Lithium Action on Adrenomedullary and Adrenocortical Functions and Serum Ionic Balance in Different Age-groups of Albino Rats

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Abstract

The aim of the current study was to investigate lithium action on adrenomedullary and adrenocortical functions and on serum ionic balance in rats. Three age-groups of male rats (juvenile: 30 days, adult: 100 days and aged: 3 years) were used. Each age-group of animal was exposed to short- (10 days) and long-term (25 days) treatments with lithium. Each age-group of rat received lithium at a dose 2 mEq/kg body weight daily for 10 and 25 days. Each daily dose (2 mEq) was divided equally into half (1 mEq) and each half was injected intraperitoneally twice (at 9 am and 9 pm) for both the durations of experiments. Control animals received physiological saline for similar duration of experiments. Thirty animals were used for each age-group and they were divided equally into 6 groups with 5 each. After termination of all the experiments rats were sacrificed and, adrenal glands were quickly dissected out and processed for epinephrine, norepinephrine and corticosterone estimations and, 3β-hydroxysteroid dehydrogenase (3β-HSDH) activity of the adrenal gland. Blood was drawn from the heart of each rat and, serum was collected and stored at −20°C until assayed for lithium, calcium, sodium, potassium and corticosterone concentrations. The findings revealed that lithium in both short- and long-term treatments was maintained well within the therapeutic range (0.3–0.8 mEq/l) in all the age-groups of rats. This alkali metal caused depletions of both epinephrine and norepinephrine concentrations from adrenal glands, and elevations of corticosterone in both adrenals and blood serum of each age-group of rat (juvenile, adult and aged). Additionally adrenal 3β-HSDH activity was also increased in all the age-groups of rats irrespective of duration of the treatments. Short-term treatment of lithium elevated only serum K+ level in juvenile and adult rats and, Ca2+ level only in adult animals. Significant elevations of serum K+ and Ca2+ levels were observed following long-term treatments of lithium in all the age-group of rats. No significant change in serum Na+ level was recorded after lithium treatment, irrespective of duration of treatments, in any age-group of rats. The findings suggest that lithium action, in respect of adrenomedullary and adrenocortical functions and, serum ionic balance, may not be largely related to the age-group of rats and that, lithium acts on adrenomedullary activity probably by stimulating the release mechanism of epinephrine and norepinephrine from the adrenal gland of rats, but stimulates adrenocortical activity by stimulating both synthesis (including 3β-HSDH activity) and release of corticosterone. Simultaneously, lithium disturbs normal ionic balance by elevating K+ and Ca2+ levels in all the age-group of rats. Thus, the antimanic drug certainly disturbs both adrenomedullary and adrenocortical functions and, serum ionic balance in all the age-group of rats.

Keywords: Lithium, adrenal cortex, adrenal medulla, corticosterone, 3β-HSDH, epinephrine, norepinephrine, sodium, potassium, calcium, rat.

Introduction

Lithium is most useful for the treatment of patients with acute mania, and prolonged treatment is generally recommended for the prophylaxis of recurrent manic-depressive states (Johnson, 1980; Kocsis, 1990). But long-term prophyl-

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Lactic lithium treatment continues to be of concern since a wide range of adverse effects have involved a number of endocrine glands (Sheard et al., 1977). Hyperthyroidism (Mannisto, 1980) and acute onset of diabetes mellitus (John et al., 1979) have been reported following lithium treatment in manic patients. Lithium treatment stimulates catecholamine release from the perfused adrenal gland of cat (Abajo et al., 1987), and induces a dose-dependent increase in plasma catecholamines in rats (O’Connor et al., 1988; Choloff et al., 1992). Synthesis and secretion of catecholamines (norepinephrine and epinephrine) are also increased by lithium treatment in the cultured bovine adrenomedullary cells (Terao et al., 1992).

Earlier works concerned with the toxic effect of lithium on adrenal cortex and recorded adrenocortical hyperactivity in rats (Krulik & Zvalsky, 1970; Jacobs, 1978). Ghosh and others (1990) have reported that lithium stimulates adrenocortical activity by increasing adrenocortical 5-ene-3β-HSDH activity and serum corticosterone level, but this alkali metal is effective only in higher doses (200 μg or 400 μg/100 gm body weight) when treated for 14 or 21 days. Lithium also stimulates adrenocorticotropin (ACTH) secretion by the rat anterior pituitary cells (corticotrophs) in primary culture and this secretory activity is dependent on calcium (Zatz & Reisine, 1985).

Information cited above reveals that there is no report on the effect of lithium on adrenal activity in juvenile or aged animals. Neither there is any information concerning the ionic balance (Ca	extsuperscript{2+}, Na	extsuperscript{+} and K	extsuperscript{+}) following lithium treatment in animals. This is pertinent because serum calcium is associated with ACTH secretion (Zatz & Reisine, 1985). Additionally, involvement of these ions with aldosterone secretion (Greenspan & Strewler, 1997) during lithium administration is not known. Thus in the present paper, lithium action on adrenomedullary and adrenocortical functions and, serum ionic balance have been investigated in juvenile, adult and aged rats.

Materials and methods

Animals

Juvenile (30 days old), adult (100 days old), and aged (3 years old) male albino rats, were collected from the inbred colony. They were housed in small groups (5 per cage; size: 30 cm × 15 cm × 15 cm) in the controlled light (12L + 12D) and temperature (25°C) conditions in the laboratory. Standard diet (Oser, 1970) and water were accessible ad libitum throughout the experiments. Animals were acclimatized to the laboratory conditions for 5 days prior to experiments.

Experimental design

Thirty rats each for juvenile (body wt: 35–40 g), adult (body wt: 95–100 gm) and aged (190–200 gm) animals were used. Each age-group of rat was equally divided into 6 groups with 5 each (juveniles: I, II, III, IV, V and VI; adults: VII, VIII, IX, X, XI and XII and, aged: XIII, XIV, XV, XVI, XVII and XVIII). Lithium chloride (Sigma, USA), dissolved in physiological saline (0.9%), was injected intraperitoneally at a dosage of 2 mEq/kg body weight per day. Each daily dose of 2 mEq/kg was divided equally and was injected twice (9 am and 9 pm) daily for the total duration of experiments. Groups II and III (juveniles), VIII and IX (adults) and, XIV and XV (aged) were treated with lithium for 10 days and, groups: V and VI (juveniles), XI and XII (adults) and, XVII and XVIII (aged) were injected for 25 days. Groups I and IV (juveniles), VII and X (adults) and XIII and XVI (aged) served as control. Furthermore, groups I, VII and XIII served as control for 10 days treatment and rests (IV, X and XVI) for 25 days treatment. Control animals received saline without lithium for the whole length of the experiments.

Animal autopsy

Animals were sacrificed by decapitation 4 hours (13:00h) after the last injection (9 am) of lithium given on day 10 or day 25 of the experiments.

Blood and tissue collection

Blood was taken from the heart of rats and, serum was collected and stored at −20°C until assayed for lithium, sodium, potassium, calcium and corticosterone estimations. Adrenal glands were quickly dissected out, weighed on a semimicro analytical balance (Mettler, Switzerland) and processed for biochemical estimations of adrenal hormones (epinephrine, norepinephrine and corticosterone) and 3β-HSDH activity.

Biochemical assay

Serum lithium level was quantitated using atomic absorption spectrophotometer (Pybus & Bowers, 1970). Serum sodium, potassium and calcium concentrations (using methyl thymol blue) (Varley et al., 1970) were measured by spectrophotometer (VARIAN DMS-100, Australia).

Sodium

Sodium was precipitated with magnesium uranyl acetate and was determined by measuring the loss in concentration of the reagent solution. The difference between reagent blank, that is, without precipitation of sodium and ‘test’ was proportional to the concentration of sodium. The ODs of the ‘blank’ and ‘test’ (unknown) were measured at 480 nm.

Potassium

After deproteinisation of serum, potassium was precipitated with sodium TPB. A stable suspension was formed and its turbidity was proportional to potassium concentration and the OD was measured at 420 nm.
Calcium

Sodium sulfite was used as a reducing agent to minimise fading of the colour. The high sensitivity of the method allowed protein precipitation to be avoided, but polyvinyl pyrrolidone was added to ensure that any protein interference became minimal. Interference by complex formation with magnesium was avoided by chelation of this ion with 8-hydroxyquinoline. Calcium produced a blue complex with methyl thymol blue in alkaline solution and, colour was measured at 612 nm.

Adrenomedullary hormones

Norepinephrine and epinephrine concentrations were not measured from the blood because norepinephrine was also produced by the extra-adrenomedullary sources such as, sympathetic nerve terminals, hypothalamus, spleen and heart (Wilson et al., 1998; Ray & Maiti, 2001). Hence, adrenomedullary hormones were estimated only from the adrenal gland.

Adrenomedullary norepinephrine (NE) and epinephrine (E) were extracted with acidified n-butanol and purified with activated alumina which acted as CAM (catecholamine) absorbant following the method of Cox and Perbach (1973). Purified samples were oxidized with NaI-I₂ solution which resulted in the formation of adrenochrome at pH 7 for NE and at pH 4 for E. In order to form a fluorescent product, the oxidation was stopped using sodium sulphite as an antioxidant. The oxidation product was then exposed to strong alkali (NaOH and Na₂-EDTA) for tautomerization of the adrenochrome to their corresponding lutins. In order to obtain peak fluorescence, sulphite and alkali were added together and left for 5 min for NE and 1 min for E. Oxidation of lutins was prevented by adding glacial acetic acid which not only stabilized the lutins, but also resulted in increased fluorescence. NE fluorescence was read at 380/480 nm 30 min after adding acetic acid, whereas E fluorescence was read at 410/500 nm immediately after adding acetic acid. Adrenal norepinephrine and epinephrine levels were assayed fluorometrically by a HITACHI spectrofluorometer (Model 650-10M) following the method of Laverty and Taylor (1968).

Corticosterone

Adrenal glands were homogenized using alcoholic saline. Tissue homogenate or serum was purified by iso-octane. Corticosterone was extracted from the sample by chloroform. Corticosterone in chloroform formed a fluorescence compound with acid-alcohol mixture (13:7) and fluorescence was read at 462/518 nm after 45 min. Corticosterone level was measured from blood serum and adrenal gland by spectrofluorometer (Glick et al., 1964).

3β-HSDH

Adrenal 5-ene-3β-HSDH activity was measured by the method of Rubin et al. (1961). Adrenal gland was homoge-
Table 1. Effects of lithium on serum lithium and ion levels in juvenile rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Lithium (mEq/L)</th>
<th>Sodium (mEq/L)</th>
<th>Potassium (mEq/L)</th>
<th>Calcium (mEq/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (I &amp; IV, 10 rats) (Saline: 2mEq/kg body wt. daily for 10 days)</td>
<td>--</td>
<td>128.04 ± 3.52*</td>
<td>5.60 ± 0.14</td>
<td>36.50 ± 2.14</td>
</tr>
<tr>
<td>Lithium treatment (II &amp; III, 10 rats) (2mEq/kg body wt. daily for 10 days)</td>
<td>0.50 ± 0.04</td>
<td>124.40 ± 4.19</td>
<td>10.15 ± 0.57</td>
<td>33.30 ± 2.15</td>
</tr>
<tr>
<td>Lithium treatment (V &amp; VI, 10 rats) (2mEq/kg body wt. daily for 25 days)</td>
<td>0.30 ± 0.01</td>
<td>133.25 ± 1.02</td>
<td>12.40 ± 0.62</td>
<td>44.80 ± 1.14</td>
</tr>
</tbody>
</table>

*Mean ± SE.
NS: Not significant.

Table 2. Effects of lithium on serum lithium and ion levels in adult rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Lithium (mEq/L)</th>
<th>Sodium (mEq/L)</th>
<th>Potassium (mEq/L)</th>
<th>Calcium (mEq/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (VII &amp; X, 10 rats)</td>
<td>--</td>
<td>125.24 ± 3.00*</td>
<td>5.08 ± 0.25</td>
<td>34.85 ± 2.14</td>
</tr>
<tr>
<td>Lithium treatment (VIII &amp; IX, 10 rats)</td>
<td>0.60 ± 0.04</td>
<td>121.50 ± 7.21</td>
<td>7.23 ± 0.92</td>
<td>50.60 ± 1.62</td>
</tr>
<tr>
<td>Lithium treatment (XI &amp; XII, 10 rats)</td>
<td>0.70 ± 0.01</td>
<td>137.65 ± 2.01</td>
<td>10.24 ± 1.12</td>
<td>43.8 ± 2.33</td>
</tr>
</tbody>
</table>

For legends, see Table 1.

Table 3. Effects of lithium on serum lithium and ion levels in aged rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Lithium (mEq/L)</th>
<th>Sodium (mEq/L)</th>
<th>Potassium (mEq/L)</th>
<th>Calcium (mEq/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (XIII &amp; XVI, 10 rats)</td>
<td>--</td>
<td>138.08 ± 4.25*</td>
<td>6.50 ± 0.14</td>
<td>32.14 ± 0.68</td>
</tr>
<tr>
<td>Lithium treatment (XIV &amp; XV, 10 rats)</td>
<td>0.30 ± 0.01</td>
<td>134.16 ± 3.22</td>
<td>6.30 ± 0.45</td>
<td>36.42 ± 1.45</td>
</tr>
<tr>
<td>Lithium treatment (XVII &amp; XVIII, 10 rats)</td>
<td>0.80 ± 0.02</td>
<td>139.25 ± 2.08</td>
<td>8.41 ± 0.92</td>
<td>46.06 ± 2.11</td>
</tr>
</tbody>
</table>

For legends, see Table 1.

Table 4. Changes in adrenal hormones and 3β-HSDH activity following lithium treatment in juvenile rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Epinephrine (µg/mg)</th>
<th>Norepinephrine (µg/mg)</th>
<th>Corticosterone Tissue (µg/gm)</th>
<th>Serum (µg/100 ml)</th>
<th>3β-HSDH (n mole/mg tissue/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (I &amp; IV, 10 rats)</td>
<td>0.92 ± 0.20</td>
<td>0.35 ± 0.08*</td>
<td>13.07 ± 2.01</td>
<td>18.63 ± 4.62</td>
<td>17.65 ± 1.10</td>
</tr>
<tr>
<td>Lithium treatment (II &amp; III, 10 rats)</td>
<td>0.43 ± 0.11</td>
<td>0.26 ± 0.06</td>
<td>24.12 ± 1.42</td>
<td>34.21 ± 7.29</td>
<td>38.62 ± 1.02</td>
</tr>
<tr>
<td>Lithium treatment (V &amp; VI, rats)</td>
<td>0.38 ± 0.08</td>
<td>0.20 ± 0.04</td>
<td>31.06 ± 1.05</td>
<td>36.41 ± 4.12</td>
<td>41.14 ± 2.10</td>
</tr>
</tbody>
</table>

For legends, see Table 1.
increased in all the age-groups of rats following 10 and 25 days of lithium treatment. Simultaneously, 5-en-3β-HSDH activity was also increased in all the age-groups of rats irrespective of duration of treatments. But changes in adrenal and serum corticosterone levels as well as 3β-HSDH activity were less intense in adults than juvenile or aged rats (Tables 4, 5 & 6).

**Discussion**

Lithium treatment, irrespective of duration of treatments for 10 days or 25 days, remained well within the therapeutic level (0.5–1.5 mEq/l or upto 2.0 mEq/l) (Klein and Davis, 1969; Prien et al., 1970; Banerji et al., 1986) in serum of all the age-groups rats. Thus, the changes observed in adrenocortical and adrenomedullary activities, and in serum mineral levels were not due to lithium toxicity in different age-groups of rat. Epinephrine level was higher than norepinephrine in both the adrenal gland and serum of all the age-groups of rats. Thus epinephrine was the predominant adrenomedullary hormone throughout the life in rats unlike poikilothersms (Bentley, 1998). Lithium administration lead to a fall in both epinephrine and norepinephrine levels from the adrenal gland of all the age-groups of rats. A similar finding of catecholamine depletion from adrenal gland was also reported following lithium treatment in pigeons and, it was pointed out that monoamineoxidase (MAO), a specific isoenzyme of catecholamine synthesis, was responsible for lithium induced catecholamine depletion from adrenal chromaffin tissues (Ghosh et al., 1997). It had been well known that catecholamines were synthesized, stored and released into circulation whenever it was necessary. Depletions of adrenal catecholamines (CAM) (norepinephrine and epinephrine) following lithium treatment could be due to a decreased synthesis of CAM, or to a disturbance in the CAM storage and/or due to an enhanced release of CAM from the adrenal gland. Lithium chloride was known to stimulate adrenomedullary hormone synthesis and secretion in cultured bovine adrenals (Terao et al., 1992) and triggered dose-dependent rise in plasma norepinephrine, epinephrine and glucose levels throughout the length of 60 min of study in rats (Chaoulouf et al., 1992). Since norepinephrine was the precursor of epinephrine (Axelord, 1975) and, as norepinephrine level was also decreased after lithium treatment, the rate of conversion of norepinephrine to epinephrine vis-à-vis epinephrine synthesis might have been stimulated by lithium. Simultaneously, lithium might have stimulated CAM release from the adrenal gland, because both the norepinephrine and epinephrine levels were depleted from the adrenal glands of lithium recipient rats. This hypothesis might also be substantiated by our findings that serum potassium (K⁺) level, including renin-
angiotensin system known to be involved in CAM release (Wilson et al., 1998), was significantly increased after lithium treatment in rats. Considering the above cited evidences into account it might be pointed out that lithium stimulated CAM release initially and synthesis later and/or both simultaneously, without affecting probably CAM storage, but it was yet to be ascertained. In contrast to our findings of CAM depletion from the adrenal gland, earlier workers had reported elevation of serum CAM after lithium treatment. Both the findings could be explained here. Glan- dular depletion of CAM could be due to its enhanced release into circulation induced by lithium which had already been pointed out earlier. Additionally release of both norepinephrine and epinephrine might be a sequel to the stress induced by lithium as adrenomedullary hormones were known to be released during stress (Wilson et al., 1998; Ray and Maui, 2001). It was pertinent to mention that whether lithium had any age dependent action on adrenomedullary hormones in rats; but our experimental results showed that probably lithium had no such effect in rats, since there was no significant difference in epinephrine and norepinephrine depletions after lithium treatment in any age-group of rat.

In rats corticosterone level remained generally higher in blood than the adrenal gland, which might be related to the optimum level necessary for normal functioning of this hormone on respective targets. In contrast to adrenomedullary CAM depletion, adrenal and plasma corticosterone levels were significantly increased following lithium treatment in juvenile, adult and aged rats. Simultaneously, 3β-HSDH activity, known to be involved in corticosterone synthesis (Banerji et al., 1986), was also increased significantly in all the age-groups of lithium recipients. Thus, lithium certainly stimulated both the synthesis as well as release of corticosterone in rats. Besides, lithium action on corticosterone was probably not related to the age-group of rats, because the degree of response of both the enzyme and corticosterone concentration was not significantly different among any age-group of rat. Rather lithium action was largely dependent upon the duration of its treatment, because short-term treatment (10 days) showed lesser response than that of long-term treatment (25 days). It was pertinent to mention that corticosterone was generally released during stress (Wilson et al., 1998; Ray and Maui, 2001). Therefore, in the present experiment an enhanced release of corticos- terone could be due to stress induced by lithium in rats. Nevertheless, the present results indicated that lithium stimulated adrenocortical activity leading to an increased synthesis and release of corticosterone in rats. Present result of increased secretion of corticosterone was also evident from earlier report that lithium induced glucocorticoid receptor mRNA level in rat brain (Peiffer & Barden, 1991). A similar result of enhanced secretion of corticosterone following lithium treatment had also been reported earlier in rats (Jacobs, 1978; Ghesri et al., 1990; Zatz & Reisine, 1985). Platman et al. (1971) also reported an increased level of cortisol in patients with manic-depressive psychosis treated with lithium carbonate. It had been known that lithium stimulated ACTH secretion by the rat anterior pituitary cells (corticotrophs) in primary culture and enhanced ACTH secretion was dependent on the availability of calcium (Zatz & Reisine, 1985). Moreover, serum calcium level was increased after lithium treatment in the present experiments. Hence, in the present study, lithium exerted its action presumably through ACTH. Nevertheless, the direct action of lithium on adreno- cortical activity could not be ruled out unless lithium recep- tor studies in corticotroph and adrenocortical cells were known in rats (Budziszewska & Lason, 1994).

Although lithium could alter potassium and calcium levels in blood, but it had no significant action on blood sodium level. Moreover, long-term treatment (25 days) was more effective than short-term (10 days) treatment in influencing ionic changes in all the age-groups of rats, because long-term treatment increased serum potassium and calcium levels in all the age-groups of rats unlike that of short-term treated rats. Short-term lithium treatment was less effective in juveniles and aged rats than adults, because lithium failed to alter serum calcium level in juveniles and, both potassium and calcium levels in aged rats; such a differential response of these ions in juvenile and aged rats might be related to sensitivity of the age- group of rats to lithium. It was pertinent to mention that lithium induced a rise in serum K⁺ level along with the elevation of serum corticosterone level in the present experiment. A simultaneous increase of both K⁺ and corticosterone levels in serum was also reported earlier (Whitworth & Vinson, 2000). However, lithium-induced K⁺ rise was explained earlier by the fact that lithium required higher quantity of K⁺ to reap- take norepinephrine by sympathetic nerve terminals (synapse) in counteracting the enhanced release of norepinephrine in manic patients (Johnson, 1980; Banerji, 1988). Nevertheless, serum K⁺ level might be considered as one of the indices of adrenocortical activity vis-a-vis corticosterone secretion, or vice-versa in rats.

It had been known that aldosterone secretion was stimulated through a rise in plasma K⁺ concentration by stimulation of enzymes responsible for converting cholesterol to pregnenolone and, corticosterone to aldosterone and, might also upregulate the number of angiotensin receptors in glomerula cells and consequently increased sensitivity of the latter cells to angiotensins. Conversely, low plasma Na⁺ might stimulate aldosterone synthesis through a stimulation of renin-angiotensin pathway (Das, 1997). Since plasma K⁺ concentration was increased after lithium treatment, stimulation of aldosterone synthesis and/or secretion induced by lithium could not be ignored in the present study. Thus, lithium stimulated serum K⁺ level through enhanced secre- tion of aldosterone (Bentley, 1998; Eliseev et al., 1989) which in turn was regulated by angiotensin (Wilson et al., 1998). In brief lithium induced K⁺ rise presumably through stimulation of angiotension-aldosterone or angiotensin- corticosterone-aldosterone pathway in rats.

Long-term lithium treatment elevated blood calcium level in all the age-groups of rats. But this antimanic drug when
treated for short-term increased blood calcium level only in adult rats, without any alteration in juvenile and aged rats; such a differential action of lithium whether related to the sensitivity of the age-group of rats, was yet to be confirmed. It had been known that thyroid calcitonin lowered blood calcium level, but parathyroid hormone (PTH) of the parathyroid gland elevated blood calcium level (Bentley, 1998). Therefore, in the current study, lithium might have involved thyroid (hypothyroidism resulted in low calcitonin production) and/or parathyroid (hyperparathyroidism resulted in an elevation of PTH) in increasing blood calcium level in lithium recipient rats, but it was yet to be known.

In essence, lithium altered adenomacular and adreno-cortical functions and blood ion balance in all the age-groups of rats. Hence, lithium, even in therapeutic dose, had significant side effects at least on adrenal gland and blood ion balance in all the age-groups of rats.

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