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

Peter Nygren, Rolf Larsson, Erik Lindh*, Sverker Ljunghall*, Jonas Rastad*, Göran Åkerström* and Erik Gylfe

Department of Medical Cell Biology, University of Uppsala, BMC Box 571, S-751 23 Uppsala and Departments of *Internal Medicine and *Surgery, University Hospital, S-751 85 Uppsala, Sweden

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Bovine parathyroid cells were used to study parathyroid hormone (PTH) release and the cytoplasmic Ca\(^{2+}\) concentration (Ca\(^{2+}\)). 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\(^{2+}\) within the 20–600 nM range. PTH release was found to increase with Ca\(^{2+}\) 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\(^{2+}\)) [1–7]. The parathyroid cell is consequently unusual among secretory cells in exhibiting an inverse relationship between Ca\(^{2+}\) 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\(^{2+}\) [8] it has now been investigated whether there is also a stimulatory component in the relation between Ca\(^{2+}\) and secretion in the parathyroid cells. It will be shown that PTH release increases with Ca\(^{2+}\) up to 200 nM but is gradually suppressed above this concentration. The discovery of dual actions of Ca\(^{2+}\) on PTH release may reflect a phenomenon of general importance for the understanding of the role of Ca\(^{2+}\) in secretion.

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 \times 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 \mu l/min. The PTH content of each cell
40 s fraction was determined radioimmunologically, using an assay detecting essentially the mid-C regional hormone [10]. To study Ca\(^{2+}\) 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 tetra-acetoxy methyl ester [7]. After loading and rinsing, 5 x 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\(^{2+}\) 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 x 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 perfusion 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 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\(^{2+}\) and PTH release we used quin-2 as both a buffer and indicator of Ca\(^{2+}\) [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\(^{2+}\) within the 20-600 nM range. The Ca\(^{2+}\)-buffering effect of quin-2 influenced Ca\(^{2+}\) 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\(^{2+}\) were always paralleled by measurements of PTH release from the quin-2-loaded cells of the same batch, it was possible to establish a relationship between Ca\(^{2+}\) and secretion. It is apparent from fig.3 that at Ca\(^{2+}\) values up to 200 nM there is a...
The dual actions of Ca\(^{2+}\) 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 \(\beta\)-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\(^{2+}\) 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 \(\mu\)M, inhibition of secretion is thus often observed [14]. Against this background we should like to propose a bimodal regulation of secretion by Ca\(^{2+}\) 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\(^{2+}\) is raised within a similar physiological range of variation. Although the mechanism behind the increased sensitivity to Ca\(^{2+}\) 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 \(\beta\)-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].

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