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European Journal of Pharmacology 447 (2002) 271–278

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Physiological and pharmacological agonists of the extracellular Ca^{2+}-sensing receptor

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Accepted 15 April 2002

Abstract

Extracellular Ca^{2+} concentration $[Ca^{2+}]_o$ is vital for a number of processes varying from blood clotting to regulation membrane permeability and excitability. For this reason $[Ca^{2+}]_o$ is under strict control of a complex homeostatic system that includes parathyroid glands, kidneys, bones and intestine. The extracellular Ca^{2+}-sensing receptor is an essential component of this system, regulating parathyroid hormone secretion, Ca^{2+} (and Mg^{2+}) excretion by the kidney, bone remodeling and Ca^{2+} reabsorption by the gastrointestinal tract. The Ca^{2+}-sensing receptor is also present in organs without an obvious link with mineral ion metabolism. This review will describe the discovery of a novel class of ion-sensing receptor(s), receptor–effector coupling and the roles of the Ca^{2+}-sensing receptor inside and outside the $Ca^{2+}{}_o$ homeostatic system. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Ca^{2+}; Ca^{2+}-sensing receptor; Polyvalent cation; Calcimimetic compound

1. Introduction

Extracellular Ca^{2+} ($Ca^{2+}{}_o$) is essential for a number of vital processes from bone formation to muscle contraction. Therefore, Ca^{2+} concentration in the extracellular fluids ($[Ca^{2+}]_o$) is tightly controlled by a sophisticated homeostatic system that includes parathyroid glands, kidney and bone. The extracellular Ca^{2+}-sensing receptor is an essential component of this system, regulating parathyroid hormone secretion, Ca^{2+} excretion by the kidney and bone remodeling. The Ca^{2+}-sensing receptor belongs to type III family of G-protein-coupled receptors, which comprises metabotropic glutamate receptors (mGlu receptors) and putative vomeronasal organ receptors. The low affinity of the receptor for Ca^{2+} (i.e. millimolar concentrations) indicates that the receptor is more sensitive to changes in net charge than to the specific ligand. Other divalent cations, trivalent cations of the lanthanide series, polyvalent cations such as spermine and spermidine and polycations such as aminoglycoside antibiotics can all activate the receptor in vitro with EC_{50}'s from the micromolar range (tri-, polyvalent cations) to millimolar concentrations (Mg^{2+}). In addition, receptor affinity for $Ca^{2+}{}_o$ can be affected by the presence of allosteric modulators, i.e. ionic strength, L-amino acids and recently developed pharmacological agents (see Fig. 1). Initially identified from bovine parathyroid glands (Brown et al., 1993), within the 5 years following its identification, Ca^{2+}-sensing receptor presence has rapidly been extended to organs where the link with mineral ion metabolism has not been elucidated (i.e. brain, stomach, eye, skin and many other epithelial cells; reviewed by Brown and MacLeod, 2001. See Table 1). Receptor function in these organs is currently largely unknown. This review will cover different types of Ca^{2+}-sensing receptor agonists and the possible role(s) of the receptor in the light of its widespread distribution.

2. Endogenous pharmacology of the Ca^{2+}-sensing receptor

2.1. $Ca^{2+}{}_o$

The Ca^{2+}-sensing receptor was initially identified from bovine parathyroid glands (Brown et al., 1993), where the receptor exerts an inhibitory action on parathyroid hormone (PTH) secretion. This inhibition is effective within the concentration range of 1–1.5 mM, with the IC_{50} for

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PII: S0014-2999(02)01849-6

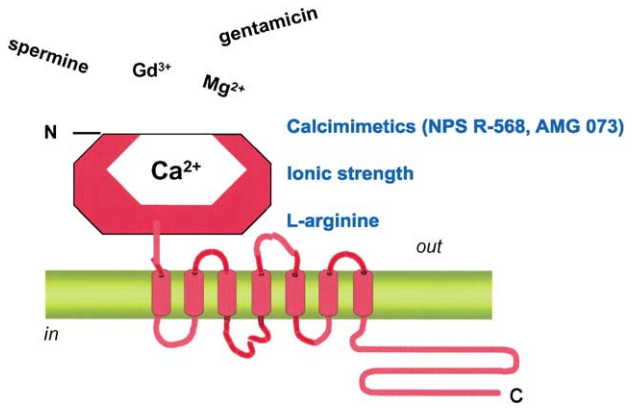

Fig. 1. Physiological and pharmacological agonists of the extracellular Ca^{2+}-sensing receptor (CaR).

$Ca^{2+}{}_o$ being ~ 1.2 mM (Brown, 1991). To determine the sensitivity of the Ca^{2+}-sensing receptor for $Ca^{2+}{}_o$, a number of studies using recombinant Ca^{2+}-sensing receptors expressed in either oocytes (Brown et al., 1993) or human embryonic kidney (HEK)-293 cells (Pearce et al., 1996) have been performed. The reported EC_{50} values from these experiments are fairly consistent with a concentration of between 3.5 and 4.2 mM in HEK cells (Ray et al., 1997; Conigrave et al., 2000), while in oocytes, the EC_{50} is in the range of 3 and 5 mM (Brown et al., 1993; Racke et al., 1993). The differences reported for Ca^{2+}-sensing receptor sensitivity to $Ca^{2+}{}_o$ could be influenced by the presence of other factors such as spermine and L-aromatic and small aliphatic amino acids, which have been shown to enhance Ca^{2+}-sensing receptor sensitivity to $Ca^{2+}{}_o$ (see below). However, since thus far only one Ca^{2+}-sensing receptor gene has been identified, there still remains the question of why certain Ca^{2+}-sensing receptor-expressing cells are responsive to very low $[Ca^{2+}]_o$ while others respond to much higher $[Ca^{2+}]_o$. For instance, elevation of $[Ca^{2+}]_o$ above 0.05 mM is sufficient to induce keratinocyte differentiation and inhibit proliferation (Bikle et al., 1996). Transfection of keratinocytes with a Ca^{2+}-sensing receptor antisense cDNA construct blocks the inhibitory effects of elevated $Ca^{2+}{}_o$ on proliferation and the stimulation of cell differentiation (Tu et al., 2001). This suggests that keratinocyte differentiation is mediated by a Ca^{2+}-sensing receptor-like molecule responding to $[Ca^{2+}]_o$ within a lower range of sensitivity. The variation of Ca^{2+}-sensing receptor sensitivity to $Ca^{2+}{}_o$ is not surprising considering that Ca^{2+}-sensing receptor-expressing cells are exposed to a wide range of divalent cation concentrations. For example, parathyroid cells are exposed to a free ionized $[Ca^{2+}]_o$ of 1–1.5 mM while bone cells may be exposed to 8–40 mM $Ca^{2+}{}_o$ (Silver et al., 1988). While intriguing, the issue of a different $Ca^{2+}{}_o$ sensitivity requires additional work.

2.2. Mg^{2+}

The effects of elevated $[Mg^{2+}]_o$ are known to mimic those of $Ca^{2+}{}_o$ in certain cell types. Secretion of PTH from parathyroid cells is inhibited by raising $Mg^{2+}{}_o$ (Brown et al., 1984; Habener and Potts, 1976), and so is agonist-stimulated cAMP accumulation in the parathyroid (Brown, 1981) and the kidney (Mathias and Brown, 1991) and renal tubular reabsorption of Mg^{2+} (Quamme, 1980). With the

Table 1
Pharmacology of the extracellular Ca^{2+}-sensing receptor (CaR) in cells endogenously expressing the receptor

Cell/Tissue	Readout	Agonists	Reference
Bovine parathyroid	↑ $Ca^{2+}{}_i$	EC_{50}'s for CaR agonists: 3 mM Ca^{2+}; 10–15 mM Mg^{2+}; 35 μM Gd^{3+}; 70 μM neomycin and 500 μM spermine. No response to Pb^{2+}, Ni^{2+}, Cd^{2+} or Al^{3+} (<500 μM). Stereoselective response to calcimimetics	Brown et al. (1993, 2001)
Human keratinocyte	IP_3 production	Increased sensitivity to $Ca^{2+}{}_o$ (0.07–0.25 mM). Stereoselective response to calcimimetics	Oda et al. (1998)
Murine Leydig cells	↑ $Ca^{2+}{}_i$	Reduced sensitivity to $Ca^{2+}{}_o$ (2.5–15 mM) and no response to Mg^{2+} (0.8–15 mM). Sensitive to Ni^{2+} (0.5–5 mM). Calcimimetics not tested	Adebanjo et al. (1998)
Sheep thyroid parafollicular cells	Ionic conductance ↑ $Ca^{2+}{}_i$	No response to Mg^{2+} (up to 10 mM). Calcimimetics not tested	McGhee et al. (1997)
Rabbit TAL	↑ $Ca^{2+}{}_i$	No response to 500 mM Gd^{3+}, 5 mM Mg^{2+} or 1 mM neomycin. Calcimimetics not tested	Desfleurs et al. (1999)
Liver	↑ $Ca^{2+}{}_i$	Greatly reduced sensitivity to Gd^{3+} (EC_{50} > 2 mM) and to spermine (EC_{50} ~ 5 mM). Stereoselective response to calcimimetics	Canaff et al. (2000)
Pancreatic β cells (endocrine)	Insulin release ↑ $Ca^{2+}{}_i$	Not sensitive to neomycin, Mg^{2+} or Gd^{3+}. Stereoselective response to calcimimetics	Straub et al. (2000)
Pancreatic acinar cells (exocrine)	↑ $Ca^{2+}{}_i$	Not sensitive to neomycin (up to 1 mM). Reduced sensitivity to Gd^{3+} (>1 mM). Calcimimetics not tested	Bruce et al. (1999)
AT-3 prostate carcinoma cells	Cell death	Reduced sensitivity to $Ca^{2+}{}_o$ (EC_{50}, 6.1 mM) and to $Mg^{2+}{}_o$ (EC_{50}, 23.4 mM). Calcimimetics not tested	Lin et al. (1998)

cloning of the Ca^{2+}-sensing receptor, it has become possible to demonstrate that not only Ca^{2+} but also Mg^{2+} can act as a Ca^{2+}-sensing receptor agonist (Brown et al., 1993). In parathyroid cells, Mg^{2+} is generally less potent than Ca^{2+} in relation to activating the Ca^{2+}-sensing receptor and producing the downstream effects seen upon Ca^{2+}-sensing receptor stimulation (Chang et al., 1998). The reasoning behind the differences between Ca^{2+} and Mg^{2+} are not fully understood but could be linked to differing capacities for receptor binding or differences in the downstream effects of the respective agonists. For example, even though Ca^{2+}_o and Mg^{2+}_o can both activate the Ca^{2+}-sensing receptor, low Mg^{2+}_o exerts a paradoxical block of PTH release, which seems to be mediated through a novel mechanism involving an increase in the activity of G alpha subunits of heterotrimeric G-proteins (Quitterer et al., 2001).

2.3. Amino acids

It is known that protein intake and Ca^{2+} excretion are inversely related (Burtis et al., 1994), and that a reduction in protein intake stimulated PTH secretion (Kerstetter et al., 1998). It has recently been shown that a number of amino acids including all three aromatic amino acids, tyrosine, phenylalanine and tryptophan, act as positive allosteric modulators of Ca^{2+}-sensing receptor in the presence but not in the absence of the receptor's native ligand, Ca^{2+}_o (Conigrave et al., 2000). The actions of the amino acids in Ca^{2+}-sensing receptor-transfected HEK cells was shown to be stereoselective, with L-amino acids being several fold more potent than D-amino acids (Conigrave et al., 2000). Individual amino acids alone displayed relatively low potencies for activating the Ca^{2+}-sensing receptor but when a mixture of amino acids was used, emulating that present in the fasting state, the sensitivity of the Ca^{2+}-sensing receptor to its polycationic agonists was greatly increased. In the case of Ca^{2+}_o, the EC_{50} was decreased by 20–40% (Conigrave et al., 2000). Physiologically, this could imply that the Ca^{2+}-sensing receptor present in the gut could act more like a nutrient sensor than a Ca^{2+} receptor per se (Conigrave et al., 2000).

2.4. Spermine

Spermine is an effective Ca^{2+}-sensing receptor agonist and, like the di- and trivalent cations, it activates phospholipase C leading to transient increases in Ca^{2+}_i following mobilisation of stored Ca^{2+} (Quinn et al., 1997). As with the aminoglycoside antibiotics, the number of charges on the polyamine is a determinant of potency as a Ca^{2+}-sensing receptor agonist. Of the polyamines, spermine contains the most number of free amino groups (four) and as a result, it is the most potent polyamine (Quinn et al., 1997). The concentration of spermine required to activate the Ca^{2+}-sensing receptor is in the high micromolar range when in the presence of low concentrations of Ca^{2+}_o, but when $[Ca^{2+}]_o$ is raised to physiological levels of ≈ 1.5 mM, concentrations of spermine required to modulate the receptor fall to ≈ 100 μM. In vivo concentrations of spermine are in the micromolar range and in specific regions such as the gastrointestinal tract, concentrations can reach 2–3 mM (Osborne and Seidel, 1990). This suggests that, under certain conditions, in the intestine the Ca^{2+}-sensing receptor could also act as a physiological receptor for polyamines thereby promoting gut differentiation (Ter Steege et al., 1997).

2.5. Amyloid β-peptides

Amyloid-β peptides are proteins produced in excess in Alzheimer's disease that have been shown to elicit Ca^{2+}-sensing receptor-like responses in both Ca^{2+}-sensing receptor-transfected HEK cells and cultured rat hippocampal pyramidal neurons (Ye et al., 1997). The amyloid β peptides effect was not observed in hippocampal pyramidal neurons collected from mice where the Ca^{2+}-sensing receptor gene was selectively disrupted (Ye et al., 1997). Whether amyloid β peptide-induced Ca^{2+}-sensing receptor activation contributes to the neuronal degeneration seen in Alzheimer's (Ye et al., 1997), or whether it has any physiological role in healthy neurons remains to be demonstrated.

2.6. Trace metals

A number of other divalent metals that are found in the body, albeit in trace amounts, have been tested for their Ca^{2+}-sensing receptor affinity. In Ca^{2+}-sensing receptor-transfected HEK cells, 5 mM Ni^{2+}, Co^{2+} and Fe^{2+} elicit activation of extracellular signal regulated kinase (ERK) and phospholipase A_2, whereas Fe^{3+} is without effect (Handlogten et al., 2000). In Ca^{2+}-sensing receptor-transfected Chinese hamster ovary cells, 1 mM Ni^{2+} failed to increase inositol accumulation, whereas 3 mM Ni^{2+} did produce a small rise and 5 mM Mn^{2+} elicited a greater effect (Ruat et al., 1996). While these metals may be thought of as endogenous due to their requirement as cofactors for many cell reactions, they are unlikely to represent physiological agonists of the receptor since the concentrations apparently required to stimulate the Ca^{2+}-sensing receptor are considerably higher than levels found in the body.

2.7. Ionic strength

The influence of ionic strength on cation sensing by the Ca^{2+}-sensing receptor has been demonstrated by Quinn et al. (1998) who showed that increases in ionic strength produced parallel changes in the sensing receptor EC_{50} for high Ca^{2+}_o-evoked increases in Ca^{2+}_i. For instance, increasing ionic strength reduces the sensitivity of the Ca^{2+}-sensing receptor to elevations in Ca^{2+}_o. The effect was demonstrated to be specific to ionic strength as neither changes in osmolarity nor the Ca^{2+}-sensing receptor ago-

nist used influenced the alterations observed with the change in ionic strength. The potential for ionic strength to influence the sensitivity of the Ca^{2+}-sensing receptor under normal physiological conditions is minimal. However, there are specific circumstances that could allow ionic strength to influence Ca^{2+}-sensing receptor function. One such instance is the concentration of sodium chloride in the urine, which may vary between ~ 50 and 300 mM. Brown and MacLeod (2001) have speculated that this variation in ionic strength would expose the Ca^{2+}-sensing receptor on the apical membrane of the inner medullary collecting duct to experience alterations in ionic strength that would be large enough to alter the receptor's EC_{50} for $Ca^{2+}{}_o$ and other Ca^{2+}-sensing receptor agonists. Furthermore, in epithelia that are involved in the transport of water and electrolytes, receptors may be exposed to changes in ionic strength that could modify Ca^{2+}-sensing receptor function (Brown and MacLeod, 2001). For example in the thick ascending limb of the loop of Henle in the kidney, sodium chloride is absorbed in excess of water, therefore, basolateral Ca^{2+}-sensing receptors could encounter ionic strength at a much higher level than that in the filtered urine which might alter the receptor's $Ca^{2+}{}_o$-sensing capacity.

3. Exogenous pharmacology of the Ca^{2+}-sensing receptor

Although the Ca^{2+}-sensing receptor and the mGlu receptors are structurally very similar, the activity of the Ca^{2+}-sensing receptor is not affected by the compounds that act upon mGlu receptors (Brown et al., 1993). The Ca^{2+}-sensing receptor is very promiscuous with relation to the ions and compounds possessing net positive charge acting as Ca^{2+}-sensing receptor agonists. In addition to the physiological ligand, extracellular Ca^{2+}, a variety of other di- and trivalent cations activate the Ca^{2+}-sensing receptor. These include, in rank order of potency: $La^{3+}>Gd^{3+}>Be^{2+}>Ca^{2+}=Ba^{2+}>Sr^{2+}>Mg^{2+}$ (Nemeth, 1990).

Determination of the regions of the receptor responsible for the binding of other Ca^{2+}-sensing receptor agonists has been performed using a variety of receptor chimera constructs using Ca^{2+}-sensing receptor and mGlu receptor (Hammerland et al., 1999). These authors showed that constructs containing the extracellular domain of the Ca^{2+}-sensing receptor and the transmembrane domain and cytoplasmic tail (COOH) of the mGlu receptor showed almost identical cation recognition when compared to the native Ca^{2+}-sensing receptor. However, agonist binding recognition in the reversed chimera, using the extracellular domain of the mGlu receptor, led to loss of Ca^{2+} and neomycin receptor activation while retaining Gd^{3+} activation. Furthermore, receptor activation by Gd^{3+} is maintained even when a truncated form of the Ca^{2+}-sensing receptor, lacking the extracellular domain, is expressed in *Xenopus* oocytes. Interestingly, the responsiveness to Gd^{3+} is increased in the construct formed between the mGlu receptor extracellular domain and the transmembrane domain and cytoplasmic tail of the Ca^{2+}-sensing receptor (Hammerland et al., 1999).

Furthermore, it has been shown that the Ca^{2+}-sensing receptor exists in the plasma membrane as a disulphide-linked dimer (Ward et al., 1998; Bai et al., 1998; see Fig. 2), dimerization being constitutive rather than a consequence of ligand binding. Thus, Ca^{2+}-sensing receptor cooperativity may have an intermolecular component in addition to an intramolecular one. In this regard, it is interesting that expression of a mutant Ca^{2+}-sensing receptor lacking the two extracellular cysteine residues thought to be responsible for disulphide-linked dimerisation (Cys^{129} and Cys^{131}), exhibits elevated sensitivity to Ca^{2+} (Zhang et al., 2001). In addition, two naturally occurring mutations of the Ca^{2+}-sensing receptor gene, E127A and F128L, found immediately adjacent to these two cysteine residues, also increase the sensitivity of the receptor and cause an inherited condition named autosomal dominant hypercalcemia (reviewed by Pollak et al., 1996). Thus, disruption of the disulphide linkages binding the Ca^{2+}-sensing receptor homodimer does appear to have a functional significance and would tend to support the idea of intermolecular cooperativity.

As well as metal ion activation, a number of basic peptides can suppress PTH secretion in isolated PT cells (Brown et al., 1991). Both peptides produced a dose-dependent inhibition of low Ca^{2+} concentration stimulated release of PTH from dispersed bovine parathyroid cells, with polyarginine acting as a more potent inhibitor with respect to polylysine. In dispersed bovine parathyroid cells, both peptides stimulated the accumulation of inositol phosphates, which was accompanied by a transient $Ca^{2+}{}_i$ spike. This $Ca^{2+}{}_i$ transient was maintained even when the extracellular calcium was removed, suggesting that the increase in $Ca^{2+}{}_i$ resulted from the release of Ca^{2+} from IP_3-dependent stores, and it was blocked by pre-incubation of the cells with extracellular Mg^{2+}, suggesting that the peptide-induced

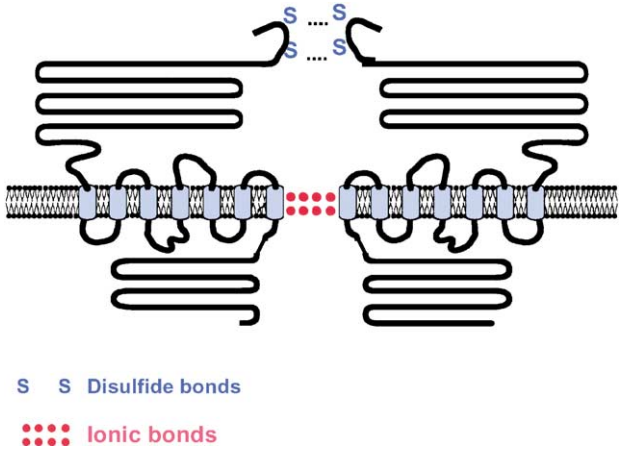

Fig. 2. Predicted structure of the extracellular Ca^{2+}-sensing receptor (CaR).

$Ca^{2+}{}_i$ mobilisation came from the same pool of stored Ca^{2+}. It therefore appears possible that these peptides may regulate parathyroid function via a similar pathway to elevated extracellular Ca^{2+}, which is through the Ca^{2+}-sensing receptor.

Along with the highly basic peptides, polyarginine and polylysine, the polyvalent aminoglycoside antibiotic neomycin has been shown to influence parathyroid gland function (Brown et al., 1991). These antibiotics are widely used in the treatment of Gram-negative infections, but their use is limited due to their potential renal toxicity. The mechanisms by which this occurs are still unclear (Josepovitz et al., 1982). Structurally, aminoglycosides such as gentamicin and tobramycin are based around a central ring known as the aminocyclitol, which is linked to two or more aminoglycoside sugars through glycosidic bonds. Each aminoglycoside contains a large number of potentially ionizable side chains, which, at physiological pH result in the drug acting as an organic polycation, thereby giving the molecule a net positive charge. In the case of neomycin, this charge is +4.37 (Josepovitz et al., 1982). The high degree of polarity of these drugs means that they are strongly hydrophilic and therefore poorly lipid soluble. The overall result of this feature is the compounds' inability to penetrate the cells membranes, which is why they are administered via parenteral routes (Laurent et al., 1990).

In the rat, the Ca^{2+}-sensing receptor is present at the luminal aspect of proximal tubular cells (Riccardi et al., 1998), which is where most of the toxicity resides. Since previously, in vitro studies have demonstrated that neomycin-induced dose-dependent $Ca^{2+}{}_i$ release in oocytes expressing the Ca^{2+}-sensing receptor (Brown et al., 1993; Riccardi et al., 1995), but not in those non-expressing the receptor (Brown et al., 1993), we examined the potential for several aminoglycosides to act as Ca^{2+}-sensing receptor agonists in the HEK-293 expression system stably expressing the human parathyroid Ca^{2+}-sensing receptor (McLarnon et al., unpublished observations). The three most common aminoglycosides, neomycin, gentamicin and tobramycin all produced dose-dependent Ca^{2+}-sensing receptor stimulation, which was characteristic of a G-protein-coupled receptor-mediated responses linked to the activation of phosphoinositide turnover. The rank order of potency of the aminoglycosides was neomycin>tobramycin $\geq$ gentamicin>kanamycin. Since neomycin, which is a mixture of the B and C isomers, has six amino groups, both tobramycin and gentamicin (mixture of isomers C1, C1a and C2) have five and kanamycin only four, we concluded that there appears to be a correlation between the number of amino groups in an aminoglycoside compound and the agonist potency at the Ca^{2+}-sensing receptor (authors' observations).

Due to the ionizable status of these drugs, the effect of pH_o on sensitivity of the Ca^{2+}-sensing receptor to these agonists is of interest. Previously, elevating pH_o from 7.4 to 8.5 has markedly enhanced the sensitivity of the receptor to its cationic activators, while reducing pH_o from 7.4 to 6.5 suppresses sensitivity (Conigrave et al., 2000). This phenomenon has been attributed to structural alterations within the extracellular domain of the receptor as a result of alterations in the pH of the extracellular media. However, with relevance to receptor sensitivity to aminoglycosides, a reduction in pH_o potentiates the activation of the Ca^{2+}-sensing receptor, an effect that is not observed with Gd^{3+} (McLarnon et al., unpublished observations).

Finally, Ward et al. (in press) have shown that in a proximal tubular-derived cell line, which endogenously expresses the Ca^{2+}-sensing receptor, aminoglycosides trigger activation of ERK1 and ERK2 as effectively as other Ca^{2+}-sensing receptor agonists, namely $Ca^{2+}{}_o$ and $Gd^{3+}{}_o$. Taken together, these observations indicate that a Ca^{2+}-sensing receptor present in the kidney could account, at least in part, for some of the toxic effects of aminoglycoside treatment.

3.1. Ca^{2+}-sensing receptor calcimimetics and calcilytics: therapeutic uses

The first class of compounds that have been generated as selective Ca^{2+}-sensing receptor pharmacological probes is the phenylalkylamines. This class of compounds, such as NPS R-467 or R-568, acts in a stereoselective manner to enhance the sensitivity of the Ca^{2+}-sensing receptor to $Ca^{2+}{}_o$ and to decrease the secretion of PTH in bovine parathyroid cells in vitro (Steffey et al., 1993). This characteristic means that NPS R-568, and related compounds, act as positive allosteric modulators to increase the sensitivity of the Ca^{2+}-receptor to activation by extracellular Ca^{2+} (Nemeth, 1996). The effects of the NPS compound are dependent upon the concentration of extracellular Ca^{2+}, since NPS R-568 fails to affect parathyroid hormone secretion in the absence of extracellular Ca^{2+}. These compounds are selective for the Ca^{2+}-sensing receptor as demonstrated using Ca^{2+}-sensing receptor expressed in *Xenopus* oocytes, where both NPS R-467 and R-568 potentiated the effect of extracellular Ca^{2+} at the receptor. Lack of Ca^{2+} in the experimental solution renders the calcimimetics redundant, an observation that is also seen in *oocytes* that express the metabotropic glutamate $mGlu_{1a}$ receptor (Hammerland et al., 1998). The calcimimetic compounds that selectively mimic or potentiate the action of extracellular Ca^{2+} at the Ca^{2+}-sensing receptor such as NPS R-568 provide valuable tools to alter the function of the Ca^{2+}-sensing receptor and thereby determine its physiological and pathophysiological significance. These calcimimetic compounds may also serve as therapeutic agents for the treatment of disorders where the Ca^{2+}-sensing receptor is under active. These compounds have so far been targeted towards parathyroid disorders, for the treatment of both primary and secondary hyperparathyroidism (Silverberg et al., 1997). The calcimimetic compound R-568 has been used in a rodent model of secondary hyperparathyroidism in order to demonstrate the importance of the

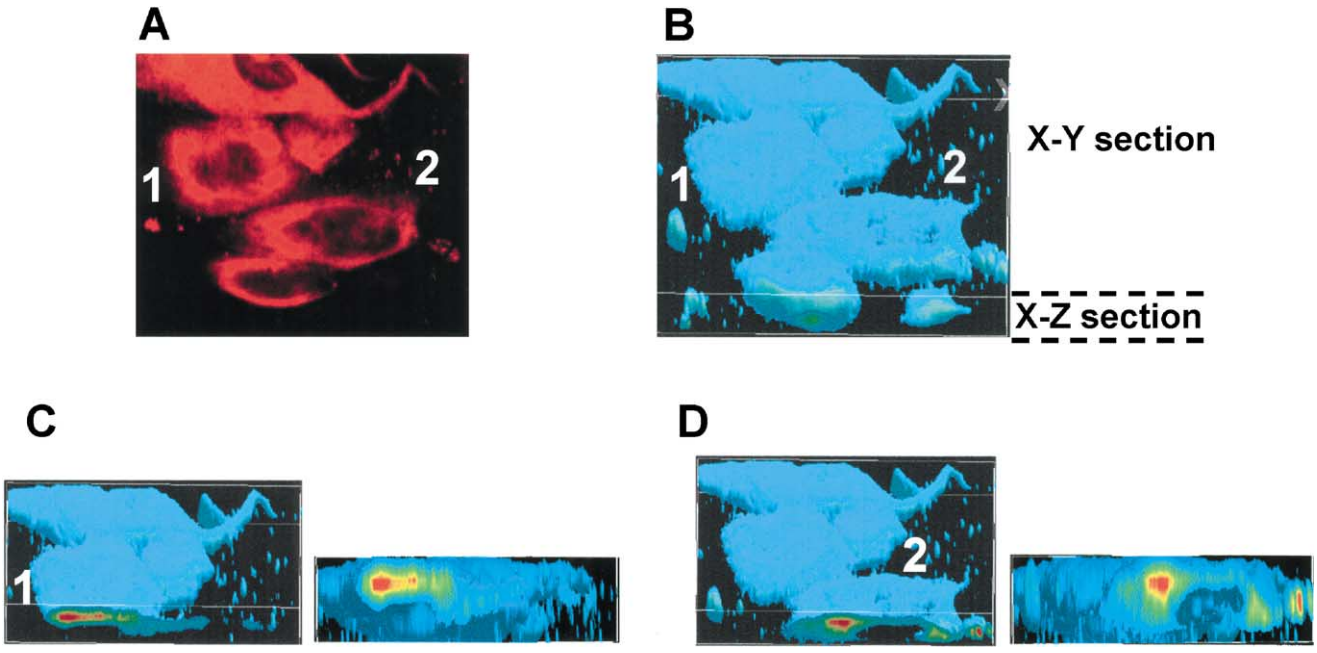

Fig. 3. Intracellular localization of the Ca^{2+}-sensing receptor (CaR) in the kidney-derived cell line opossum kidney (OK) cells: representation of two cells in the field. (A) Indirect immunofluorescence of CaR in OK cells. (B) 3-D reconstruction of CaR immunoreactivity in OK cells. (C) Section through cell 1. (D) Section through cell 2.

Ca^{2+}-sensing receptor in regulating not only PTH secretion but also parathyroid gland proliferation (Chin et al., 2000). In longer studies, daily administration of NPS R-568 to rodents prevented the development of secondary hyperparathyroidism as indexed by parathyroid cell hyperplasia and circulating levels of PTH (Wada et al., 2000).

More recently, there has been the development of the first group of compounds that act as selective Ca^{2+}-sensing receptor antagonists. The calcilytic compounds such as NPS-2143, have been demonstrated to be potent antagonists of the parathyroid gland Ca^{2+}-sensing receptor. Functionally, this compound induces an increase in the serum PTH concentration, one of the consequences of which is to lead to an increase in bone turnover. This action has raised the question of whether the anabolic action of these compounds could provide a potential tool for use in the treatment of osteoporosis (Gowen et al., 2000).

4. Concluding remarks

The current review has discussed several aspects of Ca^{2+}-sensing receptor function. Several conclusions can be drawn. The Ca^{2+}-sensing receptor is in fact a receptor for polyvalent cations, and it is possible that physiological agonists of the receptor may be different in different organs. In addition, some of these endogenous and/or pharmacological compounds exert positive cooperativity on the receptor for Ca^{2+}_o, which, in their presence, can therefore be activated by subthreshold concentrations of Ca^{2+}_o. Moreover, the Ca^{2+}-sensing receptor is expressed in regions with no apparent link with mineral ion metabolism, where receptor function can be modulated by ionic strength and by other positively charged compounds. Finally, considerable Ca^{2+}-sensing receptor immunoreactivity can also be observed intracellularly in many cell systems endogenously expressing the receptor (see Fig. 3). Whether this is due to receptor biosynthesis or whether the Ca^{2+}-sensing receptor has an additional role in intracellular Ca^{2+} homeostasis remains to be elucidated. We are therefore only beginning to understand the role of the receptor in physiological as well as pathological conditions. The availability of knockout model and of pharmacological agonists and antagonists of the receptor will greatly enhance our understanding of receptor function.

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