BRIEF NOTE

INFLUENCE OF LIVER INJURY ON TESTOSTERONE 5α-REDUCTASE ACTIVITY IN THE RAT DIENCEPHALON

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Abstract. Diencephalon testosterone 5α-reductase activity and plasma testosterone level were measured in the rat with liver injury caused by i.p. administration of CCl₄. Testosterone levels in plasma were decreased after CCl₄ injection in both group I (0.1ml/150g B.W./once a day for 2 days) and group II (0.01ml/150g B.W./twice a week for three weeks). 5α-Reductase activity in the diencephalon was decreased both on the 7th day after administration in group I and in group II, while being transiently increased on the 1st day after administration in group I. These results suggest that testosterone metabolism in the brain is inhibited during liver disorders.

Key words: liver injury, CCl₄, testosterone 5α-reductase, testosterone, estradiol-17β

Testosterone, one of the sex hormones, easily goes through the blood brain barrier into the brain (1) and is converted to dihydrotestosterone (2) by testosterone 5α-oxidoreductase (3-oxo-5α-steroid (acceptor) Δ⁴-oxidoreductase (E.C.1.3.99.5)) (5α-reductase).

The 5α-reductase activity in rat brain is different between sexes (3). The enzyme activity is much higher in male rats than in female. Furthermore, when blood testosterone was depleted in male rats after adrenalecto-testectomy, brain 5α-reductase activity showed a gradual increase for 3 days and then returned to the pre-operative level 2 h after testosterone propionate administration (4). These enzyme variations in the brain were suggested as being partly due to the changed ratio of testosterone to estradiol-17β because estradiol-17β is an inhibitor of 5α-reductase (5).

Blood testosterone levels are decreased in patients with liver cirrhosis, whereas estradiol-17β levels are increased (6). The testosterone level in brain may be lowered, whereas estradiol-17β level may be increased. In this study, we examined the effect of liver injury caused by CCl₄ on plasma testosterone levels and on brain 5α-reductase activity.
Materials and methods. Male Wistar rats were used throughout this investigation; they weighed approximately 150g at the time when the experiment began. Eight to nine rats were caged with an alternating 12 h dark-light cycle. The temperature was kept at 23 ± 0.5°C and moisture at 55 ± 1%. Food and water were available ad libitum. The first group (group I) of animals received i.p. injection of CCl₄ (0.1ml/150g B.W.) once a day for 2 days. Rats were sacrificed by decapitation the 1st, 3rd, 5th and 7th days after the last injection of CCl₄. The second group (group II) of animals received i.p. injection of CCl₄ (0.01ml/150g B.W.) twice a week for three weeks. Rats were sacrificed by decapitation 28 days after the first i.p. injection of CCl₄. Untreated rats were used as the control. Immediately after decapitation, the rat brain was removed, and the diencephalon was prepared for analysis according to the method of Schubert and Sedvall (7). Blood from the neck and trunk was collected into a test tube containing heparin-Na. Plasma was separated and stored at −80°C until assay for testosterone. The activity of diencephalon 5α-reductase was measured as previously described (4).

Plasma testosterone levels were measured using a commercial kit (Commissariat a l’Energie Atomique). The coefficient of variation for intraassay of testosterone was 5.9%.

Results were statistically analyzed using a Student’s t-test.

Results. Table 1 shows the value of lipids in the liver of rats which were administered with CCl₄. In group I, the total lipid, triglyceride and total cholesterol were increased on the first day after CCl₄ injection and only the total cholesterol was raised on the fifth day after administration. In group II, the total lipid and triglyceride were lowered.

| Table 1. Level of lipids in male rat liver after CCl₄ injection (mg/g) |
|-----------------------------|-----------------------------|-----------------------------|
|                             | Total lipid                 | Triglyceride                | Total cholesterol |
| Control                     | 42.6±2.8 (8)                | 7.8±1.6 (8)                 | 3.5±0.5 (8)       |
| Days after administration of CCl₄ |                     |                               |                   |
| 1                           | 50.8±4.8*** (7)             | 13.1±3.1*** (7)             | 4.8±0.8*** (7)    |
| 5                           | 38.7±5.4 (7)                | 7.9±1.9 (7)                 | 4.1±0.6 (7)       |
| Control                     | 45.1±2.3 (8)                | 9.1±1.4 (8)                 | 5.1±0.25 (8)      |
| Repeated administration of CCl₄ |                     |                               |                   |
|                             | 40.7±3.6** (7)              | 7.6±0.6* (7)                | 5.2±0.3 (7)       |

*P<0.05, **P<0.02, ***P<0.01 compared to untreated control group (Student’s t-test). (Mean ±SD)

On histological examination, group I showed fatty degeneration of liver cells, the severity of which decreased in the following order: on the first day, on the third day and on the fifth day. Degeneration was not recognized on the seventh day or in the untreated rat group. In group II, fatty degeneration was not
found in the liver. As regards parenchymal necrosis, the grade of severity was increased in the following order: the third day in group I, the first day in group I. In group II, striking edema was recognized in the Dese’s space.

Fig. 1 shows diencephalon 5α-reductase activity and plasma testosterone levels from the first group. On the first day after CCl₄ injection, diencephalon 5α-reductase activity was increased by 22% (P<0.05), and on the 7th day decreased by 25% (P<0.02) compared to the control values. Plasma testosterone level was decreased by 67% (P<0.01), 43% (P<0.01) and 24% (P<0.05), on the 1st, 5th and 7th days after i.p. injection of CCl₄, respectively, compared with the control value.

![Graph showing diencephalon 5α-reductase activity and plasma testosterone levels from the first group.](image)

Fig. 1. Diencephalon 5α-reductase activity (striped bars) and plasma testosterone (open bars) of male rats after CCl₄ injection for 2 days.

Results are expressed as mean ± SD (n). Asterisks represent significant differences from untreated control group.

*P<0.05, **P<0.02, ***P<0.01 (Student’s t-test).

Table 2 shows diencephalon 5α-reductase activity and plasma testosterone level from group II. Diencephalon 5α-reductase activity was decreased by 56% (P<0.01) after injection of CCl₄. Plasma testosterone level was also decreased by 54% (P<0.02).
<table>
<thead>
<tr>
<th>Treatment</th>
<th>5α-Reductase activity (pmoles/mg protein/2h)</th>
<th>Testosterone (ng/ml plasma)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>38.9 ± 11.1 (8)</td>
<td>2.84 ± 1.24 (8)</td>
</tr>
<tr>
<td>Administration of CCl₄</td>
<td>17.1 ± 9.7 * (8)</td>
<td>1.32 ± 0.88 * (8)</td>
</tr>
</tbody>
</table>

*P<0.02, **P<0.01 compared to untreated control group (Student's t-test) (Mean ± SD).

Discussion. Rommers et al. (2) confirmed that 5α-reduction of testosterone was catalyzed by 5α-reductase in the brain. Concerning 5α-reductase in the rat diencephalon, we reported that the optimal pH was 6.8 and Km value was 2.2 x 10⁻⁸M (8). Since labelled testosterone injected intraperitoneally to rats was taken up in the brain (9), there may be some relationship between the blood level and brain concentration of testosterone.

Both the histological and biochemical alterations occurred in the early stage in liver injury of rats which were given CCl₄ i.p. However, this histological change may be reversible in the acute experiment. Although it is reported that acute administration of CCl₄ produced a direct influence on the fine structure in the rat brain (10), it remains to be clarified whether CCl₄ affects testosterone-containing neurons in the brain.

In group I, the blood testosterone decreased rapidly after CCl₄ administration and significant low levels of this hormone remained even on the seventh day after injection. The 5α-reductase activity in the diencephalon was significantly increased on the first day after administration but was decreased on the seventh day. This rapid increase of 5α-reductase activity is similar to our previous report (4) concerning the enzyme activity in rats with adrenalecto-testectomy. This transient enzyme increase may be a rebound phenomenon due to rapid depletion of testosterone.

In group II, both the blood testosterone and diencephalic 5α-reductase activity were decreased. Hardly any relationship was recognized between the alteration of diencephalic 5α-reductase activity and the severity of liver pathology. Gordon et al. (11) recognized that the lowered testosterone level in blood and also the raised metabolic clearance rate of testosterone to estradiol-17β occurred in patients with cirrhosis. They (12) reported that liver 5α-reductase activity was lowered in alcholic liver disease of both human beings and baboons. Therefore, the turnover rate of estrogen may be decreased in the affected liver leading to the increased blood estrogen level on the seventh day in groups I and II. Since estradiol-17β has anti-androgen activity and is an inhibitor of 5α-reductase activity in the rat prostate (5), it may also be an inhibitor of 5α-reductase activity in the diencephalon.

Skell et al. (13) suggested that the α-adrenergic system is involved in control
of the factor responsible for "feminization" of hepatic steroid metabolism. The α-adrenergic nerve system is intricately interlocked with peptidergic neuroendocrine cells and steroid-containing cells in the hypothalamus. Furthermore, Heritage et al. (14) reported that brain stem catecholamine neurons are target sites for sex steroid hormone. Since it is postulated that steroid hormone is active as modulator of α-adrenergic system (15), change of testosterone metabolism in CNS may have an effect on this feminizing factor (13).

These results suggest that variation of testosterone metabolism in the brain is induced in patients with liver disorder. However, the physiological significance of 5α-reduction in the brain remains to be clarified.

REFERENCES