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TESTOSTERONE METABOLITES ASSOCIATED WITH CYTOSOL RECEPTORS AND NUCLEI OF ANTERIOR PITUITARY AND VARIOUS BRAIN REGIONS

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SUMMARY

Radioactive metabolites bound to the cytosol receptors and nuclei of the anterior pituitary and various brain regions were analyzed 15 min and 60 min after [^{3}H]-testosterone (T) administration in the functionally hepatectomized and eviscerated rats. It was clearly demonstrated that there was the thermolabile saturable binding protein for T and its metabolites in the cytosol of the pituitary and brain tissues.

With regard to the patterns of T metabolites bound to the cytosol receptor at 15 min after [^{3}H]-T administration, the most predominant metabolite was unchanged T and the remainder was 5α-dihydrotestosterone (DHT) in all brain regions. At 60 min after [^{3}H]-T administration unchanged T also represented the largest portion of radioactivity bound to the cytosol receptor, and DHT accounted for 17.3–11.6% of radioactivity, with the highest percentage in anterior pituitary. Estradiol 17β (E_2) accounted for the significant radioactivity in the preoptic area, hypothalamus, and other limbic systems. No significant radioactivity was detected in the metabolites other than T, DHT and E_2 in the cytosol receptor bound fractions of all brain tissues.

No sexual difference was observed in the levels and distributions of T metabolites bound to the cytosol receptors of anterior pituitary and various brain regions.

The patterns of T metabolites associated with the purified nuclei were comparable to those of the cytosol receptor bound fractions in all brain regions. That is, unchanged T was the most predominant metabolite incorporated into nuclei in all brain regions. Percent distribution of DHT was 24.8–5.8% of radioactivity associated with the nuclei, with the highest percentage in anterior pituitary, which has been reported to have the highest 5α reductase activity in the brain tissues.

In the preoptic area, E_2 represented the greater portion (36.8%) of radioactivity associated with the nucleus than in the other regions. This finding supported the hypothesis that E_2 might play the major role on the androgen induced sexual events in this area. The levels of radioactive metabolites associated with the nuclear protein 60 min after [^{3}H]-T administration was the highest in anterior pituitary followed by hypothalamus, preoptic area amygdala and cortex.

INTRODUCTION

Anterior pituitary, hypothalamus, and some limbic systems have been considered to be a target of androgen with respect to regulation of pituitary gonadotropine secretion and sexual behavior. As in the peripheral androgen target tissues, it has been shown that T was metabolized to 5α-reduced androgens (DHT and 5α-androstane 3α,17β diol) in the pituitary and brain tissues [1–4]. DHT, the active hormone in most peripheral androgen target tissues such as prostrate, seminal vesicle and so on, has been reported to be as potent as T in negative feedback control of gonadotropine secretion in human *in vivo* [5], and in rats both *in vivo* [6], and *in vitro* [7].

On the other hand, much evidence has been accumulated to show that androgen is aromatized to estrogens [8–11] and locally formed estrogen is the active hormone for initiating and maintaining the male sexual behaviour in the long-term castrated male rats [12–13]. Therefore, it is considered to be very important to determine whether transformation of T to DHT or aromatization to estrogen is the necessary step for the action of androgen in the pituitary and brain tissues.

Although the information on the mechanism of androgen action in the brain is very incomplete, the basic mechanism of androgen action may be considered to be the same as in the other androgen target tissues. That is, in a target cell, T, or its metabolites formed locally, combines with cytosol specific binding protein (receptor) to form the complex. The complex is translocated into the nucleus, binds to the chromatin and alters the transcription of the specific gene.

Identification of the metabolites of T bound to the

Systematic nomenclature for trivial names used in this paper: Testosterone (T) = 17β-hydroxy-4-androsten-3-one 5α-dihydrotestosterone (DHT) = 12β-hydroxy-5α-androstan-3-one estradiol 17β (E_2) = 1,3,5-estratriene-3,17β-diol androstenedione = 4-androstene-3,17-dione.

cytosol receptor and nucleus of the rat brain is essential to understanding the biochemical basis of the androgen induced sexual events in the brain. In this study, the metabolites of T bound to the cytosol receptors, nuclei of pituitary and various brain regions are analysed in adult rats and the meanings of the receptor systems for androgen action in the central nervous system is discussed.

EXPERIMENTAL

Animals and preparation of tissues. Adult male and female Sprague–Dawley rats, 3-4 months old, weighing 200–300 g, were used. The animals were housed under the controlled light (light on 0500–2200) and constant temperature. The animals were fed Rat Chow (Oriental Kobo Co. Ltd., Osaka, Japan) and water *ad libitum*. Animals were castrated via the scrotal route under diethylether anesthesia 16 h prior to experiment. On the day of the experiment the animals were cannulated by insertion of a polyethylene tube into the abdominal aorta, and the aorta was ligated just above the bifurcation of hepatic artery for functional hepatectomy and evisceration. Immediately after treatment, each animal was given intravenously 50 μCi of [1,2,6,7-*n*-^{3}H]-T (145 ng) with or without 10 μg of unlabeled T (approx. 70 fold) dissolved in 0.6 ml of a mixture of 0.9% saline–Tween 80 (100:1, v/v) and ethanol (5:1, v/v).

At varying times after [^{3}H]-T administration animals were sacrificed by perfusion of 100 ml of cold 0.9% saline. Immediately after sacrifice, pituitary and various brain tissues were removed and frozen on dry ice. The anterior pituitary was isolated and separated from the neurohypophysis. The hypothalamus was cut out as the block bounded anteriorly by the optic chiasma, posteriorly by the mammillary bodies, laterally by the hypothalamic sulci, and horizontally 2–3 mm under the basal surface. The preoptic area was removed as the block 3 mm anterior to chiasma opticus and a depth of 2–3 mm. The hippocampus and amygdala were dissected as illustrated in Fig. 1. The cortex was obtained from the parietal lobe.

All subsequent procedures were carried out at approximately 0°C.

Preparation of cytosol. The tissues were homogenized in 2 ml of 10 mM Tris–HCl, pH 7.4–1.5 mM EDTA buffer (TE buffer) in hand for pituitary, hypothalamus and preoptic area. Amygdala, hippocampus and cortex were homogenized in 5 ml of TE buffer in motor drive. The cytosol was prepared by centrifugation of the homogenates at an average of 105,000 *g* for 60 min in a Hitachi rotor RP-40.

Preparation of nuclei from pituitary. The pooled tissues were homogenized in 10 vol. of 0.25 M sucrose—50 mM Tris-HCl, pH 7.4—3 mM $MgCl_2$ (TM buffer) with 20 strokes of a Teflon–glass homogenizer by hand. The homogenate was filtered through a nylon mesh, then centrifuged at 800 *g* for 10 min. The crude nuclear pellet was resuspended and rehomogenized in a mixture of 1.4 ml of 0.25 M sucrose–TM buffer and 8.6 ml of 2.0 M sucrose–TM buffer, and centrifuged at an average 25,000 *g* for 20 min in a Hitachi RPS-25-3 rotor. The pellet was suspended in 5 ml of 0.25 M sucrose–TM buffer containing 0.25% Triton X-100, centrifuged at 1,000 *g* for 10 min and washed twice with 5 ml of 0.25 M sucrose–TM buffer. The pellet was used as the preparation of nuclear fraction of pituitary.

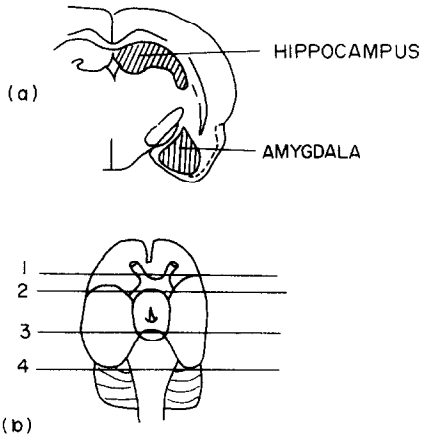

Fig. 1. Diagrams of the rat brain to illustrate the region for amygdala and hippocampus 1(a): Diagram of frontal section of rat brain. 1(b): Diagram of basis of rat brain. Amygdala was obtained between section line 2 and 3, hippocampus was obtained between section line 3 and 4.

Isolation of nuclei from the brain tissues was performed according to McEwen's method [14]. The brain tissues were homogenized in 5 ml of 0.32 M sucrose–1 mM K phosphate, pH 6.5—3 mM $MgCl_2$ (PM buffer)—0.25% Triton X-100 in a Teflon–glass homogenizer. The homogenates were centrifuged at 800 *g* for 10 min. The pellets were washed twice with 5 ml of 0.32 M sucrose–PM buffer. The crude nuclei were suspended in 2.39 M sucrose–PM buffer and 0.32 M sucrose–PM buffer to the final concentration of 2.0 M sucrose–PM buffer. The purified nuclei were obtained by centrifugation of these suspensions at 15,000 *g* for 90 min in a Hitachi RPS-25-3 rotor. Purity of the nuclei was confirmed by a phase contrasted microscopy.

Estimation of the levels of T metabolites bound to the nuclear protein. The nuclei were suspended in 1 ml of 0.5 M KCl–TE buffer, and disrupted by sonication. The sonicates were centrifuged at 10,000 *g* for 10 min. The 0.5 M KCl extracts were applied to gel filtration. The levels of T metabolites bound to the nuclear protein expressed as fmol/mg protein.

Gel filtration procedures. For separation of the macromolecule bound steroids from free steroids of the cytosol and nuclei, a column (0.9 × 15 cm) packed with fine Sephadex G-25 (Pharmacia Fine Chemicals AB, Uppsala, Sweden) was used. A Sephadex G-100 column (1.5 × 40 cm) was used for the separation of the cytosol steroid binding macromolecule and the serum sex steroid binding proteins.

Analysis and identification of radioactive steroid metabolites. 500 μg of authentic steroids (T, androstenedione, DHT, 5α-androstane 3α,17β diol and E_2) were added to the materials containing the radioactive metabolites, in order to determine the recovery and chromatographic position of each steroid. The steroid metabolites were extracted with 5 vol. of diethylether and analyzed by column chromatography on partially esterified Amberlite IRC-50 [15]. The eluent was ethanol–benzene–cyclohexane–H_2O (50:350:200:2.8, v/v). The recovery of each steroid averaged about 85%. Identification of the major radioactive metabolites (T and DHT) were confirmed by recrystallization to constant specific activity.

Effects of temperature and enzymatic digestion on the cytosol receptor steroid complex. The cytosol of the pooled anterior pituitary and various brain tissues (hypothalamus, preoptic area, and other limbic systems) was prepared from the adult male rats 60 min after [^{3}H]-T administration. The cytosol was incubated at 0, 25, 37 and 50°C for 30 min or incubated with 100 μg/ml of DNase, RNase, pronase at 37°C for 30 min and subjected to gel filtration on Sephadex G-25 for separation of macromolecule bound steroids from free steroids.

Other analytical procedures. Protein was determined by the method of Lowry *et al.* [16] with BSA as standard. Radioactivity was measured in a Searl Isocap 300 liquid scintillation spectrometer. The toluene based scintillator (4 g PPO and 100 mg POPOP in 1 l. of toluene) was used for the nonaqueous samples. For the aqueous samples, a solution containing 4 g PPO and 100 mg POPOP in 1 l. of toluene–Triton X-100 (2:1, v/v) solution was used. Counting efficiency was corrected by external standardization.

Chemicals. [1,2,6,7-*n*-^{3}H]-Testosterone (S.A. 100 Ci/mmol) was obtained from the Radiochemical Centre, Amersham, England. Nonradioactive steroids were purchased from Steraloids, Inc. (Pauling, New York, U.S.A.). Pronase, and DNase (Deoxyribonuclease, I, crude lyophylized powder from pancreas) and RNase (Type X.A from bovine pancreas) were purchased from Sigma, Inc. (St. Louis, Missouri, U.S.A.). All other chemicals were of analytical grade.

RESULTS

Effects of functional hepatectomy and evisceration on the levels of total radioactivity and distribution of T metabolites in the plasma

The animals castrated 16 h before were functionally hepatectomized and eviscerated. The levels of total radioactivity of plasma were plotted as a function of time after [^{3}H]-T administration in Fig. 2(a). In untreated animals plasma radioactivity was reduced rapidly to the concentration of 5×10^4 d.p.m./ml at 15 min after [^{3}H]-T administration; while in the treated animals the concentration of radioactivity in plasma was maintained approximately 5.5×10^5 d.p.m./ml in adult male rats and 4.0×10^5 d.p.m./ml in adult female rats, even at 60 min after [^{3}H]-T administration. Percentage of the radioactivity extractable into diethylether from the plasma is shown in Fig. 2(b).

In the treated animals the proportions of the diethylether extractable radioactivity was 85% in male rats and 70% in female rats at 60 min after [^{3}H]-T administration. On the other hand, in untreated rats this proportion was reduced markedly with time, showing that the radioactive androgens might be metabolized into the polar steroids.

Analysis of the radioactive metabolites extractable by diethylether from plasma of the treated animals was carried out as shown in Fig. 2(c). T represented 45% (in male rats) and 40% (in female rats) of the diethylether extractable radioactivity at 60 min after [^{3}H]-T administration. These results showed that this procedure was very effective for maintenance of the levels of radioactive T in the plasma.

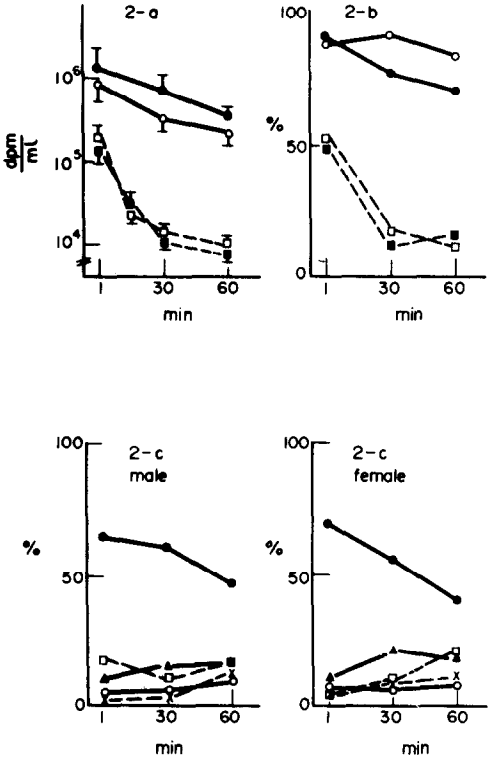

Fig. 2. Effect of functional hepatectomy and evisceration on the levels of radioactivity and distribution of T metabolites in plasma with time following the intravenous administration of [^{3}H]-T. 2(a): total radioactivity recovered. 2(b): percentage of the recovered radioactivity extractable into diethylether. ●——●; functionally hepatectomized male rats, ○——○; functionally hepatectomized female rats, ■----■; untreated male rats, □----□; untreated female rats. 2(c): Percent distribution of T and its metabolites in diethylether extracts of plasma in functionally hepatectomized and eviscerated rats ●——●; testosterone, □----□; DHT + androsterone, ○——○; androstenedione, ×----×; 5α-androstane 3α,17β diol, ▲——▲; other metabolites.

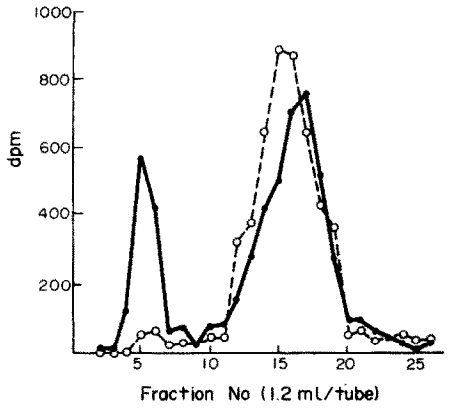

Fig. 3. Effect of nonradioactive testosterone on binding of radioactive metabolites of T to the cytosol receptor. Five adult male rats were given intravenously 50 μCi of [^{3}H]-T with or without 10 μg of nonradioactive T. 60 min later animals were sacrificed by perfusion of 0.9% saline. The cytosol of pituitary and brain tissues was prepared and subjected to Sephadex G-25. ●——●: 50 μCi of [^{3}H]-T only; O----O: 50 μCi of [^{3}H]-T with 10 μg of nonradioactive T.

Effects of perfusion of cold 0.9% saline on the contamination of serum sex steroid binding protein to the cytosol macromulecule bound steroids fractions

The serum sex steroid binding protein was eluted later than the cytosol T metabolites binding macromolecule by the gel filtration of Sephadex G-100. In the cytosol of pituitary and brain tissues from the animals perfused by 0.9% saline, the radioactive peak corresponding to the serum sex steroid binding protein was not detected. Therefore, in the following experiments the separation of the cytosol macromolecule bound steroids from the free steroids was carried out by gel filtration on Sephadex G-25 instead of Sephadex G-100.

Effect of unlabelled T on binding of radioactive metabolites of T to the cytosol-macromolecule of anterior pituitary and brain tissues

In order to determine the saturability of the binding sites for the metabolites of [^{3}H]-T in the cytosol, 10 μg of unlabelled T (approx. 70 fold) was administered simultaneously with 50 μCi of [^{3}H]-T. As shown in Fig. 3, the simultaneous administration of unlabelled T significantly inhibited the binding of radioactive metabolites of T to the cytosol macromolecule of the anterior pituitary. The same inhibition was also observed in the cytosol of the various brain regions. These findings showed that the binding sites for T metabolites in the cytosol of the brain tissues were of limited capacity.

Effects of temperature and enzymatic digestion on the cytosol-macromolecule radioactive-steroid complex

Incubation at 0 and 25°C did not affect the cytosol-macromolecule radioactive-steroid complex. However, when incubated at 37°C the cytosol-macromolecule bound radioactivity was reduced to 49.2% (in pituitary) and 59.6% (in brain tissues) of the radioactivity in the incubation at 0°C, and when incubated at 50°C, reduced to 15.6% (in pituitary) and 39.2% (in brain tissues). These results indicated that the cytosol-macromolecule steroid complex was heat labile as reported in the other steroid cytosol receptor complex.

Pronase treatment abolished this macromolecule bound radioactivity, but DNase and RNase treatment had no influence on this bound radioactivity. This finding indicated that the cytosol macromolecule was proteineous.

Analysis and identification of radioactive metabolites of T recovered from the cytosol and nucleus

Figure 4 shows a typical elution pattern of the metabolites of T recovered from the cytosol-macromolecule bound fraction of the hypothalamus by column chromatography on partially esterified Amberlite IRC-50. Good separation was obtained of the representative metabolites of T (androstenedione, DHT, T, 5α-androstane 3α,17β diol and E_2) in the brain by this chromatographic system.

The radioactive steroid preparations tentatively identified as T and DHT by chromatography were pooled from the cytosol macromolecule bound fractions of the various brain regions. The identity of radioactive T and DHT fractions was confirmed by recrystallization to constant specific activity.

T metabolites in the cytosol of anterior pituitary and various brain tissues

The results of distribution of radioactive metabolites in the cytosol of anterior pituitary and various brain tissues of adult male rats 15 and 60 min after [^{3}H]-T administration are shown in Tables 1 and 2. At 15 min after [^{3}H]-T administration, the most predominant metabolite bound to the cytosol receptor was unchanged T and the remainder was DHT in all tissues. No significant radioactivity were detected at the position of E_2 and other metabolites. At 60 min after [^{3}H]-T administration unchanged T also represented the largest amount of the total radioactivity bound to the cytosol receptor in all brain tissues. DHT accounted for 17.3–11.6% with the highest percentage of DHT in anterior pituitary. E_2 represented the significant value of radioactivity in some brain tissues. Particularly in the preoptic area, E_2 accounted for 17.9% of radioactivity bound to the cytosol receptor. In the pituitary, radioactivity of E_2 was negligible. No metabolite other than T, DHT and E_2 was detected significantly in all brain regions.

Table 3 shows the analysis of distribution of T metabolites bound to the cytosol receptor of adult female rats 60 min after [^{3}H]-T administration. This analysis revealed that no *gross* difference was observed in the levels and distributions of radioactive metabolites in both sexes. In the free steroid fractions, a significant amount of androstenedione, 5α andros-

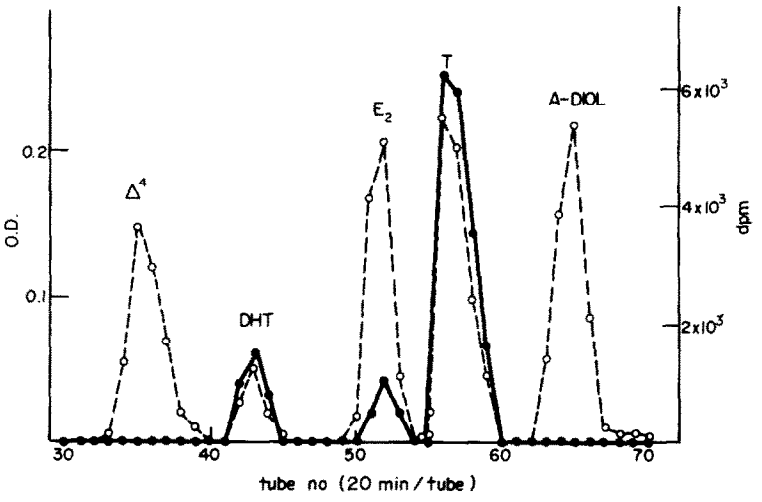

Fig. 4. Chromatographic separation of radioactive metabolites of T. 500 μg of authentic steroids [testosterone (T), 5α-dihydrotestosterone (DHT), androstenedione (Δ_4), estradiol 17 (E_2), 5α androstane 3α,17β diol (A-diol)] were added to the cytosol receptor bound fraction of hypothalamus, The steroids were extracted with 5 vol of diethylether and applied to the chromatography on partially esterified Amberlite IRC-50 using ethanol–benzene–cyclohexane-H_2O (50:350:200:2.8, v/v) as eluent. O----O: optical density of the standard steroids added. ●——●: radioactivity of the diethylether extractable T metabolites of the hypothalamus bound fraction.

tane 3α,17βdiol, and unidentified metabolites was detected in addition to T and DHT. E_2 was not detected in the free steroid fractions of any brain regions.

T metabolites associated with the purified nuclei of the anterior pituitary and brain tissues

As shown in Table 4, the patterns of T metabolites associated with the purified nuclei were similar to those observed in the cytosol receptor fractions 60 min after [^{3}H]-T administration. That is, unchanged T itself was the most predominant metabolite in all regions. DHT accounted for 24.8–5.8% with the highest levels in the anterior pituitary. In preoptic area, E_2 represented the greater portion (36.8%) of radioactivity associated with nuclei than in the other regions.

Radioactive levels associated with the nuclear protein of the anterior pituitary and various brain regions

The nuclear protein bound radioactivity was the highest in the anterior pituitary (6.0 fmol/mg protein) and in the following descending order of abundance: hypothalamus (2.1 fmol/mg protein), preoptic area

Table 1 Distribution of T metabolites in the cytosol of anterior pituitary and various brain regions of adult male rats at 15 min after [^{3}H]-T administration

Tissue		Total radioactivity	fmol/mg† protein	Percent distribution of metabolites*					
				Δ^4	DHT	T	A-Diol	E_2	Others
Pituitary	free	41,880		11.6	9.0	61.8	7.8	0	3.7
	bound	5,760	(7.3)	0.0	13.7	68.8	2.0	0	2.7
Hypothalamus	free	89,060		13.7	6.9	65.0	8.1	0	4.2
	bound	3,510	(2.5)	0.0	11.2	83.6	0.0	0	5.1
Preoptic area	free	87,680		11.5	8.2	66.4	8.8	0	5.1
	bound	3,580	(1.9)	0.0	9.9	87.3	0.0	0	2.8
Hippocampus	free	129,480		14.6	10.8	64.1	4.6	0	5.6
	bound	4,430	(1.8)	1.0	11.2	85.3	0.0	1.0	2.5
Amygdala	free	151,360		14.4	6.9	65.4	9.1	0	3.6
	bound	6,900	(2.3)	2.2	8.7	86.9	0.0	0	2.3
Cortex	free	103,900		14.6	7.5	63.4	5.9	0	7.5
	bound	4,052	(1.3)	2.0	10.5	83.8	0.0	0	4.0

Sixteen adult male rats castrated 16 h before were functionally hepatectomized and eviscerated. Each animal was given intravenously 50 μCi of [^{3}H]-T. 15 min later animals were sacrificed by perfusion of 0.9% saline. The cytosol was prepared from the pooled pituitaries and brain tissues as described in experimentals, and subjected to Sephadex G-25. Radioactive steroids were extracted with diethylether and analyzed by chromatography on Amberlite IRC-50.

* The abbreviations used are: Δ^4, androstenedione; DHT 5α-dihydro-testosterone; T, testosterone; A-diol, 5α-androstane 3α, 17β-diol; E_2, estradiol 17β; others, all other radioactive spots not identified.

† Total metabolites bound to the cytosol receptor was expressed as fmol/mg protein.

Table 2. Distribution of T metabolites in the cytosol of anterior pituitary and various brain regions of adult male rats at 60 min after [^{3}H]-T administration

Tissue		Total radio-activity	fmol/mg† protein	Percent distribution of metabolites*					
				Δ^4	DHT	T	A-Diol	E_2	Others
Pituitary	free	123,545		15.1	14.1	46.2	18.0	0.0	6.7
	bound	16,570	(5.8)	0.7	17.3	73.0	3.8	1.5	3.7
Hypothalamus	free	235,835		13.2	11.2	50.7	14.6	0.0	10.3
	bound	8,937	(2.3)	0.0	11.6	78.3	0.0	6.6	0.0
Preoptic area	free	167,990		10.3	10.0	47.6	6.7	2.7	28.2
	bound	4,950	(2.8)	4.1	15.8	61.8	0.0	17.9	0.0
Amygdala	free	119,247		15.8	9.6	53.2	16.2	0.0	5.1
	bound	6,497	(2.0)	0.7	15.5	72.4	2.4	6.5	2.5
Cortex	free	377,150		22.5	10.0	35.4	27.0	0.0	12.2
	bound	8,970	(1.2)	0.0	14.5	70.9	3.0	0.0	2.5

Thirty five adult male rats castrated 16 h before were functionally hepatectomized and eviscerated. Each animal was given intravenously 50 μCi of [^{3}H]-T. At 60 min later animals were sacrificed by perfusion of 0.9% saline. Preparation of cytosol and analysis of steroids was carried out as described in experimentals.

* The abbreviations are the same as that in the legend of Table 1.

† Total T metabolites bound to the cytosol receptor (fmol/mg protein).

(1.6 fmol/mg protein), amygdala (1.1 fmol/mg protein) and cortex (0.6 fmol/mg protein).

DISCUSSION

The information available on the receptor system for androgen in the central nervous system were limited and confusing. Recently, several authors reported the presence of the specific binding protein for androgen in the cytosol of pituitary and hypothalamus [17–22].

The present study demonstrated the presence of the thermolabile, saturable binding protein for T itself and its metabolites in the cytosol of anterior pituitary and various brain regions of rats after [^{3}H]-T administration. Furthermore, the almost same patterns of T metabolites bound to the cytosol binding protein were recovered from the nuclei of anterior pituitary and brain tissues. These findings suggested that T and its metabolites combined the cytosol specific binding protein and selectively translocated into the nuclei as in the other androgen target tissues.

Recently, evidence has been accumulated that in most androgen target tissues, such as the accessory sex organs, T is rapidly converted to DHT locally, and DHT constituted the major androgen associated with the cytosol receptor and nuclei. The active hormone in these targets is considered to be not T but DHT [23–25]. On the other hand, T itself seems to be the active hormone in some organs, such as mouse kidney [26], fetal Wolffian derivatives [27], muscle [28] and androgen dependent mammary cancer [29].

In present study, unchanged T itself was shown to be the most predominant metabolite associated with

Table 3. Distribution of T metabolites in the cytosol of anterior pituitary and various brain regions of adult female rats at 60 min after [^{3}H]-T administration

Tissue		Total radioactivity (d.p.m.)	fmol/mg† protein	Percent distribution of metabolites*					
				Δ^4	DHT	T	A-Diol	E_2	Others
Pituitary	free	61,380		15.8	6.5	54.0	8.2	0.1	15.4
	bound	6,003	(7.0)	2.8	18.7	67.5	2.4	2.9	5.6
Hypothalamus	free	83,100		17.8	2.8	48.6	6.8	0.9	23.3
	bound	3,490	(2.7)	1.5	15.2	73.3	0.0	6.9	0.3
Preoptic area	free	95,326		20.0	7.0	48.3	13.7	0.0	11.0
	bound	4,333	(2.3)	1.0	10.9	73.7	1.0	10.9	2.7
Hippocampus	free	128,208		17.2	11.3	54.3	12.9	0.0	4.2
	bound	6,830	(1.9)	0.0	16.2	78.5	0.0	5.3	0.0
Amygdala	free	155,496		17.2	10.5	59.6	7.4	0.0	5.2
	bound	7,062	(2.5)	1.8	16.0	73.8	0.0	8.4	0.0
Cortex	free	105,070		12.5	8.8	48.5	8.2	0.0	21.9
	bound	7,000	(0.8)	3.6	14.9	76.4	1.6	1.5	2.0

Seventeen adult female rats ovariectomized 16 h before were functionally hepatectomized and eviscerated. Each animal was given intravenously 50 μCi of [^{3}H]-T administration. 60 min later animals were sacrificed by perfusion of 0.9% saline. Preparation of the cytosol and analysis of steroids was carried out as described in experimentals.

* The abbreviations are the same as that in the legend of Table 1.

† Total T metabolites bound to the cytosol receptor (fmol/mg protein).

Table 4. Distribution of radioactive metabolites of testosterone recovered from purified nuclei of anterior pituitary and various brain regions of adult male rats 60 min after [^{3}H]-T

Tissue	Total radioactivity (d.p.m.)	Percent distribution of metabolites*					
		Δ^4	DHT	T	A-Diol	E_2	Others
Pituitary	11,967	0.4	24.8	71.1	1.9	1.3	0.5
Hypothalamus	4,676	1.5	10.4	77.9	1.2	8.3	0.6
Preoptic area	7,502	1.1	5.6	53.6	2.9	36.8	0.0
Hippocampus	6,438	0.4	13.1	75.4	0.0	11.1	0.0
Amygdala	4,048	0.0	8.0	78.0	1.2	11.5	0.8
Cortex	13,653	0.1	11.7	84.4	1.3	2.0	4.0

Forty five adult male rats castrated 16 h before were functionally hepatectomized and eviscerated. Each animal was given intravenously 50 μCi of [^{3}H]-T administration. 60 min later animals were sacrificed by perfusion of 0.9% saline. Preparation of nuclei and analysis of steroids was carried out as described in Experimental.

* The abbreviations are the same as that in the footnote to Table 1.

the cytosol receptor and nuclei of pituitary and various brain regions. DHT represented only one fourth or one fifth of radioactivity of T. This result is consistent with the reports by Naess *et al.* [21] and Gustaffson *et al.* [22]. Walsh *et al.* reported a family with pseudohermaphroditism due to a deficiency of 5α-reductase in the androgen target tissues [30–31].

The patients with this disease had the normal levels of serum gonadotropin and testosterone, and assumed the male gender role [32]. This suggested that the conversion of T to DHT might not be essential for the negative feedback mechanism of gonadotropin and male sexual behavior.

Lieberburg *et al.* reported that DHT was the major steroid (61% of total radioactivity) recovered from the pituitary nuclei, and in the other brain regions, DHT was recovered in an equal amount or somewhat larger portion than T at 2 h after [^{3}H]-T administration [33]. This discrepancy in the relative amount of T and DHT between Lieberburg's result and ours may be ascribed to the maintenance of the high levels of serum radioactive T by the functional hepatectomy and evisceration in our study. The percentage of DHT in the recovered metabolites increased with time after [^{3}H]-T administration. In anterior pituitary, which has been reported to have the highest 5α-reductase activity in the brain tissue [3], DHT was recovered in a larger percentage than in the other brain regions. These findings suggested that 5α-reductase activity might be the major determinant of the relative amount of T and DHT. However, the possibility of the presence of the separate receptor systems for T and DHT remains to be considered.

Martini reported that 5α androstan-3α,17β diol might have the physiological intracellular mediator of the feedback activity of T on LH secretion because of the high potency of this steroid as a suppressor of LH release [34]. However, in the present study, although 5α-androstane-3α,17β diol was present in a significant amount in the free steroid fractions, this metabolite was not detected in the cytosol receptor bound fractions and nuclei.

We could not find E_2 in the cytosol receptor bound fractions of any brain regions at 15 min after [^{3}H]-T administration as well as Naess's report [21]. However, at 60 min after [^{3}H]-T administration a significant amount of E_2 was recovered from the cytosol receptor bound fractions and nuclei of hypothalamus, preoptic area, and amygdala.

In pituitary which was reported to have no aromatization activity [11], E_2 was not detected. In preoptic area, E_2 represented the largest proportion in the brain tissues. This result supports the hypothesis that E_2 may play a major role in this area. Lieberburg *et al.* reported that the highest level of radioactivity identified as E_2 in the brain was found in the nuclei of amygdala 2 h after [^{3}H]-T administration [33]. We recovered no larger radioactivity of E_2 in the nuclei of amygdala than in the hypothalamus.

When [1,2,6,7-*n*-^{3}H]-T is aromatized to E_2, approximately 75% of ^{3}H in C-1 and C-2 is lost, and the specific activity of [^{3}H]-E_2 as T metabolite was lower than the other T metabolites. We underestimated the percentages of E_2 in the distribution of T metabolites.

Much recent work has suggested that E_2 formed locally might be the active hormone in the androgen induced male sexual behavior [12, 13, 35, 37]. But, in the mice with testicular feminization which had the marked reduced androgen receptor, but normal estrogen receptor and high levels of serum T and E_2, male sexual behavior was lost [36]. This fact indicated that the E_2 receptor system alone might not be enough for the effective regulation of male sexual behavior. Furthermore, the combined treatment of E_2 and DHT was reported to be more potent than E_2 only in restoring the male sexual behavior [38, 39]. The present study showed that T and DHT also retained in the nuclei of the preoptic area, hypothalamus, and limbic system besides E_2. This suggested the possibility of the cooperation of these T metabolites in the androgen induced sexual events in the brain.

Gustaffson *et al.* reported the absence of the cytosol receptor for androgen in the brain tissues of adult female rats and the marked difference in the patterns of T metabolites recovered from the homogenates of several brain regions [22].

However, in this study, no significant sex difference was observed in the levels and distribution of T metabolites in the cytosol receptor bound fractions and free steroid fractions of every brain region at 60 min after [^{3}H]-T administration. Therefore, the biochemical basis for sex differentiation in the central nervous system remains to be studied.

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