

Calcium Modulation of Adrenocorticotropin Levels in Women—A Clinical Research Center Study*

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ABSTRACT

In vitro calcium modulation of anterior pituitary hormone secretion has been well described. In addition, several investigations performed in human subjects have documented modulation of the circulating levels of pituitary hormones by supraphysiological calcium concentrations. Recent data from our laboratory document the existence of an extracellular calcium-sensing receptor that is thought to mediate the effects of variations in extracellular calcium on the secretion of PTH and calcitonin. We have also demonstrated the presence of this receptor in pituitary-derived, ACTH-secreting AtT-20 cells as well as in the anterior pituitary of rats and mice. In the present study we investigated the effect on anterior pituitary hormone levels of variations in serum calcium within the physiological range. We serially measured serum levels of ionized calcium (Ca_i), ACTH, cortisol, TSH, and PRL during 90-min iv infusions (on separate days) of calcium, citrate, and dextrose in 10 healthy women with a mean age

of 55 ± 5 yr. During the calcium infusion, the serum Ca_i level increased significantly from 4.32 ± 0.10 mg/dL at baseline to 4.86 ± 0.08 mg/dL at completion ($P = 0.002$), and this change was accompanied by a significant increment in the serum ACTH level from 9.87 ± 1.32 to 16.31 ± 2.84 pg/mL ($P = 0.0008$). There was no change in the serum ACTH level during the citrate infusion despite significant decrements in serum Ca_i , nor were there changes in either Ca_i or ACTH during the dextrose infusion. Finally, changes in Ca_i did not alter TSH or PRL levels. In summary, our dynamic studies are the first to demonstrate an increase in baseline serum ACTH levels in response to physiological increments in Ca_i (*i.e.* increments within the normal range). This effect was specific for increments and not decrements in serum Ca_i and was selective for ACTH, as TSH and PRL levels did not change with any of the infusions. (*J Clin Endocrinol Metab* 81: 932–936, 1996)

ALTERATIONS in the extracellular ionized calcium (Ca_i) concentration have profound effects on hormone secretion. Multiple *in vitro* studies have shown that hormone secretion is markedly inhibited in the absence of extracellular calcium; this observation suggests that there is an absolute requirement for extracellular calcium during hormone secretion (1–6). In addition, studies of human subjects have shown that physiologically relevant changes in the extracellular calcium concentration alter the secretion of some hormones, as exemplified by the inverse relation between PTH secretion and Ca_i level (7).

The ability of small, physiologically meaningful changes in extracellular calcium level to regulate PTH secretion is mediated in large part by a 120-kDa, G protein-linked, calcium-sensing receptor (CaR) expressed in parathyroid cells (8). The physiological relevance of the CaR in calcium ion homeostasis has been documented in humans by the identification of hyper- and hypocalcemic syndromes resulting from mutations in the receptor gene that reduce and increase, respectively, the biological activity of the CaR protein (9, 10). Recently, we demonstrated that this CaR is also expressed in the

mouse and rat anterior pituitary and in the mouse corticotroph cell line AtT-20 (11). The identification of this receptor in anterior pituitary cells, specifically in corticotrophs, prompted us to investigate the effect of changes in extracellular calcium levels within the physiological range on the release of ACTH and other anterior pituitary hormones.

Previous studies analyzing the influence of extracellular calcium on the regulation of anterior pituitary hormone responses in humans have most often examined the effects of hypo- and hypercalcemia on secretion, but not the effect of changes in calcium levels within the physiological range. Some, but not all, of these studies have shown that acute hypercalcemia alters the secretion of FSH, LH, GH, PRL, and TSH (12–16). One study that examined the effect of calcium on ACTH secretion in humans showed a several-fold increase in ACTH secretion with acute hypercalcemia (17).

In this study we investigated the effect of changes in extracellular calcium levels on ACTH levels, using a protocol that we had previously employed to characterize the effect of calcium on PTH dynamics. This protocol ensures variations in calcium levels within the physiological range.

Subjects and Methods

Subjects

Ten healthy women (mean age \pm SD, 55 ± 5 yr) were studied. Before enrollment in the study, each volunteer underwent a physical examination and a laboratory evaluation that included a multichannel chemistry analysis, a serum TSH determination, and a complete blood count with differential. The study was reviewed and approved by the committee for the protection of human subjects of Brigham and Women's

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Hospital. Informed written consent was obtained from each subject before participation.

Study design

The design of the protocol was identical to that described in previous studies aimed at characterizing the effect of calcium on PTH dynamics (7). Each subject was given infusions (on separate days) of citrate, calcium, and dextrose. The citrate and calcium infusions were usually administered on consecutive days, whereas the dextrose infusion was given 7–10 weeks later. Subjects were blinded to their infusions, and exactly the same procedures were followed on the 3 study days, except that a constant electrocardiogram monitor was used for the citrate infusion only.

The citrate-calcium infusion protocol required two visits to the Ambulatory Clinical Center, usually on 2 consecutive mornings: the first for a citrate infusion and the second for a calcium infusion. For each visit, the subject arrived at 0800 h, after having fasted for at least 8 h. An iv catheter was placed in a vein of each antecubital fossa and kept open with 5% dextrose in water (D5W). One iv line was used for blood sampling, and the other for the infusion of calcium or citrate.

Citrate infusion (day 1)

Citrate (anticoagulant-citrate-dextrose USP Formula A (ACD-A) containing, per 100 mL, 2.45 g dextrose, 2.2 g sodium citrate, and 0.7 g citric acid; Fenwal Laboratories, Deerfield, IL) mixed in D5W was administered via an iv infusion pump (Travenol, Deerfield, IL). Throughout the infusion, blood pressure was monitored by an automated blood pressure recorder (adult/pediatric vital sign monitor, Critikon, Tampa, FL); an electrocardiograph was obtained from a cardiac monitor (Physio Control Lifepak 7, Rowayton, CT) before each increase in the infusion rate. The citrate protocol consisted of three 30-min pulse-step intervals. During each 30-min interval, a rapid 5-min infusion of citrate was followed by a slower infusion for 25 min. Progressively increasing rates of both the fast and the slow infusions were used for three consecutive 30-min periods. The citrate dose was 42 mg/kg·h, followed by 20 mg/kg·h for the first 30-min interval; dosages for the two subsequent intervals were 70/33 and 96/44 mg citrate/kg·h, respectively. Blood samples were collected anaerobically, and serum levels of Ca_i , intact PTH, ACTH, plasma cortisol, TSH, and PRL were measured at 0, 5, 10, 20, and 30 min for each step of the infusion. Serum chemistries (including a magnesium level) and osmolality were obtained at the beginning and completion of each infusion.

Calcium infusion (day 2)

Calcium gluconate (Astra, Westboro, MA) was infused over three 30-min intervals via an iv infusion pump in a pulse-step protocol similar to the citrate infusion protocol. The doses of calcium (in milligrams per kg/h) for the fast/slow infusions were 2.4 followed by 0.75 in step 1, 3.4 followed by 1.25 in step 2, and 4.4 followed by 1.75 in step 3. The phlebotomy protocol was identical to that described for day 1.

Dextrose infusion

Dextrose (5%) was infused at volumetric rates identical to those used for calcium infusion.

Laboratory tests

Serum chemistry values were determined by the clinical chemistry laboratory; a colorimetric method was used with an Olympus AU-5061 analyzer (Olympus Corp., Lake Success, NY). The intra- and interassay coefficients of variation (CVs) for Ca in this method are 1.09% and 1.36%, respectively.

Blood for Ca_i determination was collected anaerobically and measured with an Ionetics 330 calcium analyzer (Ionetics, Costa Mesa, CA), which has a precision of 1.40% (normal range, 3.68–5.44 mg/dL).

Serum intact PTH was measured by the Allegro immunoradiometric assay (Nichols Institute, San Juan Capistrano, CA). The detection limit of the assay is 1 pg/mL (normal range, 10–65 pg/mL), and the intra- and interassay CVs are 2% and 10%, respectively.

Plasma ACTH was measured by the Allegro HS-ACTH immunometric assay (Nichols Institute). The detection limit of this assay is 1 pg/mL (normal range, 9–52 pg/mL), and the intra- and interassay CVs are 6.8% and 8.1%, respectively.

Serum TSH was measured by the Allegro HS-TSH immunoradiometric assay (Nichols Institute). The detection limit of the assay is 0.04 IU/mL, the normal range is 0.5–5.0 IU/mL, and the intra- and interassay CVs are 2.3% and 4.1%, respectively.

Serum PRL was measured by the Allegro PRL immunoradiometric assay (Nichols Institute). The detection limit of the assay is 0.14 ng/mL; the normal range is less than 20 ng/mL. The intra- and interassay CVs are 4.8% and 5.1%, respectively.

Serum cortisol was measured by the GammaCoat Cortisol RIA kit (Incstar Corp., Stillwater, MN). The detection limit of the assay is 1 $\mu\text{g}/\text{dL}$ (normal ranges: 0800 h, 9–24 $\mu\text{g}/\text{dL}$; 1600 h, 3–12 $\mu\text{g}/\text{dL}$). The intra- and interassay CVs are 4.4% and 7.3%, respectively.

Serum Ca_i was measured on the day of the infusion, whereas all other serum samples were stored at -70°F . All samples from each patient were subsequently run in duplicate in each assay.

Statistical methods

Data from the infusion studies included multiple measures (*i.e.* for Ca_i , PTH, ACTH, *etc.*) obtained by repeated sampling over time and were analyzed by a two-factor, repeated measure ANOVA, with use of a subject by day (infusion type) by time factorial design. When significant day by time interactions were observed, then additional one-factor, repeated measure ANOVAs were performed, followed by pairwise comparisons among time means using the Newman-Keuls test. The results were expressed as the mean \pm SEM unless otherwise mentioned. Differences with $P < 0.05$ were considered statistically significant.

Results

Calcium infusion

Figure 1 shows that with calcium infusion, serum Ca_i levels increased progressively from 4.32 ± 0.10 to 4.86 ± 0.08 mg/dL ($P = 0.002$), whereas with dextrose infusion, serum Ca_i levels remained constant at approximately 4.6 ± 0.1 mg/dL. To demonstrate that these changes in serum calcium levels were physiologically relevant, we measured serum PTH levels. As anticipated, PTH levels fell significantly from 36.6 ± 2.8 to 8.24 ± 0.95 pg/mL with calcium infusion ($P < 0.0001$) and were not altered by dextrose infusion. Serum osmolality was not altered by calcium infusion, with values at baseline and termination being 289.5 ± 1.6 and 289.5 ± 2.0 , respectively ($n = 6$). The baseline serum magnesium and serum phosphorus levels were 1.66 ± 0.03 milliequivalents/L and 3.22 ± 0.125 ng/dL, respectively, and did not change significantly during the calcium infusion.

Serum ACTH levels increased significantly ($P = 0.008$) when subjects received the calcium infusion, but not when they received the control dextrose infusion. On the day of calcium infusion, serum ACTH levels rose 1.7-fold from a baseline of 9.87 ± 1.32 pg/mL to a maximum of 16.31 ± 2.84 pg/mL at 80 min (Fig. 1). With dextrose infusion, serum ACTH levels remained relatively constant at approximately 11.58 ± 0.18 pg/mL. The cortisol levels did not change significantly on either the calcium infusion day or the D₅W infusion day, as assessed by one-way ANOVA. Furthermore, a two-way ANOVA comparison of the cortisol responses between the 2 infusion days showed no significant differences (Fig. 1). However, during dextrose infusion there tended to be a gradual decrease in cortisol, consistent with the diurnal variation in cortisol levels. This downward trend in cortisol was not observed on the calcium infusion day

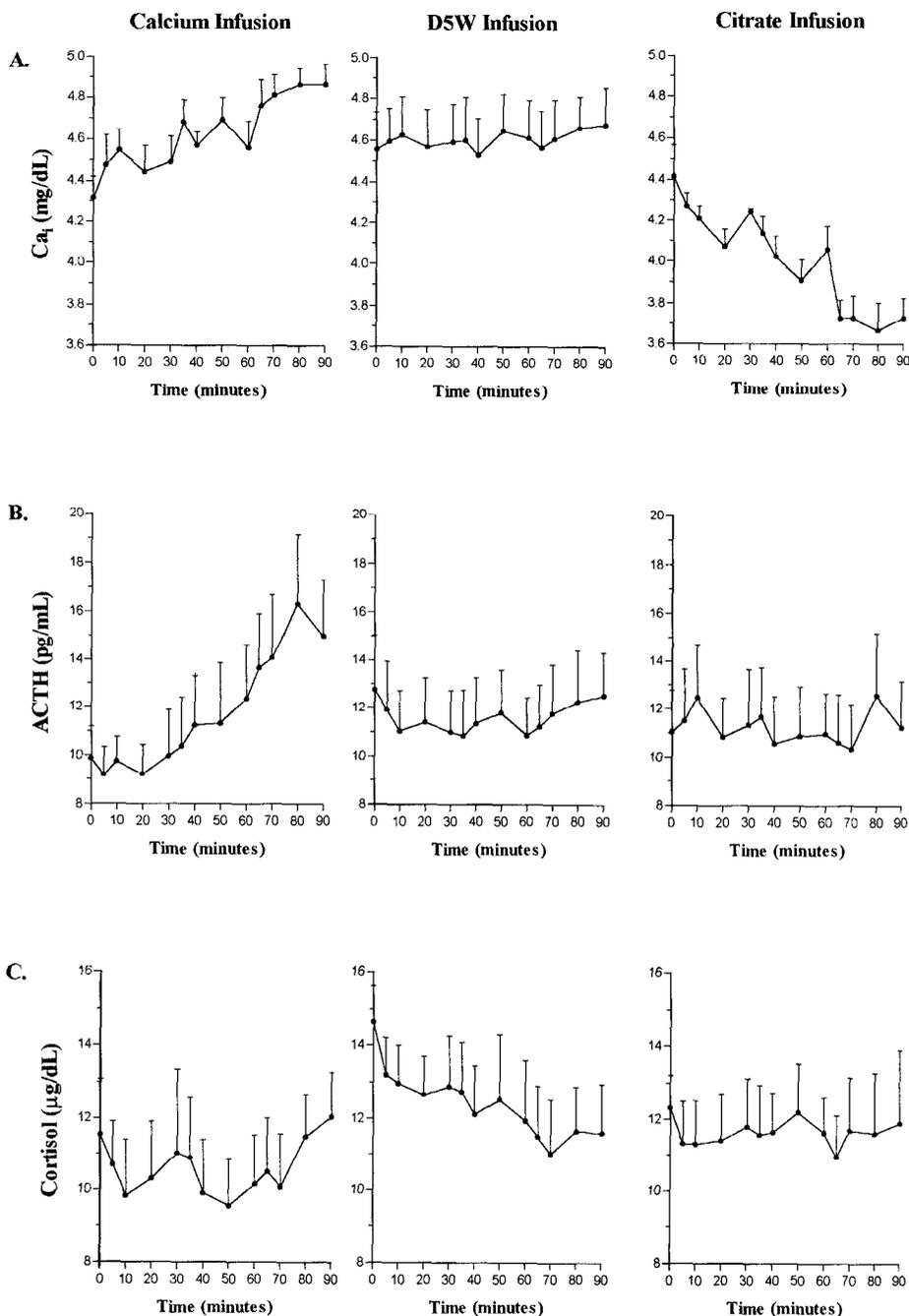


FIG. 1. Effects of calcium (left), 5% dextrose (center), and citrate (right) infusions on serum levels of Ca_i (A), ACTH (B), and cortisol (C).

when, in fact, the cortisol level increased slightly, but not significantly, when analyzed by one-way ANOVA. In the last 40 min of the calcium infusion, a period during which serum Ca_i levels rose from 4.64 ± 0.12 to 4.86 ± 0.10 mg/dL and ACTH from 11.3 ± 2.53 to 15.0 ± 2.34 pg/mL, cortisol levels rose from 9.55 ± 1.28 to 12.0 ± 1.22 μ g/dL ($P = 0.04$, by paired t test).

The ability of an increase in serum calcium levels to elevate ACTH levels appeared to reflect a specific response of the pituitary corticotroph, as neither TSH nor PRL levels were altered by the calcium infusion. Baseline and termination TSH values were 1.46 ± 0.2 and 1.22 ± 0.17 IU/mL, respectively, whereas baseline and termination PRL values were

7.43 ± 0.9 and 6.25 ± 0.88 ng/mL, respectively (Fig. 2). As anticipated, the dextrose infusion did not alter TSH or PRL levels.

Citrate infusion

To determine whether decreases as well as increases in calcium levels alter ACTH responses, we studied the effect of citrate infusion on ACTH levels. During citrate infusion, serum Ca_i levels declined significantly from a baseline of 4.41 ± 0.15 mg/dL to a minimum 3.66 ± 0.13 mg/dL at 80 min ($P = 0.0001$). As expected, serum PTH levels rose significantly from 37.7 ± 4.12 pg/mL to a maximum of 125.4 ± 11.73 pg/mL at 70 min.

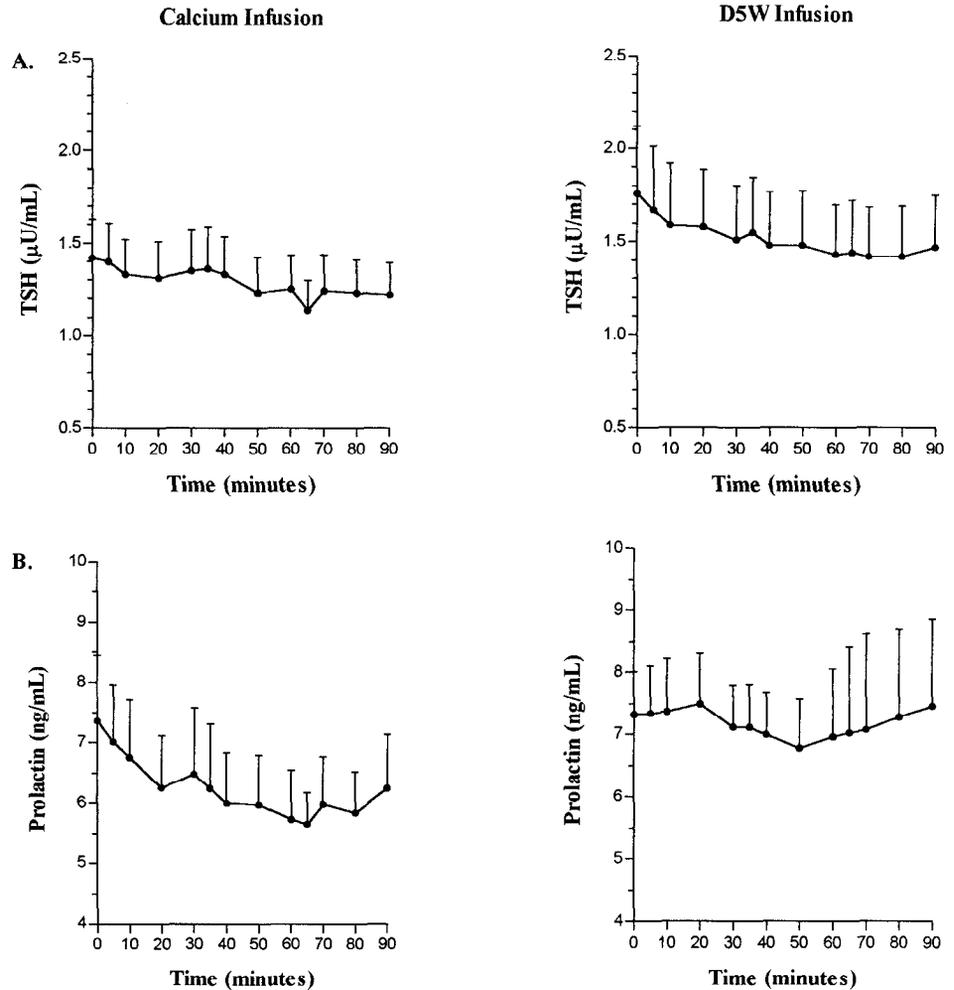


FIG. 2. Effects of calcium (*left*) and 5% dextrose (*right*) infusions on serum levels of TSH (A) and PRL (B).

Despite the decrements in Ca_i , serum levels of ACTH and plasma cortisol remained the same (Fig. 1). Likewise, levels of TSH and PRL did not change during citrate administration; baseline and termination values were 1.53 ± 0.273 and 1.17 ± 0.181 IU/mL, respectively, for TSH and 6.62 ± 0.855 and 6.84 ± 0.660 ng/mL, for PRL, respectively. Citrate infusion did not alter serum osmolality, which was 289.8 ± 2 at baseline and 289 ± 1.9 at termination ($n = 6$).

Discussion

In this report we demonstrate that iv administration of calcium gluconate sufficient to increase serum levels of Ca_i by only 12% over 90 min resulted in a substantial increase (*i.e.* by >50%) in serum ACTH levels in 10 healthy women. This effect appears to be specific because ACTH levels were not altered either by an infusion of citrate, which lowered Ca_i levels by 17%, or by an infusion of D5W, during which Ca_i levels remained constant.

This is the first report of an alteration in ACTH responses in humans by a calcium increase within the physiological range. However, our result is consistent with the results of a previous study in which a rapid infusion of calcium gluconate (0.15 mmol calcium/kg BW over 12 min) resulted in acute hypercalcemia (17). Concomitant with the develop-

ment of hypercalcemia, there was a 1.5- to 3.5-fold increase in ACTH levels as well as an increase in plasma cortisol levels. Studies in hypothyroid and hypoparathyroid subjects suggested that the calcium-mediated increase in ACTH was independent of calcitonin and PTH levels (17). Another study, in which ACTH was not measured, also showed a stimulatory effect of acute hypercalcemia on plasma cortisol levels (18). Given the well known stimulatory effect of ACTH on adrenal steroid secretion, we had expected to see an increase in plasma cortisol levels as well as in serum ACTH levels during the infusion of calcium. Our inability to detect a significant increase in cortisol may be related to a variety of factors. These include the modest increments in ACTH levels compared to the supraphysiological levels achieved with a rapid Cortrosyn (Organon, West Orange, NJ) stimulation test, the masking effect of the diurnal fall in cortisol levels, and the delay between the rise in ACTH and the rise in cortisol levels. Indeed, the tendency of the serum cortisol level to increase between 50–90 min of calcium infusion suggests that if the study were prolonged and/or if it were performed in the evening, when cortisol levels are generally low, we would detect an increase in cortisol levels with calcium infusion.

Previous studies in humans have shown that acute hy-

percalcemia can alter the levels of other anterior pituitary hormones. In six male subjects undergoing a 5-h calcium infusion, the FSH and LH responses to GnRH were enhanced by 22% and 71%, respectively, over the responses of the same subjects in a eucalcemic state (14). Some, but not all, investigators have found that acute hypercalcemia stimulates basal GH levels (12, 14) and inhibits basal PRL responses (15, 16). The induction of acute hypercalcemia has been reported to blunt the TRH-stimulated secretion of both TSH and PRL (14, 19). We found that acute changes in the calcium level within the normal physiological range did not alter basal PRL or TSH levels. Thus, ACTH appears to be much more responsive than PRL and TSH to small rapid increments in the serum calcium level. The physiological rationale for this calcium-mediated increase in ACTH is unclear. It may be that the ability of calcium to stimulate ACTH, which, in turn, should stimulate aldosterone as well as cortisol, represents a nonrenin-mediated mechanism to defend against hypercalcemia and/or volume depletion.

There are several possible mechanisms by which acute increases in extracellular calcium levels could stimulate ACTH secretion. First, extracellular calcium may alter the level of an ACTH secretagogue such as CRH or arginine vasopressin (AVP). Indeed, some studies have shown that calcium stimulates AVP secretion in rats and in patients with uremia (20, 21). Second, the ACTH increase could be secondary to a reduction in the level of an inhibitor of ACTH secretion. However, the increase in ACTH does not appear to be due to a loss of glucocorticoid feedback inhibition, because cortisol levels do not fall during calcium infusion. Third, calcium may act directly on corticotrophs to stimulate ACTH secretion.

Multiple *in vitro* studies have shown that ACTH secretion is dependent on extracellular calcium. In primary cultures of rat anterior pituitary cells, the absence of extracellular calcium reduces the ACTH response to a variety of secretagogues, including CRH, AVP, angiotensin II, norepinephrine, and oxytocin (1, 2, 4). In the mouse corticotroph cell line, AtT-20, increases in extracellular calcium between 0.5–5.0 mmol/L increased basal and CRH-stimulated ACTH secretion 3-fold (3). In corticotrophs, the effects of extracellular calcium may be mediated in part through calcium channels (3–5) and/or a recently identified CaR (8, 11). We have shown that a CaR is expressed in rat and mouse anterior pituitary glands and in AtT-20 cells (11). This pituitary CaR is similar, if not identical, to the 120-kDa G protein-coupled CaR isolated from bovine parathyroid glands that appears to be a critical regulator of PTH secretion and mineral ion homeostasis (8). In humans, mutations that activate and inhibit the CaR are associated with hypo- and hypercalcemic syndromes, respectively (9, 10). In AtT-20 cells, activation of the CaR results in increases in cytosolic calcium and inositol phosphate turnover (11). It is possible that in humans, increases in extracellular calcium activate the CaR located on corticotrophs, thus altering ACTH secretion. In addition, activation of CaRs located in the hypothalamus and other parts of the brain could indirectly influence pituitary function (22).

In summary, we observed that small acute increments in calcium within the normal physiological range raised ACTH

levels in 10 normal women. These results are in accord with *in vitro* studies showing a stimulatory effect of calcium on ACTH secretion. The mechanisms by which calcium exerts its effects are unknown, but may involve calcium channels and/or a CaR, both of which are present in corticotrophs. Finally, the stimulatory effect of calcium on ACTH was specific, as TSH and PRL secretion were not altered.

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