Effect of lithium chloride on testicular steroidogenesis and gametogenesis in immature male rats

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Abstract. The present study was performed on immature male rats aged 35 days. Subcutaneous injections of lithium chloride at a daily dose of 2.0 mg/kg for 15 days resulted in significant inhibition of spermatogenesis at stage VII of the seminiferous epithelial cycle. Spermatogonia A, preleptotene spermatocytes and step 7 spermatids were decreased in number in comparison to controls. Serum levels of FSH, LH, PRL, and testosterone were decreased. Activities of testicular Δ5-3β-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase were suppressed along with a low caudal epididymal sperm count in comparison with controls. When the treatment was prolonged for 20 and 25 days, it showed an additional significant diminution in accessory sex organ weights and number of midpachytene spermatocytes at stage VII in comparison to control animals of corresponding age. It is concluded that lithium has an adverse effect on testicular function in immature rats by reducing serum levels of FSH, LH, PRL, and testosterone. Furthermore, since hormonal changes and altered spermatogenic activities were evident when the serum concentration of lithium was within the therapeutic range, our data may have some potential clinical implications.

Lithium, an alkali metal, has been claimed to be of clinical benefit in the treatment and prophylaxis of manic depressive psychosis (1,2). A plethora of endocrine effects has been reported to be associated with lithium therapy (3). For instance, lithium treatment causes clinical hypothyroidism (3) and activation of adrenocortical functions (4).

Unfortunately, there is a lack of information concerning the effects of lithium on the reproductive system. In this regard, it is of interest to note that in mature rats, lithium exerts an adverse effect on the pituitary-gonadal axis (5), but the limited information available is controversial. Recently, we have shown that lithium administration in toad (6) and in mature rat (7) results in inhibition of testicular activity. The present work is a continuation of our study on the side effects of lithium on male reproduction. Sexually immature animals were selected in this experiment as the hypothalamic pituitary axis is more sensitive in immature than in mature animals (8). A dose of 2 mg/kg body was used since it produces serum levels of lithium which are around the therapeutic range in man.

Materials and Methods

Animals and treatment
Forty-eight immature laboratory bred male Wistar strain rats of 35 days of age and weighing 35-38 g were used for the present investigation. They were divided equally into three groups according to duration of treatment and maintained under standard laboratory conditions (14 h light: 10 h darkness, at 25±3°C) with animal diet and water available ad libitum.

Lithium chloride (LiCl) was purchased from Loba Chemical Company (Bombay, India). It was dissolved in distilled water. Eight control animals of each group received daily 1.0 ml sterile distilled water/kg sc. The other eight animals of each group were injected sc with lithium chloride at a dose of 2.0 mg · (1.0 ml distilled water)⁻¹ · kg⁻¹ · day⁻¹. Treatment was started when the rats were
35 days old and both the control and the LiCl-treated animals were killed by decapitation when 50, 55 and 60 days old at 9.00-10.00 h when serum prolactin is at its basal level (9). Blood was collected from the dorsal aorta by a heparinised syringe and centrifuged (3000 rpm), and the serum was stored at −20°C for radioimmunoassay of FSH, LH, PRL and testosterone (T). Cauda epididymis was dissected out and used for sperm count. The testes, prostate and seminal vesicles were dissected out and weighed.

Radioimmunoassay of hormones

RIA of serum FSH and LH. Serum FSH and LH were measured according to Mougdal & Madhwaraj (10) by RIA using reagents supplied by the Rat Pituitary Distribution Program and NIDDK (Bethesda, MD). Carrier-free 125I for hormone iodination was obtained from Bhaba Atomic Research Centre (Bombay, India). Pure rat FSH (NIDDK-rFSH-1-5) and LH (NIDDK-rLH-1-5) were iodinated using the chloramine T (Sigma Chemical Co, St. Louis, MO) method according to Greenwood et al. (11). NIDDK anti-rat-FSH-S-II and NIDDK anti rat-LH-S-5 were used as antiserum at final dilutions of 1:2500 and 1:10000, respectively. Goat anti-rabbit γ-globulin was used as the second antibody. It was obtained from Indo-Medicine (Friendswood, TX). Serum samples were expressed as μg/l of serum. The intraassay variations were 6% and 5% for FSH and LH respectively. All samples were run in one assay to avoid interassay variation.

RIA of serum PRL and T. Serum levels of PRL was measured by RIA according to the method of Jacobs (12) using reagents supplied by the Rat Pituitary Distribution Program and NIDDK. The intraassay variation was 6% for PRL.

Serum T was assayed according to the procedure of Auletta et al. (13). Methodological loss during extraction was monitored by adding 10000 cpm [18, 2β-3H (N)]testosterone (specific activity 50.4 Ci/mmol, New England Nuclear, Boston, MA) before extraction with 4 ml of diethylether twice. Samples were assayed in duplicate. The antiserum to testosterone was purchased from Endocrine Science (Tarzana, CA) and it had a 44% cross-reactivity with 5α-dihydrotestosterone (DHT). Free and bound testosterone were separated by using dextran-coated charcoal. The intraassay variation was 6.5%. All samples were assayed in the same assay. Since chromatographic purification of the samples was not performed, the values reported are the sum of T and DHT.

Assay of testicular Δ^3-3β-hydroxysteroid dehydrogenase (Δ^3-3β-HSD) and 17β-hydroxysteroid dehydrogenase (17β-HSD) activity

One testis from each animal was used for studying the activities of Δ^3-3β- and 17β-HSD. Testicular Δ^3-3β-HSD was assayed spectrophotometrically according to the procedure of Talalay (14). The activity of 17β-HSD was measured in UV spectrophotometer according to the procedure of Jarabak et al. (15). One unit of enzyme activity for both Δ^3-3β- and 17β-HSD was considered to be the amount causing a change in absorbance of 0.001/min at 340 nm.

Quantitative study of spermatogenesis

Paraffin sections (5 μm) of the testes were stained with periodic acid-Schiff (PAS)-hematoxylin and quantitative analysis of the seminiferous epithelium was performed on the basis of relative number of germ cell nuclei per cross-section of the seminiferous tubules at stage VII of the cycle. Count of germ cells at this stage represents spermatogenesis as a whole. Germ cell nuclei were counted in 25 random tubular-cross sections in each testis. All the nuclear counts were corrected for differences in nuclear diameter by use of the Abercrombie formula (16) and tubular shrinkage by use of the Sertoli cell correction factor (17). Theoretically, the midpachyctene spermatocyte (mPSC) to step 7 spermatid (7Sd) ratio should be 1:4 as each spermatocyte after two successive reduction divisions forms four spermatids (18). The percentage of 7Sd degeneration was calculated from the ratio. The effective percentage of spermatid degeneration caused by lithium can be shown by subtraction of the percentage of 7Sd degeneration in vehicle-injected rats from that of the lithium-injected rats.

Sperm count

Sperm samples were collected from cauda epididymis immediately after sacrifice from each rat and was placed in a small beaker containing 5 ml of 1% (W/V) fructose in 0.1 mol/l phosphate buffer saline (pH 7.4). The cauda was punctured with a fine (24") hypodermic needle and spermatooza were washed with diluent. The beaker was shaken gently to give a homogeneous concentration of spermatooza. A drop of the suspension was placed on the hemocytometer and sperm count was determined according to Majumder & Biswas (18). The procedure was repeated at least five times for each sample from each rat.

Levels of serum lithium concentration

Flame photometric determination of serum lithium 24 h after the last lithium injection produced concentrations of 0.52±0.03, 0.58±0.03 and 0.61±0.02 mmol/l when the animals were sacrificed at day 50, 55 and 60 of age, respectively.

Statistical analysis

For statistical analysis of the data, the two-tailed Student's t-test was used. Differences were considered significant when p<0.05.
Table 1.
Changes in body weight and testicular, prostatic and seminal vesicular weight in 50, 55 and 60 days old rats treated with lithium chloride from age 35 days. Values are mean ± SEM (N=8).

<table>
<thead>
<tr>
<th>Age at sacrifice</th>
<th>Treatment</th>
<th>Body weight (g)</th>
<th>Testes weight (g/kg body weight)</th>
<th>Prostate weight (mg/kg body weight)</th>
<th>Seminal vesicle weight (mg/kg body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initial</td>
<td>Final</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 days</td>
<td>Control</td>
<td>36.8±5.8</td>
<td>81.0±7.3</td>
<td>12.3±0.5</td>
<td>649±89</td>
</tr>
<tr>
<td></td>
<td>Lithium</td>
<td>36.1±6.4</td>
<td>79.9±6.2</td>
<td>11.2±0.4</td>
<td>483±74</td>
</tr>
<tr>
<td>55 days</td>
<td>Control</td>
<td>37.4±6.6</td>
<td>99.5±8.3</td>
<td>12.9±0.4</td>
<td>772±81</td>
</tr>
<tr>
<td></td>
<td>Lithium</td>
<td>38.2±5.9</td>
<td>96.4±7.1</td>
<td>11.7±0.3*</td>
<td>523±62*</td>
</tr>
<tr>
<td>60 days</td>
<td>Control</td>
<td>37.2±8.5</td>
<td>104.3±9.3</td>
<td>13.2±0.4</td>
<td>891±91</td>
</tr>
<tr>
<td></td>
<td>Lithium</td>
<td>36.1±9.6</td>
<td>97.7±10.6</td>
<td>11.4±0.6*</td>
<td>513±79**</td>
</tr>
</tbody>
</table>

p-values *<0.05, **<0.01, compared with the corresponding vehicle-treated controls.

Results

Body and organ weights
Chronic treatment of immature rats with lithium chloride for 20 and 25 days decreased the testicular, prostatic and seminal vesicular weights significantly in comparison with vehicle-treated controls, but treatment for 15 days did not alter the accessory sex organ weight (Table 1). Body weights of the lithium-treated animals in all groups did not differ from that in controls (Table 1).

Spermatogenesis
A significant reduction in the numbers of spermatogonia A (ASg), preteptolene spermatocytes (pLSc), and 7Sd at stage VII of the seminiferous epithelium cycle was observed after 15, 20 and 25 days of lithium treatment when compared with those in controls. The decrease in the number of 7Sd and the increase in the percentage of 7Sd degeneration were at the maximum after 25 days of treatment. In the group treated for 20 and 25 days, the numbers of mPSc at stage VII of the seminiferous cycle were

Table 2.
Quantitative study of spermatogenesis at stage VII of the seminiferous tubules and sperm count in immature rats treated with lithium chloride from 35 days of age and sacrificed when 50, 55 and 60 days old. Values are mean ± SEM (N=8). Abbreviations: see text.

<table>
<thead>
<tr>
<th>Age at sacrifice</th>
<th>Number of germ cells/semiferous tubule</th>
<th>% 7Sd degenerations</th>
<th>Effective 7Sd degeneration/semiferous tubule</th>
<th>Sperm count (million/ cauda epididymis)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ASg</td>
<td>pLSc</td>
<td>mPSc</td>
<td>7Sd</td>
</tr>
<tr>
<td>Control (50 days)</td>
<td>0.40±0.02</td>
<td>15.41±0.18</td>
<td>17.12±0.35</td>
<td>56.83±0.89</td>
</tr>
<tr>
<td>Lithium</td>
<td>0.35±0.01*</td>
<td>14.72±0.21*</td>
<td>15.79±0.61</td>
<td>45.63±1.17***</td>
</tr>
<tr>
<td>Control (55 days)</td>
<td>0.47±0.03</td>
<td>16.82±0.21</td>
<td>17.98±0.41</td>
<td>62.77±0.92</td>
</tr>
<tr>
<td>Lithium</td>
<td>0.38±0.02*</td>
<td>14.92±0.28*</td>
<td>16.02±0.62*</td>
<td>43.16±1.11***</td>
</tr>
<tr>
<td>Control (60 days)</td>
<td>0.54±0.02</td>
<td>17.46±0.29</td>
<td>18.82±0.42</td>
<td>65.11±0.85</td>
</tr>
<tr>
<td>Lithium</td>
<td>0.41±0.03**</td>
<td>14.98±0.26***</td>
<td>16.12±0.61**</td>
<td>39.17±0.92***</td>
</tr>
</tbody>
</table>

p-values *<0.05, **<0.01, ***<0.001 compared with the corresponding controls.
significantly decreased (Table 2). The sperm count also revealed a significant reduction in the number of spermatozoa in all lithium-treated groups (Table 2).

**Hormones**

The concentration of serum FSH, LH, PRL and T were significantly lowered in all the treated groups (Fig. 1).

**Enzymatic activities**

A significant diminution of testicular $\Delta^5$-3\beta- and 17\beta-HSD activities was observed in all the lithium-treated groups when compared with the corresponding controls (Table 3).

**Discussion**

The present findings reveal that lithium treatment of immature male rats causes a reduction in testicular steroidogenesis and gametogenesis. This occurs at a serum lithium concentration (around 0.5 mmol/l) which are therapeutic in man (5).

Prolonged administration of lithium in immature rats results in inhibition of the activities of testicular $\Delta^5$-3\beta- and 17\beta-HSD and reduction of the serum levels of LH, FSH, PRL and T. In immature rats, $\Delta^5$-3\beta- and 17\beta-HSD, which are key steroidogenic enzymes in androgen biosynthesis (19,20), are controlled partly by LH (21,22) and partly by FSH, since the latter gonadotropin possibly plays a role in induction of Leydig cell LH receptors (23,24). Furthermore, PRL augments the effects of FSH by inducing responsiveness to LH in the immature rat (25). Moreover, PRL may control lipoprotein transport in Leydig cells, thus assuring a constant supply of cholesterol for steroidogenesis (26). Besides this, PRL controls the testicular an-
The activities of testicular Δ^2-3β- and 17β-HSD enzymes are in support of the reduced levels of serum FSH, LH, PRL in immature rats after lithium treatment.

It has been reported (28) that PRL along with FSH and LH can control the weights of accessory reproductive glands. Hence, the reduction of the accessory sex organ weights in lithium-treated immature rat, sacrificed when aged 55 and 60 days, also supports the decreased level of gonadotropins and PRL.

The mechanisms by which lithium inhibits the serum level of gonadotropins, PRL and T in immature rat were not addressed by the present experiment. Our experimental results are in agreement with the findings of Banerji et al. (29) who suggested that chronic lithium treatment leads to a potentiation of dopamine receptor sensitivity, leading to an augmented inhibition of PRL release from the pituitary. Norepinephrine controls the release of hypothalamic luteinizing hormone releasing hormone and LH (30), and lithium is known to decrease the hypothalamic content of norepinephrine (31). Since lithium inhibits the activity of adenyl cyclase in different endocrine glands (32), it can also be speculated that lithium-induced inhibi-

tion of adenyl cyclase in Leydig cells could suppress testicular steroidogenesis (33).

Histological examination of the testes following lithium treatment revealed a reduction in the number of the A5g, pLSc, and 7sd in all groups. Initiation and maintenance of spermatogenesis require FSH and LH in prepubertal rats (34). Therefore, the reduction in the number of spermatogonia in all these groups confirms the decreased level of FSH and LH after lithium treatment. It has also been reported that maturation of pLSc to the zygotene stage appears to be independent of any hormonal influences (35), whereas maturation of 7sd is testosterone-dependent (36) or indirectly affected by the lack of pituitary FSH and LH (34). Moreover, PRL appears to increase the number of spermatocytes and spermatids in hereditary dwarf mice (37). Thus, reduction in all the germ cells at stage VII of spermatogenesis in 60 days old rats confirms the reduced level of PRL in the lithium-treated rat. The reduction in epididymal sperm count also strongly supports the inhibition of spermatogenesis. The decrease in the number of pLSc in all groups and of mPSc in the 60-days-old group is possibly the after-effect of reduced spermatogonial population.

In conclusion, our findings suggest that chronic treatment with lithium can reduce the levels of serum FSH, LH, PRL and T in immature male rats. In addition, our data suggest an effect of lithium at the pituitary level. If lithium would have acted only at the testicular level, then we would have seen an increased level of serum gonadotropins through a reduced feed-back effect of testosterone, especially since the pituitary-hypothalamic axis is very sensitive in immature rat (8). Moreover, our data also show that normal testicular activity in immature rats is affected by chronic administration of lithium, which may be clinically important with regard to the possible adverse effects of lithium on testicular gametogenic and steroidogenic functions.

**Table 3.**

Changes in the activities of testicular Δ^2-3β- and 17β-HSD in immature rats treated with lithium chloride from 35 days of age and sacrificed when 50, 55 and 60 days old. Values are mean ± SEM, (N=8).

<table>
<thead>
<tr>
<th>Age at sacrifice</th>
<th>Δ^2-3β-HSD units (mg of tissue)^-1.h^-1</th>
<th>17β-HSD units (mg of tissue)^-1.h^-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (50 days)</td>
<td>23.4±0.6</td>
<td>24.5±0.4</td>
</tr>
<tr>
<td>Lithium (50 days)</td>
<td>21.6±0.3*</td>
<td>21.1±0.3**</td>
</tr>
<tr>
<td>Control (55 days)</td>
<td>24.4±0.6</td>
<td>25.3±0.3</td>
</tr>
<tr>
<td>Lithium (55 days)</td>
<td>21.2±0.3**</td>
<td>22.0±0.3**</td>
</tr>
<tr>
<td>Control (60 days)</td>
<td>25.4±0.3</td>
<td>26.6±0.2</td>
</tr>
<tr>
<td>Lithium (60 days)</td>
<td>23.0±0.3**</td>
<td>23.5±0.3**</td>
</tr>
</tbody>
</table>

p-values *<0.02, **<0.001 compared with the corresponding controls.

References


20. Baillie AH, Mack WS. Hydroxy steroid dehydroge-


Received April 20th, 1990.
Accepted July 26th, 1990.

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