The Effect of Lithium on Calcium-Induced Changes in Adrenocorticotropic Hormone 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/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$_i$, there was no effect of Li on Ca$_i$-induced change in ACTH levels.

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 (7, 8) 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-pitu-
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, Roswayton, 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 in step 1, 85 mg citrate/kg in step 2; and 170 mg citrate/kg in step 3 for serum Ca, and ACTH levels were collected anaerobically at 0, 5, 10, 20, and 30 min for each step of the infusion.

Ca infusion. Ca glonuate (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, and ACTH levels were collected anaerobically at 0, 5, 10, 20, and 30 min for each step of the infusion.

Characterization of the Ca/ACTH axis

Serum ACTH and Ca 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; the maximal ACTH level, the minimal ACTH level, the midpoint of the curve (the Ca 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/ACTH dynamics.

Laboratory tests. Serum levels of Ca, phosphate (PO4), 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 was collected anaerobically and measured with an AVL 987-S electrolyte analyzer (AVL Scientific Corporation, Roswell, GA), which has an intra assay precision of 0.39% and an inter assay precision of 1.7–2.5% for Ca levels between 4.48 and 5.92 mg/dL (normal range: 4.60 to 5.32 mg/dL; N = 57). Serum ACTH levels were measured using the Allegro HS-ACTH immunomassay (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 inter assay 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 pg/mL (normal ranges: 0.00–3.1 pg/mL; 1600 h, 3–12 mg/dL). The intra- and inter assay coefficients of variation are 4.4% and 7.3%, respectively. Except for serum Ca, 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) 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. From both infusions included multiple measures (i.e., Ca, 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 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 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 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, 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 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/ACTH relationship

To evaluate the effect of changing Ca concentration per se, rather than the effect of the rate of change in Ca 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 levels after the increases in infusion rates (17). There was a statistically significant shift in the Ca/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 ± 0.07 mg/dL, the Ca levels increased from 5.22 ± 0.07 mg/dL in the Li-treated group and 5.24 ± 0.06 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,
respectively. Thus, although the maximal ACTH response was greater in the Li-treated group, for the same levels of $C_a$, within the physiological range, there was no significant difference in ACTH levels, $P > 0.05$.

**Discussion**

This study has shown that an increase in $C_a$, is accompanied by an increase in ACTH levels in both the Li-treated and control groups. For the same level of $C_a$, the ACTH levels were the same in the two groups, although the Li-treated patients had higher $C_a$ levels at the end of the Ca infusion than did the controls, precluding direct comparison between the groups at the higher $C_a$ levels. The significant 6% shift in the midpoint of the $C_a$/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, 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 $C_a$ 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 $C_a$-induced changes in ACTH levels) does not directly modulate ACTH levels under physiologic conditions, 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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**References**