Allosteric Activation of the Ca^{2+} Receptor Expressed in *Xenopus laevis* Oocytes by NPS 467 or NPS 568

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ABSTRACT

The Ca^{2+} receptor is a G protein-coupled receptor that enables parathyroid cells and certain other cells in the body to respond to changes in the concentration of extracellular Ca^{2+}. In this study, two novel phenylalkylamine compounds, NPS 467 and NPS 568, were examined for effects on *Xenopus laevis* oocytes expressing the bovine or human parathyroid Ca^{2+} receptors. Increases in chloride current (I_{Cl}) were elicited in oocytes expressing the bovine Ca^{2+} receptor when the extracellular Ca^{2+} concentration was raised above 1.5 mM, whereas Ca^{2+} concentrations > 3 mM were generally necessary to elicit responses in oocytes expressing the human Ca^{2+} receptor. NPS 467 and NPS 568 potentiated the activation of I_{Cl} by extracellular Ca^{2+} in oocytes expressing either Ca^{2+} receptor homolog, and this resulted in a leftward shift of the Ca^{2+} concentration-response curve. Neither compound was active in the absence of extracellular Ca^{2+}. Certain inorganic and organic cations known to activate the Ca^{2+} receptor were substituted for elevated levels of extracellular Ca^{2+} to increase I_{Cl} and the effects of these agonists were also potentiated by NPS 568 or NPS 467. The effects of NPS 568 were stereoselective and the *R*-enantiomer was about 10-fold more potent than the corresponding *S*-enantiomer. Neither NPS 467 nor 568 affected I_{Cl} in water-injected oocytes or in oocytes expressing the substance K receptor or the metabotropic glutamate receptor 1a. These results provide compelling evidence that NPS 467 and NPS 568 act directly upon the parathyroid Ca^{2+} receptor to increase its sensitivity to activation by extracellular Ca^{2+}. This activity suggests that these compounds are positive allosteric modulators of the Ca^{2+} receptor. As such, these compounds define a new class of pharmacological agents with potent and selective actions on the Ca^{2+} receptor.

The Ca^{2+} receptor is a cell surface G protein-coupled receptor that enables parathyroid cells and certain other cells in the body to respond to small changes in the concentration of extracellular Ca^{2+} (Brown *et al.*, 1995; Nemeth, 1996). In parathyroid cells, the Ca^{2+} receptor monitors changes in the level of serum Ca^{2+} and is coupled to the regulation of PTH secretion. Activation of the parathyroid Ca^{2+} receptor by increased levels of extracellular Ca^{2+} results in the rapid formation of inositol 1,4,5-trisphosphate, the mobilization of intracellular Ca^{2+}, and the inhibition of PTH secretion (Brown, 1991). This reciprocal relationship between extracellular Ca^{2+} levels and PTH secretion is largely responsible for maintaining systemic Ca^{2+} homeostasis. Because of its central role in this homeostatic mechanism, the Ca^{2+} receptor is a promising molecular target for drugs designed to alter circulating levels of PTH. At present, however, no potent and selective compounds are known to act at this novel receptor.

The Ca^{2+} receptor responds not only to extracellular Ca^{2+}, but also to a variety of inorganic and organic cations, such as Mg^{2+}, La^{3+}, spermine, and neomycin (Nemeth and Scarpa,

1987; Brown *et al.*, 1991a, 1991b). Although some of the organic cations, such as polylysine, activate the Ca^{2+} receptor at nanomolar concentrations, neither the inorganic nor the organic cations possess desirable pharmaceutical properties. We have synthesized a series of phenylalkylamine compounds, typified by NPS 467 and NPS 568 (Fig. 1), that mobilize intracellular Ca^{2+} and inhibit PTH secretion from bovine or human parathyroid cells *in vitro* (Steffey *et al.*, 1993). These effects are similar to those obtained by increasing the concentration of extracellular Ca^{2+}. To determine if these compounds act directly on the Ca^{2+} receptor, we have expressed the bovine or human parathyroid Ca^{2+} receptor in *Xenopus laevis* oocytes and have assessed the effects of NPS 467 and NPS 568 on Ca^{2+}-activated Cl^- currents. The results provide evidence that these phenylalkylamine compounds act to potentiate the effects of cationic agonists of the Ca^{2+} receptor, but do so differently than all other known agonists of this receptor. The results suggest that NPS 467 and NPS 568 behave as positive allosteric modulators to increase the sensitivity of the Ca^{2+} receptor to extracellular Ca^{2+}.

ABBREVIATIONS: PTH, parathyroid hormone; BoPCaR, bovine parathyroid Ca^{2+} receptor; hPCaR, human parathyroid Ca^{2+} receptor; SKR, substance K receptor; mGluR, metabotropic glutamate receptor; MBS, modified Barth's solution; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; I_{Cl}, chloride current

Materials and Methods

Preparation of cRNA. The plasmid cDNA clones used were BoPCaR (Brown *et al.*, 1993), hPCaR 4.0 (Garrett *et al.*, 1995), bovine SKR (Nakanishi, 1991), and mGluR1a isolated from rat olfactory bulb cDNA (Masu *et al.*, 1991). Plasmid DNA was linearized by *Not*I digestion, and used as template for transcription of sense-strand cRNA using T7 RNA polymerase. Transcription reactions were done as previously described (Garrett *et al.*, 1995).

Oocyte isolation and cRNA injection. Adult female *X. laevis* toads were anesthetized in 0.1% tricaine according to an animal use protocol approved by the Institutional Animal Use and Care Committee of NPS Pharmaceuticals in accordance with federal animal welfare regulations. Pieces of ovarian lobe were surgically removed and incubated for 30–60 min in Ca^{2+}-free MBS containing 1.5 mg/ml Collagenase P (Boehringer Mannheim, Indianapolis, IN). The MBS contained 88 mM NaCl, 1 mM KCl, 0.82 mM $MgSO_4$, 10 mM HEPES, and 2.4 mM $NaCO_3$, pH value 7.5. Stage V or VI oocytes were separated manually and washed with MBS containing 0.8 mM $CaCl_2$ before injection. The cRNAs of Ca^{2+} receptors, mGluR1a and SKR cRNAs were dissolved in water and 50 nl (12.5 ng/oocyte) of the RNA solution was injected into individual oocytes. Control oocytes were injected with water. After injection, oocytes were incubated at 16° in MBS containing 0.5 mM $CaCl_2$ for 2–7 days before electrophysiological recording (Goldin, 1992).

Two-electrode voltage-clamp. Voltage-recording and current-passing electrodes were filled with 3 M KCl and had resistances of 0.5–2 MΩ. Oocytes were voltage-clamped at a holding potential of −60 mV with an Axoclamp 2A amplifier (Axon Instruments, Foster City, CA) by using standard two-electrode voltage-clamp techniques (Stuhmer, 1992). Currents were recorded on a chart recorder. The standard control buffer was MBS containing 0.3 mM $CaCl_2$ and 0.8 mM $MgCl_2$, except where otherwise noted, and all concentrations shown are final. The 0 Ca^{2+} solutions contained no added Ca^{2+}, and no chelating agents were used. Test substances were applied by superfusion at a flow rate of about 5 ml/min. All experiments were done at room temperature. The activity of NPS 568 and NPS 467 was determined by their effects on agonist-evoked increases in the amplitude of I_{Cl}. Activation of I_{Cl} was quantified by measuring the peak inward current stimulated by agonist or drug, relative to the holding current at −60 mV.

Concentration-response study of agonist-mediated increases in I_{Cl}. When multiple agonist concentrations were applied to the same oocyte, the maximal increase in I_{Cl} amplitude varied considerably among different oocytes. The same degree of variability was observed in oocytes expressing BoPCaR or hPCaR and is characteristic of the oocyte expression system. Therefore, the data for each oocyte were normalized to the maximum value obtained for each series of applications. A curve was fit to the data for each experiment with the Levenberg-Marquardt algorithm using the Kaleidograph fitting program (Synergy Software, Reading, PA). The curve for each set of data was fit to the equation $I_{Cl} = A / [1 + (EC_{50} / [agonist])^{n_H}]$, where A represents the dynamic range for the stimulation of I_{Cl} and n_H is the Hill coefficient. The fitted value of the dynamic range was then used to calculate the percent of maximum response to agonists. All results expressed as percent of maximum response were pooled and fit to the equation: % of maximal response = $100 / [1 + (EC_{50} / [agonist])^{n_H}]$.

Results

In oocytes injected with cRNA encoding BoPCaR, increasing the concentration of extracellular Ca^{2+} to levels >1.5 mM activated an inward current. The reversal potential of this current was −33 ± 3 mV ($n = 4$). This reversal potential corresponds well with the equilibrium potential for Cl^- in this system (Goldin, 1992) and indicates that the channels activated by extracellular Ca^{2+} in oocytes injected with cRNA encoding BoPCaR are the endogenous Ca^{2+}-dependent Cl^- channels. These Ca^{2+} receptor-mediated increases in I_{Cl} were transient and concentration-dependent (Fig. 2). In contrast, water-injected oocytes did not respond to application of Ca^{2+} in quantities up to 20 mM (data not shown). Concentration-response characteristics of BoPCaR were determined by exposing oocytes to 1, 1.7, 3, 5.6, and 10 mM extracellular Ca^{2+} in a cumulative manner. Oocytes expressing hPCaR 4.0 typically responded only to extracellular Ca^{2+} concentrations >3 mM. These oocytes were therefore exposed to 1.7, 3, 5.6, 10, and 15 mM Ca^{2+} for concentration-response analysis. The
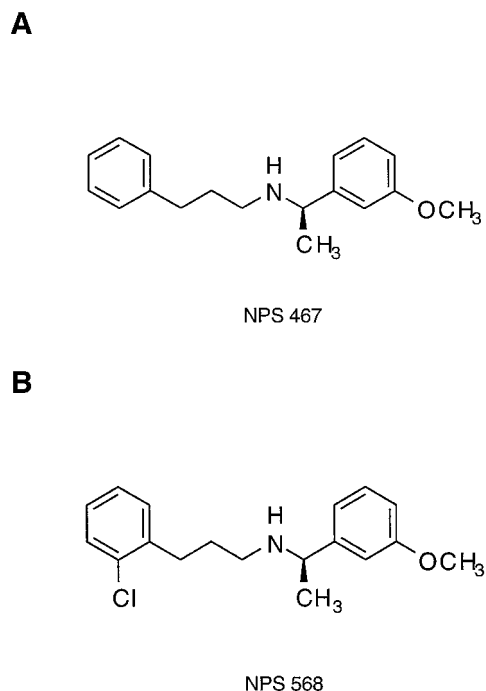
A

B

Fig. 1. The chemical structures of NPS 467 (A) and NPS 568 (B), shown as the *R*-enantromers.
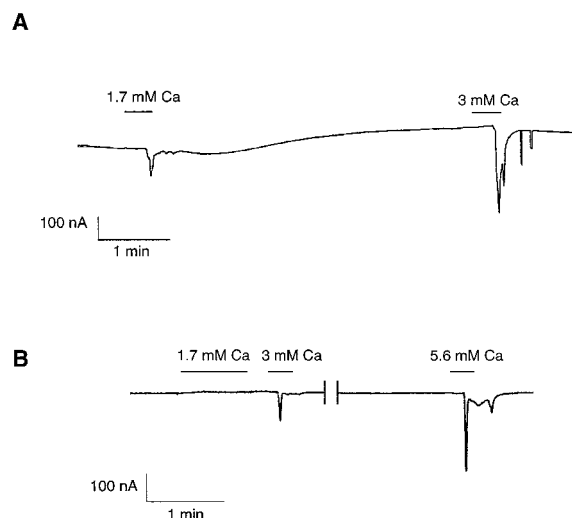
A

B

Fig. 2. Concentration-dependent increases in I_{Cl} amplitude evoked by extracellular Ca^{2+} in *X. laevis* oocytes injected 2–4 days before assay with cRNA encoding A, BoPCaR, or B, hPCaR 4.0. Tracings show I_{Cl} recorded at a holding potential of −60 mV. Oocytes were bathed in MBS containing 0.3 mM $CaCl_2$ and exposed to higher levels of Ca^{2+} for the periods indicated by *horizontal bars* above current tracings.

results of concentration-response analysis for both BoPCaR and hPCaR are shown in Fig. 3 and indicate that the bovine receptor may be somewhat more sensitive to extracellular Ca^{2+} than the human homolog when studied in this heterologous expression system.

The activation of I_{Cl} by elevated concentrations of extracellular Ca^{2+} was potentiated in the presence of 1 μM NPS R-568 (Fig. 4). In contrast, noninjected oocytes did not respond to NPS R-467 or NPS R-568 at concentrations up to 100 μM, in either the absence or the presence of added Ca^{2+} (not shown). However, when extracellular Ca^{2+} was omitted, the stimulatory activity of NPS R-467 and NPS R-568 was abolished (Fig. 5). Oocytes expressing hPCaR 4.0 also responded to application of extracellular Ca^{2+} and these responses were also potentiated by NPS R-568. These results suggest that NPS R-568 may act by sensitizing Ca^{2+} receptors to activation by extracellular Ca^{2+}. The effects of NPS R-467 and of NPS R-568 on the Ca^{2+} concentration-response relationship were determined in oocytes expressing BoPCaR in the presence of NPS R-467 or R-568 at concentrations of 1 μM, 3 μM, or 10 μM (Fig. 6A). Either compound caused a dose-dependent, leftward shift in the Ca^{2+} concentration-response curve. In oocytes expressing hPCaR 4.0, the effect of elevated extracellular Ca^{2+} was determined in the presence of 3 μM NPS R-568 and this also resulted in a leftward shift in the Ca^{2+} concentration-response curve (Fig. 6B).

The stereoselectivity in the activation of I_{Cl} by NPS 568 (which contains a single chiral carbon, Fig. 1) was examined in *X. laevis* oocytes expressing BoPCaR. Extracellular Ca^{2+} (3 mM) was applied alone, then in the presence of various concentrations of NPS S-568. After washout, Ca^{2+} was reapplied together with NPS R-568 and the response amplitudes were compared. Application of NPS R-568 (1 μM) greatly enhanced the response to extracellular Ca^{2+}, but NPS S-568 was effective only at augmenting responses at concentrations $\geq$3 μM. Overall, the potentiation of Ca^{2+} responses by 10 μM NPS S-568 was slightly less than that evoked by 1 μM NPS R-568 (Fig. 7). When 10 μM NPS S-568 was coapplied with 3 mM Ca^{2+}, responses were increased by 150 $\pm$ 42% ($n = 3$) over responses to 3 mM Ca^{2+} alone, whereas 1 μM NPS R-568 increased the response to 3 mM Ca^{2+} alone by 235 $\pm$ 67% ($n = 3$).

The parathyroid Ca^{2+} receptor can also be activated by elevated concentrations of certain other inorganic cations,

such as Mg^{2+} and Gd^{3+}, as well as organic polycations such as neomycin and spermine (Brown, 1991; Brown *et al.*, 1991a). To determine whether NPS R-568 potentiated Ca^{2+} receptor activation evoked by other cation agonists, the effects of NPS R-568 were examined on Ca^{2+} receptor-mediated responses to Mg^{2+}, Gd^{3+}, or neomycin in oocytes injected with BoPCaR or hPCaR 4.0 cRNA. Oocytes expressing BoPCaR were responsive to application of 10 mM Mg^{2+}, but a lower concentration (4 mM Mg^{2+}) did not increase I_{Cl}, nor did the same oocytes respond when challenged with a Ca^{2+}-free saline that contained 10 μM NPS R-467. However, all five oocytes tested responded when challenged with 4 mM Mg^{2+} plus 10 μM NPS R-467. (Fig. 8A). Gadolinium-evoked responses were also potentiated by NPS R-568 in oocytes ex-
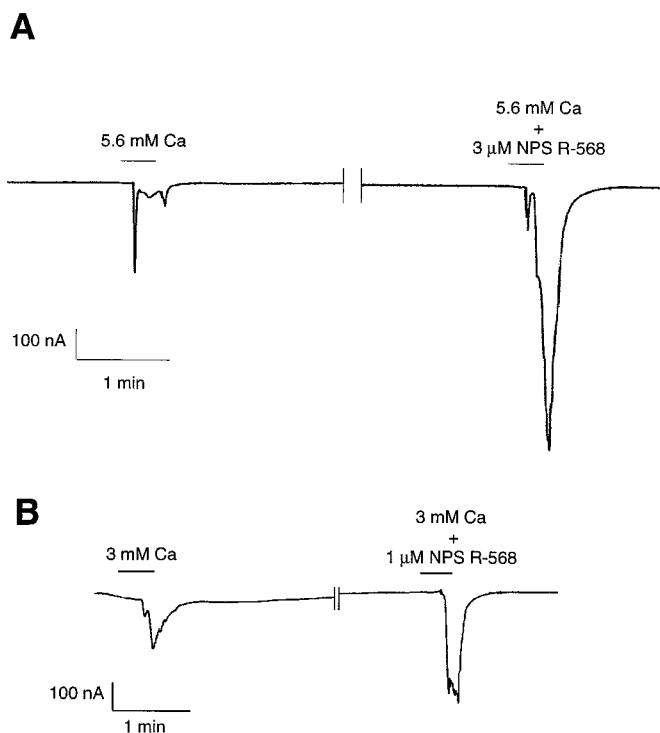
A

B

Fig. 4. NPS R-568 potentiation of Ca^{2+} receptor-dependent activation of I_{Cl} in oocyte injected with A, hPCaR 4.0, or B, BoPCaR cRNA 2–4 days earlier. Horizontal bars indicate the duration of substance application. Extracellular Ca^{2+} concentration was increased transiently to 3 mM or 5.6 mM and then, after washout, 3 mM or 5.6 mM calcium was applied in the presence of 1 μM or 3 μM NPS R-568.

Fig. 5. Ca^{2+} receptor-mediated increase in I_{Cl} by NPS R-568 requires the presence of extracellular Ca^{2+}. Tracing shows current recorded at a holding potential of −60 mV in an *X. laevis* oocyte injected with BoPCaR 4 days before assay. *Dotted horizontal bar*, duration of 1 mM Ca^{2+} application; *bold solid bar*, period during which Ca^{2+} was removed; *plain solid bar*, period when 1 μM NPS R-568 was present.
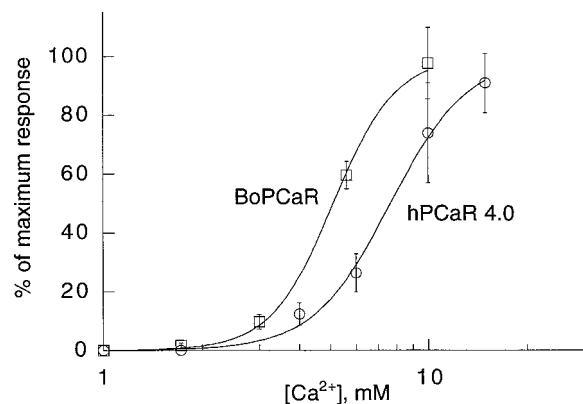

Fig. 3. Concentration-response characteristics of BoPCaR and hPCaR. Data shown are the normalized mean I_{Cl} amplitude $\pm$ standard error for 16 and 5 oocytes, respectively.
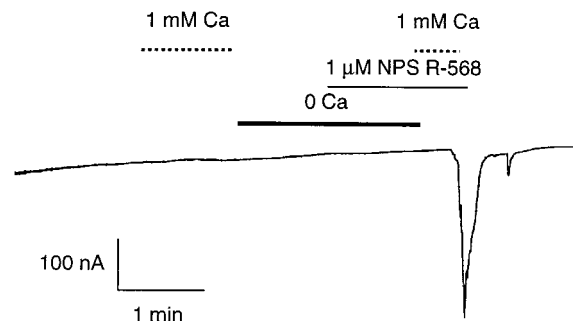
pressing BoPCaR and hPCaR 4.0 (Fig. 8B). In the absence of added Ca^{2+}, 30 μM neomycin evoked increases in I_{Cl} in all four cells tested, whereas the application of 5 μM neomycin

Fig. 6. A, Concentration-response curves for Ca^{2+} alone ($\square$) and for Ca^{2+} plus 1 μM ($\blacktriangle$), 3 μM ($\bullet$), and 10 μM ($\blacklozenge$) NPS R-568 and 10 μM NPS R-467 ($\blacksquare$) in oocytes that were injected at least 2 days earlier with BoPCaR cRNA. Data shown are the mean I_{Cl} amplitude ± standard error (n = 16, 5, 5, 4, and 4 for each concentration, respectively). EC_{50} values in millimolar for Ca^{2+}, Ca^{2+} plus 1 μM NPS R-568, Ca^{2+} plus 3 μM NPS R-568, and Ca^{2+} plus 10 μM NPS R-568 were 5.1 ± 0.1, 3.6 ± 0.2, 3.2 ± 0.1, and 1.5 ± 0.1, respectively. Hill coefficients for Ca^{2+}, Ca^{2+} plus 1 μM NPS R-568, Ca^{2+} plus 3 μM NPS R-568, and Ca^{2+} plus 10 μM NPS R-568 were 4.5 ± 0.3, 3.3 ± 0.5, 2.3 ± 0.1, and 3.4 ± 0.7, respectively. B, Concentration-response curve for Ca^{2+} alone and for Ca^{2+} plus 3 μM NPS R-568 in oocytes expressing hPCaR 4.0. EC_{50} values in millimolar for Ca^{2+} and Ca^{2+} plus 3 μM NPS R-568 were 7.6 ± 1.7 and 3.2 ± 0.2 with Hill coefficients of 3.6 ± 0.2 and 5.4 ± 1.7, respectively.

Fig. 7. NPS R-568 is a more potent activator than NPS S-568 of Ca^{2+} receptor-mediated increases in I_{Cl} amplitude. In this representative trace the oocyte was injected with BoPCaR cRNA 3 days before assay. *Horizontal bars above tracing*, substances tested and duration of their application.

did not elicit a response in any of the four oocytes. However, when 5 μM neomycin was applied together with 1 μM NPS R-568, all cells responded with large increases in I_{Cl} (Fig. 8C).

The receptor specificity of NPS R-568 was examined in oocytes injected with cRNA encoding bovine SKR or rat mGluR1a. The SKR, the mGluR1a, and the Ca^{2+} receptor are coupled to inositol triphosphate-mediated Ca^{2+} mobilization and, therefore, produce qualitatively similar responses (increases in I_{Cl} amplitude) when these receptors are activated by their cognate ligand (Masu *et al.*, 1991; Nakanishi, 1991; Brown *et al.*, 1993; Garrett *et al.*, 1995). Further, the Ca^{2+} receptor and mGluRs share limited sequence homology (Masu *et al.*, 1991; Brown *et al.*, 1993; Garrett *et al.*, 1995). Oocytes expressing SKR did not respond to 10 μM NPS 568 either alone or when added in the presence of 10 mM Ca^{2+} (n = 5). In those oocytes expressing the SKR, increases in I_{Cl} were evoked in response to substance K concentrations ranging from 0.3 to 10 nM and those responses were unaffected by NPS R-568 (10 μM). Further, the sensitivity of mGluR1a to activation by L-glutamate was not affected by NPS R-467. Responses to 3 μM L-glutamate approximated those to 3 μM L-glutamate plus 10 μM NPS R-467. (Fig. 9).
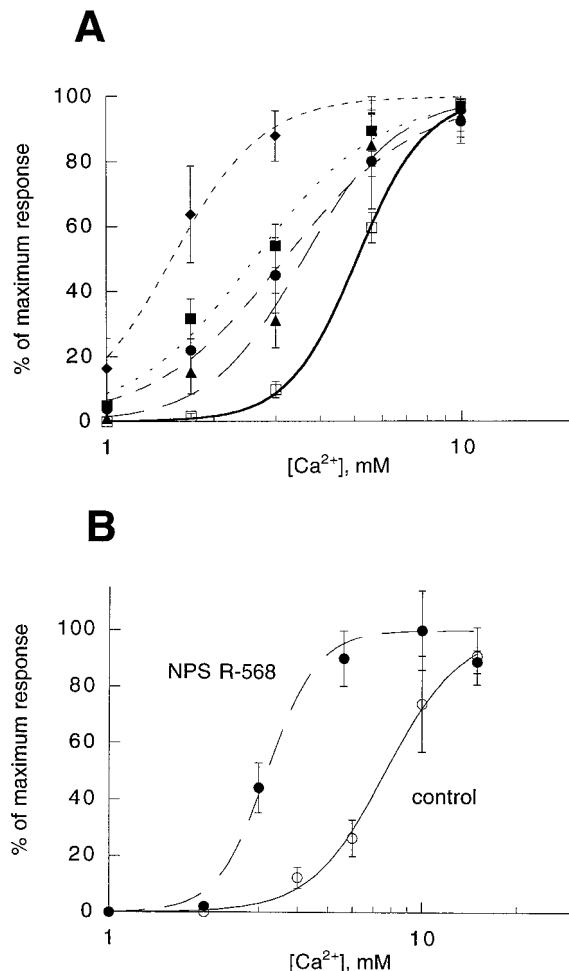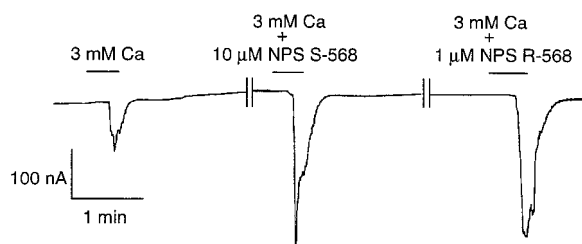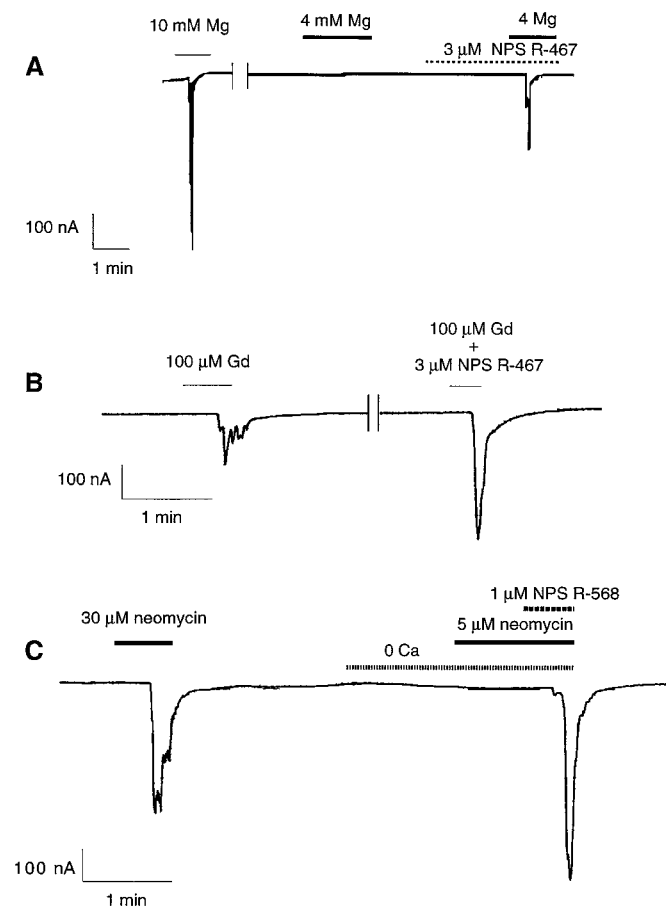

Fig. 8. A, Effects of NPS R-568 on Mg^{2+}-evoked increases in I_{Cl}. Tracing shows I_{Cl} amplitude at a holding potential of -60 mV in an *X. laevis* oocyte injected with BoPCaR cRNA 4 days earlier. Shown are changes in the concentration of Mg^{2+} in the absence of added Ca^{2+} and with the addition of NPS R-568 (*bars above tracing*). B, Effects of NPS R-467 on Gd^{3+}-evoked increases in I_{Cl} in oocytes injected with BoPCaR. In oocytes expressing hPCaR 4.0, the response to Gd^{3+} is also potentiated by NPS R-568. C. Effects of NPS R-568 on neomycin-evoked increases in I_{Cl} in the absence of extracellular Ca^{2+}.

Discussion

Expression of G protein-coupled receptors in heterologous cellular systems like *X. laevis* oocytes has been used to obtain evidence for direct actions of compounds on receptors and to further define their molecular pharmacology (Barnard and Bilbe, 1987). The *X. laevis* oocyte system is particularly useful for exploring the pharmacology of receptors that couple through phospholipase C to the mobilization of intracellular Ca^{2+} because increases in the concentration of cytoplasmic Ca^{2+} are readily assessed by measuring currents through the Ca^{2+}-activated chloride channels (Ji *et al.*, 1991; Feng *et al.*, 1996). In the present series of experiments, this system has been used to define the mechanism of action of a novel class of compounds believed to act on the Ca^{2+} receptor.

The present study has confirmed the results obtained with extracellular Ca^{2+} using the cloned bovine parathyroid Ca^{2+} receptor and has now included the human Ca^{2+} receptor, which shows some differences in sensitivity to extracellular Ca^{2+} (Brown *et al.*, 1993). Comparison of the extracellular Ca^{2+} concentration-response curves for BoPCaR and for hP-CaR 4.0 suggests that the bovine parathyroid receptor is more sensitive to Ca^{2+} than is the human receptor, although maximal responses of each receptor were similar. These differences in sensitivity to extracellular Ca^{2+} are not understood, but may be related to the fidelity of expression or to species differences. The bovine and human Ca^{2+} receptors

A

B

Fig. 9. Elevated Ca^{2+} and NPS 467 or NPS 568 do not affect SKR or mGluR1a expressed in *X. laevis* oocytes. A, Tracing shows the application of 10 mM Ca^{2+} and of 10 mM Ca^{2+} in the presence of NPS 568 to an oocyte injected 3 days earlier with cRNA encoding the SKR. After washout of Ca^{2+} and NPS 568, substance K is applied. B. Tracing shows mGluR1a responses to 3 μM L-glutamate, alone and in the presence of 10 μM NPS R-467, and to 30 μM L-glutamate for comparison. The breaks in the mGluR1a tracings represent 7 minutes. *Horizontal bars*, duration of substance application.

differ in 74 of 1078 amino acids (93% identity) and 43 of these differences are found in the cytoplasmic tail region containing amino acids 920 through 1078. The other differences are distributed throughout the remainder of the protein and many of these are conservative amino acid substitutions (Brown *et al.*, 1993; Garrett *et al.*, 1995). One possibility is that the clustering of differences within the carboxyl-terminal region (amino acids 920-1078) may account for the differences in sensitivity to agonists in this system. The mGluR1a, which shares significant homology with the Ca^{2+} receptor, exists in at least three alternative splice variants that differ in the length and sequence of the carboxyl-terminal tail. Pharmacological analysis of these splice variants has showed that although the rank order of potencies of agonists is identical for all three variants, agonists are consistently more potent on the mGluR1a than on the mGluR1b and the mGluR1c (Flor *et al.*, 1996). It may be that those differences in the carboxyl-terminal tail between the human and bovine Ca^{2+} receptors, which are unlikely to have direct effects on agonist binding, do affect the overall sensitivity of the receptor to agonist stimulation.

The ability of NPS R-467 and NPS R-568 to elicit responses in oocytes injected with cRNA encoding the Ca^{2+} receptor, but not in noninjected or water-injected oocytes, provides compelling evidence that these compounds act directly on the Ca^{2+} receptor. The response in oocytes injected with the Ca^{2+} receptor cRNA does not result simply from expression of an exogenous G protein-coupled receptor because oocytes injected with substance K receptor cRNA or mGluR1a cRNA do not respond to either compound, but readily respond to their respective ligands. Although an indirect action of these compounds is possible, it would have to result from the interactions of a molecule endogenous to the oocyte that displays no similar activity on other G protein-coupled receptors. Further, the effect of these compounds is stereoselective, as it is in authentic bovine or human parathyroid cells (Steffey *et al.*, 1993). In the aggregate then, the results offer strong evidence for an action of these compounds on the Ca^{2+} receptor.

Compounds that directly activate the Ca^{2+} receptor are called "calcimimetics." Polycations like spermine and neomycin are calcimimetics that activate the Ca^{2+} receptor in the absence of extracellular Ca^{2+}, whether expressed in *X. laevis* oocytes or in authentic parathyroid cells (Brown *et al.*, 1991a). Compounds like NPS 467 and NPS 568, however, fail to elicit responses in the absence of extracellular Ca^{2+}. Rather, they potentiate responses to extracellular Ca^{2+} as well as to other extracellular di- or trivalent cations known to act on the Ca^{2+} receptor. In each case, NPS 467 and NPS 568 shift the concentration-response curve for extracellular Ca^{2+} to the left. The most parsimonious explanation for these effects is that these phenylalkylamine compounds behave as positive allosteric modulators to increase the sensitivity of the Ca^{2+} receptor to activation by extracellular Ca^{2+}. Our recent findings that extracellular Ca^{2+} acts in the extracellular domain (Hammerland *et al.*, 1995), whereas these compounds act in the transmembrane region of the Ca^{2+} receptor (Hammerland *et al.*, 1996), are consistent with this mechanism of action. However, it remains uncertain if the phenylalkylamine compounds bind to the receptor in the absence of extracellular Ca^{2+} or if the binding of Ca^{2+} unmasks a cryptic binding site for these compounds. In either case, it is clear
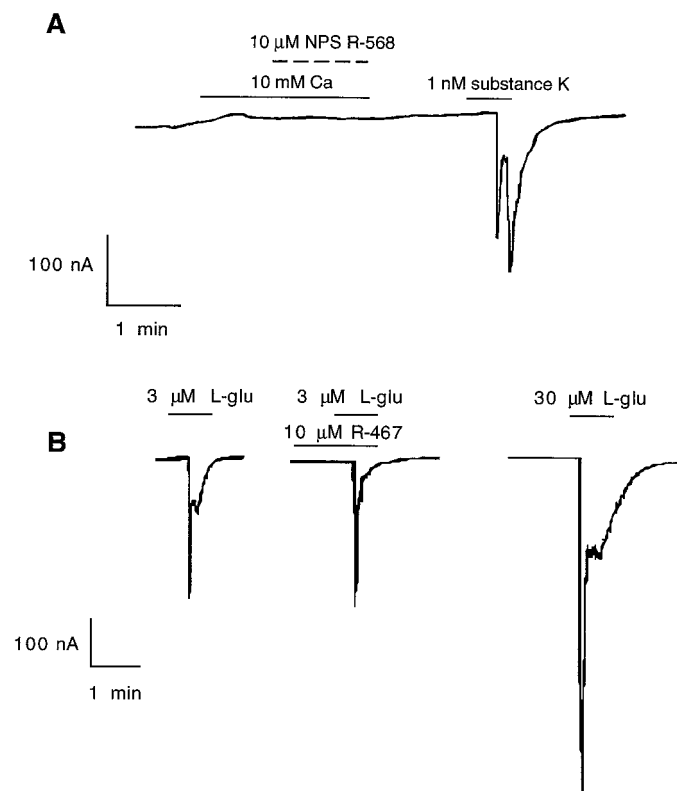
that their action is dependent on extracellular Ca^{2+} and that they potentiate responses to physiological and other Ca^{2+} receptor ligands.

Calcimimetic compounds therefore include compounds that mimic or potentiate the actions of extracellular Ca^{2+} by acting directly on the Ca^{2+} receptor. We can thus describe calcimimetics as being either Type I or Type II. Type I calcimimetics act in the absence of extracellular Ca^{2+} and the Type II calcimimetics act only in the presence of Type I calcimimetics. These structurally novel Type II calcimimetics are the first compounds that selectively target the Ca^{2+} receptor. As such, they may be suitable as drugs or drug leads to treat various bone and mineral disorders, such as hyperparathyroidism, where it is desirable to lower plasma levels of PTH (Silverberg *et al.*, 1997).

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