Activating Antibodies to the Calcium-Sensing Receptor in Two Patients with Autoimmune Hypoparathyroidism

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Autoimmune hypoparathyroidism is thought to result from immune-mediated destruction of the parathyroid glands. We encountered two patients with hypoparathyroidism and other autoimmune conditions (Graves’ disease and Addison’s disease, respectively) in whom autoimmune destruction of the parathyroid glands had not taken place. In the first, a histologically normal parathyroid gland was observed at the time of subtotal thyroidectomy; and in the second, the hypoparathyroidism remitted spontaneously. Both patients had antibodies that reacted with the cell surface of bovine parathyroid cells and human embryonic kidney (HEK293) cells transfected with the extracellular calcium-sensing receptor (CaR) but not with nontransfected HEK293 cells. The antibodies also reacted with the same bands on Western analysis of extracts of bovine parathyroid tissue and CaR-transfected HEK293 cells that were identified by an antisera to a recombinant extracellular domain of the CaR that activated the receptor based on their ability to increase inositol phosphate accumulation, activate MAPK, and inhibit PTH secretion. These results, therefore, demonstrate that patients with the biochemical findings of primary hypoparathyroidism can harbor antibodies to the CaR, which, in the two cases studied here, did not produce irreversible destruction of the parathyroid glands. (J Clin Endocrinol Metab 89: 548–556, 2004)

A U T O I M M U N E H Y P O P A R A T H Y R O I D I S M C A N o c c u r as an isolated clinical abnormality, as part of autoimmune polyendocrinopathy syndrome (APS)-1 or, less commonly, as part of APS-2 (1). APS-1 most commonly comprises mucocutaneous candidiasis, hypoparathyroidism, and Addison’s disease. APS-2 includes two or more of the following: Addison’s disease, Graves’ disease, autoimmune thyroiditis, type 1 diabetes mellitus, primary hypogonadism, myasthenia gravis, or celiac sprue.

Parathyroid histology has been available in relatively few cases of autoimmune hypoparathyroidism and has shown varying degrees of fatty replacement, atrophy, and lymphocytic infiltration (2–7). However, there was no apparent functional impact of the antibodies in the 1960s (8). Antiparathyroid antibodies have been shown to exert cytotoxic effects on cultured bovine parathyroid cells in vitro (9). Patients with adult onset hypoparathyroidism have generalized T cell activation, which could be a manifestation of cell-mediated immunity against parathyroid chief cells, leading to parathyroiditis (10). Thus, both humoral and cell-mediated immunity could contribute to loss of parathyroid cells in hypoparathyroidism.

The identity/ies of the parathyroid antigens recognized by antiparathyroid antibodies has been obscure until recently. Blizzard et al. (8) found that antiparathyroid antibodies reacted with an antigen found in normal parathyroid tissue and in some, but not all, parathyroid adenomas. More recently, Li et al. (11) found that 14 of 25 patients (18 with APS1 and seven with hypoparathyroidism and autoimmune hypothyroidism) had antibodies that reacted with the extracellular domain of the extracellular calcium (Ca2+)-sensing receptor (CaR). The CaR is the cell surface, G protein-coupled receptor through which parathyroid chief cells, thyroid C cells, and various kidney cells recognize and respond to changes in Ca2+, so as to maintain calcium homeostasis (12). However, there was no apparent functional impact of the antibodies on the CaR in this study, in which sera from several patients with autoantibodies were incubated with CaR-transfected HEK293 cells and changes in the cytosolic calcium concentration (Ca2+) were monitored.

We recently encountered two patients whose clinical presentations suggested that they might harbor antibodies to the CaR that activated the receptor. In one, a patient with longstanding hypoparathyroidism and Graves’ disease was noted to have a normal parathyroid gland at the time of subtotal thyroidectomy. In the second, a patient with newly diagnosed Addison’s disease was also hypocalcemic with an inappropriately normal serum PTH level, indicating the presence of hypoparathyroidism. The hypocalcemia subsequently remitted, however, indicating that there had not been irreversible destruction of the parathyroid glands. In

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the studies described herein, both patients were found to harbor antibodies to the CaR that increased the activity of phospholipase C and MAPK and inhibited PTH secretion. Thus, autoimmune hypoparathyroidism can result from autoantibodies to the CaR that activate the receptor, thereby inhibiting PTH secretion, rather than simply exerting a cytotoxic action on the parathyroid cell.

**Subjects and Methods**

**Patient 1**

The patient was diagnosed, at the age of 14, with hyperthyroidism attributable to Graves’ disease, in Sydney, Australia. Anti-TSH receptor antibodies were positive. He was treated with carbimazole. The same year, he had several seizures and was found to be hypocalcemic. No prior serum calcium measurements were available. At that time, PTH was 0.34 ng/ml [normal (nl.) < 0.4] (340 ng/liter [values in SI units are given in parentheses after the conventional units] (nl. < 400)]. He was treated with vitamin D and calcium supplementation and experienced no further seizures. Over the next 9 yr, antithyroid medication could not be withdrawn without a relapse of the thyrotoxicosis. At that time (23 yr of age), the thyrotoxicosis remained difficult to control because of fluctuating hypo- and hyperthyroidism. At 25 yr of age, he underwent subtotal thyroidectomy. Histological examination was consistent with treated Graves’ disease and also revealed a parathyroid gland that was read as normal by three pathologists and showed no lymphocytic infiltration, whereas there were focal accumulations of lymphocytes in the adjacent thyroid tissue. He subsequently required 1,25D replacement therapy.

Initial clinical studies were carried out at age 25. Informed written consent was obtained from the patient, and the studies were approved by the institutional ethics committee at the Royal North Shore Hospital in Sydney. Two hundred units of human PTH (1-34-Ap) tated a peak cAMP response of 33.2 nmol/100 ml (332 μmol/liter) glucomer filtrate (GF) with a control value of 0.9 nmol/100 ml (9 μmol/liter) GF, and tubular maximum phosphorus (TmPO4) GFR fell from 4.1 to 1.8 mg/dl (1.3 to 0.58 mmol/liter), indicating normal PTH sensitivity. Before the study, serum calcium was 7.8 mg/dl, nl. = 8.2–10.2 (1.95 mmol/liter, nl. = 2.1–2.6). At that time, the patient was prescribed 1.5 μg calcitriol daily, as well as supplementation with magnesium and 3 g elemental calcium. Additional laboratory tests were as follows: serum PTH 1-84, 0.39 ng/ml (nl. < 0.4) (390 ng/liter, nl. < 400) [when calcium was 7.8–8.0 mg/dl (1.95–2.00 mmol/liter)]; PTH 1-34, 205 pg/ml, nl. < 130 (205 ng/liter, nl. < 130 mmol/liter) [when calcium was 7.8–8.0 mg/dl (1.95–2.00 mmol/liter)]; intact PTH, less than 8 pg/ml (nl. = 10–65) (<8 ng/liter, nl. = 10–65) [when calcium was 8.96 mg/dl (2.25 mmol/liter)]; PTH 1–84, 0.1 ng/ml, nl. < 0.4 (100 ng/liter, nl. < 400) [when calcium was 9.0 mg/dl (2.25 mmol/liter)]; magnesium, 1.6–2.1 mg/dl, nl. = 1.5–2.6 (0.66–0.86 mmol/liter, nl. = 0.62–1.1); phosphate, 3.6 mg/dl, nl. 2.5–4.7 (12 mmol/liter, nl. = 1.2–1.5); creatinine was 0.8 mg/dl, nl. = 0.6–1.2 (70 μmol/liter, nl. = 53–106); 25-hydroxyvitamin D was 29 ng/ml, nl. = 15–53 (72 nmol/liter, nl. = 37–130); and 1,25 dihydroxyvitamin D was 43 pg/ml, nl. = 22–62 (107 pmol/liter, nl. = 55–150). A 24-h urine collection, at a time when his serum calcium was 7.8 mg/dl (1.95 mmol/liter), contained 44 mg (1.10 mg) calcium and 1.0 gm (88 mmol) creatinine. During a 6-h infusion with 400 IU PTH, 1,25 dihydroxyvitamin D increased from 24 to 45 pg/ml (60 to 112 pmol/liter), tubular maximum phosphorus (TmP/GFR decreased from 4.1 to 1.8 mg/dl (1.32 to 0.58 mmol/liter), and serum calcium increased from 8.0 to 8.2 mg/dl (2.0 to 2.05 mmol/liter). The hypocalcemia, in the presence of normal vitamin D levels and normal or slightly elevated circulating PTH levels, was not explained.

**Patient 2**

A 25-yr-old male was admitted on April 21, 2001 to the Brigham and Women’s Hospital with 6 months of progressive fatigue, weight loss, vomiting, and salt craving as well as hyperpigmentation of 2-yr duration. Past medical history revealed hypothyroidism, for which he was treated with T3 (100 μg/d). Family history was positive for celiac disease in a cousin. Physical examination was unremarkable except for hypocalcemia, which contained 44 mg (1.1 mmol) calcium and 1.0 gm (88 mmol/liter) creatinine. During a 6-h infusion with 400 IU PTH, 1,25 dihydroxyvitamin D increased from 24 to 45 pg/ml (60 to 112 pmol/liter), tubular maximum phosphorus (TmP/GFR decreased from 4.1 to 1.8 mg/dl (1.32 to 0.58 mmol/liter), and serum calcium increased from 8.0 to 8.2 mg/dl (2.0 to 2.05 mmol/liter). The hypocalcemia, in the presence of normal vitamin D levels and normal or slightly elevated circulating PTH levels, was not explained.

**Methods**

**Specimen collection and preparation.** Serum samples were collected and stored at −20 or −80 °C. Some serum samples were heated at 56 C to inactivate complement. There was no difference in the results of experiments carried out using heat-inactivated or non-heat-inactivated serum. Sera were affinity-purified as described below.

**Affinity purification of sera and antisera.** Sera from the two patients; sera from normocalcemic controls; or rabbit polyclonal, anti-CaR antisera 4637 were affinity-purified as follows: The affinity columns were coupled to a peptide corresponding to amino acid residues 344–358 of the human CaR, which had been shown to inhibit PTH secretion by thyroid cells. The columns were equilibrated with PBS or with 0.1 M sodium acetate, pH 5.0, and were loaded and washed with PBS or 0.1 M sodium acetate, pH 5.0, respectively. The columns were then eluted with 20 mmol/liter HCl (pH 2.5), and the antibodies were collected. The sera or antiserum were absorbed on the affinity column in PBS, eluted with 20 mmol/liter HCl (pH 2.5) and neutralized as recommended by the manufacturer. The purified antibodies were stored at −20 °C until use.

**Immunoperoxidase and immunofluorescence.** Cells were fixed in PBS containing 4% formaldehyde at 20 C for 10 min. Immunoperoxidase staining was performed using affinity-purified patient or control sera or affinity-purified, polyclonal anti-CaR antisera 4637 (13). Bound Iggs were detected using peroxidase-conjugated, γ-chain-specific, goat antihuman IgG or goat anti-rabbit IgG (Sigma, St. Louis, MO). Two-color immunofluorescence, to detect colocalization of anti-CaR antibodies in sera with polyclonal anti-CaR antisera 4637, was performed as before (13). Parathyroid cells were incubated with both antisera 4637 (1:200 dilution) and affinity-purified rabbit polyclonal antisera 4637 (13). The sera or antisera were absorbed on the affinity column PBS, eluted with 20 mmol/liter HCl (pH 2.5), and immediately neutralized as recommended by the manufacturer. The purified antibodies were stored at −20 °C until use.

**Western analysis.** Western blot analysis was performed as described (13). After cell lysis, nuclei and cell debris were removed by low-speed centrifugation (1000 × g for 10 min); the resultant cell lysate was used for Western blot analysis. Equal amounts of supernatant proteins were mixed with 2× sodium dodecyl sulfate (SDS)-Laemmli buffer, separated on 7.5% SDS-polyacrylamide gels or on linear 3–9% SDS-polyacrylamide...
gradient gels, and transferred to nitrocellulose filters (13). The membranes were then incubated with affinity-purified antisera 4637 (1: 1000) or with affinity-purified patient or control sera, followed by washing and incubation with horseradish peroxidase-conjugated, goat antirabbit or antihuman IgG.

Immunoprecipitation. CaR-transfected HEK293 (HEKCaR) cells were washed with ice-cold PBS and lysed with buffer containing 150 mmol/liter NaCl, 10 mmol/liter Tris-HCl (pH 7.4), 1% glycerol, 1 mmol/liter EDTA, 1 mmol/liter EGTA, 1 mmol/liter sodium o-vanadate, a cocktail of protease inhibitors, and 1% Triton X-100 (14). After centrifugation, at 10,000 × g for 10 min, supernatant protein (50 μg total lystate) was incubated with affinity-purified sera prepared from the two patients’ sera, with control serum pooled from five normocalcemic controls, or with monoclonal anti-CaR antibody (LRG) overnight at 4°C. Protein A/G-agarose beads (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) were then added for an additional 1 h. Bound precipitates were washed three times, and the pellet was eluted by boiling for 5 min with 2× Laemmli sample buffer. After SDS-PAGE, Western blot analysis was performed as described above, using anti-CaR antisera 4637.

Reactivity of anti-CaR antibodies with synthetic peptides. Immonul II microtiter plates (Dynatech, Chantilly, VA) were incubated overnight at 4°C with 1–5 μg peptide and washed three times as before (13). Patient or control sera (diluted 1:1000 in PBS-0.05% Tween-20) were then added and incubated at 37°C for 1 h. After washing and incubation overnight at 4°C with 50 μl/well of peroxidase-conjugated, goat antihuman γ-specific IgG, immunoreactivity was quantified using an ML3000 Microtiter Plate Luminometer (Dynatech).

Preparation of membranes from CaR-transfected and nontransfected HEK293 cells. Confluent monolayers of HEK293 cells, stably transfected with the CaR or nontransfected HEK293 cells, were rinsed with ice-cold PBS and scraped into lysis buffer [10 mmol/liter Tris-HCl (pH 7.4), 1 mmol/liter EDTA, 1 mmol/liter EGTA, 0.25 mol/liter sucrose, 1 mmol/liter di-thiothreitol, and a cocktail of protease inhibitors] as described previously (13). The cells were passed through a 22-gauge needle, 10 times, and nuclei and cellular debris were removed by sedimentation. The supernatants were sedimented at 45,000 × g for 1 h, and the pellets were resuspended in Eagle’s MEM containing leupeptin and a calpain inhibitor and saved at −80°C. Resuspended membranes (20 μg protein) were incubated with 100 μl patient or control sera in 500 μl Eagle’s MEM for 1 h at 37°C. After the incubations, sera were sedimented, and supernatants were diluted with Eagle’s MEM (2% final concentration) and incubated with parathyroid cells (13).

Determination of Ca2⁺-regulated PTH release. Dispersed human parathyroid cells (1 × 10⁶ cells/0.5 ml) were preincubated for 1 h at 37°C with 2% (vol/vol) patient or control sera, washed with Eagle’s MEM, and incubated with 0.5 mmol/liter Mg²⁺, and varying concentrations of Ca²⁺ (0.5–2.0 mmol/liter) for 1 h at 37°C in Eagle’s MEM with 2% serum (vol/vol) from the same patient or control used during the preincubation (13). In a second experimental design, cells were preincubated, washed, and incubated with 2% patient or control sera preabsorbed with membranes from CaR-transfected or nontransfected HEK293 cells. Supernatant PTH was measured using the Whole PTH (1–84) Specific Immunoradiometric assay kit (Scantibodies Laboratory, Inc., Santee, CA). Incubation of the three serum samples from patients 1 and 2 with parathyroid cells did alter trypan blue exclusion, which was 95–100% for both the cells treated with the patients’ sera and sera from normocalcemic controls.

Determination of CaR-stimulated inositol phosphate accumulation. Cells prelabeled with [³²P]myo-inositol were incubated with varying concentrations of CaCl₂, and 10 mmol/liter LiCl for 30 min after preincubation with affinity-purified patient or control sera (14). The affinity-purified control and patient sera were also included during the incubations after the preincubation. The reactions were terminated with 10% ice-cold trichloroacetic acid (final wt/vol). After centrifugation to remove insoluble debris, trichloroacetic acid was extracted with water-saturated diethylether, and inositol phosphates were separated on Dowex anion exchange columns (Bio-Rad Laboratories, Inc.) as described previously (14).
showed considerably less immunoreactivity with the CaR expressed in HEKCaR cells relative to antiserum 4637 (Fig. 2). Nevertheless, the patients' sera were able to immunostain CaR-transfected HEK293 cells (e.g., Fig. 1) and to immunoprecipitate the CaR extracted from HEKCaR cells, as demonstrated by subsequent Western blotting with anti-CaR antiserum 4637 using two color immunofluorescence. Dispersed bovine parathyroid cells were incubated with both antiserum 4637 (1:200 dilution) (red color in G, J, and M) and affinity-purified patient (green color in H and K) or control serum (1:100 dilution) (N), and then with secondary antibodies (see Methods). Fluorescence images were collected as described in Subjects and Methods. Photomicrographs were taken at ×1000. The yellow color in the merged images indicates colocalization of antiserum 4637 and the anti-CaR antibodies in the two patients' sera but not in the control sera (O).

**The two patients’ sera bind to CaR peptides**

To demonstrate further that the anti-CaR antibodies in the patients' sera recognized epitopes within the CaR's extracellular aminoterminus, we examined the capacity of sera from both patients to bind to synthetic peptides corresponding to residues 344–358 (4637 peptide), 214–238 (4641 peptide), and 374–391 (LRG peptide) of the human CaR. Figure 3 illustrates that the two patients' purified IgG showed substantially more binding to this synthetic peptide than did purified IgG from normocalcemic subjects that were studied concurrently. We obtained two serum samples on patient 2: one drawn while in the hospital, when his serum total calcium concentration was 1.95–2.13 mmol/liter without any treatment for his hypocalcemia (serum sample 2a); and the second, a month later, when his serum calcium concentration was 2.23 mmol/liter (serum sample 2b), and he was being treated with calcium supplementation. The reactivity of the second sample with the
CaR peptides was substantially less than that of the first sample and was not statistically significantly different from the control serum.

Stimulation of accumulation of inositol phosphates in CaR-transfected HEK293 cells

To investigate whether the patients’ anti-CaR antibodies modulated the function of the receptor, we tested the effects of affinity-purified sera from the two hypoparathyroid subjects on high Ca\(^{2+}\)\(_{o}\)-stimulated accumulation of inositol phosphates in CaR-transfected HEK293 cells, anticipating that the antibodies would activate the CaR. Figure 4 shows that, as expected, the two affinity-purified serum samples increased the accumulation of inositol phosphates at 0.5 and 1.0 mmol/liter Ca\(^{2+}\)\(_{o}\).

Stimulation of MAPK activity in CaR-transfected HEK293 cells

Figure 5 shows that affinity-purified antibodies from patients 1 and 2 stimulated MAPK at 0.5 mmol/liter Ca\(^{2+}\)\(_{o}\), relative to affinity-purified control antibodies. Panel A shows an example of a single experiment, whereas the lower panel illustrates pooled data from three independent experiments. There was a statistically significant stimulation of MAPK activity by the affinity-purified antibodies from the two patients at 0.5 mmol/liter Ca\(^{2+}\)\(_{o}\).

Patients’ sera inhibit PTH release

Because it was not feasible to obtain normal human parathyroid cells, we used parathyroid cells obtained from parathyroid adenomas or cases of primary parathyroid hyperplasia to test the effects of the patients’ sera on Ca\(^{2+}\)\(_{o}\)-
regulated PTH release. Pathological parathyroid glands from patients with primary hyperparathyroidism, although expressing lower levels of the CaR than normal bovine or human parathyroid glands, usually retain some degree of responsiveness to Ca\(^{2+}\). Figure 6 illustrates that the serum samples from the two patients inhibited PTH release substantially (both at low and at higher Ca\(^{2+}\)). In the case of patient 2, there were two serum samples (one at the time when the patient was overtly hypocalcemic, and the second at a time when his serum calcium concentration was just above the lower limit of normal while being treated with calcium supplementation). Both serum samples produced comparable degrees of inhibition of Ca\(^{2+}\)-regulated PTH release.

Preabsorption of serum from patient 2 (there was insufficient serum from patient 1 to perform the same study) with membranes prepared from CaR-transfected (but not nontransfected) HEK293 cells partially reversed the inhibitory effect of the serum on PTH release (Fig. 7).

**Discussion**

We have identified two patients with hypoparathyroidism who have autoantibodies to the CaR by several criteria. First, both patients' sera had IgG that bound to bovine parathyroid cells and colocalized on the cell surface with an authentic anti-CaR antiserum. Second, both patients' sera harbored antibodies that reacted with the cell surface of CaR-transfected HEK293 cells but not with nontransfected HEK293 cells, which do not express the receptor endogenously. The difference in immunoreactivity of the sera with CaR-transfected vs. nontransfected HEK293 cells strongly suggests that the patients' antibodies bound to the CaR.

Third, purified IgG from the patients' sera, when used for Western analysis on extracts of bovine parathyroid glands, yielded results nearly identical to those observed with an authentic anti-CaR antiserum. As with the anti-CaR antiserum, the patients' autoantibodies recognized both CaR monomers and dimers, including the characteristic immunoreactive doublet that arises from the presence of both the
immature, high mannose- and the fully mature, glycosylated forms of the receptor (16). Of interest, the patients’ sera recognized the CaR in parathyroid extracts considerably better than in extracts of HEKCaR cells. The patients’ sera clearly do react with the nondenatured CaR in HEKCaR cells, however, because they immunostained the receptor on transfected (but not on nontransfected) cells and were able to immunoprecipitate the receptor from the CaR-transfected HEK cells. Fourth, purified IgG from both patients’ sera reacted with several peptides corresponding to sequences within the CaR’s extracellular domain. Taken together, these data document that both patients harbored anti-CaR antibodies.

It should be pointed out that, because we did not sequence these two patients’ CaR genes, we cannot formally exclude the possibility that they had activating mutations of the CaR. Such a mutation would be very unlikely in patient 2, in whom hypocalcemia was only transient. Regardless, how-
ever, they both had antibodies to the CaR that activated the receptor.

In addition to reacting with the CaR on the cell surface of parathyroid and CaR-transfected cells, these anti-CaR antibodies modulated three aspects of the function of these two cell types in ways that provide strong evidence that they activate the receptor. First, affinity-purified antibodies from both patients stimulated the accumulation of total inositol phosphates in CaR-transfected HEK293 cells at 0.5 and 1 mmol/liter Ca\(^{2+}\), or an index of activation of phospholipase C. Second, affinity-purified antibodies from both patients stimulated MAPK activity at 0.5 mmol/liter, another established parameter of activation of the CaR in a variety of cell types (17–20). Third, serum samples from the two subjects inhibited PTH release from dispersed human parathyroid cells. Although the reduced PTH secretion could have conceivably resulted from nonspecific damage to the parathyroid cells, such an effect would have been expected to reduce rather than increase CaR-stimulated accumulation of inositol phosphates and activation of MAPK.

It is possible that patient 1’s anti-CaR antibodies exerted greater effects on the parathyroid than on the kidney. That is, at a time when he was mildly hypocalcemic, his urinary calcium excretion was only 1.1 mmol/24 h. If the anti-CaR antibodies activated the CaR in the kidney, one might have expected the relative or absolute hypercalciuria that is observed in patients with activating mutations of the CaR rather than the overt hypocalciuria seen in this patient. Any reasons why there might be such differential reactivity of the anti-CaR antibodies with parathyroid and kidney must remain conjectural for the moment.

Patient 2 had two serum samples, one drawn at a time when his hypoparathyroidism was first diagnosed; and the second, a month later, when he was being treated with calcium supplementation and his serum calcium concentration had just returned to levels in the mid-normal range, as it had been 8 and 16 months before the diagnosis of his Addison’s disease. It is of interest that his second serum sample, despite the fact that its activity had decreased by more than 50% as assessed by ELISA, still inhibited PTH release to an extent similar to the first sample. It should be pointed out in this regard, however, that the dilution of serum used for the ELISA was 1:1000, whereas that used for studying Ca\(^{2+}\)o-regulated PTH release was 1:50. Therefore, the more concentrated serum used for studying secretion may still have exerted a biological effect that was not observed with the 20-fold-lower concentration used in the ELISA. It is also difficult to know how to compare the relative concentrations of the anti-CaR antibody to which parathyroid cells would be exposed in vivo and in vitro. It would clearly be of interest to obtain a follow-up serum sample, now that this patient’s serum calcium has completely normalized, to assess whether or not the antibody was still present and exerted effects on CaR-mediated PTH release, MAPK activity, and the accumulation of inositol phosphates.

It should be pointed out that this patient had hypomagnesemia during the time that he was hypoparathyroid. It is possible that the hypomagnesemia, albeit mild, contributed to the genesis of the hypocalcemia (21). Conversely, it is possible that the hypomagnesemia was the result, rather than the cause, of the patient’s hypoparathyroidism. For instance, it is possible that binding of the anti-CaR antibodies to the receptor in the distal tubule would result in wasting of both calcium and magnesium. Of interest in this regard, some patients with activating mutations of the CaR exhibit hypomagnesemia (22). Regardless of the relationship of this patient’s hypomagnesemia to his hypocalcemia, however, he had anti-CaR antibodies by several criteria, which inhibited PTH secretion in vitro, even if we cannot formally prove that they caused the hypocalcemia in vivo.

Others have shown previously that patients with autoimmune hypoparathyroidism can harbor anti-CaR antibodies. Li et al. (11) demonstrated that 14 out of 25 patients with autoimmune hypoparathyroidism had anti-CaR antibodies in their serum. However, these authors were unable to document any functional impact of these sera on the Ca\(^{2+}\)o. We believe that this latter result was most likely observed because the binding of the antibodies to the CaR in vitro was too slow to produce the characteristic transient increase in Ca\(^{2+}\)o evoked by activation of the receptor by high Ca\(^{2+}\)o. The transient high Ca\(^{2+}\)o-elicited elevation in Ca\(^{2+}\)o results from activation of phospholipase C by the CaR, with subsequent production of inositol trisphosphate (IP3) and release of calcium from intracellular stores. Our assay, in contrast, was based on a more direct measurement of PLC activity; namely, quantitation of the high Ca\(^{2+}\)o-evoked production of cellular inositol phosphates.

Posillico et al. (23) showed that antibodies to the parathyroid cell surface were present in eight of 23 cases of idiopathic hypoparathyroidism. In three of these cases, the patients’ sera inhibited PTH secretion by about 50% at both low (0.5 mmol/liter) and higher (1.5 mmol/liter) levels of Ca\(^{2+}\)o. Moreover, in one of the three cases, the titer of anti-parathyroid antibody and its ability to inhibit PTH secretion decreased progressively during a period of time when the patient’s hypoparathyroidism spontaneously improved. In retrospect, it is possible that these three cases harbored activating, anti-CaR antibodies. Of interest, the three patients described by Posillico et al. and the two patients presented here all had adult-onset hypoparathyroidism. Further studies are needed to determine whether activating anti-CaR antibodies are present in patients with APS-1 and childhood onset of hypoparathyroidism.

It would be of interest to determine whether, under appropriate experimental conditions, the sera from the two patients described here could exert cytotoxic effects. Brandi et al. (9) demonstrated the presence of cytotoxic antibodies that reacted with bovine parathyroid cells in patients with autoimmune hypoparathyroidism. In any event, both of our patients had evidence that the parathyroid glands had not been damaged by immune mechanisms. In the first case, a histologically normal parathyroid gland was obtained at the time of subtotal thyroidectomy, 12 yr after hypoparathyroidism was first diagnosed. In the second case, hypoparathyroidism remitted spontaneously.

The presence of anti-CaR antibodies that exert functional actions without compromising cellular viability has diagnostic (as well as therapeutic) implications. Because the para-
thyroid glands would be intact in such cases, they would presumably retain the capacity to respond to a reduction in Ca$^{2+}_o$, with increased PTH secretion. As a diagnostic test, administration of a calcilytic could potentially be sensed by functional parathyroid tissue, even in the presence of an activating antibody, as a reduction in Ca$^{2+}_o$, thereby eliciting increased PTH secretion. Furthermore, just as administration of a calcilytic elevates Ca$^{2+}_o$, from a normal to an elevated level in the euparathyroid state, it might be possible to restore normocalcemia in a hypoparathyroid patient with an activating antibody if the calcilytic were able to antagonize the effect of the antibody (24).

In summary, we describe two patients with activating antibodies to the CaR, who exhibited hypocalemia and the clinical and biochemical picture of hypoparathyroidism. Additional studies are needed to determine the prevalence of activating, anti-CaR antibodies in patients with autoimmune hypoparathyroidism in the setting of idiopathic hypoparathyroidism, APS-1, and adult onset hypoparathyroidism with other endocrine deficiencies.

Acknowledgments

Polyclonal rabbit anti-CaR antisera 4641 (raised against amino acids 214–238 in the human CaR, referred to as 4641 peptide) and 4637 (raised against amino acids 344–358 in the human CaR, referred to as 4637 peptide), as well as monoclonal anti-CaR antibody LRG (raised against amino acids 391 in the human CaR, referred to as LRG peptide), were gifts from Dr. Karen Krapcho and Edward Nemeth (NPS Pharmaceuticals, Inc., Salt Lake City, UT) and Drs. Allen Spiegel and Paul Goldsmith [National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health (NIH), Bethesda, MD), respectively.

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