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The Effect of Lithium on Calcium-Induced Changes in Adrenocorticotrophin Levels*

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ABSTRACT

The calcium receptor (CaR) plays a central role in calcium (Ca) sensing by the parathyroid gland and other organs, including the brain. Chronic lithium (Li) therapy causes a significant alteration in Ca-sensing by the CaR-expressing parathyroid chief cells through an unknown mechanism, shifting the PTH set-point (the level of Ca that half-maximally suppresses PTH secretion) to the right. Ca is known to stimulate ACTH levels in normal subjects, and baseline ACTH levels are increased in patients with bipolar disorder. Because the stimulation of ACTH secretion by Ca likely involves the CaR, the aim of this study was to investigate the effects of Li on Ca-induced changes in ACTH levels, using Ca and citrate infusions in seven Li-treated patients and seven controls. During the Ca infusion, increments in serum-ionized Ca concentration (Ca_i) were accompanied by increments in ACTH levels that were significantly greater in the Li-treated group, $P = 0.014$, by ANOVA. Also, cortisol levels increased significantly in the Li-treated, but not the control group, during the Ca infusion, $P < 0.0001$. There was a statistically significant shift in the midpoint of the Ca_i/ACTH curve, to the right, in the Li-treated group, compared with the controls ($P = 0.042$), that was largely caused by an effect of Li on Ca_i. However, for comparable levels of Ca_i, there were no significant differences in the levels of ACTH between the two groups. Therefore, within the physiological range of Ca, there was no effect of Li on Ca_i-induced change in ACTH levels. (*J Clin Endocrinol Metab* **84:** 198–200, 1999)

LITHIUM (Li) is widely used for treating patients with bipolar disorder, and it is estimated that 0.1% of the population is currently receiving Li therapy (1–3). Li has been shown to blunt divalent cation [calcium (Ca) and magnesium Mg)]-induced changes in cytosolic Ca and Ca-induced hydrolysis of inositol phosphates (4, 5), suggesting that the locus of action of Li is distal to the Ca receptor (CaR), a G protein-coupled receptor sensing changes in Ca, which has been identified in multiple organs, including the parathyroid gland, kidney, and brain (6). We have shown that Li induces a shift in the PTH set-point (the level of Ca that half-maximally suppresses PTH secretion), to the right, both *in vivo* and *in vitro* (7, 8), suggesting a direct effect of Li on Ca-sensing at the level of the parathyroid gland.

The CaR is expressed in the ACTH-secreting pituitary cell line, AtT-20, and mediates the stimulatory action of Ca on ACTH secretion (9, 10). Moreover, we have shown that physiological increases in Ca in normal women raise ACTH levels (11), confirming previous *in vivo* work (12). In patients with bipolar disorder, dysregulation in the hypothalamic-pituitary-adrenocortical axis is present, leading to alterations in ACTH and cortisol levels (13). Reisine *et al.* (14) showed that pretreatment of the AtT-20 pituitary cell line with Ca abolished the ACTH response to Li or Ca, suggesting that Li and Ca may act through a common mechanism.

We investigated the effect of Li on Ca-modulated ACTH levels, in a study that characterized the effects of changes in serum-ionized Ca concentration (Ca_i) within physiological range on PTH dynamics in Li-treated subjects (8).

Subjects and Methods

Subjects

Seven women taking chronic Li therapy for the treatment of bipolar affective disorder and seven age-matched control subjects were studied over 2 days (8). In both groups, four subjects received a Ca infusion on the first day, followed by a citrate infusion on the second day; whereas three subjects received the citrate infusion on the first day, followed by the Ca infusion on the second day. Because of the effect of the menstrual cycle on PTH levels (15), young women had to have spontaneous, regular cycles of 28–35 days, as documented by a 3-month diary, to be eligible for the study. All premenopausal women (five in each study group) were studied during the early follicular phase (within the first 7 days) of their menstrual cycles. In addition, two postmenopausal women not taking hormone replacement therapy were studied in each group. The subjects were receiving no other medications known to affect Ca and bone metabolism. Before enrollment, each subject underwent a physical examination and a laboratory evaluation that included a multichannel serum chemistry analysis, a complete blood count with differential, and determination of iPTH level, TSH level, and 25 hydroxyvitamin D [25(OH)D] level. To avoid any confounding effect of obesity on the PTH axis (16), all subjects had a body mass index (BMI, weight in kilograms divided by height in meters squared) of less than 30. The study was reviewed and approved by the Committee for the Protection of Human Subjects of the Brigham and Women's Hospital. Informed written consent was obtained from each subject before participation.

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Study design

The design of the protocol has been described in detail in previous publications examining PTH dynamics in healthy volunteers (8, 17, 18). The protocol required two visits to the Ambulatory Clinical Center, one for a citrate infusion and the other for a Ca infusion. For each visit, the subject arrived at 0800 h in a fasting state. An iv catheter was placed in a vein of each antecubital fossa and kept open with 5% dextrose in water. One iv line was used for blood sampling and the other for infusion of Ca or citrate.

Citrate infusion. 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; Fenway Laboratories, Deerfield, IL) mixed in 5% dextrose in water, was administered via an iv infusion pump (Travenol, Deerfield, IL). Throughout the course of the infusion, blood pressure was monitored by an automated blood pressure recorder (adult/pediatric vital sign monitor, Critikon, Inc., Tampa, FL), and an electrocardiogram was obtained from a cardiac monitor (Physio Control Lifepak 7, Rowayton, CT) at each step, before the infusion rate was increased. The study consisted of three 30-min pulse-step intervals of citrate infusion. In brief, a rapid 5-min infusion of citrate was followed by a slower infusion for 25 min. Progressively increasing rates of both the fast and slow infusions were used for three additional 30-min periods. The citrate dose was 42 mg citrate/kg·h, followed by 20 mg citrate/kg·h for the first 30-min interval; dosages for subsequent intervals were 70/33, and 96/44 mg citrate/kg·h, respectively. Samples for serum Ca_i and ACTH levels were collected anaerobically at 0, 5, 10, 20, and 30 min for each step of the infusion.

Ca infusion. Ca gluconate (Astra, Westboro, MA) was infused over three 30-min pulse-step intervals, via an iv infusion pump, by a procedure similar to that used for the citrate. The doses for Ca for the fast/slow infusions were 2.4 mg/kg·h, followed by 0.75 mg/kg·h in step 1; 3.4 mg/kg·h, followed by 1.25 mg/kg·h in step 2; and 4.4 mg/kg·h, followed by 1.75 mg/kg·h in step 3. Samples for serum Ca_i and ACTH levels were collected anaerobically at 0, 5, 10, 20, and 30 min for each step of the infusion.

Characterization of the Ca_i-ACTH axis

Serum ACTH and Ca_i levels at 0, 30, 60, and 90 min of the citrate infusion and 0, 30, 60, and 90 min of the Ca infusion were fitted to a single curve for each subject, using GraphPad Prism software 1.0 (GraphPad Software, Inc., San Diego, CA) to fit sigmoidal curves to the experimentally obtained data. The computer program generated four parameters that characterized the relationship of ACTH to Ca_i: the maximal ACTH level, the minimal ACTH level, the midpoint of the curve (the Ca_i concentration at which ACTH levels are half-maximally stimulated, and the slope of the line at its midpoint). In addition, data obtained from all time points of the Ca and citrate infusions were also compared between the two groups, to evaluate further the effect of Li on Ca_i-ACTH dynamics.

Laboratory tests. Serum levels of Ca, phosphate (PO_4), Mg, and creatinine were determined by the clinical chemistry laboratory using a colorimetric method with an Olympus Corp. AU-5061 analyzer (Olympus Corp., Lake Success, NY). Blood for Ca_i was collected anaerobically and measured with an AVL 987-S electrolyte analyzer (AVL Scientific Corporation, Roswell, GA), which has an intraassay precision of 0.39% and an interassay precision of 1.7–2.5% for Ca_i levels between 4.48 and 5.92 mg/dL (normal range: 4.60–5.32 mg/dL, N = 57). Serum ACTH levels were measured using the Allegro HS-ACTH immunometric assay (Nichols Institute Diagnostics, San Juan Capistrano, CA). The detection limit is 1 pg/mL, the normal range is 9–52 pg/mL, and the intra- and interassay coefficients of variation are 6.8% and 8.1%, respectively. Serum cortisol was measured with the GammaCoat Cortisol RIA kit (INCSTAR Corp., Stillwater, MN). The detection limit of the assay is 1 μg/dL (normal ranges: 0800 h, 9–24 μg/dL; 1600 h, 3–12 μg/dL). The intra- and interassay coefficients of variation are 4.4% and 7.3%, respectively. Except for serum Ca_i samples, which were measured on the same day that they were obtained, all other serum samples were stored at −70 C before assay. All samples from each patient (except for Ca_i) were run in duplicate in the same assay.

Statistical methods. Baseline demographics were analyzed with a two-tailed *t* test. Summary measures for both study groups derived from the sigmoidal curves (including functions of maximal ACTH levels, minimal ACTH levels, midpoint, and the slope at the midpoint) were compared by *t* test. Data from both infusions included multiple measures (*i.e.* Ca_i, ACTH, and cortisol), sampled repeatedly over time, that were analyzed using repeated-measures ANOVA. Differences between the results were expressed as mean ± SEM, unless otherwise indicated. Significance was indicated for $P < 0.05$.

Results

Baseline values

The baseline Ca_i and iPTH levels were higher in the Li-treated group than in the controls. There were no differences in baseline ACTH and cortisol values between the Li-treated group and the controls. The patients had been on their present Li dose for a mean duration of 5.2 yr, with a mean Li level for six patients of 0.63 ± 0.08 mmol/L (normal range is 0.5–1.3 mmol/L); a Li level was not available for the seventh patient.

The Ca infusion: hypercalcemic stimulus

The Ca_i levels increased from 5.19 ± 0.06 to 5.78 ± 0.1 mg/dL in the Li-treated patients and from 4.92 ± 0.05 to 5.52 ± 0.07 mg/dL in the controls, $P < 0.002$ between the two groups. The increases in Ca_i at 90 min, compared with the values at the 0 min time point, were statistically significant for both groups, $P < 0.02$. Parallel to the increases in Ca_i, ACTH levels in both groups increased significantly, but more so in the Li-treated group, $P < 0.0001$. ACTH levels increased from 14.8 ± 1.6 to 26.4 ± 4.0 pg/mL in the Li-treated patients and from 13.9 ± 2.2 to 16.7 ± 2.8 pg/mL in the controls, $P = 0.002$. The cortisol increased significantly in the Li-treated group only, $P < 0.0001$.

The citrate infusion: hypocalcemic stimulus

The Ca_i levels decreased significantly from 5.18 ± 0.06 to 4.51 ± 0.03 mg/dL in the Li-treated group and from 5.0 ± 0.06 to 4.3 ± 0.04 mg/dL in the controls, $P = 0.024$ between the groups. There were no differences in the ACTH levels, between the two groups, in response to the citrate infusion.

The Ca_i/ACTH relationship

To evaluate the effect of changing Ca_i concentration *per se*, rather than the effect of the rate of change in Ca_i on ACTH levels, we used the 30-min points from both the citrate and Ca infusions to plot the curves. The 30-min time points reflect stable Ca_i levels after the increases in infusion rates (17). There was a statistically significant shift in the Ca_i/ACTH midpoint, to the right, in the Li-treated group, compared with the controls, $P = 0.042$, Fig. 1. The sigmoidal characteristics generated by the computer program in GraphPad Prism were as follows: the midpoint was 5.56 ± 0.1 mg/dL in the Li-treated group and 5.24 ± 0.6 mg/dL in the control group, $P = 0.04$; the maximum ACTH levels were 29.6 ± 5.9 pg/mL in the Li-treated group and 19.1 ± 10.2 pg/mL in the control group; the minimum ACTH levels were 11.7 ± 1.0 pg/mL in the Li-treated group and 10.1 ± 2.2 pg/mL in the control group; and the slopes of the curves were 3.1 ± 1.6 in the Li-treated group and 1.6 ± 2.4 in the control group,

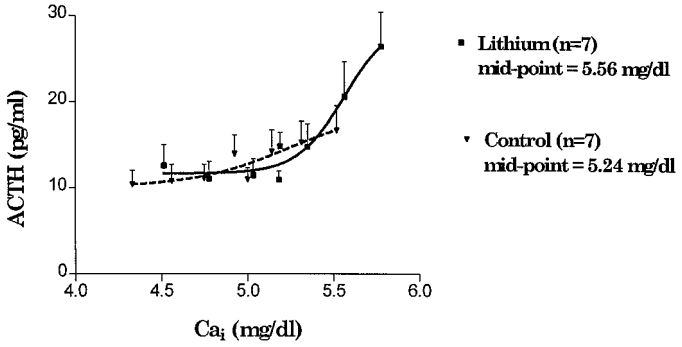

FIG. 1. The curve defining the relationship between ACTH and Ca_i levels in the Li-treated and control groups using 30-min data points (when Ca_i levels are stable from the Ca and citrate infusions). There was a significant shift in the midpoint of the Ca_i/ACTH curve to the right in Li-treated patients, $P = 0.042$

respectively. Thus, although the maximal ACTH response was greater in the Li-treated group, for the same levels of Ca_i, within the physiological range, there was no significant difference in ACTH levels, $P > 0.05$.

Discussion

This study has shown that an increase in Ca_i is accompanied by an increase in ACTH levels in both the Li-treated and control groups. For the same level of Ca_i, the ACTH levels were the same in the two groups, although the Li-treated patients had higher Ca_i levels at the end of the Ca infusion than did the controls, precluding direct comparison between the groups at the higher Ca_i levels. The significant 6% shift in the midpoint of the Ca_i/ACTH curve to the right in the Li-treated patients, relative to that in the controls, may be an effect of Li mediated through Ca on CaR-regulated pathways. This Li-induced effect on Ca-sensing in the corticotroph may be analogous to the Li-mediated effect on Ca sensing by the parathyroid gland, which shows a quantitatively similar shift to the right (7, 8). The CaR that is expressed in the murine AtT-20 corticotroph cell-line (9) is similar, if not identical, to that in bovine parathyroid cells (6). Thus, changes in extracellular Ca levels could also activate the CaR if it is also expressed in normal corticotrophs, thereby explaining the previously observed effects of increases in Ca on ACTH levels *in vivo* in normal subjects (11).

Li blunts divalent cation (Ca and magnesium)-induced changes in cytosolic Ca in parathyroid cells and the Ca-induced hydrolysis of inositol phosphates (4, 5). These results suggest that the effect of Li is distal to the CaR; yet, its precise site of action is unknown.

The exact mechanism explaining the higher maximal ACTH response to increasing Ca_i levels in the Li-treated group remains unclear and may involve other potential Li-neuroendocrine effects independent of the actions of the CaR on ACTH secretion.

Unlike the inhibitory effects of the CaR on PTH secretion, there is a stimulatory action of the receptor on ACTH secretion in AtT-20 cells, similar to that on calcitonin secretion from CaR-expressing C-cells (19). Our finding, that Li (apart from its effect on Ca_i-induced changes in ACTH levels) does not directly modulate ACTH levels under physiologic concentrations, is particularly relevant to patients with bipolar disorder, considering that there may be alterations in the hypothalamic-pituitary-adrenal axis in these patients (20).

In conclusion, our study suggests that besides the Li-induced increments in ionized Ca levels, Li has little or no impact on the levels of ACTH within a physiological range of Ca.

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