Modulation of intracellular Ca^{2+} in the parathyroid cell

Release of Ca^{2+} from non-mitochondrial pools by inositol trisphosphate

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Stimuli which enhance secretion from parathyroid cells such as low extracellular Ca^{2+} or Mg^{2+} are associated with a decrease in the cytosolic Ca^{2+} concentration as measured by quin2. Current evidence suggests that increased production of inositol 1,4,5-triphosphate (IP_3) releases Ca^{2+} from cellular stores thus increasing cytosolic Ca^{2+}. We used saponin-permeabilized dispersed bovine parathyroid cells to study the effect of IP_3 on intracellular Ca^{2+}. IP_3 released Ca^{2+} from these cells in a dose-dependent manner; half-maximal response occurred with 0.3 μM IP_3 and maximal response with 1.2 μM IP_3. Permeabilized cells incubated in the presence of the mitochondrial inhibitor antimycin A released a similar amount of Ca^{2+} suggesting that IP_3 releases Ca^{2+} from a non-mitochondrial pool. These results suggest that IP_3 regulates cytosolic Ca^{2+} in this system and may function as a second messenger controlling hormone secretion.

Inositol trisphosphate *Cytosolic Ca^{2+}* *Parathyroid cell* *Ca^{2+} electrode* *Phospholipid*

1. INTRODUCTION

Several recent studies suggest that inositol phospholipids play a role in the transduction of extracellular signals. In many cell types a variety of extracellular signals induce the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PtdIns $(4,5)P_2$) to diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3) [1]. DAG activates protein kinase C within the plasma membrane [1] whereas IP_3 is released into the cytoplasm where it presumably increases cytosolic Ca^{2+} by releasing Ca^{2+} from intracellular stores [2–4]. These stores are probably not mitochondrial but may be within the endoplasmic reticulum [4,5]. In most cell types an increase in cytosolic Ca^{2+} concentration is associated with exocytosis [6]. The parathyroid cell is unique in that stimuli which enhance secretion such as low extracellular Ca^{2+} or Mg^{2+}, are associated with a decrease in cytosolic Ca^{2+} as measured using the Ca^{2+} dye quin2, suggesting that cytosolic Ca^{2+} modulates hormone secretion [7]. The possible role of IP_3 in the Ca^{2+} homeostasis of this system is therefore particularly intriguing.

We used permeabilized dispersed bovine parathyroid cells to study the effect of IP_3 on intracellular Ca^{2+} pools. Our results indicate that IP_3 releases Ca^{2+} from non-mitochondrial pools which presumably increases cytosolic Ca^{2+}.

2. MATERIALS AND METHODS

Dispersed bovine parathyroid cells were prepared by collagenase digestion of calf parathyroid glands using a modification of the technique in [8]. Viability as assessed by trypan blue exclusion was greater than 95% and red blood cell contamination

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was less than 10%. Prior to use, the cells were washed 3 times in Ca^{2+}- and Mg^{2+}-free Hanks basal salt solution (4°C, pH 7.2), and kept on ice at a concentration of 2×10^8/ml (<2 h).

For determination of Ca^{2+} sequestration and release, 2×10^6 cells were incubated at 30°C in 0.2 ml of medium with a cation composition similar to cytosol (110 mM KCl, 10 mM NaCl, 2 mM KH_2PO_4, 1 mM $MgCl_2$) with 25 mM Hepes (pH 7.0), 1 μg/ml oligomycin, 2 mM MgATP, and an ATP-regenerating system consisting of 5.0 mM phosphocreatine, and 20 units/ml creatine kinase (basic medium). Plasma membranes were permeabilized with saponin (60 μg/ml). Experiments with functioning mitochondria and endoplasmic reticulum were performed in basic medium plus 5.0 mM succinate and 1 μM rotenone. Antimycin A (0.2 μM) was added to the basic medium to produce cells with nonfunctioning mitochondria. Medium Ca^{2+} concentration was measured with a sensitive minielectrode as described [9]. None of the compounds tested interfered with the measurement of Ca^{2+}. All experiments were repeated 3 times using different cell preparations. The traces shown are representative of all experiments.

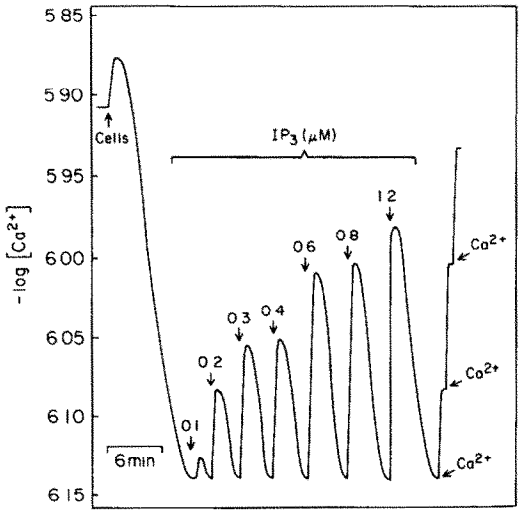

Fig.1. Effect of IP_3 on medium Ca^{2+} concentration in the presence of permeabilized bovine parathyroid cells with functioning mitochondria. Permeabilized cells were incubated in basic medium plus 5.0 mM succinate and 1 μM rotenone. Ca^{2+} release from cells was calibrated at the end of the experiment by repeated additions of 0.2 nmol Ca^{2+}.

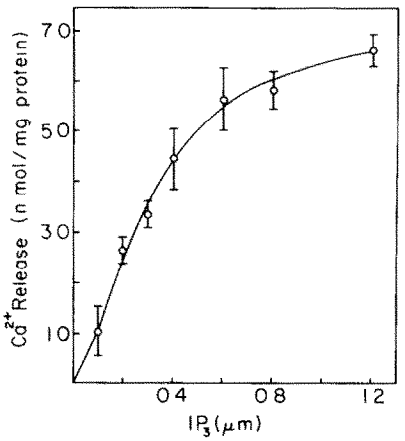

Fig.2. Concentration-dependence of IP_3-induced Ca^{2+} release. Cells were incubated as described in fig.1. Ca^{2+} release in response to IP_3 was calibrated by addition of known amounts of Ca^{2+} and is expressed per mg cellular protein. Points represent the mean ± SE of 3 experiments performed with different cell preparations.

IP_3 and inositol 1,4-bisphosphate (IP_2) were produced by alkaline hydrolysis of ox brain Ptd (4,5)P_2 and purified by paper chromatography [10] by Dr R.F. Irvine, Cambridge, England. Cellular protein was determined by the Coomassie blue method (Bio-Rad, Rockville Center, New York).

3. RESULTS

When parathyroid cells with functioning mitochondria were permeabilized, medium Ca^{2+} concentration fell within 10 min from approx. $10^{-5.9}$ M (1.25 μM) to 10^{-6} M (0.60 μM), indicating that Ca^{2+} was being sequestered into cellular stores (fig.1). These cells rapidly released Ca^{2+} into the medium in response to IP_3 in a dose-dependent manner (figs.1 and 2). Half-maximal response occurred with 0.3 μM IP_3 and maximal response with 1.2 μM IP_3. In the absence of cells, IP_3 increased medium Ca^{2+} less than 5% of that seen with cells, thus excluding contamination of the IP_3 as the source of Ca^{2+}. Maximal increase in medium Ca^{2+} concentration occurred within 45 s; thereafter, Ca^{2+} was re-accumulated into cellular stores, and medium Ca^{2+} concentration returned to baseline within 10 min. The cells responded to repeated additions of IP_3 and each Ca^{2+} release was followed by complete re-uptake.

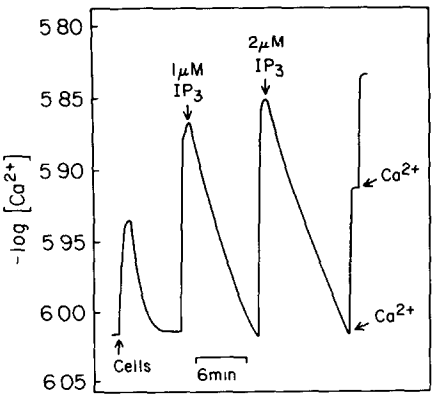

Fig.3. Effect of IP_3 on the medium Ca^{2+} concentration maintained by the non-mitochondrial compartment of permeabilized bovine parathyroid cells. Permeabilized cells were incubated in basic medium plus 0.2 μM antimycin A. Ca^{2+} release from cells was calibrated at the end of the experiment by repeated additions of 0.2 nmol Ca^{2+}

To determine if IP_3, or instead, one of its metabolites was the compound releasing Ca^{2+}, the release of Ca^{2+} in response to IP_2, the first metabolite of IP_3 was measured. IP_2 (0.8 μM) released less than 10% of the Ca^{2+} released by the same concentration of IP_3 (0.52 vs 6.11 nmol Ca^{2+}/mg cellular protein).

Permeabilized parathyroid cells incubated in the presence of the mitochondrial inhibitor antimycin A also lowered medium Ca^{2+} concentration to less than 10^{-6} M. IP_3 (1.0 μM) resulted in maximal release of Ca^{2+} from these cells (fig.3) and released the same amount of Ca^{2+} as 1.2 μM IP_3 added to cells with functioning mitochondria (7.8 ± 1.91 vs 6.64 ± 0.33 nmol Ca^{2+}/mg cellular protein).

4. DISCUSSION

These data show that IP_3 releases Ca^{2+} from cellular stores in bovine parathyroid cells. IP_3 released Ca^{2+} equally from cells incubated in the presence of the mitochondrial inhibitor antimycin A indicating that Ca^{2+} was released from a non-mitochondrial pool. The concentration of IP_3 which evoked the half-maximal Ca^{2+} release (0.3 μM) was similar to that observed in other types of permeabilized cells [2,11,12]. Permeabilized parathyroid cells responded to repeated additions of IP_3 in accord with observations in permeabilized hepatocytes [3,12] and insulinoma cells [11] suggesting that Ca^{2+} is re-sequestered in an IP_3-sensitive pool.

IP_3 may play a physiologic role in the regulation of parathyroid hormone (PTH) secretion. Estimates of IP_3 concentrations in hepatocytes using *myo*-[^{3}H]inositol labeling indicate that these concentrations are similar to those stimulating Ca^{2+} release from permeabilized bovine parathyroid cells [1]. Furthermore, Ca^{2+} release in response to IP_3 occurred within seconds which is similar to the response time of cytosolic Ca^{2+} in quin2-loaded parathyroid cells to changes in extracellular Ca^{2+} [7]. Although re-uptake of Ca^{2+} after IP_3 addition requires several minutes, this delay may reflect the time needed for a small number of cells to degrade the large amount of IP_3 contained in the incubation medium. Recent evidence suggests that Ca^{2+} re-uptake may be consequent to the degradation of added IP_3 [3,11].

IP_3 could modulate PTH secretion by several mechanisms. High extracellular Ca^{2+} may accelerate hydrolysis of Ptd(4,5)P_2 with a resulting increase in IP_3 and DAG. IP_3 would release stored Ca^{2+} and increase cytosolic Ca^{2+} thereby inhibiting PTH secretion. Alternatively, low extracellular Ca^{2+} may accelerate IP_3 degradation (and decrease hydrolysis of Ptd(4,5)P_2), lowering cytosolic IP_3 and Ca^{2+} and stimulating PTH secretion.

The role of DAG in modulating PTH secretion has only been indirectly studied by Brown et al. [13] and Morrissey [14]. These investigators reported that phorbol esters, which, like DAG presumably activate protein kinase C [1], stimulate PTH secretion from bovine parathyroid cells. This finding is the opposite of that predicted by either model proposed above. Phorbol esters are not a physiologic stimulus, however, and the role of DAG, if any, in modulating PTH secretion remains to be determined.

Clarification of the physiologic role of IP_3 in modulating cytosolic Ca^{2+} and hormone secretion in the parathyroid cell will require further studies including direct measurement of intracellular IP_3 in concert with changes in PTH secretion.

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