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Effect of Lithium Chloride on Adrenocortical Function in the Rat[1] (40013)

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Lithium salts are well-accepted therapeutic agents for the treatment of various mental disorders, particularly for the alleviation of manic depression. During the last decade, use of lithium has grown so rapidly that interest has focused on its mode of action as well as its possible influence on other biological systems. Current evidence supports a mechanism whereby lithium modifies various aspects of central biogenic amine metabolism (1, 2).

Considerable data have accumulated which demonstrate an important, although not fully understood, involvement of central biogenic amines in the modulation of the pituitary-adrenal gland axis (3-6). Experimental manipulation of brain amine levels has been shown to alter pituitary secretion of ACTH and thus adrenocortical hormone release. Since lithium treatment has been associated with altered amine levels, it is reasonable to assume that adrenal gland function may be affected as well. The few reports which deal with the effect of lithium on adrenal function are difficult to interpret (7-10).

In order to more fully ascertain the effects which lithium may have, the influence of chronic lithium chloride treatment on adrenal cortical function in the male rat was examined.

Materials and methods. Adult male Sprague-Dawley rats (Charles River Breeding Laboratories, Inc., Mass.) weighing approximately 200 g were maintained in light-controlled (fluorescent illumination, lights on 0400 hr, lights off 1800 hr), air-conditioned (22-24°) rooms with food and water available *ad libitum*. They were caged individually and allowed to acclimate to room conditions for at least 10 days prior to the experiments.

The first study was designed to examine the effect of chronic lithium chloride treatment on the rhythmic pituitary-adrenal function. Two groups of rats were used; one received lithium chloride (3 meq/kg body wt), orally, once a day (0800-1000 hr) for 2 weeks, and the other group was similarly treated with isotonic NaCl. On the day following the last drug injection, blood samples were obtained for studies of diurnal variation in plasma corticosterone (B) concentrations between 0800 and 0900 hr and 1600 and 1700 hr while the rats were in the resting state. Strict measures were employed to avoid disturbing the rats before blood sampling. No one was allowed in the animal quarters for at least 12 hr before sampling. Particular care was taken to ensure that each blood sample was obtained within 3 min after each "nonstressed" rat was removed from its cage. Each rat was then carried to an anteroom and immediately etherized; jugular blood (0.5-1.0 ml) was withdrawn into heparinized syringes. The "stress" response to ether inhalation was assessed at the same time. After the first blood sample was taken as described, the anesthetized rats were placed in individual cages in the anteroom for a 15-min period beginning with their first exposure to ether vapors. They were etherized a second time and another blood sample was collected.

The acute response to lithium was also studied. Groups of rats received one oral injection of LiCl (6 meq/kg body wt) or isotonic NaCl at 2, 12, or 24 hr prior to blood sampling. Basal B levels were measured at 0800-0900 hr.

The third segment of this investigation examined the effect of dexamethasone-21-phosphate on plasma B levels in rats chronically treated with LiCl or NaCl.

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Isotonic NaCl or diluted dexamethasone-21-phosphate (Decadron, Merck, Sharp and Dohme) (100 μg/kg body wt) was injected sc in rats at midnight on the 14th day of LiCl (3 meq/kg) or NaCl treatment. Resting and ether-stress blood samples were obtained at 0800–0900 hr.

All blood was collected in heparinized syringes. The blood was centrifuged at 4° and the plasma was separated and frozen until assayed. Plasma B was measured fluorometrically by a modification of the method described by Kendall *et al.* (11). Plasma lithium levels were measured according to the technique of Amdisen (12). Student's *t* test was used for statistical analysis of the data.

Results. Saline-treated rats exhibited a typical diurnal variation in plasma B (AM, 6.4 ± 1.4 μg/100 ml; PM, 17.4 ± 2.2). Although LiCl (3 meq/kg body wt) administered orally to male rats for 2 weeks did not abolish an AM–PM rhythm in plasma B concentration, it did cause a significant ($P < 0.01$) elevation in the morning (0800-hr) basal levels in comparison to saline-treated control rats (6.4 ± 1.4 vs 11.8 ± 1.2) (Fig. 1). The 1600-hr plasma hormone level in LiCl-treated animals (17.2 ± 1.3) did not differ from that of the controls (17.4 ± 2.2). At the end of 2 weeks, the change in body weights (Δ = final − initial) was somewhat, but not significantly, less in the LiCl-treated group.

Following ether anesthesia, the stress plasma B concentrations rose dramatically (Fig. 1) in all groups, as expected. In LiCl-treated rats, however, the 0800-hr plasma adrenal steroid concentration (44.0 ± 1.3) was elevated ($P < 0.05$) over that of its stress controls (39.4 ± 2.1); there was no comparable difference between the groups for the afternoon stress value.

The acute effect of LiCl administration on adrenocortical function is shown in Fig. 2. Young male rats given one oral dose of LiCl (6 meq/kg body wt) 2 hr prior to the 0800-hr blood draw responded with a marked elevation ($P < 0.05$) in plasma corticosterone level (32.9 ± 7.2) compared with NaCl-treated animals (17.5 ± 4.1). The difference in plasma hormone levels was evident, in spite of the presence of handling and injection stress factor which increased adrenocortical activity in both groups. When the time duration between drug or saline administration and blood sampling was extended to 12 and 24 hr, the corticosterone concentrations remained higher ($P < 0.05$) in the LiCl-treated animals. Note that the residual stress effect had dissipated by 12 hr after either injection.

In two separate assessments rats treated with LiCl or NaCl for 2 weeks were given one sc injection of either dexamethasone (100 μg/kg body wt) or saline 8 hr before sampling blood at 0800 hr (Fig. 3). The

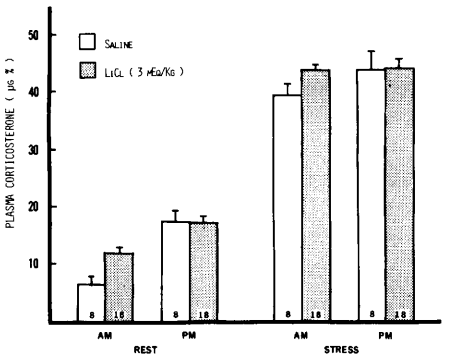

FIG. 1. The effect of chronic LiCl or NaCl treatment on "resting" and "stress" plasma B levels in the rat. Results expressed as means ± SEM; number of animals indicated in each column.

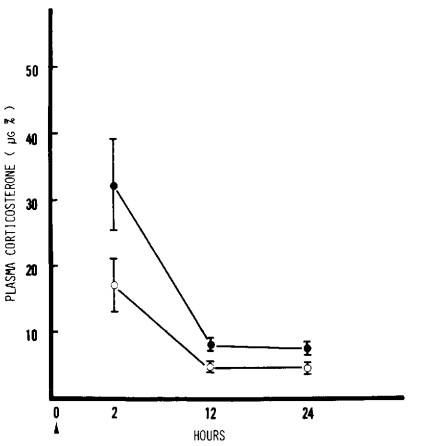

FIG. 2. Basal B secretion 2, 12, and 24 hr following an acute dose of LiCl (●) (6 meq/kg body wt) or NaCl (○). The injection time is indicated by the arrowhead; number of animals per group = 7; results expressed as means ± SEM.

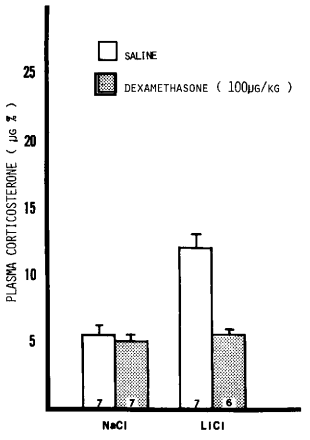

FIG. 3. The effect of one sc injection of dexamethasone (100 μg/kg body wt) on plasma B levels in rats chronically treated with LiCl or NaCl. Results expressed as means ± SEM; number of animals indicated in each column.

results for one of the two experiments are shown in Fig. 3. Notice that dexamethasone treatment did not cause an additional attenuation in resting plasma B levels in chronic NaCl-treated rats. One sc injection of 100 μg/kg body weight of dexamethasone, however, did effectively suppress the elevated plasma steroid level typically seen in long-term LiCl-treated animals (12.0 ± 1.3 vs 5.5 ± 0.4; $P < 0.01$).

Discussion. These data indicate that lithium affects the normal adrenocortical secretory pattern. In our laboratory the diurnal rhythm in adrenocortical secretion is quite typical, i.e., plasma corticosterone levels are lowest ("trough") at 0800 hr and increase steadily throughout the day to reach their highest levels ("peak") at approximately 1600 hr. Although lithium administered daily for 2 weeks to male rats did not abolish the fluctuation in plasma adrenal steroid levels (Fig. 1), it did consistently affect the rats in such a way as to cause the early-morning plasma hormone levels to be elevated midway between the normal trough and peak values. On two separate occasions we measured plasma corticosterone in lithium-treated rats at 4-hr intervals throughout a 24-hr period. The data from these studies confirmed those illustrated in Fig. 1 in that the plasma hormone level at each time point did not differ from that of saline-treated control rats except for the 0800-hr value, which was elevated as shown in Fig. 1.

Few reports exist which deal with the influence of lithium on adrenocortical secretion. However, those which have been reported (7–10) are at variance with one another and difficult to interpret. Platman and Fieve (8) and Platman *et al.* (9) have presented evidence that the morning plasma cortisol levels are increased after lithium carbonate administration. Their data were collected from patients with manic depressive disease. On the other hand, in a study using normal healthy male volunteers, Halmi *et al.* (7) reported that lithium blunts the circadian variation of serum cortisol levels by lowering peak morning values. In still another investigation (10), lithium carbonate did not have any apparent effect on adrenal function in a group of patients in clinical remission. The discrepancies in these reports are most likely due to variations in the sampling population, dose of lithium used, duration of treatment, or time of blood sampling.

We attempted to minimize variables in these studies. Each male rat of the same age and weight was treated with an identical dose of lithium for the same period of time. Blood was sampled with as little disturbance to the rats as possible. We feel that our basal plasma corticosterone levels accurately reflect the efficacy of our sampling procedure since the "resting" rats display plasma hormone levels not unlike those of animals treated with a dose of dexamethasone sufficient to suppress adrenocortical function to basal levels (13). Initially the dosage and route of administration of LiCl were given special consideration. Various concentrations of LiCl (1.5, 3.0, 6.0, and 15.0 meq/kg body wt) were administered either ip or orally at 0800–1000 hr, daily, for 2 weeks. Since all rats receiving the two higher dosages of LiCl died within 4 days, the 3.0 meq/kg body weight dose was used in all experiments unless otherwise stated. This drug regimen resulted in lithium plasma levels in the range of 0.30 ± 0.030 meq/liter as measured 24 hr after the last injection.

No difference in results was observed between the two routes of administering the drug; therefore, in order to circumvent the possible nonspecific stressful effects due to peritoneal irritation, all drug treatments were administered orally in these studies.

Ether inhalation was used in one study to assess the effect of lithium on the functional integrity of the brain–pituitary–adrenal unit and of its ability to respond with increased ACTH release. The stress plasma corticosterone level in lithium-treated rats was greater ($P < 0.05$) than that of stressed saline-treated animals during the trough period of the diurnal cycle. There was no difference between the groups in the afternoon. Despite the increased response to ether in the lithium-treated rats, however, the "stress increment" (i.e., the difference between the respective basal and stress values) was not different between the two groups. In view of the uniform stress increments in experimental and control groups, it does not appear that 3 meq/kg body weight of lithium chloride compromised adrenal responsitivity to ACTH stimulation.

On the basis of evidence presented in this report, it seems unlikely that lithium chloride has a direct effect on the adrenal cortex. If it did, one would expect a nonspecific increase in adrenal corticosterone secretion at each time point examined, rather than only at the 0800-hr sampling period as shown here. Therefore, we presume that lithium administration results in a modulation of the brain–pituitary unit to increase ACTH release. Lending credence to this rationale are some preliminary data from this laboratory which demonstrate that lithium-treated hypophysectomized rats do not have elevated plasma corticosterone levels as do similarly treated intact controls.

Dexamethasone phosphate is a well-known inhibitor of ACTH release. It remains uncertain as to whether its locus of action is at the pituitary or brain level; one can muster evidence in support of either (14). In these studies dexamethasone (100 μg/kg) given 8 hr before blood sampling effectively inhibited the elevated corticosterone level seen with lithium treatment. This could indicate that the feedback effect of dexamethasone somehow interferes with the stimulatory influence of lithium. It is not unreasonable to assume that the interaction between the two drugs occurs at a neural level. As also suggested by other (7), the dexamethasone study provides further evidence that the adrenal cortex is not the primary site of action of lithium.

Since the clinical use of lithium is rapidly increasing, interest has focused on its exact locus and mode of action. In general most evidence indicates that lithium acts through induced changes in the metabolism of neural catecholamines and/or serotonin (15–18, 20, 21). O'Connell (22) has suggested that the commonly encountered side effects and behavioral consequences of lithium therapy are causally related, at least in part, to functional alterations in biogenic amine activity in limbic system structures particularly of the hypothalamus. Noradrenaline, dopamine, and serotonin are known to play important roles in hypothalamic control mechanisms as they relate to brain–pituitary–adrenal gland function (4–6, 23). Mukherjee *et al.* (19) reported that more lithium is selectively accumulated by hypothalamic tissue than by cortex, cerebellum, and other areas. It is likely then that the therapeutic effectiveness, side effects, and possible deleterious endocrine consequences of lithium treatment reflect an action by lithium at some hypothalamic or other limbic system locus.

In this study it is conceivable that lithium chloride may have produced a general stress reaction to which the adrenal gland responded with an increase in secretion of corticosterone. In addition, the possibility that lithium may have altered corticosterone disposal rates cannot be ruled out. More detailed pharmacological information is needed to support the hypothesis that the effects caused by lithium on the adrenal gland are probably mediated by modifications in the neurochemical circuits known to influence adrenocortical function.

Summary. The effect of lithium chloride treatment on adrenocortical function was

studied. Rats given lithium orally for 2 weeks demonstrated elevated AM "resting" plasma corticosterone levels compared to saline-treated controls. There was increased plasma corticosterone 2 hr after a single lithium injection. Dexamethasone blocked the lithium-stimulated adrenal response in chronically treated rats.

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