Journal of Steroid Biochemistry, 1975, Vol. 6, pp. 1193–1199. Pergamon Press. Printed in Great Britain

LOCALIZATION OF INTRAVENOUSLY ADMINISTERED [^{3}H]-TESTOSTERONE AND ITS METABOLITES IN THE BRAIN OF THE MALE RAT: THE ABSENCE OF A MAJOR EFFECT RELATED TO THE TIME OF DAY OF THE INJECTION

DONALD L. REZEK and RICHARD E. WHALEN

Department of Psychobiology, University of California, Irvine, Irvine, California 92664, U.S.A.

(*Received* 11 *September* 1974)

SUMMARY

Different groups of three castrated male rats were injected with 40 μCi [^{3}H]-testosterone at each of six time points at 4 h intervals throughout the light-dark cycle. One half hour after the administration of the hormone, testosterone and its metabolites were extracted from several brain regions and isolated by t.l.c. Particular attention was given to testosterone and 5α-dihydrotestosterone (DHT). Testosterone was concentrated to a greater extent in the hypothalamus and cerebral peduncles than in the cortex and plasma. This effect was not altered significantly by the time of day of the injection. More DHT was found in the posterior hypothalamus and cerebral peduncles than in the cortex. There was more testosterone than DHT in all brain samples examined. There was a small time of day effect in the amount of DHT present but not in the ratio of DHT to testosterone. The DHT to testosterone ratio was greater in the posterior hypothalamus, cerebral peduncles, and cerebellum than in the cerebral cortex, the preoptic and anterior hypothalamic areas. Although too small to be statistically significant, changes in the amount of testosterone found in the brain appeared to parallel the fluctuations in DHT. It was concluded that changes in the amount of substrate present may have been responsible for the observed differences in DHT. The time of day effect seemed most obvious as a decrease in DHT in the posterior hypothalamus two to six hours after the onset of the dark phase of the light-dark cycle.

INTRODUCTION

The hypothalamus is the region of the brain that has unique biochemical characteristics that can be correlated to the area's neuroendocrine and behavioral functions [1]. This region of the brain is known to concentrate the androgen, testosterone, to a greater extent than most of the remainder of the brain. This ability of hypothalamic neurons has been documented by tissue extraction procedures [2] and autoradiographic techniques [3, 4]. *In vitro* and *in vivo* studies have shown that testosterone is metabolized by neural tissues of the rat to compounds such as 5α-dihydrotestosterone (DHT) [5, 8], androstenedione [5–8], and estrogens [9–12]. There are also regional differences in androgen metabolism in the brain and hypothalamus [9, 13].

It has been suggested that metabolism to "active" metabolites is important for the action of testosterone in the brain. There is strong evidence that the conversion to DHT is necessary for the action of the hormone in the prostate gland and other peripheral androgen dependent organs but for the brain the evidence is much less convincing. However, in the rat, Swerdloff *et al.*[14] have demonstrated that DHT is effective in producing feedback inhibition of gonadotropin release. Recently studies have also indicated that DHT and estrogens in combination can synergistically support male mating behavior in the castrated male rat [15, 16] though DHT is relatively ineffective by itself [17]. The evidence suggests therefore that the reduction of testosterone to DHT may have functional significance in the hypothalamus of the rat.

If the conversion of testosterone in the body including the brain of the animal is important, it is then important to know what changes occur in the localization of the hormone in various tissues under different conditions. One of the best methods of accomplishing this is to inject radiolabeled hormone into the blood of the live gonadectomized animal. This preparation permits an evaluation of the retention of the hormone and its metabolites by various tissues. In this study this method was used to determine regional uniqueness in the brain. Since neuroendocrine and behavioral functions of androgen action vary during the day [18], it was considered worthwhile to determine if there are changes in the localization of testosterone and its metabolites in the brain as a function of the time of day the hormone was administered.

MATERIALS AND METHODS

Animals

The animals used in this study were 90–100 day old albino male rats (Horton, Gilroy, California,

U.S.A.) group housed prior to castration. All animals were entrained to a twelve hour reversed light-dark cycle for at least thirty days. Animals were orchidectomized 48 h before being used in the experiment.

Reagents

All chemicals in the study were reagent grade and used without further purification. Steroids used as standards and carrier hormones during the extraction procedure were obtained from Mann Research Laboratories, Inc., New York, N.Y., U.S.A. (testosterone, DHT, androstenedione, and estradiol-17β) and Steraloids, Inc., Pawling, N.Y., U.S.A. (androsterone, 5α-androstane-3α, 17β-diol (3α-androstanediol), 5α-androstane-3β, 17β-diol (3β-androstanediol), etiocholanolone, and 5α-androstane-3, 17-dione (androstanedione)). Several other steroids were obtained from Steraloids to determine their relative position on t.l.c. including 5β-androstane-3α, 17β-diol, 5β-androstane-3β, 17β-diol, 17β-hydroxy-5β-androstan-3-one, 3β-hydroxy-5α-androstan-17-one, 5β-androstane-3, 17-dione, and estrone. [1α, 2α-^{3}H]-testosterone, specific activity 56 Ci/mmol, was obtained from Amersham/Searle, Arlington Heights, Illinois, U.S.A. [4-^{14}C]-testosterone, 56·1 mCi/mmol; DHT, 56·1 mCi/mmol; and androstenedione 57·5 mCi/mmol were obtained from New England Nuclear Corp., Boston, Mass., U.S.A. The radiolabeled steroids were checked by t.l.c. for purity before use.

Procedure

Under light ether anesthesia three animals were injected via the jugular vein with 40 μCi of ^{3}H-testosterone in 10% ethanol at each of six 4 h intervals during the day. The times included two hours after the onset of the dark phase of the light cycle (D + 2), six hours after the onset of the dark (D + 6), and ten hours after the onset of the dark (D + 10) and corresponding times after the lights came on: (L + 2), (L + 6), and (L + 10). Six animals, each animal representing a different time point, is referred to as a "set." There were three sets of animals in the experiment giving each time a group of three rats. Each animal was sacrificed one-half hour after the injection of the hormone.

While under light ether anesthesia the animal's thoracic cavity was opened and a blood sample was removed from the left ventricle by cardiac puncture with a heparinized syringe. Plasma was obtained by centrifugation. Following 2–3 minutes of perfusion with cold saline the animal was decapitated and the brain was removed. The whole pituitary and a sample of ventral prostate were also taken. The brain was frozen at $-20°$C prior to its dissection.

Six samples were taken from the brain. The brain was placed with the dorsal surface down and ventral-dorsal cuts were made coronally at the genu of the corpus callosum and at the anterior demarcation of the pons and sagittally at the hypothalamic sulcus (approximately 2·5 mm from midline). A sample of cerebral cortex (CX) was taken from the dorsal surface of the block and the hypothalamus was removed by a single cut 2–3 mm from the ventral surface through the region of the anterior commissure. This hypothalamic region was divided into four parts—one cut was made just posterior to the anterior commissure producing a "preoptic" sample (PO), a cut was made at the infundibular recess producing an "anterior hypothalamic" sample (AH), and a cut was made just caudal to the mammillary bodies producing a "posterior hypothalamic" sample (PH) and "cerebral peduncle" sample (CP). A sample was also taken from the cerebellar cortex (CB) since Rommerts and van der Molen[7] reported high steroid 5α-reductase activity in that part of the brain. All tissue samples were weighed on a Mettler B-6 balance to the nearest 0·01 mg.

Extraction of the metabolites

The samples were placed in test tubes containing recovery standards of 200–400 c.p.m. of [^{14}C]-testosterone, DHT, and androstenedione plus 20 μg of each of the steroid standards listed above as carriers. After the addition of 5 ml of dichloromethane, the samples were agitated for 24 h in an ice bath after the method of McEwen *et al.*[2]. The dichloromethane was then removed to another tube and evaporated under nitrogen. The remaining dichloromethane in the tissue was allowed to evaporate and then the tissue was homogenized in acetone with a ground glass tissue grinder (2 × 2 ml + 1 ml rinse). The acetone and tissue residue were also agitated in an ice bath for another 24 h and then the acetone was added to the preceding dichloromethane extract and evaporated under nitrogen. The tube and tissue residue were then washed with 5 ml of methanol which was added to the extract and evaporated. This redundant extraction procedure was used because in this initial step losses would not be compensated for by the [^{14}C] recovery standards.

100 μl plasma samples were brought to 1 ml with water and extracted 3 × with 3 ml dichloromethane.

The residues were then dissolved in 6 ml of petroleum ether and partitioned 3 × against 3 ml of 70% methanol. The methanol was evaporated to an aqueous phase that was then brought to 3 ml with distilled water and extracted 3 × with 3 ml of dichloromethane. This final extract was dried down and run on t.l.c. plates.

Thin layer chromatography

Each sample was plated on precoated activated silica gel t.l.c. plates (silica gel 60, manufactured by E. Merck, Darmstadt, Germany) with 2 × 50 μl of methanol. The plates were run for 5 cm. in system B (see Table 1) and then air dried and run twice for a full 16 cm. in system A. In two sets the testosterone zone was eluted from the original plate, replated and rerun in system C (2 ×). For all three sets the DHT zones were eluted and acetylated (0·3 ml acetic anhydride and 0·5 ml pyridine overnight) and then plated and run in system D (2 ×). Our evidence indicated

TABLE 1. THIN LAYER CHROMATOGRAPHY SYSTEMS

	Percentages of the Various Solvents in Each System					
System	Benzene	Chloroform	Cyclohexane	Ethanol	Ethyl acetate	Methanol
A	20	65		13		2
B	95					5
C	60				40	
D	71		23		6	
E			97			3
F	97			3		

that this procedure yields samples sufficiently pure for the present analysis. Using similarly prepared pooled hypothalamic extracts from different animals the ratio of [^{3}H] from the testosterone zone to [^{14}C]-testosterone recovery standard decreased only 5% from the elution of the testosterone zone of the original t.l.c. through four recrystallizations in four solvent systems. As would be expected from the possible presence of androsterone, the ratio of [^{3}H] to [^{14}C]-DHT decreased slightly over 15% when the eluted DHT zone was analyzed in the same manner. However, following acetylation of the sample there was no decrease in the ratio [^{3}H]/[^{14}C] in the DHT-acetate zone after chromatography in system D whether or not the DHT had gone through an intermediate recrystallization series. This indicates that the acetylation and rechromatography procedure gives adequate presumptive evidence for the purity and identity of the DHT.

Zones corresponding to the standards were detected on the t.l.c. plates either by U.V. light or by spraying an adjacent channel containing only the carrier steroids with a 1:1 mixture of ethanol:sulfuric acid and developing the plates for several minutes at 100°C. The zones corresponding to the origin, the region more polar than the androstanediols, the androstandiols, androstenedione, and androstanedione were then scraped from the original plates into scintillation vials, eluted, and fluor added as previously reported [19]. From the plates on which the radioactivity from the testosterone zones were replated, the regions corresponding to the mobility of testosterone and estradiol-17β were scraped for counting. Similarly on the plates on which the acetylated DHT zones were replated, zones corresponding to DHT acetate and androsterone acetate were scraped and prepared for counting.

Samples were counted in both tritium and ^{14}C windows of a Beckman 150 liquid scintillation counter set to count for 50 min or 3%. The AQC (automatic quench correction) feature of the instrument was set so that 0·5% of the tritium counts would fall in the ^{14}C window. [^{14}C] standards were used to determine the proportion of [^{14}C] counts in the tritium window.

Androstanediol analysis

After the original counting of the vials containing the radioactivity from the androstanediol zones, to determine if 3α- or 3β-androstanediol predominated, the toluene was evaporated from the scintillation fluid from the pooled PO, AH, PH, and CP samples of

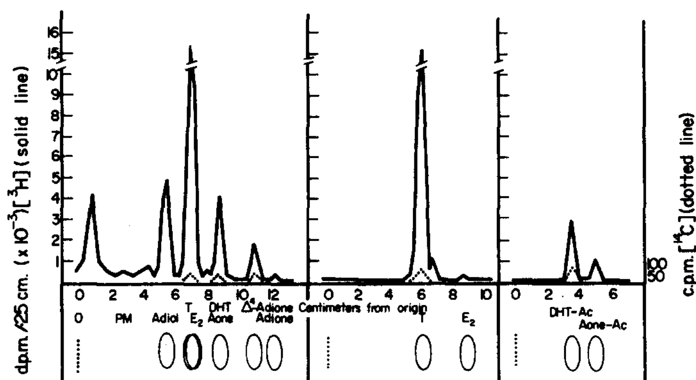

Fig. 1. The three panel diagram represents the standard t.l.c. plating and replating used in this experiment. For illustration the sample used was a combined extract from several brains after the samples used in the study had been removed. The solid line represents the separation of the [^{3}H] metabolites and the dotted line represents the [^{14}C] recovery standards. The ovals below indicate the mobility of non-radioactive standards run on the same plates. The first panel is the initial t.l.c. and the second and third are the replating of the testosterone zone from the original and of the acetate derivatives of the DHT zone respectively. O = origin; PM = polar metabolites; Adiols = 3α-and 3β-androstanediols; T = testosterone; Δ^{4}Adione = androstenedione; Adione = androstanedione; DHT-Ac = dihydrostestosterone acetate; Aone-Ac = androsterone acetate; and E$_2$ = estradiol. The standard solvent front was 16 cm from the origin.

Table 2, The Mobilities of Several Steroids Relative to that of Testosterone on the First Thin Layer Chromatogram

Steroid	Mobility
5β-androstane-3α,17β-diol	0.53
5α-androstane-3β,17β-diol	0.77
5α-androstane-3α,17β-diol	0.78
5β-androstane-3β,17β-diol	0.89
testosterone	1.00
etiocholanolone	1.04
estradiol-17β	1.05
5β-androstane-17β-ol-3-one	1.13
5α-androstane-3β-ol-17-one	1.15
5α-androstane-17β-ol-3-one (DHT	1.27
androsterone	1.25
androstenedione	1.53
estrone	1.56
5β-androstane-3,17-dione	1.67
5α-androstane-3,17-dione	1.71

all the animals using a rotary evaporator. The scintillation phosphors were separated from the steroid by first partitioning the residue between benzene and 70% methanol. The methanol was then evaporated down to the aqueous phase and extracted with dichloromethane. The extract was then evaporated. This residue was placed on a silica gel column and most of the phosphor eluted with benzene. The androstanediols were eluted with benzene:methanol 95:5. This eluate was run on a silica gel t.l.c. plate in system C and then eluted and rerun in system E. The final separation was on aluminum oxide t.l.c. plates (type T, manufactured by E. Merck, Darmstadt, Germany) in system F. The zones corresponding to authentic 3α- and 3β-androstanediol standards were scraped and the relative radioactivity in the two zones determined.

Calculations

To find the d.p.m./mg tissue first the c.p.m. attributable to ^{14}C were subtracted from the counts in the tritium window minus background. The net tritium c.p.m. were converted to d.p.m. using the external standard channels ratio technique. Correction was made for loss during recovery. The proportion of the [^{14}C]-androstenedione added at the beginning of the extraction procedure to that recovered from the t.l.c. plate was used as a measure of recovery for the zones on the original t.l.c. plate that were not replated. Since the testosterone and DHT zones were replated the proportions of [^{14}C]-testosterone and DHT (acetate) were used as a measure of recovery through the whole extraction and replating procedure for the zones on their respective plates. This corrected d.p.m. was then divided by the weight of the tissue in mg.

RESULTS

The d.p.m./mg with the mobilities of testosterone and DHT for the various tissues at the six time points are presented in Table 3. The distribution of the metabolites given as a percentage of the total d.p.m./mg recovered from the t.l.c. plates is presented in Table 4. Approximately 4×10^{-5} % of the injected radioactivity was found per mg of brain tissue in the dichloromethane soluble extract.

Testosterone

More radioactivity with the mobility of testosterone was found in the hypothalamus and ventral midbrain than in the cerebral or cerebellar cortices. The pituitary and prostate accumulated even greater amounts of testosterone than did the brain. No large changes were seen for the different times during the day (see Table 3).

The d.p.m./mg with the mobility of testosterone were subjected to a 2-way analysis of variance (ANOVA) with repeated measures to determine if regional and time of day effects exist in the localization of this hormone in the brain. The statistical test demonstrated that area specific differences in localization within the brain exist ($F_{(5,60)} = 9{\cdot}24$, $P < 0{\cdot}001$). However differences among the various times of the day were not significant. From the data presented in Table 2, it is evident that regional differences are more consistent than are the differences among the various times of the day. The Newman–Keuls ranking procedure with $q_{0{\cdot}05}$ $(r,60)$ indicated that CX=CB < AH=PH=CP. For the statistical procedures used refer to Winer[20].

Separate ANOVA's were conducted on the blood and prostate samples. The test showed a significant time of day effect for the amounts of testosterone in the blood $F_{(5,12)} = 16{\cdot}17$, $P < 0{\cdot}001$. No difference was demonstrable with the amount of testosterone present in the prostate among the various times. No time of day effect was apparent in the amount of testosterone in the pituitary.

Dihydrotestosterone

The relative distribution of DHT was different from that of testosterone. The greatest amount of DHT in d.p.m./mg tissue was found in the area of the cerebral peduncles in the midbrain with less found rostrally throughout the hypothalamus. Less DHT was found in the cerebral cortex than in the hypothalamus or cerebellar cortex.

As indicated by the data in Fig. 2 and Table 2 the greatest change among the various time points was apparent between D + 2 and D + 6. This was seen most clearly in the PH samples. There seemed to be a decrease in the variance between the two points as well.

When the d.p.m./mg from the DHT-acetate zone were analyzed, it was found that like testosterone there was a regional effect but there was also a time of day effect. The pituitary was also included in this analysis. For the time of day effect $F_{(5,84)} = 4{\cdot}83$, $P < 0{\cdot}005$ and by area $F_{(6,84)} = 18{\cdot}21$, $P < 0{\cdot}001$.

The area differences in the presence of DHT were quite evident. There was what appeared to be an

TABLE 3. Testosterone and DHT by the Time of Day

	Time of Day Relative to the Onset of the Light or Dark					
Testosterone (d.p.m./mg ± S.E.)						
	D+10	L+2	L+6	L+10	D+2	D+10
PO	16.9±2.0	20.3±3.3	17.5±2.4	15.6±2.4	15.8±2.1	16.6±2.4
AH	22.3±2.0	22.6±2.6	18.6±2.2	18.7±2.7	18.8±2.6	18.0±2.4
PH	18.2±1.4	22.2±3.4	16.4±0.9	16.9±1.9	16.4±2.2	16.8±2.7
CP	20.5±1.9	25.6±3.2	22.3±3.0	19.8±3.6	18.0±2.0	18.6±2.9
CX	14.4±1.6	17.5±2.6	11.0±0.9	13.6±2.7	12.8±2.0	14.0±4.0
CB	11.8±1.2	13.1±2.3	10.0±0.6	12.3±1.8	17.4±6.0	10.9±1.2
Brain Mean	17.4±1.6	20.2±1.8	16.0±1.9	16.2±1.2	16.5±0.9	15.8±1.2
Pituitary	38.2±2.0	45.3±4.8	29.3±3.2	44.9±4.8	42.9±5.5	36.4±5.2
Prostate	29.0±4.2	39.0±3.4	33.3±3.4	31.2±4.6	20.1±2.8	27.1±7.1
Plasma	10.6±1.4	12.0±2.2	9.4±0.7	10.3±2.5	8.2±0.6	9.8±2.1
DHT (d.p.m./mg tissue ± S.E.)						
PO	1.2±0.4	2.0±0.3	1.3±0.1	1.8±0.2	2.4±0.2	2.1±0.3
AH	3.0±0.6	2.8±0.3	2.0±0.2	2.2±0.1	2.6±0.3	2.1±0.3
PH	2.9±0.7	4.0±0.8	2.5±0.2	2.8±0.1	2.8±0.4	2.7±0.2
CP	5.1±0.7	6.2±0.7	4.7±0.5	4.9±0.5	5.4±0.1	5.4±0.9
CX	1.1±0.6	1.8±0.8	1.3±0.3	1.3±0.3	1.4±0.2	1.2±0.3
CB	2.1±0.7	3.0±0.8	2.2±0.2	1.5±0.5	2.8±0.2	2.1±0.4
Brain Mean	2.6±0.6	3.3±0.7	2.3±0.5	2.4±0.5	2.9±0.5	2.6±0.6
Pituitary	2.2±0.7	3.2±1.0	0.7±0.4	2.5±1.5	0.9±0.6	2.7±1.0
Prostate	45.4±10.4	27.8±5.2	30.6±3.6	28.9±3.3	23.2±9.8	48.4±9.5
Plasma	0.2±0.1	0.6	0.1±0.1	0.3±0.1	0.2±0.1	0.2±0.1

anterior-posterior gradient throughout the area of the hypothalamus. Little DHT was found in the preoptic area while more was found in the mesencephalic "cerebral peduncle" samples than in any other part of the brain or in the pituitary. Here the Newman–Keuls procedure demonstrated that CX < PH < CP with $q_{0\cdot05}(r, 84)$.

In the brain or pituitary, however, the level of DHT did not approach the amount of DHT relative to testosterone found in the prostate. In the prostate 34·3 ± 4·0 c.p.m./mg had the mobility of DHT. The ratio of DHT to testosterone was 1·33 ± 0·23 as compared to brain ratios of less than 0·3 (see Fig. 3).

Analysis by ratios

Ratios are commonly used to analyze the amount of substance taken up or produced by a tissue. In the brain the d.p.m./mg tissue relative to that of the blood plasma or relative to the cerebral cortex has been used. When this type of analysis is done to compare the various regions, the results are similar to those obtained with the analysis of d.p.m./mg. For the tissue to plasma ratios the Newman–Keuls procedure indicated that CX=CB < AH=PH=CP and CX=CB < PO < CP ($q_{0\cdot05}(r, 72)$). Similarly considering the cortex as a control tissue, ratios of tissue to cortex (CX) were also examined. It was found that CX=CB < PO=AH=PH=CP. This evidence supports the conclusion that the hypothalamus and cerebral peduncle region concentrated testosterone to a greater extent than did the cortical areas examined.

The ratio of DHT to testosterone can also be used to differentiate among tissues. The use of this method gives a different type of area specificity than the other measures do. When this ratio was analyzed no time of day effect was observed in the brain ($F_{(5,72)}$ = 1·32); however distinct area differences were noted,

TABLE 4. PERCENTAGE OF d.p.m./mg RECOVERED FROM THE PRINCIPAL ZONES[1]

Sample	n	d.p.m./mg from zones	Origin	Unidentified Polar	Adiol	T	DHT[2]	Androsterone[2]	Δ^4-Adione	Adione
PO	18	32.8±1.8[3]	4.7±0.6	16.9±1.1	6.1±0.3	53.0±2.0	6.0±0.8	3.3±0.6	8.5±0.7	1.5±0.3
AH	18	37.1±1.9	5.1±0.7	15.7±1.0	6.0±0.2	54.0±1.3	6.5±0.5	2.6±0.4	7.9±0.7	1.9±0.4
PH	18	36.0±1.7	5.6±0.8	14.7±0.8	7.7±0.7	49.9±1.7	8.3±0.6	4.2±0.5	8.1±0.8	1.4±0.3
CP	18	48.8±2.6	10.4±2.4	14.4±0.8	7.8±0.3	43.2±2.0	11.2±0.6	2.9±0.6	8.1±1.2	2.0±0.4
CX	18	28.6±1.9	5.3±0.8	17.7±1.2	7.9±0.3	48.6±1.9	4.6±0.5	6.4±1.3	8.0±0.7	0.8±0.7
CB	6	31.9±3.8	4.3±0.6	13.3±0.7	8.7±0.5	52.9±2.7	9.8±1.1	4.1±0.7	6.2±1.1	0.8±0.2
Pit	18	61.4±3.4	8.1±1.2	12.9±0.8	4.6±0.5	64.6±1.7	3.4±0.6	0.9±0.3	3.3±0.7	0.5±0.2
Prostate	17	121.6±8.1	5.1±0.6	7.3±0.7	23.1±1.3	23.7±1.8	27.1±2.0	6.2±0.8	5.3±1.2	1.1±0.1
Plasma	18	29.2±2.5	20.5±1.8	27.6±1.3	8.0±0.4	35.3±2.2	0.6±0.2	2.3±0.5	6.3±0.6	0.3±0.1

1. The percentages and d.p.m./mg do not include small amounts of radioactivity that had the mobility of estrogen.
2. As separated as acetates upon replating.
3. Mean ± S.E.

Adiol = androstanediols; T = testosterone; Δ^4-Adione = androstenedione; and Adione = androstanedione.

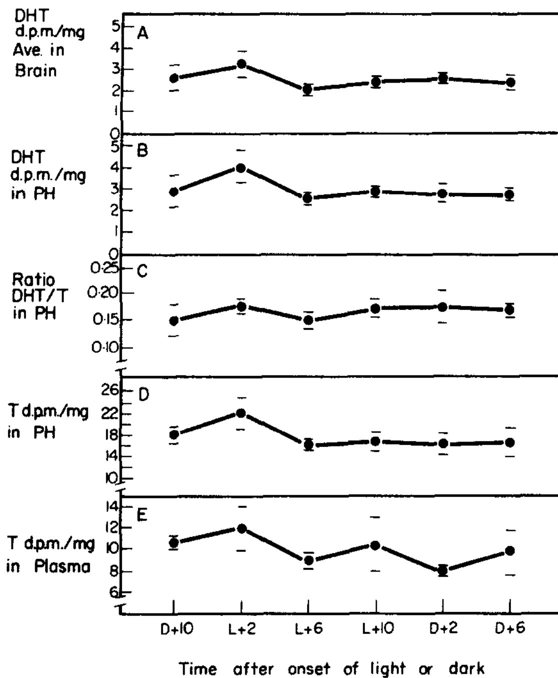

Fig. 2. This series of five panels illustrates the time of day effect with regard to the amount of DHT and testosterone found in the brain. A gives an average of the amount of DHT (±S.E.) found across all the brain areas examined. In B the PH is presented as an example of one brain area showing the effect. C demonstrates that the effect disappears in the DHT/testosterone ratio. Panel D shows the parallel though insignificant changes in testosterone in the PH. Panel E shows the changes in the blood levels of d.p.m./mg radioactivity with the mobility characteristics of testosterone. It is not clear if the changes in the blood concentration of testosterone may partially explain the observed changes in the brain.

$F_{(5,72)} = 21{\cdot}45$, $P < 0{\cdot}001$. The Newman–Keuls test at $P < 0{\cdot}05$ showed that CX=PO=AH < CB < CP. The relationship is seen in Fig. 3.

An analysis of the ratio of the DHT in the brain to the testosterone in the blood was also performed. This was done to determine if major fluctuations occurred during the day in the amount of DHT in the brain to the amount of substrate in the circulation of the animal. No significant diurnal changes were found. As with the other ratios significant regional differences were encountered however.

Other radioactivity

Quantities of radioactivity with the mobility of androstanediol, androsterone, androstenedione, and a trace with the mobility of androstanedione were also present in the samples (see Table 3). Separating 3α- and 3β-androstanediol revealed that greater than 90% was 3α-androstanediol.

Androstenedione showed no change related to the time of day. The CP again contained more radioactivity with the characteristic mobility of this hormone than did other brain tissue. The presence of androsterone was indicated since the androsterone acetate zone contained radioactivity.

Besides the non-dichloromethane extractable metabolites in the blood that were not quantified, a fair amount of polar metabolites were also found on the first t.l.c. (see Table 4). Smaller amounts were found in other tissues. This is contrasted with the much greater amount of DHT found in the brain, pituitary, and prostate than in the blood.

In those samples where estradiol-17β was separated by t.l.c., a small amount of radioactivity with the mobility of estradiol-17β was present in some samples but the amount was too low from which to draw any conclusions.

DISCUSSION

Many of the area differences in metabolism found in this investigation are substantiated by *in vitro* studies by Denef *et al.*[13]. The hypothalamus appears to produce more DHT in posterior areas than in anterior areas with the highest production being in the cerebral peduncles and the mesencephalon. The cerebellar cortex produces more DHT than does the cerebral cortex as would be anticipated from the differences in the 5α-reductase levels in these tissues [7].

Four relationships of testesterone to DHT can be distinguished in the brain. One is exemplified by the cerebral cortex where minimal testosterone and minimal DHT are present. Another is exemplified by the cerebellar cortex where the amount of testosterone is still low but relatively more DHT is found. Another is represented by the preoptic and anterior hypothalamic areas where there is more testosterone than in the cortex while there is little DHT and last by the posterior hypothalamus and mesencephalon where there is a good deal of both testosterone and DHT. These differences agree with *in vitro* metabolism studies [7, 13]. It may therefore be possible to classify areas of the brain by the interaction of uptake and metabolism characteristics of testosterone and DHT of particular regions. An even more complicated condition may be found to exist when other metabolites are considered.

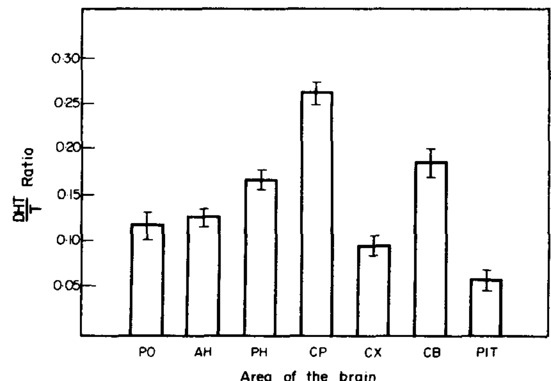

Fig. 3. The ratios (±S.E.) of d.p.m./mg of radioactivity with the mobility of DHT to that of T (testosterone).

We have described small but consistent differences in the localization of testosterone and DHT among the regions of the brain. Since the differences are small it is not surprising some studies have failed to find them [5, 21]. When using radiolabeled tracers it is probable that factors such as the time between the injection and sacrifice, the amount of hormone administered and the degree of isolation of the androgens measured all contribute to the disparities among the various studies. The existence of the differences has been substantiated however by the use of methods other than injecting radioactive hormones. When the amount of endogenous hormone in various organs of the noncastrated rat is measured, regional differences in the brain can also be detected. Robel *et al.*[22] measured the levels of endogenous testosterone and DHT by gas chromatography and found higher levels of these compounds in the hypothalamus than in the cortex. Ten times as much testosterone and at least five times as much DHT were found in the hypothalamus compared to the parietal cortex. These are even greater differences than those found in the present study. The ratio of DHT to testosterone in the hypothalamus (approximately 0·15) was similar to the ratios reported here.

The findings reported here may be interpreted in light of our knowledge of the localization of the regulation of male rat mating behavior and neuroendocrine function in the hypothalamus. Area specificity in the response to androgens has been reported. Davidson[23] and Lisk[24] have implanted testosterone directly into the brains of male rats and have found that implants in the preoptic-anterior hypothalamic zone are more effective than those in other areas in producing male mating behavior. Johnston and Davidson[25] have also implanted DHT propionate in the brain in the areas that testosterone propionate was effective and were not able to stimulate male mating behavior as effectively as with the testosterone propionate. However, both of these substances, when implanted into the median eminence were able to block gonadotropin secretion. Implants into other areas of the hypothalamus in animals have been less effective in accomplishing this [26, 27]. These findings indicate both a regional and hormone specific action with regard to mating and neuroendocrine functions. The functional specificity of certain brain regions could be related to the regional hormone-metabolite specificity in the brain.

Although there was a statistically significant time of day effect in the amount of DHT present in the brain, the observed differences were rather small. The changes may have been due to several factors including changes in the amount of the substrate testosterone available to the tissue or actual changes in the metabolic activity of the tissue. Further investigation would be required to elucidate the actual cause of the changes in the localization of the hormone and its metabolites in the tissue. The magnitude of the time of day effect is not considered to be of sufficient import to be pursued here.

Acknowledgements—This work was supported by grant HD-00893 from the National Institute of Child Health and Human Development to R.E.W. and by a NDEA Title IV Predoctoral Fellowship to D.L.R.

REFERENCES

1. Davidson J. M.: In *Reproductive Biology* (Edited by H. Balin and S. Glasser). Excerpta Medica, Amsterdam (1972) pp. 897–901.
2. McEwen G. S., Pfaff D. W. and Zigmond R. E.: *Brain Res.* **21** (1970) 17–28.
3. Pfaff D. W.: *Science* **161** (1968) 1355–1356.
4. Sar M. and Stumpf W. E.: *Endocrinology* **92** (1973) 251–256.
5. Stern J. M. and Eisenfeld A. J.: *Endocrinology* **88** (1971) 1117–1125.
6. Jaffe R. B.: *Steroids* **14** (1969) 483–491.
7. Rommerts F. F. G. and van der Molen H. J.: *Biochim. biophys. Acta* **248** (1971) 489–502.
8. Massa R., Stupnicka E., Kniewald Z. and Martini L.: *J. steroid Biochem.* **3** (1972) 385–399.
9. Naftolin F., Ryan K. J. and Petro Z.: *Endocrinology* **90** (1972) 295–298.
10. Reddy V. V. R., Naftolin F. and Ryan K. J.: *Endocrinology* **94** (1974) 117–121.
11. Weisz J. and Gibbs C.: *Endocrinology* **94** (1974) 616–620.
12. Weisz J. and Gibbs C.: *Neuroendocrinology* **14** (1974) 72–86.
13. Denef C., Magnus C. and McEwen B. S.: *J. Endocr.* **59** (1973) 605–621.
14. Swerdloff R. S., Walsh P. C. and Odell W. D.: *Steroids* **20** (1972) 13–22.
15. Larsson K., Sodersten P. and Beyer C.: *Horm. Behav.* **4** (1973) 289–299.
16. Feder H. H., Naftolin F. and Ryan K. J.: *Endocrinology* **94** (1974) 136–141.
17. Whalen R. E. and Luttge W.: *Horm. Behav.* **2** (1971) 117–125.
18. Taya T. and Igarashi M.: *Endocr. japon.* **21** (1974) 211–215.
19. Whalen R. E., Luttge W. G. and Green R.: *Endocrinology* **84** (1969) 217–222.
20. Winer B. J.: *Statistical Principles in Experimental Design.* McGraw Hill, New York (1962).
21. Perez-Palacios G., Perez A. E., Cruz M. L. and Beyer C.: *Biol. Reprod.* **8** (1973) 395–399.
22. Robel P., Corpechot C. and Baulieu E. E.: *FEBS Lett.* **33** (1973) 218–220.
23. Davidson J. M.: *Endocrinology* **79** (1966) 783–794.
24. Lisk R. D.: *Endocrinology* **80** (1967) 754–761.
25. Johnston P. and Davidson J. M.: *Horm. Behav.* **3** (1972) 345–357.
26. Lisk R. D.: *Acta endocr., Copenh.* **41** (1962) 195–204.
27. Davidson J. M. and Sawyer C. H.: *Proc. Soc. exp. Biol. Med.* (*N.Y.*) **107** (1961) 4–7.