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REVIEW

# The calcium-sensing receptor and calcimimetics in blood pressure modulation

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Calcium is a crucial second messenger in the cardiovascular system. However, calcium may also be an extracellular first messenger through a G-protein-coupled receptor that senses extracellular concentration ($Ca^{2+}_o$), the calcium-sensing receptor (CaR). The most prominent physiological function of the CaR is to maintain the extracellular $Ca^{2+}$ level in a very tight range by regulating the circulating levels of parathyroid hormone (PTH). This control over PTH and $Ca^{2+}$ levels is partially lost in patients suffering from primary and secondary hyperparathyroidism. Allosteric modulators of the CaR (calcimimetics) are the first drugs in their class to become available for clinical use and have been shown to successfully treat certain forms of primary and secondary hyperparathyroidism. In addition, several studies suggest beneficial effects of calcimimetics on cardiovascular risk factors associated with hyperparathyroidism. Although a plethora of studies demonstrated the CaR in heart and blood vessels, exact roles of the receptor in the cardiovascular system still remain to be elucidated. However, several studies point toward a possibility that the CaR might be involved in the regulation of vascular tone. This review will summarize the current knowledge on the possible functions of the CaR and calcimimetics on blood pressure regulation.

## LINKED ARTICLES

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## Abbreviations

7TM, seven-transmembrane; AA, arachidonic acid; AC, adenylate cyclase; AKT, protein kinase B; ATF-2, activating transcription factor-2; ATP, adenosine trisphosphate; $Ca^{2+}_o$, extracellular calcium concentration; cAMP, cyclic adenosine monophosphate; CaR, calcium-sensing receptor; ERK, extracellular regulated kinase; FHH, familial hypocalciuric hypercalcaemia; Gi and Gq11, alpha subunit of i and q subtype of the heterotrimeric G proteins; GPCR, G-protein-coupled receptor; GRKs, G-protein-coupled kinases; IP3, inositol-1,4,5-triphosphate; JNK, jun amino terminal kinase; MAPK, mitogen-activated protein kinase; MEK, MAPK kinase; NSHPT, neonatal severe hyperparathyroidism; P13K, phosphatidylinositol 3-kinase; P14K, phosphatidylinositol 4-kinase; p38, p38 MAPK; PHPT, primary hyperparathyroidism; PIP2, phosphatidylinositol-4,5-biphosphate; PKC, protein kinase C; PTH, parathyroid hormone; SEK1, stress-activated protein kinase ERK kinase 1; SHPT, uremic secondary hyperparathyroidism; VSMCs, vascular smooth muscle cells

## Introduction

Calcium (Ca) is a non-redundant intracellular messenger in the cardiovascular system. Lowering the calcium entry into the cells of the vessels and heart by blockers of calcium channels is widely used in treatment of hypertension and arrhythmias. And as such it is well known that calcium acts as a second messenger through the actions of calcium channels, exchangers and pumps. However, after the cloning of the calcium-sensing receptor (CaR), a seven-transmembrane

(7TM) and G-protein-coupled receptor (GPCR) in 1993, it is possible that calcium may also act as a first messenger (Brown *et al.*, 1993). The CaR activation mainly by extracellular calcium concentration ($Ca^{2+}_o$) in the physiological status elicits an array of cellular responses, including inositol trisphosphate ($IP_3$) production, and thereby increases in intracellular $Ca^{2+}$ concentration ($Ca_i^{2+}$). The CaR also activates mitogen-activated protein kinases (MAPKs) and phosphatidylinositol 4-kinase (PI4K).

The most important physiological function of the CaR is to maintain and regulate systemic calcium homeostasis. The CaR has been shown to be a unique regulator of metabolic processes in parathyroid, kidney and bone (Tfelt-Hansen *et al.*, 2003b). Thus, the receptor is an attractive target for the treatment of several disorders, and the therapeutic prospects in CaR ligands have been underlined by the recent introduction of an allosteric modulator of the CaR, cinacalcet (Sensipar® and Mimpara® in USA and Europe respectively), in the clinical treatment of secondary hyperparathyroidism by uraemia and parathyroid cancer leading to primary hyperparathyroidism (PHPT) (Tfelt-Hansen *et al.*, 2005a). However, the receptor is also widely expressed at lower levels in tissues not involved in calcium homeostasis, and modulates various cellular functions, including secretion of peptides, ion-channel/transporter activity, gene expression, proliferation, differentiation, apoptosis and chemotaxis (Tfelt-Hansen *et al.*, 2005a). In the cardiovascular system, a functional CaR has been shown to be present in the heart as well as in blood vessels (Bukoski *et al.*, 1997; Wang and Bukoski, 1998; Ohanian *et al.*, 2005; Tfelt-Hansen *et al.*, 2005b; Weston *et al.*, 2005; Ziegelstein *et al.*, 2006). Following a brief introduction to the structure, signal transduction and physiological function of the CaR, this review will primarily focus on the potential roles of the CaR in modulation of blood pressure, both directly through the CaR expressed in blood vessels and through other mechanisms. Furthermore, effects of cinacalcet, the first and only allosteric modulator of a GPCR to enter the drug market, on hypertension will be discussed.

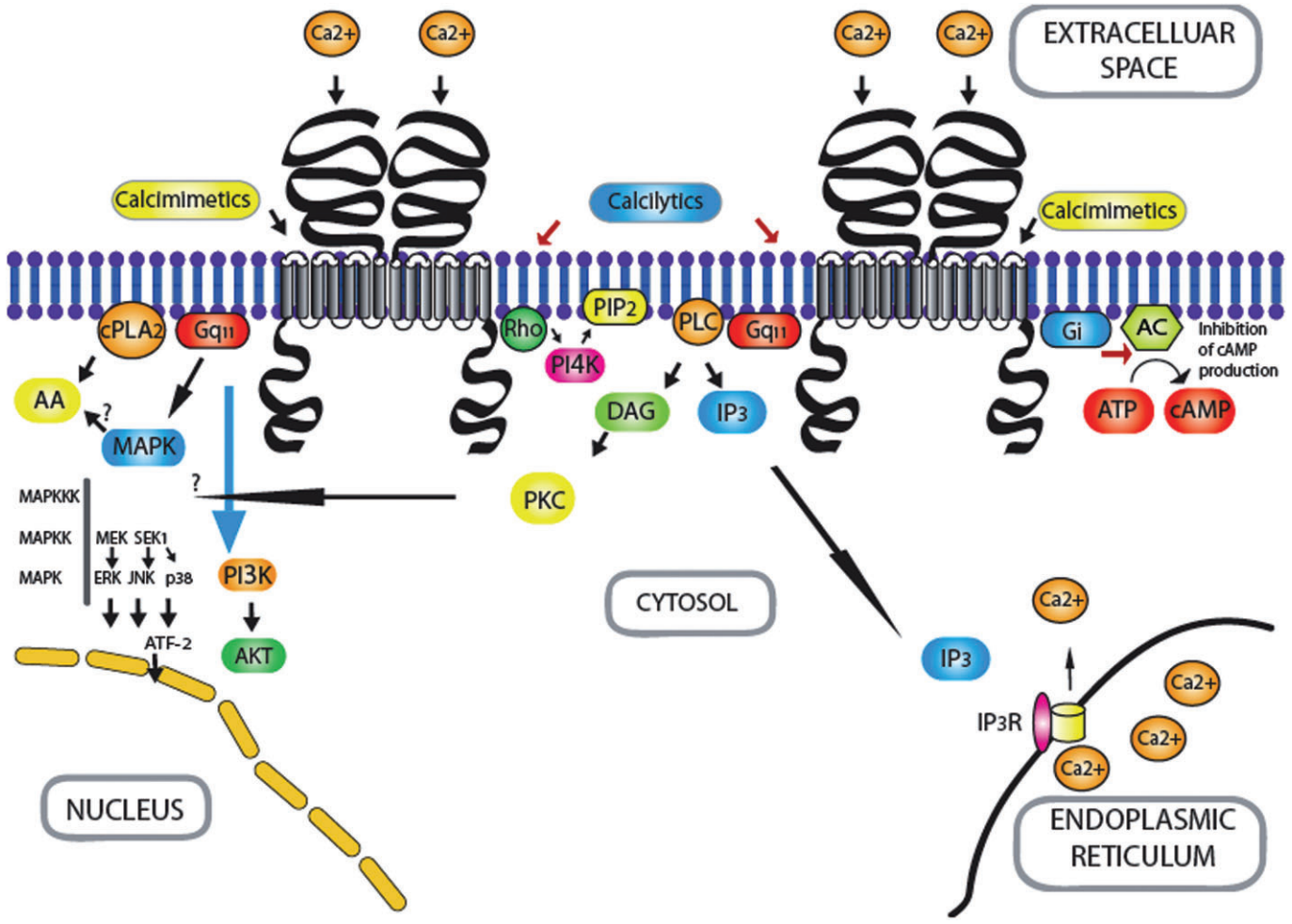
## Molecular structure and signalling pathways of the CaR

The family C of GPCRs, to which the CaR belongs, comprises at least three different subfamilies: Group I includes the metabotropic glutamate receptors, mGluRs1–8 (Jingami *et al.*, 2003); Group II contains the CaR, the novel GPRC6A (Wellendorph and Brauner-Osborne, 2004), a subgroup of putative pheromone receptors and the taste receptors (Hoon *et al.*, 1999); and Group III includes the metabotropic $GABA_B$ receptors (Kaupmann *et al.*, 1997). All these receptors share a common feature, the exceptionally large extracellular domain, which is structurally related to the Venus-flytrap domain of the bacterial periplasmic binding proteins (Felder *et al.*, 1999). The CaR has three structural domains: a large, 612 amino acid residues, extracellular amino (N)-terminal domain; a 7TM domain (TMD) of 250 amino acid residues; and a 216-amino-acid-containing intracellular carboxy (C)-terminus domain. The extracellular amino (N)-terminal domain of the CaR is the main site of $Ca_o^{2+}$ binding, but TMD also participates in calcium sensing (Brauner-Osborne *et al.*, 1999; Hammerland *et al.*, 1999). The cell-surface CaR is present in a homodimeric configuration, which is crucial for its normal function (Bai *et al.*, 1999).

Binding of $Ca_o^{2+}$ or other agonists to the CaR commonly elicits activation of complex intracellular signals, which specifically modulate various cellular functions. The nature of intracellular pathway activated by the CaR markedly depends on the cell type in which the receptor is expressed. The CaR, like other 7TMs, acts through G-proteins. In most cells, the CaR interacts with $G\alpha_{q/11}$ subunits of heterotrimeric G proteins, resulting in activation of phospholipases $A_2$, C and D (Handlogten *et al.*, 2001). Stimulation of PLC results in generation of $IP_3$, which releases $Ca^{2+}$ from intracellular stores in the endoplasmic reticulum, leading to increases in $Ca_i^{2+}$ (Figure 1). Another molecule generated upon PLC activation, diaglycerol, provides the signals for activation of the serine/threonine kinase protein kinase C. In parallel, CaR activates PI4K, which is an enzyme that carries out the first step in inositol lipids biosynthesis, independently of heterotrimeric G proteins, but by a Rho-dependent mechanism (Huang *et al.*, 2002). In some cells, the CaR interacts with a *Pertussis* toxin sensitive inhibitory G protein, $G\alpha_i$, which results in inhibition of adenylate cyclase and a decrease in cellular cyclic adenosine monophosphate (cAMP) levels. The CaR has also been linked by several signalling pathways to various MAPKs such as MAPK kinase 1, 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 (Tfelt-Hansen *et al.*, 2003a; 2004; 2006; Tfelt-Hansen and Brown, 2005). In addition to G-proteins, the CaR binds the scaffolding protein, filamin A (Awata *et al.*, 2001). This interaction is functionally important, since its impairment attenuates CaR-mediated ERK activation. Furthermore, the CaR can interact with β-arrestins and G-protein-coupled kinases (GRKs), which are typically involved in homologous desensitization of GPCRs. Although the CaR is believed to desensitize very slowly, β-arrestins and GRKs appear to mediate the CaR desensitization. However, β-arrestins and GRKs have also been shown to act as signal transducers themselves independent of the G proteins (Lefkowitz and Shenoy, 2005). Future studies are needed to investigate whether this also is true for the CaR.

## Ligands and allosteric modulation of the CaR

The CaR is a promiscuous receptor, which in addition to $Ca_o^{2+}$, also responds to many other cations (e.g. $Mg^{2+}$, $Gd^{3+}$), naturally occurring polyamines (e.g. spermine and spermidine) and some antibiotics (e.g. neomycin) (Tfelt-Hansen and Brown, 2005). The affinity of these molecules to the CaR varies. In general, molecules with high-charge density are more efficient agonists of the CaR than those with lower charge density (Quinn *et al.*, 1997). Moreover, the CaR appears to be highly susceptible to allosteric modulation by a wide range of endogenous ligands and environmental conditions. For example, lowering of ionic strength renders the




## Figure 1

Signalling pathways activated by calcium-sensing receptor (CaR). CaR is activated by $Ca^{2+}_o$, calcimimetics and numerous other agents. Please refer to text for detailed information. Black and blue arrows signify stimulation and red arrows signify inhibition. Abbreviations: arachidonic acid (AA), adenylate cyclase (AC), protein kinase B (AKT), activating transcription factor-2 (ATF-2), adenosine trisphosphate (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).

receptor more sensitive to $Ca_o^{2+}$ and other agonists and elevating of ionic strength produces the converse effect. Changes in extracellular pH can also affect sensitivity of the CaR to its agonists (Quinn *et al.*, 2004). Acidic pH decreases the potency of $Ca_o^{2+}$ and other agonists to activate the CaR, and vice versa for alkaline pH.

CaR signalling has also been shown to be potentiated by numerous L-α-amino acids, in particular the aromatic amino acids L-phenylalanine, L-tyrosine, L-histidine and L-tryptophan (Conigrave *et al.*, 2000). The L-α-amino acids potentiate CaR signalling at physiologically relevant concentrations, and they have been shown to inhibit parathyroid hormone (PTH) secretion from human parathyroid cells in an acute and reversible manner (Conigrave *et al.*, 2004). Several pharmacological CaR modulators, which bind to the TMD, have also been developed. Based on their pharmacological properties on CaR signalling, these synthesized allosteric modulators are divided into two classes: calcimimetics and calcilytics. Calcimimetics are synthetic CaR activators specifically developed for the medical treatment of hypercalcaemia. AMG073, also termed Cinacalcet, is currently the drug of choice and widely use for the medical management of uremic secondary hyperparathyroidism (SHPT) (Dong, 2005). Negative allosteric modulators of the CaR are named calcilytics. In addition to the therapeutical potential in osteoporosis treatment, calcilytics are an important tool for researchers in studying functional properties of the CaR.

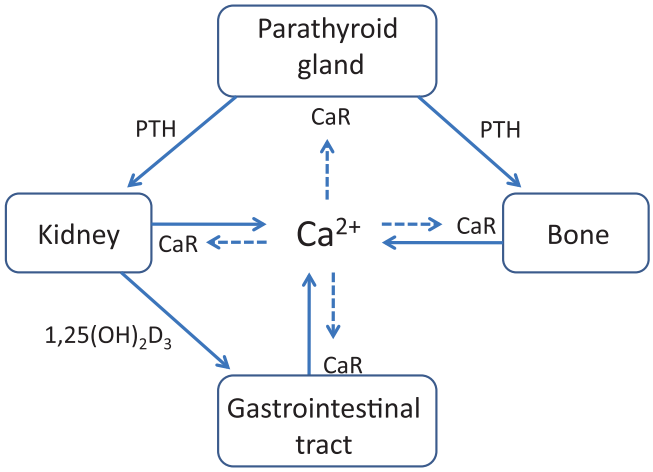
## CaR in normal physiology and pathophysiology

The major function of the CaR is to regulate free ionized $Ca^{2+}_o$. The body maintains a constant $Ca^{2+}$ concentration by

the concerted action of $Ca^{2+}$ absorption in the intestine, reabsorption in the kidney and exchange from bone. There are three, so-called calciotropic hormones: PTH, calcitonin and $1,25(OH)_2$ $D_3$ (vitamin D), which regulate the $Ca^{2+}$ transport processes. The CaR expressed in the parathyroid glands and kidney has a central role in $Ca^{2+}$ homeostasis. 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 $Ca^{2+}$ reabsorption and bone resorption by activated osteoclasts, both leading to an increase in $Ca^{2+}$. Furthermore, the relative hypocalcaemia also results in decreased secretion of calcitonin from thyroid C cells mediated by the CaR, preventing inhibition of bone resorption by calcitonin (Copp, 1994), although the potential of calcitonin on the calcium homeostasis is thought to be weak in human. Both PTH and low $Ca^{2+}$ induce synthesis of $1,25(OH)_2$ $D_3$ in the proximal tubular cells of the kidney. The vitamin D metabolite stimulates intestinal $Ca^{2+}$ absorption through the binding with vitamin D receptor. In the gastrointestinal tract, hypercalcaemia stimulates gastric acid secretion as well as gastrin release, while apical CaR activation by $Ca^{2+}$ and polyamines modifies colonic fluid secretion. Moreover, the bone resorption-induced local increases in $Ca^{2+}$ may decrease osteoclastic bone resorption and stimulate osteoblastic bone formation through the CaR expressed in these cells. Thus, Ca handling is concerted by the parathyroid gland, kidney, bone and gastrointestinal tract through the CaR, PTH and vitamin D in human (Figure 2) (Chang *et al.*, 2008). Since heterozygous CaR knockout mouse represents hypercalcaemia, excessive secretion of PTH and enlarged parathyroid glands (Ho *et al.*, 1995), the most relevant role of the CaR seems to be Ca homeostasis through the regulation of PTH production and secretion and the action of PTH in the kidney and bone. Recent study in terms of tissue-specific CaR knockout supports this notion.

In the kidney, filtered Ca is reabsorbed by proximal tubules (60–70%), thick ascending limbs (20–25%) and distal tubules (8–10%). As the results, the voided urine contains about 1% of the filtered Ca in healthy adults. In proximal tubules, Ca reabsorption is mainly passive through paracellular route between adjacent cells. In medullary and cortical thick ascending limbs, basal Ca absorption is mediated by the paracellular pathway whereas active Ca absorption, which is regulated by PTH and calcitonin, proceeds through the transcellular route. In distal convoluted tubules, Ca is transported entirely through the transcellular pathway. In the kidney, the CaR is widely expressed along the nephron and functions: (i) $Ca^{2+}$ and inorganic phosphate homeostasis; (ii) mono- and divalent cation transport; (iii) urinary acidification; (iv) urine concentration; and (v) renin release.

The CaR protein is expressed along most of all the nephron segments; apical membranes in proximal tubules and collecting ducts, and basolateral cell membranes in thick ascending limbs (Riccardi *et al.*, 1998). In cortical ascending limbs where type 1 PTH receptor is abundantly expressed, apical CaR activation inhibits PTH-stimulated $Ca^{2+}$ absorption (Motoyama and Friedman, 2002), suggesting that hypercalciuria suppresses PTH-sensitive $Ca^{2+}$ reabsorption. Basolateral CaR also plays a role in NaCl reabsorption by modulating function of apical $Na^+$-$K^+$-$2Cl^-$ cotransporter NKCC2 (SLC12A2) and the renal outer medullary potassium




## Figure 2

Calcium ion homeostasis is tightly regulated by the calcium-sensing receptor (CaR). During fasting state, trivial decrease in serum $Ca^{2+}$ is sensed by the CaR expressed on the parathyroid gland, kidney and bone (dotted line). Low $Ca_o^{2+}$ level (black arrows) stimulates parathyroid hormone (PTH) secretion from the parathyroid gland (solid line), thereby activating bone resorption and renal reabsorption of $Ca^{2+}$. PTH-induced synthesis of active vitamin D stimulates $Ca^{2+}$ absorption from the intestine. Thus, serum $Ca^{2+}$ level should be normalized to the basal level. In contrast, at postprandial state, serum $Ca^{2+}$ elevated by its absorption from the gastrointestinal tract is sensed by the CaR of these tissues (dotted line). High $Ca_o^{2+}$ level suppresses PTH secretion from the parathyroid gland (solid line), followed by the suppression of $Ca^{2+}$ mobilization from the other tissues and recovery to the basal $Ca_o^{2+}$ level.

$K^+$ channel. When the CaR is activated, paracellular $Na^+$, $Ca^{2+}$ and $Mg^{2+}$ transport is inhibited, producing a 'Bartter-like' phenotype (Vargas-Poussou *et al.*, 2002; Watanabe *et al.*, 2002). By contrast, in distal tubules, CaR activation inhibits the plasma membrane $Ca^{2+}$-ATPase (Blankenship *et al.*, 2001), suggesting that hypercalcaemia suppresses Ca reabsorption through the CaR. In addition, in collecting ducts, the CaR activation by high $Ca^{2+}_o$ inhibits vasopressin action and promotes urinary $H^+$ secretion through down-regulation of aquaporin 2 and $H^+$-ATPase, suggesting that the CaR serves to mitigate calcium precipitation and stone formation by blunting the urinary concentrating capacity as well as urinary acidification (Sands *et al.*, 1997; Renkema *et al.*, 2009).

Unlike most secretory cells, renin secretion from the juxtaglomerular cells is inversely related to $Ca^{2+}_o$ and $Ca^{2+}_i$ levels (Atchison *et al.* 2010). Recent several reports suggest that increased $Ca^{2+}_o$ inhibits cAMP-stimulated renin secretion through the CaR (Ortiz-Capisano *et al.*, 2007; Maillard *et al.*, 2009). Calcimimetic compound R-568 blunted renin release and reduced forskolin-stimulated renin increase in mice juxtaglomerular cells (Watanabe *et al.*, 2002; Maillard *et al.*, 2009). This was confirmed by an *in vivo* study, where the response was independent of the changes in $Ca^{2+}_o$ and PTH. Also, other calcimimetic cinacalcet decreased cAMP level as well as renin release in primary culture of juxtaglomerular cells most likely through stimulating a calcium calmodulin-activated phosphodiesterase 1 (PDE1) (Ortiz-Capisano *et al.*,

2009). These findings might be one of the plausible mechanisms of CaR-mediated vasorelaxation discussed later.

The role of the CaR in Ca handling is supported by human diseases with CaR mutation. Functionally important mutations in the CaR have been found to cause disorders in calcium homeostasis due both to changes in the set point for PTH secretion and to the control of renal calcium excretion. Relative hypocalciuria is shown in loss-of-function CaR mutations even when PTH-dependent hypercalcaemia is present. This condition is called familial hypocalciuric hypercalcaemia (FHH; OMIM 145980) and neonatal severe hyperparathyroidism (NSHPT; OMIM 239200). Heterozygous and homozygous CaR knockout mouse represents the similar phenotype of FHH and NSHPT respectively. In contrast, gain-of-function mutations, as found in autosomal dominant hypocalcaemia (OMIM 601298), lead to relative hypercalciuria in spite of hypocalcaemia. In some cases with Bartter syndrome, CaR activating mutations have been reported and now known as Bartter syndrome type V (Vargas-Poussou *et al.*, 2002)

Polymorphisms in the CaR gene affect: (i) PTH secretion in healthy population (Scillitani *et al.*, 2004), hypercalciuria (Vezzoli *et al.*, 2007) and haemodialysis patients (Yano *et al.*, 2000); (ii) PTH suppressibility in renal failure patients with secondary hyperparathyroidism (Yokoyama *et al.*, 2002); and (iii) risk of stone formation in PHPT (Vezzoli *et al.*, 2002).

Inactivating and activating autoantibodies to the CaR, respectively, decrease and increase the sensitivity to serum $Ca_o^{2+}$ level (Kifor *et al.*, 1997; 2001).

Furthermore, patients with either PHPT or SHPT show significant reduced expression of the CaR mRNA and protein in the parathyroid gland (Gogusev *et al.*, 1997; Corbetta *et al.*, 2000; Yano *et al.*, 2000; 2003), which at least in part provides possible explanation for the observed increase in PTH secretion. Several clinical trials with cinacalcet and other calcimimetics have shown that the compounds promptly and effectively reduce the elevated serum PTH and calcium levels in PHPT and SHPT (Block *et al.*, 2004; Peacock *et al.*, 2005).

PHPT is the result of an increase in the mass of parathyroid tissue, and can be cured by the removal of the enlarged parathyroid gland. Calcimimetics may provide a non-invasive way to normalize serum $Ca^{2+}$ levels, providing a non-surgical alternative. SHPT occurs in chronic kidney disease as a result of phosphorus retention and of reduced $Ca^{2+}_o$ and calcitriol concentrations in serum, which in combination lead to increased PTH secretion. Traditional therapy for SHPT has been based on vitamin D analogues and calcium-containing phosphate binders (Bover *et al.*, 2009b). Calcium-containing phosphate binders rise $Ca^{2+}_o$ and lower phosphate, and vitamin D analogues increase serum concentration of both $Ca^{2+}$ and phosphate and lowers PTH. Thus, patients treated with these compounds often display hypercalcaemia, and in case of vitamin D also hyperphosphatemia, possibly leading to increased cardiovascular events and mortality (Raggi and Kleerekoper, 2008). In addition to lowering serum PTH level, cinacalcet also reduces $Ca^{2+}_o$ and $Ca^{2+} \times$ phosphorous product and have beneficial effects on cardiovascular risk factors and events in patients with renal failure (Cunningham *et al.*, 2005; Bover *et al.*, 2009a,b; Block *et al.*, 2010). Animal study clearly showed that cinacalcet effectively prevented vascular calcification in renal failure model whereas vitamin D developed it with similar level of PTH reduction (Kawata *et al.*, 2008). More information regarding the mutations, polymorphisms and diseases in the CaR can be obtained from the website (http://www.casrdb.mcgill.ca/) or other references (Tfelt-Hansen *et al.*, 2003b).

Apart from its role in mineral ion homeostasis, the CaR has many other functions in a variety of tissues not directly involved in systemic mineral ion metabolism. Expression of the CaR has been documented in many tissues such as brain, pancreas and blood vessels, and in many different cell types throughout the body.

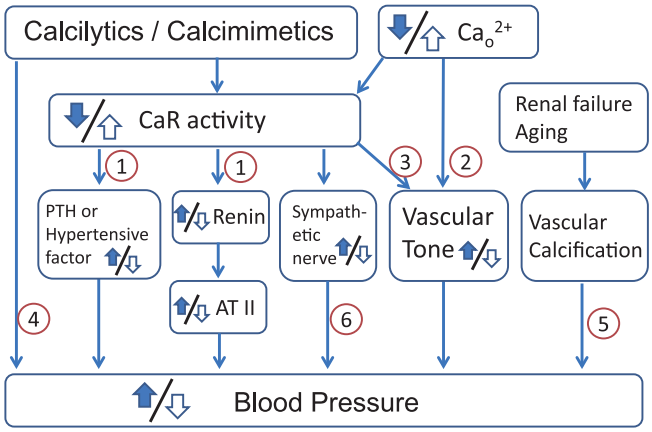
In accordance with the broad expression of the CaR, the receptor might also possess other important physiological roles such as regulation of gut hormone secretion (Conigrave and Brown, 2006) and control of arterial blood pressure (Smajilovic and Tfelt-Hansen, 2008), although these roles still remain to be studied in details.

## CaR and calcimimetics in hypertension

It has been reported that increasing dietary calcium lowers blood pressure in models of hypertension (Hatton and McCarron, 1994). Multiple lines of evidence suggest that the CaR might participate in the modulation of blood pressure. Several *in vivo* studies demonstrated effects of calcimimetics on hypertension and vascular calcification. Calcimimetics have been shown to decrease blood pressure in uremic and spontaneously hypertensive rats but not in normotensive rats (Ogata *et al.*, 2003; Odenwald *et al.*, 2006). Similar hypotensive effects were achieved with parathyroidectomy (Ogata *et al.*, 2003). In addition to the long-term reductions in blood pressure, calcimimetics induce acute hypertension in normal and uremic rats, which is followed by a marked and sustained hypotensive effect in uremic rats (Odenwald *et al.*, 2006; Fryer *et al.*, 2007). Furthermore, calcilytics have shown to increase blood pressure in normotensive rats in the presence of parathyroid glands (Rybczynska *et al.*, 2006). The effect of the calcilytics is probably not related to the reduced PTH, since PTH as well as PTH-related peptide lowers blood pressure (Schluter and Piper, 1998). However, it might be probable that activation of the CaR in parathyroid glands suppress secretion of potent parathyroid hypertensive factor, thereby leading to a decrease in blood pressure. Accordingly, low $Ca^{2+}_o$ was reported to stimulate unidentified parathyroid hypertensive factor secretion in parathyroid gland organ cultures (Sutherland and Benishin, 2004).

Relationships between the change in $Ca^{2+}_o$ and blood pressure may be resulted from: (i) hormonal effects, including renin, PTH and unidentified factor; (ii) effect of $Ca^{2+}_o$ on vascular tone, which is not related to the CaR; (iii) direct effect of the CaR on vascular tone; (iv) drug-specific effect, which is independent of PTH, $Ca^{2+}_o$ and the CaR; (v) vascular calcification; and (vi) other mechanisms (Figure 3).

Activation of the CaR suppress renin secretion as described above (Ortiz-Capisano *et al.*, 2007; Maillard *et al.*, 2009), and thereby decrease levels of angiotensin II and aldosterone, leading to a decrease in blood pressure. A recent study demonstrated the lack of the hypertensive effect of calcilytic NPS 2143 in the presence of a calcium channel inhibitor and type 1 angiotensin II (AT1) receptor antagonist




## Figure 3

Mechanisms of blood pressure regulation by extracellular calcium and/or calcium-sensing receptor (CaR). Relationships between the change in $Ca^{2+}_o$ and blood pressure may be resulted from: (i) hormonal effects, including renin, parathyroid hormone (PTH) and unidentified factor; (ii) effect of $Ca^{2+}_o$ on vascular tone, which is not related to the CaR; (iii) direct effect of the CaR on vascular tone; (iv) drug specific effect, which is independent of PTH, $Ca^{2+}_o$ and the CaR; (v) vascular calcification; and (vi) other mechanisms such as through the sympathetic nervous system. Calcilytics and elevated $Ca^{2+}_o$ decrease the CaR activity, followed by elevating PTH (and maybe parathyroid-derived hypertensive factor), renin and activating vascular tone and possibly sympathetic nerve to lead an elevation of blood pressure (black arrows). On the other hand, calcimimetics and decreased $Ca^{2+}_o$ increase the CaR activity, followed by the opposite response (white arrows). AT II, angiotensin II.

(Rybczynska *et al.*, 2010), probably indicating mechanisms other than hormonal alteration.

In addition, mechanisms independent of the interaction with the CaR might also contribute to effects of calcimimetics and calcilytics. Nakagawa *et al.* (2009) demonstrated similar hypotensive effects of enantiomers R-568 (calcimimetic) and S-568 in rats. Since S-568 has no or very little activity on the CaR, the hypotensive effect of R-568 was most likely not mediated via the CaR. Furthermore, *ex vivo* studies in isolated arteries demonstrated CaR-independent relaxant effects of calcimimetics, predominantly acting by inhibiting $Ca^{2+}$ influx through L-type $Ca^{2+}$ channels into vascular smooth muscle (Smajilovic *et al.*, 2007). Further studies are needed to elucidate CaR-dependent versus CaR-independent effects of the calcimimetics (Smajilovic *et al.*, 2007; Thakore and Ho, 2011).

In addition to animal studies, genetic studies suggested that the CaR might play a role in human vascular disease. Polymorphism in human CaR gene is associated with increased frequency of coronary heart disease, myocardial infarction and cardiovascular death (März *et al.*, 2007). Moreover, a recent study showed that common variations in the CaR are associated with blood pressure in young African Americans (Jung *et al.*, 2009), suggesting the involvement of the CaR in the control of blood pressure. Although the authors suggest that the effect on blood pressure was through the CaR gene variants effect on sodium retention, further study is necessary.

In addition to blood pressure modulation, the CaR has also been associated with vascular calcification. Arterial calcification is very common in patients with chronic kidney disease and contributes to the increased cardiovascular risk (London *et al.*, 2003). A combined analysis have suggested that calcimimetics have beneficial effects on cardiovascular risk factors and events in patients with renal failure (Cunningham *et al.*, 2005). In animal models of chronic kidney disease, treatment with calcimimetics did not induce vascular calcification in contrast to traditional therapy (Henley *et al.*, 2005) and had beneficial effects on arterial remodelling (Koleganova *et al.*, 2009). Kawata *et al.* (2008) demonstrated that parathyroidectomy also suppressed vascular calcification, suggesting that the effect of calcimimetic is most likely via suppressing PTH secretion without increasing $Ca^{2+}_o$ and $Ca^{2+}$ × phosphorous product levels. Although vascular calcification affects blood pressure, it takes substantially long time to progress the pathological changes. Thus, vascular calcification cannot fully explain acute or subacute alteration in vascular tone and blood pressure by change in $Ca^{2+}_o$.

Other mechanism of the CaR-mediated vascular tone is through the peripheral and central nervous system. Although knowledge of the CaR in the nervous system has been limited, a recent study showed that the CaR in perinatal mouse sympathetic neurons is functional to regulate axonal and dendritic growth (Vizard *et al.*, 2008), suggesting the presence of potential mechanisms of the CaR-mediated vascular tone other than described above.

Calcimimetics and calcilytics have a direct effect on the CaR in the blood vessels (Weston *et al.*, 2005; Smajilovic *et al.*, 2007). Here, we will discuss direct effects of change in $Ca^{2+}_o$ and CaR activity in the vasculature on vascular tone. Besides being expressed in the organs involved in calcium homeostasis, the CaR has also been found to be functionally expressed in tissues uninvolved in calcium homeostasis, including cardiovascular tissues. The presence of the CaR has been demonstrated in several different blood vessels. First, expression of the CaR protein was reported in perivascular nerves in rat mesenteric, coronary, renal and cerebral arteries, and $Ca^{2+}_o$ induced relaxation in these vessels (Bukoski *et al.*, 1997; Wang and Bukoski, 1998). Later, the CaR expression was found in rat subcutaneous small arteries and biphasic effect of $Ca^{2+}_o$ was observed (Ohanian *et al.*, 2005). In this report, low $Ca^{2+}_o$ induced small vasoconstriction whereas high (3–10 mM) $Ca^{2+}_o$ induced vasorelaxation. In addition, other CaR agonists such as $Mg^{2+}_o$ and neomycin induced concentration-dependent relaxation similar to that observed with high $Ca^{2+}_o$, indicating further involvement of the CaR. The CaR was detected in the whole homogenates from rat subcutaneous small arteries, but exact localization was not investigated. Several later reports showed presence of the CaR in vascular smooth muscle cells (VSMCs) and endothelial cells from different types of blood vessels. However, there have been conflicting reports regarding whether the CaR is present in the VSMCs. Wonneberger *et al.* (2000) found CaR mRNA transcripts in the gerbil spiral modiolar artery and demonstrated biphasic increase in intracellular $Ca^{2+}$ in response to elevations in the concentration of $Ca^{2+}_o$. In addition, using $Ca^{2+}_o$ and other CaR agonists ($Gd^{3+}$, $Ni^{2+}$), they induced a biphasic vasoconstriction. Since the increase in intracellular $Ca^{2+}$ was paralleled by the biphasic vasoconstric-

tion, they suggested that the CaR is most likely localized in the VSMCs. However, Farzaneh-Far *et al*. (2000) failed to detect CaR transcripts in the cultured VSMCs from rat aorta, and suggested a presence of a receptor that is functionally related to, but molecularly distinct from, the CaR. One potential candidate could be a closely related protein, GPRC6A (34% amino acid identity), which is activated by amino acids and positively modulated by $Ca^{2+}_o$ (Wellendorph and Brauner-Osborne, 2004). GPRC6A has been demonstrated in both endothelial cells and VSMCs from rat mesenteric arteries (Harno *et al*., 2008). Activation of the receptor induced endothelium-dependent hyperpolarization of the VSMCs. Furthermore, in the presence of GPRC6A agonists, the hyperpolarisations produced by calindol, the positive allosteric modulator of the CaR, were potentiated. Thus, this allosteric modulator might interact with both CaR and GPRC6A. This might also explain some of the CaR-independent effects of calcimimetics and calcilytics discussed above.

However, we found the CaR in cultured VSMCs from rat aorta (Smajilovic *et al*., 2006). Our data are supported by recent reports that demonstrated the CaR in human and bovine VSMCs, including aortic VSMCs. Alam *et al*. (2009) found that the expression of the CaR was markedly reduced in calcified areas of atherosclerotic arteries and in VSMCs cultured to have the mineralized phenotype. Furthermore, it has been demonstrated that incubating VSMCs in the presence of 1.8 mM $Ca^{2+}_o$, which is present in a regular culture medium, and 2.5 mM $Ca^{2+}_o$ for 24 h decreases the CaR expression compared to that observed in 1.2 mM $Ca^{2+}_o$ (Alam *et al*., 2009). Thus, level of the CaR expression appears to be regulated by several factors, such as culture conditions and phenotype of the cells, and could therefore account for the inconsistent detection of the CaR expression in the VSMCs in the previous studies. It should also be kept in mind that level of the CaR expression may also vary between species and different arteries.

Weston *et al*. (2005) demonstrated that the CaR present in the endothelial layer of rat mesenteric and porcine coronary arteries activates intermediate conductance $Ca^{2+}$-sensitive $K^+$ channels ($IK_{Ca}$), resulting in $K^+$-induced hyperpolarization of VSMCs. Although hyperpolarization is usually associated with relaxation, stimulation of the CaR with a specific positive modulator, Calindol, did not have any effect on phenylephrine-precontracted mesenteric arteries. In a novel report, they demonstrated that this was due to phenylephrine-induced increases in $K^+$, the so-called '$K^+$ clouds', emanating from large conductance $Ca^{2+}$-sensitive $K^+$ channels ($BK_{Ca}$) present on contracted VSMCs, which make the system less able to respond to further increases in $K^+$ (Weston *et al*., 2008). Therefore, any further increase in $K^+$ after the opening of endothelial cell $IK_{Ca}$ channels via CaR activation would generate minimal vasorelaxation, which might not be detected. Once the $K^+$ clouds were inhibited using iberiotoxin, a selective inhibitor of $BK_{Ca}$ channels, CaR-induced vasodilation was detected. Same year Dora *et al*. (2008) in an elegant paper provided data that the CaR may play a role in modulating the nature of the endothelium-derived hyperpolarizing factor response. Recently, the first evidence for presence of the CaR in human cardiovascular tissue, namely aortic endothelial cells, has been provided (Ziegelstein *et al*., 2006). Stimulation of the receptor stimulated production of nitric oxide (NO) in these cells, suggesting further a possible role of the CaR in vasodilation. Taken together, these results indicate that the CaR may have a physiological role in the modulation of vascular tone, and thereby blood pressure. We do not believe that CaR is a potential drug target in treatment of hypertension but it seems likely that patients with hyperparathyroidism may benefit from the blood pressure lowing effects of the positive CaR modulator.

## Conclusion

It is now well established that the CaR is functionally present in blood vessels of many types and species, and several reports demonstrated its involvement in vasodilation. Moreover, multiple studies demonstrated hypotensive effects of calcimimetics in rats. The exact mechanisms for the effects of calcimimetics on the blood pressure remain to be elucidated, and may be through direct effects on the CaR expressed in blood vessels or indirect through the kidney or parathyroid glands. In view of increasing clinical use of calcimimetics in treatment of hyperparathyroidism understanding of CaR's role in blood pressure modulation is essential.

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## Conflict of interest

The authors have no conflict of interest.

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