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Antibodies to an α subunit of skeletal muscle calcium channels regulate parathyroid cell secretion

(calcium agonists/parathyroid hormone/ion channels/pertussis toxin)

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ABSTRACT **We have shown previously that Ca^{2+}-channel agonists, which open Ca^{2+} channels, inhibit parathyroid hormone (PTH) secretion from dispersed bovine parathyroid cells, whereas Ca^{2+}-channel antagonists, which close Ca^{2+} channels, stimulate PTH release. We now have tested the effects of mouse antibodies specific for purified α subunits of rat skeletal muscle Ca^{2+}-channel proteins on PTH secretion by bovine parathyroid cells *in vitro*. Mouse antisera (MC-2, MC-3, MC-4) blocked the secretion of PTH from parathyroid cells incubated with 0.5 mM Ca^{2+} ions. Affinity-purified MC-4 antibodies inhibited PTH release in a concentration-dependent manner. Incubation of parathyroid cells with pertussis toxin markedly reduced MC-4-dependent inhibition of PTH secretion. Parathyroid cell membrane proteins were fractionated by $NaDodSO_4$/polyacrylamide gel electrophoresis under either reducing or nonreducing conditions and immunoblotted with MC-4 antiserum. Antibodies bound to one major band of protein with M_r ≈150,000. These results suggest that the antibodies bind to Ca^{2+}-channel α subunits and act as agonists that open the channels and inhibit PTH release.**

Voltage-sensitive Ca^{2+} channels, facilitating flux of Ca^{2+} ions across the cell membrane, control diverse cell functions such as hormone or neurotransmitter secretion and muscle contraction (1, 2). The L type of Ca^{2+} channel is sensitive to 1,4-dihydropyridine compounds: agonists of this class promote the open state of the channel and antagonists block the channel (3, 4). We have studied the L type of Ca^{2+} channel in the parathyroid cell, which is negatively regulated by Ca^{2+} (5). 1,4-Dihydropyridine agonists, which open the Ca^{2+} channel and permit entry of extracellular Ca^{2+} into the parathyroid cell, inhibit parathyroid hormone (PTH) release. 1,4-Dihydropyridine derivatives that are Ca^{2+}-channel antagonists stimulate secretion (6).

We prepared polyclonal, monospecific mouse antibodies against highly purified preparations of α subunits of voltage-sensitive Ca^{2+} channels (7) from rat muscle transverse tubules (T-tubules) and tested the effects of the antibodies on parathyroid cell secretion. The results suggest that antibodies bind to α subunits of voltage-sensitive Ca^{2+} channels and activate the channels, which in turn leads to an inhibition of PTH secretion from cells.

METHODS

Cell Preparations. Bovine parathyroid cells were dispersed by incubation with collagenase (1.8 mg/ml) and pancreatic DNase I (0.5 mg/ml) (8). Dispersed cells were suspended in Eagle's minimum essential medium containing 0.02 M Hepes (pH 7.40) in place of $NaHCO_3$, 0.1% (wt/vol) heat-inactivated bovine serum albumin, 0.5 mM $MgSO_4$, and $CaCl_2$ as noted. Cells were centrifuged through medium containing 2% heat-inactivated bovine serum albumin to remove debris. Cell viability, determined by trypan blue exclusion, was greater than 95%. Cells were incubated with antibodies as noted in a shaking water bath for 90 min at 37°C and the medium was separated from the cells and stored at −20°C until assayed for PTH by radioimmunoassay. Where stated, cells were treated with pertussis toxin (0.1 μg/ml; Calbiochem) for 4 hr at 25°C and then washed twice before further testing (8).

PTH Radioimmunoassay. Samples were assayed for PTH by a midregion radioimmunoassay (9). Samples were diluted with 0.05 M Veronal buffer (pH 8.6) containing 20% (wt/vol) charcoal-stripped human plasma. Synthetic PTH (bovine, residues 1–84; Bachem, Torrance, CA) was used as standard, and sample or standard was added to ^{125}I-labeled PTH-(44–68) peptide and NG1 antiserum (a gift of Larry Mallette, Baylor College of Medicine, Houston). The mixtures were incubated overnight at 4°C and then dextran-coated charcoal was added. The bound phase was assayed for ^{125}I by scintillation counting. Values were interpolated using a weighted four-parameter logistic model (10). The coefficient of variation is 5% near the middle of the standard concentration curve.

Membrane Preparation. Bovine parathyroid glands were minced and homogenized as described (11). Minced tissue was homogenized in 3 volumes of 10 mM Tris/HCl (pH 7.5) containing 100 mM KCl, 5 mM $MgCl_2$, 1 mM EGTA, 10% (vol/vol) glycerol, and protease inhibitors (phenylmethylsulfonyl fluoride, 0.4 mM; aprotinin, 0.1 unit/ml; soybean trypsin inhibitor, 1 mg/ml, and pepstatin A, 10 mg/ml). The tissue was homogenized in a Waring blender four times at high speed for 10 sec, the homogenate was centrifuged at 1000 × *g* for 20 min, and the supernatant fraction was centrifuged at 150,000 × *g* for 30 min. The membrane pellets were suspended in homogenization buffer and stored at −80°C. Protein was determined by the method of Bradford (12).

$^{45}Ca^{2+}$ Uptake. Parathyroid cells (150,000 cells) were incubated in 2.0 ml of solution A [140 mM NaCl/5.0 mM KCl/0.5 mM $CaCl_2$ (1 μCi of $^{45}CaCl_2$; 1 μCi = 37 kBq)/0.5 mM $MgCl_2$/10 mM glucose/10 mM Hepes, pH 7.4] at 37°C. $^{45}Ca^{2+}$ uptake by cells reached equilibrium within 10 min. Antiserum to Ca^{2+}-channel α subunits (1:10,000 dilution) was added to the cells in a shaking water bath. At the times indicated, ice-cold Ca^{2+}-free solution A with 50 mM EGTA (disodium salt) was added and the incubation mixtures were filtered over Whatman GF/B glass-fiber filters under vacuum. Cells retained on the filter were washed twice with 2 ml

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Abbreviations: G protein, guanine nucleotide-binding signal-transduction protein; PTH, parathyroid hormone; T-tubule, transverse tubule; WGA, wheat germ agglutinin.

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(each wash) of Ca^{2+}-free solution A and radioactivity was determined by liquid scintillation spectrometry.

T-Tubule Preparation. Membrane fractions enriched in T-tubule vesicles were prepared from back and hindleg muscle tissues of 150- to 250-g male Sprague–Dawley rats. The procedure and the characterization of the T-tubule membrane fractions will be reported in detail elsewhere (T. Beeler, H.C., and M. Daniels, unpublished work).

Purification of α Subunits of Voltage-Sensitive Ca^{2+} Channels. Ca^{2+} channels from rat T-tubule membranes were purified as described by Curtis and Catterall (7), with the following modifications. Rat T-tubule membranes (50 pmol of [^{3}H]nitrendipine binding sites per mg of protein) were incubated with 10 nM (+)-[^{3}H]PN200-110 {isopropyl 4-(2,1,3-benzoxadiazol-4-yl)-1,4-dihydro-5-([^{3}H]methoxycarbonyl)pyridine-3-carboxylate} at 4°C for 60 min. The labeled membranes were centrifuged and suspended by homogenization in buffer containing 1% (wt/vol) digitonin, 185 mM KCl, 1.5 mM $CaCl_2$, 10 mM Hepes/Tris (pH 7.4), and the same protease inhibitors used for preparing parathyroid cell membranes. The suspension was incubated for 40 min on ice and then was centrifuged at 100,000 × *g* for 30 min. The supernatant fraction containing solubilized {[^{3}H]PN200-110·Ca^{2+} channel} complexes was recovered and fractionated by column chromatography on wheat germ agglutinin (WGA)-agarose (E-Y Lab, San Mateo, CA), DEAE-Sepharose (Pharmacia), and then WGA-agarose. The eluate from the second WGA-agarose column was purified further by sucrose density gradient centrifugation and the peak fractions containing {[^{3}H]PN200-110·Ca^{2+} channel} complexes were pooled and subjected to preparative $NaDodSO_4$/PAGE (3% polyacrylamide stacking gel and 5–15% acrylamide gradient separation gel). The portion of each gel containing the α subunits of voltage-sensitive Ca^{2+} channels was excised, protein was electroeluted, and the solution was lyophilized.

Preparation of Mouse Antibodies to α Subunits of Voltage-Sensitive Ca^{2+} Channel. Six-week-old female BALB/c mice were immunized intraperitoneally with purified Ca^{2+}-channel α subunit (20 μg of protein per mouse) emulsified with Freund's complete adjuvant; three booster injections of the same preparation in Freund's incomplete adjuvant were given (20 μg of protein per mouse) at 2-week intervals. The last immunization was with the same amount of antigen dissolved in phosphate-buffered saline. Antisera were obtained 3 days later.

Immunoblot Analysis. Proteins in membrane preparations from bovine parathyroid cells were solubilized with 2% $NaDodSO_4$ in 62.5 mM Tris/HCl (pH 6.8) containing 10% glycerol and either 20 mM dithiothreitol or 20 mM *N*-ethylmaleimide, fractionated by $NaDodSO_4$/PAGE, and transferred by electrophoresis to nitrocellulose sheets (13). The nitrocellulose sheets were incubated with blocking solution [10 mM Tris/HCl (pH 8.0) containing 3% gelatin, 0.05% Tween 20, and 150 mM NaCl] for 1 hr at 24°C, then incubated overnight with solutions containing mouse antisera. The bound antibody–antigen complexes were detected with alkaline phosphatase coupled to goat IgG directed against mouse IgG (Promega Biotec, Madison, WI), using nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate as substrates.

Affinity Purification of MC-4 Antibody. Ca^{2+}-channel proteins from T-tubule membranes, which had been partially purified on WGA-agarose columns, were fractionated by preparative $NaDodSO_4$/PAGE (5–16% linear gradient of acrylamide) under nonreducing condition (with 20 mM *N*-ethylmaleimide) and then transferred onto nitrocellulose sheets by electrophoresis. A vertical strip was cut out, and the area containing α subunits was visualized as described above. By aligning the stained vertical strip with the remainder of the nitrocellulose sheets, horizontal strips corresponding to immobilized α subunits were identified, excised, and incubated overnight at room temperature with MC-4 antiserum after nonspecific binding sites were blocked with the blocking solution. The bound antibodies were eluted with 100 mM glycine/HCl (pH 3.0), neutralized with 1 M NaOH, and concentrated.

RESULTS

Three polyclonal, monospecific mouse antisera directed against α subunits of voltage-sensitive Ca^{2+} channels from rat skeletal muscle were tested for effects on PTH secretion. These antisera were found to be specific for the nonreduced form of skeletal muscle Ca^{2+}-channel α-subunit proteins (M_r 175,000) on immunoblot analysis. When the nitrocellulose transfer sheets, which contained purified Ca^{2+}-channel proteins solubilized from rat T-tubule membranes and fractionated by $NaDodSO_4$/PAGE in the presence of either dithiothreitol or *N*-ethylmaleimide, were analyzed, the antisera recognized only the α-subunit protein band with an apparent M_r of 175,000 on the blots prepared under nonreducing conditions (data not shown). Under reducing conditions, the antigenicity of the protein was destroyed.

Mouse antisera were examined for their effects on PTH secretion from parathyroid cells at two Ca^{2+} concentrations, 0.5 mM (which stimulates PTH secretion) and 2.0 mM (which normally decreases PTH secretion) (Fig. 1). Mouse antisera (MC-2, MC-3, or MC-4, each diluted 1:1000) decreased PTH secretion from cells in medium containing 0.5 mM Ca^{2+} but had little or no effect at 2 mM Ca^{2+}. Normal BALB/c mouse serum at the same dilution did not alter PTH release at 0.5 or 2.0 mM Ca^{2+}.

The relation between MC-4 antiserum concentration and inhibition of PTH secretion from parathyroid cells is shown in Fig. 2. MC-4 antiserum diluted 1:100,000–1,000,000 had little or no effect on PTH secretion. However, a biphasic inhibition of PTH secretion from cells was observed at higher concentrations of antiserum. MC-4 antiserum, diluted 1:1000–50,000, decreased PTH secretion by approximately 25%, whereas a 1:100 dilution of antiserum resulted in 50% inhibition from cells incubated in medium containing 0.5 mM Ca^{2+}.

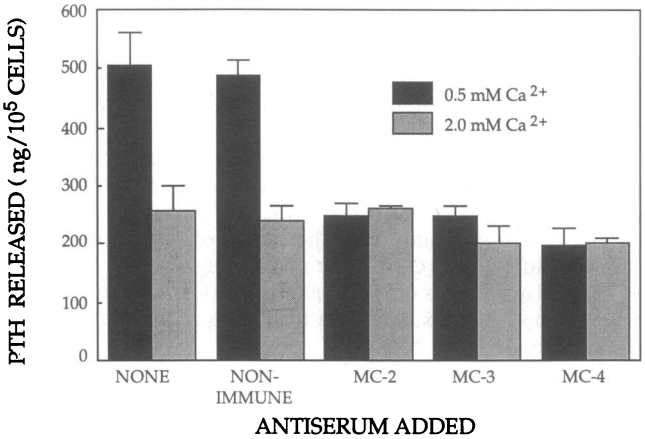

FIG. 1. Effects of polyclonal antiserum on parathyroid cell secretion. Dispersed bovine parathyroid cells were incubated either with monospecific mouse antisera (diluted 1:1000) directed against α subunit of rat T-tubule Ca^{2+} channels or with normal BALB/c mouse serum (nonimmune). In control cells (no antiserum added) basal PTH release was 507 ± 55 ng per 10^5 cells and was suppressed by 50% at 2.0 mM Ca^{2+} (gray bars) as compared to cells in 0.5 mM Ca^{2+} (black bars). The mean values + SEM of duplicate experiments, each analyzed in duplicate, are shown.

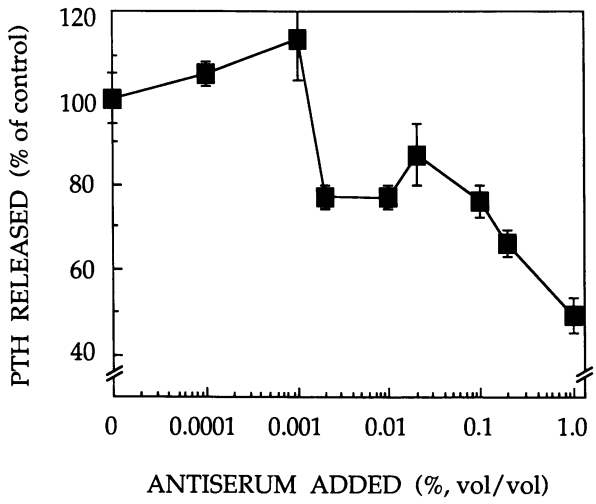

FIG. 2. Effect of MC-4 antiserum concentration on PTH secretion by parathyroid cells. Antiserum MC-4 was added to cells in medium containing 0.5 mM Ca^{2+}, and levels of PTH released were determined. The amount of PTH released by control cells (100%) (no MC-4 antiserum added) was 650 ± 52 ng per 10^5 cells. Each value represents the mean values ± SEM of quintuplicate samples analyzed in duplicate.

Affinity-purified MC-4 antibodies inhibited PTH secretion from parathyroid cells in medium containing 0.5 mM Ca^{2+}, but not in medium containing 2 mM Ca^{2+} (Fig. 3). Antibodies directed against α subunits of voltage-sensitive Ca^{2+} channels were removed from MC-4 antiserum by prior incubation of the antibodies with purified α subunits of rat T-tubule Ca^{2+} channels that had been immobilized on a nitrocellulose sheet. The absorbed antiserum did not affect PTH release when diluted 1:10,000 (Table 1) or when diluted 1:50,000 or 1:100,000 (data not shown).

$^{45}Ca^{2+}$ uptake by dispersed parathyroid cells reaches a plateau within 10 min (data not shown). After cells were

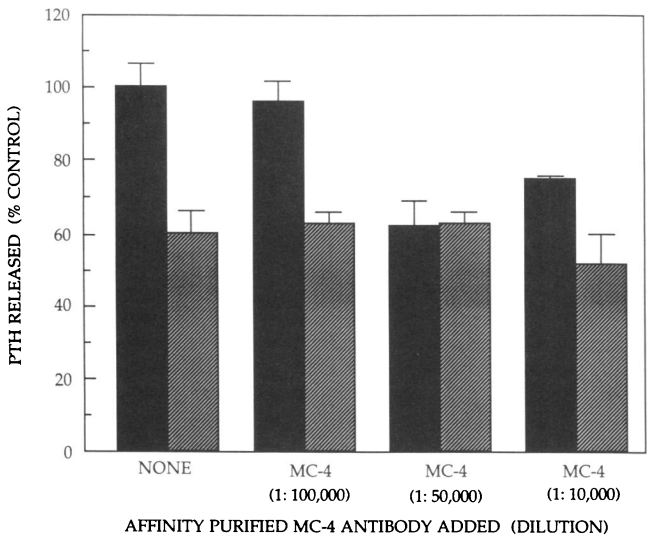

FIG. 3. Inhibition of parathyroid hormone release by MC-4 antibodies affinity-purified by association with highly purified rat skeletal muscle α subunit of voltage-sensitive Ca^{2+} channels. The antibody–α-subunit complexes were washed and dissociated, and antibody was recovered. Affinity-purified MC-4 antibody was added to dispersed cells (dilutions shown were normalized to the original antiserum) in medium containing 0.5 mM Ca^{2+} (black bars) or 2 mM Ca^{2+} (hatched bars), and PTH secretion was determined. Basal release of PTH by control cells (100%) was 2260 ± 152 ng per 10^5 cells. Each value represents quadruplicate samples analyzed in duplicate.

Table 1. Prior absorption of MC-4 antiserum with purified α subunits of rat skeletal muscle Ca^{2+} channels

Antiserum added (1:10,000 dilution)	PTH released, % basal	
	0.5 mM Ca^{2+}	2.0 mM Ca^{2+}
None	100 ± 6	42 ± 3
MC-4	75 ± 3	51 ± 2
Absorbed MC-4	100 ± 4	40 ± 3

MC-4 antiserum was absorbed for 24 hr with purified α subunits of rat skeletal muscle Ca^{2+} channels that had been immobilized on nitrocellulose sheets. Bovine parathyroid cells were dispersed and incubated without MC-4, with MC-4, or with absorbed MC-4 for 60 min at 37°C. PTH was determined by radioimmunoassay of cell supernatants after separation by centrifugation. Basal release (no antiserum added, 0.5 mM Ca^{2+}) of PTH by control cells (100%) was 1620 ± 80 ng per 10^5 cells. The data shown represent the average of values from three experiments. In each experiment triplicate samples were analyzed in duplicate (n = 18).

equilibrated with $^{45}Ca^{2+}$, MC-4 antiserum (1:10,000 dilution) was added and $^{45}Ca^{2+}$ uptake by the cells was determined as a function of incubation time (Fig. 4). MC-4 antiserum resulted in a marked increase in $^{45}Ca^{2+}$ uptake by cells, whereas normal mouse serum was without effect.

The possibility that a guanine nucleotide-binding signal-transduction protein (G protein) might play a role in activation of voltage-sensitive Ca^{2+} channels of bovine parathyroid cells was tested (Table 2). Dispersed bovine parathyroid cells were incubated with pertussis toxin, and PTH release from cells was determined. Pertussis toxin reversed most of the MC-4 antibody-dependent inhibition of PTH release, which suggests that a pertussis toxin-sensitive G protein such as $G_i\alpha$ or $G_o\alpha$ is required for Ca^{2+}-channel activation by antibody molecules.

Parathyroid cell membrane proteins were solubilized and fractionated by $NaDodSO_4$/PAGE, electrophoretically transferred to nitrocellulose sheets, and incubated with MC-4 antiserum. The bound antibodies on nitrocellulose sheets were visualized as described in *Methods*. One major band of antigen with apparent M_r 150,000 was detected under reducing or nonreducing conditions, as well as other minor protein bands that also were present on the nitrocellulose blot incubated with nonimmune serum (Fig. 5). Specific antibody binding to the M_r 150,000 protein band also was apparent after fractionation of parathyroid cell membrane proteins and immunoblotting with MC-2 and MC-3 antisera (data not shown).

DISCUSSION

Many electrically excitable cells contain voltage-sensitive Ca^{2+} channels (3, 4, 14–16). Ca^{2+}-channel antagonists, by blocking Ca^{2+} channels, inhibit insulin release in pancreatic cells (17), gonadoliberin-induced release of luteinizing hormone in anterior pituitary cells (18), and thyroliberin-induced release of thyrotropin in GH_4C_1 pituitary cells (19, 20). The aldosterone response to angiotensin II and K^+ ions also are blocked by Ca^{2+}-channel antagonists in adrenal glomerulosa cells (21). The Ca^{2+}-channel agonists, on the other hand, raise intracellular Ca^{2+} content and stimulate insulin release from pancreatic tissue (22, 23). The parathyroid cells are unusual because Ca^{2+} influx inhibits secretion of PTH (5). Ca^{2+}-channel agonists, which open Ca^{2+} channels and allow influx of extracellular Ca^{2+}, inhibit PTH release. Ca^{2+}-channel antagonists, which close the channels, stimulate PTH secretion (6).

We tested the effects of polyclonal antisera, directed against purified α subunits of Ca^{2+} channels from rat skeletal muscle T-tubules, on parathyroid cell secretion. Antibodies to the α subunits of dihydropyridine-sensitive Ca^{2+}

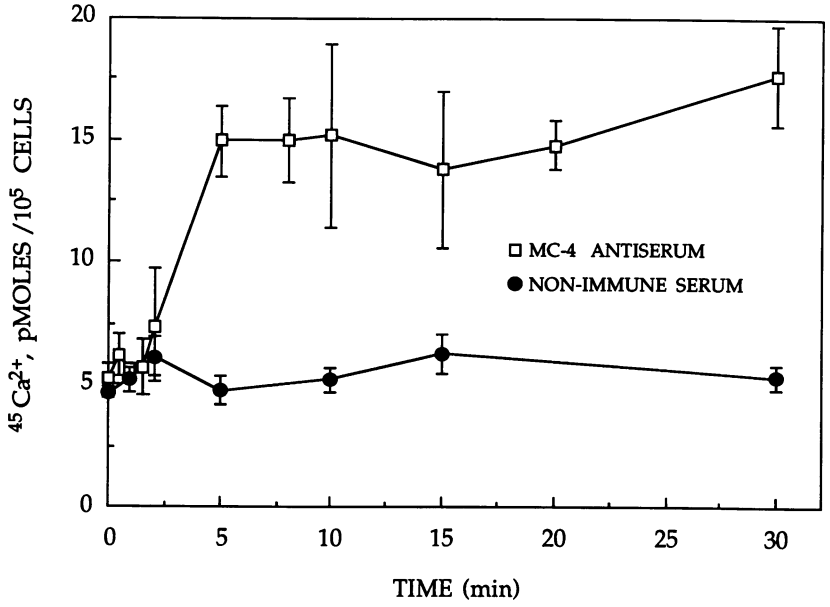

FIG. 4. $^{45}Ca^{2+}$ uptake by parathyroid cells. Dispersed parathyroid cells were incubated with $^{45}Ca^{2+}$ for 10 min. Then, MC-4 antiserum (1:10,000 dilution; □) or normal BALB/c mouse serum at the same dilution (●) was added at time zero. Each value represents the mean value ± SEM of quintuplicate determinations.

channels were found to function as agonists for these channels in parathyroid cells. We studied in depth one such antiserum (MC-4) exhibiting Ca^{2+}-channel agonist-like properties. The MC-4 antiserum inhibited PTH release at 0.5 mM Ca^{2+}, which suggests that the antibodies activate the Ca^{2+} channels, allowing influx of extracellular Ca^{2+} and thereby inhibiting PTH secretion. The relationship between extracellular Ca^{2+} concentration and intracellular Ca^{2+} levels and rates of parathyroid cell secretion has been described (24). Increases in the concentrations of extracellular Ca^{2+} resulted in increases in Ca^{2+} influx, which is coupled to inhibition of PTH secretion. Incubation of cells with MC-4 antiserum also resulted in a marked increase in uptake of $^{45}Ca^{2+}$ by parathyroid cells. Normal mouse serum did not affect either PTH secretion or $^{45}Ca^{2+}$ uptake. We might predict a more rapid effect; however, the rate of Ca^{2+} accumulation in cells is consistent with Ca^{2+} influx via activated Ca^{2+} channels (25). Ca^{2+} efflux was not determined and it is possible that the rise in intracellular $^{45}Ca^{2+}$ in parathyroid cells after exposure to MC-4 antiserum is due to equilibration of extracellular $^{45}Ca^{2+}$ with a larger pool of cytoplasmic Ca^{2+}. The marked (≈4-fold) increase in intracellular $^{45}Ca^{2+}$ in parathyroid cells after exposure to the antiserum makes this unlikely. The mechanism of receptor–effector coupling in the parathyroid cell remains to be elucidated. Previous reports have suggested the involvement of the G proteins in the regulation of ion channels and the second messengers (26, 27). In AtT-20/D16-16 pituitary cells, a G protein mediates somatostatin-induced inhibition

Table 2. Interaction of pertussis toxin and MC-4 antiserum on PTH release

Experiment	Addition(s)	PTH secreted, ng per 10^5 cells
1	None	1066 ± 38.8
	MC-4	678 ± 27.0
	MC-4 + pertussis toxin	942 ± 98.2
2	None	1073 ± 38.0
	MC-4	654 ± 29.8
	MC-4 + pertussis toxin	977 ± 36.7
3	None	1041 ± 44.7
	MC-4	709 ± 41.1
	MC-4 + pertussis toxin	979 ± 45.8

Bovine parathyroid cells were dispersed and incubated with or without pertussis toxin (0.1 μg/ml) for 4 hr at room temperature. Cells were washed and MC-4 antiserum was added at 1:10000 dilution. Cells were incubated at 37°C for 60 min and PTH was determined in cell supernatants by radioimmunoassay. Each value represents the mean ± SEM of triplicate samples assayed in duplicate.

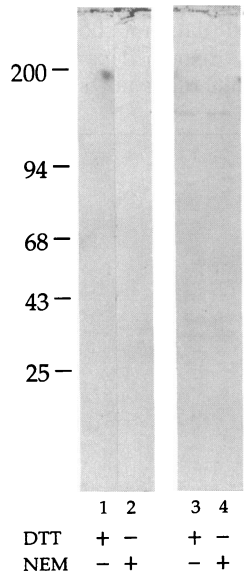

FIG. 5. Immunoblot analysis of parathyroid cell membranes. Parathyroid cell membranes were fractionated by $NaDodSO_4$/PAGE (5–15% gradient) under nonreducing [20 mM *N*-ethylmaleimide (NEM)] or reducing [20 mM dithiothreitol (DTT)] conditions and transferred to nitrocellulose paper. Sheets were incubated overnight with MC-4 antiserum and stained with alkaline phosphatase coupled to goat IgG directed against mouse IgG, using nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate as substrates. Little or no nonimmune mouse serum bound to parathyroid cell membranes (lanes 1 and 2). Affinity-purified mouse antibody to α subunits of Ca^{2+} channels specifically bound to a M_r 150,000 protein in parathyroid cell membranes (lanes 3 and 4). Values and markers at left represent the M_r ($\times 10^{-3}$) and positions of standard proteins in an adjacent lane.

of voltage-dependent Ca^{2+} currents (28) and a GTP-binding protein regulates voltage-sensitive Ca^{2+} channels in NG108-15 neuroblastoma–glioma hybrid cells (29). G proteins link atrial muscarinic acetylcholine receptors to K^+ channels (30, 31). Pertussis toxin catalyzes ADP-ribosylation and inactivation of $G_o\alpha$ and $G_i\alpha$ proteins. In bovine parathyroid cells, Ca^{2+} inhibition of PTH release is mediated by a pertussis toxin-sensitive G protein (8). In addition, pertussis toxin abolishes Ca^{2+}-channel activation by (+)-202-791, a dihydropyridine derivative (6). We have now shown that incubation of parathyroid cells with pertussis toxin reverses most of the antibody-dependent inhibition of PTH secretion, which suggests that $G_i\alpha$ or $G_o\alpha$ signal-transduction proteins mediate the activation of Ca^{2+} channels by antibodies. Further study will be necessary to confirm this hypothesis.

Recent reports from two laboratories indicate that the α subunit of rabbit skeletal muscle Ca^{2+} channels consists of two polypeptides, α_1 and α_2, that are similar in size but have different biochemical properties (32, 33). The α_1 subunit (M_r 170,000–175,000) is not a glycoprotein that contains terminal *N*-acetylglucosamine or sialic acid residues in the carbohydrate chains, and the electrophoretic mobility of this subunit remains unchanged after disulfide bonds are reduced. The α_1 subunit of skeletal muscle Ca^{2+} channels contains specific binding sites for dihydropyridine (32, 34) and phenylalkylamine (35) derivatives and is phosphorylated by cAMP-dependent protein kinase (36). Depolarization of GH_3 pituitary cells results in phosphorylation of Ca^{2+} channels and activation of the channels (37). The M_r 143,000 α_2 subunit is a heavily glycosylated protein and is linked by disulfide bonds to a protein of $M_r \approx 25{,}000$. Our findings suggest that the M_r 150,000 protein in parathyroid cell membrane is similar to the α_1 subunit of the skeletal muscle Ca^{2+} channel. The affinity-purified antibody recognizing the M_r 150,000 protein blocks Ca^{2+} inhibition of PTH release. Prior absorption of the antiserum with purified α subunits of rat skeletal T-tubule Ca^{2+} channels removed the antibody molecules that decrease secretion of PTH by parathyroid cells.

After this work was completed, we saw a recent article by Malouf *et al.* (38), who reported antibody-dependent opening of T-tubule Ca^{2+} channels in planar membrane bilayers *in vitro*. Their antibody precipitated proteins of apparent M_r 175,000, 90,000, 55,000, and 34,000. In the current study we have shown that antibodies that specifically interact with Ca^{2+}-channel subunits activate the channels in intact parathyroid cells.

We conclude that parathyroid cells possess a M_r 150,000 protein analogous to the α_1 subunit of the dihydropyridine-sensitive Ca^{2+} channel. The M_r 150,000 protein is recognized on immunoblots by antibody directed against α subunits of skeletal muscle Ca^{2+} channels. The antibodies that recognize this protein activate voltage-sensitive Ca^{2+} channels of parathyroid cells. These findings suggest that the antibodies open Ca^{2+} channels, allowing influx of Ca^{2+} ions, which in turn inhibits PTH secretion. In addition, the results with pertussis toxin suggest that the function of the L-type Ca^{2+} channel in parathyroid cells is linked to a signal-transducing G protein(s).

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