis in neonatal cardiomyocytes, suggesting the CaR's involvement in regulation of the cell cycle. Although adult cardiomyocytes lose the ability to proliferate, cell proliferation can take place in neonatal cardiomyocytes [38, 39]. Moreover, DNA synthesis is observed in neonatal cardiomyocytes undergoing hypertrophy, perhaps due to partial progression through the cell cycle [39, 40]. The CaR-mediated decrease in DNA synthesis seen in our studies was not correlated with changes in cell number, pointing toward CaR's protective role against cardiac hypertrophy. On the contrary, it has been suggested by a Chinese group that the CaR may promote hypertrophy,

who investigated this possibility in a model of neonatal rat cardiomyocyte angiotensin II-induced hypertrophy [41]. They noted an increase in angiotensin II-induced hypetrophic response in the presence of Gd^{3+} compared to angiotensin II alone. Moreover, these authors claimed that the CaR induces neonatal rat cardiomyocyte apoptosis through activation of MAPKs and caspase 9 signaling pathways [42, 43]. However, in their studies, Gd^{3+} was the only agonist used to show the effects of the CaR. It is possible that the effects of Gd^{3+} may take place through the CaR, but we find it more likely that the observed effects are due to unspecific effects of Gd^{3+}. The toxicity of gadolinium on cardiac and cochlear cells has been demonstrated previously [44-46]. The effects in these cells are related to altered electrical conduction. In chondrocytes, gadolinium ion blocks intracellular calcium currents after mechanical stimulation, probably by blocking mechanosensitive calcium channels [47]. Therefore, additional studies using other CaR agonists and approaches are needed to determine the roles of the CaR in cardiac hypertrophy and apoptosis.

In addition to the rat heart, immunohistochemical staining of tissue sections from a sheep heart revealed CaR protein in endocardial endothelium, myocardial microvasculature, and cardiac fibroblasts [4]. Although the CaR has been demonstrated in rat cardiomyocytes, it appeared not to be expressed in cardiomyocytes from sheep. To follow up on these interesting data, we investigated the possibility that the CaR is expressed in the rat cardiofibroblast. Surprisingly, we were not able to detect its transcripts in rat or human cardiac fibroblasts grown in cell culture (data not published). These results might be explained by species variation or by the difference between cell culture systems versus tissue specimens.

Data is less conflicting on the presence the CaR in endothelial cells, where the receptor has been shown to be expressed in arteries from many organs and species [48-50]. For example, the presence of CaR was neatly demonstrated in endothelial cells from porcine coronary arteries [49]. Stimulation of the receptor with a specific positive allosteric modulator, Calindol, but not its less potent S-enantiomer, induced endothelium-dependent hyperpolarization of vascular smooth muscle cells (VSMC). The hyperpolarization to calindol was significantly reduced in the presence of a negative allosteric modulator of the CaR, Calhex. Furthermore, a specific inhibitor of intermediate conductance Ca^{2+}-sensitive potassium channels (IK_{Ca}) abolished calindol-induced hyperpolarization. Therefore, it is interesting that the CaR has previously been shown to activate Ca^{2+}-sensitive potassium channels in porcine brain [51]. Taken together, these results suggest that the CaR in the endothelial layer of porcine coronary arteries activates IK_{Ca}, resulting in K^+ -induced hyperpolarization of the VSMCs. Thus it seems likely that the CaR is a regulator of coronary vascular tone and thus blood flow.

5. CONCLUSION

It is now evident that that the CaR is functionally expressed in cardiac tissue, specifically in cardiomyocytes and coronary endothelial cells. Presently, there are conflicting data regarding the presence of the CaR in cardiofibroblasts, the most numerous cells in the heart. In the cardiomyocytes, all studies to date have shown that the CaR might regulate hypertrophy. In the coronary arteries, the CaR present in the endothelial cells probably regulates coronary vascular tone through potassium channels and thus is a regulator of flood flow. However, additional studies are needed to determine the exact expression pattern of the CaR and its signaling mechanisms to understand its precise role in both normal heart physiology and pathophysiology.

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