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Functional expression of the parathyroid cell calcium receptor in *Xenopus* oocytes

Frederick K. Racke[a], Lance G. Hammerland[b], George R. Dubyak[a], Edward F. Nemeth[b,*]

[a]*Department of Physiology and Biophysics, Case Western Reserve University School of Medicine, Cleveland, OH 44106, USA*
[b]*NPS Pharmaceuticals, Inc., 420 Chipeta Way, Salt Lake City, UT 84108, USA*

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Various studies suggest the existence of a plasma membrane receptor on parathyroid cells that senses changes in the concentration of extracellular Ca^{2+}. To test this hypothesis, *Xenopus leavis* oocytes were injected with poly(A)$^+$-enriched mRNA from bovine parathyroid cells and examined for their ability to respond to increases in the concentration of extracellular Ca^{2+} or other polycations. Cytosolic Ca^{2+} concentrations were measured indirectly by recording Cl^- currents through the endogenous, cytosolic Ca^{2+}-activated Cl^- channel. Increasing the concentration of extracellular Ca^{2+} (from 0.7 to 5 mM) or Mg^{2+} (from 0.8 to 10 mM) elicited oscillatory increases in the Cl^- current. Responses to either divalent cation were not observed in oocytes injected with water or with mRNA prepared from HL-60 cells or rat liver. Responses elicited by extracellular Mg^{2+} persisted when extracellular Ca^{2+} was reduced to low micromolar levels. La^{3+}, Gd^{3+}, or neomycin B also evoked oscillatory increases in the Cl^- current in oocytes under conditions of low extracellular Ca^{2+} levels. These extracellular polycations all cause the mobilization of intracellular Ca^{2+} in oocytes injected with parathyroid cell mRNA like they do in intact parathyroid cells. The injection of parathyroid cell mRNA thus confers on oocytes the ability to detect and respond to changes in the concentration of extracellular polycations. The data provide compelling evidence for the existence of a cell surface Ca^{2+} receptor protein(s) on parathyroid cells that regulates cellular function.

Parathyroid cell; Calcium; Calcium receptor; Receptor expression; *Xenopus* oocyte

1. INTRODUCTION

The parathyroid cell is one of the specialized cells in the body known to respond to changes in the concentration of extracellular Ca^{2+}. Indeed, the level of plasma Ca^{2+} is the primary physiological stimulus regulating secretion of parathyroid hormone (PTH). Increasing the concentration of extracellular Ca^{2+} elicits corresponding increases in the concentration of intracellular Ca^{2+} ($[Ca^{2+}]_i$) in parathyroid cells, and this is associated with an inhibition of parathyroid hormone secretion [1]. The increases in $[Ca^{2+}]_i$ elicited by extracellular Ca^{2+} arise from the mobilization of intracellular Ca^{2+} and the influx of extracellular Ca^{2+} and, since first demonstrated, have been suggested to result from the ability of extracellular Ca^{2+} to activate a cell surface Ca^{2+} receptor [2]. The mobilization of intracellular Ca^{2+} in parathyroid cells can be evoked by a variety of extracellular di- and trivalent cations [3,4] and by certain organic polycations [5,6]. Biochemical studies show that these extracellular polycations cause the rapid formation of inositol 1,4,5-trisphosphate and diacylglycerol in parathyroid cells consistent with receptor-dependent mobilization of intracellular Ca^{2+} [7–9]. These physiological and biochemical studies are complimentary and, together with studies using monoclonal antibodies [10], suggest the presence on the surface of parathyroid cells of a Ca^{2+} receptor that enables these cells to detect and respond to small changes in the concentration of extracellular calcium [11,12].

Despite these diverse studies, there is still no unequivocal evidence for a Ca^{2+} receptor protein on the surface of parathyroid cells and the very existence of such a receptor is still controversial. In the present study, we have tested for genetic material in parathyroid cells that encodes a Ca^{2+} receptor protein(s). *Xenopus* oocytes were used as a functional receptor expression system because they are an efficient cellular model that can express a variety of exogenous membrane receptors coupled to the mobilization of intracellular Ca^{2+} [13] and because increases in $[Ca^{2+}]_i$ are readily monitored by recording Cl^- currents through an endogenous Cl^- channel that is activated by cytosolic Ca^{2+} [14]. We report the presence, in bovine parathyroid cells, of mRNA which imparts to *Xenopus* oocytes the ability to detect and respond to changes in the level of extracellular Ca^{2+} and various other inorganic and organic polycations.

2. EXPERIMENTAL

Bovine parathyroid glands were obtained from a local abattoir; parathyroid cells were enzymatically dissociated, purified, and cultured overnight as previously described [15]. Total RNA was obtained by guanidinium thiocyanate-phenol extraction and purified on a CsCl gradient. Poly (A)$^+$-enriched mRNA was obtained using oligo d(T) cellulose chromatography and diluted to a final concentration of 1 $\mu g/\mu l$.

*Corresponding author. Fax: (1) (801) 583 4961.

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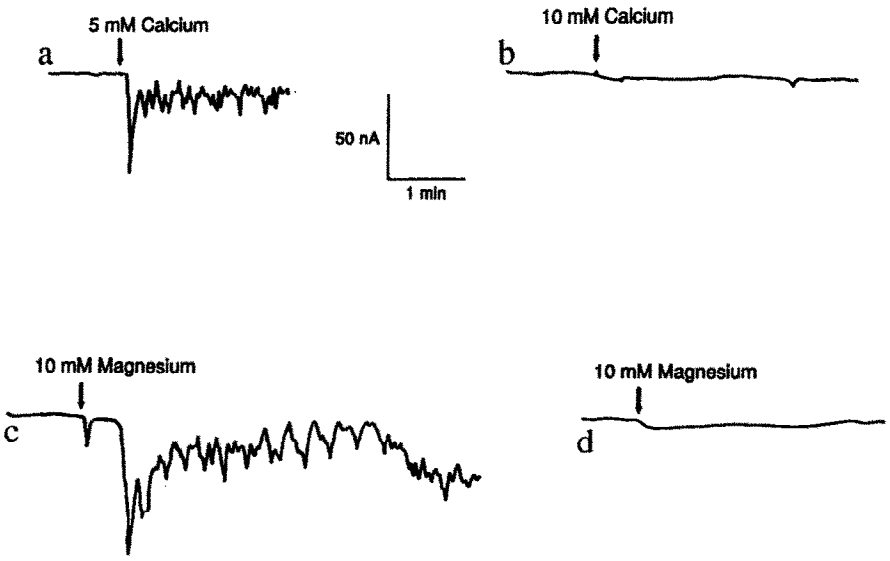

Fig. 1. Extracellular Ca^{2+} or Mg^{2+} evoke increases in Cl^- current in *Xenopus* oocytes injected with parathyroid cell mRNA. Oocytes were injected with bovine parathyroid cell poly(A)$^+$-enriched mRNA (50 ng; left trace) or water (50 nl; right trace) and incubated 2 days. The oocytes were bathed in MBS containing 0.71 mM Ca^{2+} and 0.82 mM Mg^{2+}, and at the times indicated by the arrows, concentrated solutions (5–10 μl) of $CaCl_2$ (traces a and b) or $MgCl_2$ (traces c and d) were added to obtain the final concentration shown. Traces a and c are representative of those seen in 12 separate oocytes injected with 2 different parathyroid cell mRNA poly (A)$^+$ enriched mRNA preparations. Traces b and d are representative of those seen in 2 other oocytes injected with water or oocytes injected with rat liver mRNA.

Ovarian lobes were surgically excised from adult female *Xenopus laevis* toads anesthetized with 0.1% tricaine. Pieces of ovarian lobes were incubated for 30 min in Ca^{2+}-free modified Barth's solution (MBS; 15 mM HEPES, 88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, and 0.82 mM $MgSO_4$ containing 1.5 mg/ml collagenase type IA (Worthington)). Stage 5 or 6 oocytes were separated manually and washed extensively in MBS containing 0.41 mM $CaCl_2$, 0.3 mM $Ca(NO_3)_2$, and 0.82 mM $MgSO_4$. Individual oocytes were incubated (19°C) overnight in this same solution supplemented with 2.5 mM pyruvate, 50 U/ml penicillin, and 50 μg/ml streptomycin.

Oocytes were injected using a WPI pneumatic Picopump (PV820) with glass pipettes having tip diameters between 5 and 7 μM. Injected substances included 50 nl of poly (A)$^+$-enriched mRNA (1 μg/μl) from bovine parathyroid cells, HL60 cells, or rat liver, 50 nl of purified substance K receptor cRNA (10 ng/μl), or 50 nl of water. Oocytes were then incubated 2–3 days at 19°C in MBS supplemented with pyruvate and antibiotics.

Chloride currents were measured using standard two electrode voltage clamp techniques. Glass microelectrodes had tip resistances of 1.5 to 3 MΩ when filled with 3 M KCl. Oocytes were clamped to a potential of −60 mV using an Axoclamp 2A amplifier (Axon Instruments). Current traces were recorded directly on a strip- chart recorder. The oocytes were initially bathed in 1 ml of MBS containing 0.7 mM Ca^{2+} and 0.8 mM Mg^{2+}. In some experiments, addition of test substances was made by the removal of 200 μl of the bathing solution and replacement with MBS containing 5-fold concentrated solutions of test substance. In most experiments, the oocytes were placed in a small chamber having a volume of 0.5 ml and superfused (5 ml/min) with MBS containing the desired final concentration of test substance. In those experiments designed to assess the mobilization of intracellular Ca^{2+}, the concentration of extracellular Ca^{2+} was reduced to low levels by omitting Ca^{2+} from the MBS. The estimated concentration of extracellular Ca^{2+} in this buffer, estimated by titration with fura-2, was 20 μM.

3. RESULTS

Authentic parathyroid cells respond not only to extracellular Ca^{2+} but additionally to a wide variety of extracellular inorganic di- and trivalent cations. All these other inorganic polycations cause increases in $[Ca^{2+}]_i$ in the nominal absence of extracellular Ca^{2+} in bovine parathyroid cells and thus elicit the mobilization of intracellular Ca^{2+}. To test for the functional expression of a Ca^{2+} receptor in *Xenopus* oocytes, the ability of these inorganic and organic polycations to increase Cl^- currents when extracellular Ca^{2+} was reduced to low micromolar levels was assessed.

Xenopus oocytes were injected with bovine parathyroid cell mRNA, water, or mRNA from rat liver or HL-60 cells. After incubation for 2 to 3 days, oocytes injected with parathyroid cell mRNA responded to increases in the concentration of extracellular Ca^{2+} (from 0.7 to 5 mM) with rapid oscillatory increases in the Cl^- current (Fig. 1a). Such responses to extracellular Ca^{2+} were seen with each of three different poly(A)$^+$-enriched mRNA preparations from bovine parathyroid cells. With each preparation of parathyroid cell mRNA, 63% of the injected oocytes (312 out of 489 cells) responded to an elevation of extracellular Ca^{2+} to 5 mM. In contrast, extracellular Ca^{2+} did not cause changes in the Cl^- current in oocytes injected with water (0 out of 20 cells) or mRNA from rat liver (0 out of 5 cells) or HL-60 cells (0 out of 8 cells; Fig. 1b).

Increasing the concentration of extracellular Mg^{2+} from 0.8 to 10 mM likewise evoked increases in the Cl^- current that tended to oscillate (Fig. 1c). This response to extracellular Mg^{2+} was observed only in oocytes injected with parathyroid cell mRNA (Fig. 1d). The Cl^- responses to extracellular Ca^{2+} or Mg^{2+} were reversible.

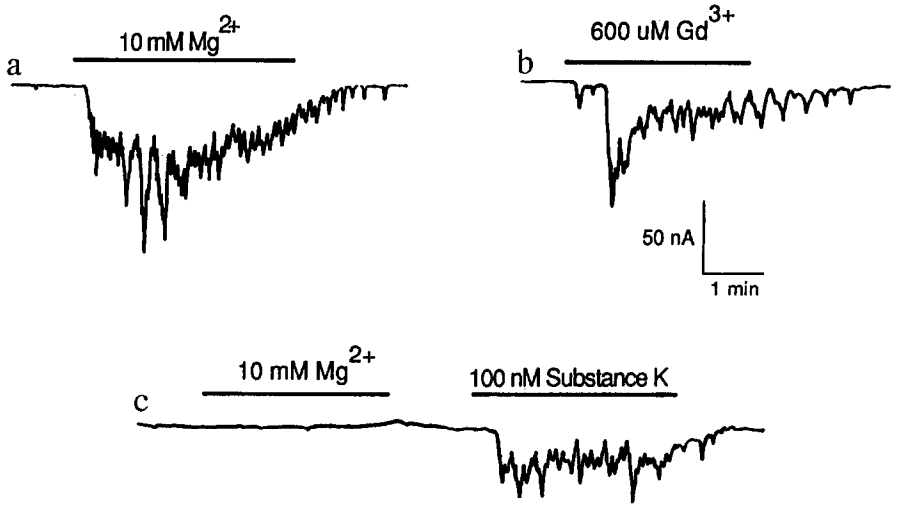

Fig. 2. Extracellular di- and trivalent cations mobilize intracellular Ca^{2+} in *Xenopus* oocytes expressing parathyroid cell mRNA. Oocytes were injected with parathyroid cell mRNA (50 ng; traces a and b) or cRNA encoding the substance K receptor (0.5 ng; trace c) and incubated for 3 days. Oocytes were initially superfused with MBS containing 10–20 μM Ca^{2+} before switching to Ca-free MBS containing the indicated concentration of Mg^{2+} (a), Gd^{3+} (b) or Mg^{2+} followed by substance K (c) and washed several times. Traces a and b are representative of the responses seen in 8 other oocytes and trace c is representative of results obtained in 4 other oocytes.

Following stimulation, reduction of the extracellular divalent cation concentration by superfusion of the oocytes in MBS containing normal concentrations of Ca^{2+} and Mg^{2+} resulted in a cessation of the oscillatory Cl^- currents (e.g. Fig. 2). These evoked current responses likely reflect the ability of extracellular divalent cations to increase $[Ca^{2+}]_i$ in oocytes injected with parathyroid cell mRNA and thereby activate the endogenous cytosolic Ca^{2+}-activated Cl^- channel [14].

To determine if the response to extracellular Mg^{2+} resulted from the mobilization of intracellular Ca^{2+}, the concentration of extracellular Ca^{2+} was reduced to 20 μM. This concentration of Ca^{2+} is far below the threshold concentrations that cause cytosolic Ca^{2+} responses in authentic parathyroid cells (0.5 to 3 mM) or Cl^- current responses in oocytes injected with parathyroid cell mRNA (2 to 3 mM). Oocytes injected with parathyroid cell mRNA still responded to extracellular Mg^{2+} (10 mM) when extracellular Ca^{2+} was reduced to this low level, consistent with the mobilization of intracellular Ca^{2+} (Fig. 2a). In the presence of a low concentration of extracellular Ca^{2+}, Mg^{2+} failed to evoke responses in oocytes that were injected with cRNA encoding the substance K receptor, although these oocytes responded readily to the addition of substance K (Fig. 2c).

The trivalent cations La^{3+} and Gd^{3+} were also examined for effects on parathyroid cell mRNA-injected oocytes. These trivalent cations are generally impermeant in mammalian cells and are believed to act on the cell surface of parathyroid cells to cause the mobilization of intracellular Ca^{2+}. The addition of either La^{3+} or Gd^{3+} caused oscillatory increases in the Cl^- current similar to that evoked by extracellular Ca^{2+} or Mg^{2+}. Moreover, the response to these trivalent cations persisted when the concentration of extracellular Ca^{2+} was reduced to low micromolar levels (Fig. 2b). The ability of extracellular Mg^{2+}, La^{3+}, and Gd^{3+} to evoke increases in the Cl^- current under this condition suggests strongly that these inorganic polycations cause the mobilization of intracellular Ca^{2+} specifically in oocytes expressing parathyroid cell mRNA.

The magnitude of the responses to di- and trivalent cations varied considerably from oocyte to oocyte, even when injected with the same preparation of parathyroid cell mRNA. Nonetheless, by examining a large number of oocytes, concentrations causing a half-maximal response (EC_{50}'s) could be approximated. The apparent EC_{50}'s for extracellular Ca^{2+} and Mg^{2+} were 5 mM and 10 mM, respectively, whereas those for La^{3+} and Gd^{3+} were similar, about 80 μM. These approximate EC_{50}'s are higher than those observed in bovine parathyroid cells but are in the appropiate concentration range, with divalent cations causing responses at millimolar concentrations and trivalent cations eliciting responses at micromolar levels. Purified cRNAs of cell surface receptors expressed in oocytes typically show a slightly decreased sensitivity to receptor agonists [13].

In addition to extracellular di- and trivalent cations, parathyroid cells also respond to certain organic polycations such as spermine and neomycin B. These organic molecules seem to act similarly to extracellular Ca^{2+} to mobilize intracellular Ca^{2+} and inhibit PTH secretion. In oocytes injected with parathyroid cell mRNA, there was a large increase in the Cl^- current elicited by neomycin B which, like the responses to extracellular Ca^{2+} and other inorganic polycations, oscillated around an increased conductance state and ceased upon superfusion with MBS lacking neomycin B (Fig.

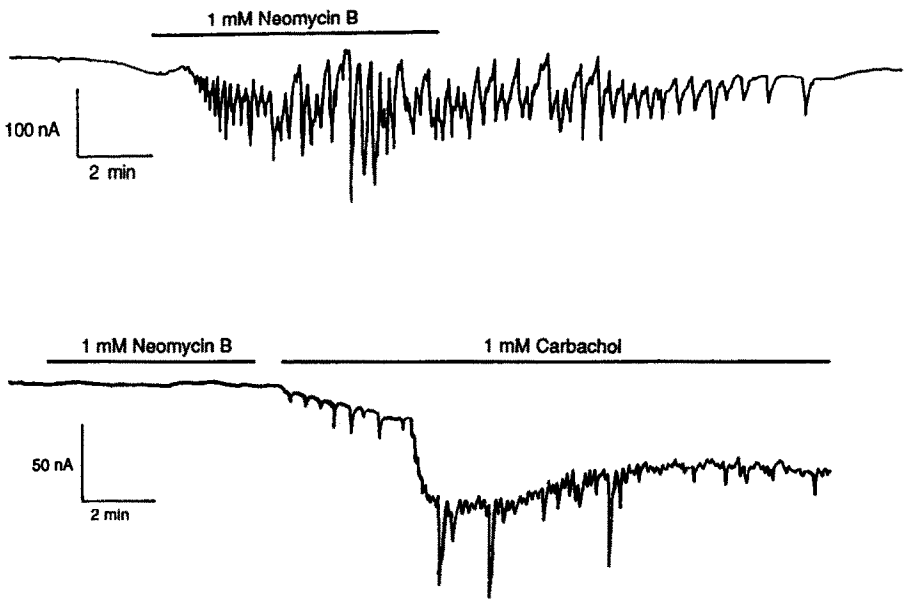

Fig. 3. Oocytes expressing parathyroid mRNA respond to neomycin B. Oocytes injected with parathyroid mRNA (top trace) or water (bottom trace) were incubated 3 days and placed in MBS. Where indicated, 1 mM neomycin B or 1 mM carbachol was added to the bathing medium.

3a). Neomycin B (100 mM or 1 mM) failed to cause any change in Cl^- current in water-injected oocytes or in oocytes injected with rat liver mRNA.

About 40% of oocytes are known to respond to carbachol, an effect mediated by an endogenous muscarinic receptor linked to the mobilization of intracellular Ca^{2+} [16]. In five oocytes examined, one showed inward currents in response to carbachol yet all five failed to respond to neomycin B (1 mM). This finding demonstrates that the failure to respond to neomycin B does not result from the lack of the appropriate molecular machinery underlying the mobilization of intracellular Ca^{2+}. Rather, the results show that the response to neomycin B depends on the expression of a protein(s) encoded specifically by parathyroid cell mRNA, and this is true also for the inorganic polycations examined.

4. DISCUSSION

Although extracellular Ca^{2+} has long been known to act directly on the parathyroid cell to regulate PTH secretion, the mechanism(s) used by these cells to sense the ambient calcium concentration has remained elusive. Most of the data derived from physiological and biochemical studies supports the presence, on the surface of parathyroid cells, of a Ca^{2+} receptor that is coupled to the activation of a phospholipase C resulting in the formation of inositol 1,4,5-trisphosphate and the mobilization of intracellular Ca^{2+} [11,12]. Increases in $[Ca^{2+}]_i$ arising from the mobilization of intracellular Ca^{2+} are associated with the inhibition of PTH secretion [2]. The putative Ca^{2+} receptor on parathyroid cells is therefore functionally and mechanistically akin to other membrane receptors that initially transduce extracellular signals into functional cellular responses. The essential difference is that the physiological ligand for the Ca^{2+} receptor is an inorganic ion rather than an organic molecule.

The present findings demonstrate that *Xenopus* oocytes gain responsivity to various extracellular inorganic and organic polycations when injected with parathyroid cell mRNA. There is no endogenous sensitivity of oocytes to the tested polycations because oocytes injected with water or mRNA from rat liver or HL-60 cells do not respond to these polycations. *Xenopus* oocytes are known to possess endogenous responsivity to some extracellular divalent cations such as Mn^{2+} and Ni^{2+} [17], so it is significant that the responses observed in the present study were only observed in oocytes injected with parathyroid cell mRNA. Oocytes that were not injected with parathyroid cell mRNA were capable of responding to other ligands, either through endogenous receptors (muscarinic) or through exogenously expressed receptors (substance K). All these results show that the response to extracellular Ca^{2+} and other polycations is conferred specifically by parathyroid cell mRNA.

The Cl^- current responses in oocytes elicited by extracellular Mg^{2+}, La^{3+}, Gd^{3+} or neomycin B persisted when the concentration of extracellular Ca^{2+} was reduced to low micromolar levels and likely result from the mobilization of intracellular Ca^{2+}. Additional experiments reveal that the magnitude of the responses evoked by these extracellular polycations is little affected when Ca^{2+} influx is blocked in oocytes (B. Hung and E.F. Nemeth, unpublished observations). Together, these results suggest that the major action of extracellular polycations in parathyroid cell mRNA-

injected oocytes is to cause the mobilization of intracellular Ca^{2+}. The mobilization of intracellular Ca^{2+} is seemingly the major mechanism associated with the regulation of PTH secretion in authentic parathyroid cells; influx of extracellular Ca^{2+} appears not to be tightly coupled to PTH secretion. In fact, extracellular Mg^{2+} does not promote influx of extracellular Ca^{2+} in bovine parathyroid cells yet inhibits PTH secretion similarly to extracellular Ca^{2+} [2]. Thus, the principle mechanism used by extracellular polycations to regulate PTH secretion has been imparted to *Xenopus* oocytes by injection of mRNA from parathyroid cells. Because the transmembrane and intracellular signaling mechanisms are present in oocytes that fail to respond to extracellular Ca^{2+}, as assessed by their ability to respond to carbachol or substance K, it must be the initial recognition mechanism that is specifically supplied by parathyroid cell mRNA. What is uncertain is whether this initial recognition mechanism consists of a single protein. Reduction of the response to a single clone or multiple clones will be necessary to resolve this issue.

Although precise concentration-response curves were not constructed for all polycations tested, it is noteworthy that the apparent rank order of potency paralleled that observed in bovine parathyroid cells (neomycin B $\approx La^{3+} \approx Gd^{3+} > Ca^{2+} > Mg^{2+}$). This suggests that the pharmacological profile of the protein expressed in oocytes is similar to that of authentic parathyroid cells. It is significant that neomycin B elicited responses only in oocytes injected with parathyroid cell mRNA. Although organic polycations were originally suggested to act directly on the Ca^{2+} receptor [5], alternative actions are possible [18]. However, the specificity of the response to neomycin B suggests that it is the recognition mechanism that is the site of action of this compound, rather than some interaction with phospholipids or G-proteins.

In the aggregate, the present results demonstrate the presence of mRNA in parathyroid cells that imparts to *Xenopus* oocytes the ability to detect and respond to changes in the concentration of extracellular Ca^{2+} and other polycations. Such findings indicate the presence of a Ca^{2+} receptor protein(s) on the surface of parathyroid cells.

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