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Thapsigargin Shifts the CA Set Point of Parathyroid Cells to Lower Extracellular [CA]

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The hypothesis that cytosolic calcium concentration ($[Ca^{2+}_{cyt}]$) is the primary regulator of parathyroid hormone (PTH) secretion is supported by a number of studies that show an inverse relationship between them. One agent shown to inhibit PTH secretion is thapsigargin, a sesquiterpene lactone that raises $[Ca^{2+}_{cyt}]$ by inhibiting the Ca-ATPase that pumps Ca^{2+} from the cytosol into the lumen of the endoplasmic reticulum. Thapsigargin may act on the parathyroid cell other than to inhibit the Ca-ATPase, however, in ways that might also affect PTH secretion. We have tested its effects on functional parameters, such as protein synthesis, the exocytic machinery, and the ability of parathyroid cells to respond to different concentrations of extracellular Ca^{2+} ($[Ca^{2+}_{ex}]$). In particular, we have determined whether the inhibition of PTH secretion by thapsigargin is independent of or is modulated by changes in $[Ca^{2+}_{ex}]$. The results revealed no effects of thapsigargin on protein synthesis or the exocytic mechanisms within 2 h of treatment, and showed that $[Ca^{2+}_{ex}]$ can modulate PTH secretion in the presence of thapsigargin. Its inhibition of PTH secretion, therefore, appears to rest on its ability to shift $[Ca^{2+}_{cyt}]$ to higher levels, but the possibility that it interacts with the Ca receptor has not been eliminated. The results support the hypothesis that the primary regulator of steady-state PTH secretion is $[Ca^{2+}_{cyt}]$.

Key Words: Parathyroid; PTH; calcium; thapsigargin; secretion.

Introduction

Parathyroid hormone (PTH), through its calcemic actions in bone and kidney, maintains the concentration of ionized calcium in the extracellular fluid ($[Ca^{2+}_{ex}]$) at physiological levels. In turn, the principal regulator of PTH secretion from the parathyroid glands is $[Ca^{2+}_{ex}]$. At low $[Ca^{2+}_{ex}]$, parathyroid glands secrete at a maximal rate; secretion is minimal at normo- or hypercalcemia. The secretory rate is a continuous, sigmoidal function of $[Ca^{2+}_{ex}]$, and that concentration wherein secretion is halfway between the maximum and minimum rates has been defined as the calcium "set point" *(1)*. In addition to the steady-state secretory control by $[Ca^{2+}_{ex}]$, β-adrenergic agonists, such as dopamine or isoproterenol, acutely stimulate *(2,3)* and α-adrenergic agonists inhibit exocytosis of PTH *(4)*.

Increases or decreases in $[Ca^{2+}_{ex}]$ are reflected in corresponding increases and decreases in the concentration of cytoplasmic ionized calcium ($[Ca^{2+}_{cyt}]$) in parathyroid cells *(5)*. Both are inversely related to the rate of PTH release *(6)*. In addition, the ionophores A23187 and ionomycin inhibit PTH secretion *(7)*; calcium channel agonists inhibit PTH release, whereas channel antagonists stimulate release *(8)*. These findings support the hypothesis that $[Ca^{2+}_{cyt}]$ is a major second messenger for changes in $[Ca^{2+}_{ex}]$, and is an important intracellular regulator of PTH secretion *(9)*. Increases in $[Ca^{2+}_{ex}]$ activate the phosphatidylinositol phosphate transduction pathway *(10,11)*, an effect that is consistent with the existence of an extracellular calcium receptor, such as those recently cloned *(12,13)*.

Shoback et al. *(14)* used thapsigargin to provide further evidence that the major intracellular regulator of PTH secretion is $[Ca^{2+}_{cyt}]$. Thapsigargin is a sesquiterpene lactone that increases $[Ca^{2+}_{cyt}]$ by inhibiting the Ca^{2+}-ATPase of the endoplasmic reticulum *(15,16)*, and thereby blocks the ATP-dependent transport of Ca^{2+} from the cytoplasm to the lumen of the endoplasmic reticulum. In short incubations of parathyroid cells, Shoback et al. *(14)* showed that thapsigargin increased $[Ca^{2+}_{cyt}]$ and inhibited the secretory rate of PTH in a dose–responsive manner without altering the cellular levels of inositol phosphates *(14)*. They did not, however, investigate whether thapsigargin might have inhibited PTH secretion by means of an inhibition of protein synthesis as shown to occur in other cell types by Wong et al. *(17)* or perhaps by inhibition of cAMP-sensitive exocytosis.

In the present study, we have examined whether there might be mechanisms alternative to that of increased $[Ca^{2+}_{cyt}]$ by which thapsigargin could inhibit secretion.

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Among the possibilities are that thapsigargin inhibits PTH synthesis or transport through the cell, its exocytosis, or the ability of the parathyroid cell to sense $[Ca^{2+}_{ex}]$. Our results are consistent with the hypothesis that during 1- to 2-h experiments, thapsigargin's inhibition of PTH secretion is owing to its ability to increase $[Ca^{2+}_{cyt}]$, although the possibility that thapsigargin may interact with the extracellular Ca receptor cannot be excluded. In concert with the expectation that thapsigargin should increase $[Ca^{2+}_{cyt}]$ and inhibit PTH secretion to a degree determined by both its own concentration and that of the $[Ca^{2+}_{ex}]$ at which studies are performed, we found that the inhibition of PTH secretion by thapsigargin can be overcome by decreasing $[Ca^{2+}_{ex}]$. When the entire range of $[Ca^{2+}_{ex}]$ from near zero to 2 m*M* was examined, the results showed that thapsigargin shifts the Ca set point for PTH secretion to lower $[Ca^{2+}_{ex}]$.

Results

Effects of Ca and Thapsigargin on $[Ca^{2+}_{cyt}]$

To determine whether thapsigargin raised $[Ca^{2+}_{cyt}]$ in our adult bovine parathyroid cells, freshly isolated cells were loaded with Indo-1 AM as described in Materials and Methods. The Indo-1-loaded cells (6×10^5) were placed in a cuvet containing 3 mL of Ca-free Hank's Balanced Salt Solution (HBSS), placed in the fluorimeter and brought to 37°C. After baseline recording, additions of Ca, thapsigargin, or other agents were made. With $[Ca^{2+}_{ex}]$ nominally zero, basal $[Ca^{2+}_{cyt}]$ was 170 n*M*. When $[Ca^{2+}_{ex}]$ was raised to 0.5 m*M*, $[Ca^{2+}_{cyt}]$ increased to 370 n*M*. When thapsigargin at a final concentration of 1 μ*M* was added to the cells at 0.5 m*M* Ca, the $[Ca^{2+}_{cyt}]$ rose to 575 n*M*. The addition of 7.5 μ*M* ionomycin increased $[Ca^{2+}_{cyt}]$ above our maximum detection limit. All changes in $[Ca^{2+}_{cyt}]$, regardless of cause, occurred within 30 s. The results established that at a $[Ca^{2+}_{ex}]$ of 0–0.5 m*M*, $[Ca^{2+}_{cyt}]$ was increased by addition of Ca, 1–5 μ*M* thapsigargin, or ionomycin. The presence of Indo-1 in the cells did not affect the ability of $[Ca^{2+}_{ex}]$ to regulate the secretion of PTH, as determined by radioimmunoassay (RIA) (data not shown).

Effects of Thapsigargin on Secretion

Bovine parathyroid cells were incubated at 0.7 or 2.5 m*M* Ca in the presence or absence of 1 μ*M* thapsigargin. After 2-, 4-, or 20-h incubations, the cells were sedimented, and samples of the media removed. PTH secretion was estimated by RIA of the media. Figure 1 shows that secretion of PTH at 2.5 m*M* Ca was only 25–30% of that at 0.7 m*M* Ca. When 1 μ*M* thapsigargin was present at 0.7 m*M* Ca, PTH secretion was inhibited to the same extent as by 2.5 m*M* Ca. Secretion was linear for 4 h, and slowed somewhat thereafter. The effects of thapsigargin and of Ca were preserved throughout 20 h of incubation.

To determine the rate of onset of the inhibitory effect of thapsigargin, shorter incubations of 15 and 30 min were performed. The results showed that at 0.7 mM Ca, thapsigargin inhibited secretion within 15 min (control = 4.32 ± 0.6, thapsigargin = 2.61 ± 0.2 ng PTH/10^6 cells; $n = 6$/group, $p < 0.025$). The degree of inhibition was the same as that by 2.5 m*M* Ca (2.0 ± 0.06 ng PTH/10^6 cells), and thapsigargin did not further inhibit the secretion of PTH at 2.5 m*M* Ca.

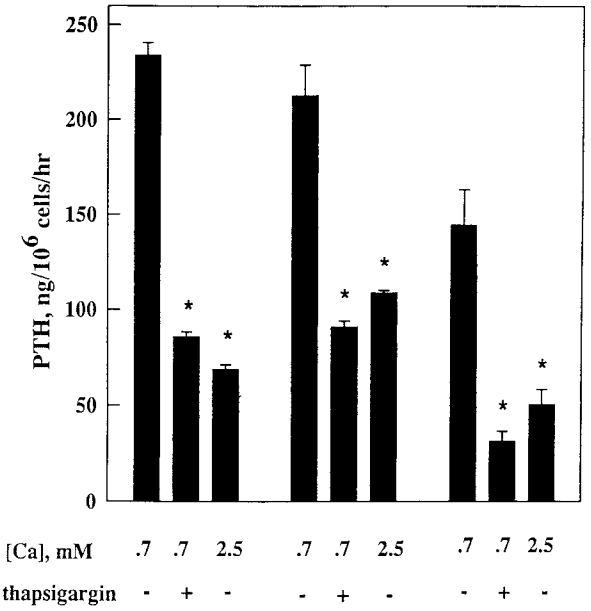

Fig. 1. Effect of 1 μ*M* thapsigargin on PTH secretion. 2×10^6 cells/mL were incubated at 37°C at 0.7 or 2.5 m*M* Ca for 2 h (left), 4 h (center), and 20 h (right). The cells were sedimented, and samples of the media were assayed for immunoreactive PTH. Data in this and all following figures are presented as mean ± SE ($n = 5$/group; $^*p < 0.01$ vs the controls at 0.7 m*M* Ca).

To determine whether thapsigargin inhibited PTH secretion by reducing the cellular levels of PTH, cells were incubated at 37°C for 2 h at 0.7 m*M* Ca in the presence of none, 0.1, 0.33, and 1.0 μ*M* thapsigargin, and with no thapsigargin at 2.5 m*M* Ca. After sedimentation of the cells, media were removed, and cell pellets were homogenized with electrophoresis sample buffer. Portions of each extract were diluted, and RIA for PTH was performed. The results showed no effect of either thapsigargin or 2.5 m*M* Ca on the cellular content of PTH. In the order listed above, the cellular contents of PTH in μg/10^6 cells were: 13.0 ± 0.4, 14.6 ± 1.5, 13.2 ± 2.2, 15.6 ± 0.6, and 15.9 ± 1.4; $n = 6$ per group, no significant differences.

Effect of Thapsigargin on Protein Synthesis

Most previous reports concluded that thapsigargin did not alter the synthetic rate of proteins in the various cell types tested, but Wong et al. *(17)* reported that thapsigargin induced extensive inhibition of protein synthesis (within 10 min) in GH_3 pituitary cells and HepG2 cells. In order to clarify this question in parathyroid cells, we examined short-term protein synthesis. Cells were preincubated for 1.5 h at 0.7 and 2.5 m*M* Ca, with or without 1 μ*M* thapsigargin,

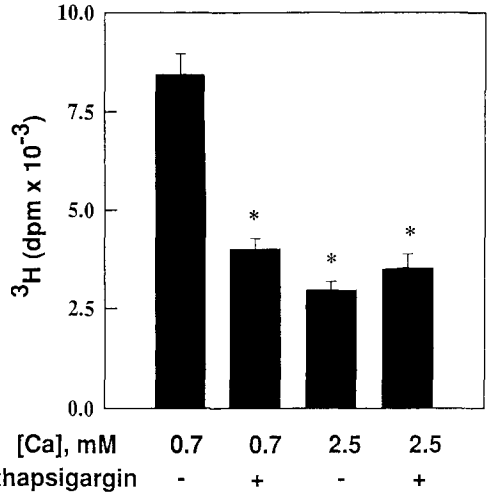

Fig. 2. Effect of 1 μ*M* thapsigargin on release of newly synthesized protein. 2×10^6 cells/mL were incubated at 37°C for 2 h in the presence or absence of thapsigargin. 50 μCi/mL of [^{3}H]leucine were added to each sample, and the incubations were continued for an additional 3 h. The cells were sedimented; samples of the media were precipitated by trichloroacetic acid, and the acid-insoluble radioactivity was determined. (n = 6/group; *$p < 0.01$ vs the controls at 0.7 m*M* Ca). There was no effect of thapsigargin on release of ^{3}H at 2.5 m*M* Ca.

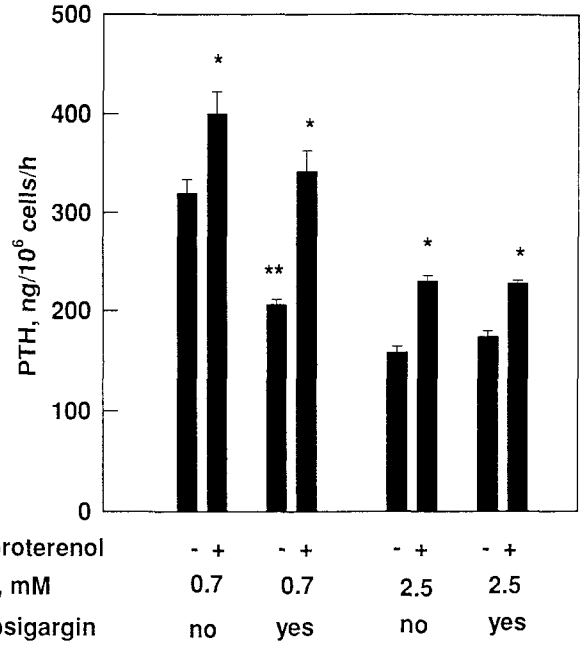

Fig. 3. The failure of thapsigargin to inhibit isoproterenol-stimulated PTH secretion. Samples of 1×10^6 cells/mL were incubated at 37°C for 1 h in the presence or absence of 1 μ*M* thapsigargin, 10^{-6} *M* isoproterenol, and 0.7 or 2.5 m*M* Ca. The cells were sedimented, and media were radioimmunoassayed for PTH. (n = 5/condition; *$p < 0.01$ vs respective control without isoproterenol; **$p < 0.01$ vs control without thapsigargin).

and then [^{3}H]leucine was added to the media for 15 min. The cell pellets were homogenized in urea/HCl/cysteine, and then proteins were precipitated. Radioactivity in the newly synthesized proteins was then determined. Under the conditions used, there was no significant effect of thapsigargin on the incorporation of [^{3}H]leucine into trichloroacetic acid-precipitable proteins at either [Ca]. At 0.7 m*M* Ca, control = 7.1 ± 0.3, and thapsigargin = 6.8 ± 0.3 cpm × 10^3. At 2.5 m*M* Ca, control = 5.9 ± 0.3, and thapsigargin = 5.7 ± 0.45 cpm × 10^3. RIA of the media following the 15-min incubations confirmed that at 0.7 m*M* Ca, thapsigargin inhibited secretion of immunoreactive PTH to the same degree as did incubation at 2.5 m*M* Ca, i.e., by 71 and 74% respectively, compared to the 0.7 m*M* Ca control incubations.

To determine whether thapsigargin altered the synthesis of any particular subset of proteins in parathyroid cells, cells were preincubated at 0.7 m*M* Ca and 2.5 m*M* Ca, with and without 1 μ*M* thapsigargin for 1.5 h, and then incubated with [^{35}S]methionine for 15 min. The cells were sedimented, hot electrophoresis sample buffer was added to the pellets, and then they were homogenized, and processed for SDS-PAGE and autoradiography. Observation of the autoradiograms revealed no selective effect of either Ca or thapsigargin on the radioactivity in any protein band (data not shown).

To determine whether thapsigargin, like high [Ca^{2+}_{ex}], would inhibit the secretion of newly synthesized proteins, cells were preincubated for 2 h in the presence or absence of thapsigargin at 0.7 and 2.5 m*M* Ca. The cells were then incubated with 50 μCi/mL of [^{3}H]leucine for an additional 3 h. The cells were pelleted, and protein in the media was precipitated with trichloroacetic acid. The amounts of secreted, acid-insoluble radioactivity were measured by scintillation counting. At 0.7 m*M* Ca, secretion of acid-insoluble ^{3}H from parathyroid cells was two to three times greater than it was from cells incubated either with thapsigargin or at 2.5 m*M* Ca (Fig. 2). The results showed that thapsigargin inhibited the release of newly synthesized protein in the same way as did 2.5 m*M* Ca, and to the same degree as it did that of immunoactive PTH.

Effect of Thapsigargin on Exocytosis

The possibility was considered that thapsigargin inhibited PTH secretion by directly disabling the mechanism for exocytosis of secretory granules. To test this, we determined whether thapsigargin would inhibit β-adrenergic stimulation of acute PTH secretion. We used isoproterenol, a potent secretagog for the regulated secretory pathway in parathyroid cells *(18)*. Parathyroid cells were incubated for 1 h at 0.7 or 2.5 m*M* Ca in the presence or absence of isoproterenol and of thapsigargin. Cells were then sedimented, and samples of the media were assayed for PTH by RIA. The results showed that thapsigargin inhibited PTH secretion at 0.7 m*M* Ca, but did not prevent the surge of exocytosis induced by isoproterenol at either 0.7 or 2.5 m*M* Ca (Fig. 3). Thus, thapsigargin did not inhibit PTH by disabling the exocytotic mechanism.

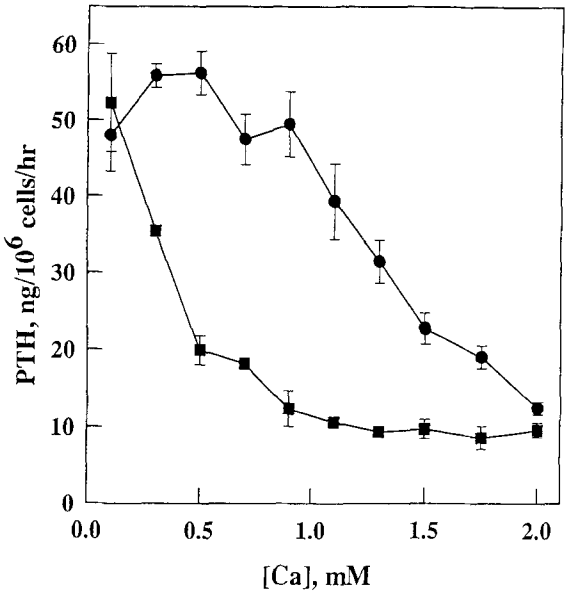

Fig. 4. The effects of 5 μ*M* thapsigargin on PTH secretion at different [Ca]. 2×10^6 cells/mL were incubated for 2 h, and media were assayed for immunoreactive PTH. Data are presented as means ± SE ($n = 6$/data point). Data points without error bars have SE smaller than the data point. ●, Control incubations; ■, thapsigargin.

Effects of Thapsigargin on the Sensitivity of Parathyroid Cells to [Ca^{2+}_{ex}]

In order to determine whether thapsigargin interfered with the ability of parathyroid cells to respond to different [Ca^{2+}_{ex}], cells were incubated at numerous [Ca] between 0.1 and 2.5 m*M* in the presence or absence of thapsigargin. The cells were incubated for 2 h, and samples of the media were measured for PTH by RIA. Figure 4 shows the results of one experiment that demonstrates the ability of parathyroid cells to respond to different [Ca^{2+}_{ex}] in the presence of 5 μ*M* thapsigargin. The effect of thapsigargin was to lower the [Ca^{2+}_{ex}] at which inhibition of PTH secretion occurred. The [Ca] at which PTH secretion was at the midpoint of its maximum and minimum rates (i.e., the Ca set points for PTH secretion) was between 1.1 and 1.25 m*M* in the absence of thapsigargin. In the presence of thapsigargin, the Ca set point and the entire secretory response curve were shifted to lower [Ca].

In some experiments, the maximal and minimal rates of PTH secretion at the lowest and highest [Ca^{2+}_{ex}], respectively, were decreased by thapsigargin. When data were expressed as ratios of PTH secretion at any [Ca^{2+}_{ex}] to that at the Ca set point, however, the relative increases or decreases over set point were the same; this indicated that the decreased secretion was not owing to an effect on the ability of the cells to respond to different [Ca^{2+}_{ex}]. The reason for this overall inhibition of secretion is not known, but the results are consistent with the proposal that after several hours of treatment with thapsigargin, the rate of protein production decreases. This was supported during our experiments on the effects of thapsigargin on protein synthesis. We observed 20–25% decreases in the amount of [^{3}H]-labeled protein in cells incubated with thapsigargin for 4 h (data not shown).

Discussion

We conducted experiments to address possible alternatives regarding how and under what circumstances thapsigargin inhibited the secretion of PTH. We used RIA to measure PTH cellular content, and observed that thapsigargin did not cause any change in PTH levels. To determine whether thapsigargin decreased the rate of protein synthesis in parathyroid cells, we examined the rates of incorporation of ^{3}H-leucine or ^{35}S-methionine. Analysis of either the total amounts of radioactivity incorporated into acid-insoluble fractions or the relative incorporation of radioactive amino acids into individual protein bands following SDS-PAGE indicated that thapsigargin did not significantly inhibit protein synthesis during short incubations. When longer periods of amino acid incorporation were examined, however, variability in the effects of thapsigargin on incorporation of [^{3}H]leucine was observed. In some experiments, significant decreases in amino acid incorporation were observed after incubations of 2 or 4 h, whereas other experiments showed no significant change. The variability may be accounted for in part by the known effect of high [Ca] (and thereby high [Ca^{2+}_{cyt}]) to increase the rate of intracellular degradation of unsecreted PTH *(19)*. In addition, variable degrees of depletion of Ca from the lumen of the endoplasmic reticulum may have generated uncontrolled effects on synthesis and processing of PTH and other secreted proteins. A23187 and thapsigargin eliminate the Ca gradients required to maintain the structure of the endoplasmic reticulum, resulting in vesiculation of the endoplasmic reticulum and inhibition of protein transport *(20,21)*. It is also possible that removal of Ca from Ca binding chaperone proteins, like calreticulin and calnexin, may interfere with the folding or transport of proteins that reside inside or in the membrane of secretory vesicles *(22)*.

Ca-regulated PTH secretion may follow a constitutive-like, as opposed to a classic regulated secretory pathway *(18)*. Ca does not appear to regulate exocytosis, since isoproterenol stimulates PTH release independently of [Ca^{2+}_{ex}] *(23)*. Instead, Ca^{2+} controls by unknown means the percentage of secretory vesicles that reach the plasma membrane from the *trans*-Golgi network. If thapsigargin were functioning uniquely to increase [Ca^{2+}_{cyt}], therefore, it should not inhibit exocytosis. Our present results support this hypothesis in that thapsigargin inhibited steady-state PTH release, but had no discernible effect either on the cellular content of PTH or on the exocytosis of PTH elicited by isoproterenol, at either high or low [Ca] (Fig. 3).

The experiment shown in Fig. 4 demonstrated that thapsigargin did not interfere with the cell's ability to sense or to respond to changes in $[Ca^{2+}_{ex}]$. The most likely explanation for the effects of thapsigargin on the sensitivity of parathyroid cells to $[Ca^{2+}_{ex}]$ is that it increased the level of $[Ca^{2+}_{cyt}]$ by an amount determined by its concentration inside the cell, the degree of inhibition of the Ca^{2+}-ATPases responsible for pumping Ca from the cytosol into the lumen of the endoplasmic reticulum, and $[Ca^{2+}_{ex}]$. The increased $[Ca^{2+}_{cyt}]$ at any given $[Ca^{2+}_{ex}]$ is most likely responsible for the shift of the PTH secretion vs $[Ca^{2+}_{ex}]$ curve to the left, although it is also possible that thapsigargin modulated the sensitivity of the extracellular Ca receptor to sense $[Ca^{2+}_{ex}]$. The Ca set point studies were important to the ability to distinguish a primary and direct effect of thapsigargin on the secretory apparatus from its effect on $[Ca^{2+}_{cyt}]$. The observation that thapsigargin did not inhibit PTH secretion by disturbing the secretory machinery or the pathway of secretory vesicles is supported by the fact that in many set point experiments that were performed, the maximum secretory rate of PTH at about 0.1 m*M* Ca was not reduced by thapsigargin.

The finding that thapsigargin shifted the secretory set point for Ca to lower $[Ca^{2+}_{ex}]$ would be predicted for an agent that specifically inhibits the Ca^{2+}-ATPases of the endoplasmic reticulum. Although perhaps not important to the resting $[Ca^{2+}_{cyt}]$ of most cells, the endoplasmic reticulum and its Ca^{2+}-ATPases may be important determinants of $[Ca^{2+}_{cyt}]$ in parathyroid cells, since their resting $[Ca^{2+}_{cyt}]$ are higher than those of most cells of the body *(5,14)*. The results of others have shown that physiological agents capable of modulating endoplasmic reticulum Ca^{2+}-ATPases could also potentially serve as second messengers capable of modulating $[Ca^{2+}_{cyt}]$ *(24)*. One can thus speculate that in some cases in which the Ca set point of patients with abnormal parathyroid function has been increased, the cause might be increased activity of the Ca^{2+}-ATPases of the endoplasmic reticulum.

Materials and Methods

Chemicals

Collagenase I, purified collagenase (CLSPA grade), and elastase were obtained from Worthington Biochemical (Lakewood, NJ). Thapsigargin was purchased from Calbiochem (San Diego, CA). Indo-1 AM and the penta-potassium salt of Indo-1 were purchased from Molecular Probes, Inc. (Eugene, OR). RPMI-1640 Select Amine Kit was purchased from Gibco Labs (Grand Island, NY). All materials for gel electrophoresis were purchased from Bio-Rad (Richmond, CA). Scintiverse II scintillation cocktail was purchased from Fisher Chemical (Fairlawn, NJ). HBSS, ionomycin and other chemicals were purchased from Sigma Chemical (St. Louis, MO). [^{3}H]leucine was purchased from American Radiolabeled Chem., Inc. (St. Louis, MO). $Na^{125}I$ and [^{35}S]methionine were purchased from Dupont-NEN (Boston, MA).

Cell Preparation

Parathyroid glands were obtained from a slaughterhouse and were brought to the laboratory on ice. The glands were trimmed of extraneous tissues, sliced, and digested. We followed a digestion procedure *(25,26)* using elastase (100 μg/mL) and bacterial collagenase (400–600 U/mL) previously subjected to Amicon PM30 filtration membrane to remove impurities with $M_r < 30{,}000$. In some cases, purified CLSPA collagenase was used instead of the cruder preparation. The cells were placed in medium containing 1.5 m*M* Ca prior to experiments. In this article, [Ca] is used to denote total extracellular Ca, whereas $[Ca^{2+}_{ex}]$ denotes ionized Ca. A concentrated solution of $CaCl_2$ was added to Ca-free culture medium to attain the required Ca concentrations, and previous examination of the media with Ca^{2+}-sensing electrodes indicated that in our incubation media, $[Ca^{2+}_{ex}]$ is approx 85% that of [Ca] (unpublished results).

Examination of Cytosolic Calcium

Our procedure was adapted and modified from those of Owens *(27)* and Williams and Fay *(28)*. Briefly, 3×10^6 cells/mL were placed in culture medium not containing phenol red at 1 m*M* Ca. The cells were oxygenated and Indo-1 AM was added at a final concentration of 5 μ*M*. The cells were incubated for 30 min at room temperature with gentle agitation, and then washed by sedimentation at least three times in HBSS containing 1 m*M* Ca (no phenol red) to remove the excess Indo-1. The final cell pellet was resuspended in HBSS. Fluorimetric examination for $[Ca^{2+}_{cyt}]$ was performed using an Alphascan fluorimeter (Photon Technology Int., Inc., South Brunswick, NJ). To obtain a calcium standard curve that could be translated into $[Ca^{2+}_{cyt}]$, we followed the procedure of Williams and Fay *(28)*. The excitation wavelength was 455 nm, and the emission wavelengths set at 410 and 490 nm. Indo-1 shifts its emission maximum from 490 nm (nominally Ca-free medium) to 410 nm when the dye is saturated with calcium *(29)*. The concentration of Ca within the cells $[Ca^{2+}_{cyt}]$ was derived by comparing the ratios of fluorescence emissions at 410–490 nm of cell suspensions to those of the Ca standards.

Measurements of Synthesis and Secretion of Newly Synthesized Proteins

To compare rates of amino acid incorporation into newly synthesized proteins, fresh parathyroid cell suspensions were preincubated at 37°C in medium containing either 0.7 or 2.5 m*M* Ca in the presence or absence of 1 μ*M* thapsigargin. Leucine concentration was 10% of that normally present in the medium. [^{3}H]leucine was then added, and incubation continued for 15-min periods. The labeled cells were sedimented, and then homogenized in 8 *M* urea/0.2 *M* HCl/0.1 *M* cysteine. Radioactive proteins were precipitated with

10% trichloroacetic acid. Precipitates were solubilized with NaOH. Several cycles of precipitation and solubilization were performed. Radioactivity was measured in samples of the final NaOH extracts using a Packard 1900 CA liquid scintillation spectrometer (Downer's Grove, IL).

Gel Electrophoresis of Newly Synthesized Proteins

[^{35}S]methionine was used to examine electrophoretic patterns of newly synthesized proteins. In this case, preincubation media contained 10% of the normal content of methionine to ensure optimal isotope uptake and linear rates of incorporation. Following incubations with [^{35}S]methionine, cells were sedimented, dissolved in hot electrophoresis sample buffer (15% mercaptoethanol, 0.4% SDS), and then subjected to SDS-PAGE *(30)* using 12.5% polyacrylamide gels and a minigel apparatus (Bio-Rad Labs, Hercules, CA). Following electrophoresis, the gels were fixed in acetic acid and methanol, and then rinsed by several changes of distilled water. The gels were dried, and autoradiography was performed at –70°C using Kodak XOMAT XAR-5 (Sigma Chemical) X-ray film.

RIA of PTH Secretion

Immunoactive PTH in incubation media and cell extracts was estimated using goat antibovine PTH antibody 1811 at a dilution of 1:70,000 as previously described *(25)*.

Presentation of Data

All data are presented as mean ± SE. The statistical significance was obtained using the Student's *t*-test.

References

1. Brown, E. M., Gardner, D. G., Brennan, M. F., Marx, S. J., Spiegel, A. M., Attie, M. F., et al. (1977). *Am. J. Med.* **66,** 923–931.
2. Brown, E. M., Watson, E. J., Leombruno, R., and Underwood, R. H. (1983). *Metabolism* **32,** 1038–1044.
3. Chu, L. L. H., MacGregor, R. R., and Hamilton, J. W. (1983). *Mol. Cell. Endocrinol.* **33,** 157–168.
4. Brown, E. M., Hurwitz, S. H., and Aurbach, G. D. (1978). *Endocrinology* **103,** 893–899.
5. Shoback, D., Thatcher, J., Leombruno, R., and Brown, E. (1983). *Endocrinology* **113,** 424–426.
6. Shoback, D. M., Thatcher, J., Leombruno, R., and Brown, E. M. (1984). *Proc. Natl. Acad. Sci. USA* **81,** 3113–3117.
7. Brown, E. M., Gardner, D. G., and Aurbach, G. D. (1980). *Endocrinology* **106,** 133–138.
8. Fitzpatrick, L. A., Brandi, M. L., and Aurbach, G. D. (1986). *Biochem. Biophys. Res. Commun.* **138,** 960–965.
9. Pocotte, S. L., Ehrenstein, G., and Fitzpatrick, L. A. (1991). *Endocr. Rev.* **12,** 291–301.
10. Brown, E., Enyedi, P., LeBoff, M., Rotberg, J., Preston, J., and Chen, C. (1987). *FEBS Lett.* **218,** 113–118.
11. Epstein, P. A., Prentki, M., and Attie, M. F. (1985). *FEBS Lett.* **188,** 141–144.
12. Brown, E. M., Gamba, G., Riccardi, D., Lombardi, M., Butters, R., Kifor, O., et al. (1993). *Nature* **366,** 575–580.
13. Racke, F. K., Hammerland, L. G., Dubyak, G. R., and Nemeth, E. F. (1993). *FEBS Lett.* **333,** 132–136.
14. Shoback, D. M., Chen, T. H., Pratt, S., and Lattyak, B. (1995). *J. Bone Miner Res.* **10,** 743–750.
15. Jackson, T. R., Patterson, S. I., Thastrup, O., and Hanley, M. R. (1988) *Biochem. J.* **253,** 81–86.
16. Thastrup, O., Cullen, P. J., Drobak, B. K., Hanley, M. R., and Dawson, A. P. (1990). *Proc. Natl. Acad. Sci. USA* **87,** 2466–2470.
17. Wong, W. L., Brostrom, M. A., Kuznetsov, G., Gmitter-Yellen, D., and Brostrom, C. O. (1993). *Biochem. J.* **289,** 71–79.
18. Muresan, Z. and MacGregor, R. R. (1994). *Mol. Biol. Cell* **5,** 725–737.
19. Cohn, D. V. and MacGregor, R. R. (1981). *Endocrinol. Rev.* **1,** 1–26.
20. Lodish, H. F. and Kong, N. (1990). *J. Biol. Chem.* **265,** 10,893–10,899.
21. Sambrook, J. F. (1990). *Cell* **61,** 197–199.
22. Hebert, D. N., Foellmer, B., and Helenius, A. (1996). *EMBO J.* **15,** 2961–2968.
23. Morrissey, J. J. and Cohn, D. V. (1979). *J. Cell Biol.* **82,** 93–102.
24. Camacho, P. and Lechleiter, J. D. (1993). Science **260,** 226–229.
25. MacGregor, R. R., Sarras, Jr., M. P., Houle, A., and Cohn, D. V. (1983). *Mol. Cell. Endocrinol.* **30,** 313–328.
26. Ridgeway, R. D. and MacGregor, R. R. (1988). *Am. J. Physiol.* **254,** E279–286.
27. Owens, C. S. (1988). *Cell Calcium* **9,** 141–147.
28. Williams, D. A. and Fay, F. S. (1990). *Cell Calcium* **11,** 75–83.
29. Grynkiewicz, G., Poenie, M., and Tsien, R. Y. (1985). *J. Biol. Chem.* **260,** 3440–3450.
30. Laemmli, U. K. (1970). *Nature* **227,** 680–685.