Novel Role of the Calcium-Sensing Receptor in Blood Pressure Modulation

Sanela Smajilovic, Jacob Tfelt-Hansen

The calcium-sensing receptor (CaR) was cloned in 1993 from bovine parathyroid gland. Subsequently, the receptor was reported in a wide range of tissues and cell types. It belongs to the superfamily C of the G protein–coupled receptors, which are also known as 7 transmembrane receptors.

The CaR is a promiscuous receptor that, in addition to extracellular calcium (Ca$^{2+}$), recognizes many other ligands. CaR ligands are divided into type I agonists, which are direct agonists, and type II, which act as allosteric modulators that change the affinity of the receptor to calcium and other cations. The newly developed calcimetics are type II agonists that bind to the transmembrane domain and positively modulate the CaR. These drugs have been introduced recently in the treatment of uremic secondary hyperparathyroidism.

The binding of Ca$^{2+}$ or other CaR agonists to the CaR elicits complex intracellular signals through modulation by the CaR of a wide array of intracellular signaling proteins, including G proteins and phospholipase C, which stimulates inositol triphosphate production. Downstream of or in parallel with phospholipase C, the CaR also activates mitogen-activated protein kinases (MAPKs).

The most important physiological function of the CaR is to maintain and regulate systemic calcium homeostasis. In parathyroid glands, the CaR inhibits secretion of parathyroid hormone (PTH), the key calcium-regulating hormone. The CaR is also expressed in bone, kidney, and intestine, the major calcium-translocating organs. In the kidney, the receptor regulates renal sodium and ion transport, although the exact mechanisms of action are not fully established.

In addition to organs involved in calcium homeostasis, the receptor is also widely expressed in other tissues, including blood vessels and cardiomyocytes. The CaR protein was first reported in perivascular nerves of isolated rat arteries and proposed to modulate vascular tone. Recently, the CaR has also been detected in endothelial cells and vascular smooth muscle cells (VSMCs) and shown to induce hyperpolarization of the smooth muscle cells. Furthermore, several novel studies reported the CaR as a possible regulator of blood pressure. In this review, we summarize the status of research concerning possible roles of the CaR in blood pressure regulation and discuss possible mechanisms for its actions. A brief overview on the CaR structure, signaling, and function is provided, followed by a more detailed discussion of the expression and function of the receptor in the cardiovascular system and its role in vascular tone regulation.

**Structure and Signaling of the CaR**

The CaR consists of 3 structural domains: a large, extracellular domain of ≈600 amino acids and 7 transmembrane and intracellular domains each containing ≈200 amino acids (Figure 1). The receptor is present in a homodimeric configuration, which is crucial for its normal function. The main Ca$^{2+}$ binding site is in the extracellular domain of the CaR, but the transmembrane domain also participates in the calcium sensing. The extracellular domain contains several N-linked glycosylation sites and cysteine residues, which are important for cell-surface expression and function of the receptor. In the intracellular domain, the CaR has 5 protein kinase C phosphorylation sites, which are involved in downregulation of the receptor activity and thereby are a negative feedback mechanism.

In addition to Ca$^{2+}$, the CaR responds to many other cations, naturally occurring polyamines, and some antibiotics (Table). Furthermore, the CaR is also sensitive to changes in ionic strength and pH. In addition to the direct agonists, several allosteric modulators that change the affinity of the receptor to Ca$^{2+}$ and other direct agonists have been identified. Positive allosteric modulators are the L-amino acids and small pharmacological drugs known as “calcimetics,” Drugs that negatively modulate the CaR likewise in an allosteric fashion are termed “calcilytics.”

Elevations in the Ca$^{2+}$ or exposure to other CaR agonists activate the CaR and may convert into activation of many signaling pathways. The nature and selection of such pathways depend markedly on the cell type in which the receptor is expressed, and the spatial temporal activation of the different signaling pathways are, at present, poorly understood. The CaR, like other G protein–coupled receptors, acts mainly through G proteins. In most cell types, the CaR interacts with G$q_0$ or G$q_{11}$, subunits of heterotrimeric G proteins, resulting in activation of phospholipases C and A$_5$. This interaction also provides the...
signals for activation of protein kinase C, which, in turn, negatively modulates the activity of the receptor as negative feedback. In parallel, the CaR activates phosphatidylinositol 4-kinase, which is an enzyme that facilitates the first step in inositol lipids biosynthesis, independent of the G proteins, but by a \( p \)-dependent mechanism. In addition to Goq and Go11, the CaR can interact with a pertussis toxin–sensitive inhibitory G protein, Goi, which results in inhibition of adenylly cyclase and a reduction in cAMP levels.

The CaR has also been reported to activate MAPKs, such as the extracellular signal-regulated kinases, p38 MAPK and Jun amino-terminal kinase, which account for many distal effects of the CaR, such as proliferation, differentiation, regulation of peptide secretion, and ion channel activity. In addition, the CaR has been shown to transactivate epidermal growth factor receptor. Thus, MAPKs could be activated directly by the CaR or indirectly mediated by epidermal growth factor receptor transactivation.

The CaR has been found recently to interact with 2 scaffolding proteins, filamin A and \( \beta \)-arrestins. Filamin A appears to be important for extracellular signal regulated kinase activation by the CaR and protects the membrane-bound CaR protein against degradation. \( \beta \)-Arrestins and G protein–coupled kinases, a third CaR interacting protein, are typically involved in homologous desensitization of G protein–coupled receptors. Although the CaR is believed to desensitize very slowly, \( \beta \)-arrestins and G protein–coupled kinases appear to mediate CaR desensitization. In favor of this notion, \( \beta \)-arrestin 2 null mice appear to have a leftward shift in the calcium-PTH relationship, and this inverse sigmoidal relationship is mediated by the CaR, as mentioned above. Thus, \( \beta \)-arrestins are also important for regulating CaR function in the parathyroid glands in vivo. Lately, \( \beta \)-arrestins and G protein–coupled kinases have also been shown to act as signal transducers themselves independent of the G proteins. This is mediated by activation of MAPKs, adding to the complexity of the downstream signaling mechanism of the G protein–coupled receptors. Future studies are needed to investigate whether this also is the case for the CaR.

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

<table>
<thead>
<tr>
<th>Direct Agonists</th>
<th>Positive Allosteric Modulators</th>
<th>Negative Allosteric Modulators</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{Ca}^{2+} )</td>
<td>L-type amino acids</td>
<td>Calcilytics</td>
</tr>
<tr>
<td>Other cations (eg, ( \text{Gd}^{3+} ), ( \text{Mg}^{2+} ))</td>
<td>Calcimimetics</td>
<td></td>
</tr>
<tr>
<td>Polyamines (eg, spermine)</td>
<td></td>
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<tr>
<td>pH</td>
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<tr>
<td>Ionic strength</td>
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**CaR in the Calcium Homeostasis**

\( \text{Ca}^{2+} \) homeostasis is tightly regulated, mainly by 3 so-called calciotropic hormones: PTH, calcitonin, and 1,25(OH)\(_2\) vitamin D\(_3\). The inverse sigmoidal relationship between \( \text{Ca}^{2+} \) and secretion of the PTH and the positive relationship between \( \text{Ca}^{2+} \) and secretion of the calcitonin are both mediated by the CaR. The CaR expressed in the parathyroid glands and kidney has a central role in \( \text{Ca}^{2+} \) homeostasis. A reduction in \( \text{Ca}^{2+} \) plasma concentration results in a CaR-mediated increase in PTH secretion from the parathyroid cells because of the inverse sigmoidal relationship between \( \text{Ca}^{2+} \) and secretion of the PTH mediated by the CaR. The increased PTH level promotes distal renal tubular \( \text{Ca}^{2+} \) reabsorption and
bone resorption by the lining cells, both of which increase Ca\(^{2+}\) levels.\(^{37}\) Furthermore, the relative hypocalcemia also leads to reduced release of calcitonin from thyroid C cells mediated by the CaR, preventing inhibition of bone resorption by calcitonin.\(^{39}\) Both PTH and a low Ca\(^{2+}\) level induce synthesis of 1,25(OH)\(_2\) vitamin D\(_3\) in the proximal tubular cells of the kidney. This active vitamin D metabolite stimulates intestinal Ca\(^{2+}\) absorption. The reverse of these events occurs in hypercalcemia.

In the kidney, the CaR is expressed in many segments of the nephron.\(^{39}\) In brief, the functions of the CaR along the nephron are as follows: (1) to diminish the inhibitory effect of PTH on renal phosphate reabsorption in the proximal tubule;\(^{40}\) (2) to inhibit renal calcium excretion in the cortical thick ascending limb of the loop of Henle;\(^{41}\) and (3) to reduce urinary concentrating capacity in the inner medullary collecting duct.\(^{42}\) Lately, CaR expression was also demonstrated in the juxtaglomerular cells of the mouse kidney and shown to suppress the release of renin, the critical enzyme in the formation of angiotensin, and the potent vasoconstrictor angiotensin II.\(^{43}\)

In addition to the parathyroid glands and the kidney, bone and intestine are the 2 other important organs involved in the maintenance of Ca\(^{2+}\) homeostasis. In vitro studies have reported the presence of CaR in cells of bone and intestine.\(^{44}\) Therefore, the presence of the CaR in these 4 organs, all of which are important in Ca\(^{2+}\) homeostasis, may allow Ca\(^{2+}\) to act as a fourth calcitropic hormone, or as a “first messenger.”

Expression of the CaR in the Cardiovascular Tissues

The CaR has conclusively been demonstrated in 2 main components of the cardiovascular system: heart and blood vessels\(^{45}\) (Figure 2). A functional CaR is expressed in neonatal and in adult rat cardiomyocytes, and its activation induces increases in intracellular calcium (Ca\(^{2+}\)) concentrations and intracellular inositolphosphate concentrations, indicating that the CaR is linked to the phospholipase C pathway.\(^{26,46}\) Furthermore, CaR in the cardiomyocytes activates extracellular signal-regulated kinase 1/2, which are components of the MAPK signaling pathway known to be activated by the CaR. Recently, Klein et al\(^{47}\) demonstrated the CaR protein in the heart from sheep using commercially available CaR-specific antibodies. The CaR was localized to endocar-

DNA synthesis is observed in cardiomyocytes undergoing hypertrophy, perhaps because of a partial progression through the cell cycle.\(^{98,49}\) Activation of the CaR in rat neonatal cardiomyocytes induces a reduction in DNA synthesis, without affecting the cell number, suggesting that CaR might protect against cardiac hypertrophy.\(^{26}\) Furthermore, increased expression of the CaR protein in the rat heart was reported during ischemia/reperfusion.\(^{50}\) Additional studies are needed to determine the exact expression pattern of the CaR and its signaling mechanisms to understand its role in heart physiology and pathophysiology.

The presence of the CaR has also been reported in blood vessels of many types and species. More than 10 years ago, the CaR protein was detected in perivascular nerves in rat mesenteric, renal, coronary and cerebral arteries.\(^{51,52}\) The authors did not detect CaR mRNA in the arteries but did find it in dorsal root ganglia and suggested that the CaR protein might be transported from the dorsal root ganglia to the perivascular nerve network of peripheral arteries.\(^{53}\) Lately, evidence of a functional CaR in medial and endothelial layers of the blood vessels has been provided. The CaR was detected in whole homogenates from rat subcutaneous small arteries\(^{53}\) and in endothelial cells from rat mesenteric arteries, porcine coronary arteries,\(^{6}\) and aorta from rat and sheep.\(^{57,54}\) Recently, the CaR was shown in immortalized endothelial cells from human aorta, and stimulation of the receptor induced production of NO, the most potent vasodilator.\(^{55}\) The CaR was shown previously to stimulate NO production in other cells.\(^{56,57}\) The CaR was also found in VSMCs from the gerbilline spiral modiolar artery,\(^{58}\) from rat aorta,\(^{7}\) and from human aorta.\(^{59}\) However, it should be noted that some investigators have been unable to demonstrate the presence of the CaR in aortic VSMCs.\(^{47,60}\) We found the CaR to be present in cultured aortic VSMCs from rats; however, in our hands, immunohistochemical staining of rat aortic tissue sections with the monoclonal anti-CaR LRG antibody made at the National Institutes of Health\(^{61}\) showed the CaR in the endothelium but not in the medial layer of the vessel (Figure 2).\(^{54}\) Moreover, Klein et al\(^{47}\) did not find the CaR in the VSMCs in aorta sections from sheep.

The overall picture is that the CaR is present in the perivascular nerves of the adventitia, as well as in endothelial cells and perhaps VSMCs. Potential roles for the CaR in modulating vascular tone of the blood vessels are discussed below.

Potential Roles of the CaR in Regulating Blood Pressure

The CaR plays an essential role in extracellular Ca\(^{2+}\) homeostasis by regulating the rate of PTH secretion and the rate
of calcium reabsorption by the kidney. Quite a few observations link Ca\(^{2+}\) homeostasis with blood pressure.\(^{62}\) The practicing physician Addison\(^{63}\) was first to associate calcium directly with clinical hypertension. Eighty years ago he reported that oral calcium supplementation could lower blood pressure in hypertensive individuals. Several subsequent studies confirmed this hypothesis.\(^{64}\) Moreover, in animal models of hypertension, parathyroidectomy and elevated dietary Ca\(^{2+}\) intake prevent the development of hypertension.\(^{65–67}\) Furthermore, epidemiological studies confirm an inverse correlation between Ca\(^{2+}\) intake and blood pressure in specific human populations.\(^{68,69}\)

Several potential mechanisms that may explain the effect of calcium on blood pressure have been proposed, including changes in the secretion of calcium-regulating hormones, such as PTH and parathyroid hypertensive factor. However, there has been little or no consideration to the hypothesis that changes in Ca\(^{2+}\) concentration may modify the blood pressure through the CaR. Nevertheless, several studies in vitro, as well as in vivo, point toward this possibility.

It has been known for a long time that Ca\(^{2+}\) can induce relaxation of isolated arteries. In 1911, Cow\(^{70}\) observed that Ca\(^{2+}\) reduces reactivity of isolated arteries. More than 50 years later, Webb and Bohr\(^{71}\) showed that high Ca\(^{2+}\) induces relaxation after contraction induced by norepinephrine, but the exact molecular mechanism for this phenomena has remained unknown. In 1997, 4 years after the cloning of the CaR, Bukoski et al\(^{5}\) suggested that Ca\(^{2+}\) induces relaxation of the isolated arteries by activating CaR in perivascular nerves, resulting in the release of a nerve-derived hyperpolarizing vasodilator, possibly a cannabinoid. Later, Ohanian et al\(^{63}\) showed that Ca\(^{2+}\), Mg\(^{2+}\), and neomycin (all CaR agonists) induce relaxation in isolated rat subcutaneous small arteries, which express the CaR. Recently, we have shown that a calcimimetic, AMG 073, induces relaxation on isolated rat aorta.\(^{54}\) Although the relaxation was also observed when endothelium function was inhibited by N\(^{G}\)-nitro-L-arginine methyl ester and indomethacin, the effect was greater in the intact vessels. Wu and Bohr\(^{72}\) demonstrated a similar finding for calcium-induced relaxation many years ago. Recently, an elegant study by Weston et al\(^{6}\) showed that stimulation of the CaR with a specific positive modulator, Calindol, induces endothelium-dependent hyperpolarization of the VSMCs in rat mesenteric and porcine coronary arteries. Calindol-induced hyperpolarization was abolished by a specific inhibitor of the intermediate conductance Ca\(^{2+}\)-sensitive potassium channels, TRAM-34. In favor of this hypothesis, the CaR was shown previously to activate various ion channels in the brain, including nonselective cation channels\(^{73,74}\) and calcium-sensitive K\(^{+}\) channels.\(^{75}\) Edwards and Weston\(^{76}\) proposed that the CaR in the endothelial layer of the arteries activates intermediate conductance Ca\(^{2+}\)-sensitive potassium channels, resulting in K\(^{+}\) -induced hyperpolarization of the VSMCs. Although hyperpolarization is usually associated with relaxation, Calindol did not induce relaxation of phenylephrine-precontracted mesenteric arteries in this study. They hypothesized that this could be because of phenylephrine-induced increases of K\(^{+}\) from smooth muscle cells, the so-called “K\(^{+}\) clouds,” which make the system less able to respond to further increases in K\(^{+}\). This prevents hyperpolar-

Figure 3. Schematic figure of the proposed effects of the calcium-sensing receptor on blood pressure. Black arrows signify known effects; red arrows signify proposed effects. See text for details.
izing after K⁺ eflux from endothelial-cell K⁺ channels. In a novel report, they showed, by using K⁺-sensitive electrodes, that this K⁺ cloud was a reality. Moreover, once the cloud was abolished, Calindol-induced relaxation of precontracted vessels were obtained. Furthermore, they demonstrated diminished relaxed effects of Calindol in arteries from a rat model of type 2 diabetes, which could be explained by a reduced expression of the CaR in these vessels. These observations are supported by a recent study showing a relaxant effect of Ca²⁺ on phenylephrine-precontracted mesenteric arteries that was sensitive to both TRAM-34 and to endothelial cell damage, pointing toward the CaR as the mediator of the effects of calcium and Calindol. Moreover, Ca²⁺ increased the sensitivity to acetylcholine.

All together, these results indicate that the CaR may have a physiological role in the modulation of blood pressure (Figure 3). This hypothesis is supported by several in vivo studies.

A calcimimetic, NPS R-568, has been reported to significantly decrease blood pressure in uremic and spontaneously hypertensive rats but not in normotensive rats. Similar effects on blood pressure were achieved by parathyroidectomy, and administration of NPS R-568 did not intensify the hypotensive effect of the parathyroidectomy. Accordingly, administration of a calcilytic NPS-2143 increases blood pressure in normotensive rats in the presence of parathyroid glands. The effect of the calcimimetic is probably not directly related to the reduced PTH secretion, because PTH causes vasodilation and lowers blood pressure. More likely, activation of the CaR in parathyroid glands by the calcimimetic might suppress secretion of parathyroid hypertensive factor. In favor of this hypothesis, low Ca²⁺ was shown to increase parathyroid hypertensive factor secretion in parathyroid gland organ cultures from normotensive and hypertensive rats. Although the results indicate that the hypotensive effect of NPS R-568 depends on the presence of parathyroid glands, direct effects of the calcimimetic, either via the CaR and/or other mechanisms, on blood vessels cannot be excluded. Furthermore, a modulation of the renin-angiotensin system may also be involved, because activation of the CaR was shown to suppress renin secretion in mice. However, there were no changes in plasma angiotensin-converting enzyme activity and aldosterone levels after administration of the calcimimetic in uremic rats.

In addition to the long-term reductions in blood pressure, the calcimimetics induce acute hypertension in both uremic and normal rats. Thus, the calcimimetics cause an initial blood pressure increase in normal and uremic rats, which is followed by a marked and sustained hypotensive effect in uremic rats. The mechanisms for the actions of the calcimimetics and calcium on the blood pressure remain to be elucidated but may be through direct effects by the presence of the CaR on the endothelium cells and perivascular nerves or indirect through the kidney or parathyroid glands.

Conclusions

It is now evident that the CaR is expressed in blood vessels of many types and species, and several investigators suggest that the receptor might be involved in the regulation of blood pressure. This could provide a mechanism for an old observation that Ca²⁺ induces vasodilation in isolated blood vessels, as well as for beneficial effects of dietary calcium on hypertension.

Several studies demonstrated that calcimimetics, which positively modulate the CaR, lower blood pressure in rats. Moreover, clinical studies have shown that calcimimetics have a beneficial effect on important cardiovascular outcomes in patients with uremic secondary hyperparathyroidism.

Increasing clinical use of calcimimetics in the treatment of hyperparathyroidism and a potential use of calcilytics in the treatment of osteoporosis make it essential to understand the role of the CaR in vascular physiology. We suggest that the presence of the CaR in many components of the cardiovascular system makes calcium a first messenger that modulates the system. Despite these promising data, the conclusive evidence for the CaR as a modulator of blood pressure lacks and should be of high priority for the field. Future studies should reveal the exact mechanisms by which the CaR may regulate vascular tone and, thus, blood pressure.

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None.

References

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