Cytosolic Ca^{2+} and the regulation of secretion in parathyroid cells

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The concentration of intracellular Ca^{2+} ($[Ca^{2+}]_i$) was measured in dissociated bovine parathyroid cells loaded with quin-2 or fura-2. In quin-2-loaded cells, increases in the concentration of extracellular Ca^{2+} elicited slow, monophasic increases in $[Ca^{2+}]_i$, whereas in fura-2-loaded cells, extracellular Ca^{2+} evoked rapid, transient increases which were followed by lower, yet sustained increases in $[Ca^{2+}]_i$. Cytosolic Ca^{2+} transients arose from the mobilization of cellular Ca^{2+} and could be evoked by a variety of divalent cations. Transient, but not sustained increases in $[Ca^{2+}]_i$ were associated with an inhibition of hormone secretion. Secretion was still inhibited, however, when cytosolic Ca^{2+} transients were blocked by buffering with quin-2, suggesting that changes in $[Ca^{2+}]_i$ might not be the essential factor regulating secretion in parathyroid cells.

(Parathyroid cell) *Secretion* *cytosolic Ca^{2+}* *Quin-2* *Fura-2* *Receptor*

1. INTRODUCTION

Calcium levels in blood and extracellular fluids are controlled primarily by alterations in the rate of secretion of parathyroid hormone (PTH). Secretion of PTH, in turn, is regulated by changes in the extracellular Ca^{2+} concentration, and it is now clear that increased concentrations of extracellular Ca^{2+} inhibit PTH secretion [1]. But it is far from certain how this extracellular Ca^{2+} signal is detected by the parathyroid cell and how this recognition event is subsequently transformed into an intracellular signal(s) which controls secretion. Previous studies using the fluorescent Ca^{2+} indicator quin-2 to assess the concentration of intracellular Ca^{2+} ($[Ca^{2+}]_i$) have reported an inverse relationship between $[Ca^{2+}]_i$ and secretion of PTH [2–4], suggesting that cytosolic Ca^{2+}, in contrast to its general activator role in other secretory cells [5,6], may act as an inhibitory intracellular signal in parathyroid cells.

Here, novel measurements of $[Ca^{2+}]_i$ in parathyroid cells provide some insight into how these cells might detect increases in the ambient Ca^{2+} concentration. These measurements reveal that an increase in the extracellular Ca^{2+} concentration elicits two different responses in parathyroid cells. One of these responses is associated with the generation or modulation of an intracellular signal(s) that regulates PTH secretion, although this signal may not be cytosolic Ca^{2+}.

2. EXPERIMENTAL

Dissociated bovine parathyroid cells were prepared by collagenase digestion of minced glands [4]. Following purification on Percoll gradients, the cells were washed and resuspended in a buffer which contained (mM): NaCl, 126; KCl, 4; $MgSO_4$, 1; KH_2PO_4/K_2HPO_4, 0.7; $CaCl_2$, 2; Na-Hepes, 20 (pH 7.45–7.47), and was supplemented with 1 mg/ml glucose and 1% bovine serum albumin. Cells were incubated (37°C) with the

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ester of quin-2 or fura-2 for 15 min, washed, and incubated for an additional 15 min in ester-free buffer. Indicator-loaded cells were then washed several times in buffer containing 0.5 mM $CaCl_2$ and equilibrated at 22°C for 10–15 min before use. The fluorescence of indicator-loaded cells was measured in a custom-built spectrofluorimeter equipped with a thermostatted cuvette holder and magnetic stirrer using excitation and emission wavelengths of 399 and 499 nm, respectively. Fluorescence signals were calibrated after correction for extracellular leakage of indicator as in [4] using K_d values for quin-2 and fura-2 of 115 and 224 nM, respectively.

For secretion studies, control or indicator-loaded cells were incubated for 20 min (37°C) in the presence or absence of test substance. Following incubation, the cells were rapidly sedimented by centrifugation and the amount of PTH in samples of the supernatant was determined by radioimmunoassay [4].

3. RESULTS AND DISCUSSION

Although quin-2 is a useful intracellular Ca^{2+} indicator, the high intracellular concentrations of this indicator that are necessary to obtain a fluorescence signal result in significant Ca^{2+} buffering within the cell and thereby possibly damp rapid, transient changes in $[Ca^{2+}]_i$ [7]. The fluorescent indicator fura-2 has approx. 30-times more Ca^{2+}-dependent fluorescence intensity than quin-2 [8], so much lower intracellular concentrations of indicator are required to obtain usable Ca^{2+}-dependent fluorescence signals. The different patterns of change in $[Ca^{2+}]_i$ reported by quin-2 and by fura-2 in parathyroid cells are patently obvious (fig.1). In dissociated bovine parathyroid cells loaded with quin-2, small increases (0.5 mM) in the extracellular Ca^{2+} concentration produced relatively slow, monophasic increases in $[Ca^{2+}]_i$ (fig.1, [4]). In cells loaded with fura-2, however, a rather different pattern emerged: rapid and transient increases in $[Ca^{2+}]_i$ followed by lower yet sustained increases in $[Ca^{2+}]_i$ (fig.1). Incubation with the ester of quin-2 routinely resulted in intracellular concentrations of 0.8–1 mM, whereas the intracellular concentrations of fura-2 varied between 75 and 150 μM. The different patterns of change in $[Ca^{2+}]_i$ seen in fura-2- and in

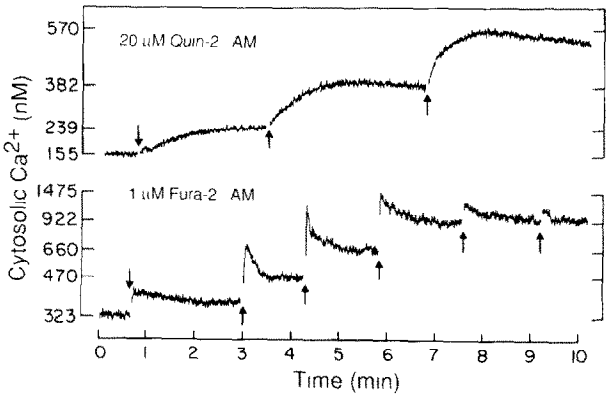

Fig.1. Effects of increases in the concentration of extracellular Ca^{2+} on $[Ca^{2+}]_i$ in parathyroid cells as reported by quin-2 (top) or fura-2 (bottom). Cells were equilibrated at 37°C in buffer containing 0.5 mM $CaCl_2$ and at each of the arrows the extracellular Ca^{2+} concentration was increased by 0.5 mM. Both traces were obtained from the same preparation of cells which was divided into aliquots and incubated with the indicated concentration of the acetoxymethyl ester (AM) of quin-2 or fura-2. The results shown for fura-2-loaded cells are representative of the pattern seen in each of 22 separate cell preparations.

quin-2-loaded cells appeared to result from the greater buffering capacity of quin-2 rather than from some intrinsic property of either indicator. Thus, cells loaded with both fura-2 and quin-2 showed only slow, monophasic increases in $[Ca^{2+}]_i$ in response to extracellular Ca^{2+}. Moreover, when cells were loaded with lower concentrations of quin-2 (about 200 μM intracellularly), rapid and transient increases in $[Ca^{2+}]_i$ were detected which mimicked those seen in fura-2-loaded cells [9]. Finally, steady-state levels of cytosolic Ca^{2+} were always higher in cells loaded with fura-2, a difference most marked at extracellular Ca^{2+} levels of 0.5 mM, where $[Ca^{2+}]_i$ was 180 ± 4 (n = 19) and 323 ± 6 nM (n = 13) in quin-2- and fura-2-loaded cells, respectively. These various pieces of evidence suggest that the concentrations of quin-2 necessary to measure cytosolic Ca^{2+} in fact result in the presence of a detrimental intracellular Ca^{2+} buffer, thereby decreasing or abolishing the rapid and transient increases in $[Ca^{2+}]_i$ elicited by increases in extracellular Ca^{2+}.

In a variety of secretory cells responding to their appropriate stimuli, receptor activation often

results in rapid cytosolic Ca^{2+} transients [10]. Therefore, the existence of similar transients in the parathyroid cell responding to its physiological stimulus suggests, as a working hypothesis, that there is a receptor on the surface of the cell which, when activated by extracellular Ca^{2+}, triggers transient increases in $[Ca^{2+}]_i$. Since some other divalent cations behave like extracellular Ca^{2+} and depress PTH secretion (see below), this putative receptor might not be all that selective for Ca^{2+}. Indeed, increases in the extracellular concentration of Mg^{2+} or the addition of Sr^{2+} or Ba^{2+}, all evoked rapid cytosolic Ca^{2+} transients akin to those evoked by Ca^{2+}. These other divalent cations, however, did not cause large steady-state increases in $[Ca^{2+}]_i$ like those obtained by increasing extracellular Ca^{2+} [11].

The failure of other divalent cations to elicit sustained increases in $[Ca^{2+}]_i$ suggests that extracellular Ca^{2+}, perhaps by influx across the plasma membrane, may contribute to steady-state Ca^{2+} levels in parathyroid cells. Consistent with this view was the ability of La^{3+} to affect differentially transient and sustained increases in $[Ca^{2+}]_i$ induced by extracellular Ca^{2+}. Pretreatment with La^{3+} (20 μM) depressed the sustained increase in $[Ca^{2+}]_i$ induced by extracellular Ca^{2+} without affecting the rapid cytosolic Ca^{2+} transient (fig.2). When La^{3+} was added to cells bathed in a high concentration of Ca^{2+}, $[Ca^{2+}]_i$ fell promptly to levels comparable to those seen in the presence of low extracellular Ca^{2+} (fig.2). The results suggest that La^{3+} acts in the parathyroid cell, as in many other cells [12], by blocking Ca^{2+} influx. Alternatively, or additionally, La^{3+} may act by displacing Ca^{2+} from specific binding sites on the cell surface [12] which are somehow coupled to the regulation of steady-state $[Ca^{2+}]_i$. In either case, it is clear that steady-state cytosolic Ca^{2+} levels are determined specifically by extracellular Ca^{2+}, whereas cytosolic Ca^{2+} transients can be elicited by various divalent cations independently of changes in extracellular Ca^{2+}.

Many receptor-dependent stimuli that elicit a rapid and transient increase in $[Ca^{2+}]_i$ generally do so by causing the mobilization of cellular Ca^{2+} [13]. Cytosolic Ca^{2+} transients in the parathyroid cell elicited by divalent cations are refractory to in-

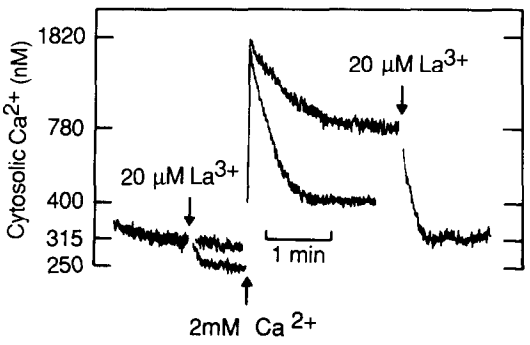

Fig.2. Preferential inhibitory effects of La^{3+} on steady-state $[Ca^{2+}]_i$ in fura-2-loaded parathyroid cells. Cells were initially equilibrated in phosphate-free buffer containing 0.5 mM $CaCl_2$. Two superimposed traces are shown. The trace in which La^{3+} was added prior to Ca^{2+} shows a lower steady-state $[Ca^{2+}]_i$ immediately following the cytosolic Ca^{2+} transient.

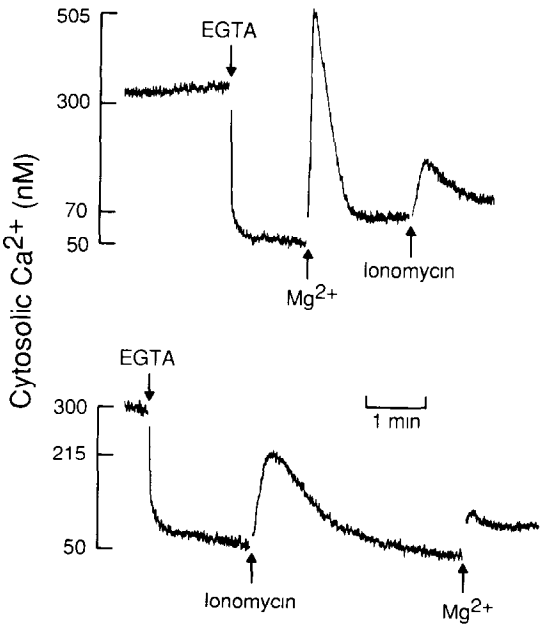

Fig.3. Mg^{2+}-induced cytosolic Ca^{2+} transients persist in the absence of extracellular Ca^{2+}. Fura-2-loaded cells were equilibrated in buffer containing 0.5 mM $CaCl_2$ before the addition of sufficient EGTA (0.67 mM) to reduce the extracellular Ca^{2+} to <1 μM. The subsequent addition of 7 mM $MgCl_2$ (to achieve a final concentration of 8 mM) elicited a rapid and transient increase in $[Ca^{2+}]_i$ (top). The addition of ionomycin (1 μM) also evoked a transient rise in $[Ca^{2+}]_i$ and inhibited the response to Mg^{2+} (bottom).

hibition by La^{3+} and likewise reflect rapid and transient mobilization of cellular Ca^{2+}. Thus, when extracellular Ca^{2+} was reduced to $<1\ \mu M$ by the addition of EGTA, increasing the concentration of Mg^{2+} (or adding Sr^{2+}, not shown) still evoked a transient 10-fold increase in $[Ca^{2+}]_i$ (fig.3). Pretreatment with ionomycin, to deplete cellular stores of Ca^{2+}, inhibited the Mg^{2+}-induced cytosolic Ca^{2+} transient by 91% (fig.3).

The use of fura-2 to monitor $[Ca^{2+}]_i$ thus provides a new view of parathyroid cell physiology which prompts a reconsideration of recent notions concerning cytosolic Ca^{2+} and PTH secretion [2,14,15]. It is now clear that increasing extracellular Ca^{2+} elicits two mechanistically distinct changes in $[Ca^{2+}]_i$ – and of concern is whether both or either of these changes are necessary to cause an inhibition of secretion. Of the various divalent cations examined, only Ca^{2+} caused a large, sustained increase in $[Ca^{2+}]_i$, yet all divalent cations tested inhibited secretion of PTH. Thus, increasing extracellular Ca^{2+} from 0.5 to 2 mM inhibited PTH secretion by 62.4 ± 2.6% (n = 3). Comparable degrees of inhibition were also obtained with 8 mM Mg^{2+} (65.7 ± 2.1%), 1 mM Ba^{2+} (53.9 ± 6.2%), or 4 mM Sr^{2+} (70.4 ± 9.2%). It therefore appears that steady-state increases in $[Ca^{2+}]_i$ are not necessary to cause inhibition of PTH secretion – a conclusion which we reached previously [4] and which now seems more secure.

The above findings render the cytosolic Ca^{2+} transient, or some mechanism associated with it, as a likely event leading to an inhibition of PTH secretion. To examine the possible role of these transients in regulating secretion, we took advantage of the ability of quin-2 to buffer, and thereby impair, cytosolic Ca^{2+} transients. In unloaded or fura-2-loaded cells, the addition of 8 mM Mg^{2+} inhibited PTH release by 58 and 56%, respectively, and, in the latter cells, evoked a transient, 8.5-fold increase in $[Ca^{2+}]_i$ (fig.4). Such Ca^{2+} transients were inhibited by 97% in cells loaded with quin-2, yet this buffering action failed to affect the inhibitory actions of Mg^{2+}, which depressed secretion by 56% (fig.4). This rather surprising observation provides some evidence for supposing that cytosolic Ca^{2+} per se may not be the intracellular signal acting to suppress secretion of PTH.

We believe that the results obtained with fura-2 furnish a tentative model for exploring the

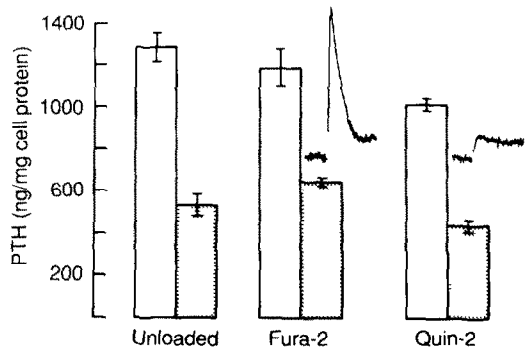

Fig.4. Inhibition of PTH secretion by Mg^{2+} in unloaded (control) or indicator-loaded parathyroid cells. Cells were loaded (or not) with quin-2 or fura-2 and resuspended in buffer containing 0.5 mM $CaCl_2$. Control and indicator-loaded cells were incubated in the presence (stippled columns) or absence (open columns) of 8 mM $MgCl_2$. Each value is the mean ± SE of 3 determinations. Accompanying the PTH release data for indicator-loaded cells are fluorescent traces obtained from the same cell preparations that show the corresponding effects of an increased extracellular Mg^{2+} concentration on $[Ca^{2+}]_i$.

mechanism of stimulus-secretion coupling in parathyroid cells. The model holds, in its initial and likely oversimplified form, that there exists a receptor on the surface of parathyroid cells which is promiscuous and responds to a variety of divalent cations. Activation of this receptor, perhaps by directly binding divalent cations, leads to the rapid mobilization of cellular Ca^{2+}, resulting in a transient increase in $[Ca^{2+}]_i$. Although such cytosolic Ca^{2+} transients are clearly associated with an inhibition of secretion, it is equally clear that secretion can be altered independently of transient (or sustained) increases in $[Ca^{2+}]_i$. The results point to the possibility that some other, as yet unidentified, intracellular signal(s) plays an important role in regulating secretion of PTH.

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REFERENCES

[1] Habener, J.F., Rosenblatt, M. and Potts, J.T. (1984) Physiol. Rev. 64, 985–1053.

[2] Shoback, D.M., Thatcher, J., Leombruno, R. and Brown, E.M. (1984) Proc. Natl. Acad. Sci. USA 81, 3113–3117.

[3] Larsson, R., Wallfelt, C., Abrahamson, H., Gylfe, E., Ljunghall, S., Rastad, J., Rorsman, P., Wide, L. and Akerstrom, G. (1984) Biosci. Rep. 4, 909–915.

[4] Nemeth, E.F., Wallace, J. and Scarpa, A. (1986) J. Biol. Chem. 261, 2668–2674.

[5] Douglas, W.W. (1974) Biochem. Soc. Symp. 39, 1–28.

[6] Rubin, R.P. (1982) Calcium and Cellular Secretion, Plenum, New York.

[7] Rink, T.J. and Pozzan, T. (1985) Cell Calcium 6, 133–144.

[8] Grynkiewicz, G., Poenie, M. and Tsien, R.Y. (1985) J. Biol. Chem. 260, 3440–3450.

[9] Nemeth, E.F. and Scarpa, A. (1986) Biophys. J. 49, 230a.

[10] Gill, D.L. (1985) Adv. Cyclic Nucleotide Protein Phosphorylation Res. 19, 307–324.

[11] Nemeth, E.F. and Scarpa, A. (1986) Endocrinology, in press.

[12] Weiss, G.B. (1974) Annu. Rev. Pharmacol. 14, 343–354.

[13] Berridge, M.J. and Irvine, R.F. (1984) Nature 312, 315–321.

[14] Brown, E.M., Leombruno, R., Thatcher, J. and Burrowes, M. (1985) Endocrinology 116, 1123–1132.

[15] LeBoff, M.S., Shoback, D., Brown, E.M., Thatcher, J., Leombruno, R., Beaudoin, D., Henry, M., Wilson, R., Pallotta, J., Marynick, S., Stock, J. and Leight, G. (1985) J. Clin. Invest. 75, 49–57.