Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 3113–3117, May 1984
Cell Biology

Relationship between parathyroid hormone secretion and cytosolic calcium concentration in dispersed bovine parathyroid cells

(extracellular calcium/hormonal secretion/second messengers)

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Communicated by R. H. Wasserman, January 23, 1984

ABSTRACT **The parathyroid cell is unusual among exocytotic systems in that low extracellular Ca^{2+} concentrations stimulate, while high Ca^{2+} concentrations inhibit, parathyroid hormone (PTH) release, suggesting that this cell might have unique secretory mechanisms. In the present studies, we used the Ca^{2+}-sensitive fluorescent dye QUIN-2 to examine the relationship between cytosolic Ca^{2+} concentration and PTH release in dispersed bovine parathyroid cells. The secretagogue dopamine, which enhances PTH release 2- to 3-fold in association with 20- to 30-fold increases in cellular cAMP, had no effect on the cytosolic Ca^{2+} level (261 ± 28 vs. 236 ± 22 nM for control cells at 1 mM extracellular Ca^{2+}; $P > 0.05$). Dibutyryl-cAMP, which produces a comparable stimulation of PTH release, likewise did not modify the level of cytosolic Ca^{2+}. Removal of extracellular Ca^{2+} produced a further decrease of the cytosolic Ca^{2+} to 82 ± 10 nM. However, PTH secretion persisted at a near maximal rate despite this decrease of extracellular and cytosolic Ca^{2+} and was 95 ± 2.5% of the rate of hormonal release at 0.5 mM extracellular Ca^{2+}. In contrast, addition of the divalent cation ionophore ionomycin to parathyroid cells at 1.0 mM extracellular Ca^{2+} inhibited PTH secretion in association with an increase in cytosolic Ca^{2+} from 230 ± 13 nM to 570 ± 50 nM. Moreover, the magnitude of the ionomycin-induced reduction in PTH secretion (64 ± 4% relative to the secretory rate at 0.5 mM Ca^{2+}) was equivalent to the inhibition of PTH release caused by 1.5 mM extracellular Ca^{2+} (64 ± 6%), which increased the cytosolic Ca^{2+} to similar levels (450 ± 48 nM). Thus, the parathyroid cell differs from secretory cells thought to operate by stimulus–secretion coupling in the following ways: changes in PTH release can occur without detectable alterations in the cytosolic Ca^{2+} concentration, maximal rates of PTH secretion occur at cytosolic Ca^{2+} concentrations that fail to support exocytosis in other cell types, and increases in the cytosolic Ca^{2+} concentration due to ionomycin inhibit rather than stimulate PTH release. Therefore, the control of PTH secretion by Ca^{2+} and other secretagogues may involve previously undefined mechanisms whereby hormonal release is relatively independent of the cytosolic Ca^{2+} at low levels of this parameter and is inversely related to cytosolic Ca^{2+} at higher levels of intracellular Ca^{2+}.**

The theory of stimulus–secretion coupling postulates that an increase in the cytosolic Ca^{2+} concentration underlies the activation of exocytosis in a variety of tissues (1, 2). The parathyroid gland is unusual among secretory systems in that parathyroid hormone (PTH) release is stimulated by low extracellular Ca^{2+} concentrations and inhibited by high extracellular Ca^{2+} concentrations (3). Moreover, we recently used the intracellular, Ca^{2+}-sensitive fluorescent dye QUIN-2 (4) to show that an increase in extracellular Ca^{2+} concentration from maximally stimulatory (0.5 mM) to maximally inhibitory levels (2.0 mM) is associated with an increase in calculated cytosolic Ca^{2+} concentration from 180 nM to 650 nM (5). This inverse relationship between PTH release and cytosolic Ca^{2+} suggested that the secretory process in the parathyroid cell might differ markedly from classical stimulus–secretion coupling. In the present studies, we used QUIN-2 to define further the unique properties of the parathyroid cell by demonstrating that (*i*) agents increasing intracellular cAMP stimulate secretion without any measurable change in cytosolic Ca^{2+}, (*ii*) PTH secretion persists despite decreasing the cytosolic Ca^{2+} concentration to levels (<100 nM) that do not support exocytosis in other systems, and (*iii*) high cytosolic Ca^{2+} concentrations *per se* inhibit hormonal release even at levels (>1 μM) that maximally stimulate exocytosis in other cells.

MATERIALS AND METHODS

Dispersed parathyroid cells were prepared from freshly minced parathyroid tissue from neonatal calves by digestion with DNase and collagenase as detailed (6). Trypan blue exclusion was routinely >95%. Parathyroid cells prepared in this fashion maintain high ratios of cellular potassium to cellular sodium (7) and have high levels of ATP (8). Moreover, PTH release is linear for several hours and is inhibited up to 80% by Ca^{2+} concentrations similar to those that inhibit PTH secretion *in vivo* (5). Cell preparations contained no visible fat cells by Sudan staining or in cytocentrifuge preparations.

Bovine parathyroid cells (20×10^6 cells per ml) were incubated with the acetoxymethyl ester of QUIN-2 (Lancaster Synthesis, Lancaster, England) at a final concentration of 10–20 μM in Eagle's minimal essential medium (Earle's salts with bicarbonate, Ca^{2+}, and Mg^{2+} deleted) supplemented with 0.02 M Hepes (adjusted to pH 7.47 with NaOH), 0.2% bovine serum albumin, 1.0 mM $CaCl_2$, and 0.5 $MgSO_4$ ("standard medium") at 37°C for 20 min (5). The cellular suspension was diluted 10-fold with standard medium and incubated an additional 20–60 min. The cellular pellet was washed three times with a saline solution containing 0.025 M Hepes (adjusted to pH 7.47 with NaOH), 125 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 0.5 mM $MgSO_4$, 1 gm of dextrose per liter, 1 mM Na_2HPO_4, and 0.1% bovine serum albumin ("saline"). The washed cellular pellet was resuspended in 3 ml of the standard medium with 0.1% bovine serum albumin for experiments with dopamine and dibutyryl-cAMP or in 3 ml of the above saline containing 0.05% bovine serum albumin for studies with ionomycin. In 33 experiments, cells loaded with 10–15 μM QUIN-2 acetoxymethyl ester had cellular QUIN-2 concentrations of 0.68 ± 0.07 mM.

The saline solution ($CaCl_2$, and $MgSO_4$ deleted) was first pretreated with Chelex-resin (Bio-Rad) and then supplemented with 0.5 mM $MgSO_4$ for use in experiments with subphysiologic Ca^{2+} concentrations. Total Ca^{2+} concentration

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Abbreviation: PTH, parathyroid hormone.

in Chelex-treated saline was ≤ 1 μM by atomic absorption spectrometry. The extracellular Ca^{2+} concentration in cellular suspensions was varied by the addition of 0.15 M $CaCl_2$. Direct determination of the Ca^{2+} concentrations in these solutions by atomic absorption spectrometry showed that they were within 3% of the calculated values.

The fluorescence of parathyroid cellular suspensions (7–10 $\times$ 10^6 cells per ml) was monitored in thermostatted cuvettes (37°C) in a Perkin–Elmer MPF3 spectrofluorimeter (excitation, 339 nm; emission, 492 nm). Cellular suspensions were mechanically stirred during all experiments except when recording was interrupted for 10–20 sec during the addition of dopamine, dibutyryl-cAMP, $CaCl_2$, or ionomycin and resuspension of cells with a plastic pipette. Fluorescence was monitored after the addition of secretagogues for 3–10 min or until the signal was stable as specified below. The calibration of fluorescence (F) signals at the end of a study was achieved by cellular lysis with the detergent Triton X-100 (0.06–0.12%) in Ca^{2+} concentrations ≥ 1 mM (F_{max}) and after addition of 10 mM EGTA and alkalinization with 1 M Tris base to render the pH > 8.3 (9) (F_{min}, free $Ca^{2+} \leq 1$ nM). Cytosolic Ca^{2+} was determined from the equation:

$$[Ca^{2+}] = 115 \text{ nM} \frac{(F - F_{min})}{(F_{max} - F)} \quad (4).$$

The dissociation constant for QUIN-2 used in this calculation (115 nM) assumes a cytosolic free-magnesium concentration that arbitrarily has been assigned a value of 1 mM (9). Corrections were made for any changes in the autofluorescence of unloaded parathyroid cells at the same cell concentration due to the additives used in each experiment. Autofluorescence was generally 20–30% of F_{max} and decreased by 10–20% after cellular lysis. Thus, changes in autofluorescence were only 2–5% of F_{max}. Less than 10% of the intracellular dye was lost during the course of these experiments, and loss of dye was unaffected by the extracellular Ca^{2+} concentration or the presence of ionomycin.

PTH release was assessed by incubation of QUIN-2-loaded cells (1–2 $\times$ 10^6 cells per ml) in 0.2–0.3 ml of saline or standard medium at various extracellular Ca^{2+} concentrations in parallel with fluorescence studies in 5-ml polypropylene scintillation vials (Sarstedt, Princeton, NJ) at 37°C for 10, 15, or 60 min in the presence or absence of secretagogues. In secretory experiments with ionomycin, cells were incubated directly in 0.3 ml of saline in 1-ml polypropylene microfuge tubes (Sarstedt, Princeton, NJ) at 37°C for 15 min. Supernatant samples (0.175 ml) for PTH determination were frozen after sedimentation of the cellular pellet at 250 $\times$ g for 2 min in a desk-top centrifuge (Sorvall GLC-2B). Radioimmunoassay for PTH was performed in duplicate on triplicate incubation vials as described (10) using an anti-bovine PTH antiserum raised in a sheep, GW-1, which recognizes intact hormone and COOH-terminal fragments of PTH. We (11) and others (12) have found that degradation of PTH after its release from the parathyroid cell cannot account for the effects of Ca^{2+} and other secretagogues on PTH release. Previous studies have indicated that dispersed bovine parathyroid cells loaded with QUIN-2 exclude trypan blue >95%, have cellular levels of ATP and potassium similar to control cells, and show secretory dynamics for PTH that are indistinguishable from those of control parathyroid cells (5).

Ionomycin was a generous gift from Timothy Rink. Stock solutions of ionomycin (2 mM) and QUIN-2 (10 mM) were stored at −20°C in anhydrous dimethyl sulfoxide. Dopamine and dibutyryl-cAMP were purchased from Sigma. Other reagents were of the best grade commercially available. Data are presented as means ± SEM, and statistical probability was assessed by the t test. Statistical significance was at the $P < 0.05$ level.

RESULTS

Responses of Cytosolic Ca^{2+} and PTH Release to Dopamine and Dibutyryl-cAMP in Dispersed Bovine Parathyroid Cells. Bovine parathyroid cells show a parallel increase in cellular cAMP and PTH release in response to dopamine (13). In addition, dibutyryl-cAMP also stimulates PTH release (14, 15), and dopamine augments cAMP-dependent protein kinase activity (10) and cellular phosphorylation (16), suggesting that the increase in secretory activity is related to cAMP-dependent mechanisms. To examine whether dopamine and dibutyryl-cAMP might modulate PTH release independently of changes in the cytosolic Ca^{2+} concentration, we determined the effects of these agents on cytosolic Ca^{2+} at doses (10 μM and 3 mM, respectively) that stimulate PTH release 2- to 3-fold in dispersed bovine parathyroid cells. Cellular fluorescence in QUIN-2-loaded cells was monitored initially for 5–10 min at 1.0 mM extracellular Ca^{2+}. Dopamine or dibutyryl-cAMP was then added, and recording was continued for an additional 10 min (see Fig. 1). Cytosolic Ca^{2+} at 1.0 mM extracellular Ca^{2+} was 236 ± 22 nM (n = 6) and did not change significantly after addition of dopamine (261 ± 28 nM; n = 6) or dibutyryl-cAMP (268 ± 32 nM; n = 4).* Neither dopamine nor dibutyryl-cAMP altered the autofluorescence of unloaded parathyroid cells or maximal fluorescence due to QUIN-2. Addition of 2.0 mM Ca^{2+} at the end of the experiment raised cytosolic Ca^{2+} to 600–700 nM (Fig. 1), comparable to the cytosolic Ca^{2+} concentration found in the absence of dopamine or dibutyryl-cAMP (5).

In contrast, PTH release from QUIN-2-loaded parathyroid cells incubated at 1.0 mM extracellular Ca^{2+} for 10 min was stimulated 2.6-fold by 10 μM dopamine (2.16 ± 0.27 vs. 0.83 ± 0.12 ng per 10^5 cells per 10 min; n = 12). In previous studies, 3 mM dibutyryl-cAMP stimulated PTH release 2.3-fold in dispersed bovine parathyroid cells (15); in the present studies, the stimulation of secretion in control and QUIN-2-loaded cells averaged 1.8-fold. Therefore, dopamine stimulates PTH release in the absence of any measurable change in cytosolic Ca^{2+} concentration and at levels of cytosolic Ca^{2+} (≈200 nM) that are near resting values in other exocytotic systems (17, 18).

Response of Cytosolic Ca^{2+} and PTH Release to Subphysiologic Extracellular Ca^{2+} Concentrations. To assess whether PTH release persists at even lower levels of cytosolic Ca^{2+}, we compared the effects of subphysiologic concentrations of extracellular Ca^{2+} on cytosolic Ca^{2+} and PTH release. Reducing extracellular Ca^{2+} to concentrations ≤ 1 μM with Chelex-treated solutions produced a decrement in cytosolic Ca^{2+} concentration of QUIN-2-loaded parathyroid cells to 82 ± 10 nM (n = 9), which remained stable at that level for at least 10 min. This value was significantly lower ($P < 0.001$) than that of parathyroid cells incubated at 0.5 mM extracellular Ca^{2+} (179 ± 8 nM; n = 13). PTH release from QUIN-2-loaded parathyroid cells incubated in parallel, on the other hand, was unchanged by the reduction in extracellular calcium (95 ± 2.5% of the value at 0.5 mM extracellular Ca^{2+}; a

*In several experiments, dibutyryl-cAMP was added to bovine parathyroid cells after prior exposure to dopamine or vice versa. Since the order of addition of these agents had no effect on cytosolic Ca^{2+}, data from both types of experiments were pooled for analysis. In most experiments, cell incubations with dopamine were carried out with standard medium because dopamine-stimulated secretion was greater in this solution than in saline solution. In an additional eight experiments, the effects of dopamine on cytosolic Ca^{2+} concentration were assessed in saline at 0.5 or 1.0 mM Ca^{2+}. This agent likewise had no effect on cytosolic Ca^{2+} concentration in this medium (188 ± 13 nM for control cells vs. 208 ± 20 nM for dopamine-treated cells).

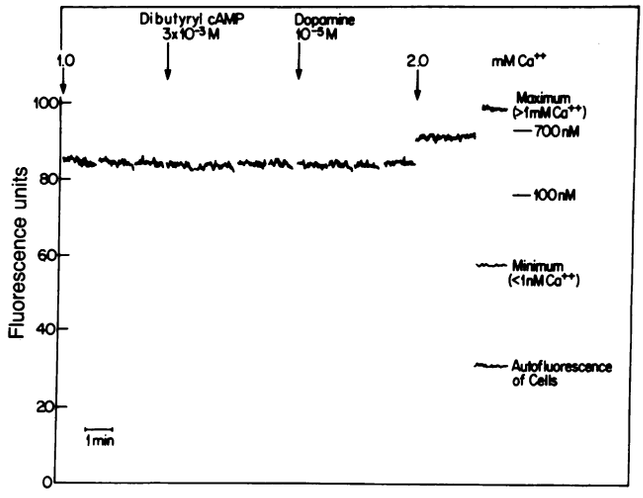

FIG. 1. Effect of dibutyryl-cAMP and dopamine on the fluorescence of QUIN-2-loaded parathyroid cells. After loading with the dye as described, a suspension of dispersed bovine parathyroid cells (7×10^6 cells per ml) was incubated initially in standard medium with 1.0 mM $CaCl_2$. When the fluorescence of this suspension had been stable for several minutes, 3 mM dibutyryl-cAMP was added at the point indicated by the arrow. Dopamine was subsequently added, and the fluorescence was monitored again for 10 min. At the end of the experiment, the cells were lysed with Triton X-100, and calibration was carried out as described. Maximal and minimal fluorescence of QUIN-2-loaded cells and the autofluorescence of unloaded cells from the same preparation of cells are shown on the right. The experiment shown is representative of six experiments testing the effects of dopamine and four for dibutyryl-cAMP on the fluorescence of QUIN-2-loaded cells.

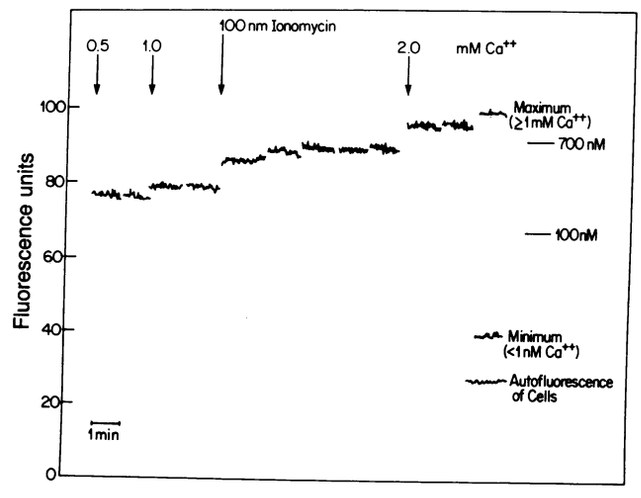

FIG. 2. Effect of ionomycin on the fluorescence of QUIN-2-loaded bovine parathyroid cells. Cellular loading with QUIN-2 was carried out as described in Fig. 1. Fluorescence was initially monitored in standard medium with 0.5 mM $CaCl_2$ for 5 min. The extracellular Ca^{2+} concentration was then increased to 1.0 mM, and cellular fluorescence was followed until stable. Ionomycin (100 nM) was subsequently added, and fluorescence was again monitored for 6–15 min. Finally the extracellular Ca^{2+} concentration was increased to 2.0 mM, and fluorescence was monitored for 5 min. The experiment was terminated by cellular lysis, and calibration was carried out as described in Fig. 1. The results shown are typical of those obtained from experiments in which the effects of ionomycin were examined at 1.0 mM (n = 12) and 2.0 mM (n = 10) extracellular Ca^{2+}.

composite of the effects of varying Ca^{2+} concentrations as well as the divalent cation ionophore ionomycin on cytosolic Ca^{2+} concentration and PTH release is shown in Fig. 3). Cells incubated with 0.1 mM Ca^{2+} and 0.5 mM Mg^{2+} also released PTH at a rate equivalent to those incubated with 0.5 mM Ca^{2+} (93.5% of the latter) and had a cytosolic Ca^{2+} level intermediate between that at <1 μM and 0.5 mM Ca^{2+} (135 ± 18 nM; n = 5). Although a PTH antiserum that recognizes COOH-terminal fragments of PTH as well as the intact hormone (10) was used to measure PTH in these secretory studies, we have demonstrated previously with HPLC (11) that release of intact bovine PTH-(1–84) persists unabated at subphysiologic Ca^{2+} concentrations.

Effects of Ionomycin on Cytosolic Ca^{2+} and PTH Release in Dispersed Bovine Parathyroid Cells. We previously found that increasing the extracellular Ca^{2+} from 0.5 to 2 mM elevates cytosolic Ca^{2+} concentration 3- to 4-fold in association with a 75–80% inhibition of PTH secretion (5)—the inverse of classical stimulus–secretion coupling. However, these studies did not establish whether there was a causal relationship between the changes in cytosolic Ca^{2+} concentration and PTH release. Moreover, it is possible that a further rise in cytosolic Ca^{2+} might stimulate secretion as in other exocytotic systems. In these studies, therefore, we utilized the divalent cation ionophore ionomycin to examine the effects on PTH release of raising cytosolic Ca^{2+} at a constant extracellular Ca^{2+} concentration.

A fluorescence tracing from a typical experiment using ionomycin is shown in Fig. 2. In all experiments, fluorescence was monitored for 6–15 min after the addition of ionomycin, and an average cytosolic Ca^{2+} over the period of observation was used for subsesquent analysis. Ionomycin in the concentrations used in these studies did not alter autofluorescence of unloaded parathyroid cells or maximal fluorescence due to QUIN-2.

At an extracellular Ca^{2+} level of 1 mM, 100 nM ionomycin increased the cytosolic Ca^{2+} of QUIN-2-loaded parathyroid cells from 230 ± 13 nM to 570 ± 50 nM (n = 12; $P < 0.001$) (Fig. 3). The value of cytosolic Ca^{2+} in the presence of ionomycin at 1 mM extracellular Ca^{2+} is comparable to the cytosolic Ca^{2+} concentrations observed at 1.5 and 2.0 mM extracellular Ca^{2+} (450 ± 48 and 646 ± 68 nM, respectively; n = 13). When the extracellular Ca^{2+} concentration in ionomycin-treated cells was increased to 2.0 mM, the mean cytosolic Ca^{2+} concentration increased further to greater than 1 μM, with 7 of 10 experiments yielding values between 1.3 μM and 2.4 μM (Fig. 3).

In parallel experiments, PTH release in QUIN-2-loaded parathyroid cells incubated with 100 nM ionomycin at 1 mM extracellular Ca^{2+} was inhibited 64 ± 4% relative to maximal secretory rates at 0.5 mM Ca^{2+} (Fig. 3). This degree of suppression was statistically greater than that observed with 1 mM extracellular Ca^{2+} alone (24 ± 3.7%; n = 12; $P < 0.01$). Moreover, the suppression of PTH release with ionomycin was equivalent to that achieved with 1.5 mM extracellular Ca^{2+} (64 ± 6%). Incubation of cells with 30 nM ionomycin at 1.0 mM Ca^{2+} increased cytosolic Ca^{2+} to a level slightly lower than that observed with 100 nM ionomycin (485 ± 40 nM; n = 5) and inhibited PTH release correspondingly less (53 ± 4%; n = 8) (not shown). In cells exposed to 2 mM extracellular Ca^{2+} and 100 nM ionomycin, PTH release was inhibited 81 ± 2% (n = 10), which was nearly identical to the degree of suppression at 2.0 mM extracellular Ca^{2+} without the ionophore (80 ± 2%; n = 12). PTH release from dispersed parathyroid cells was not affected by the concentrations of dimethyl sulfoxide used in these experiments (0.2–0.3%), and the effects of ionomycin on PTH release were fully reversible after washing the cells free of this agent. The data at 0.5, 1.0, 1.5, and 2.0 mM Ca^{2+} are in agreement with those we obtained previously (5) and are included for the purposes of comparison.

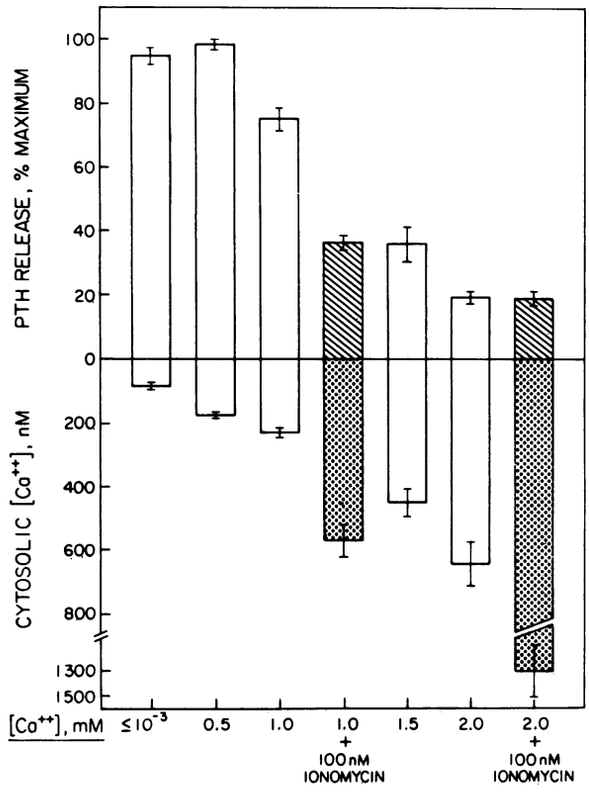

FIG. 3. Effects of varying extracellular Ca^{2+} concentrations and ionomycin on cytosolic Ca^{2+} concentration and PTH release in QUIN-2-loaded bovine parathyroid cells. (*Upper*) PTH release (mean ± SEM) was determined as described in *Materials and Methods* after incubation at 37°C for 15 min in experiments testing the effects of ionomycin (hatched bars) or for 60 min in experiments comparing PTH release at concentrations ≤ 1 μM and at 0.5 mM extracellular Ca^{2+} (open bars). PTH release is linear with time at 0.5 mM extracellular Ca^{2+} for up to 3 hr. Results for the two types of experiments were analyzed together and expressed as the percentage of maximal PTH release at 0.5 mM Ca^{2+} for the appropriate period of incubation. Effects of 100 nM ionomycin on PTH release were determined at 1.0 (n = 7) and 2.0 mM (n = 5) extracellular Ca^{2+}. (*Lower*) Cytosolic Ca^{2+} concentrations (nM, mean ± SEM) are shown at corresponding extracellular Ca^{2+} concentrations (open bars) as well as at 1.0 and 2.0 mM extracellular Ca^{2+} in the presence of 100 nM ionomycin (stippled bars).

DISCUSSION

Tsien, Rink, and co-workers have developed the Ca^{2+}-sensitive, fluorescent dye QUIN-2 as a probe of the intracellular Ca^{2+} concentration in suspensions of small cells (4, 9). After passive diffusion of the acetoxymethyl ester of QUIN-2 across the plasma membrane and hydrolysis of the ester by ubiquitous cellular esterases, the free tetracarboxylic dye is trapped intracellularly. The latter binds Ca^{2+} in 1:1 stoichiometry and undergoes a several-fold increase in fluorescence upon binding of this divalent cation. Estimates of the free intracellular Ca^{2+} concentration are made by calibrating the fluorescence of intact cells loaded with QUIN-2 with the Ca^{2+}-sensitive component of the fluorescence of the same cellular suspension after lysis (4, 9). Intracellular QUIN-2 is probably located predominantly in the cytosol (9), and values of intracellular Ca^{2+} obtained with QUIN-2 have been denoted as cytosolic free-Ca^{2+} concentration (4, 9). However, a contribution of other cellular pools of the dye to total cellular fluorescence has not been totally excluded.

The parathyroid cell appears to be very unusual in its relationship between the cytosolic Ca^{2+} concentration and PTH release. In our previous studies (5) we used QUIN-2 to show that increasing the extracellular Ca^{2+} concentration from 0.5 mM to 2 mM increases the cytosolic Ca^{2+} concentration from ≈200 nM to ≈600 nM in parallel with an inhibition of PTH secretion. In the present studies, we further defined the relationship between PTH release and cytosolic Ca^{2+} in the following ways. First, secretagogues thought to enhance PTH secretion by changes in cellular cAMP, such as dopamine, produce no significant increases in the cytosolic Ca^{2+} concentration. Second, levels of cytosolic Ca^{2+} that are not known to support exocytosis in other systems (17, 18) (<100 nM) promote maximal or near maximal rates of hormonal secretion. Third, increases in extracellular Ca^{2+} are associated with significant increases in cytosolic Ca^{2+} concentration, which are closely linked to an inhibition rather than stimulation of hormonal secretion. The effects of dopamine and/or increases in cellular cAMP on cytosolic Ca^{2+} in the parathyroid cell differ from those in other cells recently studied with QUIN-2 to measure cytosolic Ca^{2+} concentration. In pituitary cells, where dopamine may lower cellular cAMP (19), it prevents the thyrotropin-releasing hormone-induced rise in cytosolic Ca^{2+} (20), whereas in platelets, agents raising cAMP prevent agonist-induced increases in cytosolic Ca^{2+} (21, 22). The lack of effect of dopamine on cytosolic Ca^{2+} concentration in bovine parathyroid cells suggests that this agent stimulates PTH secretion through Ca^{2+}-independent pathways, presumably involving cAMP-dependent phosphorylation (16). We cannot rule out the possibility that dopamine increases the Ca^{2+} concentration in a small intracellular pool with an attendant increase in PTH release because of stimulus–secretion coupling. However, the divalent cation ionophore A23187 inhibits dopamine-stimulated PTH release (23), suggesting that the augmented PTH secretion due to dopamine is inhibited rather than stimulated by an increase in the cytosolic Ca^{2+} concentration. Rink *et al.* (18) have recently suggested that a "calcium-independent" form of secretion also occurs in platelets.

That PTH secretion is related to the cytosolic Ca^{2+} concentration in an unusual manner is also supported by the effects of very low extracellular Ca^{2+} concentrations on cytosolic Ca^{2+} concentration and PTH release. The former is reduced acutely by ≈2-fold to levels less than 100 nM, while the latter does not change significantly. The release of intact PTH(1-84), in fact, is stimulated slightly by these low extracellular Ca^{2+} concentrations (11). The "resting" cytosolic calcium concentration in many cell types is thought to be of the order of 100–200 nM (4, 9, 17–19, 20–22), and the activation of exocytosis is associated with several-fold increases in the cytosolic Ca^{2+} concentration (17, 18, 24). Thus, the secretory process in the parathyroid cell may involve secretory mechanisms that do not require the levels of cytosolic Ca^{2+} usually required to support exocytosis. It has been suggested that at least a portion of PTH release occurs via a form of "bypass" secretion (25, 26); the mechanistic and morphologic details of this form of secretion remain to be defined.

Previous indirect studies using divalent cation ionophores (23, 27, 28) and maneuvers putatively modifying sodium–calcium exchange in the parathyroid cell (7, 29) have suggested that high cytosolic Ca^{2+} concentrations inhibit PTH release. Moreover, we have recently found that the inhibition of PTH release by high extracellular Ca^{2+} concentrations is associated with a 3- to 4-fold rise in the cytosolic Ca^{2+} concentration (5). In the present studies, we have extended these observations to show that ionomycin-induced elevations in cytosolic Ca^{2+} concentration inhibit PTH release to a degree consistent with the inhibition of secretion by concentrations of extracellular Ca^{2+} that raise the cytosolic Ca^{2+} to a similar extent. These results suggest that high extracellular Ca^{2+} concentrations inhibit PTH release through the concomitant increase in the cytosolic Ca^{2+} concentration and that a change in the extracellular Ca^{2+} concentration *per se* is un-

necessary. These studies also show that using the ionophore to raise cytosolic Ca^{2+} levels to >1 μM likewise inhibits secretion. Thus, it is unlikely that high extracellular Ca^{2+} concentrations by themselves fail to achieve a sufficiently high cytosolic Ca^{2+} concentration to unmask a stimulatory effect on secretion of very high cytosolic Ca^{2+} concentrations.

In lymphocytes (9), platelets (unpublished observations), and adrenal glomerulosa and fasciculata cells (5), increases in the extracellular Ca^{2+} concentration within the physiologic range produce modest, if any, changes in the cytosolic Ca^{2+} concentration. Thus, the parathyroid cell not only shows an inverse relationship between PTH release and the cytosolic Ca^{2+} concentration but also possesses mechanisms by which small changes in the extracellular Ca^{2+} concentration produce large changes in cytosolic Ca^{2+} (5). These unusual properties are presumably essential to the normal physiological role of the parathyroid cell—to produce large changes in PTH release that are inversely related to small changes in the extracellular Ca^{2+} concentration. The mechanisms by which the extracellular Ca^{2+} concentration regulates the cytosolic Ca^{2+} concentration and the latter, in turn, modulates PTH secretion remain to be determined.

The authors gratefully acknowledge the contributions of Drs. Timothy Rink, Roger Tsien, Barton Holmquist, and B. L. Vallee to the completion of this study. Expert secretarial assistance was provided by Ms. Diane Rioux and Mrs. Nancy Orgill. E.M.B. is supported by Public Health Service Grants 2RO1 AM25910, 2RO1 AM30028, and 1KO1 AM00627 as well as by the Milton Fund.

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