Parathyroid hormone secretion in the absence of extracellular free Ca^{2+} and transmembrane Ca^{2+} influx

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The involvement of extracellular Ca^{2+} and Ca^{2+} influx across the plasma membrane in parathyroid hormone (PTH) secretion was investigated in vitro using a new preparation of bovine parathyroid cells. Incubation of these cells in the presence of 25 μM or 2.5 μM free ambient Ca^{2+} induced a maximal rate of PTH secretion. Low free Ca^{2+} secretion is not associated with changes in membrane permeability, requires metabolic energy, and is reversible. The Ca^{2+} channel blocker D600 had no effect on either ^{45}Ca-influx or PTH secretion in these cells. These results, showing that extracellular Ca^{2+} and Ca^{2+} influx across the plasma membrane are not required for PTH secretion by parathyroid cells, emphasize the differences in the cellular mechanisms underlying the secretion of PTH *vs* that of other secretory cells.

Calcium channels *Calcium influx* *Cell isolation* *Exocytosis*
Parathyroid cell *PTH secretion*

1. INTRODUCTION

At variance with many endocrine glands, PTH secretion from parathyroid glands has an unusual dependence on extracellular Ca^{2+}: lowering the ambient $[Ca^{2+}]$ within the physiological range causes an increase in secretion of PTH both in vivo [1] and in vitro [2,3]. The mechanism underlying this response is unknown, and may be distinct from the secretory process occurring in most other endocrine cells, which requires Ca^{2+} influx and elevated cytosolic Ca^{2+} concentrations to trigger exocytosis [4,5].

The involvement of extracellular Ca^{2+} in PTH secretion was investigated in this communication using a newly developed preparation of bovine parathyroid cells with enhanced purity, intactness, and viability [2]. Experimental protocols leading to a dramatic decrease in secretion from other endocrine tissues and cells, such as the absence of added Ca^{2+} in the suspending medium [6–8] or the presence of Ca^{2+}-blocking agents [9–11], did not inhibit the stimulation of PTH secretion in isolated parathyroid cells. These findings, unique to parathyroid cells, are further indication that the stimulus–secretion coupling process underlying PTH release is largely different from that common to other secretory cells.

2. MATERIALS AND METHODS

2.1. *Cell isolation procedure*

Freshly isolated bovine parathyroid cells were prepared as in [2].

2.2. *Cell buffers*

The standard cell buffer contained 141 mM

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Abbreviations: PTH, parathyroid hormone; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethane sulfonic acid; RIA, radioimmunoassay; NaCN, sodium cyanide; BSA, bovine serum albumin; EGTA, ethylene glycol-bis(β-aminoethyl ether)*N,N'*-tetraacetic acid

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NaCl, 5.3 mM KCl, 20 mM Hepes (pH 7.47), 5 mM glucose, 0.8 mM $MgSO_4$, 2 mM $CaCl_2$, and 1% BSA. True concentrations of ionized Ca^{2+} were measured with a Ca^{2+} electrode, as in [12]. An ionized Ca^{2+} level of 25 μM was present in buffer containing 1% BSA and no added Ca^{2+}. When 100 μM Na_2EGTA was added, 2.5 μM free Ca^{2+} was present.

2.3. *Assays*

(1) Measurement of bovine PTH and cellular protein were as in [2]. 'Basal secretion' was defined as PTH released in buffer containing 2 mM free Ca^{2+}, and 'low calcium-stimulated secretion' as that occurring in 0.8 mM free Ca^{2+}.

(2) Cellular K^+ was measured by atomic absorption spectroscopy, as in [2].

(3) ^{45}Ca-uptake was initiated by resuspending centrifuged parathyroid cells in fresh media at 37°C containing $^{45}Ca^{2+}$ (2 μCi/μmol), with other additions noted in text. Aliquots (100 μl) of incubating cells were withdrawn and diluted into 6 ml ice-cold medium (with 5 mM Na_2EGTA, without $CaCl_2$ or BSA), filtered onto glass fiber filters (Schleicher and Schuell no.25), then washed with 9 ml of medium. The dried filters were assayed for $^{45}Ca^{2+}$ content, and uptake was expressed as nmol Ca^{2+}/mg cell protein.

3. RESULTS AND DISCUSSION

The inverse relationship between PTH release and physiological free $[Ca^{2+}]$ in isolated parathyroid cells was as in [2]; in fig.1A the secretory response in extended ranges of ambient Ca^{2+} is shown. At medium $[Ca^{2+}]$ comparable to those occurring during hypercalcemia in vivo, basal PTH secretion persisted as the secretory response became insensitive to further increases in extracellular Ca^{2+}. As the external $[Ca^{2+}]$ was reduced to 0.4–0.6 mM and below, the stimulated rate of PTH release reached a maximum and plateaued. When parathyroid cells were exposed to buffers containing either 2.5 μM or 25 μM free Ca^{2+}, maximal stimulation of PTH secretion was

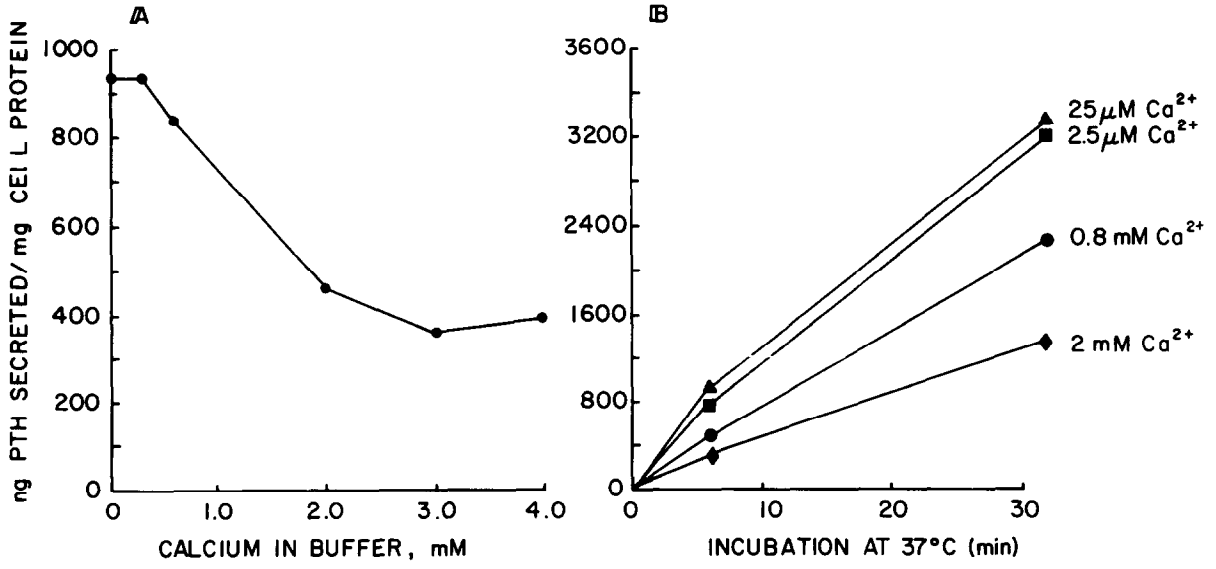

Fig.1. Dependence of PTH secretion on an extended range of ambient free $[Ca^{2+}]$. In fig.1A,B, cellular incubations were initiated by resuspending parathyroid cells in buffers which contained varying ionized $[Ca^{2+}]$ between 25 μM and 4 mM, as determined by a Ca^{2+} electrode (section 2). (A) PTH release as a function of the ambient $[Ca^{2+}]$ after 10 min incubation at 37°C. The experiment was stopped by centrifugation of the cell suspensions, and the cell-free supernatants were stored frozen for subsequent PTH RIA. (B) Rates of PTH secretion in buffers containing 2.5 μM, 25 μM, 0.8 mM and 2 mM free Ca^{2+}. Media samples were removed after 10 and 30 min incubation, as in (A).

unabated and could be detected within 5 min of incubation (fig.1B). The stimulation of PTH secretion by a very low extracellular [Ca^{2+}] contrasts with findings in many other endocrine cells [6–8], and is viewed as a first indication that extracellular Ca^{2+} influx is not involved in triggering PTH release.

Secretion of PTH in 25 μM Ca^{2+} is functionally similar to that occurring in 0.8 mM Ca^{2+}, and hormone release under either condition probably occurs through the same energy-dependent exocytotic mechanism. Fig.2B shows that PTH secretion in either 25 μM or 0.8 mM Ca^{2+} was inhibited by ~80% when parathyroid cells were suspended in glucose-free buffers containing 2 mM NaCN, and could be partially restored by readdition of glucose. These results show that secretion from parathyroid cells in buffers containing 25 μM Ca^{2+} has an apparent dependence on intracellular ATP, and can be supported by either glycolysis or oxidative phosphorylation, similar to other endocrine glands [4,13].

Fig.2A shows that the secretory rate stimulated by 25 μM Ca^{2+} can be reversed to basal levels by addition of Mn^{2+}, a potent suppressor of PTH release in this system [2]. The percent inhibition of PTH secretion caused by either 0.75 or 1.5 mM Mn^{2+} was comparable for parathyroid cells suspended in buffers containing either 25 μM or 0.8 mM Ca^{2+}. These results show that PTH secretion can be turned off, as well as on, in the virtual absence of external Ca^{2+}, and emphasize the functional similarity of PTH secretion in 25 μM Ca^{2+} to that occurring in 0.8 mM Ca^{2+}.

Stimulus–secretion coupling in a number of secretory cells is mediated through membrane depolarization [14–16], and Ca^{2+} channel antagonists inhibit secretion by preventing Ca^{2+} influx through voltage-sensitive Ca^{2+} channels [10–11]. Fig.4 shows that 50 μM D600 did not af-

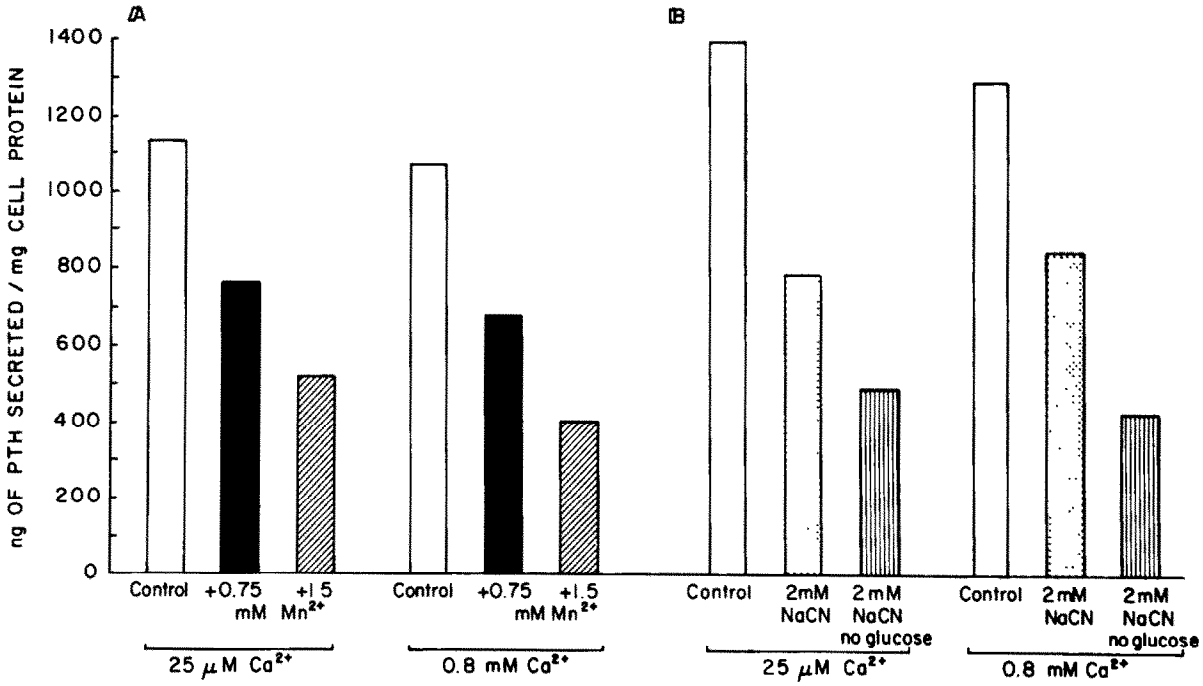

Fig.2. Comparison of PTH release in 25 μM and 0.8 mM Ca^{2+}: effects of Mn^{2+} or NaCN. The inhibitory effects on PTH release of either Mn^{2+} (A) or NaCN (B) were examined. Cellular incubations were initiated by resuspending parathyroid cells in buffers containing either 25 μM or 0.8 mM free Ca^{2+}. Control cell suspensions received no other additions. Experimental suspensions contained: (A) in addition either 0.75 or 1.5 mM Mn^{2+}; (B) either 2 mM NaCN or 2 mM NaCN without glucose. After 15 min cellular incubation, media aliquots were removed and treated as in fig.1.

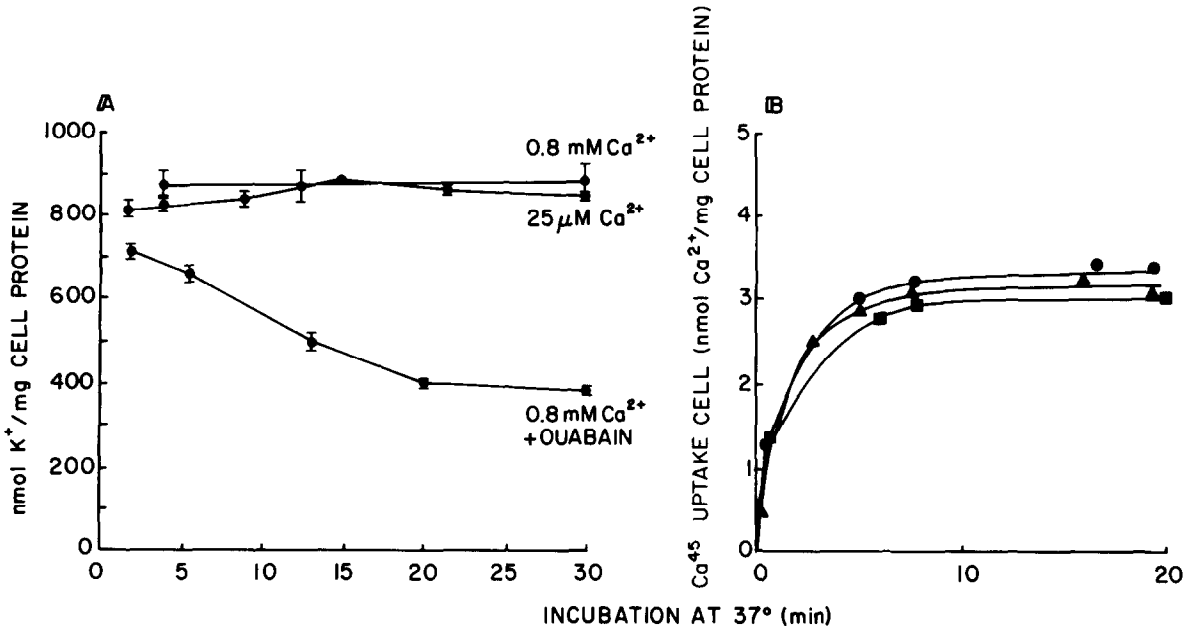

Fig.3. Measurements of cell membrane permeability changes during and after exposure to 25 μM Ca^{2+} buffer. (A) The experiment was initiated by resuspending parathyroid cells in buffers containing either 25 μM Ca^{2+}, 0.8 mM Ca^{2+}, or 0.8 mM Ca^{2+} with 100 μM ouabain. Duplicate 50 μl aliquots were withdrawn at successive time intervals for K^+ determination (section 2) and data points are expressed ± SEM. Corrections for K^+ in the extracellular space were made as in [2]. (B) Parathyroid cells were preincubated at 37°C for 30 min in buffers containing either 25 μM Ca^{2+} (▲), 0.8 mM Ca^{2+} (●), or 2.0 mM Ca^{2+} (■). The 3 cell suspensions were then centrifuged and their supernatants aspirated. The graph shows their subsequent ^{45}Ca-uptake in 2 mM Ca^{2+}-buffer. The experiment was initiated by separately resuspending each pellet in 700 μl 2 mM Ca^{2+}-buffer containing ^{45}Ca (2 μCi/μmol). Aliquots (100 μl) were processed at successive time intervals for ^{45}Ca content (section 2).

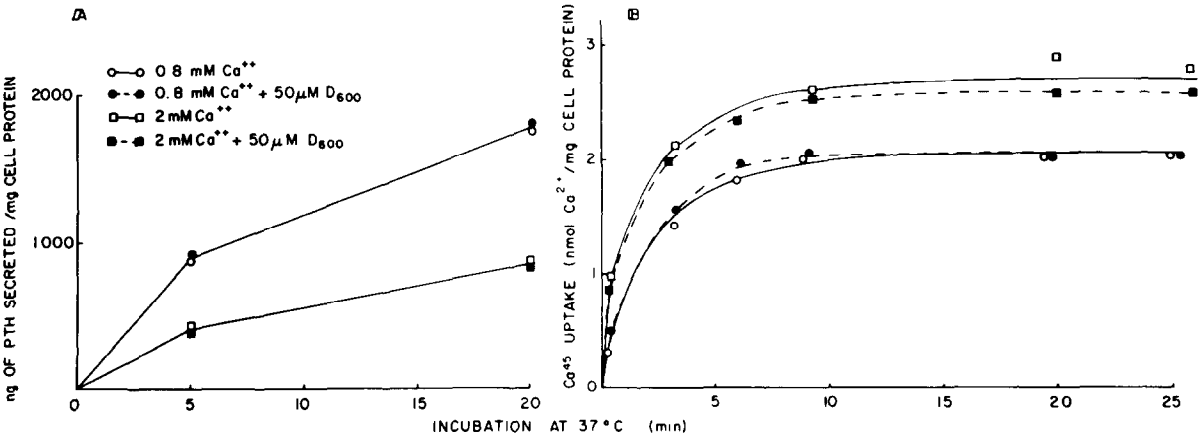

Fig.4. Effect of D600 on PTH secretion and Ca^{45} uptake. (A) Rates of basal and low Ca^{2+}-stimulated PTH secretion in the presence and absence of 50 μM D600. The experiment was initiated by resuspending centrifuged parathyroid cells in either 0.8 mM Ca^{2+} (○), 0.8 mM Ca^{2+} and 50 μM D600 (●), 2.0 mM Ca^{2+} (□), or 2.0 mM Ca^{2+} and 50 μM D600 (■). After 20 min cellular incubation, media aliquots were withdrawn and treated as in fig.1. (B) ^{45}Ca-uptake for cells suspended in the same 4 buffers, each containing ^{45}Ca (2 μCi/μmol). ^{45}Ca uptake was initiated by resuspending centrifuged parathyroid cells (2 mg cell protein/tube) in 400 μl of each of the 4 buffers. During a 25 min incubation at 37°C, 100 μl aliquots were removed and processed for ^{45}Ca content (section 2).

fect either basal or low calcium-stimulated PTH secretion, or the rates of ^{45}Ca-uptake into parathyroid cells. Electrophysiological measurements showing that the low calcium stimulus causes hyperpolarization of the parathyroid cell membrane [17,18] are compatible with the observation that D600 does not affect PTH secretion, and provide further evidence that the mechanism of PTH secretion does not involve the operation of potential-dependent Ca^{2+} channels.

Damage to cell membranes caused by exposure to low Ca^{2+} is generally evidenced by non-specific increases in the plasma membrane permeability to K^+ and to Ca^{2+} [19,20]. For secretory cells, such membrane damage may result in hormone release which occurs through non-physiological leakage rather than exocytosis. Fig.3A shows that parathyroid cells in 25 μM Ca^{2+} maintained their intracellular K^+ contents at the same value as control cells in 0.8 mM Ca^{2+} during a 30 min incubation, in contrast to the rapid loss of intracellular K^+ from cells incubated in the presence of ouabain. These results, demonstrating the lasting integrity of the parathyroid cell membrane in buffers virtually devoid of Ca^{2+}, make the possibility of hormone leakage unlikely.

Incubation of endocrine cells and tissues in Ca^{2+}-free buffer increased the membrane permeability of mast cells [21], pancreatic β cells [22], and adrenal chromaffin cells [7] to Ca^{2+}, resulting in a massive stimulation of hormone release when physiological [Ca^{2+}] were reintroduced. Using a similar experimental protocol (fig.3B), parathyroid cells were preincubated for 30 min in 25 μM, 0.8 mM, or 2 mM Ca^{2+}, and subsequently each group was resuspended in 2 mM Ca^{2+} containing a trace of ^{45}Ca. In the 3 cell suspensions, the subsequent rates of ^{45}Ca-uptake and/or exchange were identical, indicating that the Ca^{2+} flux rates across the plasma membrane had not been affected by the previous incubation conditions. Under these same conditions PTH secretion reversed to basal rates as well (not shown). These results show that the permeability of the parathyroid cell membrane is unaffected by the absence of Ca^{2+} in the medium, and that the PTH secretory mechanism is not irreversibly altered by exposure to 25 μM Ca^{2+}

These results emphasize the contrasting nature of PTH secretion with hormone release from a number of other endocrine glands, and indicate that PTH secretion is independent of plasma membrane Ca^{2+} influx. Further measurements must be made to determine the relationship of the cytosolic free [Ca^{2+}] to PTH exocytosis. An increase in the cytosolic [Ca^{2+}], induced by Ca^{2+} release from a Ca^{2+}-sequestering organelle, remains a possible mechanism for triggering PTH secretion. Alternatively, PTH release may be stimulated by a decrease in intracellular free Ca^{2+}, opposite to other secretory cells. PTH secretion may be regulated by other events not necessarily related to changes in free cytosolic Ca^{2+} levels.

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