

Depolarizing Response of Rat Parathyroid Cells to Divalent Cations

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ABSTRACT Membrane potentials were recorded from rat parathyroid glands continuously perfused in vitro. At 1.5 mM external Ca^{++} , the resting potential averages -73 ± 5 mV (mean \pm SD, $n = 66$). On exposure to 2.5 mM Ca^{++} , the cells depolarize reversibly to a potential of -34 ± 8 mV (mean \pm SD). Depolarization to this value is complete in ~ 2 –4 min, and repolarization on return to 1.5 mM Ca^{++} takes about the same time. The depolarizing action of high Ca^{++} is mimicked by all divalent cations tested, with the following order of effectiveness: $\text{Ca}^{++} > \text{Sr}^{++} > \text{Mg}^{++} > \text{Ba}^{++}$ for alkali-earth metals, and $\text{Ca}^{++} > \text{Cd}^{++} > \text{Mn}^{++} > \text{Co}^{++} > \text{Zn}^{++}$ for transition metals. Input resistance in 1.5 mM Ca^{++} was 24.35 ± 14 M Ω (mean \pm SD) and increased by an average factor of 2.43 ± 0.8 after switching to 2.5 mM Ca^{++} . The low value of input resistance suggests that cells are coupled by low-resistance junctions. The resting potential in low Ca^{++} is quite insensitive to removal of external Na^+ or Cl^- , but very sensitive to changes in external K^+ . Cells depolarize by 61 mV for a 10-fold increase in external K^+ . In high Ca^{++} , membrane potential is less sensitive to an increase in external K^+ and is unchanged by increasing K^+ from 5 to 25 mM. Depolarization evoked by high Ca^{++} may be slowed, but is unchanged in amplitude by removal of external Na^+ or Cl^- . Organic (D600) and inorganic (Co^{++} , Cd^{++} , and Mn^{++}) blockers of the Ca^{++} channels do not interfere with the electrical response to Ca^{++} changes. Our results show remarkable parallels to previous observations on the control of parathormone (PTH) release by Ca^{++} . They suggest an association between membrane voltage and secretion that is very unusual: parathyroid cells secrete when fully polarized, and secrete less when depolarized. The extraordinary sensitivity of parathyroid cells to divalent cations leads us to hypothesize the existence in their membranes of a divalent cation receptor that controls membrane permeability (possibly to K^+) and PTH secretion.

INTRODUCTION

It is well known that the rate of parathyroid hormone secretion (PTH) is inversely related to serum Ca^{++} levels. In vivo (Mayer and Hurst, 1978;

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Patt and Luckhardt, 1942) and *in vitro* (Brown et al., 1976; Morrissey and Cohn, 1978; Sherwood et al., 1970) experiments have demonstrated that a slight decrease (on the order of tenths of millimoles) in external Ca^{++} concentration evokes a strong secretory response from parathyroid cells. Conversely, an increase in extracellular Ca^{++} reduces the rate of PTH secretion. The curve relating secretion to Ca^{++} concentration is very steep, with the full secretion rate at 0–0.5 mM and maximal suppression at 2–2.5 mM (Brown et al., 1976; Habener and Potts, 1976; Morrissey and Cohn, 1978). The steepness of the curve suggests the existence in parathyroid cells of an unusual and very sensitive Ca-sensing mechanism, the nature of which has not been discovered.

In many secretory cells, external Ca^{++} is essential for secretion. Physiological stimulation results in Ca^{++} entry into the cell interior, which is responsible for triggering exocytosis (Douglas, 1968; Petersen, 1980; Rubin, 1970). This general scheme may not apply to parathyroid cells. An increase in external Ca^{++} , as well as Mg^{++} , decreases PTH secretion (Brown et al., 1976; Habener and Potts, 1976; Sherwood et al., 1970) and full secretory activity can be observed in the absence of external Ca^{++} (Fischer et al., 1982; Pintado and Wallace, personal communication). In addition, application of the calcium ionophore A23187, which presumably increases cytosolic Ca^{++} (Reed and Lardy, 1972), inhibits PTH secretion (Brown et al., 1980; Fischer et al., 1982; Habener et al., 1977).

Membrane electrical activity plays an important role in stimulus-secretion coupling in many gland cells (Petersen, 1980). Electrophysiological studies on the parathyroid have been performed only recently and the two reports available show contradictory findings. In mouse parathyroid gland, *in vitro* intracellular recordings by Bruce and Anderson (1979) show that cells hyperpolarize when the external Ca^{++} concentration decreases. In contrast, it has been reported that goat parathyroid cells maintained in culture depolarize in low Ca^{++} and that voltage-dependent Ca^{++} entry may trigger PTH exocytosis (Sand et al., 1981).

We have performed experiments in rat parathyroid glands *in vitro* and have found a very clear and reversible depolarization in response to increased external Ca^{++} in the physiological range for rats (Roth and Raisz, 1964). Our results thus agree qualitatively with those found in the mouse (Bruce and Anderson, 1979). Depolarization is also observed after addition of all divalent cations tested, both alkali-earth and transition metals, including the Ca^{++} channel blockers Cd^{++} , Mn^{++} , and Co^{++} . The depolarization evoked by increased Ca^{++} is accompanied by an increase in input resistance and may be due to a suppression of K^+ permeability. The possibility that a divalent cation receptor located on the external surface of the membrane controls membrane permeability and, in turn, PTH secretion, is considered.

METHODS

Preparation

Experiments were performed on parathyroid glands obtained from adult, male

Wistar rats 100–200 g in weight. Animals were killed with ether and the gland was excised under a dissecting microscope. Parathyroid glands in the rat are located bilaterally in the lateral border of the thyroid gland and appear as pale ovoids ~1–1.5 mm in diameter partially embedded in the thyroid tissue (Greene, 1935). The parathyroid gland and part of the thyroid tissue adjacent to it were transferred to the recording chamber and secured to the bottom by small stainless-steel pins. The thin connective capsule covering the gland was removed with the aid of a stereomicroscope.

Experimental Setup

The recording chamber and part of the experimental setup are schematically represented in Fig. 1. The chamber was made of lucite and divided into three

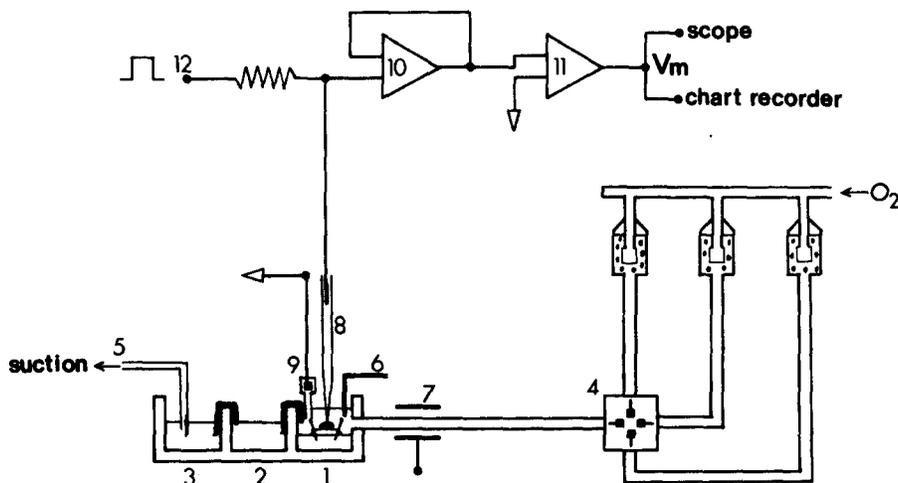


FIGURE 1. Schematic drawing of the apparatus used for intracellular recording in the continuously perfused parathyroid gland. 1: recording chamber. 2: intermediate reservoir. 3: reservoir where the suction (5) is applied. Fluid flows between 1, 2, and 3 by way of cotton wicks. 4: stopcock to select between five different solutions. 6: microthermistor permanently attached to the recording chamber. 7: heating filament that warms up the inflow solution. 8: glass microelectrode. 9: 3 M KCl agar bridge. 10: voltage follower. 11: stage for electronic compensation of electrode capacitance and resistance. 12: square current pulses (obtained from a constant current pump) that can be injected through the microelectrode.

compartments. The gland was placed in the first compartment (1), sitting on a layer of Sylgard (Dow Corning Corp., Midland, MI). This compartment contained the inlet for the external fluid. A thin wall of plastic attached to the bottom of the chamber (not illustrated in the figure) was placed as a baffle between the gland and the inlet aperture to direct the solution stream away from the microelectrode. The recording compartment had a volume of ~0.5 cm³ and the bathing solution flow rate was 1 ml/min. Solution flowed from compartment 1 to a buffer compartment (2) and from this to a third compartment (3), where suction was applied. Flow between compartments was by way of cotton wicks. A silk thread glued at the outlet needle (5) was used to break the meniscus, making the suction regular and smooth.

The external perfusion system was mounted separately from the recording table. Any one of five solutions could be applied while recording in a given parathyroid cell. Switching was accomplished without interrupting the solution flow, and the level of solution in the recording compartment was thus kept constant. The dead space of the perfusion system was <0.1 ml and its washout time was 1 or 2 s. The temperature in the recording compartment was monitored with a microthermistor (6) and controlled by a circuit of our own design. This circuit passed the appropriate current through a coil (7) placed around the solution inlet, thus maintaining the recording compartment at 37°C.

Solutions

Solution compositions are shown in Table I. Modified Krebs solutions with variable Ca^{++} concentrations (between 0.5 and 2.5 mM) were made by mixing solutions A and B. In experiments where the effects of divalent cations other than Ca^{++} or Mg^{++} were tested, SrCl_2 , BaCl_2 , MnCl_2 , CdCl_2 , CoCl_2 , or ZnCl_2 were substituted equimolarly for NaCl. Cl-free solutions (98% Cl-free) with variable K^+ concentrations were made by mixing solutions E, F, G, and H. In

TABLE I
Composition of Solutions (in mM)

	A Krebs 0.5 Ca	B Krebs 2.5 Ca	C Na-free 1.5 Ca	D Na-free 2.5 Ca	E Cl-free, 0 K, 2 Ca	F Cl-free, 0 K, 4 Ca	G Cl-free, 100 K, 2 Ca	H Cl-free, 100 K, 4 Ca
NaCl	120	117	—	—	—	—	—	—
KCl	5	5	5	5	—	—	—	—
K-glutamate	—	—	—	—	—	—	100	100
Na-fumarate	2.7	2.7	—	—	2.7	2.7	2.7	2.7
Na-pyruvate	4.9	4.9	—	—	4.9	4.9	4.9	4.9
Na-glutamate	4.9	4.9	—	—	130	127	30	27
MgSO_4	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1
CaCl_2	0.5	2.5	1.5	2.5	2.0	4.0	2.0	4.0
Glucose	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8
HEPES	10	10	—	—	10	10	10	10
Trizma (pH 7.5)	—	—	142.5	141.5	—	—	—	—

some experiments variable amounts of D600 from a 1-mM stock solution were added to the modified Krebs solution until reaching final concentrations between 30 and 150 μM . The pH of all solutions was adjusted to 7.4 except for the Na-free solutions (C and D), where Trizma, pH 7.5, was used as buffer and as Na substitute. Solutions were continuously bubbled with 100% O_2 and had an osmolality of 270–290 mosmol/kg. The junction potentials of all solutions were measured with the electrode placed in the recording chamber. In solutions with differences only in divalent cation concentration, the junction potential differed by <1 mV. When switching from a modified Krebs solution to a Na-free solution, a junction potential of +4 mV was observed.

Recording

Parathyroid cells were impaled with glass microelectrodes filled with 3 M KCl and having 100–200 M Ω DC resistance. The microelectrodes were pulled with a vertical puller from thick-walled borosilicate glass (OD = 2 mm, ID = 0.7 mm) with an inner filament for ease of filling. The microelectrodes were electrically connected to an FET operational amplifier (AD515; Analog Devices, Inc.,

Norwood, MA) wired as a voltage follower and placed close to the preparation. External voltage was sensed by a 3 M KCl agar bridge placed in the recording compartment of the chamber. The membrane potential was simultaneously monitored by an oscilloscope and a digital meter, and recorded on a chart recorder (Brush Instruments, Gould Inc., Cleveland, OH) with a frequency response of ~ 100 Hz. Square pulses of 200 ms duration and variable amplitude (between 0.1 and 0.25 nA) obtained from a constant-current pump were injected through the microelectrode to measure input resistance. Stray capacitance and voltage drop across the microelectrode were electronically compensated. The recording chamber and micromanipulator were placed on a heavy granite table sitting on inner tubes for shock absorption. The recording table was electrically shielded to prevent noise.

Experimental Procedure

Cells were successfully impaled as deep as 250–300 μm from the surface of the gland. Most impalements only lasted for a few seconds, but occasionally the electrode stayed in the cell and a stable value of the membrane potential was reached in 2 or 3 min. Good impalements were characterized by a sudden change in the membrane potential. In all cases, the recorded voltage was balanced to zero before entering the gland. After an impalement, a check for voltage drift was made by withdrawing the electrode from the gland. In every cell from which a stable recording could be made, the response to changes in external Ca^{++} concentration was tested. This was used as an indication that the recordings were made in a qualitatively homogeneous population of parathyroid cells. After this test, the rest of the experimental protocol was performed. We have recorded more than 60 parathyroid cells for periods from 10 min to >3 h. In long penetrations it was possible to test several solutions with different ionic compositions. In many other cells, stable recordings lasting several minutes were obtained, but the electrode came out of the cell before a solution change could be accomplished.

RESULTS

Resting Potential in Parathyroid Cells

Most cells were impaled while the gland was bathed with a solution containing 1.5 mM Ca^{++} . In many of these cells, the resting potential stabilized in the first minute after the penetration, reaching an average value of -73 ± 5 mV (mean \pm SD, $n = 66$).

Membrane Potential Depends on External Ca^{++}

In all parathyroid cells exposed to more than one solution, the magnitude of the membrane potential was strongly dependent on the concentration of external Ca^{++} . This is illustrated in Fig. 2 by a continuous recording of membrane voltage from a single cell. The cell was initially impaled in Krebs 2.5 mM Ca^{++} , yielding a resting potential of -27 mV. Solution changes are indicated by the arrows. The records show very clearly that membrane potential depends strongly and reversibly on external Ca^{++} . The cell depolarizes in response to an increase in external Ca^{++} after a

delay required for washout and equilibration. Once the membrane potential started to change, the time required to attain the new steady state level was between 1.5 and 3 min, depending on each particular cell.

Membrane voltage is sensitive to external Ca^{++} in a narrow concentration range, between 1 and 2.5 mM Ca^{++} . Ca^{++} concentrations of <1 mM were only tested in a few cells because, for unknown reasons, perhaps chance, it was difficult to obtain stable intracellular recordings. In the lower part of Fig. 2, switching from 1.5 to 0.5 mM Ca^{++} solution does

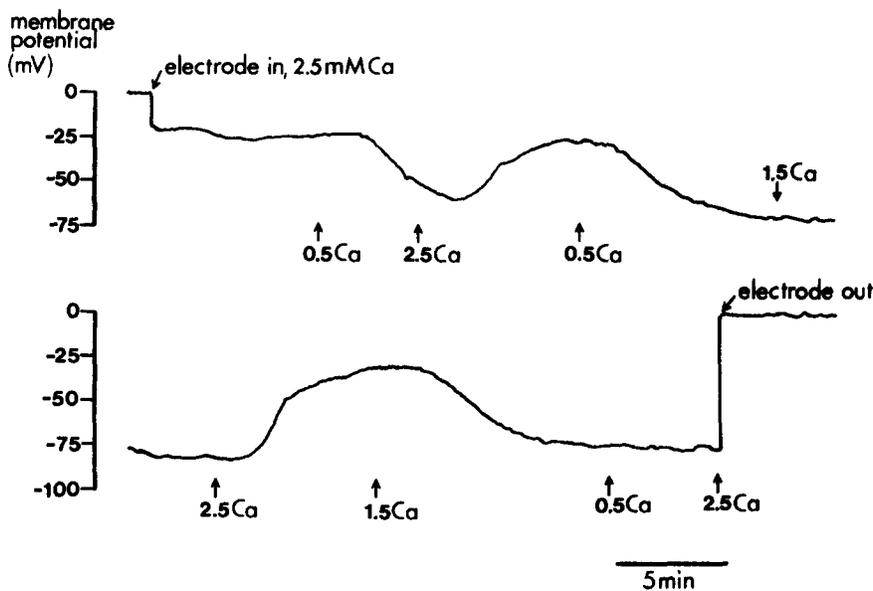


FIGURE 2. Modifications of membrane potential by changes of external Ca^{++} concentration. The traces are a continuous intracellular recording from a parathyroid cell exposed to different external Ca^{++} concentrations at the time indicated. The cell was impaled in a solution containing 2.5 mM Ca^{++} . The steepest voltage response occurs between 1.5 and 2.5 mM Ca^{++} . In 0.5 mM Ca^{++} , the membrane potential is about the same as in 1.5 mM Ca^{++} , or slightly more positive. Different Ca^{++} concentrations were obtained by mixing solutions A and B of Table I. Osmolality of all solutions was between 270 and 290 mosmol/kg, pH 7.4, temperature 37°C.

not have an appreciable effect on the membrane potential. Some cells even showed a slight depolarization when the bathing solution was changed from 1.5 to 0.5 mM Ca^{++} .

The mean resting potential of several cells as a function of external Ca^{++} concentration is shown in Fig. 3. The values in A are from cells continuously recorded for 10–120 min and exposed to at least two solutions with different Ca^{++} concentrations. Extreme values of potential were -34 ± 8 mV (mean \pm SD) in 2.5 mM Ca^{++} and -80.5 ± 2 mV (mean \pm SD) in 1.0 mM Ca^{++} .

Fig. 3B shows the same curve compiled from penetrations maintained for only 1–10 min, too short a time for a solution change to be accomplished. The Ca^{++} concentration in the bathing solution for each penetration is given on the axis. The measurements by the two methods are in excellent agreement and clearly demonstrate that in parathyroid cells a 1.5 mM change in external Ca^{++} in a definite range (between 1 and 2.5 mM) evokes a mean potential change of 46 mV. The relationship between membrane potential and external Ca^{++} in that range is very steep.

One of the most striking characteristics of this electrical response is its nearly perfect reversibility. Some cells were exposed several times to

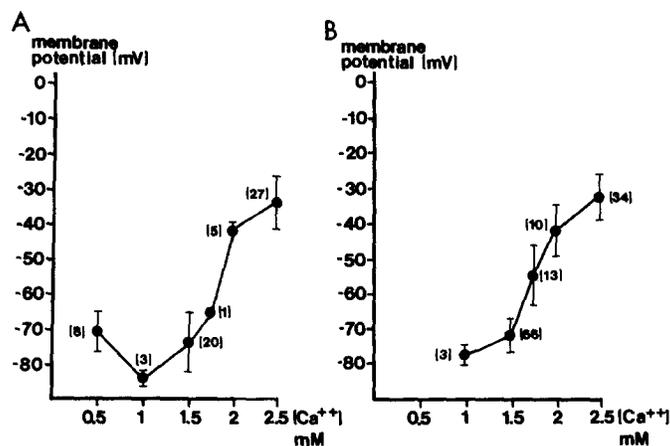


FIGURE 3. Membrane potential in parathyroid cells as a function of external Ca^{++} . (A) Data from single penetrations that were held long enough (10 min to 3 h) to record resting potential in at least three solutions with different Ca^{++} concentrations. Each point represents the mean \pm SD of 3–27 measurements as given by the numbers in parentheses. (B) Data from penetrations too brief to allow a change of the bathing solution. The penetrations were performed in the Ca^{++} concentration specified on the axis, and the resting potential remained stable for 1–10 min. Each point represents the mean \pm SD of 1–66 measurements, and the numbers of measurements are in parentheses. Composition of solutions is the same as in Fig. 2.

solutions with 1.5 and 2.5 mM Ca^{++} and the membrane potential was measured at a given time after the solution change. In a typical cell the mean membrane potential values of eight measurements done 5–6 min after switching to the new solution were -79.7 ± 2.4 mV (mean \pm SD) in 1.5 mM Ca^{++} and -32.2 ± 2.5 mV (mean \pm SD) in 2.5 mM Ca^{++} . The extremely small dispersion around the mean indicates a nearly perfect reversibility of the response.

On three occasions stable intracellular recordings from the tissue surrounding the parathyroid gland (presumably from thyroid cells) were obtained. The resting potential of these cells was about -65 mV and did

not show any appreciable modification in response to changes in external Ca^{++} concentration in the range of 0.5–2.5 mM Ca^{++} .

Absence of Spike Activity

The electrical response evoked by increasing or decreasing external Ca^{++} always showed the qualitative features referred to above and no spiking activity was ever seen. In some cells that showed a very low resting potential (–40 mV in 1.5 mM Ca^{++}), irregular jumps and spikes were observed in the first seconds after the impalement that were probably due to an imperfect penetration of the cell, since either the microelectrode came out of the cell shortly after the impalement or the membrane sealed around the electrode and the “spikes” disappeared as membrane potential increased.

Calcium Effects Are Mimicked by Other Divalent Cations

Other alkali-earth cations have the same qualitative effect on parathyroid cell membrane potential as Ca^{++} . This is illustrated in Fig. 4A, which shows a continuous intracellular recording from a single cell. The initial impalement was done in a solution containing 1.5 mM Ca^{++} , yielding a resting potential of –76 mV. At the time indicated by the arrows, the bathing solution was changed to one containing, in addition to 1.5 mM Ca^{++} , 1 mM of another divalent cation. All the cations caused a reversible depolarization, but with different time courses and magnitudes. The effectiveness for the response was $\text{Ca}^{++} > \text{Sr}^{++} > \text{Mg}^{++} > \text{Ba}^{++}$. None of these divalent cations was as effective as Ca^{++} in depolarizing parathyroid cells. Sr^{++} and Ba^{++} responses were, respectively, ~60 and ~30% of the depolarization observed with Ca^{++} . Mg^{++} yielded an intermediate value. The difference in the time course is also very apparent. Whereas the depolarization induced by 1 mM additional Ca^{++} reached the steady state in 2.5 min, the Ba^{++} -induced depolarization was three times smaller and required ~6 min to attain the steady state. The same experimental protocol shown in Fig. 4A has been repeated in three different cells with similar results.

We have also studied the effect of other divalent cations, and an example is shown in Fig. 4B. The record belongs to another parathyroid cell impaled in 1.5 mM Ca^{++} , with an initial membrane potential of –75 mV. The experimental protocol was the same as before: in addition to 1.5 mM Ca^{++} , 1 mM of a test divalent cation was added to the bathing solution. In all cases the cell depolarized when the test cation was added. In this case the effectiveness of the response is $\text{Ca}^{++} > \text{Cd}^{++} > \text{Mn}^{++} > \text{Co}^{++}$. Cd^{++} and Mn^{++} seem to be slightly more effective than Sr^{++} , and the effectiveness of Co^{++} is about the same as with Ba^{++} . The effects of Cd^{++} , Mn^{++} , and Co^{++} have been tested in two different cells with identical qualitative results. Zn^{++} was tested in a cell (not illustrated) and evoked a depolarization of about the same magnitude as Co^{++} . Fig. 4A and B shows unequivocally that membrane potential in parathyroid cells is affected not only by external Ca^{++} but by all the divalent cations tested.

Input Resistance and Cell-to-Cell Coupling

Input resistance has been measured in several cells by passing hyperpolarizing square current pulses through the recording microelectrode. For these measurements the voltage drop across the microelectrode resistance

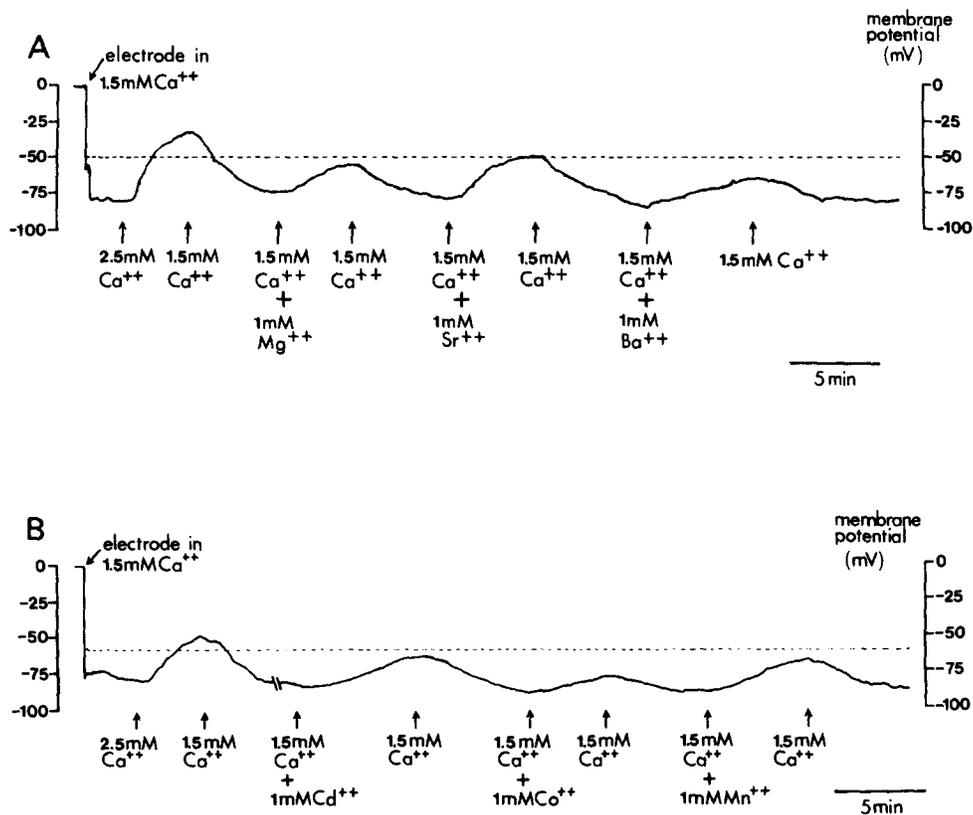


FIGURE 4. Effect of various divalent cations on membrane potential of parathyroid cells. *A* shows a continuous intracellular recording from a cell bathed with solutions containing the indicated concentration of divalent cations. The cell was impaled in 1.5 mM Ca^{++} , and switches to other solutions are indicated by the arrows. Solution composition is the same as in Fig. 1, except that MgCl_2 , SrCl_2 , BaCl_2 , or additional CaCl_2 were substituted equimolarly for NaCl . The dashed horizontal line at -50 mV membrane potential is drawn for convenience of comparison. *B* shows a continuous recording of another cell exposed to the indicated transition elements. The time between the parallel bars in the record was 2.5 min, and the dashed line at -57 mV membrane potential is drawn for convenience.

was subtracted out electronically. The subtraction circuit was adjusted before penetrating the cell; the adequacy of the subtraction was tested after the electrode was withdrawn. The measurement was discarded if electrode resistance changed significantly during the penetration.

The mean value for input resistance was $24.3 \pm 14 \text{ M}\Omega$ (mean \pm SD, 10 cells) at 1.5 mM Ca^{++} . Taking into account the small size of parathyroid cells ($\sim 8 \mu\text{m}$ diam; Roth and Raisz, 1964), the expected input resistance for a single cell is $\sim 500 \text{ M}\Omega$, assuming a membrane resistance of $1,000 \Omega \text{ cm}^2$. For a single cell of this diameter, a $24\text{-M}\Omega$ input resistance would imply an unreasonably low value for membrane resistance, $48 \Omega \text{ cm}^2$. The input resistance thus strongly suggests that in the whole gland, cells are coupled by low-resistance junctions. Input resistance would then reflect both membrane and junctional resistance of the coupled cells (see Discussion).

Input Resistance Changes During the Depolarization Induced by High Ca^{++}

The electrical response to Ca^{++} in parathyroid cells is accompanied by a change in input resistance, but, as illustrated in Fig. 5, the correlation

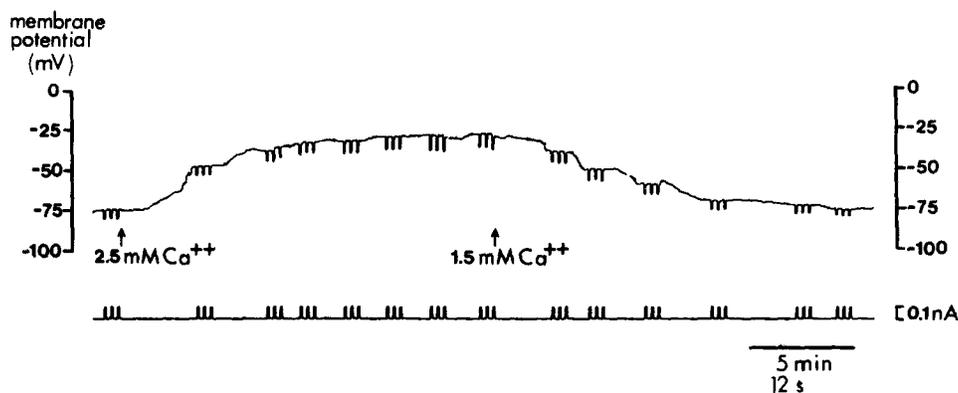


FIGURE 5. Input resistance increases on exposure to 2.5 mM Ca^{++} . The cell was impaled in a solution containing 1.5 mM Ca^{++} , and solution changes occurred at the arrows. The composition of solutions was the same as in Fig. 2. Input resistance was measured by injecting hyperpolarizing square current pulses of 0.1 nA amplitude and 200 ms duration, and the voltage drop across the microelectrode was electronically subtracted. The input resistance is thus proportional to the voltage deflection during the current pulse. The 12-s time scale is applicable during the pulse trains, and the 5-min scale at other times.

between the two variables is not very precise. Input resistance was determined from the voltage deflection (upper trace) recorded during the injection of hyperpolarizing current pulses of 0.1 nA amplitude (lower trace). It can be seen that input resistance increases by a factor of 2 or 3 during the depolarization induced on changing from 1.5 to 2.5 mM Ca^{++} . After switching back to the 1.5 mM Ca^{++} solution, input resistance decreases as the cell progressively hyperpolarizes and, in this case, falls somewhat below the initial value.

In cells where input resistances in 1.5 and 2.5 mM Ca^{++} were measured, it was higher in 2.5 mM Ca^{++} in every case, and the average factor was

2.4 ± 0.8 (mean \pm SD, 10 cells). In most cases the time course of the input resistance change did not exactly parallel the voltage change. It is of course possible that the resistance change is a result of the V_m change caused by Ca^{++} , rather than being a direct effect of Ca^{++} .

Complete Replacement of External Na^+ Does Not Prevent the Depolarization Induced by High Ca^{++}

To investigate which ions participate in the electrical response induced by Ca^{++} , intracellular potentials have been recorded in cells perfused with solutions in which one permeant external ion was replaced by another that was presumed to be less permeant. The effect of complete replacement of external Na^+ by Tris^+ was tested in three cells, and in all cases similar qualitative results were obtained. An example is shown in Fig. 6.

In this cell, membrane potential was continuously recorded for ~ 35 min and it was possible to switch eight different times to solutions with different ionic compositions. The impalement was done in a Na-free solution containing 1.5 mM Ca^{++} and the initial resting potential was -68 mV, which is close to the usual value found at this Ca^{++} concentration in cells perfused with the modified Krebs solution. Switching to a full- Na^+ solution with the same Ca^{++} concentration (1.5 mM Ca^{++}) caused only small fluctuations in the membrane potential. The introduction of 2.5 mM Ca^{++} , Na-free solution evoked a depolarization of ~ 35 mV, which was reversed on returning to 1.5 mM Ca^{++} . At this time the introduction of a full- Na^+ solution with 2.5 mM Ca^{++} induced a depolarization to the same voltage as before, but with a faster time course than in the absence of Na^+ . The second run with the same solutions caused the same effects: relatively slow depolarization by Na-free 2.5 mM Ca^{++} , slow repolarization by 1.5 mM Ca^{++} , and fast depolarization by 2.5 mM Ca^{++} .

Although full- Na^+ and Na-free solutions have been compared in only three cells, we think it is safe to say that at 1.5 mM Ca^{++} , external Na^+ does not make an important contribution to the resting potential in parathyroid cells. The magnitude of the depolarization induced by 2.5 mM Ca^{++} is about the same in the presence or absence of Na^+ in the external solution. However, the depolarization induced by Na-free, 2.5 mM Ca^{++} probably has a slower time course than the one observed with full-Na, 2.5 mM Ca^{++} . This difference in time course, although very clear for the cell shown in Fig. 6, was not so apparent in the other two cells, where Na^+ -free and full- Na^+ solutions were compared, and therefore no definitive conclusion can be drawn at present. In cells perfused with Na^+ -free solutions, the repolarization after switching to 1.5 mM Ca^{++} was slower than in cells never exposed to Na^+ -free solutions. This slower repolarization may be due to temporary inactivation of the Na-K pump in cells partially depleted of internal Na^+ after several minutes of being perfused with Na^+ -free solution.

Depolarization Induced by Ca^{++} Is Not Abolished by Ca^{++} Channel Blockers

Since the depolarization induced by Ca^{++} was present even in the absence

of external Na^+ , the hypothesis that a Ca^{++} conductance could be partially responsible for this response was tested in several cells. It has been shown previously (see Fig. 4) that several divalent cations reputed to be Ca^{++} channel blockers (Cd^{++} and Co^{++} , and, often, Mn^{++}) simulate the effects of Ca^{++} in parathyroid cells. In this section we show that neither inorganic nor organic Ca^{++} channel blockers modify the depolarization induced by 2.5 mM Ca^{++} .

The effects of two inorganic Ca^{++} channel blockers are shown in Fig. 7. Part A compares the depolarization induced by 2.5 mM Ca^{++} with and without 2 mM Co^{++} . The cell was impaled in 1.5 mM Ca^{++} (not illustrated

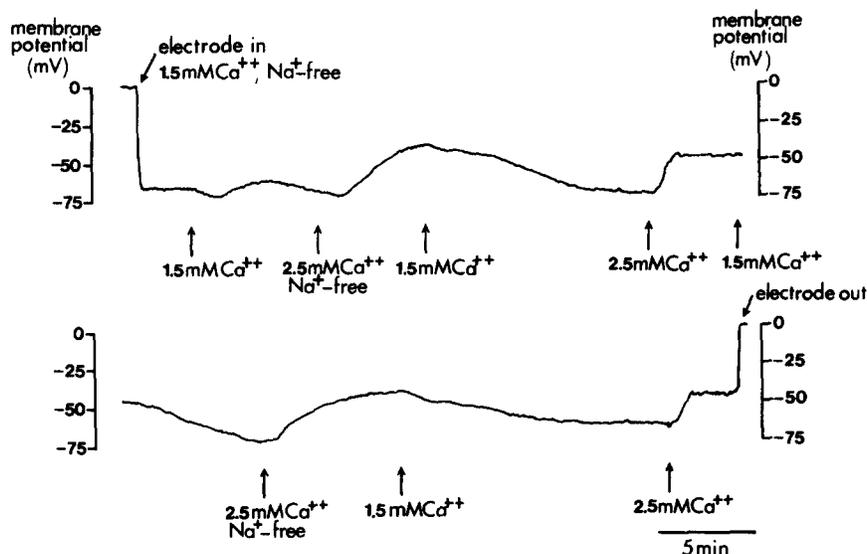


FIGURE 6. Effect of complete replacement of external Na^+ on the depolarization induced by high Ca^{++} . The cell was initially impaled in a 1.5 mM Ca^{++} , Na^+ -free solution, and changes to other solutions are indicated by the arrows. Both panels are continuous in time and belong to the same cell. The composition of solutions is given in Table I. The time courses of the depolarization and repolarization evoked by high and low Ca^{++} , respectively, in the absence of Na^+ are discussed in the text.

in the figure), and several minutes later a solution with 2.5 mM Ca^{++} evoked a reversible depolarization of 33 mV. A second run in 2.5 mM Ca^{++} , but with the addition of 2 mM Co^{++} , yielded a depolarization of 40 mV. The effect of Ca^{++} and Co^{++} was additive and the depolarization was larger. In part B, from another cell, 2.5 mM Ca^{++} depolarizes by ~40 mV even in the presence of 0.5 mM Cd^{++} .

The effects of several concentrations of D600 have been studied in three cells and two of them are illustrated in Fig. 8. In A, the depolarization evoked by 2.5 mM Ca^{++} can be compared in the absence and in the presence of 30, 80, and 100 μM D600. The record in B is from

another cell, where a very large concentration (150 μM) of D600 was used. The response was only slightly smaller than in the control records taken before and after the addition of D600. The effect of 40 μM D600 in 1.5 mM Ca^{++} was tested in two cells, and no appreciable change of membrane potential was observed. The records in Fig. 8 clearly show that large concentrations of D600 have very little, if any, effect on membrane potential depolarization induced by high Ca^{++} . These results suggest that in parathyroid cells, a Ca^{++} current through the usual voltage-

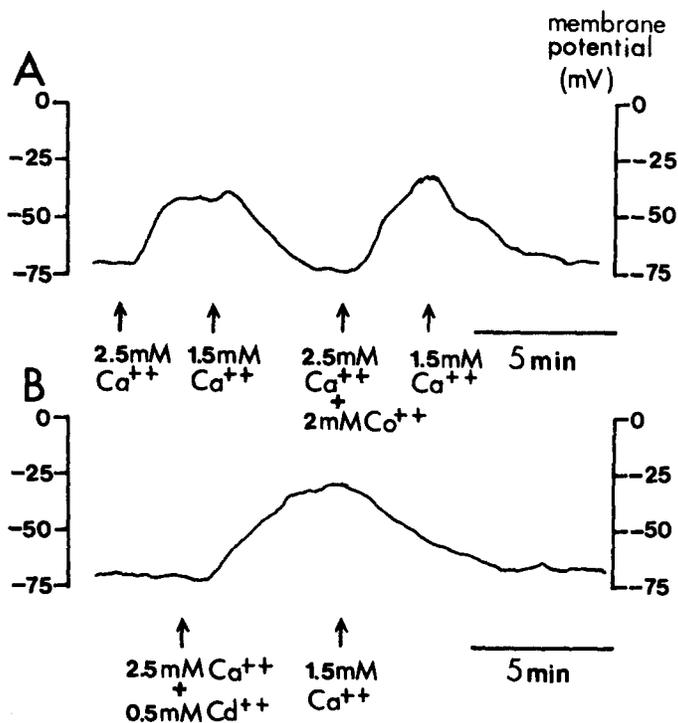


FIGURE 7. Effects of inorganic Ca^{++} channel blockers on the depolarization evoked by high Ca^{++} . *A* shows the membrane potential of a cell initially impaled in 1.5 mM Ca^{++} , with solution changes at the arrows. *B* is from a different cell, initially penetrated in 1.5 mM Ca^{++} . The composition of solutions is the same as in Fig. 2, except that CoCl_2 and CdCl_2 were substituted equimolarly for NaCl .

dependent Ca^{++} channels does not participate in the depolarization seen in high Ca^{++} . This is further supported by the observation that Ba^{++} is less effective than Ca^{++} , even though Ba^{++} is more effective than Ca^{++} in carrying current through Ca channels (Hagiwara et al., 1974).

Effects of Chloride Substitution

In several experiments, 98% of the external Cl^- was replaced by glutamate and the effects of variable concentrations of Ca^{++} and K^+ on parathyroid

cell membrane potential were studied. Complete replacement of Cl^- was done to test whether its presence was necessary for the voltage changes induced by Ca^{++} and to avoid possible hysteresis in the response when studying the effects of alterations in external K^+ (Hodgkin and Horowicz, 1959). The Ca^{++} concentrations used in these solutions were higher than those used in the modified Krebs solution because high concentrations of glutamate decrease Ca^{++} activity. Ca^{++} concentrations used for the low- and high- Ca^{++} solutions were 2 and 4 mM, respectively. These concentra-

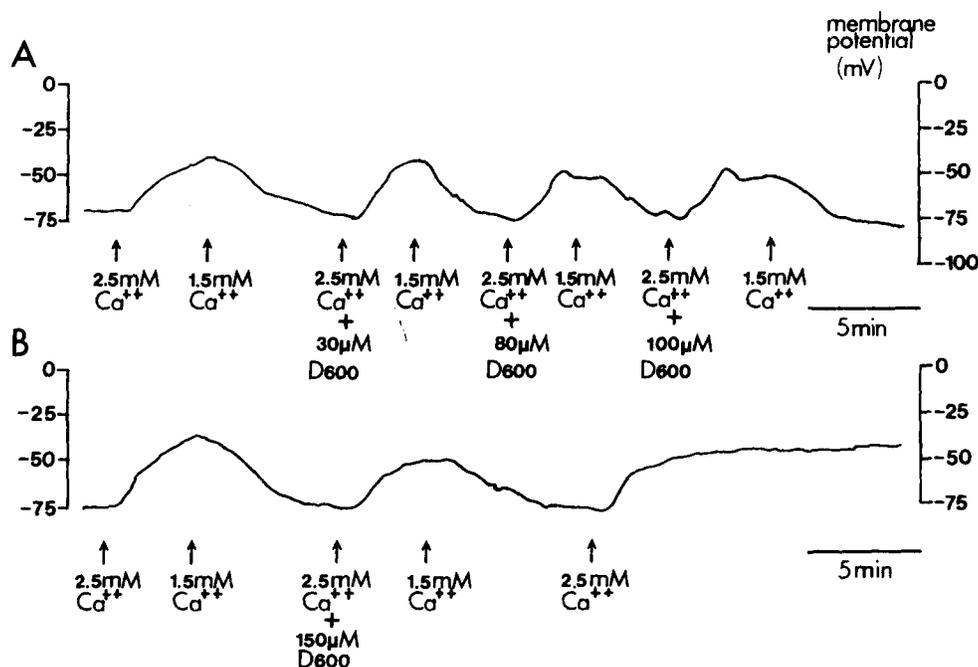


FIGURE 8. The organic Ca^{++} channel blocker D600 does not affect the response to Ca^{++} changes. *A* is a continuous recording from a cell initially impaled in 1.5 mM Ca^{++} with changes to new solutions indicated by the arrows. *B* is from another cell, where a larger concentration of D600 was tested. The composition of solutions is the same as in Fig. 2. Variable quantities of D600 from a 1-mM stock solution were added to the 2.5 mM Ca^{++} solution to obtain the desired final concentration of D600.

tions were chosen empirically because they yielded membrane potential values about the same as 1.5 and 2.5 mM Ca^{++} in the Krebs solution. An estimate with a calcium electrode gave similar calcium activities for both sets of solutions.

Removal of Cl^- does not seem to affect appreciably the resting potential measured in either low or high Ca^{++} at a constant (5 mM) K^+ concentration. Mean values of potential were -78.6 ± 6.6 mV (mean \pm SD, $n = 14$) in 2 mM Ca^{++} and -39.0 ± 7.3 mV (mean \pm SD, $n = 13$) in 4 mM Ca^{++} . Thus, the mean depolarization induced by high Ca^{++} was ~ 39 mV,

which is not very far from the value measured with the Krebs solutions (40 mV) at the same concentration of potassium.

Effects of Alteration in External Potassium

The effects of varying external K^+ at low and high Ca^{++} are illustrated by the records of Fig. 9. Part A shows a continuous intracellular recording

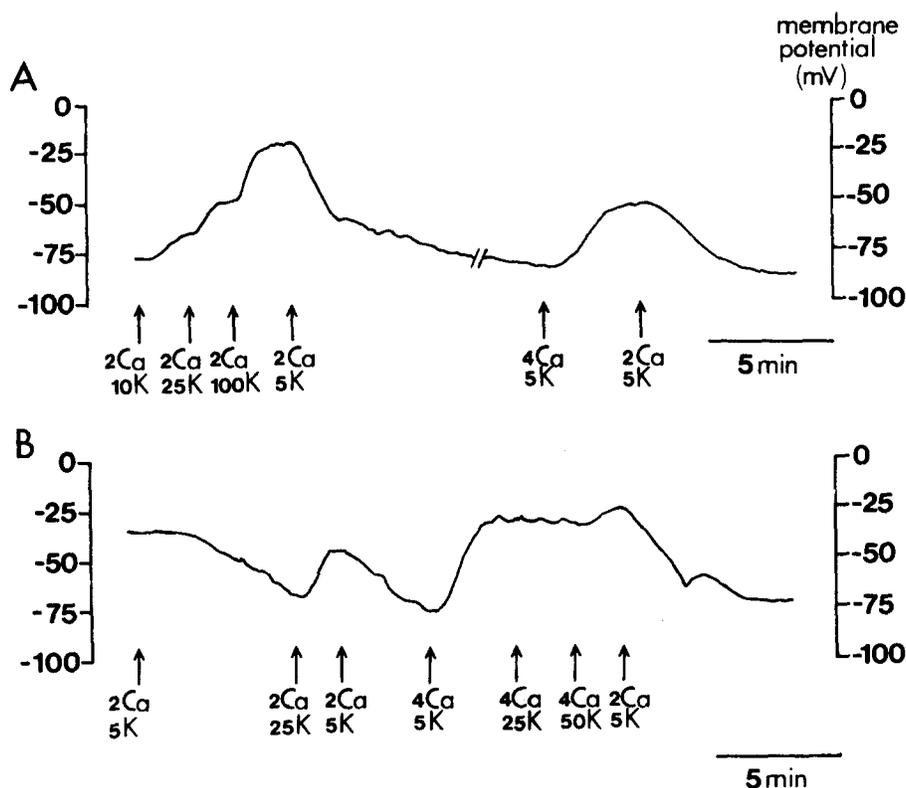


FIGURE 9. Effects of external Cl^- replacement and variable external K^+ concentrations on the electrical activity in parathyroid cells. A is a recording from a cell initially impaled in a 2 mM Ca^{++} , 5 mM K^+ , Cl^- -free solution with changes to new solutions at the time indicated by the arrows. The time between the parallel bars in the record was 2 min. B is from another cell first impaled in a 4 mM Ca^{++} , 5 K^+ , Cl^- -free solution with changes to new solutions at the time pointed out by the arrows. The composition of solution is given in Table I. In all cases, 98% of external Cl^- was substituted by glutamate.

of a cell initially impaled in 2 mM Ca^{++} , 5 mM K^+ solution, with a resting potential of -76 mV. Successive changes to solutions with 10, 25, and 100 mM K^+ at the same Ca^{++} concentration are indicated by the arrows. The cell depolarizes upon an increase in external potassium to a level determined by the concentration. After switching back to the 2 mM Ca^{++} ,

5 mM K^+ solution, the cell repolarizes to approximately the original level. In all cells depolarized by elevated external K^+ , the repolarization showed a slower time course than the depolarization. Fig. 9A also shows that the same cell was depolarized reversibly by 4 mM Ca^{++} at a constant (5 mM) K^+ concentration by ~ 36 mV.

Fig. 9B illustrates a continuous intracellular recording from another cell, which was initially impaled in 5 mM K, 4 mM Ca and had a resting potential of -33 mV. V_m went negative by 34 mV on changing to 5 mM K, 2 mM Ca. The addition of 25 mM K, 2 mM Ca reversibly depolarized the cell by 28 mV. After recovery in 5 mM K, 2 mM Ca, the cell was subsequently depolarized by 43 mV in 5 mM K, 4 mM Ca, reaching a membrane potential of about -30 mV. At this point the addition of 25 and 50 mM K^+ , keeping constant the external Ca^{++} , had little, if any, effect on the membrane potential. The reintroduction of 5 mM K, 2 mM Ca solution repolarized the cell to the previous resting potential level (-74 mV). Fig. 9B also shows that the time course of membrane depolarization and hyperpolarization induced by Ca^{++} is very similar to the voltage changes observed by the introduction or the withdrawal of high K^+ solution.

The records in Fig. 9 are typical examples of the response of parathyroid cells to changes in external Ca^{++} and K^+ in Cl-free solutions, and they suggest that external Cl^- does not make an important contribution to either the resting potential or the Ca^{++} -induced electrical response. In low Ca^{++} , the membrane potential is highly sensitive to external K^+ , but once the cells are depolarized by high Ca^{++} , external K^+ seems to have little effect on the membrane potential.

The effects of external Ca^{++} and K^+ in Cl-free solutions have been studied in many cells and a summary is given in Fig. 10. In the figure, membrane potential in 2 mM (filled symbols) and 4 mM (open symbols) Ca^{++} has been plotted vs. the logarithm of external K^+ . The vertical line represents the standard deviation of the mean and the numbers of observations are in parentheses. In low Ca^{++} , the membrane potential is very sensitive to external K^+ . Fitting by eye, the straight part of the curve (above 25 mM K^+) yields an ~ 61 mV change in membrane potential for a 10-fold change in external K^+ , which is similar to the value predicted by the Nernst equation for a potassium electrode at $37^\circ C$. Extrapolating the straight line to 0 mV membrane potential gives an estimate of the intracellular potassium concentration of 160 mM. At high Ca^{++} , the number of measurements is smaller, but it is very clear that membrane potential is less sensitive to external K^+ . Thus, it seems that at low Ca^{++} , parathyroid cells behave as a potassium electrode and P_K is the dominant permeability. At high Ca^{++} , the ratio of P_K to other permeabilities probably decreases and thus membrane potential is less sensitive to external potassium. The possible participation of different ionic permeabilities in the voltage change seen in parathyroid cells will be analyzed in the Discussion.

Effects of Isoproterenol

It is known that the beta agonist isoproterenol, at concentrations between 1 and 10 μM , increases PTH secretion rate in dispersed bovine parathyroid cells (Brown et al., 1977). We have studied the effects of variable concentrations of isoproterenol (between 10 and 40 μM) added to the modified Krebs solution in four cells whose electrical response to external Ca^{++} had previously been observed. These concentrations of isoproterenol did not have an important effect on the membrane potential either at low or at high Ca^{++} . In one cell there was no voltage change at all. In another cell membrane potential hyperpolarized by 6–7 mV, and in the

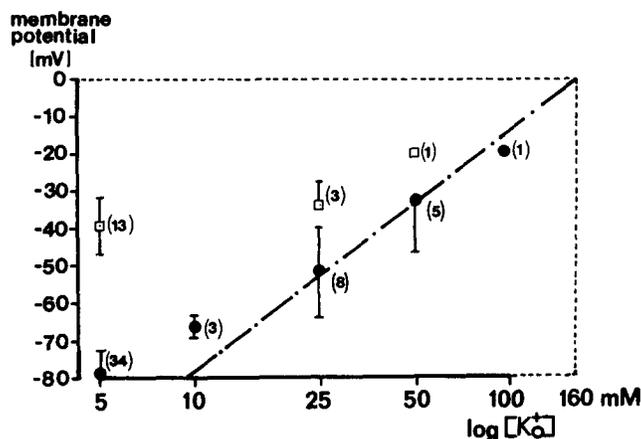


FIGURE 10. Dependence of membrane potential (ordinate) on the external concentration of K^+ represented in a semilogarithmic scale (abscissa). Filled circles are measurements in low Ca^{++} (2 mM) and open squares are measurements in high Ca^{++} (4 mM). Every point represents the mean and the vertical bars represent the standard deviation of several measurements obtained from records like those shown in Fig. 10. The number of measurements is in parentheses. The straight line has been drawn by eye and has a slope of ~ 61 mV per 10-fold increase in external K^+ concentration. The intercept of this line with the 0-mV axis gives an estimate of the internal concentration of K^+ .

other two cases a 4–5-mV depolarization was observed. Although the number of observations is small and no definitive conclusion can be drawn, our data suggest that the mechanism for isoproterenol stimulation of parathyroid cells is different from that evoked by lowering divalent cations (see Discussion).

DISCUSSION

The effects of external calcium concentration on parathyroid cell membrane potential have been reported only twice, with contradictory conclusions. Mouse parathyroid cells hyperpolarize on lowering external Ca^{++}

(Bruce and Anderson, 1979), but cultured goat parathyroid cells are said to do the opposite, having a resting potential of -29.2 mV in 0.8 mM Ca^{++} and -44.7 mV in 2.4 mM Ca^{++} (Sand et al., 1981). Our measurements on rat parathyroid agree qualitatively with observations in the mouse, but the magnitude of our resting potentials is ~ 1.5 times larger than reported for mouse, and twice as large as the reported values for cultured goat cells.

There have been no reports of spontaneous spike activity, regardless of Ca^{++} concentration, and we have found none, even though our cells had resting potentials of very healthy magnitude. In cultured cells, regenerative responses are said to be triggered by injection of depolarizing current through the microelectrode (Sand et al., 1981). The high electrode resistance and low input resistance of the gland prevented us from stimulating effectively through the microelectrode.

Parathyroid cells in the rat are ~ 8 μm in diameter (Roth and Raisz, 1964). Using $1,000$ Ω cm^2 as a guess of membrane resistance (Cole, 1968), the input resistance would be 498 $\text{M}\Omega$ were the cells not coupled to each other by junctions of lower resistance. For goat parathyroid cells in culture, the reported figure is 503 ± 218 $\text{M}\Omega$ in 2.4 mM Ca^{++} and 290 ± 198 $\text{M}\Omega$ at 0.8 mM Ca^{++} . We report a much lower value for input resistance for cells in the gland, 24.3 ± 14 $\text{M}\Omega$. This strongly suggests that in the parathyroid, as in other glands (Petersen, 1980), cells are interconnected by low-resistance junctions. Freeze-fracture replicas of rat parathyroid cells have confirmed the existence of numerous gap junctions (Ravazzola and Orci, 1977; López-Barneo and Franzini-Armstrong, 1982).

Possible Origins of the Depolarization Induced by Divalent Cations

It is first necessary to consider possible but uninteresting explanations of the sensitivity of membrane potential to Ca^{++} concentration. The large magnitude of the resting potential in low Ca^{++} makes it unlikely that we have problems with the seal between membrane and microelectrode. Further, the input resistance is higher in 2.5 mM Ca^{++} , when the cell is depolarized. The depolarization is thus unlikely to result from a failure of the membrane-electrode seal. A second possibility is that raising Ca^{++} causes sufficient Ca^{++} entry to reduce coupling between cells (De Mello, 1975; Iwatsuki and Petersen, 1977; Rose and Loewenstein, 1975). This could lead to depolarization of the impaled cell by the shunt resistance around the electrode, when the impaled cell is no longer supported by its neighbors. This possibility can be discarded on the grounds that Mg^{++} , which is normally present in the cell at high concentration and does not cause uncoupling (De Mello, 1975; Iwatsuki and Petersen, 1977), has an effect on membrane potential similar to Ca^{++} . Transition elements also depolarize parathyroid cells, although they are very impermeant and do not directly affect cell coupling. On occasion, they even compete with Ca^{++} and restore cell coupling (Petersen, 1980). Thyroid follicular cells

from the rat are coupled and have an input resistance value of 11 M Ω (Green and Petersen, 1981). Both in the mouse (Bruce and Anderson, 1979) and in the rat (present experiments), the resting potential of thyroid cells was not affected by changes in external Ca⁺⁺ in the range of interest. Thus, both of these uninteresting explanations are unlikely.

The potential changes observed are much too large to be accounted for as a direct result of a change in the equilibrium potential of Ca⁺⁺. Even if the membrane were exclusively Ca⁺⁺ permeable, changing Ca⁺⁺ from 1.5 to 2.5 mM would change the membrane potential by only 6.7 mV, much less than the observed change. Nor does it seem likely that Ca⁺⁺ channels of the type reported in other tissues play a part in the response. The selectivity of the Ca⁺⁺ channel for alkali-earth divalent cations (Hagiwara et al., 1974; Hagiwara, 1975) contrasts with our findings in parathyroid cells, where no qualitative distinction between the four cations (Ca⁺⁺, Sr⁺⁺, Mg⁺⁺, and Ba⁺⁺) was seen. Ba⁺⁺ is not very effective in depolarizing parathyroid cells although it permeates well through the Ca channel (Hagiwara et al., 1974; Reuter, 1973). In addition, Co⁺⁺, Cd⁺⁺, and Mn⁺⁺, which are Ca⁺⁺ channel blockers (Baker et al., 1973; Kohlhardt et al., 1973; Hagiwara, 1975), mimic the effects of Ca⁺⁺ rather than blocking the response. Finally, the Ca⁺⁺ channel blocker D600 (Kohlhardt et al., 1972) is without effect on the response to raising Ca⁺⁺.

Thus, it seems that parathyroid cells possess an unusual calcium-sensing mechanism of extraordinary sensitivity, not observed in other biological preparations. A similar but much smaller response is seen in the resting secretory membrane of freshwater lamellibranch mantles. A 10-fold decrease in external calcium (from 6 to 0.6 mM) hyperpolarizes by 6 mV (Sorenson et al., 1980).

The order of effectiveness of divalent cations in depolarizing parathyroid cells is very similar to the affinity of Ca-binding proteins and calmodulin-like receptors for several divalent cations. It is known that Mn⁺⁺, Sr⁺⁺, Mg⁺⁺, and Ba⁺⁺ can bind to a brain Ca⁺⁺ regulatory protein (Wolff et al., 1977). In molluscan neurons, activation of potassium permeability by internal Ca⁺⁺ (an effect probably mediated by a Ca⁺⁺ receptor located in the inner side of the membrane) is simulated by other divalent cations (Meech, 1974; Meech and Standen, 1975). Ca⁺⁺ is the most effective ion, followed by Cd⁺⁺, Sr⁺⁺, and Mn⁺⁺. The cations Ba⁺⁺, Co⁺⁺, Mg⁺⁺, and Zn⁺⁺ have been found to be very ineffective (Gorman and Hermann, 1979). About the same order has been found in the exchange of various divalent cations for troponin-bound ⁴⁵Ca⁺⁺ (Fuchs, 1971).

These findings suggest that the interaction of divalent cations with parathyroid cells is not based on their different permeability through the membrane and point toward the possible existence of a divalent cation receptor in the external surface of these cells. This receptor could be a Ca-binding protein with a different affinity for each of the divalent cations that affect membrane voltage. Modifications in membrane permeability

by conformational changes of the receptor after it binds to one or several divalent cations would result in the electrical events associated with alterations in the external concentration of divalent cations. Recent morphological studies on rat parathyroid glands using the freeze-fracture technique (López-Barneo and Franzini-Armstrong, 1982; C. Franzini-Armstrong, unpublished observations) have revealed the existence in the cell membrane of particles that are densely packed, regularly distributed throughout the plasmalemma, and unusually uniform in size. One might speculate that these unusual particles are receptors, whose dense packing confers on these cells their extraordinary Ca^{++} sensitivity.

Input Resistance and Membrane Resistance

The input resistance (R_i) in a tissue that is electrically interconnected by gap junctions depends on both junctional (R_j) and membrane resistance (R_m). The change that we observe on raising external Ca^{++} could arise from altering either factor. We know no mechanism by which extracellular Mg^{++} or transition elements could raise junctional resistance, and therefore we suspect that divalent cations alter membrane resistance.

The magnitude of the R_m change signaled by a 2.4-fold increase in R_i depends on the ratio R_j/R_m (cf. Holman and Neild, 1979, for a somewhat different conclusion). To approach this problem we used a simple model of the tissue, which was adequate for our purposes. The model (Fig. 11A) assumes that the penetrated cell (I) is a sphere of radius 4 μm , surrounded by spherical shells of 8 μm thickness. The shells contain successive generations of neighbors. Fig. 11B illustrates the electrical circuit that corresponds to this three-dimensional array assuming that (a) R_m (resistance of the impaled cell) and R_j (resistance of its junctions to all of its nearest neighbors) are the same for all cells, and (b) the resistivity of extracellular and intracellular media is negligible (Bennett, 1966; Socolar and Loewenstein, 1979). To facilitate computations, only three shells of cells (in addition to the impaled one) have been considered. The values of $R_{m1,2,\text{and}3}$ and $R_{j1\text{and}2}$ (see Fig. 11B) used are given in the figure legend.

Solving this equivalent circuit yields the plot shown in Fig. 11C. The ordinate represents the ratio R_i/R_m at different values of R_j/R_m , as given on the abscissa in a semilog scale. The curve shows that when R_j/R_m is large, R_i/R_m approaches unity, since the impaled cell is well isolated from its neighbors and input resistance reflects only the membrane resistance of the impaled cell. When R_j/R_m is very small, R_i/R_m again approaches unity because the recording is being made from what is in essence a large single cell. For intermediate values of R_j/R_m , it can be seen that R_i/R_m is much smaller than unity and therefore a given increase in R_m is signaled by a smaller change in R_i . The same qualitative relationship between R_m , R_j , and R_i is obtained with a two-dimensional model, but the minimal R_i/R_m occurs at a different ratio of junctional to membrane resistance.

Permeability Changes Involved in the Response to High Calcium

In the absence of other information, the voltage change induced by high

Ca^{++} could be due to either an increase of a depolarizing conductance or a decrease in a polarizing conductance, e.g., P_K . Neglecting the contribution of anions, it can be calculated by the Goldman (1943) equation

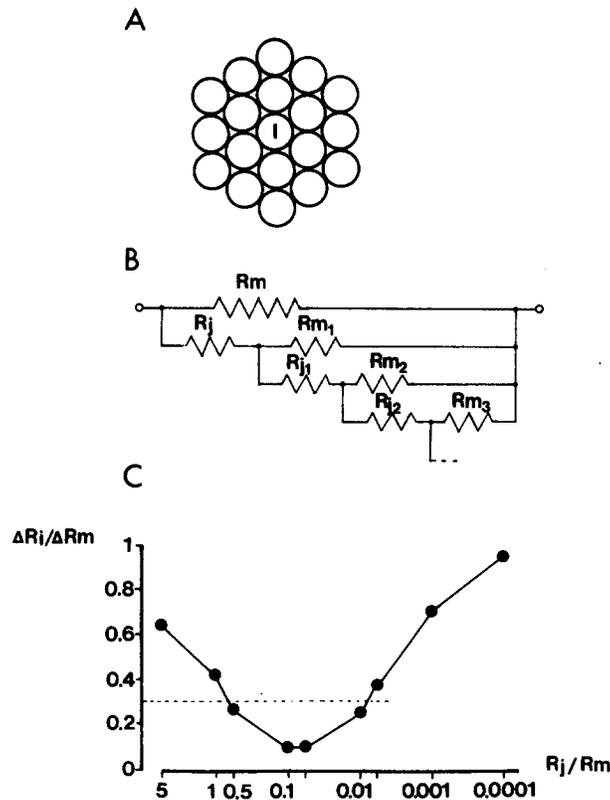


FIGURE 11. Cell-to-cell coupling in the parathyroid gland. *A* shows a model of cell topology in the parathyroid gland. The impaled cell (I) is surrounded by spherical shells of closely packed cells. The first, second, and third shells contain 25, 97, and 216 cells. *B* shows the equivalent electrical circuit of this model. R_m is the membrane resistance of a single cell, and R_j is the parallel resistance of the junctions to all of its neighbors. R_{m1} , R_{m2} , and R_{m3} are the parallel membrane resistance of the first, second, and third shell surrounding the penetrated cell and are, respectively, $R_m/25$, $R_m/97$, and $R_m/216$. R_{j1} and R_{j2} are the resultant junctional resistance between the first and the second shells and between the second and the third shells. These values are $25R_j/97$ and $25R_j/216$, respectively. *C* illustrates that the ratio of the change in input resistance to the change in membrane resistance (R_i/R_m) depends on the ratio R_j/R_m (abscissa). The interrupted line is drawn at a ratio R_i/R_m of 0.3 (a given change in R_m results in a change in R_i that is smaller by a factor of 0.33).

that if a depolarization of 39 mV (-73 mV at 1.5 Ca^{++} and -34 mV at 2.5 mM Ca^{++}) were due to a decrease in P_K , a 7.9-fold increase in membrane permeability would be observed that would be reflected in an

R_m change of about the same size. This calculation is done assuming 10 mM as the intracellular concentration of Na and 160 mM (estimated from the plot in Fig. 10) as the intracellular concentration of K^+ .

In a coupled tissue, an 8-fold increase in R_m can result in a 2.4-fold R_i change, only 30% as large as the R_m change, as discussed above. The interrupted line in Fig. 11C shows that with the three-dimensional model, two different R_j/R_m ratios could give an R_i/R_m ratio of this value. R_j/R_m near 0.01 seems a reasonable ratio for a well-coupled tissue. Therefore, it is not impossible that the depolarization seen in high Ca^{++} is due to a suppression of K^+ permeability. Clarification of this point must await more explicit data on R_m , and this will necessarily involve electrically isolated cells.

Relationship Between Membrane Electrical Events and PTH Secretion

In the mouse (Bruce and Anderson, 1979) and in the rat (present experiments), the relationship between membrane potential and external Ca^{++} is very similar to the relationship between PTH release and external Ca^{++} observed in the cow, both in vivo (Mayer and Hurst, 1978) and in vitro (Brown et al., 1976; Habener and Potts, 1976). PTH release and membrane potential as a function of external Ca^{++} are illustrated in Fig. 12. Part A has been redrawn from Habener and Potts (1976) and part B is based on our experimental data. Both curves are very steep and comparable, although there is a shift of 0.2–0.3 mM along the Ca^{++} concentration axis when they are superimposed. This shift may be related to differences between animal species or to differences in calcium activity between solutions used in biochemical and electrophysiological experiments.

We are not aware of the existence of information relating PTH release and external Ca^{++} in the rat. However, the Ca^{++} range in which a steep change in membrane voltage is seen (1–2.5 mM) is very similar to the range over which reversible morphological changes are seen in cultured rat parathyroid glands (1.3–2.7 mM; Roth and Raisz, 1964).

Other divalent cations also have parallel effects on membrane voltage and PTH secretion. Mg^{++} is 2.5 times less efficient than Ca^{++} in decreasing PTH secretion (Habener and Potts, 1976; Sherwood et al., 1970), and we find that it is ~ 2.4 times less potent than Ca^{++} in depolarizing parathyroid cells. It has recently been observed that Mn^{++} and Sr^{++} are very effective in inhibiting PTH secretion and that Ba^{++} is a poor Ca^{++} substitute in decreasing PTH release (Wallace and Scarpa, 1982). This corresponds quite well with their depolarizing potency in our experiments.

Other points of parallelism between membrane voltage and secretion are that ouabain inhibits PTH secretion, possibly by depolarizing the cells (Brown et al., 1981; Fischer et al., 1982), and that complete removal of external Na^+ (which we have shown does not substantially affect the membrane potential) does not alter the modulation by external Ca^{++} of

PTH secretion in dispersed cells from the cow (Fischer et al., 1982; Wallace and Scarpa, 1982). Taken together, these data strongly suggest that in parathyroid cells, hyperpolarization is associated with increased secretion, and depolarization with decreased secretion.

A separate mechanism, mediated by cAMP, may also exist. Isoproterenol is a potent secretagogue for PTH release (Brown et al., 1977; Fischer et al., 1982), although it does not seem to modify membrane potential. Isoproterenol activation of PTH release, in contrast to low- Ca^{++} -activated secretion (Fischer et al., 1982), is accompanied by a large increase in

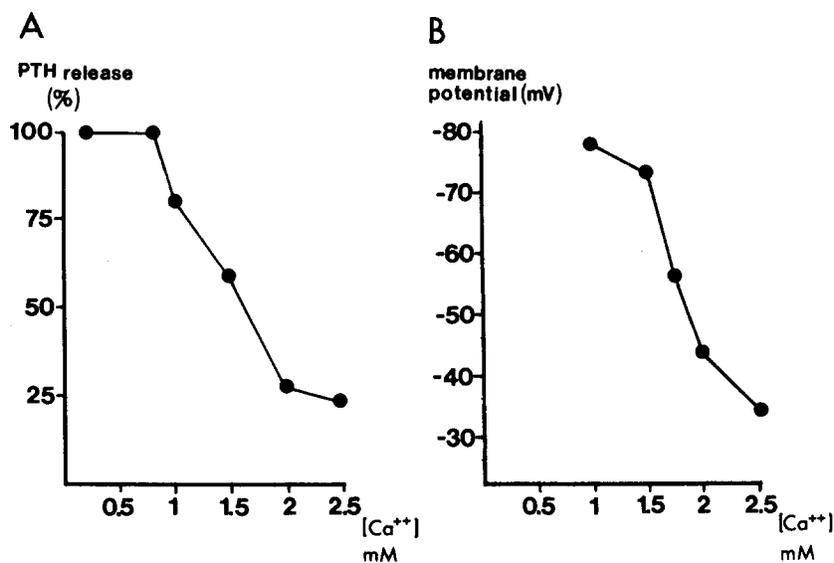


FIGURE 12. Ca^{++} alterations affect PTH secretion and membrane potential in parallel. A has been redrawn from the data of Habener and Potts (1976) on slices of calf parathyroid glands maintained in vitro. The percent of immunoreactive PTH is given in the ordinate and Ca^{++} concentration in the abscissa. The Mg^{++} concentration was held constant at 0.8 mM. Points represent the means of four to six measurements. B is from our results on the rat. The Mg^{++} concentration was held constant at 1.1 mM. Points represent the means of 3–66 measurements.

intracellular cAMP (Brown et al., 1977; Fischer et al., 1982).

This correspondence between membrane voltage and secretion contrasts with the usual sequence of events found in other glands, where the physiological stimulants for secretion evoke a membrane depolarization that opens voltage-dependent Ca^{++} channels. Ca^{++} entry through these channels seems to be responsible for triggering exocytosis, and removal of divalent cations from the external solution turns off secretion (Petersen, 1980; Rubin, 1970). There seem to be some exceptions to this usual pattern. For example, juxtaglomerular cells hyperpolarize by 8–10 mV

after addition of adrenaline, which is a stimulant for renin secretion (Fishman, 1976). In these cells, complete removal of external Ca^{++} and increase of Mg^{++} stimulate renin release (Fray, 1977).

In addition to the electrophysiological findings and the effect of divalent cations on PTH secretion, other evidence supports the view that the mechanism for stimulus secretion-coupling in the parathyroid gland is somehow unusual. Fully reversible secretory activity can be observed in dispersed cells in the absence of Ca^{++} . Further, large doses (up to 10^{-4} M) of the Ca^{++} channel blocker D600 have no effect on PTH secretion activated by low Ca^{++} (Wallace et al., 1983). Finally, it is known that the divalent cation ionophore A23187, which presumably increases cytosolic Ca^{++} (Reed and Lardy, 1972), inhibits PTH secretion (Brown et al., 1980; Fischer et al., 1982; Habener et al., 1977).

In summary, membrane potential of parathyroid cells is steeply dependent on external Ca^{++} in the physiological range. It seems highly likely that the membrane potential change is related to a change in secretory activity (Fig. 12). The relation between membrane voltage, calcium, and secretion is very unusual and suggests the existence of a stimulus-secretion coupling mechanism that has no precedent in the literature. The mechanism of this coupling and the permeabilities involved in the Ca^{++} -induced depolarization remain to be solved.

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