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Bimodal regulation of secretion by cytoplasmic Ca^{2+} as demonstrated by the parathyroid

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Bovine parathyroid cells were used to study parathyroid hormone (PTH) release and the cytoplasmic Ca^{2+} concentration (Ca_i^{2+}). When the extracellular Ca^{2+} concentration was decreased from 3.0 to 0.5 mM, perifused cells reacted with rapid stimulation of PTH release. However, a further reduction of extracellular Ca^{2+} to < 10 nM resulted in prompt inhibition. Both effects were readily reversible. Using the intracellular Ca^{2+} indicator quin-2 also as a buffer for calcium it was possible to control Ca_i^{2+} within the 20–600 nM range. PTH release was found to increase with Ca_i^{2+} up to 200 nM but was gradually suppressed above this concentration.

Parathyroid hormone release; cytoplasmic Ca^{2+}; Quin 2; Secretion; Bimodal regulation

1. INTRODUCTION

The major physiological stimulus of parathyroid hormone (PTH) release is a lowering of extracellular Ca^{2+} which translates into a decreased cytoplasmic concentration of the ion (Ca_i^{2+}) [1–7]. The parathyroid cell is consequently unusual among secretory cells in exhibiting an inverse relationship between Ca_i^{2+} and secretion. However, when lowering extracellular Ca^{2+} far below the physiological range we have consistently observed inhibition of PTH release [5–7]. Using intracellular quin-2 as a buffer and indicator for Ca_i^{2+} [8] it has now been investigated whether there is also a stimulatory component in the relation between Ca_i^{2+} and secretion in the parathyroid cells. It will be shown that PTH release increases with Ca_i^{2+} up to 200 nM but is gradually suppressed above this concentration. The discovery of dual actions of Ca_i^{2+} on PTH release may reflect a phenomenon of general importance for the understanding of the role of Ca^{2+} in secretion.

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2. MATERIALS AND METHODS

Parathyroid glands obtained from adult cattle within a few minutes after slaughter were used for preparation of cell suspensions [6]. The medium used in the experiments was a 20 mM Hepes buffer (pH 7.4) containing 0.1% human serum albumin, 0.5 mM Mg^{2+}, Ca^{2+} in the < 10 nM (Ca^{2+}-deficient + 0.5 mM EGTA) to 3.0 mM range and physiologically balanced in other cations with Cl^- as the sole anion [9]. The dynamics of PTH release was studied by perifusing the isolated cells. A suspension of 15×10^6 cells in 1 ml medium was mixed with a 1.5 ml slurry of Biogel P-4 (50 mg/ml) and then added to a perifusion chamber consisting of a vertical 1 cm chromatographic column. The cells were perifused at 37°C with medium containing 3.0, 0.5 mM or < 10 nM Ca^{2+} at a rate of 600 μl/min. The PTH content of each

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40 s fraction was determined radioimmunologically, using an assay detecting essentially the mid-C regional hormone [10]. To study Ca_i^{2+} the dispersed cells were loaded with 0.5–2.0 mM of the Ca^{2+} indicator quin-2 by incubation for 30–40 min at 37°C in a Ca^{2+}-deficient medium containing 0.5 mM EGTA and 12.5–50 μM quin-2 tetraacetoxy methyl ester [7]. After loading and rinsing, 5×10^6 cells were suspended in 1.3 ml medium containing < 10 nM Ca^{2+}. The cell suspension was incubated with constant stirring at 37°C in a 1 cm cuvette placed in a Perkin-Elmer LS 5 spectrofluorometer with excitation and emission wavelengths set at 339 and 492 nm, respectively. Ca_i^{2+} was calculated essentially as in [11] assuming a K_d for the Ca^{2+}–quin-2 complex of 115 nM [8]. Quin-2-loaded cells from the same batch were also used for determination of PTH release. For the latter purpose 5×10^5 cells were incubated for 2 h at 37°C in 0.5 ml medium containing < 10 nM–3.0 mM Ca^{2+}.

3. RESULTS AND DISCUSSION

In previous studies PTH release was inhibited when lowering the extracellular Ca^{2+} concentration from 0.5 mM to <25 nM by the addition of EGTA [5–7]. Using a similar approach Brown et al. [12] failed to observe any differences in PTH release in the < 10 nM–1 mM Ca^{2+} range during incubation for up to 1 h. An irreversible drop in secretion after prolonged incubation at <20 nM Ca^{2+} was attributed to toxic actions of EGTA. In the present investigation it was ascertained from studies of the kinetics of PTH release in a perifusion system that the inhibition of secretion was not due to any noxious effect of EGTA or low Ca^{2+}. As shown in fig.1, a lowering of extracellular Ca^{2+} from 3.0 to 0.5 mM rapidly stimulated PTH release. However, a further decrease by omission of Ca^{2+} and addition of 0.5 mM EGTA resulted in a prompt inhibition. Both effects were readily reversed when the extracellular Ca^{2+} concentration was subsequently increased to 0.5 and 3.0 mM. Moreover, control experiments showed that PTH release was not influenced by 0.5 mM EGTA per se, and that prolonged exposure to < 10 nM extracellular Ca^{2+} did not damage the parathyroid cells. Secretion during 2 h in the presence of 1 mM Ca^{2+} and 0.5 mM EGTA was thus identical to that

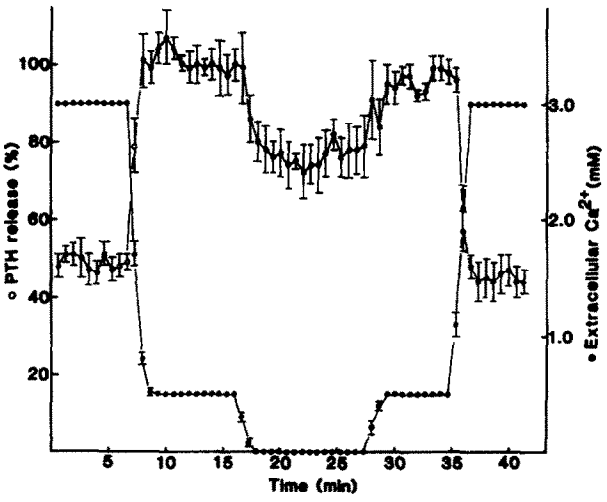

Fig.1. Effects of different Ca^{2+} concentrations on PTH release from perifused parathyroid cells. PTH secretion (○) is expressed as percentage of the average hormone release during the first period of perifusion at 0.5 mM Ca^{2+} in each experiment. The Ca^{2+} concentration of the perifusate was measured indirectly by including phenol red as a spectrophotometric indicator in the medium containing 0.5 mM Ca^{2+} (●). Results are means ± SE for 5 experiments.

at 0.5 mM Ca^{2+} alone, and incubation for the same period of time in a Ca^{2+}-deficient medium containing 0.5 mM EGTA did not change the pattern of PTH release during subsequent incubations at 0.5–3.0 mM Ca^{2+} (not shown).

To elucidate the relationship between Ca_i^{2+} and PTH release we used quin-2 as both a buffer and indicator of Ca_i^{2+} [8]. By loading the cells with various amounts of quin-2 in a Ca^{2+}-deficient medium followed by exposure to different extracellular Ca^{2+} concentrations it was possible to control Ca_i^{2+} within the 20–600 nM range. The Ca^{2+}-buffering effect of quin-2 influenced Ca_i^{2+} only in a medium deficient in Ca^{2+} but not when the extracellular concentration was 0.5 mM or higher. Fig.2 shows a typical recording of the quin-2 fluorescence when extracellular Ca^{2+} was increased from < 10 nM to supraphysiological concentrations. Since the determinations of Ca_i^{2+} were always parallelled by measurements of PTH release from the quin-2-loaded cells of the same batch, it was possible to establish a relationship between Ca_i^{2+} and secretion. It is apparent from fig.3 that at Ca_i^{2+} values up to 200 nM there is a

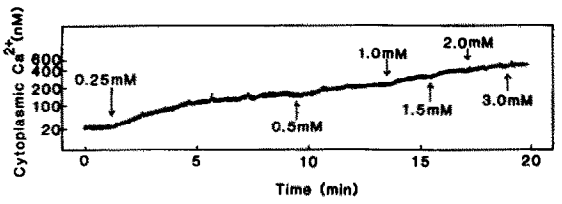

Fig.2. Effect of increasing concentrations of extracellular Ca^{2+} on Ca_i^{2+} of parathyroid cells. A typical fluorescence trace is shown with approximate Ca_i^{2+} values and extracellular Ca^{2+} concentrations indicated.

steep, strong, and highly significant positive correlation to PTH release, whereas the previously established negative relationship [1–7] was confirmed for concentrations exceeding 200 nM.

The dual actions of Ca_i^{2+} on PTH release are reminiscent of the effect of extracellular Ca^{2+} on glucose- and cAMP-stimulated insulin secretion [13]. When the voltage-dependent Ca^{2+} channels of the pancreatic β-cells are opened by glucose depolarization, insulin release increases steeply with extracellular Ca^{2+} up to 2.5 mM but is then gradually inhibited as the concentration rises to 15 mM. Also other secretory cells in which a rise of Ca_i^{2+} under physiological conditions results in stimulation of exocytosis seem to exhibit the inhibitory component. When electro-permeabilized secretory cells are exposed to Ca^{2+} concentrations above 10 μM, inhibition of secretion is thus often observed [14]. Against this background we should like to propose a bimodal regulation of secretion by Ca_i^{2+} with a stimulatory component more sensitive to Ca^{2+} than the inhibitory one. Since in the parathyroid cells, both components are considerably more sensitive to Ca^{2+} than in other secretory cells, it is not surprising that secretion is inhibited rather than stimulated when Ca_i^{2+} is raised within a similar physiological range of variation. Although the mechanism behind the increased sensitivity to Ca_i^{2+} in the parathyroid cells remains to be elucidated, it is noteworthy that activation of the cAMP- and Ca^{2+}-phospholipid-dependent protein kinases markedly sensitizes the insulin secretory machinery to calcium [13]. The demonstration of the inhibitory action of extracellular Ca^{2+} on the pancreatic β-cells was consequently facilitated by raising cAMP [13]. Moreover, it was recently shown that permeabilized parathyroid cells lose the characteristic Ca^{2+} inhibition of the release process [15,16], but that it can be restored by GTP analogues [16].

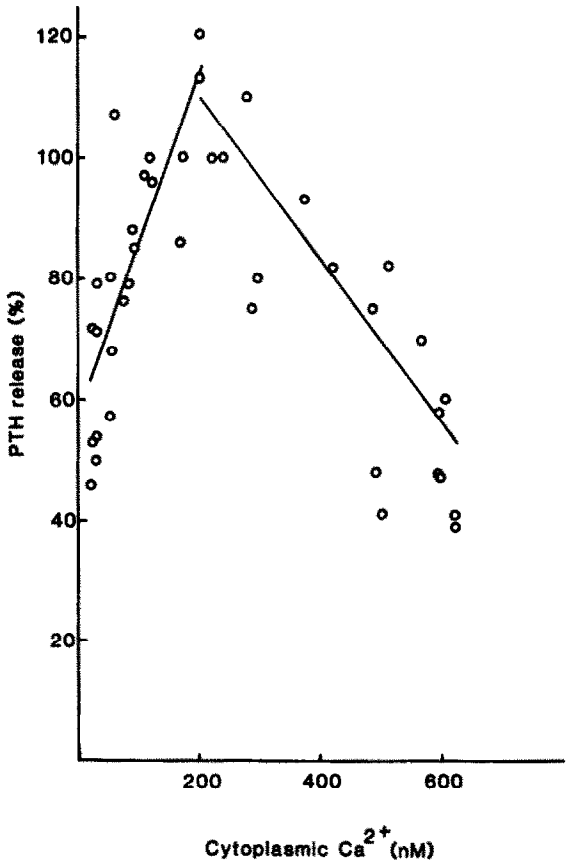

Fig.3. Relationship between Ca_i^{2+} and PTH release from quin-2 loaded parathyroid cells. PTH release is expressed as the percentage of secretion at 0.5 mM Ca^{2+}. Correlations between Ca_i^{2+} and PTH release were estimated below and above a Ca_i^{2+} value of 200 nM using the 2 observations at this limit for both calculations. The correlations obtained were strong and highly significant ($P < 0.001$) with r values of 0.81 and -0.88 and slopes of 0.29 and -0.14 below and above 200 nM respectively.

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