Effects of Bromocriptine (CB-154) Treatment on Copulatory Behavior in Hyperprolactinemic Adult Male Rats

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Adult male rats received pituitary transplantations to induce hyperprolactinemia. Some of those had bromocriptine (CB-154) treatment, and the other had vehicle (saline) as control. The grafted animals with vehicle exhibited significantly more suppression than sham operated animals. While the hyperprolactinemic rats treated with CB-154 showed no significantly more suppression of copulatory behavior than sham operated control. In the grafted animal without CB-154 treatment, the concentration of serum PRL was significantly higher than in sham operated controls. There were no significant differences in adrenal weight and serum levels of gonadotropin between grafted and control groups. These results may imply that PRL has direct actions on central nervous system (CNS) and inhibits copulatory behavior in hyperprolactinemic male rats.—KEY WORDS: CB-154, copulation, hyperprolactinemia

Chronic elevation of serum prolactin (PRL) level, hyperprolactinemia (hyperPRL) induced by prolactin-secreting adenoma or ectopic pituitary glands, results in suppression of gonadotropin secretion, increases in adrenal weights [3] and deficits in copulatory behavior in experimental animals [6,8,9,12]. It has been repeatedly demonstrated that chronic hyperPRL inhibits copulatory behavior in male rats [6,8,12]. The effects of hyperPRL on male sexual activity include significant inhibition of both sexual arousal and erectile function [7].

In human, hyperPRL is caused by or associated with, a variety of pathogenic states: pituitary adenoma, hypothalamic disorders, hypogonadism and hypothyroidism, and is detected in patients with infertility [17,18], impotence and hypogonadism [19]. Therapy for hyperPRL has been successful in many cases treated by either transsphenoidal surgery [14] or medical therapy with dopamine agonists, ergotic drugs, especially bromocriptine (CB-154) [19]. Dopamine agonists are very effective in decreasing serum PRL levels and in inducing tumor shrinkage of patients bearing PRL-secreting adenomas [4,13]. CB-154 treatments have been reported to restore sexual function in hyperprolactinemic men [17,18] and to increase copulatory behavior in hyperprolactinemic male rats [5].

The present study was undertaken to confirm the suppressive effects of hyperPRL induced by grafting pituitary glands and clarify the effects of CB-154 treatment on male copulatory behavior in hyperprolactinemic rats.

Wistar–Imamichi male rats obtained from Imamichi Institute for Animal Reproduction (Tsuchiura) were maintained on a 14-hr light and 10-hr dark lighting schedule (light off at 19:00). Animals were housed in a temperature-
controlled room (22–27°C). They received a standard laboratory diet (MB-1, Funabashi Farm Co. Ltd., Chiba) and tap water *ad libitum*. CB-154 (Parlodel, Sandoz, Co., Ltd.) was dissolved in 0.9% saline and the suspension was injected subcutaneously with a dose of 1mg/rat between 12:00–14:00 every–day. Hyperprolactinemia was induced at 10 weeks of age by grafting two whole pituitaries from male donors with the same age in the same strain under the left kidney capsule [1,2]. Twenty male rats were assigned to four groups as follows: (1) 5 males which were grafted two whole pituitaries under the left kidney capsules at the age of 10 weeks and injected vehicle (saline) for 7 days prior to tests of copulatory behavior; (2) 5 males which were grafted fat under the same place as control (sham operation) and injected vehicle for 7 days; (3) 5 males which were grafted two pituitaries and received CB-154 administration for 3 days; (4) 5 males which were grafted two pituitaries and received CB-154 administration for 7 days. Four weeks after surgery, tests of copulatory behavior were conducted.

Test of copulatory behavior were conducted between 1 and 3 hr after onset of darkness under dim red illumination from a 40-w red light, by direct observation of male paired with estrogen-progesterone primed ovariectomized females. Adult females were ovariectomized and rendered behaviorally estrous by injections of 1-estradiol 3-benzoate (Sigma Chemical. Co., Ltd. 10µg/0.1ml sesame oil) 48hr prior to testing, and progesterone (Sigma Chemical. Co., Ltd. 500µg/0.1ml sesame oil) 4hr prior to testing. Each test lasted for a period of 1hr. Tests were conducted in a semi-circular observation cage (radius 40cm, height 50cm) with sawdust on the floor [16]. Each male was allowed on adaptation period of 5 min prior to introduction of a receptive female into the observation cage. Upon introduction of the female, the occurrence of mount, intromission, and ejaculation was recorded by a trained observer. Subsequently, the data analysis was performed on the following parameters of copulatory behavior: (1) Mount latency (ML); the time from introduction of the female to first mount (2) Intromission latency (IL); the time from introduction of the female to first intromission (3) Ejaculation latency (EL); the time from first intromission to ejaculation (4) Postejaculatory interval (PEI); the time from the ejaculation to the next intromission (5) Mount frequency (MF); the number of mounts within 1 hr (6) Intromission frequency (IF); the number of intromissions within 1 hr (7) Ejaculation frequency (EF); the number of ejaculations within 1hr (8) Hit rate (HR); intromission frequency/intromission frequency + mount frequency

They were sacrificed by decapitation between 20:00~21:00 in a few hours after the tests. The decapitation was carried out in 30 seconds after taking animals from each cages to prevent PRL variation [10,15]. Blood was collected and centrifuged at 3,000g for 15 minutes for the analysis of serum hormone. The serum was stored at -50°C until analyzed by RIA for determination of serum PRL, LH and FSH. After the decapitation, the testes, seminal vesicle, epididymis, prostate and adrenals were removed and weighted.

Radioimmunoassay (RIA): Serum concentrations of PRL, LH and FSH were measured by RIA using the method of Furudate et al. [11] with reagents provided from NIADDK. The standard references used were, rPRL-RP-3 for PRL, rLH-RP-2 for LH and rFSH-RP-2 for FSH. The intra- and inter-assay coefficients of variation were 9.6 and 15.9 for PRL, 3.5 and 5.3 for LH, and 5.3 and 9.8 for FSH, respectively. The effects of CB-154 treatment on serum hormone levels and the weights relative body weight of the testes, seminal vesicles, epididymis, prostate and adrenals were assessed using Student's t-test or Cochran-Cox test when comparing two groups. For the behavioral data, treatment effects were analysed by Mann–Whitney U tests.

The grafted animals with vehicle exhibited significant suppression of copulatory behavior, compared with sham-operated animals. CB-154 treatment resulted in a consistent improvement of copulatory behavior in the grafted animals (Table 1). A significant increase (P <0.05) in MF and a significant decrease (P <0.05) in both EF and HR were recognized in the animals with pituitary transplants and vehicle injections, when compared with those in sham-operated animals. The hyper-prolactinemic rats treated with CB-154 displayed no significant suppression of copulatory behavior in comparison with sham operated controls, whereas the mild prolongation of PEI
EFFECTS OF CB-154 ON COPULATORY BEHAVIOR

Table 1. Copulatory behavior in male rats grafted or sham-operated after CB-154 or vehicle treatment (3 or 7 days)

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>ML (sec.)</th>
<th>IL (sec.)</th>
<th>EL (sec.)</th>
<th>PEH (sec.)</th>
<th>MF</th>
<th>IF</th>
<th>EF</th>
<th>HR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham + vehicle</td>
<td>5</td>
<td>31.0 ± 7.4</td>
<td>100.2 ± 48.0</td>
<td>170.0 ± 34.1</td>
<td>363.2 ± 21.4</td>
<td>30.8 ± 6.5</td>
<td>35.2 ± 3.6</td>
<td>6.4 ± 0.5</td>
<td>55.4 ± 6.5</td>
</tr>
<tr>
<td>Graft + vehicle</td>
<td>5</td>
<td>28.6 ± 6.8</td>
<td>481.2 ± 282.5</td>
<td>306.6 ± 78.5</td>
<td>413.6 ± 38.2</td>
<td>62.0 ± 11.5</td>
<td>32.4 ± 8.4</td>
<td>3.4 ± 1.0</td>
<td>29.1 ± 6.9</td>
</tr>
<tr>
<td>Graft + CB-154 (3 days)</td>
<td>5</td>
<td>21.0 ± 5.1</td>
<td>19.6 ± 4.3</td>
<td>112.6 ± 18.4</td>
<td>408.9 ± 22.4</td>
<td>19.4 ± 2.7</td>
<td>32.8 ± 1.7</td>
<td>6.8 ± 0.4</td>
<td>63.4 ± 3.0</td>
</tr>
<tr>
<td>Graft + CB-154 (7 days)</td>
<td>5</td>
<td>34.8 ± 6.6</td>
<td>72.2 ± 23.5</td>
<td>240.4 ± 117.1</td>
<td>429.7 ± 12.3</td>
<td>33.4 ± 10.4</td>
<td>26.2 ± 4.5</td>
<td>5.8 ± 0.7</td>
<td>50.2 ± 8.8</td>
</tr>
</tbody>
</table>

All values represent Mean ± SE. Mann-Whitney U test: P < 0.05 a vs. b, c vs. d, e vs. f, g vs. h P < 0.01 c vs. d, i vs. j, b vs. k, d vs. l, f vs. m

Table 2. Serum levels of PRL, LH and FSH in male rats grafted or sham-operated after CB-154 or vehicle treatment (3 or 7 days)

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>PRL (ng/ml)</th>
<th>LH (ng/ml)</th>
<th>FSH (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham + vehicle</td>
<td>5</td>
<td>3.50 ± 0.15a</td>
<td>0.89 ± 0.13</td>
<td>12.65 ± 0.39</td>
</tr>
<tr>
<td>Graft + vehicle</td>
<td>5</td>
<td>7.79 ± 1.28b</td>
<td>0.62 ± 0.12</td>
<td>11.85 ± 0.46</td>
</tr>
<tr>
<td>Graft + CB-154 (3 days)</td>
<td>5</td>
<td>1.04 ± 0.05c</td>
<td>0.97 ± 0.14</td>
<td>12.38 ± 0.50</td>
</tr>
<tr>
<td>Graft + CB-154 (7 days)</td>
<td>5</td>
<td>1.22 ± 0.15d</td>
<td>1.11 ± 0.17</td>
<td>12.98 ± 0.51</td>
</tr>
</tbody>
</table>

All values represent Mean ± SE. Statistical analysis was determined by Student's t-test. P < 0.05 a vs. b, a vs. c, a vs. d P < 0.01 b vs. c, b vs. d

Table 3. Weights relative to body weight (mg/100g B.W.) of the testes, epididymis, seminal vesicle, prostate gland and adrenal gland of each treatment group

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Testes</th>
<th>Epididymis</th>
<th>Prostate gland</th>
<th>Seminal vesicle</th>
<th>Adrenal gland</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham + vehicle</td>
<td>5</td>
<td>632 ± 12.3</td>
<td>215 ± 10.5</td>
<td>180 ± 12.3</td>
<td>170 ± 15.3</td>
<td>14.3 ± 0.4</td>
</tr>
<tr>
<td>Graft + vehicle</td>
<td>5</td>
<td>614 ± 13.0</td>
<td>213 ± 5.8</td>
<td>227 ± 18.6</td>
<td>258 ± 52.1</td>
<td>15.9 ± 0.7</td>
</tr>
<tr>
<td>Graft + CB-154 (3 days)</td>
<td>5</td>
<td>612 ± 18.2</td>
<td>210 ± 13.1</td>
<td>166 ± 19.2</td>
<td>187 ± 10.1</td>
<td>14.9 ± 0.4</td>
</tr>
<tr>
<td>Graft + CB-154 (7 days)</td>
<td>5</td>
<td>638 ± 12.5</td>
<td>218 ± 2.2</td>
<td>188 ± 7.4</td>
<td>163 ± 4.1</td>
<td>16.5 ± 0.4</td>
</tr>
</tbody>
</table>

All values represent Mean ± SE. Stastical analysis was determined by Student's t-test.

(P < 0.05) in the animals with CB-154 treatment for 7 days was observed. Furthermore, the grafted animals with CB-154 treatment for 3 days showed significant decrease in both EL (P < 0.01) and MF (P < 0.01) in addition to a significant increase in both EF (P < 0.01) and HR (P < 0.01), compared with those in the grafted animal with vehicle.

The results of serum hormone and weights relative to body weight of each organ are shown in Tables 2 and 3. It has been confirmed that there was no significant difference in the concentration of serum testosterone between treatment and control groups in the previous study [12]. The concentration of serum PRL in grafted animals was significantly higher than that in control animals. Serum level of PRL in the grafted animals with CB-154 treatments was significantly decreased when compared to grafted animals with vehicle (P < 0.01) and sham operated controls (P < 0.05). Although hyperPRL has been reported to be associated with an increase in the adrenal weight and with the suppression of gonadotropin secretion [3],...
there were no significant differences in the weight relative to body weight to adrenal glands and in the serum levels of gonadotropin between control and grafted groups.

The present study clearly demonstrated that the hyperPRL induced by pituitary grafts resulted in a significant suppression of copulatory behavior and the CB-154 treatment restored the suppressed copulatory behavior to its normal level. In the present experiment, CB-154 treatment to hyperprolactinemic rats for 3 days was more effective on copulatory behavior than treatment for 7 days. This result was inconsistent to the report by Doherty et al. [5]. The effects of CB-154 treatment for 7 days and 14 days on the grafted male CD-F rats were investigated in their studies in which a long term treatment of CB-154 was found to be more effective on copulatory behavior. It might be supposed that such a discrepancy was mainly due to the strain difference in sensitivity to CB-154. Besides, dopamine agonist tolerance may develop in the grafted animals with CB-154 for 7 days. The fact that in their studies the grafted animals showed a reduction in serum gonadotropin level may participate with such differences. Without altering serum concentrations of FSH and LH, the short term (3 days) treatment is effective enough to improve copulatory behavior in male rats with pituitary transplants. These results may imply that PRL has direct actions on the central nervous system (CNS) and inhibits copulatory behavior in hyperprolactinemic male rats.

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References

高プロラクチン血症により抑制された雄ラットの交尾行動に対する Bromocriptine (CB-154) 处置の回復効果

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下垂体移植雄ラットに bromocriptine (CB-154) を投与し、対照群には vehicle（生理食塩水）を投与して交尾行動の観察を行った。高プロラクチン血症群では偽手術群と比べて交尾行動の有意な抑制が認められた。一方、CB-154処置群では偽手術群と比べて交尾行動の有意な抑制は認められなかった。下垂体を移植し生理食塩水を投与した群では偽手術群に比べて血清プロラクチン濃度が有意 (P<0.05) に上昇した。下垂体移植群と偽手術群の間には副腎重量と性腺刺激ホルモンの血清濃度において有意差は認められなかった。これらの結果はプロラクチンが中枢神経系に直接作用し、交尾行動を抑制することを示唆するものと思われる。