Sex-specific Occurrence of Androgen Receptors in Rat Brain*

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The metabolism and binding of [1,2,6,7-\textsuperscript{3}H]testosterone in male and female rat brain has been studied in an attempt to find an explanation for the relative androgen unresponsiveness characterizing the female hypothalamo-pituitary axis involved in regulation of hepatic steroid metabolism.

The most significant sex differences in the pattern of [\textsuperscript{3}H]testosterone metabolites recovered from several brain regions (including pituitary, pineal gland, and hypothalamus) after intraperitoneal administration of [\textsuperscript{3}H]testosterone were the predominance of testosterone and androstenedione in male brain compared to the quantitative importance of 5a-androstane-3a,17\beta-diol, 5a-androstane-3\beta,17\beta-diol, epitestosterone, and dihydroepitestosterone in female brain. One possible explanation for the androgen unresponsiveness of female rats is, therefore, the faster metabolism of testosterone to inactive compounds in female brain.

Experiments both in vivo and in vitro showed the presence of high affinity, low capacity binding sites for [\textsuperscript{3}H]testosterone in male pituitary, pineal gland, and hypothalamus (K\textsubscript{d} values in the region of $1 \times 10^{-10}$ to $1 \times 10^{-11}$ M and number of binding sites 1.0 to 1.4 x 10^{-10} mol per mg of protein). The steroid-macromolecular complexes generally had a pI of 5.1, were excluded from Sephadex G-200, were heat-labile, and were sensitive to protease. Competition experiments indicated the following order of ligand affinities: testosterone > 5a-dihydrotestosterone and estradiol > androstenedione >> corticosterone. No steroid-binding proteins of similar nature were found in pituitary, pineal gland, or hypothalamus from female rats. On the basis of these results it is suggested that the androgen unresponsiveness of female rats referred to above relates to the absence of receptor protein for androgens in female rat brain.

In support of this hypothesis, 2% day-old female rats, which are known to be affected by androgens with regard to liver enzyme activities, were shown to contain receptor proteins for androgen in the brain.

In conclusion, the relative androgen unresponsiveness of the female hypothalamo-pituitary axis is probably explained by the absence of receptor proteins for androgen in female hypothalamus and pituitary. The fast metabolism of testosterone in female rat brain also serves to decrease the availability of active androgen to potential receptor sites. It may be speculated that the presence of androgen receptors in male brain is the result of neonatal programming ("imprinting") by testicular androgen.

Administration of androgen to castrated rats results in significant changes in activities of hepatic enzymes active on steroid hormones (1, 2). In these experiments, male rats respond much better to androgen than female rats, and it has been shown that the greater androgen responsiveness in male rats is due to neonatal imprinting by androgens (3). Recently, results in our own laboratory have shown that an intact hypothalamo-pituitary axis is necessary for androgen regulation of hepatic enzyme activities (4, 5), and it seems reasonable to believe that androgen action on liver enzymes is mediated via central mechanisms. In view of this, it appears adequate to search for the explanation for the relative androgen unresponsiveness of female rats, as regards liver enzymes, in a sex difference in androgen action in brain. Information on this point in literature is relatively scanty and confusing. Whereas some authors deny the existence of specific binding sites for androgen in rat brain (6-8), other groups have described the occurrence of androgen receptors in pituitary (9-11), hypothalamus (9, 11), pineal gland (12), and cortex (11) of male rats. Furthermore, diverging opinions exist on the nature of the active metabolites of testosterone in rat brain, and both 5a-dihydrotestosterone (10, 11, 13) and estrogens (14) have been suggested as mediators of androgen action.

Considering the present confusion in the field of mechanism of action of androgens in the brain we have undertaken a combined in vivo and in vitro study aimed at finding an explanation for the relative androgen unresponsiveness in female rats. In view of the uncertainty as to the nature of the androgen metabolites present in brain tissue we have characterized the metabolites of intraperitonally administered [1,2,6,7-\textsuperscript{3}H]testosterone in several brain regions showing androgen uptake. Furthermore, isoelectric focusing has been used...
as a sensitive method to detect androgen-binding proteins in cytosol from some brain regions.

**MATERIALS AND METHODS**

Steroids—[1,2,6,7-3H]Testosterone (specific radioactivity, 84 Ci per mmol) and [1,2,6,7-3H]Androstenedione (specific radioactivity, 83 Ci per mmol) were purchased from the Radiochemical Centre, Amersham, England, and [1,2-3H]Epitestosterone (specific radioactivity, 30 Ci per mmol) from New England Nuclear, Boston, Mass., unlabeled testosterone, 5α-dihydrotestosterone, androstenedione, epitestosterone, 5α androstan-3α,17β-diol, 5α androstan-3β,17β-diol, progesterone, one, estradiol, and corticosterone were generously supplied by Dr. J. Babcock (Upjohn Co., Kalamazoo, Mich.).

5α-[3H]Androstan-3β-17-dione was biosynthesized from [1,2,6,7-3H]Androstenedione by incubation with liver nuclei from female rats (15). Sodium borohydride reduction of 5α-[3H]Androstan-3β,17-dione yielded a mixture of 5α-[3H]Dihydrotestosterone and 5α-[3H]Dihydroepitestosterone; this mixture was resolved by radiogas chromatography of the silylated products (see below).

Animal Experiments—Sprague-Dawley rats, 8 weeks old, were used in all experiments. When castrated rats were used, the operation was performed under ether anesthesia 14 to 16 h before the experiment.

In one series of experiments castrated male and female rats were given an intraperitoneal injection of 250 μCi of [1,2,6,7-3H]Testosterone in 120 μl of acetone. Thirty minutes after the animals were anesthetized, the brain perfused, taken out, and dissected. The thalamus was taken out, limited by the hippocampus lateral to the midline. The midbrain was removed by a transection through the pyriform lobe at the same level as the caudal border of the mammillary bodies. A similar incision was made half-way between the thalamus chorioides and sulcus rhinalis running parallel to these grooves. The brain was then divided into two parts by a sagittal section through the midline. Each of the halves were transected according to Fig. 1. The section in front of the genu of the corpus callosum and the section through the thrid ventricle and the anterior commissure left a slice from which a piece of the cerebral cortex was taken. The septal area together with the nucleus tractus diagonalis were separated from the rest of this slice by the corpus callosum, the lateral ventricle, and a section from the lateral ventricle to a point at the inferior surface 2 mm lateral to the midline. The midbrain was removed by a transection from behind the mammillary body to in front of the anterior collicle. A horizontal section separated the thalamus from the preoptic area, so that the latter region could be taken out bounded laterally by the fissa chorioides. The amygdala and the overlying cortex were removed by a horizontal section through the sulcus rhinalis. They were separated from each other and from caudal regions by the incisions made earlier. The thalamus was taken out, limited by the hippocampus and the internal capsule. The hippocampus was loosened and removed.

The wet weights of the brain specimens were determined, and the samples were combusted in a tissue combustion device (Oxymat, Intertechnique, Plaisir, France) prior to measurement of radioactivity with the use of a Packard liquid scintillation spectrometer model 2420.

Calculation of radioactivity in disintegrations per min was performed under ether anesthesia 14 to 16 h before the experiment. When castrated rats were used, the operation was performed under ether anesthesia 14 to 16 h before the experiment.

**Identification of Radioactive Metabolites**—In a second series of experiments, four to six castrated male and female rats were each given 250 μCi of [1,2,6,7-3H]Testosterone as described above and anesthetized 30 min later. Blood was collected by the ophthalmic venous plexus method (16). The brain was perfused, taken out, and dissected as described above. Brain specimens from one rat were pooled with corresponding specimens from other rats of the same sex. Ten volumes of acetone/ethanol, 1/1 (v/v) were added to each pool of samples, the tissue was homogenized, and the homogenate was kept in a shaking water bath at 37° overnight. The homogenate was centrifuged at 20,000 × g, and the supernatant was evaporated to dryness in vacuo. The residue was dissolved in distilled water and passed through a 10-g XAD-2 column as previously described (17). Aliquots of the methanol eluate from the column were taken for measurement of radioactivity and for identification of radioactive metabolites by thin layer and radiogas chromatography. This type of experiment was performed three times with each sex.

Thin layer chromatography was performed on precoated silica gel plates (250 μm, Merck AG, Darmstadt, West Germany). Chromatography in the solvent system ethyl acetate/96% (v/v) ethanol/15 mM ammonium hydroxide, 30 μl (v/v), which separates free steroids, steroid monosulfates, disulfates, and glucuronides (18), and subsequent scanning of the thin layer plates for radioactivity with a Berthold thin layer scanner model H (Berthold, Wildbad, West Germany) revealed that practically all radioactivity chromatographed like unconjugated steroids. Chromatography in the solvent system ethyl acetate/chloroform, 1/4 (v/v), separated [3H]Testosterone and its metabolites. External radioactivity reference steroids were chromatographed on the same chromatoplates as the biological samples. The relative amounts of radioactive steroid metabolites were measured from scanner and radiogas chromatograms.

Radogas chromatography of (trimethyl)silylated samples was performed on a Hewlett-Packard model 402 gas chromatograph equipped with a Barber-Colman radioactivity monitoring system model 5190. The stationary phases used were 1.5% SE-30 and 1% OV-17.

A steroid was considered identified if it had the same thin layer chromatographic mobility and the same retention time, relative to testosterone on SE-50 and on OV-17, as the reference steroid.
cases, aliquots of the void volume were analyzed by isoelectric focusing. Dissociation constants and number of binding sites for androgen receptor proteins were determined according to Scatchard (21). Specific binding was measured as the difference between binding in the presence and absence of a 100-fold excess of unlabeled steroid (nonspecific and total binding, respectively). Addition of unlabeled steroid generally resulted in total displacement of the protein-bound radioactivity. Incubation at 0–4°C resulted in significantly lower specific steroid binding. The ligand specificity of the androgen receptors was investigated in vitro by incubating cytosol with [1,2,6,7-3H]testosterone in a concentration equal to Km, plus a competing unlabeled steroid in a concentration about 100 times that of [1,2,6,7-3H]testosterone.

**Protein Binding of Androgen in Rat Blood**—In some experiments, blood was collected from castrated male and female rats 30 min after intraperitoneal injection of 250 μCi of [1,2,6,7-3H]testosterone. An aliquot of aortic serum was diluted with TKR buffer and chromatographed on a Sephadex G-25 column. Binding studies were also carried out in vitro with serum from male and female rats under the same experimental conditions as were used in binding studies with cytosol preparations from brain.

**Formation of 17α-Hydroxy-Δ4 Steroids in Rats**—In order to investigate the occurrence of 17α-hydroxysteroid reductase activity in the rat, 20% (w/v) homogenates in Eagle’s medium of the following tissues were prepared from male and female rats: brain, pancreas, adrenals, liver, skeletal muscle, spleen, kidneys, submaxillary glands, lungs, skin, and blood. The homogenates, 10 ml, were incubated without added cofactors for 30 min at 37°C with 1 μCi of [1,2,6,7-3H]androstenedione diluted with unlabeled steroid to give final substrate concentrations of 10⁻¹, 10⁻², or 10⁻³ M. The incubations were terminated by the addition of 40 ml of chloroform/methanol, 2/1 (v/v). The mixture was shaken well and was allowed to stand for 12 to 16 h. After centrifugation, the supernant was taken off, reduced to dryness, and analyzed by thin layer and radiographs chromatography.

**RESULTS**

### Uptake of Androgen in Different Regions of Brain—Table I shows the average retention of radioactivity found in various regions of the brain after administration of [1,2,6,7-3H]testosterone to castrated male and female rats. The highest uptake of radioactivity was found in the pineal gland and in the pituitary. Other regions with a relatively high uptake were the septal area, pons plus medulla oblongata, and the hypothalamus.

#### Characterization of Metabolites of [1,2,6,7-3H]Testosterone in Brain of Male and Female Rats—Fig. 2 summarizes the identifications and relative amounts of metabolites of [1,2,6,7-3H]testosterone in pituitary, pineal gland, pons plus medulla oblongata, hypothalamus, midbrain, septal area plus nucleus tractus diagonalis, amygdala, cortex, thalamus, cerebellum, hippocampus, and blood. Fig. 3 shows representative radiographs chromatograms. In most regions of male rat brain, unmetabolized testosterone constituted the major radioactive compound. Another metabolite of great quantitative importance in male brain was androstenedione and in the pituitary, pineal gland, and hypothalamus, testosterone and androstenedione made up more than 80% of the total radioactivity. Small amounts of 5α-dihydrotestosterone, 5α-androstane-3α,17β-diol, and 5α-androstane-3β,17β-diol were also identified in male rat brain.

The pattern of [1,2,6,7-3H]testosterone metabolites in female rat brain was quite different from that of male. Thus, testosterone and androstenedione were of much smaller quantitative importance whereas 5α-androstane-3α,17β-diol and 5α-androstane-3β,17β-diol occurred in relatively large amounts. The most striking difference, however, was the sex-specific presence of epitestosterone and 5α-dihydroepitestosterone in female rat brain. These metabolites were present in practically all regions of the brain and constituted 20 to 40% of the total radioactivity. The experiments aimed at determining the site of 17α-hydroxysteroid reductase in the rat showed formation of epistosterone one from androstenedione only in male liver and female brain. Despite intensive search no evidence was found for the presence of estrone or estradiol in either male or female rat brain.

The findings described prompted us to investigate the occurrence and nature of receptor proteins for androgens in male and female rat brain. The pituitary, pineal gland, and hypothalamus were selected for further studies since these organs displayed a relatively high uptake of androgen and since they showed marked sex differences in their patterns of radioactive metabolites.

#### Protein Binding of [1,2,6,7-3H]Testosterone in Pituitary In Vivo and In Vitro—Isoelectric focusing of the void volume from Sephadex G-25 chromatography of pituitary cytosol from castrated male rats given [1,2,6,7-3H]testosterone 30 min prior to death yielded a reproducible radioactive peak with a pl of 5.1 (Fig. 4). Chromatography of the cytosol on Sephadex G 200 revealed that the labeled steroid-macromolecular complex was excluded from the column. Analysis of the radioactivity in the void volume of the Sephadex G-25 and G-200 columns showed in both cases that [3H]testosterone made up more than 95% of the radioactivity.

The optimal conditions for labeling of macromolecules in the pituitary cytosol with [1,2,6,7-3H]testosterone were found to be incubation for 30 min at 37°C. Incubation for longer times than 30 min did not increase the binding of [1,2,6,7-3H]testosterone to protein, as judged by chromatography on Sephadex G-25. Under the incubation conditions used, less than 20% of the incubated [1,2,6,7-3H]testosterone was metabolized; the radioactivity eluted in the void volume during Sephadex G-25 chromatography consisted exclusively of [3H]testosterone. Analysis of the incubation results according to Scatchard (21) indicated the presence of a high-affinity low-capacity testosterone-binding macromolecule in pituitary cytosol (Fig. 5). The Kd with [1,2,6,7-3H]testosterone as ligand was calculated to be 0.9 × 10⁻¹⁰ mol per mg of protein. When the in vitro-labeled [3H]testosterone-macromolecular complex was analyzed by isoelectric focusing,
one single radioactive peak (pI 5.1) was seen. Displacement experiments where the effect of various unlabeled steroids on high affinity binding of [1,2,6,7-\textsuperscript{3}H]testosterone to pituitary cytosol was investigated (Table II) showed that corticosterone did not compete for the binding sites whereas androstenedione, 5\alpha-DHAT, and estradiol competed relatively efficiently, although not to the same degree as unlabeled testosterone. The competition experiment with 5\alpha-DHAT indicates that the binding site on the macromolecule binds testosterone with somewhat higher affinity than 5\alpha-DHAT.

The [\textsuperscript{3}H]testosterone·macromolecular complex formed in vitro by incubation of male pituitary cytosol was unstable to heat and showed a rapid dissociation at 50\degree (Table III). Furthermore, the complex was sensitive to treatment with protease. These results indicate that the testosterone-binding macromolecule in male pituitary cytosol is a protein.

When pituitary cytosol from castrated female rats given [1,2,6,7-\textsuperscript{3}H]testosterone 30 min before death was analyzed by gel filtration on Sephadex G-25, the void volume always contained less than 20\% of radioactivity per mg of protein when compared to the corresponding fraction from male pituitary cytosol. Isoelectric focusing of the macromolecular-bound fraction of female pituitary cytosol did not show any focused radioactive peak. When cytosol from female pituitaries were incubated with [1,2,6,7-\textsuperscript{3}H]testosterone under the same conditions as in incubations with male preparations, only small amounts of radioactivity bound to macromolecules (corresponding to about 10\% of the amount, calculated per mg of protein, normally bound in incubations with male cytosol). Furthermore, it was not possible to displace the radioactivity bound by adding excess amounts of unlabeled testosterone to

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### Table

| SEX | PITUITARY | PINEAL GLAND | PONS + MEDULLA OBLONGATA | HYPOTHALAMUS |
|-----|-----------|--------------|---------------------------|-------------|
| A   | T         | 5\alpha 3\alpha | T 5\alpha 3\alpha         | T           |
| T   | 3\beta 5\beta | 3\beta 5\beta | T 5\alpha 3\alpha         | T           |
| 5\alpha-DHAT | 3\alpha-17\beta-diol | 3\beta, 5\alpha-3\beta,17\beta-diol | ET, epitestosterone, 5\alpha-ET, 5\alpha-DHAT-epitestosterone | T 5\alpha 3\alpha |

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**Fig. 2.** Relative distribution of metabolites of [1,2,6,7-\textsuperscript{3}H]testosterone in different regions of the brain of castrated male and female rats. A, androstenedione; T, testosterone; 5\alpha, 5\alpha-DHAT; 3\alpha, 3\alpha-17\beta-diol; 3\beta, 3\beta-17\beta-diol; ET, epitestosterone; 5\alpha-ET; 5\alpha-DHAT-epitestosterone. Three sets of experiments were run. The dark portion of the columns represents the lowest value and the dark plus light portion the highest value found.
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FIG. 3. Radiogas chromatograms of extracts from the pituitary of castrated male (a) and female (b) rats given 250 µCi of [1,2,6,7-3H]testosterone 30 min before death. A, androstenedione; T, testosterone; 3α, 5α-androstane-3α,17β-diol; 3β, 5α-androstane-3β,17β-diol; ET, epitestosterone; 5αET, 5α-dihydroepitestosterone.

FIG. 4. Isoelectric focusing of cytosol from pituitaries of castrated male rats given 250 µCi of [1,2,6,7-3H]testosterone 30 min before death. The cytosol was chromatographed on a Sephadex G-25 column prior to electrofocusing. Each fraction contained 0.3 ml. •—•, radioactivity; x—x, pH.

the incubations, indicating that no high affinity, low capacity binding of [3H]testosterone takes place in female pituitary cytosol.

Protein Binding of [1,2,6,7-3H]Testosterone in Pinal Gland In Vivo and In Vitro—Analysis of cytosol of male pineal gland labeled in vivo or in vitro with [1,2,6,7-3H]testosterone by isoelectric focusing showed a homogeneous radioactive peak with a pI of 5.1. This peak exclusively consisted of [3H]testosterone. Scatchard analysis of in vitro binding data revealed a 8 of 0.8 x 10^{-10} M and a number of binding sites of 1.0 x 10^{-14} mol per mg of protein. Competition experiments indicated that

the testosterone-binding protein in male pineal gland had a similar ligand specificity as that present in male pituitary (Table II) except that estradiol seemed to have a somewhat higher affinity to the androgen receptor in pineal gland. No high affinity, low capacity binding of testosterone could be detected in female pineal gland cytosol, either in vivo or in vitro.

Protein Binding of [1,2,6,7-3H]Testosterone in Hypothalamus In Vivo and In Vitro—Isoelectric focusing of cytosol from male hypothalami labeled in vivo with [1,2,6,7-3H]testosterone showed a reproducible pattern of two radioactive peaks, a major peak with a pI of 5.1 and a minor one with a pI of 4.0 (Fig. 6). These peaks were shown to contain only [3H]testosterone. A similar pattern of peaks was obtained following electrofocusing of male hypothalamic cytosol labeled in vitro with [1,2,6,7-3H]testosterone. The 8 for the binding of [1,2,6,7-3H]testosterone to the hypothalamic receptor protein, as determined by Scatchard analysis, was 1.1 x 10^{-10} M and the number of binding sites 1.4 x 10^{-14} mol per mg of protein. Competition experiments indicated that the testosterone receptor in male hypothalamus had a similar ligand specificity as those in male pituitary and pineal gland. Female hypothalamic cytosol
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Heat inactivation of [3H]testosterone-macromolecular complex formed in vitro by incubation of male pituitary cytosol

Cytosol incubated with [1,2,6,7-3H]testosterone for 30 min at 37° was chromatographed on Sephadex G-25. The void volume was divided into aliquots which were incubated for 10 min at 0, 37, 50, 60, and 80°. The incubation at 0° was a control for the experiment. Macromolecular binding was assayed by gel filtration on Sephadex G-25 and the results are expressed as a percentage of the control incubation.

| Temperature | Macromolecular binding (%) |
|-------------|-----------------------------|
| 0° (control) | 100                         |
| 37          | 84                          |
| 50          | 17                          |
| 60          | 3                           |
| 80          | 0                           |

FIG. 6. Isoelectric focusing of cytosol from hypothalami of castrated male rats given 250 μCi of [1,2,6,7-3H]testosterone 30 min before death. The cytosol was chromatographed on a Sephadex G-25 column prior to electrophoresis. Each fraction contained 0.3 ml. ●, radioactivity; x—x, pH.

Brain cortex labeled in vivo with [1,2,6,7-3H]testosterone showed a complex pattern of radioactive peaks that was not reproducible in different experiments. In vitro incubations of [1,2,6,7-3H]testosterone with cytosol from male brain cortex showed the presence of high affinity, low capacity binding sites. \( K_d \) was calculated to 1.0 x 10^{-10} M, and the number of binding sites to 1 x 10^{-14} mol per mg of protein.

Table III

| Ligand and brain region | Temperature | Macromolecular binding (%) |
|-------------------------|-------------|----------------------------|
|                         | 0° (control)| 100                        |
|                         | 37          | 84                         |
|                         | 50          | 17                         |
|                         | 60          | 3                          |
|                         | 80          | 0                          |

Protein Binding of [1,2,6,7-3H]Epitestosterone in Brain In Vitro—In view of the finding of [3H]epitestosterone in brain from female rats, it was considered of interest to investigate the protein binding in vitro of [1,2,6,7-3H]epitestosterone in cytosol from male and female rats. Scatchard analysis of the results indicated that pituitary and hypothalamus from both male and female rats contain high affinity, low capacity binding sites for [1,2,6-3H]epitestosterone with similar \( K_d \) values (about 3 x 10^{-8} M) and number of binding sites (0.3 x 10^{-14} mol per mg of protein).

Table IV

| Ligand and brain region | Male rat | Female rat |
|-------------------------|----------|------------|
|                         | \( K_d \) | Number of binding sites | \( K_d \) | Number of binding sites |
| [1,2,6,7-3H] Testosterone | \( 0.9 \times 10^{-8} \) | 1.2 x 10^{-14} | b | b |
| Pituitary               | \( 0.8 \times 10^{-8} \) | 1.0 x 10^{-14} | b | b |
| Pituitary               | \( 1.1 \times 10^{-8} \) | 1.4 x 10^{-14} | b | b |
| Hypothalamus            | \( 1.0 \times 10^{-10} \) | 1.0 x 10^{-14} | b | b |
| Hypothalamus from 2-day-old rats | \( 0.5 \times 10^{-10} \) | 0.9 x 10^{-14} | 0.5 x 10^{-15} | 0.9 x 10^{-14} |
| [1,2-3H] Androsterone   | \( 9.0 \times 10^{-10} \) | 1.4 x 10^{-14} | b | b |
| Hypothalamus            | \( 3.0 \times 10^{-9} \) | 0.3 x 10^{-14} | 3.0 x 10^{-10} | 0.3 x 10^{-14} |
| Hypothalamus            | \( 7.0 \times 10^{-9} \) | 0.3 x 10^{-14} | 3.0 x 10^{-10} | 0.3 x 10^{-14} |

* No binding sites detected.
* Not analyzed.

Protein Binding of [1,2,6,7-3H]Testosterone in Brain Cortex—Analysis by isoelectric focusing of cytosol from male and female rats given [1,2,6,7-3H]testosterone did not show any focused radioactive peak. Binding studies in vitro gave no indication for the presence of any specific androgen-binding protein in serum. In view of these findings it may be concluded that the steroid-binding proteins measured in male brain do not originate from blood.
DISCUSSION

The present investigation has demonstrated a marked sexual difference in rat concentration of high affinity, low capacity binding sites in rat pituitary, hypothalamus, and pineal gland. Whereas these tissues in male rats showed the presence of a receptor protein for testosterone, it was not possible to detect a macromolecule of similar nature in corresponding brain regions from female rats. According to current concepts of mechanism of action of steroid hormones, these compounds are bound to specific receptor proteins in the cytosol of target organs and the steroid-receptor complex is transported into the cell nucleus where transcription of mRNA is facilitated. Absence of receptor protein from a steroid target tissue is combined with unresponsiveness to the hormone in question (22). Probable primary target sites for androgen action on the liver enzymes are the hypothalamus or the pituitary (cf. above), or both, and the present findings therefore offer one possible explanation for the relative androgen unresponsiveness of female liver enzyme activities. A further indication that testosterone receptor concentration in the brain may determine responsiveness of liver enzymes to androgen was obtained from experiments on 28-day-old male and female rats showing presence of quantifiable amounts of testosterone receptor in pituitary tissue from both sexes. These results correlate well with previous findings on androgen responsiveness of liver enzyme activities in developing rats; at 28 days of age male and female rats respond equally well to treatment with androgen (25).

In a previous study we have shown that androgen responsiveness in male rats is developed as a result of neonatal imprinting by testicular androgen (24). The present investigation has indicated that the underlying mechanism may be irreversible induction of a receptor for testosterone in brain. Neonatal androgen imprinting is known to occur in the hypothalamus (25); whether the androgen receptor in male pituitary and pineal gland is induced as a result of direct imprinting on these tissues or whether it is regulated by the hypothalamus remains to be shown.

Irreversible programming of sex steroid receptor concentration in adult life by androgen action in the neonatal period may be a general phenomenon. Treatment of female rats with testosterone propionate shortly after birth decreased the uptake of estradiol in hypothalamus and uterus on Day 60 of life (26). On the other hand, feminization of newborn male rats with cyproterone resulted in increased estradiol binding in the hypothalamus (27). When guinea pigs were treated with cyproterone acetate pre- and postnatally and were castrated early in life they developed a relative androgen unresponsiveness compared to postpubertally castrated control animals with respect to testosterone stimulation of prostate, seminal vesicle, and preputial gland growth (28). Insufficient production of androgen during the neonatal period has been suggested to play a role in the etiology of testicular feminization in the human and in animals, a disease associated with generalized absence of androgen receptors in all organs (22). Again the question may be asked whether receptor concentration in peripheral target organs is regulated by direct androgen imprinting or whether it is regulated by the imprinted hypothalmo-hypophyseal system.

In addition to the differences in specific protein binding, sex differences were also observed in the pattern of [3H]testosterone metabolites recovered from several brain regions including pituitary, pineal gland, and hypothalamus. The most conspicuous differences were the predominance of testosterone and androstenedione in male brain compared to the quantitative importance of 5a-androstane-3α,17β-diol, 5α-androstane-3β,17β-diol, epitestosterone, and dihydroepiandrosterone in female brain. It may be suggested that an additional explanation for the androgen unresponsiveness in female rats is the faster metabolism of testosterone to inactive compounds in female brain. On the other hand, the reason why testosterone appears to be more protected from metabolism in male brain may be binding of testosterone to specific high affinity protein-binding sites.

The sex-specific occurrence of epitestosterone and dihydroepiandrosterone in female brain is probably related to the sex-specific presence of 17α-hydroxysteroid reductase in female brain. Epitestosterone is known to be inactive as androgen, and in confirmation of this we did not find any change in liver enzyme activities in castrated male or female rats when these were given epitestosterone propionate. In spite of this, epitestosterone was found to bind to high affinity, low capacity sites in both male and female brain tissue. It is possible that conversion of testosterone to epitestosterone and dihydroepiandrosterone in female brain constitutes a process of physiological significance yet to be elucidated.

The physical characteristics and ligand affinities and specificities of the testosterone receptors in male pituitary, pineal gland, and hypothalamus were similar and also agree well with reports from other laboratories (9–12). It may well be that the testosterone receptors studied in various brain regions are identical. The physiological ligand for the receptor appears to be testosterone rather than 5α-dihydrotestosterone, as suggested by some authors (10, 13). This conclusion is in agreement with previous results indicating that testosterone itself may be the predominant active androgen principle in vivo in most androgen target organs and that conversion to 5α-dihydrotestosterone generally is not a prerequisite for androgen activity (15).

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