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Intracellular Ionized Calcium and Increasing Doses of Lithium Chloride Therapy in Healthy Sprague–Dawley Rats

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ADEGBOYEGA, P. A. AND A. O. OKORODUDU. *Intracellular ionized calcium and increasing doses of lithium chloride therapy in healthy Sprague-Dawley rats.* PHARMACOL BIOCHEM BEHAV **49**(4) 1087–1091, 1994.—The use of lithium salts in the prophylaxis and treatment of several psychiatric and neurologic disorders continues to be well accepted despite the apparent lack of understanding regarding its mode of action at the molecular level. This lack of delineation in the mechanism of action is supported by numerous conflicting publications. Despite the lack of understanding, a role for calcium in the manifestation of lithium's action is a constant singular consensus. Intracellular ionized calcium ($[Ca^{++}]_i$) is involved in the proper functioning of cells because of its role in the second messenger pathway. It is therefore essential to evaluate the effect of lithium on intracellular calcium metabolism in a well-defined system. In this study, platelets loaded with Fura-2-Acetoxymethyl were used to evaluate the effect of intraperitoneally administered lithium chloride at 0, 2.5, 5.0, 7.5, and 10 mmol/kg body wt. on $[Ca^{++}]_i$. The results showed a slight relative increase in serum Ca^{++} that correlated well with the dose of LiCl administered to the rats. The baseline $[Ca^{++}]_i$ were comparable in the study groups, but the response to thrombin stimulation was more pronounced at LiCl doses of 2.5, 5.0, and 7.5/kg body wt. compared with control and rats treated with 10 mmol LiCl/kg body wt. This finding suggests a dose-dependent response of $[Ca^{++}]_i$ to LiCl treatment. The observation may therefore explain the variations that have been reported in $[Ca^{++}]_i$ studies with respect to LiCl therapy using different doses.

Lithium Intracellular calcium Thrombin Platelets Rats

THE serendipitous discovery in 1949 of the calming effect of lithium salts on a variety of hyperactive psychiatric patients (25) was one of the most significant discoveries in the history of pharmacotherapy. Lithium salts have since been used as antimanic agents for the prophylaxis (5) and treatment of bipolar disorders, such as manic-depressive psychosis (44). The successful use of lithium in controlling the hyperactivity of patients with a variety of diagnoses, including schizophrenia and organic brain damage, has also been reported. In addition, lithium is considered by some investigators to be the drug of choice for the prevention of chronic cluster headaches; and it may also be effective in episodic or periodic forms of cluster headaches (20,26). Although the effectiveness of lithium salts as an antimanic agent is well established, the exact biochemical mechanism by which lithium ions exert a therapeutic effect is yet to be defined (23,28,33,44). It is known that lithium as a monovalent cation competes at ion channels in cell membranes with other monovalent and divalent cations including sodium, magnesium, and calcium. This competition at the ions channels is noteworthy because some of these ions—for example, calcium—play fundamental roles in the regulation of many basic cellular processes, both within and outside the central nervous system. Available data show that there is an overall disturbance of calcium metabolism in bipolar affective disorders, and lithium produces its effect by interacting with calcium-regulated neurochemical systems (30,31). The advent of fluorescent intracellular chelators has made it possible to directly measure intracellular ionized calcium concentration $[Ca^{++}]_i$. However, it is not yet possible to study changes in $[Ca^{++}]_i$ within the intact brain. Therefore, previous studies involving the direct measurement of $[Ca^{++}]_i$ made use of blood platalets because they provide a reliable peripheral model of the central neurons.(17,18,32,36,37). The significant functional similarities between platalets and central neurons

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include the accumulation, storage, and release of biogenic amines (36–38), analogous α-adrenergic receptors (22), abnormal serotonin reuptake, monoamine oxidase levels or adrenergic receptors in depression, and similar blockades of biogenic amines reuptake by tricyclic antidepressants (38). These findings strongly support the hypothesis that changes in the central nervous system are reflected in platalets (17). To explore the theory that lithium acts by altering $[Ca^{++}]_i$ dynamics, we carried out a controlled study of the changes in baseline and thrombin-stimulated $[Ca^{++}]_i$ in platelets of rats treated with different doses of lithium chloride.

METHOD

Study Population

We randomly divided 25 male Sprague-Dawley rats, weighing 250–300 g (Harlan, Houston, TX), into five equal groups, A-E. They were maintained under similar conditions and allowed to acclimatize to the new environment for a period of 7 days before starting the experimental procedures. The rats had access to drinking water and food throughout the acclimatization and experimental periods. Beginning at day 8, and continuing for 7 days afterward, rats in each group were given daily intraperitoneal injections of normal saline (group A), and 2.5, 5.0, 7.5, and 10.0 mmol LiCl/kg body wt. in normal saline for groups B-E.

Biochemical Studies

Specimen collection. On day 7 of the treatment period, the rats were fasted overnight (12 h), but had access to drinking water. The following morning (day 8), the rats were anesthetized with methoxyflurane fume, and blood samples were collected from the dorsal aorta after the abdomen was surgically opened. Blood for the intracellular studies was collected into 10-ml polypropylene syringes containing 2 ml acid citrate dextrose buffer (ACD: 85 mmol citric acid, 110 mmol dextrose, pH 4.9). The blood samples for the extracellular measurement of total calcium, ionized calcium, total protein, albumin, creatinine, and blood urea nitrogen (BUN) were collected using heparinized capillary tubes (Radiometer, Copenhagen, Denmark) and Microtainer tubes (Becton Dickinson Vacutainer Systems, Rutherford, NJ).

Extracellular assays. Whole blood ionized calcium was measured within 15 min after sampling using an AVL 980 calcium and pH analyzer (AVL Scientific, Atlanta, GA). To minimize the effect of pH changes on ionized calcium, the capillary blood collectors were filled with blood and then placed in ice granules before analysis. Serum total calcium, total protein, albumin, creatinine, and BUN were measured using a Kodak Ektachem 700XR Analyzer (Eastman Kodak Company, Rochester, NY).

Intracellular calcium measurement. Platelets were isolated from the ACD anticoagulated blood by centrifugation at 200 × *g* for 10 min using the method of Brass et al. (10). The isolated platelets were resuspended in a platelet buffer (5.0 mmol Hepes, 137 mmol NaCl, 2.0 mmol, KCl, 1.0 mmol $MgCl_2$, 5.5 mmol glucose; pH 7.4) and incubated at 37°C until a characteristic pearlescent appearance indicating discoid, unactivated platelet was observed. Platelet counts were accomplished using a Technicon H-1 System (Miles, Tarrytown, NY), and the platelets were adjusted to a count of $2 \times 10^5/\mu l$.

We used the assay for intracellular ionized calcium $[Ca^{2+}]i$ by Tsien et al. (42). A total of 2 ml of the platelet suspension was loaded with 3 μmol of Fura-2AM per ml of platelet suspension and incubated for 30 min at 37°C, performed twice. After the incubation period, extracellular Fura-2AM was removed by washing three times and resuspending in platelet buffer to a final count of $1 \times 10^5/\mu l$. The relative fluorescence intensity (RFI) of the continuously stirred 1.5-ml platelet suspension was then measured at 370°C using a Hitachi F-2000 Fluorescence Spectrophotometer (Hitachi Instruments, Danbury, CT). Excitation wavelengths were 340 and 380 nm at an emission wavelength of 510 nm, with a band-pass setting of 10 nm. Resting $[Ca^{2+}]i$ and peak $[Ca^{2+}]i$ were determined before and after stimulation with 7 U bovine plasma thrombin (Sigma Chemical Co., St. Louis, MO), as indicated in Fig. 1. The $_iCa^{2+}$ concentrations were calculated automatically by the F-2000 Fluorescence Spectrophotometer's built-in software using the following equation (41): $[Ca^{2+}]_i = K_d (F - F_{min})/(F_{max} - F)$, where F = fluorescence as a function of $[Ca^{2+}]_i$; F_{min} = fluorescence at very low $[Ca^{2+}]_i$; F_{max} = fluorescence at very high $[Ca^{2+}]_i$; and K_d = effective dissociation constant.

Fluorescence measured after the addition of 7 U thrombin at 50 s defined the peak $[Ca^{2+}]_i$. Maximum fluorescence was measured after the addition of 5 μl 10% Triton X100, and the subsequent addition of 5 μl of 0.5 molar ethylenediaminetetraacetic acid defined the minimum fluorescence.

RESULTS

We evaluated the effects of LiCl therapy on pertinent serum analytes. Table 1 shows the effect of 1 week of lithium treatment on serum total calcium, ionized calcium, total protein, albumin, creatinine, and BUN. Treatment with LiCl in this study had no significant effect on the renal function of the experimental animals, as indicated by the comparison of the creatinine and BUN values relative to the control group ($df = 8$, $p < 0.05$). There were mild elevations in the serum total calcium concentrations of the lithium-treated rats relative to the control ones. This increase in total calcium was not statistically significant ($df = 8$, $p < 0.05$). Also, at the 10 mmol/kg body wt. dose of the LiCl, a statistically significant increase in serum ionized calcium was observed ($df = 8$, $p < 0.05$). The results for the other measured analytes were not significantly different relative to the control group (A).

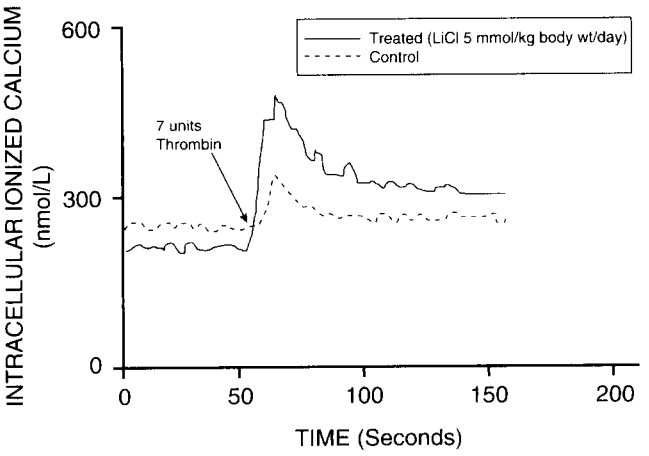

FIG. 1. Representative traces of thrombin-induced changes in platelet intracellular free calcium concentrations [(Ca 2^+)$_i$].

TABLE 1

SERUM CONCENTRATIONS TOTAL AND IONIZED CALCIUM, TOTAL PROTEIN, ALBUMIN, CREATININE, AND BUN (MEAN ± SD) 24 H AFTER LAST LiCL DOSAGE WAS ADMINISTERED

Parameter	Group A (Control)	Group B (LiCl = 2.5 mM/ kg per day)	Group C (LiCl = 5 mM/ kg per day)	Group D (LiCl = 7.5 mM/ kg per day)	Group E (LiCl = 10 mM/ kg per day)
Total calcium (mmol/l)	2.29 ± 0.16	2.37 ± 0.06	2.49 ± 0.16	2.38 ± 0.03	2.50 ± 0.17
Ionized calcium (pH = 7.4, mmol/l)	1.39 ± 0.09	1.41 ± 0.03	1.41 ± 0.06	1.43 ± 0.04	1.65 ± 0.10
Total protein (g/dl)	5.10 ± 0.24	5.04 ± 0.18	5.13 ± 0.25	5.08 ± 0.15	5.15 ± 0.37
Albumin (g/dl)	2.82 ± 0.16	2.62 ± 0.16	3.00 ± 0.19	2.64 ± 0.09	2.92 ± 0.45
Creatinine (mg/dl)	0.64 ± 0.08	0.58 ± 0.04	0.73 ± 0.11	0.56 ± 0.05	0.68 ± 0.10
BUN (mg/dl)	15.60 ± 1.62	14.8 ± 0.84	17.25 ± 0.43	14.2 ± 1.64	17.80 ± 2.93

Except for a statistically significant increase in the ionized calcium for group E rats (LiCl dose = 10 mmol/kg per day), the results are not significantly different at $p < 0.05$, $df = 8$ for the comparison of each study group with the control rats. BUN = blood urea nitrogen. $n = 5$ per group.

Figure 1 illustrates the intracellular calcium measurements before and after thrombin stimulation, using control and LiCl-treated (5 mmol/kg body wt. per day) rats. The correlation between the baseline and thrombin-stimulated $[Ca^{2+}]_i$ was examined relative to increasing doses of LiCl; Fig. 2 illustrates the findings. The baseline cytosolic $[Ca^{2+}]_i$ were relatively identical in all study groups, except for the noticeable slight decrease in the LiCl-treated rats. Statistical evaluation of the means for the baseline $[Ca^{2+}]_i$ of the experimental groups (B–E) relative to the control showed no significant difference ($df = 8$, $p < 0.05$). However, the response to stimulation was more marked in the rat platelets at LiCl doses of 2.5, 5.0, and 7.5 mmol/kg body wt. (experimental groups B–D). These increased stimulation peaks were statistically different from the peak stimulation for the control group A ($df = 8$, $p < 0.05$). At the highest dose dosage of 10.0 mM/kg body wt. per day (group E), the responses to stimulation were not different from those of control group A. The results show that under

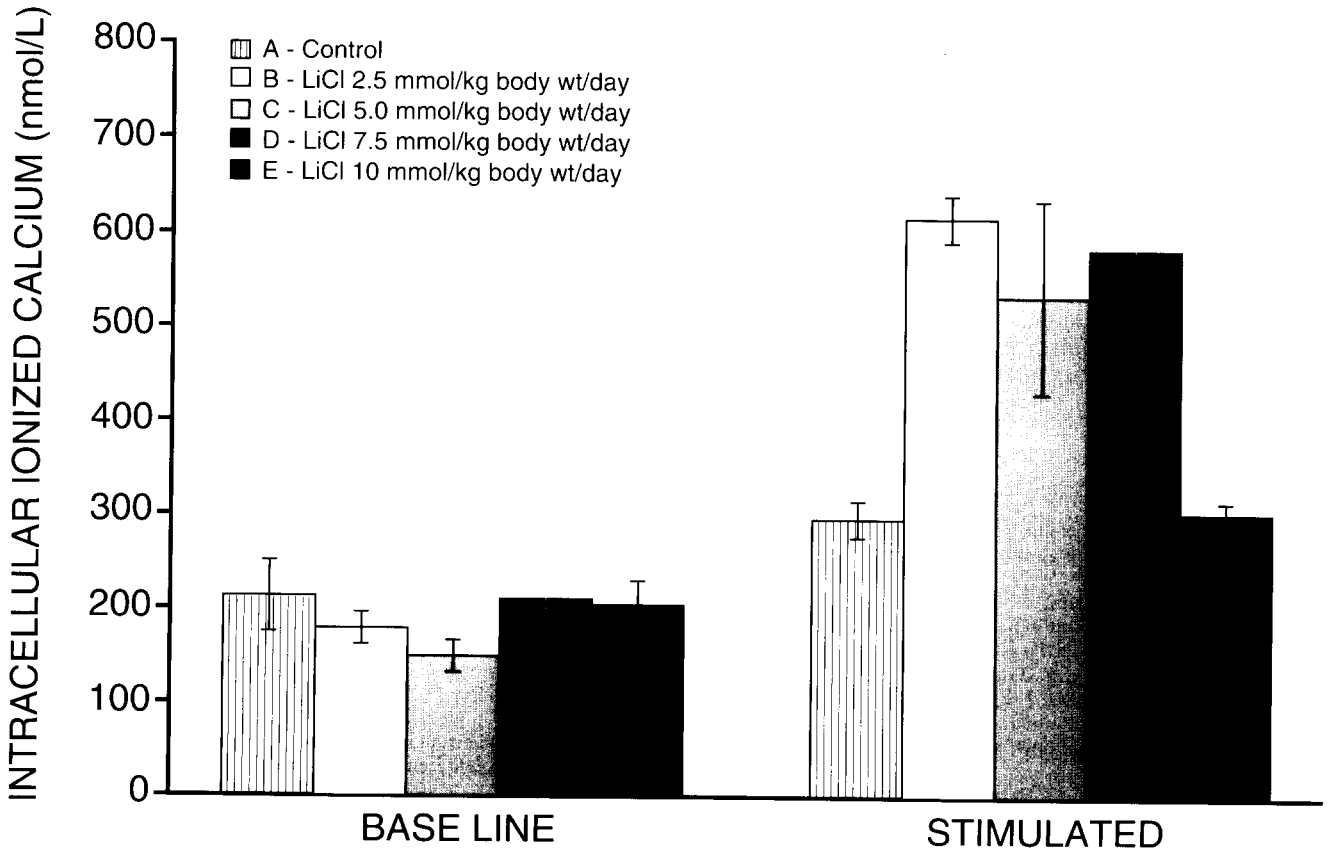

FIG. 2. Baseline and thrombin-stimulated cytosolic platelet calcium of the controls and LiCl-treated animals. $n = 5$ in each group; all experiments were performed in duplicate.

the same experimental conditions, the percentage stimulation of $[Ca^{2+}]_i$ at LiCl doses of 2.5, 5.0, and 7.5 mmol/kg body wt. per day was much greater than that obtained in the controls and also at a dose of 10 mmol/kg body wt. per day. The stimulation was highest at an LiCl dose of 2.50 mmol/kg body wt. per day, with a gradual decrease as the dose and day increased.

DISCUSSION

Investigations into the biology of the affective disorders, as well as the pharmacologic mechanism of action of lithium, have centered on biogenic amines and electrolytes (3). Previous studies have suggested that lithium induces hypercalcemia, but the results of such studies are conflicting (4). Some groups have reported an increase in serum calcium during lithium treatment (2,4,8,12,13,21,29,34,40), whereas others have failed to confirm these changes (24,43). Various reports have suggested the possibility that lithium exerts its effect through an influence on calcium metabolism. Lithium-induced hypercalcemia has also been reported to be accompanied by elevated circulating parathyroid hormone (PTH) in some patients (11,14,15,34). This finding led to the hypothesis that lithium may act, at least in part, by resetting the parathyroid "set-point" or "calciostat" through an intracellular mechanism that decreases the ability of the parathyroid cell to sense serum calcium levels accurately (34,45).

Our findings are consistent with the hypotheses that lithium induces mild hypercalcemia in most lithium-treated individuals, and that measurement of ionized calcium may be more sensitive than total calcium for detecting hypercalcemia in patients receiving lithium (40). The increase serum ionized calcium level that we found in this study was mild, and was in agreement with the reported findings of Toffaletti et al. (40). However, the elevation of ionized calcium was not as high as that reported by Fujimura et al. (21). In the study by Fujimura et al., the control rats and rats treated with 0.5 and 2.0 mmol/kg per day LiCl for 6 days were reported to have serum-ionized calcium levels of 2.57 ± 0.02, 2.59 ± 0.02, and 2.63 ± 0.01 mmol/l. These values were greater than those recorded for the serum nonionized calcium in that same study, and cannot be explained because ionized calcium is normally about 50% of the total serum calcium.

In the last several years, the search for a clear understanding of the pathophysiology of affective disorders and the exact mechanism of the pharmacologic actions of lithium and other therapeutic agents with antimanic actions has focused on changes in intracellular calcium dynamics. Elevations of baseline and agonist-stimulated $[Ca^{2+}]_i$ have been reported in the blood platelets of patients with untreated bipolar affective disorder (9,18,19,27). Lithium therapy has also been shown to restore the elevated baseline $[Ca^{2+}]_i$ to near-normal values in this group of patients (18).

However, a recent study by Tan et al. reported that eurythmic patients treated with lithium had higher baseline $[Ca^{2+}]_i$ than did nontreated healthy controls (39). In that same study, the lithium-treated patients' platelets also had an increased response to agonist-stimulated increases in $[Ca^{2+}]_i$. It was therefore suggested that chronic lithium treatment may reset both baseline and stimulated $[Ca^{2+}]_i$ at higher levels. However, this theory was not substantiated with a baseline measurement from the patients before treatment. Considering the published data from similar and other related studies, the elevated baseline $[Ca^{2+}]_i$ in the lithium-treated patients in study by Tan et al. was probably much higher before the commencement of lithium therapy. Results from our rat studies show that treatment of rats with LiCl for 1 week causes a decrease in the baseline $[Ca^{2+}]_i$ and an increased response to agonist stimulation of $[Ca^{2+}]_i$. These changes were, however, more pronounced at a dosage of 2.5–7.5 mmol/kg body wt. per day than at 10 mmol/kg per day. The decrease in baseline $[Ca^{2+}]_i$ in our study supports recent reports from human studies that indicate that lithium may function by decreasing the level of $[Ca^{2+}]_i$ (14,45). At the subcellular level, hyperactivity of intracellular second-messenger signaling mechanisms, especially those of intracellular free calcium and phosphoinositide cycle, may be the main disturbance underlying mania and bipolar affective disorder. Lithium acting as a homeostatic drug seems to be selective against hyperactive receptors, in which it acts to drive them back toward their normal operating range (7,17). Lithium probably does this through modulation of the phosphoinositol metabolism (1,6,7,16,30,35). Our findings suggest a dose-dependent response of $[Ca^{2+}]_i$ to lithium therapy. This may account for its narrow therapeutic range, and also explain the contradictory reports in the literature.

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