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THE ROLE OF HYPOTHALAMIC AND HYPOPHYSEAL 5α-DIHYDROTESTOSTERONE, ESTRADIOL AND PROGESTERONE RECEPTORS IN THE MECHANISM OF FEEDBACK ACTION

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SUMMARY

There is accumulating evidence for the role of estrogen receptors in the hypothalamus and hypophysis in the feedback action of the hormone on the central nervous system. Limited information, however, is available on androgen receptors in the brain. In this communication, 5α-dihydrotestosterone receptors in the hypothalamus and hypophysis were studied further.

The highly thermolabile 8 S protein(s) from male rat hypothalami and hypophysis showed high affinity for 5α-dihydrotestosterone (dissociation constant, $0{\cdot}74–2{\cdot}4 \times 10^{-9}$ M) with a limited number of binding sites (10^{-14} mol/mg cytosol protein). From competition experiments the binding protein(s) seemed to be specific for 5α-dihydrotesterone. The 8 S receptor macromolecule became 4 S subunits under high salt conditions.

The hypothalamic 5α-dihydrotestosterone receptor was also demonstrated in male mice, hamsters, guinea pigs, rabbits and cats, indicating its possible universal existence.

In contrast with the concentration of estrogen receptors in the preoptic-anterior hypothalamus, androgen 5α-dihydrotestosterone receptors tended to concentrate in the middle hypothalamus and the median eminence. A small but definite amount of receptors was noted in the amygdala.

Purified nuclei from male rat hypothalami and adenohypophyses were found to possess KCl extractable receptor molecules for 5α-dihydrotestosterone. From the above-mentioned results it is postulated that androgens can act in the brain through its interaction with 5α-dihydrotestosterone receptors in a way similar to that in the peripheral target organs.

Androgen 5α-dihydrotestosterone receptors were isolated from hypothalamic cytosols of developing male rats. They gradually developed at successive stages after birth: an appearance of a very small peak at 7 days was followed by its rapid increase at 14 days, and then rather strong binding was observed from 21 to 28 days old.

Abundance of hypothalamic estrogen receptors, together with the aromatization of androgens to estrogens, suggest a possible role of the estrogen receptors in the action of androgens and/or estrogens in males. It is implied that more complicated control systems are involved in the feedback action of the hormones on the brain.

Progesterone receptors seemed to be absent or, if any, in a very low concentration in the hypothalamus of ovariectomized estradiol-primed rats.

1. INTRODUCTION

The existence of estradiol receptor macromolecules has been well demonstrated in the hypothalamus and the hypophysis, as tabulated in Table 1 [1–11, 51, 52, 62–64].

It is now thought that the interaction of estrogens with these receptors is a key event in its feedback action on the central nervous system (CNS) [1, 12–15].

The brain, especially the hypothalamus, is also sensitive to androgen in its gonadotropin inhibiting action [16–18] and its determining organization of the neural tissues that mediates sexual behavior [19, 20]. This has been further substantiated by uptake data, after injection of [^{3}H]-testosterone, that the radioactive androgen accumulates in the hypothalamus and hypophysis [21–29], and by the presence of testosterone "receptor" isolated from castrated male rat hypothalamus and hypophysis by gel filtration [30–34].

2. ANDROGEN (DHT) RECEPTORS IN THE HYPOTHALAMUS AND HYPOPHYSIS

Estradiol is unaltered in the hypothalamus and the hypophysis [13], which is similar to the uterus and other peripheral target organs. In contrast, testosterone is metabolized into 5α-dihydrotestosterone (DHT), which is considered as an active androgen in the mechanism of action of androgens in target organs [35–38].

Earlier work of Jaffe [39] demonstrated the presence of DHT in the brain after injection of labelled testosterone. One hour after intravenous administration of [^{3}H]-testosterone, the hypothalamus and pituitary showed higher concentrations of DHT than did the cerebrum [25]. The hypothalamus and the hypophysis were able to convert testosterone to 5α-dihydrotestosterone [40–43]. These results, together with the presence of 5α-reductase in the hypothalamus and hypophysis [42], strongly suggest the existence of local metabolism of testosterone in the brain tissues.

In this context, it is interesting to learn whether the hypothalamus and the hypophysis contain receptors for DHT. Very recently, Kato and Onouchi have provided evidence for the existence of receptor molecules for DHT isolated from cytosols of rat

Table 1. Properties of estrogen receptors in the hypothalamus and hypophysis

	Sedimentation coefficient (S)	Dissociation constant (Kd)	Maximal binding capacity (mol per mg protein)	References
(Hypothalamus)				
Rat				
Cytosol				
Adult				
Female	8·4 ± 0·07 S	~ 10^{-9} M	~ 10^{-14}	Kato[1, 2, 3]
	8·4 ± 0·4 S (median emience)			Kato[1, 2, 3]
	Two peaks (No S values given)			Mowles *et al.*[4]
	8 S	4·41 × 10^{-10} M	1·72 × 10^{-14}	Korach & Muldoon[63]
Male	8 S			Kato *et al.*[5] Maurer[62]
	8 S	4·03 × 10^{-10} M	1·71 × 10^{-14}	Korach & Muldoon[63]
Immature				
Female	8 S, trace (7 days old)			Kato *et al.*[5]
	8 S (14 days old)			
	8·6 ± 0·46 S (21 days old)			
	8·6 ± 0·24 S (28 days old)			
	8·4 ± 0·24 S (35 days old)			
	4 S, 8 S			Plapinger & McEwen[51, 52]
	8 S	4·80 × 10^{-10} M	2·13 × 10^{-14}	Korach & Muldoon[63]
Male	8 S (21, 28 days old)	5·00 × 10^{-10} M	2·8 × 10^{-14}	Kato (this paper)
	8 S	6·37 × 10^{-10} M	1·34 × 10^{-14}	Korach & Muldoon[63]
Nuclear				
Adult				
Female	7 S			Mowles *et al.*[30]
	6 S			Kato[29]
Cow				
Cytosol	No S value given			Kahwanago *et al.*[27]
Rabbit				
Cytosol	No S value given			Chader & Villee[28]
Nuclear	No S value given			Chander & Villee[28]
(Hypophysis)				
Rat				
Cytosol				
Female	8 S			Kato *et al.*[8]
	8·6 ± 0·2 S	1·40 × 10^{-9} M	0·24 pmol	Notides[9]
	4·5 S			Mowles *et al.*[4]
	8 S		0·36 pmol	Korach & Muldoon[10]
	8 S (immature, 28-day-old)	~ 10^{-9} M	~ 10^{-14}	Kato *et al.* (unpublished)
Male	8 S			Kato *et al.* (unpublished)
	8 S		0·37 pmol	Korach & Muldoon[10]
Nuclear				
	6 S			Kato *et al.*[11]
	7 S			Mowles *et al.*[4] Anderson *et al.*[64]
Cow				
Cytosol	No S value given			Kahwanago *et al.*[6]

hypothalamus [44, 46] and hypophysis [45] by sucrose density gradient centrifugation. Ginsburg *et al.*[47] have also demonstrated the presence of DHT receptors in the brain tissues by the use of gel filtration.

1. *Hypothalamic cytosol 5α-dihydrotestosterone receptors*

The 105,000 *g* supernatant, cytosol, was obtained from hypothalami and hypophyses of 28-day-old and adult intact and castrated male rats, which were dissected out as previously described [3, 13]. The cytosol (0·29 ml) was incubated for 30 min or 1 h at 0–4°C with purified [1, 2-^{3}H]-5α-dihydrotestosterone (49 Ci/mmol, New England Nuclear Corp.) in the absence or in the presence of unlabelled steroid hormones. The mixture was then layered on 5–20% sucrose density gradient in 0·01 M Tris–HCl buffer (pH 7·4) containing 0·0015 M EDTA and 0·003 M 2-mercaptoethanol, and centrifuged in an ultracentrifuge. The optical absorbance at 280 nm of each fraction was measured. An appropriate volume of the cytosol fraction was also taken for the measurement of protein by the method of Lowry *et al.* Apparent sedimentation coefficients (S) were determined by the use of crystalline BSA, alcohol dehydrogenase from yeast, and beef liver catalase as standard substances according to the method of Martin and Ames. Radioactivity (0·2 ml aliquots) was measured in a scintillation counter in a Triton–toluene–PPO–POPOP system.

a. *Isolation and properites.* A representative sedimentation pattern of the hypothalamic cytosol from 28-day-old male rats following *in vitro* addition of [^{3}H]-DHT is shown in Fig. 1. There was a discrete peak of radioactivity in the 8·6 S region. In contrast, the cytosol from the cerebral cortical tissues incubated with labelled DHT did not show any distinct peak of radioactivity.

Radioactive materials collected from the peak of

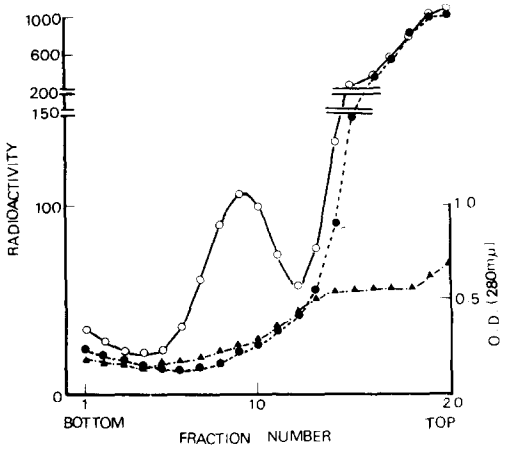

Fig. 1. Sucrose density gradient pattern of the cytosol from the hypothalamus or the cerebral cortex obtained from 28 day old male rats. The hypothalamic cytosol (0·29 ml containing 3·44 mg of protein) was incubated with [^{3}H]-5α-dihydrotestosterone ($8·8 \times 10^{-10}$ M) for 1 h at 0–4°. The cerebral cortical cytosol obtained from the homogenate of 0·5 g wet weight/ml of Tris-buffer was incubated with labelled 5α-dihydrotestosterone ($3·0 \times 10^{-10}$ M) for 1 h at 0–4°. —○—, Hypothalamic cytosol; —●—, cerebral cortical cytosol; —▲—, optical absorbance at 280 nm for the hypothalamic sytosol. Adapted from Kato and Onouchi[44].

the gradients of the hypothalamic cytosol were extracted with chloroform–methylene chloride and ether. The radioactive steroids were identified by thin layer chromatography on silica gel, developed by a mixture of acetone–benzene (1:4, v/v) or benzene–methanol (9:1, v/v) as previously described [13]. More than 90% of the radioactivity from the peak of the gradients of the hypothalamic cytosol was found to have the same R_f as standard DHT in the thin layer system utilized, indicating that virtually all of the radioactivity was unaltered DHT.

As shown in Fig. 2, the 8 S component became saturated with increasing concentrations of [^{3}H]-DHT. From analysis of the data on saturation kinetics by the Lineweaver–Burk plot, the dissociation constant and the number of binding sites of the hypothalamic binding component were estimated to be approximately $7·4 \times 10^{-10}$ M and $0·9 \times 10^{-14}$ mol/mg cytosol protein, respectively. These indicate that the component with a relatively small quantity of binding sites possesses a high affinity for DHT.

When the 8 S peak of labelled DHT of hypothalamic cytosols from 28-day-old male rats was collected and followed by treatment with high salt (0·4 M KCl), a binding peak was found in the 4 S region (Fig. 3). The conversion of 8 to 4 S components suggested a possible relationship between subunit and aggregate forms.

When the hypothalamic cytosol was incubated with [^{3}H]-DHT (7×10^{-10} M) in the presence of unlabelled steroid hormones (1×10^{-8} M), unlabelled DHT completely abolished the binding of [^{3}H]-DHT to the hypothalamic component but 5β-DHT did not. Other androgenic substances such as androstenedione, dehydroepiandrosterone and epiandrosterone or cortisol did not show any definite

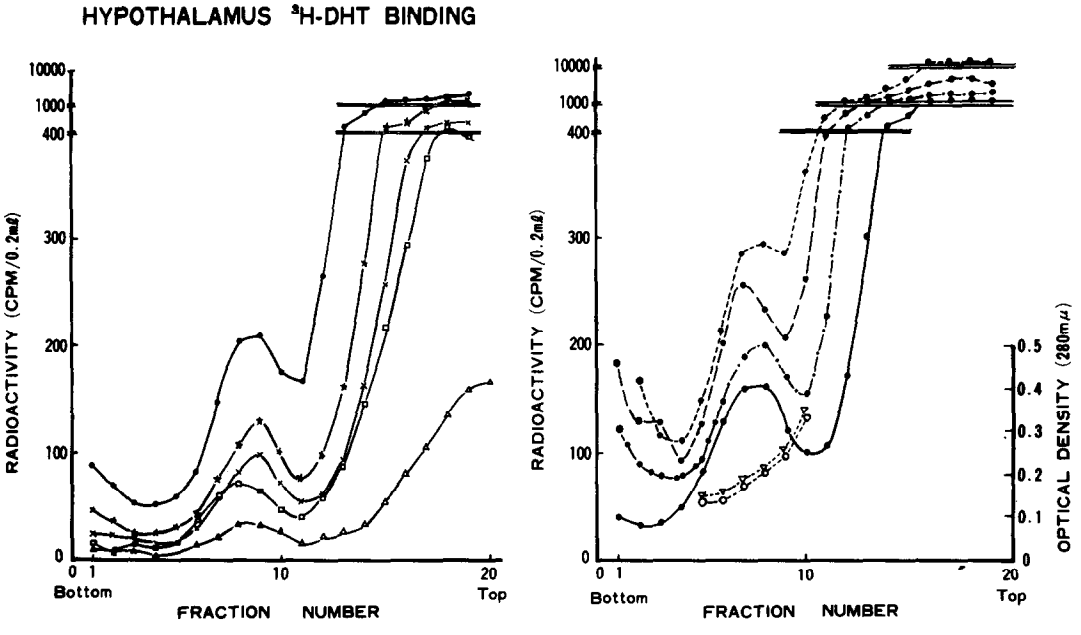

Fig. 2. Sucrose density gradient patterns of 28 day old male rat hypothalamic cytosol incubated *in vitro* with varying concentrations of [^{3}H]-5α-dihydrotestosterone. The cytosol (0·29 ml) containing 3·11 mg of protein and labelled 5α-dihydrotestosterone, was layered on 4·8 ml of 5–20% sucrose density gradient followed by centrifugation at 34,000 rev./min for 16 h in a RPS40T2-rotor in a Hitachi 65P ultracentrifuge. ●——●, $9·94 \times 10^{-9}$ M; ●—●, $5·04 \times 10^{-9}$ M; ●—●, $2·79 \times 10^{-9}$ M; ●——●, $1·51 \times 10^{-9}$ M on the left figure, $1·01 \times 10^{-9}$ M on the right figure; ✱——✱, $0·72 \times 10^{-9}$ M; ×——×, $0·31 \times 10^{-9}$ M; □——□, $0·19 \times 10^{-9}$ M; △——△, $0·09 \times 10^{-9}$ M; ○···○, OD_{280}nm for sample with $2·79 \times 10^{-9}$ M; ▽···▽, OD_{280}nm for sample with $0·09 \times 10^{-9}$ M.

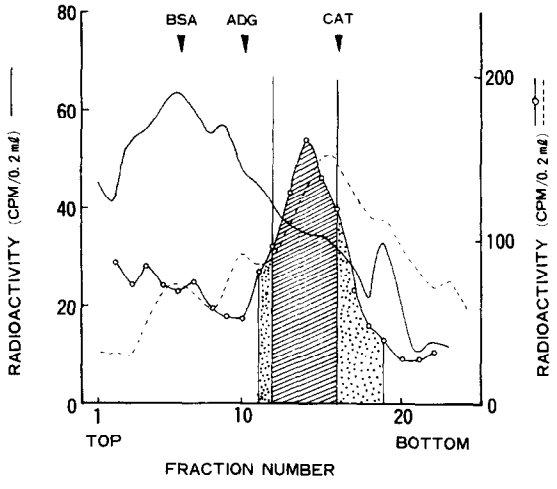

Fig. 3. Density gradient pattern of hypothalamic 4 S receptor labelled with [^{3}H]-DHT. The 8–9 S peak of labelled DHT of hypothalamic cytosols (—○—) from 28-day-old male rats were collected (/////) and concentrated, followed by incubation with [^{3}H]-DHT in a medium containing high salt (0·4 M KCl) (—).

effect on the [^{3}H]-DHT binding. Only 17β-estradiol, among estrogenic compounds such as estrone, estriol and diethylstilbestrol, decreased the binding of [^{3}H]-DHT in a competitive way (Fig. 4). Cyproterone, an anti-androgen, lowered the binding, but clomiphene, an antiestrogen, did not. On the sedimentation pattern obtained from [^{3}H]-testosterone (45 Ci/mmol, NEN, $0{\cdot}1 \times 10^{-9}$ M) or [^{3}H]-progesterone (35·5 Ci/mmol, NEN, $0{\cdot}3\text{–}5 \times 10^{-9}$ M) with the hypothalamic cytosol, there was no appreciable peak of radioactivity in the region of 8·6 S. Unexpectedly, unlabelled testosterone competed considerably and progesterone depressed the binding moderately. The androgen binding component from 28-day-old male rat hypothalamus seemed to have a specificity for DHT relative to other hormones.

On sedimentation patterns of hypothalamic cytosols of intact adult male rats, there was a peak of labelled DHT in the 8–9 S region, which became more evident by castration. Thus, endogenous androgen seems to occupy the binding sites of receptor molecules in the hypothalamus. Furthermore, even in very old castrated male rats, a single definite peak of 8–9 S was found.

These results suggest that the cytosol of the hypothalamus of male rats, immature or mature, posesses a macromolecular component, receptor, capable of binding 5α-DHT with a high affinity. This is consistent with the uptake data which have shown a predilection for androgen in the hypothalamus and with the finding that testosterone is converted to 5α-DHT, an active form of androgen, in the hypothalamus. It is possible that this 5α-DHT receptor is related to the mechanism of action of the hormone on the brain in male rats.

b. *Possible universal existence of DHT receptors in the hypothalamus of various animals.* The DHT binding components with sedimentation coefficients of 8–9 S were also detected by sucrose density gradient centrifugation from hypothalamic cytosols of male mice, hamsters, guinea-pigs and rabbits. The dissociation constant and the number of binding sites of the hypothalamic components for these animals were in the same order as those for male rats. In addition, intact and castrated male cat hypothalamus seemed to contain a DHT-binding receptor of 8–9 S.

From these results, the hypothalamic cytosols from various animals seem to contain an androgen receptor which resembles DHT receptor in the rat hypothalamic cytosol. This indicates a possible universal existence of receptor molecules for DHT in the hypothalamus. The biological significance of hypothalamic receptors, thus, might be more generalized in the mechanism of action of androgens on the brain.

c. *Intracerebral localization of DHT receptors.* An attempt was made to isolate DHT receptors by sucrose density gradient centrifugation from hypothalamic cytosols labelled *in vitro* with [^{3}H]-5α-DHT or [^{3}H]-estradiol obtained from 28-day-old rats in order to compare the distribution of the respective receptors in the hypothalamus. The preoptic-anterior hypothalamic region, the middle hypothalamus and the posterior hypothalamus, the median eminence and the amygdaloid complexes and other brain tissues were dissected, as previously described [3, 13]. The cytosols were incubated *in vitro* with [^{3}H]-5α-DHT for 1 h at 0°C [44] or with [^{3}H]-estradiol for 30 min at 0°C [5].

Representative sedimentation patterns of hypothalamic cytosol incubated with [^{3}H]-DHT is shown in Fig. 5. Total radioactive DHT bound (TDb) in

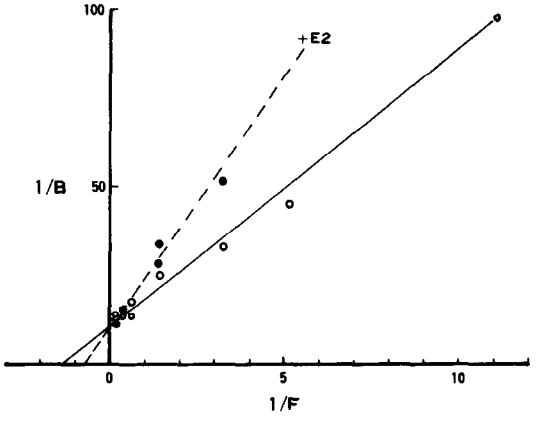

Fig. 4. The Lineweaver–Burk plot for the determination of the dissociation constant (Kd) and the number of binding sites (NBS) of DHT binding component of hypothalamic cytosol from 28-day-old male rats in the absence (–○–) or presence (–●–) of unlabelled 17β-estradiol (10^{-9} M). The hypothalamic cytosol (0·29 ml containing 3·10 mg protein) was incubated with various concentrations of [1, 2-^{3}H]-5α-dihydrotestosterone ($0{\cdot}09\text{–}9{\cdot}94 \times 10^{-9}$ M). It is noted that both curves have the same Y intercept.

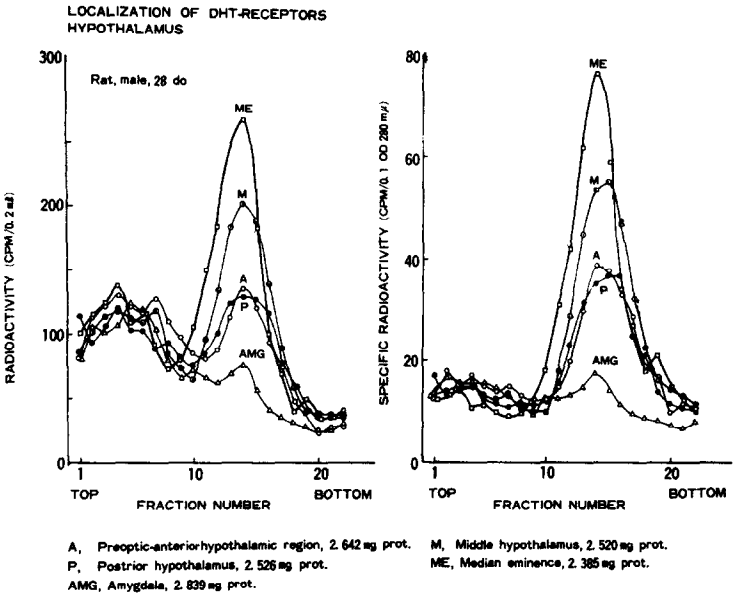

Fig. 5. Density gradient sedimentation patterns of the cytosol from the preoptic-anterior hypothalamus, the middle hypothalamus, the posterior hypothalamus, the median eminence and the amygdaloid complexes of 28-day-old male rats. The cytosol (0·29 ml containing 2·39–2·64 mg protein) was incubated *in vitro* with [^{3}H]-DHT for 1 h at 0°C. Details are given in the text.

the 8–9 S in the various parts of the hypothalamus of male rats and the specific radioactivity were in the following decreasing order; the middle hypothalamus > the posterior hypothalamus > the anterior hypothalamus. The median eminence contained the highest concentration of DHT receptors. A small but definite peak of labelled DHT was found in the amygdaloid complexes. In sharp constrast, estradiol receptors in the hypothalamus of 28-day-old female rats were in the following order; the preoptic-anterior hypothalamus > middle hypothalamus > posterior hypothalamus, which is almost the same as that in adult overiectomized rat hypothalamus [3].

Androgen (DHT) receptors tend to concentrate in the median eminence and the middle hypothalamic region with their wider distribution in the whole hypothalamus, in contrast with the highest concentration of estrogen receptors in the preoptic-anterior hypothalamus and the median eminence. It is interesting that the androgen receptors, in small but definite amounts, are present in the amygdaloid complexes.

d. *Development of androgen (DHT) receptors in the hypothalamus.* The hypothalamic estradiol receptors increase gradually after birth in female rats, then rapidly between 14 and 21 days of age, and reach a maximum at 28 days of age. Receptor "availability" is variable during pubescence. Some role of a "matured" hypothalamic estrogen receptor in the mechanism of the onset of puberty is suggested [5, 48–52].

A differential uptake mechanism on testosterone binding in the CNS has been demonstrated in the adult and postnatal developing female rats [53]. It has been claimed that high affinity and low binding capacity "classical" testosterone receptors are not present in the hypothalamus of the very young female rat [54–56]. Little is known, however, about the development of androgen receptors in the hypothalamus at postnatal stages. The sedimentation profiles of the cytosol fractions from the hypothalami of male rats at 7, 14, 21 and 28 days of age are illustrated in Fig. 6. In the very young rat (7 days

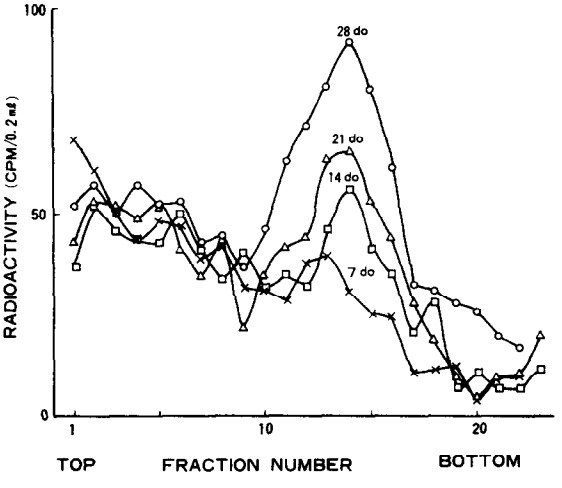

Fig. 6. Representative sedimentation profiles in sucrose density gradient of hypothalamic cytosol from male rats at 7, 14, 21 or 28 days of age. The cytosol was incubated with [^{3}H]-DHT. It is noted that androgen (DHT) receptors gradually develop in the hypothalamus of male rats at successive stages after birth.

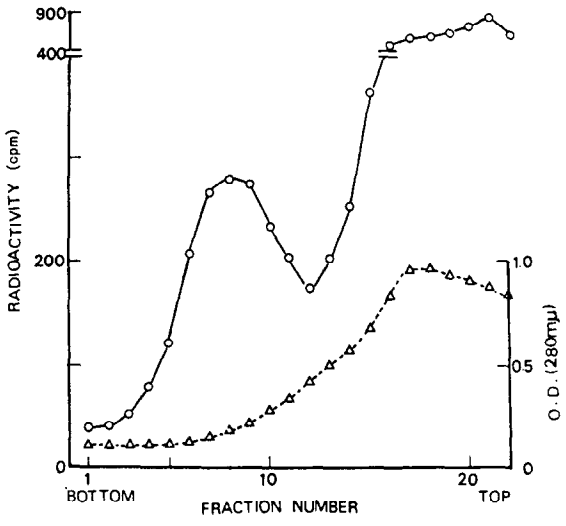

Fig. 7. Sucrose density gradient pattern of the cytosol from the hypophyses from 28 day old male rats. The hypophyseal cytosol (0·29 ml containing 2·28 mg of protein) was incubated with [^{3}H]-5α-dihydrotestosterone ($7·2 \times 10^{-10}$ M) for 1 h at 0–4°. —○—, the hypophyseal cytosol; —△—, optical absorbance at 280 nm for the hypophyseal cytosol. Adapted from Kato and Onouchi[45].

of age) there was a very small peak of labelled androgen in the 8–9 S region. At 14 days of age, a definite peak of 8–9 S appeared. The hypothalami of 21-day-old rats showed a single more definite peak of radioactivity in the same region; its sedimentation pattern was similar to that at 28 days of age in which labelled DHT binds the receptors strongly.

These results demonstrate that the androgen (DHT) receptors can be isolated from hypothalamic cytosols of developing male rats. They further indicate gradual development of receptors for androgens, possibly DHT, in the hypothalamus of male rats at successive stages after birth.

2. *Hypophyseal cytosol DHT receptors* [45]

a. *Isolation and properies.* A representative sedimentation pattern of hypophyseal cytosol incubated *in vitro* with [^{3}H]-5α-DHT is shown in Fig. 7. There was a discrete peak of radioactivity in the 8 S region. More than 86% of the radioactive material from the peak of the gradients of the hypophyseal cytosol was found in the area corresponding to standard DHT in the thin layer system utilized, indicating that virtually all of the radioactivity was unaltered 5α-DHT. On analysis of the data on saturation kinetics by the Lineweaver–Burk plot, the dissociation constant and the number of binding sites of the hypophyseal binding component were estimated to be approximately $1·9–2·4 \times 10^{-9}$ M and $2·4–5·3 \times 10^{-14}$ mol/mg cytosol protein, respectively. These results indicate that the hypophyseal component with a small number of binding sites possesses a high affinity for DHT.

When the hypophyseal cytosol was incubated with [^{3}H]-5α-DHT ($4·3–7·2 \times 10^{-10}$ M) in the presence of unlabelled steroid hormones (10^{-8} M), unlabelled 5α-DHT completely abolished the binding of [^{3}H]-5α-DHT to the hypophyseal component but its inactive isomer, 5β-DHT, did not. Testosterone and progesterone competed to lesser extent. Other androgenic substances such as androstenedione, dehydroepiandrosterone and epiandrosterone, however, did not show any definite effect on the [^{3}H]-5α-DHT binding. Neither cortisol nor diethylstilbestrol showed an effect. The androgen binding component, therefore, seemed to have a specificity for 5α-DHT relative to other steroid hormones. These results suggest that the cytosol of the hypophysis of male rats

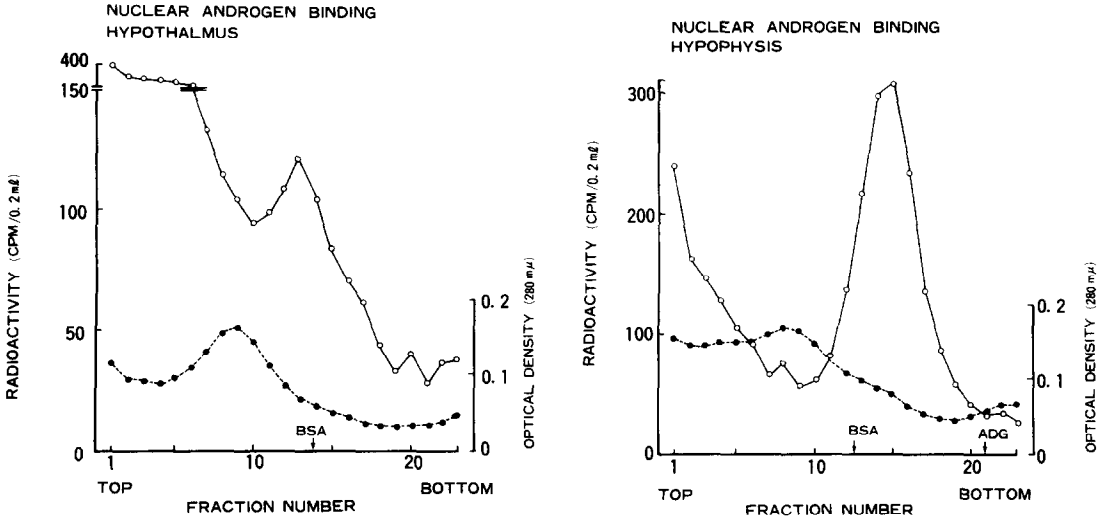

Fig. 8. Representative density gradient sedimentation pattern of KCl extractable DHT receptor molecules from purified nuclear pellet from hypothalami or adenohypophyses of castrated adult male rats. The slices were *in vitro* with [^{3}H]-DHT for 30 min. at 37°C.

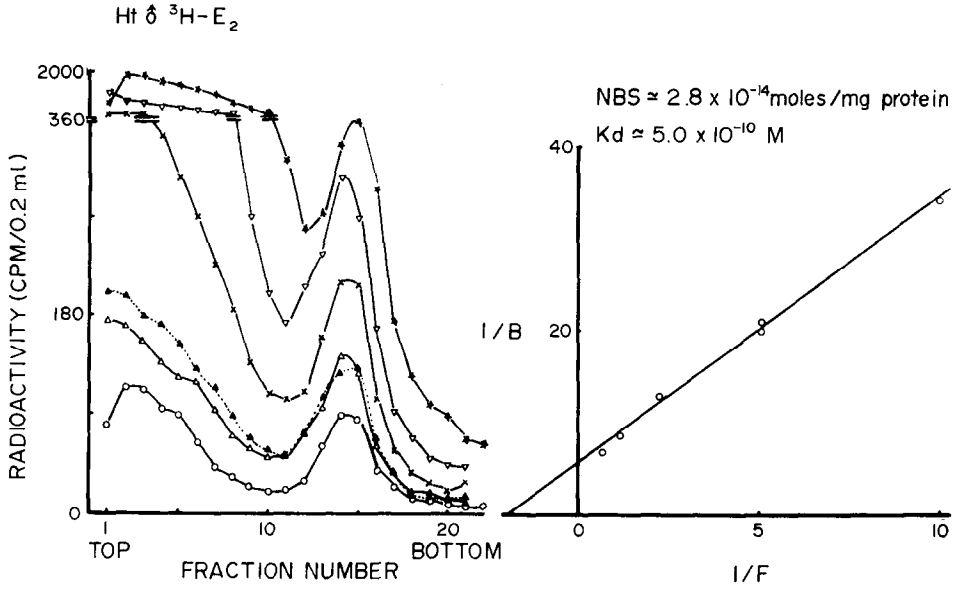

Fig. 9. Density gradient sedimentation pattern of hypothalamic cytosol from 28-day-old male rats labelled *in vitro* with [^{3}H]-estradiol. The cytosol was incubated with [^{3}H]-estradiol for 30 min. at 0°C. It is noted that estradiol receptors are abundant in the hypothalamus of male rats.

possesses a macromolecular component, receptor, capable of binding DHT. It is possible that this DHT receptor is related to a direct action of androgen on the hypophysis in male animals.

3. NUCLEAR DHT RECEPTOR COMPLEXES IN THE HYPOTHALAMUS AND THE ANTERIOR HYPOPHYSIS

Hypothalamic and anterior hypophyseal slices collected from 30 to 50 castrated male rats were incubated with [^{3}H]-DHT at 37°C for 30 min, and the purified nuclear pellet was extracted with 0·4 M KCl. Androgen–receptor complexes of 4–6 S could be detected from the hypothalamus and the anterior hypophysis, but no nuclear receptor complexes were formed in liver and cerebral cortical slices. Representative sedimentation profiles of nuclear androgen receptor complexes in the hypothalamus and hypophysis are shown in Fig. 8. Nuclear complexes were formed at 37°C, but not at 0°C. Formation of neonuclear androgen-receptor complex(es) seemed to be temperature-dependent. Thus, in the hypothalamo-hypophyseal unit, androgen may act through its interaction with DHT receptors in a way similar to that of estrogen [1, 2, 8, 11, 15].

According to Whalen and Rezek[57], 0·5 h after the administration of [^{3}H]-testosterone into castrated male adult rats, testosterone, DHT and androstenedione were found in the anterior hypothalamus and posterior hypothalamus. These results, together with the findings on the ability of the brain to metabolize testosterone to DHT and the presence of 5α-reductase in the hypothalamus and hypophysis [39–43], strongly suggested the existence of local metabolism of testosterone in these brain tissues.

Since cytosol and nuclear DHT receptor molecules can be isolated from the hypothalamus and hypophysis, it is tempting to speculate that DHT metabolized locally and/or taken up from blood can interact with the DHT receptors in the hypothalamus and hypophysis, which may play a role in the mechanism of action of androgen on the CNS (44–46].

4. ANDROGEN- AND ESTROGEN RECEPTORS AND THEIR RELATION TO ANDROGEN FEEDBACK ACTION

Naftolin *et al.*[58–60] have demonstrated that the hypothalamus has an ability to aromatize androgen to estrogens. Testosterone and androstenedione are metabolized in the hypothalamus to estradiol and estrone, respectively [61]. It has been proposed that androgen effects may be mediated *via* aromatized products such as estrogens.

In this context, it is interesting that estrogen receptors are abundant in the hypothalamus of male rats in an amount comparable to that in female rats (Fig. 9). Estrogen aromatized in the hypothalamus can be "picked up" by high-affinity estrogen receptor molecules. In addition to the possible role of DHT

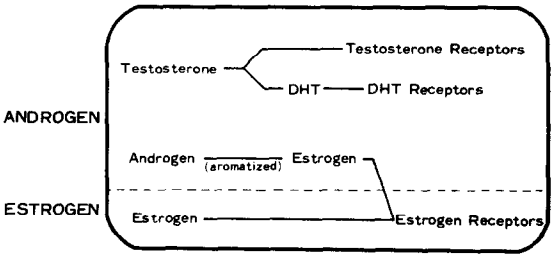

Fig. 10. Possible routes of the feedback action of androgens and estrogens on the brain of males.

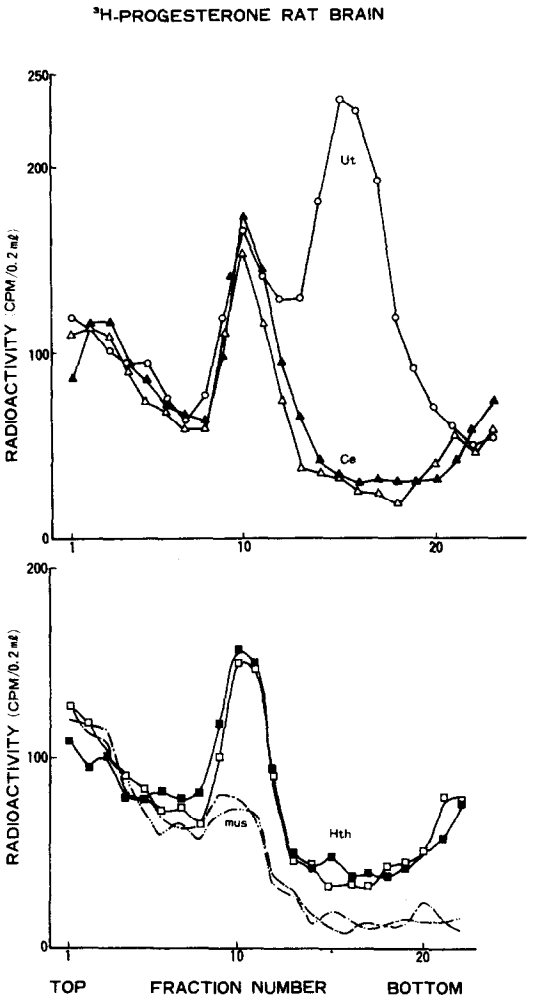

Fig. 11. Sucrose density gradient patterns of hypothalamic or uterine cytosols labelled with [^{3}H]-progesterone from ovariectomized estrogen-primed rats. The cytosol was incubated for 2 h at 0°C with [^{3}H]-progesterone in the absence (○,△,□,—·) or the presence (▲,■,—··) of progestational compounds.

and testosterone receptors in the mechanism of feedback action of androgen on the CNS, it is possible that estrogen receptors in the hypothalamus are the basis of androgen effects *via* the central aromatizing ability. Receptors for androgens and estrogens in males and their possible role in the mechanism of androgen feedback action on the brain are illustrated in Fig. 10. Which is actually the most important of the possible routes of the feedback action of the steroid hormone, however, needs further study.

5. PROGESTERONE BINDING RECEPTORS IN RAT HYPOTHALAMUS

Although progesterone receptors have been well demonstrated in the peripheral target organs, conflicting data still exist on progesterone receptors in the brain. Seiki and Hattori[65] and Iramain *et al.*[66] claimed the presence of progesterone receptors in the hypothalamus of rats and guinea-pigs, but very recently Etgers *et al.*[67] reported the absence of the receptors in the hypothalamus of castrated estrogen-primed guinea-pigs. Cytosols obtained from the hypothalamus or the uterus of castrated estradiol-primed rats were incubated with [^{3}H]-progesterone and ultracentrifuged through a sucrose gradient. As shown in Fig. 11, there was no specific binding in the hypothalamus in the 7 S region, in sharp contrast with the presence of a large 7 S peak in the uterus. The 4 S binding seemed to be non-specific, because no inhibition was found by progestational compounds. This suggests the absence of progesterone receptors or, if any, a very low concentration in the hypothalamus of rats. Confirmation of the absence or the presence of progesterone receptors in the hypothalamus is very important for elucidation of the mechanism of its central action. The present situation for steroid hormone receptors in the brain is summarized in Table 2.

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Table 2. Steroid hormones receptor in the brain

	Estradiol	Progesterone	Testosterone	Corticosterone
Specific uptake	Yes	Possibly yes	Yes	Yes
Convertion to metabolites	No	Possibly yes	Converted to DHT* estrogens	—
Receptor(s) isolated	Present	Possibly present, but with inconsistent data	Receptors for DHT and testosterone	Present
Specific region of the highest concentration	Preoptic-anterior hypothalamus, median eminence and anterior hypophysis		Hypothalamus and hypophysis	Hippocampus

* DHT, 5α-dihydrotestosterone.

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