Calcium-activated calcium permeability in parathyroid cells

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The Ca^{2+} receptor mechanism of the parathyroid cell was studied using La^{3+} as a probe. La^{3+} was found to bind to the cell surface without further penetration. Measurements of ^{45}Ca fluxes and the cytoplasmic Ca^{2+} concentration (Ca_i^{2+}) revealed a stimulatory component in the action of La^{3+} on Ca^{2+} permeability resulting in a rise in Ca_i^{2+}. These effects mimicked those obtained when raising the extracellular Ca^{2+} concentration from 0.5 to 3.0 mM, but the actions of La^{3+} and Ca^{2+} were not additive. The results suggest the existence of a novel Ca^{2+} permeability physiologically activated by binding of Ca^{2+} to an external receptor.

Ca^{2+} channel *Ca^{2+} flux* *cytoplasmic Ca^{2+}* *Lanthanide* *Parathyroid hormone* *Secretion*

1. INTRODUCTION

The physiological release of parathyroid hormone (PTH) is inhibited by a rise of extracellular Ca^{2+}, an effect which is mediated by an increased cytoplasmic Ca^{2+} activity (Ca_i^{2+}) [1-7]. Another unusual feature is that inhibition of secretion is associated with depolarization. A number of divalent cations and even trivalent lanthanum mimic the inhibitory [8] and depolarizing [9,10] actions of Ca^{2+}, but the mechanisms involved in signal transduction are still largely unknown. We have suggested that Ca^{2+} entry occurs through channels activated by Ca^{2+} itself, and that this influx causes the depolarization [11]. Here, it is shown that, despite being restricted to the cell exterior, La^{3+} can activate Ca^{2+} influx and raise Ca_i^{2+} in parathyroid cells.

2. MATERIALS AND METHODS

Parathyroid glands were obtained from adult Sprague-Dawley rats or cattle within a few minutes after slaughter. The basal medium used in all experiments contained 5 mM Tris (pH 7.4), 139 mM NaCl, 4.7 mM KCl, 0.5 mM $MgCl_2$ and 0.5 mM $CaCl_2$. ^{45}Ca uptake and efflux were studied essentially as in [11,12]. To remove extracellular and superficially bound ^{45}Ca in the uptake experiments, the tissue pieces were washed for 60 min at room temperature in a medium containing 2.0 mM $LaCl_3$. Prior to the use of an La^{3+}-washing procedure, it was ascertained in experiments involving ^{45}Ca uptake and efflux as well as Ca_i^{2+} that at the reduced temperature La^{3+} effectively inhibits Ca^{2+} fluxes and that there is no stimulatory component (to be published). By determining the tissue content of ^{45}Ca after each efflux experiment, it was possible to express the results as fractional outflow rate (% of tissue content of ^{45}Ca per min). The La^{3+} stimulation of ^{45}Ca efflux observed in the present study could not be attributed to displacement of ^{45}Ca from the plasma membrane, which is depleted of radioactivity after 60 min of perifusion in non-radioactive medium containing calcium. In identical perifusions at room temperature there was only inhibition (to be published). To monitor changes in Ca_i^{2+}, suspen-

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sions of parathyroid cells were prepared from cattle and rats [6]. The cells were loaded with the Ca^{2+} indicators quin-2 (cattle) or fura-2 (rats) by incubations for 30–40 min at 37°C in medium containing 25 μM quin-2 tetraacetoxy methyl ester or 1 μM fura-2 tetraacetoxy methyl ester [6]. Suspensions of the cells from cattle were studied in a spectrofluorometer [5,6]. Only in experiments with no quenching ions present were calculations of Ca_i^{2+} values possible [13]. The fura-2-loaded rat cells were allowed to attach to the bottom of a culture chamber [14]. The chamber was placed within a thermostatted box (37°C) in an inverted Nikon Diaphot microscope equipped for epifluorescence microfluorometry. Loss of intracellular fluorescence due to photobleaching or leakage of the indicators was negligible during the observation periods. The subcellular location of lanthanum was checked by electron microscopy. La^{3+} was found to bind to the cell surface without further penetration (not shown).

3. RESULTS

Table 1 shows the effects of La^{3+} on the uptake of ^{45}Ca. During 60 min of incubation, 0.5 or 2.0

Table 1

Effects of La^{3+} on ^{45}Ca uptake by the parathyroid gland

$[La^{3+}]$ (mM)	$[Ca^{2+}]$ (mM)	Incubation time (min)	^{45}Ca uptake (mmol/kg dry tissue)	Effect of La^{3+}
0 (control)	0.5	15	0.54 ± 0.12 (10)	
0.02	0.5	15	0.70 ± 0.10 (10)	0.15 ± 0.05[b] (10)
0 (control)	3.0	15	1.61 ± 0.27 (12)	
0.02	3.0	15	1.54 ± 0.12 (12)	−0.07 ± 0.24 (12)
0 (control)	0.5	60	0.91 ± 0.23 (11)	
0.02	0.5	60	1.02 ± 0.18 (11)	0.11 ± 0.05[a] (11)
0 (control)	0.5	60	1.19 ± 0.10 (10)	
0.5	0.5	60	0.64 ± 0.10 (10)	−0.54 ± 0.12[c] (10)
2.0	0.5	60	0.34 ± 0.07 (5)	−0.82 ± 0.19[a] (5)
0 (control)	3.0	60	4.41 ± 0.34 (10)	
0.5	3.0	60	2.33 ± 0.28 (10)	−2.08 ± 0.19[c] (10)
2.0	3.0	60	1.05 ± 0.31 (5)	−2.98 ± 0.36[c] (5)

Pieces of rat parathyroid tissue were incubated for 15 or 60 min at different La^{3+} concentrations in medium containing 0.5 or 3.0 mM ^{45}Ca (40 and 6.7 Ci/mol, respectively). The intracellular radioactivity was measured after subsequent washing for 60 min at room temperature in a medium containing 2.0 mM $LaCl_3$. Results are given as means ± SE for the number of experiments indicated within parentheses. [a] $p > 0.05$; [b] $p < 0.01$; [c] $p > 0.005$

mM La^{3+} inhibited the uptake of 0.5 or 3.0 mM ^{45}Ca. At 0.5 mM Ca^{2+} there was also a stimulatory component in the La^{3+} action on ^{45}Ca uptake observed when exposing the cells for 15 or 60 min to only 20 μM of the trivalent cation. The La^{3+}-stimulated uptake of ^{45}Ca was not additive to that obtained by increasing the extracellular Ca^{2+} concentration from 0.5 to 3.0 mM. The dual actions of La^{3+} on Ca^{2+} permeability became particularly evident when studying the kinetics of ^{45}Ca efflux (fig.1). The introduction of 0.5 mM La^{3+} into the perifusion medium thus resulted in transient stimulation of ^{45}Ca efflux followed by an inhibition which was reversed upon omission of La^{3+}. The stimulatory phase was considerably diminished after increasing the Ca^{2+} concentration of the perifusion medium from 0.5 to 3.0 mM. Fig.2 shows the actions of Ca^{2+} and La^{3+} on Ca_i^{2+} as measured with quin-2 in suspensions of bovine parathyroid cells and with fura-2 in single rat parathyroid cells. In the quin-2 loaded cells a rise of the extracellular Ca^{2+} concentration from 0.5 to 3.0 mM resulted in an increased Ca_i^{2+}. It is apparent that La^{3+} and Mn^{2+} promptly quenches the fluorescence from extracellular quin-2 and that only Mn^{2+} reaches the intracellular indicator. In the case of La^{3+}, the initial drop in fluorescence was thus followed by an increase reaching stability after 1 min. When single fura-2-loaded rat parathyroid cells were studied in a microscope fluorometer there was no interference from extracellular indicator. Both a rise of the Ca^{2+} concentration and addition of La^{3+} thus resulted only in increased fluorescence. The La^{3+}-induced increase in Ca_i^{2+} apparently depended on the influx of Ca^{2+}, since it was diminished after reducing the extracellular Ca^{2+} concentration from 0.5 to ~0.07 mM. Moreover, there was no additional effect of La^{3+} after raising extracellular Ca^{2+} to 3.0 mM.

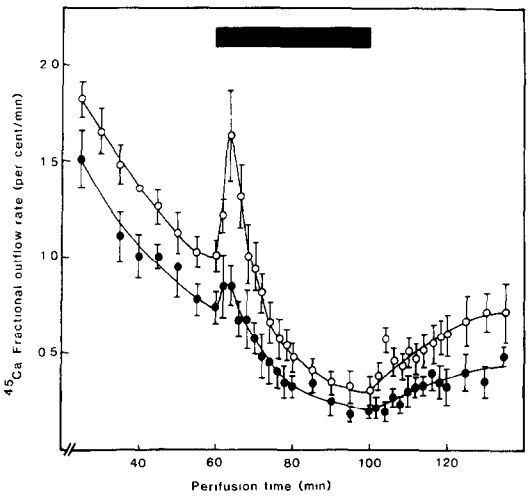

Fig.1. Effect of La^{3+} on ^{45}Ca efflux from the parathyroid gland. Pieces of rat parathyroid tissue were loaded with 3.0 mM ^{45}Ca (167 Ci/mol) for 90 min at 37°C in 100 μl medium. The perifusion medium contained 0.5 (○) or 3.0 (●) mM Ca^{2+} and was delivered at a rate of about 40 μl/min. During the period indicated by the horizontal bar, 0.5 mM La^{3+} was also present. Results are given as means ± SE for 5 experiments.

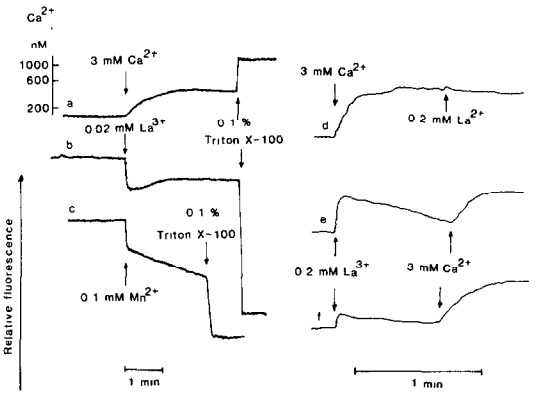

Fig.2. Effects of La^{3+} on Ca_i^{2+} of parathyroid cells. In a–c, 5×10^6 quin-2-loaded and rinsed cells from cattle were suspended in 1.3 ml of medium containing 0.5 mM Ca^{2+}. The cell suspensions were incubated with constant stirring at 37°C in a 1 cm cuvette placed in a spectrofluorometer with excitation and emission wavelengths set at 339 and 492 nm, respectively. In a, with no quenching ions present, it was possible to calculate Ca_i^{2+} values, which are shown. In d–f, single fura-2-loaded rat parathyroid cells were studied in a microscope fluorometer at 37°C with excitation at 339 nm and emission >470 nm. The medium initially contained 0.5 (d,e) or ~0.07 (f) mM Ca^{2+}. Note the different time scales for a–c and d–f.

4. DISCUSSION

In most secretory cells exocytosis is believed to be triggered by an increase in Ca_i^{2+}. Also in the parathyroid cells there is a stimulatory component in the action of Ca_i^{2+}, which becomes maximal

already at low Ca_i^{2+} values [6,7]. Nevertheless, there is no doubt that under physiological conditions the dominating effect of raised Ca_i^{2+} is inhibition of PTH secretion [2,4,6,7].

Mg^{2+} inhibition of PTH release has been found to be accompanied by increased Ca_i^{2+} depending on the presence of extracellular calcium [1,3]. Attempts have been made to explain the rises in Ca_i^{2+} after exposure to Ca^{2+} or Mg^{2+} by depolarization with subsequent influx of Ca^{2+} through voltage-dependent channels [1]. However, although both cations depolarize the parathyroid cells [9,10], we have demonstrated an absence of voltage-dependent Ca^{2+} channels [11], and K^+ depolarization is, indeed, associated with decreased Ca_i^{2+} and enhanced secretion [4,6]. It is difficult to study whether divalent cations other than Mg^{2+} inhibit PTH release indirectly via Ca^{2+} or if they interact with the exocytotic machinery after entering the cells. A major problem is that the ions interfere with the measurements of Ca_i^{2+}, by reacting with intracellular Ca^{2+} indicators like quin-2 and fura-2.

In contrast to divalent cations the trivalent lanthanides are generally restricted to the extracellular space [15], and it was apparent from our electron microscopic controls and the Ca_i^{2+} data that this is also the case for parathyroid cells. Since La^{3+} shares the ability of divalent cations to depolarize the parathyroid cells [9] and inhibit PTH release [8], it can be used as a probe for testing our proposal that there is a Ca^{2+} permeability activated by external calcium [11]. La^{3+} has been widely used to displace Ca^{2+} from binding sites, to block Ca^{2+} fluxes and to inhibit Ca^{2+}-dependent processes [15]. In accordance with such actions a high concentration of La^{3+} (5 mM) was found to inhibit ^{45}Ca uptake by parathyroid cells incubated at different concentrations of Ca^{2+} for 15 min [16]. Inhibition of Ca^{2+} permeability was also apparent from the present measurements of ^{45}Ca uptake and efflux. However, there was a transient stimulatory component in the action of La^{3+} which became the dominating effect after reduction of the extracellular La^{3+} concentration to the micromolar range. The La^{3+} stimulation of Ca^{2+} permeability resulted in an increase in Ca_i^{2+}, dependent on influx of Ca^{2+}. Moreover, it was evident from the measurements of ^{45}Ca uptake and efflux as well as Ca_i^{2+} that the stimulatory actions of Ca^{2+} and La^{3+} on Ca^{2+} permeability were not additive, suggesting that the two cations operate through a common mechanism.

Exposure of parathyroid cells to low concentrations of La^{3+} mimicked the effects of raising extracellular Ca^{2+} within the physiological control range for PTH release, both with regard to ^{45}Ca fluxes [11] and Ca_i^{2+}. Considering that La^{3+} is restricted to the exterior of the parathyroid cells, the present data strongly support the concept of a Ca^{2+} permeability activated by binding of Ca^{2+} to an external receptor. This Ca^{2+}-activated Ca^{2+} permeability is not necessarily unique to parathyroid cells. It can even be expected to occur in other cells with a Ca^{2+}-sensing function, like the calcitonin-secreting cells and possibly those releasing renin [17] and glucagon [18]. Also cell types dominated by other Ca^{2+} channels might have Ca^{2+}-activated Ca^{2+} permeability. La^{3+} has thus been found to stimulate Ca^{2+}-dependent release of adrenomedullary catecholamine [19] and pancreatic amylase [20] as well as to enhance ^{45}Ca uptake into the pancreatic acinar cells [20,21]. It is interesting to note that verapamil under different experimental conditions either enhanced or slightly diminished the La^{3+}-induced release of catecholamine [19]. We have previously shown that this 'calcium antagonist' and its derivative D-600 can both stimulate and inhibit the Ca^{2+} permeability of the parathyroid cells depending on the extracellular Ca^{2+} concentration [6].

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