High Extracellular Ca\textsuperscript{2+} Hyperpolarizes Human Parathyroid Cells via Ca\textsuperscript{2+}-activated K\textsuperscript{+} Channels*

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Stina Välimäki\textsuperscript{‡,§}, Anders Höög\textsuperscript{¶}, Catharina Larsson\textsuperscript{‡}, Lars-Ove Farnebo\textsuperscript{¶,‖}, and Brännström

From the Departments of \textsuperscript{‡}Molecular Medicine, \textsuperscript{§}Surgical Sciences, and \textsuperscript{¶}Oncology and Pathology, Karolinska Hospital, Karolinska Institutet, SE-171 76 Stockholm, Sweden

Membrane potential has a major influence on stimulus-secretion coupling in various excitable cells. The role of membrane potential in the regulation of parathyroid hormone secretion is not known. High K\textsuperscript{+}-induced depolarization increases secretion from parathyroid cells. The paradox is that increased extracellular Ca\textsuperscript{2+}, which inhibits secretion, has also been postulated to have a depolarizing effect. In this study, human parathyroid cells from parathyroid adenomas were used in patch clamp studies of K\textsuperscript{+} channels and membrane potential. Detailed characterization revealed two K\textsuperscript{+} channels that were strictly dependent of intracellular Ca\textsuperscript{2+} concentration. At high extracellular Ca\textsuperscript{2+}, a large K\textsuperscript{+} current was seen, and the cells were hyperpolarized (−50.4 ± 15.4 mV), whereas lowering of extracellular Ca\textsuperscript{2+} resulted in a dramatic decrease in K\textsuperscript{+} current and depolarization of the cells (−0.1 ± 8.8 mV, \( p < 0.001 \)). Changes in extracellular Ca\textsuperscript{2+} did not alter K\textsuperscript{+} currents when intracellular Ca\textsuperscript{2+} was clamped, indicating that K\textsuperscript{+} channels are activated by intracellular Ca\textsuperscript{2+}. The results were concordant in cell-attached, perforated patch, whole-cell and excised membrane patch configurations. These results suggest that [Ca\textsuperscript{2+}]\textsubscript{i}, regulates membrane potential of human parathyroid cells via Ca\textsuperscript{2+}-activated K\textsuperscript{+} channels and that the membrane potential may be of greater importance for the stimulus-secretion coupling than recognized previously.

Extracellular free calcium ([Ca\textsuperscript{2+}]\textsubscript{o}), regulates the secretion of parathyroid hormone (PTH)\textsuperscript{1} from parathyroid cells through a cell surface Ca\textsuperscript{2+} sensing receptor (CaR) (1–3). Low [Ca\textsuperscript{2+}]\textsubscript{o}, stimulates the secretion of PTH, whereas high [Ca\textsuperscript{2+}]\textsubscript{i}, inhibits the secretion via activation of CaR. The second messenger mediating the inhibition is the free intracellular Ca\textsuperscript{2+} ([Ca\textsuperscript{2+}]\textsubscript{i}) (4). In this respect, the parathyroid cell differs from the majority of other endocrine cells, in which secretion is stimulated by high [Ca\textsuperscript{2+}]\textsubscript{i}. The molecular mechanisms by which [Ca\textsuperscript{2+}]\textsubscript{i}, regulates the secretion of PTH are not well understood.

In various endocrine cells, the membrane potential is one of the key players in the cellular signaling, and depolarization of the cell membrane triggers hormone secretion. It is generally considered that resting membrane potential in the majority of excitable and endocrine cells is determined by K\textsuperscript{+} conductance and that high extracellular K\textsuperscript{+} induces membrane depolarization and thereby hormone secretion. Interestingly, high K\textsuperscript{+}-induced depolarization of the parathyroid cell increases PTH secretion (5). In contrast, increased [Ca\textsuperscript{2+}]\textsubscript{o}, which causes inhibition of PTH secretion, has also been shown to cause membrane depolarization (6–8).

The majority of previous electrophysiological studies have been performed in bovine or rodent parathyroid cells, and some studies indicate that highly relevant differences may exist between species. Furthermore, only two studies have applied modern electrophysiological techniques on human parathyroid cells (9, 10). It has been shown that the parathyroid cell possesses several types of K\textsuperscript{+} channels, although the role of these K\textsuperscript{+} channels is not completely understood (9–12).

The aim of the present study was to investigate the relationship between [Ca\textsuperscript{2+}]\textsubscript{o}, [Ca\textsuperscript{2+}]\textsubscript{i}, K\textsuperscript{+} channels, and membrane potential in human parathyroid cells with patch clamp technique. Detailed characterization of K\textsuperscript{+} currents revealed a strict dependence of [Ca\textsuperscript{2+}]\textsubscript{i}. At low [Ca\textsuperscript{2+}]\textsubscript{i}, only a small K\textsuperscript{+} current could be detected, and the cells were depolarized, whereas elevation of [Ca\textsuperscript{2+}]\textsubscript{i}, resulted in dramatic enhancement of K\textsuperscript{+} current, and the cells were hyperpolarized. Taken together, these findings demonstrate that extracellular Ca\textsuperscript{2+} regulates membrane potential of human parathyroid cells via Ca\textsuperscript{2+}-activated K\textsuperscript{+} channels.

EXPERIMENTAL PROCEDURES

Parathyroid Samples—Parathyroid adenoma cells were obtained from 14 patients operated on for primary hyperparathyroidism. The median age of the patients at the time of the operation was 63 years (range 27–83). The preoperative median concentration of serum calcium was 2.78 mmol/liter (range 2.48–3.13; reference range 2.20–2.60), and the preoperative median concentration of serum PTH was 144 ng/liter (range 57–418; reference range 12–55). The median adenoma weight was 400 mg (range 100–2300). The routinely performed histopathological evaluation of the parathyroid samples included hematoxylin-eosin staining and Oil Red O fat staining on frozen sections. Informed consent was obtained from all patients, and the study was approved by the local ethics committee of the Karolinska Hospital.

Preparation of Cells—Pieces of parathyroid adenomas were transplanted immediately after surgery to the laboratory at room temperature in sterile tubes. The tissue pieces were placed in sterile plates containing serum-free keratinocyte medium (Keratinocyte-SFM, Invitrogen) containing 5 ng/ml epidermal growth factor, 50 \( \mu \)g/ml bovine pituitary extract, 100 units/ml penicillin G sodium, and 100 \( \mu \)g/ml streptomycin sulfate (Invitrogen). The Ca\textsuperscript{2+} concentration of this medium is 0.09 mM. This serum-free medium has been shown previously to be suitable for culturing human parathyroid cells for a considerable time, enabling physiological studies (13). Possibly remaining adipose or connective tissue was macro-

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† To whom correspondence should be addressed: Dept. of Surgical Sciences, Karolinska Institutet, Karolinska Hospital P9:03, SE-171 76 Stockholm, Sweden. Tel.: 46-8-517-73727; Fax: 46-8-33-15-87; E-mail: lars-ove.farnebo@kirurgi.ki.se.

‡ The abbreviations used are: PTH, parathyroid hormone; CaR, Ca\textsuperscript{2+}-sensing receptor; pF, picofarads.
thus demonstrating their parathyroid origin.

To confirm the parathyroid origin of these cells, a panel of antibodies was employed, including one raised against the N-terminal part of the human PTH (amino acids 1–34, NovoCastra, Newcastle upon Tyne, UK, clone OP-4, mouse IgG1, diluted 1:600). Single parathyroid cells were then plated into Nunclon™ Surface plates (NUNC™, Nalge Nunc International, Roskilde, Denmark) using the following procedure. First, one droplet of medium was added into a plate. The tissue was then held with forceps in contact with medium when mincing the tissue with a sterile scalpel. This enabled the cells to grow in a confluent manner and be separated from the underlying tissue.

Immunostaining—To demonstrate the parathyroid identity of the cells, immunocytochemistry was performed using an antibody raised against the N-terminal part of the human PTH (amino acids 1–34, NovoCastra, Newcastle upon Tyne, UK, clone OP-4, mouse IgG1, diluted 1:600). Single parathyroid cells were then plated into Nunclon™ Surface plates (NUNC™, Nalge Nunc International, Roskilde, Denmark) using the following procedure. First, one droplet of medium was added into a plate. The tissue was then held with forceps in contact with medium when mincing the tissue with a sterile scalpel. This enabled the cells to grow in a confluent manner and be separated from the underlying tissue.

Electrophysiology—Single- and whole-cell currents were recorded with the patch clamp technique (14), using an Axopatch 200 patch clamp amplifier (Axon Instruments Inc., Foster City, CA). Whole-cell currents were recorded using the conventional or perforated patch whole-cell configuration of the patch clamp technique, and whole-cell currents are expressed as total current divided by cell capacitance, if not stated otherwise. Single channel studies were performed using inside-out and cell-attached configurations. Current traces are displayed according to the convention, upward deflections denoting outward currents. All experiments were performed at room temperature (22–24 °C).

Solutions—The extracellular solution contained 138 mM NaCl, 5.6 mM KCl, 1.2 mM MgCl₂, 5 mM HEPES-NaOH, and 2.6 mM CaCl₂. The pH of this solution was 7.4. The standard intracellular solution consisted of 2.6 mM KCl, 10 mM EGTA, 25 mM KOH, and 5 mM HEPES-KOH at pH 7.15. The free Ca²⁺ concentration of this solution is calculated to be <10 nM using the MaxChelator software version 2.10, which uses the method described by Bers et al. (15). A total of 100 μl of 1 M CaCl₂ was added to 10 ml of intracellular solution to produce the required free Ca²⁺ concentration of 10 μM. In the perforated patch experiments, the pipette solution contained 10 mM KCl, 76 mM K₂SO₄, 10 mM NaCl, 1 mM MgCl₂, 10 mM HEPES-NaOH at pH 7.35 (KOH), and 200 μg/ml amphotericin B dissolved in dimethyl sulfoxide (Me₂SO). The final concentration of Me₂SO was less than 0.1%. All chemicals were of analytical grade and obtained from Sigma.

Data Analysis—For analysis of mean current, the channel recordings were filtered at 2 kHz (-3 db value, 8-pole Bessel filter; Frequency Devices, Haverhill, MA), digitized at 10 kHz and stored in a computer, using an Axon Instrument analogue digital converter (TL-1). The data are presented as mean values ± S.E. Channel activities were compared using Student’s t test or analysis of variance for multiple groups, and p values less than 0.05 were regarded as significant.
RESULTS

To further characterize the function of K⁺/H⁺ channels in parathyroid cells, a series of patch clamp experiments were performed on human parathyroid adenoma cells. The configurations applied were whole-cell, cell-attached, excised patch, and perforated patch. The findings are summarized in Figs. 1–4.

**The Activity of K⁺/H⁺ Current in Human Parathyroid Adenoma Cells Depends on \([Ca^{2+}\)]_{i}—** In the whole-cell configuration, human parathyroid cells were voltage-clamped at \(-80 \text{ mV} \) and subsequently depolarized according to the protocol presented in Fig. 1. The K⁺ currents were measured at two different \(Ca^{2+}\) concentrations in the pipette, corresponding to \([Ca^{2+}\)]_{i}. In the presence of 10 \( \mu \text{M} \) \([Ca^{2+}\]), the K⁺ current was significantly higher than the K⁺ current in the presence of low \(<10 \text{ nM}\) \([Ca^{2+}\]), \((105 \pm 29 \text{ pA/\mu F} \text{ versus } 8.3 \pm 4.3 \text{ pA/\mu F}; p < 0.01; n = 20; \) measured at +70 mV; Fig. 1, A and B). The K⁺ current was almost completely blocked by the addition of extracellular 10 mM tetraethylammonium (data not shown). When \([Ca^{2+}\] was clamped, changes in \([Ca^{2+}\] did not alter K⁺ currents \((p = 0.56; \text{Fig. 1C})\). At low \([Ca^{2+}\] (<10 nM), fundamentally no K⁺ current was seen even at 2.6 mM \([Ca^{2+}\]o. At high \([Ca^{2+}\] (10 \( \mu \text{M}\)), a distinct K⁺ current was activated both in the presence of 0.5 and in the presence of 2.6 mM \([Ca^{2+}\]o (Fig. 1C). Taken together, this indicates that human parathyroid adenoma cells display K⁺ channels, which have \(Ca^{2+}\) binding site/sites located on the inside of the cell membrane, and that these channels are activated by \([Ca^{2+}\]. Since the internal milieu of the cell is modulated in this configuration with the possible loss of crucial intracellular signal transduction molecules, we continued the characterization of the nature and function of the K⁺ channels in the following experiments.

**Human Parathyroid Adenoma Cells Have at Least Two Types of \(Ca^{2+}\)-activated K⁺ Channels**—In a previous study on bovine cells, a big conductance 175-pS K⁺-selective \(Ca^{2+}\)-activated channel maximally activated at \(-160 \text{ mV}\) \([Ca^{2+}\], has been reported (11). In human parathyroid adenoma cells, a 35-pS...
and a small 12-pS K⁺ channel have been described that are both sensitive to [Ca²⁺]ᵢ. (9). Here we used the excised patch configuration to study single K⁺ channels and to further characterize the Ca²⁺-activated K⁺ channels seen in the whole-cell configuration. In Fig. 2, single K⁺ channels from human parathyroid adenoma cells are displayed. In the presence of low [Ca²⁺]ᵢ, i.e., 5 mM [Ca²⁺]ᵢ, only few K⁺ channels were seen in parathyroid adenoma cells (Fig. 2, B and C). Single channels were only possible to evaluate at low [Ca²⁺]ᵢ and +80 mV, and the single channel conductances of these channels were 168 ± 10 pS and 48 ± 4 pS, respectively (Fig. 2D). The channel open times for the big and small conductance channels were 2.1 ± 0.9 mS and 9.8 ± 7.0 mS. Both K⁺ channels appear to be voltage-sensitive since they were activated at low [Ca²⁺]ᵢ and +80 mV (Fig. 2C). The addition of high [Ca²⁺]ᵢ solution enhanced K⁺ channel activity dramatically at both +80 mV and −80 mV (Fig. 2, B and C). Current-voltage relation for K⁺ currents in inside-out recordings displayed an out-

Fig. 3. Extracellular Ca²⁺ regulates K⁺ channel activity in cell-attached recording. An extracellular solution with a total of 50 mM K⁺ was used to suppress parathyroid cell membrane potential. As shown in A, at 2.6 mM [Ca²⁺]₀, K⁺ channels are active. Lowering of [Ca²⁺]₀ to 0.5 mM suppresses K⁺ channel activity and a base-line drift, indicating a change of membrane potential, is seen. Intermediate [Ca²⁺]₀ (1.25 mM) causes recovery of channel activity but not to the same extent as that seen in the presence of 2.6 mM. B, a voltage-clamp protocol comparable with Fig. 2A (with the difference that the steps were run from −80 mV to +80 mV in the pipette) was applied. C, in expanded current traces, a small negative shift of the membrane potential at 2.6 mM [Ca²⁺]₀ since symmetrical K⁺ solutions were used. In i, zero current was measured at pipette potential 0 mV, equivalent with a membrane potential of 0 mV, whereas in ii, zero current was at −20 mV, indicating a membrane potential of −20 mV. In B and C, arrowheads indicate zero current, and i and ii represent current traces at 0.5 and 2.6 mM [Ca²⁺]₀, respectively.
wardly rectifying behavior (Fig. 2C). These experiments demonstrate that there are at least two types of \(\text{K}^+\) channels present in the parathyroid adenoma cell membrane, both of which are activated by \(\text{Ca}^{2+}\).

\([\text{Ca}^{2+}]_o\) Activates \(\text{K}^+\) Currents via \(\text{Ca}^{2+}\) and Hyperpolarizes Membrane Potential—To investigate whether the \(\text{K}^+\) current was sensitive to changes in \(\text{Ca}^{2+}\), whereas the internal cell milieu was maintained, the cell-attached and perforated patch configurations were used. Since increases in \(\text{Ca}^{2+}\) lead to increases in \(\text{Ca}^{2+}\), via activation of CaR (1, 2), one would expect an increase of the \(\text{K}^+\) currents when elevating \(\text{Ca}^{2+}\).

In Fig. 3, the cell-attached configuration was used. An excess of \(\text{K}^+\) was added to extracellular solution to decrease the influence of endogenous membrane potential on \(\text{K}^+\) currents. In the extracellular solution, \(\text{[K}^+]_o\) was 50 mM, whereas the pipette solution contained 150 mM \(\text{K}^+\). Pipette potential was held at 0 mV. At 2.6 mM \(\text{[Ca}^{2+}]_o\), \(\text{K}^+\) channel activity was seen, whereas lowering of \(\text{[Ca}^{2+}]_o\) to 0.5 mM resulted in significant decrease of channel activity. Intermediate \(\text{[Ca}^{2+}]_o\) caused recovery of channel activity, but not to the same extent as seen in the presence of 2.6 mM (Fig. 3A). Base-line drift seen in low \(\text{[Ca}^{2+}]_o\) is likely to be due to a change of membrane potential and was noted in all recordings. Since 50 mM \(\text{K}^+\) in extracellular solution was used, this approach allows estimation of membrane potential even in the cell-attached configuration, in which no access to the cell interior is obtained. At 0.5 mM \(\text{[Ca}^{2+}]_o\), zero current was measured at pipette potential 0 mV, equivalent with a membrane potential of 0 mV. At 2.6 mM \(\text{[Ca}^{2+}]_o\), zero current was measured at 20 mV, indicating a membrane potential of 20 mV (Fig. 3C).

Further support of this observation was gained by using the perforated patch configuration. Using this technique, the cells were current-clamped, and membrane potential was measured while changing the \(\text{[Ca}^{2+}]_o\). As expected and in agreement with our results in the whole-cell and cell-attached configurations, the \(\text{K}^+\) currents increased significantly from 5.6 ± 5.6 pA/pF to 33.0 ± 6.9 pA/pF when \(\text{[Ca}^{2+}]_o\) was elevated from 0.5 mM to 2.6
mm (*, p < 0.05; n = 6; measured at +70 mV, Fig. 4B). At low [Ca\textsuperscript{2+}]\textsubscript{o}, the cells were depolarized, whereas an increase of [Ca\textsuperscript{2+}]\textsubscript{o} lead to hyperpolarization of the cell membrane (Fig. 4C). Compiled data showed that in the presence of low [Ca\textsuperscript{2+}]\textsubscript{o}, the cell membrane was depolarized to −0.1 ± 8.8 mV, whereas following increase of [Ca\textsuperscript{2+}]\textsubscript{i}, to 2.6 mM, the cells were hyperpolarized to −50.4 ± 13.4 mV (***, p < 0.001; n = 12; Fig. 4).

**DISCUSSION**

Two important elements influencing the stimulus-secretion coupling in various excitable and endocrine cells are [Ca\textsuperscript{2+}]\textsubscript{o}, and membrane potential. The parathyroid cell is an exceptional secretory cell since increases in [Ca\textsuperscript{2+}]\textsubscript{o} are associated with inhibition of PTH secretion. This contrasts the general principle by which elevated [Ca\textsuperscript{2+}]\textsubscript{i} stimulates secretion. The parathyroid cell is also remarkable because high [Ca\textsuperscript{2+}]\textsubscript{o}, which inhibits PTH secretion, has been postulated to cause depolarization of the parathyroid cells (6–8). Therefore, further studies are obviously needed to understand the relation between [Ca\textsuperscript{2+}]\textsubscript{i}, membrane potential, and PTH secretion. Another reason for renewed investigation of the role of the membrane potential is a most interesting finding of Oeting et al. (16), which seems to have gone unnoticed by other investigators. This group showed that permeabilization of parathyroid cells by microelectrodes or indirectly using a voltage-sensitive dye (6) lead to 2.6 mM, the cells were hyperpolarized to 85 mV, whereas low-

We further show that at high extracellular Ca\textsuperscript{2+}, a large K\textsuperscript{+} current is seen, and the cells are hyperpolarized, whereas lowering of extracellular Ca\textsuperscript{2+} results in a dramatic decrease in K\textsuperscript{+} current and depolarization of the cells. The finding that human parathyroid adenoma cells are depolarized in low [Ca\textsuperscript{2+}]\textsubscript{o}, is compatible with the study by Dempster et al. (5), who showed that high K\textsuperscript{+} increases hormone secretion from the parathyroid cells. Also in line with our results is the study by Oetting et al. (16), which showed that the inhibiting effect of [Ca\textsuperscript{2+}]\textsubscript{o}, on PTH secretion disappeared when membrane potential was absent. Together these data clearly indicate that the stimulation of PTH secretion is coupled to the depolarization as in other endocrine cells. In insulin-secreting β-cells, the stimulation of insulin secretion is induced by the closure of K\textsubscript{ATP} channels, depolarization of the β-cell plasma membrane, and opening of the voltage-gated Ca\textsuperscript{2+}-channels (20). In rat lacticotrophs, erg-like inward-rectifying K\textsuperscript{+} current and Ca\textsuperscript{2+}-activated K\textsuperscript{+} current are coupled to the depolarization stimulating prolactin secretion (21), whereas in rat corticotrophs, corticotropin-releasing hormone triggers membrane depolarization via a protein kinase A-dependent closure of K\textsuperscript{+} channels, ultimately leading to the release of adrenocorticotropic (22). In conclusion, we propose that the molecular mechanism for inverted relation between [Ca\textsuperscript{2+}]\textsubscript{o} and PTH secretion is the Ca\textsuperscript{2+}-activated K\textsuperscript{+} channel, which controls the membrane potential.

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