Parathyroid cells express dihydropyridine-sensitive cation currents and L-type calcium channel subunits

WENHAN CHANG,1 STACY A. PRATT,1 TSUI-HUA CHEN,1 CHIA-LING TU,1 GABOR MIKALA,2 ARNOLD SCHWARTZ,3 AND DOLORES SHOBACK

1Endocrine Research Unit, Department of Medicine, Veterans Affairs Medical Center, University of California, San Francisco, CA 94121; 2Institute of Molecular Pharmacology and Biophysics, University of Cincinnati Medical Center, Cincinnati, Ohio 45267; 3Division of Clinical Pharmacology, First Department of Internal Medicine, Imre Haynal University of Health, H-1135 Budapest, Hungary

Received 27 July 2000; accepted in final form 7 March 2001

Parathyroid cells express dihydropyridine-sensitive cation currents and L-type calcium channel subunits. Am J Physiol Endocrinol Metab 281: E180–E189, 2001.—Parathyroid cells express Ca2+-conducting currents that are activated by raising the extracellular Ca2+ concentration ([Ca2+]o). We investigated the sensitivity of these currents to dihydropyridines, the expression of voltage-dependent Ca2+ channel (VDCC) subunits, and the effects of dihydropyridines on the intracellular free [Ca2+]i ([Ca2+]i) and secretion in these cells. Dihydropyridine channel antagonists dose dependently suppressed Ca2+-conducting currents, and agonists partially reversed the inhibitory effects of the antagonists in these cells. From a bovine parathyroid cDNA library, we isolated cDNA fragments encoding parts of an α1A-subunit and a β3-subunit of L-type Ca2+ channels. The α18-subunit cDNA from the parathyroid represents an alternatively spliced variant lacking exon 29 of the corresponding gene. Northern blot analysis and immunocytochemistry confirmed the presence of transcripts and proteins for α1- and β3-subunits in the parathyroid gland. The addition of dihydropyridines had no significant effects on high [Ca2+]o-induced changes in [Ca2+]i, whereas Ca2+ influx across the membrane is probably responsible for sustained changes in [Ca2+]i.

Studies by Fitzpatrick and colleagues (16, 18, 26) have suggested a role for L-type Ca2+ channels in regulating intracellular Ca2+ mobilization and PTH release. Other groups, however, found no effects of dihydropyridines on the same parameters (23). Expression of classic L-type channels in parathyroid cells is further contested by the observation that membrane depolarization, which activates L-type Ca2+ channels, has no significant effect on [Ca2+]i or parathyroid function (28, 37). These aspects of parathyroid cell signal transduction remain controversial. The current studies were undertaken to address whether parathyroid cells express L-type Ca2+ channels and whether these channels participate in high [Ca2+]o-regulated cellular functions.

On the basis of electrophysiological and pharmacological criteria, voltage-dependent Ca2+ channels (VDCCs) are classified into L-, N-, T-, P/Q-, and R-types (1). Each of these channels consists of a pore-forming α1-subunit and accessory proteins, including α2δ-, β-, and γ-subunits (7, 8, 32, 36). The structure of the α1-subunit determines ion selectivity, voltage sensitivity, and binding specificity to its ligands (7, 33, 36, 38). L-type VDCCs are characterized by their sensitivity to changes in membrane potential and high affinities for 1,4-dihydropyridines, phenylalkylamines, and benzothiazepines (1, 33, 36). Three major subtypes of L-type α1-subunits have been described, including: 1) the α1C in heart, smooth muscle, and neurons; 2) the α1S in skeletal muscle; and 3) the α1D in neuroendocrine cells (1, 33, 35). Each α1-subunit contains four membrane-associated motifs (I-IV), and each motif is comprised of six membrane-spanning domains (S1-S6). Recent stud-

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Am J Physiol Endocrinol Metab
ies have identified the dihydropyridine binding sites in the S5 and S6 domains of motif III (III-S5 and III-S6) and the S6 domain of motif IV (IV-S6) of L-type α₁-subunits (8, 33). The sensors of membrane potential are also localized to the S6 domain in each motif (I-IV) (2, 36). The genomic structure of L-type α₁-subunits is relatively complex. The human skeletal α₁S-subunit gene, for example, spans 90 kb and consists of 44 exons (20). Alternatively spliced transcripts of this gene have been identified and possibly contribute to the molecular and functional diversity of L-type α₁-subunits in different tissues (15, 25).

We previously identified and characterized nifedipine-sensitive cation-selective currents in parathyroid cells, whose activity increased with rising [Ca²⁺]₀ (9). Although these currents can conduct Ca²⁺, they are not voltage gated like other dihydropyridine-sensitive L-type channels (9). These findings suggested that the channels that bind nifedipine and conduct Ca²⁺ currents in parathyroid cells may differ from classic L-type channels. To define further the pharmacology and molecular identity of high [Ca²⁺]₀-induced, nifedipine-sensitive cation currents in these cells, we examined the effects of different dihydropyridine agonists and antagonists on these currents and isolated cDNA fragments encoding L-type channel subunits. We found that the high [Ca²⁺]₀-activated Ca²⁺ currents were modulated in a dose-dependent manner by (+)- and (−)-202–791 and R- and S-BAY K 8644. Clones isolated from a bovine parathyroid cDNA library showed substantial homology to human α₁S- and β₃-subunits of L-type Ca²⁺ channels. The expression of RNA transcripts and protein of L-type channel subunits in parathyroid cells was further confirmed by Northern analysis and immunocytochemistry. We further found that dihydropyridines had no significant effects on high [Ca²⁺]₀-induced increases in [Ca²⁺], and PTH release, suggesting that L-type Ca²⁺ channels in parathyroid cells do not participate in the immediate responses to the changes in [Ca²⁺]₀.

MATERIALS AND METHODS

**Materials**

R- and S-BAY K 8644 were purchased from Calbiochem (La Jolla, CA). (+)- and (−)-202–791 were obtained from Calbiochem and Novartis Pharma (Basel, Switzerland). Media were prepared by the Cell Culture Facility of the University of California, San Francisco. Antibodies against the α₁-subunit of L-type Ca²⁺ channels (anti-pan-α₁) were raised against a peptide (pan-α₁, amino acid sequence: DNPDYL-TRDWSILGP-HHLD) within an intracellular domain between motif IV and the COOH terminus and were affinity-purified by Alomone Labs (Jerusalem, Israel). This epitope is otherwise noted. All sequencing was done by the Biomolecular Core Facility of the University of California, San Francisco. Human parathyroid glands were obtained at the time of surgery for primary hyperparathyroidism according to protocols approved by the Committee on Human Research at the University of California, San Francisco.

**Preparation of Parathyroid Cells**

Bovine parathyroid cells were isolated after collagenase and DNase digestion of parathyroid gland fragments, as previously described (9). For electrophysiological studies, isolated cells were plated on no. 1 round coverglasses and incubated for 30 min at 37°C before recording (9). For measurements of [Ca²⁺], cells were cultured on no. 1 round coverglasses for 12–18 h in MEM with FCS (2%) and penicillin/streptomycin (100 U/ml).

**Whole Cell Recording**

Whole cell voltage clamping was performed using glass pipettes with an electrical resistance of 1–4 MΩ, as previously described (9). Membrane potential (Vₘ) was controlled, and membrane current (Iₘ) was detected by an Axo-Patch amplifier (Axon Instruments, Foster City, CA). Channel activity was assessed by calculating the membrane conductance (Gₘ) derived from the slope of the Iₘ-Vₘ plots. Iₘ-Vₘ plots were created using the following voltage-clamping protocol (Fig. 1, i). Cells were held at −60 mV; then a series of 150-ms test voltage pulses were applied at 2-s intervals in increments of 20 mV from −100 to +120 mV. The current traces were recorded from 20 ms before to 25 ms after each applied voltage pulse. The downward and upward deflections represent the inward and outward currents, respectively. Arrows in Figs. 1–3 represent zero current level. Membrane currents used for making Iₘ-Vₘ plots are the arithmetic means of the currents recorded during the voltage pulses. Representative experiments are shown in Figs. 1–3, and all experiments were performed on ≥3 cells at room temperature, unless otherwise specified.

**Electrode solutions.** Recordings were performed with a whole cell electrode solution containing (mM): 140 Cs-MES, 5 MgCl₂, 10 EGTA, 10 HEPES (pH 7.4), 4 MgATP, 0.3 GTP, and a nucleotide-regenerating system (NRS: 14 mM phosphocreatine and 50 U/ml creatine phosphokinase) (9).

**Bath solutions and extracellular bath perfusion.** All bath solutions (BS) contained 10 mM HEPES (pH 7.4) and 10 mM tetraethylammonium (TEA) to block endogenous K⁺ currents (9). Various [Ca²⁺] in the BS (0.7–90 mM) were achieved by the addition of Ca acetate. Acetate was used as the anion charge-carrier to minimize the activity of endogenous CI⁻ currents (9). Osmolarity of the BS was adjusted to ≈330 mosM/l with sucrose as needed. Each BS is specified by the concentration of its major cation species as follows

90 Ca/10 TEA acetate BS = (in mM) 90 Ca²⁺, 10 TEA⁻,
190 acetate⁻, 10 HEPES (pH 7.4)
0.7 Ca/10 TEA acetate BS = (in mM) 0.7 Ca²⁺, 10 TEA⁻,
11.4 acetate⁻, 10 HEPES (pH 7.4), and 267 sucrose

Recording and cell perfusion were done in a Lucite perfusion chamber with a volume of 0.8 ml, as previously described (9). Channel antagonists and agonists were premixed with appropriate BS and then delivered to the recording chamber. All recordings were initiated ≥10 min after delivery of a given BS.

**Screening of PTH cDNA Library**

A bovine parathyroid λ ZAP II cDNA library, prepared from newborn calf parathyroid poly A⁺ RNA (Stratagene,
**RT-PCR**

Total and poly A+ RNA were isolated from newborn calf parathyroid glands and human parathyroid adenomas with an RNA Stat-60 kit (10). The primers used for bovine (upper: 5′-ACAAACGCTGACAGAAATG-3′; lower: 5′-ACACGAAAGCCGGAAGAA-3′) and human (upper: 5′-TGGGCTTCATATCACTTT-3′; lower: 5′-GGGTTAGCCACTCTCTCTG-3′) α1S-subunits were derived from the sequences of the bovine parathyroid cDNAs identified in this study (PT α1-1) and the human skeletal muscle homologue (20) (GenBank: L33789). PCR was performed on the first strand cDNA reverse transcribed from bovine or human parathyroid poly A+ RNA (0.5 μg), as described. The following reaction temperatures and durations were used: 95°C for 1 min, 55°C for 1 min, and 72°C for 1 min, each for 30 cycles. The reaction ended with a 7-min extension at 72°C. PCR products were electrophoresed on agarose gels, visualized by ethidium bromide staining, subcloned into pT-Adv (Clontech Laboratories, Palo Alto, CA) or PCR II-TOPO (Invitrogen, Carlsbad, CA) vectors by use of TA cloning methods according to the manufacturer’s instructions, and sequenced.

**Immunocytochemistry**

Immunocytochemistry of bovine parathyroid sections was performed as described (10) using the anti-pan α1 antiserum (4 × 10⁻⁵ M). To assess specificity, sections were treated with either antiserum preincubated with excess peptide (2.4 × 10⁻⁵ M) or nonimmune rabbit IgG. After diaminobenzene (DAB) staining, cells or sections were counterstained with aqueous hematoxylin (10). Experiments were repeated four times on tissue sections from two animals.

**Measurement of [Ca²⁺], and PTH Release**

[Ca²⁺] was determined using an InCyt Im2 imaging system (Intracellular Imaging, Cincinnati, OH) with a ×40 Nikon Fluor objective. Briefly, cells were loaded with fura 2-AM (3 μM) in buffer A [20 mM HEPES (pH 7.4), 120 mM NaCl 5 mM KCl, 1 mM MgCl₂, 1 mg/ml pyruvate, 1 mg/ml glucose, and 1.0 mM CaCl₂] at 37°C for 30–40 min. After three washes with buffer A, cells were incubated at 37°C for 15–30 min before recording. Fluorescent emission (510 nm) for the excitation of a COHU high-performance charge-coupled device camera (COHU, San Diego, CA), digitized, and stored in a microcomputer. The 340/380 excitation ratio (R₃₄₀₃₈₀) of emitted fluorescence was calculated.

PTH release was measured from cells treated with vehicle (0.1% ethanol) or dihydropyridines (10⁻⁶ M) for 30 min at 37°C (31).

**Statistics**

Data, normalized to baseline activity in individual experiments, were combined and reported as means ± SE. Statistical significance was determined by ANOVA with a f-test using Microsoft Excel computer software (Microsoft, Seattle, WA).

**RESULTS**

**Dihydropyridine Agonists and Antagonists Modulate Ca²⁺-Conducting Currents**

In previous studies, we characterized two types of Ca²⁺-conducting currents in bovine parathyroid cells. Type 1 Ca²⁺ currents increased with rising [Ca²⁺], and were blockable by Cd²⁺, La³⁺, Gd³⁺, and nifedipine.

---

**Fig. 1.** Inhibition of the Ca²⁺-conducting currents in bovine parathyroid cells by dihydropyridine (−202–791). A: whole cell patch clamp recordings were made (as described in MATERIALS AND METHODS) from a cell perfused with 0.7 Ca/10 TEA acetate bath solution (BS, ii) and 90 Ca/10 TEA acetate BS (iii–viii) in either the absence (iii) or presence of (−202–791) (iv–viii, 10⁻¹⁰ to 0 M) and La³⁺ (viii, 3 × 10⁻⁵ M). Panel i shows voltage-clamping protocol. B: membrane conductance (Gₘ) derived from the slopes of membrane current (Iₘ) – membrane potential (Vₘ) plots shown in A. Arrows, 0 current level.
Type 2 currents, which were insensitive to Cd\(^{2+}\) and nifedipine, could be blocked by La\(^{3+}\) and Gd\(^{3+}\) (9, 10).

To determine whether type 1 currents were sensitive to other dihydropyridines, we tested the effects of (+)- and (−)-202–791 and R- and S-BAY K 8644 on high [Ca\(^{2+}\)]\(_{o}\)-induced Ca\(^{2+}\) currents. As shown in Fig. 1, the dihydropyridine antagonist (−)-202–791 suppressed \(I_m\) and \(G_m\) in a dose-dependent manner (10\(^{-10}\) to 3 × 10\(^{-7}\) M), with an IC\(_{50}\) of \(\approx 10^{-9}\) M. In the presence of a maximum dose of (−)-202–791, the residual type 2 currents that accounted for \(\approx 50\%\) of total currents were further blocked by La\(^{3+}\) (3 × 10\(^{-3}\) M). The reversal potential (\(V_r\)) derived from \(I_m\)-\(V_m\) plots, did not shift significantly with (−)-202–791 (data not shown), suggesting that this blocker did not affect the ion selectivity of these currents. These results are compatible with our previous findings for nifedipine and indicate that dihydropyridines affect only type 1 currents.

The effects of (−)-202–791 on the Ca\(^{2+}\)-conducting currents could be partially reversed by its isomeric counterpart (+)-202–791, as demonstrated in Fig. 2. In a representative experiment, addition of (−)-202–791 (10\(^{-8}\) M) suppressed \(I_m\) and \(G_m\) by \(\approx 60\%\) (Fig. 2A, i and ii, and B). Subsequent perfusion of the cell with (+)-202–791 (3 × 10\(^{-6}\) M) partially restored \(I_m\) (Fig. 2A, iii) and \(G_m\) (Fig. 2B). Recovery of \(I_m\) and \(G_m\) was not due to damage to the membrane-pipette seal, because both parameters could be suppressed by Cd\(^{2+}\), a blocker of type 1 currents (Fig. 2A, iv, and B), and La\(^{3+}\) (Fig. 2B).

R- and S-BAY K 8644 also modulated type 1 currents in parathyroid cells. As shown in Fig. 3, the \(I_m\) and \(G_m\) induced by high [Ca\(^{2+}\)]\(_{o}\) (i and ii) were suppressed by R-BAY K 8644 (10\(^{-6}\) M) (iii). The inhibitory effects of R-BAY K 8644 were reversed by addition of S-BAY K 8644 (10\(^{-8}\) M), an L-type channel agonist (Fig. 3iv). Again, the recovery of \(I_m\) and \(G_m\) by S-BAY K 8644 was not the result of leakage of the membrane seal, because these parameters could be further suppressed by nifedipine (10\(^{-5}\) M; Fig. 3v) and Gd\(^{3+}\) (3 × 10\(^{-3}\) M; Fig. 3vi).

These studies strongly suggest that dihydropyridine-sensitive L-type channels are present in parathyroid cells. The insensitivity of Ca\(^{2+}\)-conducting currents to membrane potential, however, suggested that the channel subunits interacting with dihydropyridines and responsible for conducting Ca\(^{2+}\) currents in these cells may differ from classic L-type VDCCs.

**Cloning of Parathyroid Channel Subunit cDNAs**

To determine the identity of putative L-type channel subunits in parathyroid cells, we screened a parathyroid cDNA library for the presence of L-type \(\alpha_{1}\)- and \(\beta_{2}\)-subunit cDNAs. We isolated three partial \(\alpha_{1}\)-subunit cDNAs with sizes of \(\approx 3.3\) kb (PT\(_{\alpha_{1}}\)-1), \(\approx 0.8\) kb (PT\(_{\alpha_{1}}\)-2), and \(\approx 0.3\) kb (PT\(_{\alpha_{1}}\)-3). Sequencing revealed that clones PT\(_{\alpha_{1}}\)-2 and PT\(_{\alpha_{1}}\)-3 were part of the PT\(_{\alpha_{1}}\)-1 clone (data not shown). The nucleotide and predicted amino acid sequences of PT\(_{\alpha_{1}}\)-1 were 86 and 88% identical to those of the human skeletal muscle \(\alpha_{1S}\)- subunit, respectively (Fig. 4A and data not shown). Using the same library, we also isolated a partial
β3-subunit cDNA (~488 bp) whose predicted amino acid sequence was 99% identical to the human VDCC β3-subunit (Fig. 4B).

The amino acid sequences derived from the PT α1-1 cDNA clone revealed the presence of putative dihydropyridine-binding domains (Fig. 4A, III-S5, III-S6, and IV-S6 domains) and membrane potential sensors (III-S6 and IV-S6 domains). One striking difference between the PTα1-1 clone and the human skeletal α1S homologue is a deletion of 19 amino acids within the linkers between the IV-S3 and IV-S4 domains. These amino acids are encoded by 57 nucleotides, consisting of exon 29 of the α1S-subunit gene (20). To confirm whether such a deletion resulted from alternative transcript splicing or was a cloning artifact, we performed RT-PCR using poly A⁺ RNA isolated from bovine parathyroid and human parathyroid adenoma tissue. In four experiments from three separate RNA preparations, we consistently amplified α1-subunit cDNA lacking exon 29 from bovine (Fig. 5A) and human (Fig. 5B) parathyroid tissues. These findings indicated that the parathyroid gland predominantly expresses the alternatively spliced variant of the L-type α1-subunit.

**RNA and Protein Expression of the L-type α₁ and β₃-Subunit in PTH**

To examine the sizes of the full-length transcripts for the parathyroid α₁- and β₃-subunits, we performed Northern blot analysis. With probes made from the PTα1-1 and the human β₃-subunit cDNA, we identified, respectively, a 6-kb and 3.8-kb transcript in the parathyroid (Fig. 6). The sizes of these transcripts were comparable to those of the rabbit skeletal muscle α₁-subunit and human β₃-subunit transcripts, respectively (12, 14, 34).

To assess the protein expression of α₁-subunits in parathyroid cells, we performed immunocytochemistry using an anti-pan-α₁ antiserum raised against an epitope that is conserved among α₁C-, α₁S-, and α₁D-subunits. Brown DAB staining for the α₁-subunit was localized to parathyroid cells and smooth muscle cells of nearby arterioles and venules (Fig. 7, a and b).

---

Fig. 4. Deduced amino acid sequences of α₁ (PTα1-1)- and β₃ (PTβ3)-subunit cDNAs isolated from a parathyroid cDNA library as described in MATERIALS AND METHODS. A: sequence alignment of the PTα1-1 subunit (bPT, top row) and human skeletal α1S-subunit (h-SkM, bottom row); Genebank accession number: L33798. B: sequence alignment of the PTβ3-subunit (bPT, top row) and human β₃-subunit (h-Emb, bottom row); Genebank accession number: X76555.
Preincubation of antibodies with pan-α1 peptide prevented staining, indicating specificity of the antibody (Fig. 7c). These findings further confirmed the presence of L-type Ca\(^{2+}\) channels in parathyroid cells.

Effects of Dihydropyridines on the Intracellular Ca\(^{2+}\) Concentration and PTH Secretion in Parathyroid Cells

To test whether the dihydropyridine-sensitive type 1 current contributes to the high [Ca\(^{2+}\)]\(_o\)-induced increases in [Ca\(^{2+}\)]\(_i\) in parathyroid cells, we measured [Ca\(^{2+}\)]\(_i\) by microfluorimetry in the presence and absence of (−)202−791 and R-BAY K 8644. As shown, adding (−)202−791 (10\(^{-6}\) M) had no significant effect on the sustained (>5 min) increase in [Ca\(^{2+}\)]\(_i\) induced by raising [Ca\(^{2+}\)]\(_o\) from 0.5 to 2.0 mM (Fig. 8A) or to 5.0 mM (data not shown). Increases in [Ca\(^{2+}\)]\(_i\) were, however, blocked by either La\(^{3+}\) (10\(^{-5}\) M) (Fig. 8A) or Gd\(^{3+}\) (data not shown). Pretreatment of cells with (−)202−791 (10\(^{-6}\) M) for 30–60 min also did not cause significant effects on [Ca\(^{2+}\)]\(_i\) (Fig. 8B). The increase in [Ca\(^{2+}\)]\(_i\) induced by high [Ca\(^{2+}\)]\(_o\) was blocked by the cation channel blocker Gd\(^{3+}\) (10\(^{-3}\) M) (Fig. 8B). Similar results were obtained using another L-type channel antagonist, R-BAY K 8644 (data not shown). These observations indicate that acute increases in [Ca\(^{2+}\)]\(_i\) in parathyroid cells due to high [Ca\(^{2+}\)]\(_o\) are not mediated by the activation of the dihydropyridine-sensitive channels. Instead, other La\(^{3+}\)- or Gd\(^{3+}\)-sensitive channels may be involved in mediating Ca\(^{2+}\) entry in these cells.

We next tested the effects of dihydropyridines on PTH release. Whereas high [Ca\(^{2+}\)]\(_o\) suppressed PTH release, the addition of (−)202−791 or R-BAY K 8644 did not affect PTH release at any concentration tested (10\(^{-9}\) to 10\(^{-6}\) M) (Table 1 and data not shown). In addition, (−)202−791, S-BAY K 8644, and nifedipine did not alter PTH release (data not shown), suggesting that L-type Ca\(^{2+}\) channels may not play a role in modulating PTH secretion.

DISCUSSION

Changes in [Ca\(^{2+}\)]\(_o\) regulate PTH secretion by interacting with CaRs in the membranes of parathyroid cells (5). High [Ca\(^{2+}\)]\(_o\) inhibits PTH release, whereas maximal secretion occurs at low [Ca\(^{2+}\)]\(_o\). Studies with ionomycin and thapsigargin, which mobilize intracellular Ca\(^{2+}\), suggest that increasing [Ca\(^{2+}\)]\(_i\) is an important step in suppressing PTH release (27, 30). In parathyroid cells, initial increases in [Ca\(^{2+}\)]\(_i\) that re-
Several studies have investigated the nature of the channels responsible for high $[Ca^{2+}]_o$-induced $Ca^{2+}$ influx in parathyroid cells. Certain observations suggested an L-type $Ca^{2+}$ channel as a potential candidate (26). Fitzpatrick et al. (17) showed that the L-type channel agonist (+)202–791 suppressed and the antagonist (~)202–791 enhanced PTH secretion in dispersed adult bovine parathyroid cells. This group (Fitzpatrick et al., 18) also detected by use of an L-type channel antiserum a protein in parathyroid cell lysates with a size ($\approx$150 kDa) comparable to the skeletal $\alpha_{1S}$-subunit. Furthermore, incubation of cells with this same antiserum reduced PTH secretion, suggesting that this antiserum could bind to and activate the putative $Ca^{2+}$ channel. In support of this possibility, increased isotopic $Ca^{2+}$ flux was also demonstrated after preincubation of cells with this antibody (18).

Other evidence linking L-type channels to parathyroid function was reported by Cooper et al. (13), who showed that BAY K 8644 increased $[Ca^{2+}]_i$ and $Ca^{2+}$ influx in parathyroid cells. In the current investigation, we recorded membrane currents that were sensitive to dihydropyridines in isolated parathyroid cells. Our immunocytochemistry and Northern blotting provided evidence that protein and mRNA encoding putative L-type $\alpha_1$- and $\beta$-subunits were expressed in these cells. Finally, cloning and RT-PCR data confirmed the presence of an alternatively spliced skeletal $\alpha_{1S}$-subunit in these cells. Taken together, our observations and those of other investigators support the idea that parathyroid cells express a skeletal muscle-like isoform of the L-type $Ca^{2+}$ channel and that these channels might mediate changes in $[Ca^{2+}]_i$ and PTH release.

Our functional studies, however, yielded somewhat unexpected results, given the work of others summarized above and our own electrophysiology experiments. We were unable to demonstrate statistically significant effects of dihydropyridines on high $[Ca^{2+}]_o$-induced increases in $[Ca^{2+}]_i$ in parathyroid cells or PTH release with a variety of acute and short-term incubation protocols. These observations confirm those of Muff et al. (23), who found no effects of (+)202–791 on PTH release or $[Ca^{2+}]_i$.

At present, we have no explanation for the inconsistencies in studies with dihydropyridines among different laboratories. They may be attributed to differences in methods and reagents. Variations could also be due to the ages of the animals from which parathyroid tissues were obtained. Our studies and those of Muff et al. (23) employed glands from newborn calves, whereas Fitzpatrick and coworkers (16–18) used adult bovine parathyroid glands. The expression of L-type $\alpha_1$-subunits may be developmentally regulated in certain tissues. For instance, $\alpha_1$-subunit expression increases in the adult brain compared with embryonic brain (15).

Whether the expression of L-type $Ca^{2+}$ channel subunits increases with age in the parathyroid and whether these subunits become functional in mediating $Ca^{2+}$ influx only in cells from adult glands are possibilities that will require further investigation.

The partial $\alpha_1$-subunit cDNA we cloned from the parathyroid appears to be an alternatively spliced product of the skeletal muscle $\alpha_{1S}$-subunit gene (20). This CDNA lacks the 57 nucleotides that correspond to exon 29 of the human homolog of this gene and that
encode 19 amino acids within the linker between IV-S3 and IV-S4 domains. Such a deletion would shorten this extracellular loop from 32 to 13 amino acids. Similar spliced products have been previously identified in skeletal muscle-like BC3H1 cells, mouse ovary, rabbit intestinal smooth muscle, and rat brain (15, 25). Our RT-PCR experiments detected only cDNAs lacking exon 29 in bovine and human parathyroid tissues, suggesting that the spliced variant is the predominant one expressed in the parathyroid. The significance of such a structural modification on channel function in any system, however, remains unclear.

Parathyroid cells are nonexcitable. Membrane depolarization by high K\(^{+}\) does not induce Ca\(^{2+}\) influx (28, 37). The lack of a response to depolarizing concentrations of K\(^{+}\) could be due to the lack of expression of classic VDCCs (i.e., dihydropyridine-responsive channels) or the expression of channels with altered voltage-sensing properties. Our electrophysiological data support the latter possibility (9). Using a traditional whole cell patch-clamp configuration, we recorded dihydropyridine-sensitive membrane currents in parathyroid cells that were voltage independent. Their affinity for dihydropyridines, however, was comparable to that of classic VDCCs. The nifedipine-sensitive currents in parathyroid cells were cation nonselective, in contrast to typical VDCCs in excitable cells, which exhibit significant selectivity for Ca\(^{2+}\). We did not record other voltage-sensitive Ca\(^{2+}\)-conducting currents with the same protocols. It is therefore likely that the channels that bind dihydropyridines in parathyroid cells are not sensitive to changes in membrane potential.

Clearly, because their electrophysiological properties differ, the molecular characteristics of the responsible channel subunits in parathyroid cells must diverge from classic L-type VDCCs. By surveying the deduced partial amino acid sequence of the parathyroid \(\alpha_1\)-subunit cDNA, we were able to identify the putative dihydropyridine-binding domains (i.e., III-S5, III-S6, and IV-S6) and membrane potential-sensing domains (i.e., III-S4 and IV-S4). The expression of

<table>
<thead>
<tr>
<th>Table 1. Effects of different [Ca(^{2+})](_{o}) and dihydropyridines on PTH release</th>
</tr>
</thead>
<tbody>
<tr>
<td>([\text{Ca}^{2+}]_{o}), mM |</td>
</tr>
<tr>
<td>Treatment | 0.5</td>
</tr>
<tr>
<td>% of Control (n) | 100.0 ± 3.4</td>
</tr>
<tr>
<td>(P) value | &lt;0.01</td>
</tr>
</tbody>
</table>

Treatment values are means ± SE. [Ca\(^{2+}\)]\(_{o}\), extracellular calcium concentration; PTH, parathyroid hormone. Vehicle indicates cells were incubated with 0.1% ethanol.
dihydropyridine-binding regions supports data showing that these drugs bind to parathyroid cell membranes (21). The presence of voltage-sensing domains may, however, contradict our electrophysiological results, because the dihydropyridine-blockable currents we recorded were not classically voltage gated. Because the 3.3-kb parathyroid α1-subunit cDNA we cloned represents only ~50% of the full-length transcript, it is likely that modifications in the as-yet-unknown portions of the cDNA could potentially alter the ability of the channels to sense changes in membrane potential. The confirmation of this possibility requires the cloning of the full-length parathyroid α1-subunit cDNA. Alternatively, the protein product of the full α1-subunit, whose partial clone we identified, may not mediate the voltage-insensitive currents in these cells. Other as-yet-unidentified channel subunits may be responsible for conducting these currents, or other channel regulators or subunits may modify the voltage dependency of the multisubunit channel complex in parathyroid cells.

A major unresolved issue inherent in these data is the difference between the electrophysiological and microfluorimetric findings. Electrophysiological recordings clearly show that dihydropyridine agonists and antagonists activate or block, respectively, cation currents in parathyroid cells that are responsive to changes in [Ca$^{2+}$]$_i$, and that can be carried by Ca$^{2+}$ (9). Yet, surprisingly, these same agents did not detectably alter [Ca$^{2+}$]$_i$, either in the basal state or in response to raising [Ca$^{2+}$]$_o$ under a variety of short-term protocols. Our intracellular Ca$^{2+}$ determinations were done on groups of cells, from which single cells responding with even a 20% change in [Ca$^{2+}$]$_i$; could have been reliably detected. No significant differences in [Ca$^{2+}$]$_i$ over as long as 60 min were seen. Although we cannot explain why dihydropyridines had no effect on [Ca$^{2+}$]$_i$, we think that differences in methodology may be important to consider. 1) Patch-clamping is a more sensitive method for recording ion channel activity than is microfluorimetry. Because we performed electrophysiological recordings in the presence of high [Ca$^{2+}$]$_o$ (90 mM), which greatly enhanced type 1 currents, we could demonstrate clearly a blockade of the currents by dihydropyridines. In microfluorimetry, Ca$^{2+}$ fluxes induced by smaller but more physiological increments of [Ca$^{2+}$]$_o$ (from 0.5 to 2.5 or 5.0 mM) could have occurred. Perhaps, however, the changes in [Ca$^{2+}$]$_i$ were too small to alter [Ca$^{2+}$]$_i$ over the entire cytosol. Other aspects of Ca$^{2+}$ mobilization activated by CaRs, such as the release of intracellular Ca$^{2+}$ and Ca$^{2+}$ influx via dihydropyridine-insensitive channels, could also have masked subtle changes in [Ca$^{2+}$]$_i$, brought about by dihydropyridines in single-cell microfluorimetry experiments. 2) With intact cells, the recording conditions for microfluorimetry, Ca$^{2+}$ may not be the predominant ion carried by these dihydropyridine-sensitive currents. Hence, [Ca$^{2+}$]$_i$ may not change with these drugs. In support of this possibility, our previous studies clearly show that these currents can be conducted by divalent as well as monovalent cations. Given the lack of available parathyroid-derived cDNAs for the other channel subunits, it is not possible to address definitively the basis for different results from electrophysiological vs. microfluorimetric studies.

The differences between the two types of data emphasize the importance of using electrophysiological approaches as a guide for hypothesis generation and protocol development. Ultimately, physiological principles must always be tested in intact cells, and eventually in animals, to assure that they are valid with respect to physiological events in vivo, including hormone secretion and second-messenger generation. A more complete understanding of the functions served by dihydropyridine channel subunits in nonexcitable tissues will eventually require more complete information on the subunit structure and molecular properties of the ion channels expressed in the parathyroid.

We appreciate the assistance of Dr. Orlo Clark in the procurement of human parathyroid tissue and the support of Vivian Wu in the preparation of this manuscript.

During these studies, Dr. Shoback was supported by a Merit Review from the Department of Veterans Affairs, the Northern California Arthritis Foundation, and National Institutes of Health (NIH) Grant DK-43400, and Dr. Schwartz was supported by NIH Grant HL-43231.

REFERENCES


DIHYDROPYRIDINE-SENSITIVE Ca\(^{2+}\) CHANNELS IN PARATHYROID CELLS


