Studies on the effect of prolactin treatment on testicular steroidogenesis and gametogenesis in lithium-treated rats

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Abstract. The effect of PRL supplementation in lithium-treated rats on spermatogenesis, testicular Δ^3-3β-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase activities, and serum levels of FSH, LH, PRL and testosterone were studied on the 22nd day of the experiment. Subcutaneous injections of lithium chloride at a dose of 2.0 mg·kg^{−1}·day^{−1} for 21 days resulted in a significant inhibition of spermatogenesis at stage VII of the seminiferous epithelial cycle, along with remarkable diminution of serum levels of the above hormones and suppression of the activities of the above two testicular steroidogenic enzymes. Administration of bovine PRL at a dose of 0.25 mg·kg^{−1}·day^{−1} plus lithium treatment resulted in a remarkable protection of spermatogenic and steroidogenic activities of the testes, along with restoration of serum levels of FSH and testosterone. It is concluded that PRL can markedly protect the testicular dysfunction induced by lithium chloride treatment in rats.

Although the therapeutic usefulness of lithium for the treatment of manic depressive psychosis (1,2) remains indubitable, there is a wide range of adverse effects on metabolic and endocrine functions following lithium treatment in psychotic patients (3). Lithium causes clinical hypothyroidism (3), activation of adrenocortical functions (4) and is a male reproductive toxicant (5-9).

Unfortunately, there is an apparent paucity of information concerning the effects of lithium on the reproductive system. In this regard, it should be noted that in mature rats, lithium inhibits the plasma levels of LH, FSH, PRL and testosterone (5,6), but these studies, however, have not provided consistent results. Recently we have shown that lithium administration in toad (7) and in mature (8) and immature rats (9) results in inhibition of testicular steroidogenesis and spermatogenesis. Since there is a close relationship between PRL and testicular activities (10,11) and chronic lithium treatment results in a remarkable inhibition of plasma PRL (8,12,13), the present investigation was undertaken to examine the effect of PRL on lithium-induced testicular dysfunction. A dose of 2 mg/kg was used, since it is the minimum dose that produces toxic effects on testicular activity, whereas 1 mg/kg has no significant effect (8). Moreover, the dose of 2 mg/kg produces serum levels of lithium which are around the therapeutic range in man. The duration of 21 days was selected as this is the minimum duration of lithium treatment for testicular steroidogenesis and gametogenesis to be affected (14).

Materials and Methods

Animals and treatment

Thirty adult male albino rats of Wistar strain, 90 days old and weighing 150-170 g, were used for the experiments. The animals were maintained under standard laboratory conditions (14 h light: 10 h dark and 30±1°C), with animal diet and water available ad libitum. Animals were divided into 3 equal groups according to mode of treatment.

Lithium chloride (LiCl) was purchased from Loba Chemical Company (Bombay, India). It was dissolved in sterile distilled water. Ten animals received 0.1 ml sterile
distilled water and were considered as controls. Twenty animals were injected with LiCl at a concentration of 20 g/l distilled water sc at a dose of 2.0 mg kg\(^{-1}\) day\(^{-1}\) for 21 days. Ten animals of the LiCl-treated group received bovine PRL at a concentration of 25 g/l 0.9% saline (Sigma Chemical Co, USA) sc at a dose of 0.25 mg kg\(^{-1}\)day\(^{-1}\) 8 hours after LiCl treatment for 21 days. Ten other LiCl-treated animals received only vehicle at the time of PRL supplementation; 24 h after the last LiCl injection, the animals were killed by decapitation. Blood was collected from the dorsal aorta by a heparinized syringe, centrifuged (3000 rpm) and the serum was stored at -20°C for radioimmunoassay of FSH, LH, PRL and testosterone. Testes were dissected out and weighed. One testis was placed in Bouin’s fluid for spermatogenic study and the other one was used for biochemical estimation of steroidogenic enzyme activities.

Quantitative study of spermatogenesis
Bouin’s fixed testes were embedded in paraffin wax. 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 tubule at stage VII of the cycle. Count of germ cells at this stage represents spermatogenesis as a whole. Germ cell nuclei were counted in 20 round tubular cross-sections in each testes. All the nuclear counts were corrected for difference in nuclear diameter by Abercrombie’s formula (15) and tubular shrinkage by the Sertoli cell correction factor (16). Theoretically, the midpachytene spermatocyte (mPSc) to step 7 spermatid (7 Sd) ratio should be 1:4 as each spermatocyte after two successive reduction divisions forms four spermatids (17). The percentage of 7 Sd 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 the lithium-injected rats.

Assay of testicular \(\Delta^5\)-3β-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase activity
One testis from each animal was used for studying the activities of \(\Delta^5\)-3β-hydroxysteroid dehydrogenase (\(\Delta^5\)-3β-HSD) and 17β-hydroxysteroid dehydrogenase (17β-HSD). Testicular \(\Delta^5\)-3β-HSD was assayed spectrophotometrically according to the procedure of Talalay (18). The activity of 17β-HSD was measured in UV spectrophotometer according to the procedure of Jarabak et al. (19). One unit of enzyme activity for both \(\Delta^5\)-3β and 17β-HSD was considered to be the amount causing a change in absorbance of 0.001/min at 340 nm.

Radioimmunoassay of hormones
**RIA of serum FSH and LH.** Serum FSH and LH were measured according to Moudgal & Madhwaraj (20) by RIA using reagents supplied by the Natl. Pituitary Distribution Program and NIDDK (Bethesda, MD). For hormone iodination, carrier-free \(^{125}\)I was obtained from Bhabha Atomic Research Centre (Bombay, India). Pure rat FSH (NIAMDD-rFSH-1-5) and LH (NIAMDD-rLH-1-5) were iodinated using chloramine-T (Sigma Chemical Co, St. Louis, MO) according to the method of Greenwood et al. (21). NIAMDD anti-rat-FSH-S-II and NIAMDD-anti rat LH-S-5 were used as antigens at a final dilution of 1:2500 and 1:10 000, respectively. Goat anti-rabbit γ-globulin was used as the second antibody. It was obtained from Indo-Medicine, (Friendswood, TX). Values were expressed as mg/l of serum. The intra-assay variations were 4 and 5% for FSH and LH, respectively. All samples were run in one assay to avoid the inter-assay variation.

**RIA of serum PRL and testosterone.** Serum levels of PRL were measured by RIA according to the method of Jacobs (22) supplied by the Natl. Pituitary Distribution Program and NIAMDD (Bethesda, MD). The intra-assay variation was 5%.

Serum testosterone was assayed according to the procedure of Auletta et al. (23). Methodological loss during extraction was monitored by adding 10 000 cpm (1a, 2β, \(^3\)H(N)) testosterone (specific activity 50.4 C/mmoll, New England Nuclear, Boston, MA) before extraction with 4 ml of diethylether two times. 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. Free and bound testosterone were separated by using dextrancoated charcoal. The intra-assay variation was 6%. All samples were run at the same time. Since chromatographic purification of the samples was not performed, the values reported are the sum of testosterone and 5α-dihydrotestosterone.

**Levels of serum lithium concentration**
Flame photometric determination of serum lithium, 24 h after the last lithium injection, produced mean concentrations of 0.58-0.71 mmol/l (N=20) (24).

**Statistical analysis**
For statistical analysis of the data, ANOVA followed by multiple two-tailed t-test was performed. Differences were considered significant when p<0.05.

**Results**

**Body and organ weight**
Twenty one days of LiCl treatment resulted in a decreased testicular weight in comparison to vehicle-treated animals. Administration of PRL to lithium-treated animals revealed a significant restoration of testicular weight in comparison to lithium-
Table 1.
Effect of PRL on testicular weight, testicular $\Delta^5$-3\(\beta\)- and 17\(\beta\)-HSD activities in lithium-treated rats.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Testicular weight (mg/kg body weight)</th>
<th>$\Delta^5$-3(\beta)$-HSD activity (U/mg of tissue/h)</th>
<th>17(\beta)$-HSD activity (U/mg of tissue/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle-treated</td>
<td>14500±433(^a)</td>
<td>29.58±0.32(^a)</td>
<td>28.92±0.48(^a)</td>
</tr>
<tr>
<td>Lithium-treated</td>
<td>11500±504(^b)</td>
<td>20.12±0.41(^b)</td>
<td>19.69±0.63(^b)</td>
</tr>
<tr>
<td>Lithium + PRL-treated</td>
<td>13700±490(^a)</td>
<td>28.43±0.56(^a)</td>
<td>26.87±0.59(^a)</td>
</tr>
</tbody>
</table>

Each value represents mean ± SEM of 10 animals in each group. The results obtained were compared by analysis of variance and multiple comparison two-tailed t-test at a level of p<0.05. In any vertical column, the means with different superscripts differ significantly from each other.

treated animals not receiving PRL (Table 1). Body weights of the lithium-treated animals in all groups did not differ from that in controls.

Spermatogenesis
Lithium treatment resulted in a significant reduction in the number of spermatogonia A (ASg) and step 7 spermatid (7Sd) in comparison to control animals. Administration of PRL along with LiCl revealed a marked protection in the number of ASg and 7Sd degeneration (Table 2).

Hormones
The serum concentrations of FSH, LH, PRL and testosterone were decreased in lithium-treated animals in comparison to control animals. Supplementation of PRL from the start of the experiment to lithium-treated animals resulted in increased serum levels of FSH and testosterone compared with lithium-treated animals (Fig. 1).

Steroidogenic enzymes
A significant diminution of testicular $\Delta^5$-3\(\beta\)$- and 17\(\beta\)$-HSD activities was observed in lithium-treated animals compared with control animals and the PRL-supplemented group (Table 1).

Discussion
Supplementation of PRL to lithium-treated animals results in significant protection of testicular weight, spermatogenesis at stage VII, testicular steroidogenic activity, and serum levels of FSH and testosterone. Decreased testicular weight, testicular steroidogenic enzyme activity, and spermatogenesis, and low serum levels of FSH, LH, PRL and testosterone in lithium-treated animals are consistent with our previous findings and those of others (5-9). It must be emphasized that lithium-induced

Table 2.
Effect of PRL on quantitative study of spermatogenesis at stage VII in lithium-treated rats.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Number of germ cell seminiferous tubule cross-section</th>
<th>mPSc/7Sd</th>
<th>% 7Sd degeneration</th>
<th>Effective 7Sd degeneration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ASg</td>
<td>pLSc</td>
<td>mPSc</td>
<td>7Sd</td>
</tr>
<tr>
<td>Vehicle-treated</td>
<td>0.62(^a)</td>
<td>17.52(^a)</td>
<td>17.32(^a)</td>
<td>59.62(^a)</td>
</tr>
<tr>
<td></td>
<td>± 0.01</td>
<td>± 0.38</td>
<td>± 0.45</td>
<td>± 1.62</td>
</tr>
<tr>
<td>Lithium-treated</td>
<td>0.40(^b)</td>
<td>16.85(^a)</td>
<td>16.62(^a)</td>
<td>41.68(^b)</td>
</tr>
<tr>
<td></td>
<td>± 0.03</td>
<td>± 0.42</td>
<td>± 0.51</td>
<td>± 1.11</td>
</tr>
<tr>
<td>Lithium + PRL-treated</td>
<td>0.58(^a)</td>
<td>17.92</td>
<td>17.81(^a)</td>
<td>57.93(^a)</td>
</tr>
<tr>
<td>PRL-treated</td>
<td>± 0.03</td>
<td>± 0.52(^a)</td>
<td>± 0.42</td>
<td>± 1.18</td>
</tr>
</tbody>
</table>

Each value represents mean ± SEM of 10 animals in each group. The results obtained were compared by analysis of variance and multiple comparison two-tailed t-test of p<0.05. In any vertical column, the means with different superscripts differ significantly from each other.
Fig. 1.
Effect of PRL supplementation on serum levels of FSH, LH, PRL and testosterone in lithium-treated rats. Each bar represents mean ± srm of 10 animals in each group [] control, [] lithium, [] lithium + PRL (ANOVA followed by multiple t-test at the level of p<0.05).

reduction in serum testosterone reflects parallel changes in serum LH levels, as LH play a major role in testicular steroidogenesis (25). As FSH may act synergistically with LH, the notable suppression in serum testosterone following LiCl treatment is probably due to a reduction in serum levels of both FSH and LH (26). The mechanism involved in the reduction in serum PRL after lithium treatment is not known, but a potentiation of dopamine receptor sensitivity has been suggested, leading to an inhibition of PRL release from the pituitary (12). The low serum level of PRL is also one of the causes for the diminution of serum testosterone in lithium-treated animals, as PRL potentiates the effect of LH on testicular androgen synthesis (10,11,27).

The mechanism by which exogenous PRL protects the testicular dysfunction in lithium-treated rats cannot be determined from the present experiment. It has been reported that treatment with bovine PRL or PRL-producing ectopic pituitary grafts caused a significant rise in peripheral FSH levels in genetically dwarf mice, rats and hamsters (27,28). The failure of PRL to restore the serum LH levels in lithium-treated rats cannot be explained properly, though it is consistent with a previous report (27). Moreover, it has been indicated that PRL affects the testicular steroidogenesis by increasing the availability of precursors for androgen biosynthesis (29), perhaps by regulating the activity of cholesterol ester synthetase in the testes in a manner similar to that described in the ovary (30). Beside this, PRL increases testicular activities...
of Δ3-3β- and 17β-HSD in hereditary dwarf mice (10,31) as well as significantly increases serum androgen levels in hypophysectomized rats (11). It has been reported that Leydig cells have specific PRL receptors (32). In addition, it is suggested that the sensitizing effect of PRL on the action of LH on the testes is very important (33). Therefore, the protection of testicular Δ3-3β-HSD and 17β-HSD activities and the restoration of the serum testosterone levels towards the control value in lithium-treated rats are due to a direct effect of PRL on the testes as well as to a stimulation of the FSH secretion from the pituitary, as FSH may also play a role in the regulation of testicular steroidogenesis (34).

A quantitative study of the spermatogenesis was carried out at a single stage of the spermatogenic cycle, stage VII, as the cellular association at this stage is composed of elements positioned equidistant in the entire process of spermatogenesis. Therefore, counts of germ cells at the particular stage of the cycle are representative of the condition of spermatogenesis as a whole. The reduction in the number of ASg in lithium-treated rats is possibly due to an increased rate of degeneration of spermatogonia, as FSH inhibits the normal degeneration of spermatogonia (25) and the diminution of pituitary FSH secretion may be the reason for the degeneration of spermatogonia. Russel et al. (34) reported that FSH is required to obtain quantitatively normal spermatogenesis in pubertal rats. The reduction in the number of 7Sd is probably due to the low level of testosterone, because the conversion of pachytene spermatocyte to spermatid requires testosterone (35,36) or is indirectly affected by the lack of pituitary FSH and LH (37). The mechanism by which PRL restores the spermatogonic activity in lithium-treated rats is not definitely known, but it may be suggested that PRL exerts these effects by modulating pituitary FSH secretion as well as by stimulating testicular androgen synthesis.

In conclusion, for the first time our data provide evidence that PRL administration leads to restoration of testicular activities which are affected by lithium treatment.

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References


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