Lithium Chloride Stabilizes Systolic Blood Pressure and Increases Adrenal Catecholamines in the Spontaneously Hypertensive Rat

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O'CONNOR, E. F., S. K. NAYLOR, R. H. COX AND J. E. LAWLER. Lithium chloride stabilizes systolic blood pressure and increase adrenal catecholamines in the spontaneously hypertensive rat. PHYSIOL BEHAV 44(1) 69-74, 1988.—The effects of daily, intraperitoneal injections of LiCl (3 mEq/kg) on systolic blood pressure (SBP) and adrenal catecholamine levels were measured in spontaneously hypertensive rats (SHR) and in normotensive Wistar-Kyoto rats (WKY). Control animals from each strain were injected with equivalent volumes (0.1 ml/100 g b.wt.) of 0.9% saline (0.15 mEq/kg). SBP in LiCl-treated SHR was significantly lower (p<0.05) than that of saline-treated SHR (177±7 vs. 196±4 mm Hg, respectively) after one week. After two weeks SBP was lower in LiCl SHR than in saline controls, but this difference was not significant. While SBP of both LiCl and saline treated WKY was not significantly different (146±4 vs. 147±8 mm Hg, respectively), SBP in both WKY groups remained lower than the SBP for either group of SHR. LiCl induced a significant weight loss in the SHR, but not in the WKY. Adrenal norepinephrine and epinephrine were significantly (p<0.05) higher in LiCl-treated rats of both strains; dopamine was also higher in LiCl-treated rats of both strains, but significant only between SHR-LiCl and SHR controls. It appears that LiCl's effect in slowing the development of hypertension is independent of its action on adrenal catecholamines. The SHR's increased sensitivity to LiCl, relative to weight loss and SBP, may reflect differences in genetic or physiological status of the animal compared to WKY. These differences may be associated with alterations in membrane ion transport systems.

Hypertension SHR Adrenal catecholamines LiCl

LITHIUM salts, such as lithium chloride (LiCl), are widely used as a therapy for mania, depression, and for some compulsive behaviors such as anorexia nervosa. LiCl is also used in rats as an aversive stimulus in learning paradigms (7, 22, 27). As such, solutions of lithium salts and their effects on locomotor activity and aversive learning have become standards against which other putative aversive agents have been compared (37). Despite the wide use of lithium salts, the biochemical mechanisms and neuronal substrates affected by lithium are not clear. These neurochemical bases underlying LiCl's beneficial effects may be revealed by observations of its biochemical, physiological and behavioral effects in laboratory rats, particularly in those rats showing sensitivity to lithium.

Numerous data show that LiCl affects adrenergic systems; the sympathetic nervous system appears to be particularly sensitive at several levels. Specifically, LiCl is known to affect transynaptic stimulation (29) and the uptake, release and degradation of NE in nerve terminals of the sympathoadrenal system (5, 9, 39). Decreases in locomotor activity (7, 26, 33) and the facilitation of aversive learning after LiCl (22,23), particularly in passive avoidance, are similar to those seen following intraperitoneal (IP) epinephrine or nor-epinephrine (16, 20, 24, 38), and suggest that the sympathoadrenal system may mediate some of LiCl's effects on behavior and activity.

Recently, several investigators (3, 12, 30) have shown that LiCl reduces or stabilizes blood pressure in the spontaneously hypertensive rat (SHR). Since the sympathoadrenal system contributes to the development and maintenance of hypertension in the SHR (13,28), we decided to examine the effects of LiCl on the sympathoadrenal system,
as indexed by the catecholamine content of the adrenal medullae of SHR and normotensive Wistar-Kyoto (WKY) rats subjected to chronic LiCl injection.

METHOD

Ten male SHR and ten male WKY rats eight weeks of age were received from Taconic Farms (Germantown, NY). Upon arrival, the rats were caged in pairs in a constant environmental room under a 12/12 hr light/dark cycle and allowed free access to water and chow. After a two-week period of quarantine, the heart rates and blood pressures were measured indirectly using the tail cuff procedure (Narco Biosystems, Houston, TX). At 9 weeks of age, rats of each strain were assigned to one of two groups. One group received IP injections of 3.0 M LiCl in a volume of 0.1 ml per 100 g b.wt. to yield a dose of 3.0 mEq Li+ kg b.wt. The other group was given an equivalent volume of 0.9% (0.15 mEq) NaCl. These doses are similar to those used to evaluate the behavioral effects of LiCl in the rat (7). Rats were weighed and injected daily between 15:00 and 16:00 hours for three weeks. All blood pressure measurements were made before injections and at least 19 hr after the previous injection. The site of injection on the abdomen was alternated between the right and the left side to reduce irritation. Blood pressure, heart rate and body weight were followed for an additional three weeks during which the animals continued to receive LiCl or NaCl. At the end of this time, the rats were killed by decapitation using a small guillotine. Five to ten ml of trunk blood was collected in a chilled heparinized 50 ml tube and centrifuged at 3000xg for five minutes to separate plasma from red cells. An aliquot of the plasma (200-500 μl) was taken for immediate determination of Na+, K+, Li+ and osmolality (pOsm) using an Orion Research 1020 Ion Analyzer and a Wescor 5130A Vapor Pressure Osmometer, respectively. The remaining plasma was frozen for later biochemical analyses.

The adrenal glands were removed, frozen immediately on dry ice and stored at -80°C for later analysis. The tissues were weighed and then homogenized by sonication in 1.0 ml of chilled 0.4 N perchloric acid solution (PCA) containing 1.0 g/l Na2EDTA and 0.5 g/l sodium metabisulfite. Following centrifugation of the homogenate (11,000×g, 4 minutes), the supernatant was transferred to a 1.5 ml Eppendorf tube. An aliquot of this extract was diluted 1:100 with column buffer made 0.01 M with PCA. The diluted sample was placed in an autosampler (Micromeritics, Norcross, GA) which injected 50 μl samples onto a column in a liquid chromatograph equipped with electrochemical detection (Bioanalytical Systems Model LC-304TD, West Lafayette, IN). The separation of the adrenal catecholamines was achieved using a mobile phase consisting of 0.2 M NH4H2PO4, pH 3.5 buffer and containing a 0.05 g/l Na2EDTA and with 0.2 g/l sodium octyl sulfate as the ion pairing agent. The solid phase was Ultrasphere 3 μm, C-18 resin in a 100 mm by 4.6 mm i.d. stainless steel column. The mobile phase was pumped over the column at a flow rate of 1.0 ml/minute. After separation, the sample components passed through a thin layer electrochemical transducer maintained at +0.5 volts versus an Ag/AgCl reference. The output of the electrochemical detector was displayed and integrated by a Hewlett Packard 3390 recording integrator (Corvallis, OR).

Sample components were identified using three different methods: by flow voltammetry; by comparing sample component retention times with those of authentic norepinephrine (NE), epinephrine (E) and dopamine (DA) (Sigma, St. Louis, MO); and by spiking samples with authentic compounds. The amount of DA, NE and E in a sample was determined by interpolation of a standard curve. Injection or handling losses were corrected using an internal standard, epinephrine (Sigma), added to the samples. All data are reported as means (±SEM). Raw data and ratios of the catecholamines were analyzed using analysis of variance followed where appropriate with Newman-Keuls to locate significance. Significance was accepted at p<0.05.

RESULTS

Mean baseline systolic blood pressure in the 10-week-old SHR and WKY was 170.8±11.2 mm Hg and 138.0±6.3 mm Hg, respectively, which is similar to other findings (17). Five weeks later, at the end of the experiment, systolic pressure had increased significantly in the untreated SHR but was unchanged in the other 3 groups (see Fig. 1). Heart rate was
LiCl IN THE SHR

TABLE 1
PLASMA CONCENTRATIONS OF Na+, K+ (mEq/L) AND PLASMA OSMOLALITY (pOsm; mosmols/kg) IN SHR AND WKY

<table>
<thead>
<tr>
<th>Strain</th>
<th>pNa+</th>
<th>pK+</th>
<th>pOsm</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY</td>
<td>133.44 ± 2.76</td>
<td>4.87 ± 0.13</td>
<td>291.20 ± 1.40*</td>
</tr>
<tr>
<td>WKY+LiCl</td>
<td>133.42 ± 2.34</td>
<td>7.02 ± 0.63*</td>
<td>307.41 ± 3.97?</td>
</tr>
<tr>
<td>SHR</td>
<td>132.04 ± 1.33</td>
<td>5.04 ± 0.75</td>
<td>304.00 ± 2.84</td>
</tr>
<tr>
<td>SHR+LiCl</td>
<td>134.58 ± 2.16</td>
<td>6.35 ± 0.58</td>
<td>321.43 ± 2.29?</td>
</tr>
</tbody>
</table>

* p<0.05; ? p<0.01, relative to respective genotypic control.

Values are means ± S.E.M. (N=5/group).

TABLE 2
ADRENAL CATECHOLAMINES EPINEPHRINE (E), NOREPINEPHRINE (NE) AND DOPAMINE (DA) (µg/GLAND), AND SYSTOLIC BLOOD PRESSURE (SBP) (mmHg) IN SHR AND WKY

<table>
<thead>
<tr>
<th>Strain</th>
<th>E</th>
<th>NE</th>
<th>DA</th>
<th>SBP</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY</td>
<td>37.08 ± 1.78*</td>
<td>5.87 ± 0.45</td>
<td>0.33 ± 0.03*</td>
<td>142.5 ± 6.0*</td>
</tr>
<tr>
<td>WKY+LiCl</td>
<td>45.39 ± 4.92</td>
<td>7.94 ± 0.68*</td>
<td>0.37 ± 0.04</td>
<td>140.6 ± 2.9</td>
</tr>
<tr>
<td>SHR</td>
<td>28.91 ± 2.15</td>
<td>6.36 ± 0.19</td>
<td>0.24 ± 0.01</td>
<td>185.0 ± 4.2*</td>
</tr>
<tr>
<td>SHR+LiCl</td>
<td>40.33 ± 1.73*</td>
<td>9.51 ± 0.62*</td>
<td>0.37 ± 0.02*</td>
<td>175.6 ± 11.7</td>
</tr>
</tbody>
</table>

* p<0.05; ? p<0.01, in comparison with the respective nonlithium control group. * p<0.05, in comparison with the value for WKY. Values are means ± S.E.M. (N=5/group).

The effect of LiCl on plasma parameters is listed in Table 1. Basal plasma osmolality was significantly higher in SHR than in WKY. Although LiCl induced a significant increase in pOsm (+16.6±0.8 mosmols in SHR and 16.2±0.2 mosmols in WKY), it was not significantly different between genotypes. Plasma K+ increased in SHR and WKY treated with LiCl but was significant only in the WKY. Plasma Na+ was not altered by IP injection of LiCl.

The effects of LiCl on adrenal catecholamine levels are presented in Table 2. Control WKY had significantly higher E and DA values than did control SHR. NE values were higher in SHR compared to WKY controls, but this difference was not significant.

LiCl treatment was associated with significantly higher adrenal NE in both strains relative to that found in their respective genotypic controls. Additionally, SHR treated with LiCl showed significant increases in the levels of all three catecholamines compared to their saline controls. The increases in E, NE and DA in the SHR were significantly greater than those increases in WKY. A comparison of the ratios of NE to E and of DA to NE suggested that the observed increases were associated with a general increase in the production of all three catecholamines rather than the stimulation of any given step in catecholamine synthesis.

DISCUSSION

The findings of this study indicate that daily IP injections of LiCl over a three week period stabilized blood pressure in the SHR but did not affect blood pressure in the WKY. In addition, several other differences, notably in body weight and in adrenal catecholamine content, were apparent between LiCl-treated SHR and WKY.

The SHR appeared to be more sensitive to LiCl compared to WKY relative to weight loss. This observation is similar to that reported by Das and Bhargava (12). The weight loss after LiCl which we observed in this study may have been related to diuresis; we indirectly observed that LiCl induced a greater diuresis in SHR than in the WKY; this may have been the basis for the observed differences in weight loss.

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The ratios were not significantly different between any of the treatment groups.

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to clarify this particular question. Polyuria and polydipsia, characteristics of lithium treatment in the rat, are not accompanied by decreased fluid volume, if the rat is permitted free access to water. In fact, the plasma water content of rats given Li and that of control rats has not been found to be different (11). However, if water is withheld from animals treated with lithium, they do dehydrate and show a body weight loss (8).

Plasma osmolality (pOsm) increases with lithium in both the SHR and WKY. This phenomenon has been observed in other strains treated with lithium (8). If the increase in pOsm were due to volume contraction, it would suggest a reduction of fluid volume of about 5.6% in both SHR and WKY which should have been reflected as a difference in hematocrit. In our animals, hematocrit values were not significantly different between any of the groups, suggesting that volume contraction, if it did occur, was minimal. A more systematic study of the differential effects of lithium in SHR and WKY should identify a renal act as an important component associated both with LiCl action and genetic hypertension. In fact, several studies (14, 18) have shown that red cell Na+—Li+ countertransport is higher in hypertensive rats than in normotensive rats. We suggest that this difference in permeability may be the basis for the difference between SHR and WKY in some aspects of their responsiveness to lithium. More work is necessary to document that the difference in transport of lithium and sodium seen in the red cell exists in other tissues as well, e.g., smooth muscle, renal tubules.

LiCl also increased adrenal catecholamines in both SHR and WKY. This finding is in agreement with reports of increased plasma NE in patients on lithium therapy (10). While the exact mechanisms underlying these increases are unknown, several possibilities exist. Increased adrenal catecholamines could result from: 1) an increase in catecholamine synthesis, 2) a decrease in the release of the catecholamines, 3) an increase in the uptake of catecholamines by the adrenal medulla, 4) a decrease in the catabolism of the catecholamines in the adrenal medulla, or 5) a combination of these.

Synthesis of the catecholamines depends on the activity of the enzymes tyrosine hydroxylase (THO) and dopamine-beta hydroxylase (DBH) which are associated with the synthesis of DOPA and NE, and can be induced transynaptically. A third enzyme, phenylephrine n-methyl-transferase (PNMT), is induced by activation of the pituitary-adrenocortical axis and cortisol (1) and is associated with the production of epinephrine.

Since the content and, by implication, the production of all three catecholamines increased in LiCl, it appears likely that LiCl induced increases in catecholamines through both transynaptic (sympathoadrenal) and cortisol (pituitary-adrenal cortex) related processes. In fact, LiCl has been shown to increase the activity of THO and PNMT in the adrenal medulla of rats (15), and LiCl is also associated with increased plasma ACTH and cortisol (34). Additional evidence demonstrates that either hormone can act directly on the adrenal medulla to increase PNMT synthesis and activity (4, 40).

The increase in adrenal NE suggests that LiCl stimulates DBH. This is contrary to a previous finding reported by Banerji (4) who found that LiCl decreased plasma DBH in rats. Since plasma DBH and NE is thought to originate primarily from the release of sympathoadrenal synaptic contents in blood and thus reflects sympathoadrenal activity, Banerji’s finding would suggest a decrease in sympathoadrenal activity. In the present study, we report that chronic LiCl increased the levels of both NE and DA in the adrenal. This suggests that the activities of both TOH and DBH were increased, and that sympathoadrenal stimulation by lithium contributed to the increase.

LiCl has also been shown to facilitate synapticosomal reuptake of NE (9) and to promote the inhibitory action of NE on its own release from sympathetic ganglia (39). While these actions of LiCl were not examined in our study, it is possible that the increased adrenal catecholamines could result from LiCl’s stimulation of NE and E uptake and inhibition of their release at the adrenal medulla. Certainly none of the possible actions of lithium can be excluded and further work is necessary to resolve which of the processes mediates LiCl’s effects on the adrenal medullary catecholamines.

While increasing the adrenal medullary catecholamine content in both SHR and WKY, LiCl apparently stabilized BP in the SHR and had no effect on BP in the WKY. This observation is consistent with the original finding of a decrease of BP in SHR with LiCl reported by Baetge et al. (3) and by Koda et al. (30). Differences in the interpretation of the results rather than the protocols used explain our observations of a decrease and ours of a stabilization of BP in the SHR with LiCl.

Koda et al. (30) and Baetge et al. (3) gave LiCl (1.7 g/kg diet) in the food and measured BP after 21 days of diet, finding a significant reduction of BP in SHR treated with LiCl. Their BP measurements were made 24 hr after a cannula was implanted and, while measuring BP directly, may have been obtained too soon after surgery. Further, their rats had lower plasma lithium concentrations than did our animals. Our findings are also consistent with those of Das and Bhargava (12) who found that LiCl (4 mEq/kg b.wt.) given for nine days stabilized BP in the SHR while not affecting BP in the WKY. Lower doses (0.5 and 2.0 mEq/kg) had no effect on BP in either genotype.

LiCl’s antihypertensive action on BP is surprising in light of its known endocrine effects. LiCl is associated with increases in plasma renin activity (19), aldosterone (31), ACTH and cortisol (36), and vasopressin and angiotensin II (35). Each of these compounds would be expected to increase BP. In fact, Bach et al. (2) reported a rapid induction of hypertension in rats by daily IP LiCl (4 mg/165 g b.wt.).

One possible mechanism by which LiCl could exert an antihypertensive effect, in light of its action as a releaser of norepinephrine, is suggested by Menkes et al. (32), who reported that LiCl dampens the response of tracheal smooth muscle to excitatory agents, especially those whose action is mediated by the second messenger, inositol phosphate. On a wider application, lithium may have the same effect on vascular smooth muscle, decreasing the reactivity of the tissue to circulating hormones or neurotransmitters. In the WKY, such an effect might not be noticeable under normal conditions, but it might be uncovered by examining reactivity to exogenous pressor agents, such as phenylephrine.

The results of our study also suggest that the adrenal medulla and its products may mediate LiCl’s effects on physiology and behavior. Additional support for this hypothesis is suggested by both indirect and direct evidence.

Intraperitoneal LiCl increases plasma glucose and induces an intolerance to exogenous glucose. Adrenalectomy abolishes these responses while cortisol replacement has no effect (17). Prior administration of dihydroergotamine, an alpha adrenergic antagonist, also blocks the effect of LiCl.

In behavioral experiments, LiCl potentiates acquisition
and retention of aversive learning tasks (23) as does IP E (25,34). Conversely, adrenalectomy impairs aversive learning and this impairment can be corrected by alpha adrenergic agonists, but not cortisol (6). Both LiCl (37) and E (17) decrease locomotor activity. This evidence suggests that adrenal catecholamines, especially E, mediate the effects of LiCl on physiology and behavior. While implicating such mediation, the evidence does not exclude the possibility that other mechanisms are interposed between LiCl and the adrenal catecholamines or between adrenal catecholamines and the target tissues. For example, LiCl is known to stimulate the release of ACTH from the anterior pituitary. ACTH, other mechanisms are interposed between LiCl and the adrenal medulla. Since we did not examine catecholamine synthesis mediated by cortisol, has been shown to stimulate the release of adrenal medullary catecholamines directly (40).

The participation of the adrenal medulla and its products in hypertension is not clear. Our findings of increased adrenal catecholamines in both WKY and SHR, without concomitant increases in blood pressure in lithium-treated rats, of higher blood pressures in control SHR, and of a lack of significant differences between untreated SHR and WKY relative to adrenal catecholamines suggest an independence or a dissociation of blood pressure and adrenal catecholamines. This is in agreement with Grobecker et al. (21) who reported that the activity of adrenal catecholamines is significantly less in the SHR compared to the WKY during the early development of hypertension. They also reported significantly increased adrenal TOH and DBH activity only after the hypertension was well developed. PNMT activity in SHR did not differ from WKY during this later stage. Evidence for the adrenal catecholamines supporting hypertension is given by de Champlain and van Ameringen (13) who demonstrated that adrenalectomy reduced blood pressure in both SHR and WKY strains, and that this effect could be mimicked using an alpha adrenergic blocker. They further demonstrated that in rats of either strain the adrenal medulla may have a compensatory, but not a primary, role in cardiovascular regulation, as indicated by the severe reduction of blood pressure following adrenalectomy in rats previously sympathectomized with 6-hydroxydopamine.

While our finding of increased adrenal catecholamines after LiCl suggests increased sympathoadrenal activity, other possibilities also exist. As discussed earlier, LiCl might impair transynaptic stimulation of the adrenal medulla as Klingman (29) reported for the superior cervical ganglion. LiCl may also facilitate the reuptake of NE and E by the adrenal medulla. Since we did not examine catecholamine content of other peripheral and sympathetically innervated tissues, or the plasma content of catecholamines after LiCl, we cannot further resolve LiCl's actions on the sympathoadrenal system. An examination of the NE and E content in heart, spleen and plasma is needed to identify LiCl's actions on the sympathoadrenal system. These additional studies will help to clarify LiCl's behavioral and physiological actions.

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REFERENCES