Effects of Antiprostatic Agents on $5\alpha$-Dihydrotestosterone Binding to Rat Hypophyseal and Hypothalamic Cytosol Macromolecules

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Synopsis

The binding of $5\alpha$-dihydrotestosterone to the hypophyseal and hypothalamic cytosol macromolecules prepared from castrated male rats was observed. The effects of antiprostatic agents on $5\alpha$-dihydrotestosterone binding to both hypophyseal and hypothalamic cytosol macromolecules was examined. Cyproterone acetate and chlomadinone acetate showed the significant inhibiting effects on $5\alpha$-dihydrotestosterone binding to 7-8 S macromolecules of cytosol from both hypophysis and hypothalamus. SCH 13521 and AA 560 did also affect $5\alpha$-dihydrotestosterone binding to 7-8 S macromolecules of cytosol from both tissues.

An increasing number of steroidal and nonsteroidal antiandrogens have attracted the interest of investigators, because the therapeutic drug for prostatic cancer, which has less side effects and more effectiveness than estrogen, has been ardently awaited. We have already reported the effects of steroidal and nonsteroidal antiandrogens on the binding of $5\alpha$-dihydrotestosterone to rat prostatic cytosol macromolecules (Yamanaka et al., 1978). Recent reports have indicated the presence of $5\alpha$-dihydrotestosterone receptor in the cytosol fractions prepared from male rat hypophysis and brain (Kato and Onouchi, 1973a; Naess et al., 1975a; Kato and Onouchi, 1973b; Mercier et al., 1976; Lieberburg et al., 1977). The present study was designed to search for the effects of steroidal and nonsteroidal compounds on the binding of $5\alpha$-dihydrotestosterone to rat hypophyseal and hypothalamic cytosol macromolecules.

Materials and Methods

Sample preparation

Male Horizman rats, 42-49 days old, were killed by decapitation without anaesthesia 24 hr after castration. The preparation of the tissue was carried out at 0°C-2°C. The tissue block containing the hypothalamus was bounded anteriorly by the optic chiasma, laterally by the hypothalamic sulci, posteriorly by mammillary bodies. The hypophyseal or hypothalamic tissues were gently homogenized with a Potter-Elvehjem homogenizer in cold 50 mm Tris-HCL (pH 7.4)-1.5 mm EDTA disodium salt-2 mm 2-Mercaptoethanol. The homogenate was centrifuged at 105,000×g for 60 min. at 2°C. The 105,000×g supernatant was concentrated using Diaflomembrane (Minikon), to get an appropriate protein concentration (8-12 mg/ml).
Measurement of total binding of cytosol to 5α-dihydrotestosterone

Cytosol (400 μl) was mixed with 10 μl of an ethanol solution of 3H-dihydrotestosterone (1,2-3H) 5α-dihydrotestosterone (Specific activity, 48 Ci/m mole, The Radiochemical Centre, Amersham, England) and 100 μl of glycerol, to give a final concentration of 2×10^-9 M and 10% (v/v), respectively, and was incubated overnight at 0°C by gentle shaking either in the absence or in the presence of unlabelled steroids or test compounds dissolved in 5 μl of ethanol. After incubation an equal volume of 0.25% dextran-2.5% charcoal-10% (v/v) glycerol was added, and then the samples were vortexed for 5 sec and centrifuged immediately at 8,000×g for 10 min. Aliquots of the supernatant were dissolved in 1.0 ml of distilled water, 1.0 ml of ethyl alcohol and 10 ml of toluene containing 0.4% PPO and 0.01% POPOP, and then vortexed for 30 sec, to extract the labelled steroid from the aqueous phase. Radioactivity was counted in a Packard Tri Carb liquid scintillation spectrometer, Model 3380, with a counting efficiency of 37-48%.

Sucrose gradient centrifugation

Sedimentation coefficient of 5α-dihydrotestosterone binding macromolecules were determined according to the method of Martin and Ames (1961), using bovine serum albumin (Sigma, U.S.A.) and beef liver catalase (Sigma, U.S.A.) as marker protein. After treatment with dextran-charcoal solution, 200 μl of the supernatant was layered over preformed 5-20% (v/v) sucrose gradients in 50 mm Tris-HCL (pH 7.4)-2 mm 2-Mercaptoethanol-10% (v/v) glycerol. Gradients were centrifuged for 21 hr at 178,900×g at 2°C in a Hitachi RPS 65T roter. Fractions (100 μl each) were collected using a density gradient fractionator (ISCO, Model 640), and radioactivities were measured using a Packard Tri Carb liquid scintillation spectrometer, Model 3380.

Protein assay

Cytosol protein was determined by the method of Lowry et al. (1951), using bovine serum albumin as standard.

Results

The influence of steroidal and nonsteroidal compounds on the binding of 5α-dihydrotestosterone to rat hypophyseal and hypothalamic cytosol macromolecules

Table 1. shows the influence of 5α-dihydrotestosterone, estrogens (estradiol-17β, estriol, estrone), cortisol, steroidal antiandro- gens (cyproterone acetate, chlormadinone acetate) and nonsteroidal antiandrogens (SCH 13521, AA 560) on the binding of 5α-dihydrotestosterone to hypophyseal and hypothalamic cytosol macromolecules from castrated male rats. The binding in both hypophysis and hypothalamus to 5α-dihydrotestosterone were inhibited to various degrees by addition of these compounds examined, but there were no remarkable differences of inhibition rates between hypophysis and hypothalamus. Cyproterone acetate and chlormadinone acetate showed the significant inhibiting effects on the binding. SCH 13521 and AA 560 did affect the binding of 5α-dihydrotestosterone to hypophyseal and hypothalamic cytosol macromolecules.

Table 1. Inhibition of the 5α-dihydrotestosterone binding to the hypophyseal and hypothalamic cytosol from castrated rats.

<table>
<thead>
<tr>
<th>Additions</th>
<th>Hypophysis (%)</th>
<th>Hypothalamus (%)</th>
</tr>
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<tbody>
<tr>
<td>5α-dihydrotestosterone</td>
<td>76</td>
<td>72</td>
</tr>
<tr>
<td>Cyproterone acetate</td>
<td>62</td>
<td>51</td>
</tr>
<tr>
<td>Chlormadinone acetate</td>
<td>62</td>
<td>56</td>
</tr>
<tr>
<td>SCH 13521</td>
<td>41</td>
<td>32</td>
</tr>
<tr>
<td>AA 560</td>
<td>74</td>
<td>54</td>
</tr>
<tr>
<td>Estrone</td>
<td>35</td>
<td>22</td>
</tr>
<tr>
<td>Estradiol 17β</td>
<td>72</td>
<td>57</td>
</tr>
<tr>
<td>Estriol</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>Cortisol</td>
<td>16</td>
<td>8</td>
</tr>
</tbody>
</table>

Data are shown as the average of three experiments performed independently. Incubation was performed with cytosol in 2×10^-6 M 3H-dihydrotestosterone in the absence or presence of 2×10^-6 M compounds.

Sedimentation profiles of rat hypophyseal and hypothalamic cytosol incubated with 3H-5α-dihydrotestosterone

Fig. 1. shows the 3H-radioactivity profiles obtained after sucrose gradient centrifugation of castrated male rat hypophyseal and hypothalamic cytosol, which was incubated with 3H-5α-dihydrotestosterone and then treated with dextran-coated charcoal.
EFFECTS OF ANTIPROSTATIC AGENTS

Fig. 1. Sedimentation pattern of cytosol 5α-dihydrotestosterone binding macromolecules from castrated rat hypothalamus and hypophysis in a linear 5-20% sucrose gradients in the absence or presence of unlabelled 5α-dihydrotestosterone. Cytosol was prepared from either 60 hypothysis or hypothalami of castrated male rats in 50 mM Tris-HCl (pH 7.4)-1.5 mM EDTA disodium salt-2 mM 2-Mercaptoethanol (protein concentration: 12.44 mg/ml, 18.58 mg/ml, respectively) and incubated at 0°C overnight with 2 x 10^{-9} M 3H-5α-dihydrotestosterone in the absence (-) or presence (---) of unlabelled 5α-dihydrotestosterone 2 x 10^{-6} M. After the dextran coated charcoal treatment (see Materials and Methods), aliquots (200 μl) were then layered on sucrose gradients in 50 mM Tris-HCl (pH 7.4)-1.5 mM EDTA disodium salt-2 mM 2-Mercaptoethanol-10% (v/v) glycerol and centrifuged at 178,900 x g for 21 hrs at 2°C in Hitachi RPS 65T roter.

Fig. 2. illustrates a series of sucrose gradient sedimentation patterns of cytosol incubated with 3H-dihydrotestosterone in the absence or presence of an excess amount of antiandrogens. A 1000-fold concentration of cyproterone acetate or chlormadione acetate did diminish the radioactive peak at 7-8 S in both the hypphyseal and hypothalamic cytosol (Fig. 2—B (hypothalamus), Fig. 2—D (hypophysis)). It was also observed that 1000-fold concentration of nonsteroidal antiandrogens (SCH 13521, AA 560) decreased the radioactive peak at 7-8 S regions in the cytosol from hypphyseal and hypothalamus (Fig. 2—A (hypothalamus), Fig. 2—B (hypophysis)).

Discussion

The action of the steroidal antiandrogens appeared to be mediated through competition with androgens at the level of target organ receptors (Newmann et al., 1966; Fang and Liao, 1969; Belham and Neal, 1971; Mangan and Mainwaring, 1972). SCH 13521, which is a substituted anilide, is a nonsteroidal antiandrogen (Neri et al., 1972a; Neri, et al., 1972b). The mechanism of action of this compound is not completely clear, but one mechanism of the action of this compound might be involved with the competition of SCH 13521 itself and/or
metabolites of this compound for the intracellular 5α-dihydrotestosterone receptor (Varkarakis et al., 1975; Shimazaki et al., 1977). AA 560 is also a derivative of anilide and its antiandrogenic action was reported to be about three times as strong as SCH 13521 (Takahashi et al., 1977). We have previously observed that 1,000-fold concentration of SCH 13521 did not affect the 3H-dihydrotestosterone binding to 7–8 S macromolecules in the cytosol from rat ventral prostate (Yamanaka et al., 1978).

In this paper it was shown that incubation with a 1,000-fold concentration of SCH 13521 decreased the 3H-5α-dihydrotestosterone binding to 7–8 S macromolecules in the cytosol from rat hypophysis and hypothalamus. It was very interesting that there was a difference between the brain and the ventral prostate in the competition between 3H-5α-dihydrotestosterone and SCH 13521 for cytosol 5α-dihydrotestosterone receptor.

Several investigators have reported that there exist androgen receptors in the cytosol from male rat hypophysis and hypothalamus and it was assumed that these binding macromolecules could play a part in the regulation of gonadotropins secretion. The present in vitro studies indicated that both SCH 13521 and AA 560 affected the bind-

Fig. 2. Sedimentation pattern of cytosol 5α-dihydrotestosterone binding macromolecules from rat hypothalamus and hypophysis in a 5–20% sucrose gradients in the absence or presence of unlabelled antiandrogens. Cytosol preparation and sucrose gradient centrifugation are the same as given in the legend to Fig 1. Hypothalamus (A and B): Hypothalamic cytosol (protein concentration 13.30 mg/ml) were incubated at 0°C overnight with (A) 2×10⁻⁶ M SCH 13521 (—○—) or 2×10⁻⁶ M AA 560 (—△—), and (B) 2×10⁻⁶ M cyproterone acetate (C.A.) (—○—) or 2×10⁻⁶ M chlormadinone acetate (C.M.A.) (—△—). Hypophysis (C and D): Hypophyseal cytosol (protein concentration 14.68 mg/ml) were incubated at 0°C overnight with (C) 2×10⁻⁶ M SCH 13521 (—○—) or 2×10⁻⁶ M AA 560 (—△—), and (D) 2×10⁻⁶ M cyproterone acetate (C.A.) (—○—) or 2×10⁻⁶ M chlormadinone acetate (C.M.A.) (—△—).
ing 5α-dihydrotestosterone to cytosol macromolecules from male rat hypophysis and hypothalamus. Thus, nonsteroidal antiandrogens such as SCH 13521 and AA 560 might increase the secretion of gonadotropin via the break of negative feedback. In fact, Prout et al., (1975) reported that administration of SCH 13521 to male patients with stage D prostatic cancer evoked an increase in secretion of luteinizing hormone, although not markedly. Takahashi et al., (1977) reported that when both SCH 15321 and AA 560 were administered to male rats, serum testosterone concentration increased significantly. Therefore, if the above mentioned assumption were true, the administration of a compound which has an ability to inhibit the binding of 5α-dihydrotestosterone to cytosol macromolecules from male hypophysis and brain would have to result in increased secretion of gonadotropin and also, paradoxically, in increased production of testosterone. Such a phenomena was already reported after treatment of cyproterone (Newmann et al., 1970).

We showed in this paper that both cyproterone acetate and chlormadinone acetate affected the binding of 5α-dihydrotestosterone to cytosol macromolecules from male rat hypophysis and hypothalamus. Unexpectedly, these steroidal antiandrogens have been reported to have no effect on secretion of gonadotropin with higher doses (Johnson and Naqvi, 1969; Newmann et al., 1968; Geller et al., 1977). Concerning this discrepancy, Neass et al. (1975b) cited the possibility that when an antiandrogen which, in addition, had potent progestational properties was administered, its progestational activity might exceed the antiandrogenic effect of this compound and cause a decreased gonadotropin output. Another possibility is that progestational compound may interact with the androgen receptors and thereby function as a weak androgen.

Naftolin et al. (1972) suggested that aromatization of androgens was required for the hormonal feedback effect on the gonadotropin secretion. On the other hand, Swerdloff et al. (1972) concluded that conversion of androgens to estrogens was not always required for the inhibition of the hypothalamic pituitary axis. Further studies for the detailed mechanism of feedback control of gonadotropins secretion in male subjects are ardently awaited.

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References

Naess, O., V. Hansson, O. Djoeseland and A. Atttramadal (1975b). ibid. 97, 1355.