Sex Hormone Metabolism in the Brain: Influence of Central Acting Drugs on 5α-Reduction in Rat Diencephalon

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Synopsis

Rats after adrenalectomy-testectomy showed a gradual increase in diencephalon 3-oxo-5α-steroid: (acceptor) Δ⁴-oxidoreductase (5α-reductase) activity for 3 days. The activity then returned near to the normal range on the 4th postoperative day. When rats were given testosterone propionate (TP) 3 days after adrenalectomy-testectomy, diencephalon 5α-reductase activity returned to the preoperative range 2 hr after TP administration. Diencephalon 5α-reductase activity showed a highly significant increase (p<0.01) after a single administration of carbamazepine, reserpine, diazepam, phenytoin, phenobarbital or disulfiram. A significant increase (p<0.05) was also found after a single administration of methylphenidate, caffeine or methamphetamine. Plasma testosterone decreased concurrently after administration of all these agents, except diazepam. Diencephalon enzyme activity decreased significantly after repeated disulfiram administrations (p<0.01) but increased significantly after methamphetamine administrations (p<0.05). Plasma testosterone showed a tendency to decrease after repeated methamphetamine administrations but tended to increase after repeated disulfiram administrations.

Materials and Methods

Central acting drugs

Animals and treatment

Male Wistar rats were used. The animals were housed in a room with an alternating 12 hr 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 rats weighed about 150 g at the time the experiment began.

The drug administration schedule is shown in Table 1. The agents were dissolved in saline or suspended in olive oil and administered intraperitoneally, once or repeatedly for 14 consecutive days.

The control animals were given the saline or olive oil vehicle alone with the same regimen as the experimental animals.

The rats in the acute experimental group were killed by decapitation 4 hr after drug injection. Chronic experimental rats were decapitated 24 hr after the last injection. Diencephalon tissue and plasma were collected from both groups.

Adrenalectomy-testectomy was performed in the rats of another group under Nembutal anesthesia. Testosterone propionate (TP) and 5α-dihydroxytestosterone (DHT) were dissolved in a mixture of propylene glycol: ethanol (8:2). Rats were intraperitoneally administered TP or DHT at 1 mg/100 g B. W., 3 days after the operation. The control rats were given the vehicle alone. The animals were killed by decapitation 2 hr after administration of steroid hormones or vehicle.

Immediately after decapitation, the rat brain was removed, and the diencephalon was prepared for analysis according to the method of Schubert and Sedvall (1972). Blood from the vessels of the neck and thorax was collected into a polypropylene test tube containing heparin-Na, and the plasma was separated.

Determination of diencephalon 5α-reductase activity

The isolated diencephalon was weighed and homogenized with 9 volumes of phosphate buffer (pH 6.8) using a teflflon glass homogenizer. All procedures were conducted between 0°C and 4°C. Protein concentration was determined by the method of Lowry et al. (1951).

A 0.15–0.20 μCi of 4-14C-testosterone (specific activity 60 mCi/mole, Radiochemical Center, Amer sham, England) dissolved in ethanol was transferred to test tubes, and the ethanol was evaporated by nitrogen gas. The radioactive steroid was dissolved in 0.05 ml of propylene glycol, mixed with 252 nmols of NADPH (Sigma, St. Louis, Mo.) and 1 ml of diencephalon homogenate (about 8 mg protein), and the total volume was adjusted to 2.5 ml with phosphate buffer (pH 6.8). The diencephalon homogenate kept in boiling water bath for 5 min was used as a blank. This reaction mixture was incubated at 38°C for 2 hr by continual shaking in air, and the reaction was stopped by the addition of 10 ml of chloroform: ether mixture (3:1).

Following incubation, the reaction mixture was extracted twice with 10 ml of chloroform: ether mixture (3:1) each time. The combined extracts were washed once with distilled water and were dehydrated with anhydrous Na2SO4, and the solvent was evaporated to dryness under reduced pressure. The extract was then dissolved in chloroform and transferred to an 8 × 250 mm column of Florisil (3 g, activated at 60°C for 4 hr). The column was washed with 20 ml of chloroform and eluted with 25 ml of 2% methanol-chloroform. The eluate was evaporated to dryness and re-dissolved in 2% methanolchloroform and subjected to thin layer chro-

Table 1. Doses of central acting drugs used in rats

<table>
<thead>
<tr>
<th>Agent</th>
<th>Trade name</th>
<th>Dose, i. p. (mg/100 g B. W.)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Single</td>
</tr>
<tr>
<td>Diazepam</td>
<td>Cercine</td>
<td>0.25</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>Wintermin and Contomin</td>
<td>4</td>
</tr>
<tr>
<td>Dipiperon</td>
<td>Propitan</td>
<td>8</td>
</tr>
<tr>
<td>Reserpine</td>
<td>Apoplon</td>
<td>0.2</td>
</tr>
<tr>
<td>Imipramine</td>
<td>Tofranil</td>
<td>4</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>Philopon</td>
<td>0.6</td>
</tr>
<tr>
<td>Methylphenidate</td>
<td>Ritalin</td>
<td>1</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>Aleviatin sodium</td>
<td>5</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>Disulfiram</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Caffeine, anhyd.</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Saline</td>
<td></td>
<td>0.1 ml</td>
</tr>
<tr>
<td>Olive oil</td>
<td></td>
<td>0.2 ml</td>
</tr>
</tbody>
</table>
matography (TLC) on silica gel-G in a chloroform: ether system (3:1). X-ray film was applied to the chromatographic plate for 2 weeks and developed. Silica gels (corresponding to exposed areas of the film plate) on the thin layer plate were individually scraped into a counting vial containing toluene scintillator, and the radioactivity was counted in a scintillation spectrometer (Aloka LSC 653 type).

The enzyme activity of 5α-reductase was expressed as pmoles/mg protein/2 hr from the sum of the metabolic products (4-14C-5α-dihydrotestosterone and 4-14C-5α-androstan-3α, 17β-diol) after incubation with 4-14C-testosterone. The optimal pH of 5α-reductase was 6.8, and Km value was 2.2×10⁻⁶M.

Identification of the metabolic products (4-14C-5α-dihydrotestosterone and 4-14C-5α-androstan-3α, 17β-diol) was performed by TLC, gas-liquid chromatography (GLC) and estimation of constant specific activity. The metabolic products corresponding to dihydrotestosterone (DHT) and 5α-androstan-3α, 17β-diol showed the same Rf values with the authentic steroids on TLC, using eight kinds of development system. The metabolic products, DHT and 5α-androstan-3α, 17β-diol, were also identified by GLC (FID), using 2% OV-17 and 2% XE-60 columns. 5α-DHT and 5β-DHT could be separated by both the columns. 5α-androstan-3α, 17β-diol and other androstandiols could be separated by 2% OV-17 column.

Determination of testosterone in plasma
Rat plasma was mixed with about 1,000 dpm of 3H-testosterone (1, 2, 6, 7, 5H-testosterone, specific activity 100 Ci/m mole, Radiocchemical Center, Amersham) for correction of recovery, shaken and maintained at room temperature for 15 min. The plasma was extracted once in about 6 ml of ether, and the extract was washed with 1 ml of distilled water and evaporated under nitrogen gas.

Sephadex LH-20 (2 ml) was swollen overnight with a mixture of n-hexane: benzene: methanol (90:5:5) and packed in a siliconized column of 7 mm in inner diameter. The dried residue was dissolved in the same solvent and transferred to the column and eluted. The first 7.5 ml of eluate was discarded and the next 8 ml collected. This fraction of eluate was evaporated to dryness under nitrogen gas, and the residue was dissolved in 1 ml of methanol. One-fifth of the solution was used for correction of recovery.

Radioimmunoassay of testosterone was performed according to a slightly modified method of Nieschlag and Loriaux (1972) using testosterone-3-oxime-BSA. The radioactivity was measured by adding a dioxan scintillator at room temperature (PGO 5 g, naphthalin 100 g and dioxan 1,000 ml) in a liquid scintillation spectrometer (Aloka LSC-653 type).

Recovery was examined by adding 0.6 ng of testosterone to five 1 ml aliquots of rat plasma and it was 105±10% (coefficient of variation 9.5%). The testosterone concentration (mean±S.D.) of four samples from a plasma pool of three rats was 1.85±0.11 ng/ml (coefficient of variation, 5.9%).

Results
Diencephalon 5α-reductase activity was maximal 3 days after adrenalectomy-testectomy (Fig. 1). This elevated 5α-reductase activity returned to the pre-operative level 2 hr after TP administration, whereas enzyme activity of the DHT group remained high, as in the solvent administered controls (Fig. 2).

Fig. 3 shows diencephalon 5α-reductase activity 4 hr after drug administration. The increase in enzyme activity was highly significant (p<0.01) with carbamazepine, reserpine, diazepam, phenytoin, phenobarbital and disulfiram, and significant (p<0.05) with methylphenidate, caffeine and methamphetamine, compared with the normal saline control group.

![Fig. 1. Activity of 5α-reductase in diencephalon of adrenalectomized-testectomized rats.](attachment:fig1.png)

The figure in parentheses indicates the number of cases.
The bars represent S. D.
* p<0.01, compared to the untreated group.
Fig. 2. Influence of testosterone propionate on $5\alpha$-reductase activity in diencephalon of adrenalec-tomized-testecotomized rats.

The half bars represent one S. D.

AxGx = Adrenalectomized-testecotomized rat
TP = Testosterone propionate
DHT = $5\alpha$-dihydrotestosterone

*p<0.01, compared to the untreated group.

Fig. 4 shows diencephalon $5\alpha$-reductase activity following the repeated drug administration. A significant decrease (p<0.01) in enzyme activity was found in the disulfiram group but a significant increase (p<0.05) was present in the methamphetamine group.

Discussion

Sholiton et al. (1966) reported in their study that testosterone was coverted to $\Delta^4$-androstenedione by rat brain homogenates. Kaneyuki (1974) confirmed that this conversion was catalyzed by testosterone $17\beta$-dehydrogenase. Sholiton and Werk (1969) also found that testosterone underwent $5\alpha$-reduction in the rat brain. Kohsaka et
al. (1976) confirmed that 5α-reduction was catalyzed by 5α-reductase in the rat brain. These data indicate that testosterone is metabolized in the brain.

Male rats 3 days after adrenalectomy-testectomy showed a maximal increase of diencephalon 5α-reductase activity which returned to the control normal range 4 days after the operation. This phenomenon remains unexplained. Administration of testosterone propionate (TP) 3 days after the operation, when 5α-reductase activity was supposed to be maximal, prevented increased activity. Massa et al. (1972), and Kniewald and Milkovic (1973) reported the effect of testosterone on brain 5α-reduction in testectomized rats. Their results are compatible with our present findings on 5α-reductase activity.

In the previous paper (Kohsaka et al., 1976), we determined diencephalon 5α-reductase activity in rats after intraperitoneal administration of phenytoin, imipramine or chlorpromazine for 14 consecutive days. In the present study, we examined the following drugs: diazepam (anti-anxiety drug), chlorpromazine, dipiperon and reserpine (antipsychotic drugs), imipramine (antidepressant), methamphetamine and caffeine (psychostimulants), phenytoin, phenobarbital and carbamezepine (antiepileptics), and disulfiram (alcohol deterrent).

Fig. 5 summarized the results obtained in both the present and previous studies.

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**Fig. 4.** Effects of repeated administrations of a central acting drug on 5α-reductase activity of rat diencephalon.

The figure in parentheses indicates the number of cases.
The half bars represent one S. D.
*p<0.01, **p<0.05, compared to the saline or olive oil group.
In groups showing a significant increase of $5\alpha$-reductase activity after a single injection, the plasma testosterone concentration tended to decrease, except for the diazepam group. In groups showing a significant decrease in $5\alpha$-reductase activity after repeated injections, the plasma testosterone tended to increase. The methamphetamine group, which showed a significant increase in enzyme activity, showed decreased plasma testosterone.

Central acting drugs producing variations in enzyme activity were studied on the basis of similar chemical structure, but we could not find any relationship between these drugs and changes in enzyme activity. Among drugs used in our studies, chlorpromazine and reserpine are reported to depress secretion of gonadotropin in the hypothalamus (Takewaki, 1962; de Wied, 1967). Since phenytoin exerts its direct action on adrenals and increases corticoid secretion, it is possible that phenytoin increases the secretion of adrenal androgen (Woodbury, 1954). On the other hand, chlorpromazine (Hollister, 1964; Bloom, 1965) and imipramine (Kuhn, 1958) may produce liver dysfunction, and therefore, testosterone metabolism may be influenced by this liver dysfunction. However, whether all central acting drugs used in this investigation affect testosterone-metabolizing enzyme directly in the liver remains to be clarified. Nevertheless, our data indicate that $5\alpha$-reductase activity may be influenced by blood testosterone, and that administration of central acting drugs may produce brain changes in testosterone concentration due to varia-

![Fig. 5. The relationship between diencephalic $5\alpha$-reductase activity and plasma testosterone levels in rats after administration of central acting drugs.](Image)

- **Single administration**
  - Ratio of diencephalon $5\alpha$-reductase activity: after drug administration (SA)-to-untreated rats (CA).
  - Ratio of plasma testosterone level: after drug administration (ST)-to-untreated rats (CT).

- **Repeated administration**

* Values obtained in the previous study (Kohsaka, Kaneyuki and Shohmori, 1977).
tions in 5α-reductase activity.

From another point of view, central acting drugs that produced significant enzyme activity are known to influence monoamine metabolism in the central nervous system. Chlorpromazine blocks dopamine receptor sites (Goldberg and Yates, 1969). Phenytoin acts as a monoamine oxidase inhibitor (Azzaro et al., 1973) and increases brain 5-hydroxytryptamine level (Green and Grahame-Smith, 1975). Amphetamine increases the release of monoamine in the brain and concurrently blocks re-uptake with a resultant elevation of free norepinephrine content (Glowinski and Axelrod, 1965), and disulfiram is an inhibitor of dopamine-β-hydroxylase and increases dopamine content and decreases norepinephrine content in the brain (Goldstein et al., 1964).

The relationship is not clear between brain monoamine metabolism changes produced by these drugs and variations in 5α-reductase activity. However, since dopamine stimulates LH-RH secretion (Rotsztejn et al., 1977) and steroid hormones affect monoamine metabolism (Gudelsky et al., 1977), it is possible that a close relationship exists between brain monoamines and steroid hormones.

References