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THE DISTRIBUTION, NUCLEAR UPTAKE AND METABOLISM OF [^{3}H]DIHYDROTESTOSTERONE IN THE BRAIN, PITUITARY GLAND AND GENITAL TRACT OF THE MALE RHESUS MONKEY

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Summary—Three adult male rhesus monkeys were castrated and administered 2 mCi [^{3}H]dihydrotestosterone ([^{3}H]DHT) intravenously. Brain and peripheral organs were removed after 60 min and were examined either by thaw-mount autoradiography or by subcellular fractionation and high performance liquid chromatography. Neurons that accumulated radioactivity in their nuclei were distributed widely in many regions of the brain including the preoptic area, hypothalamus, septal area-bed nucleus, amygdala, thalamus, and brain stem. Several brain areas which were labeled after [^{3}H]DHT injection had not been labeled in earlier experiments after [^{3}H]testosterone ([^{3}H]T) injection. The major metabolite of [^{3}H]DHT in extranuclear fractions from brain was [^{3}H]androstanediol, but [^{3}H]DHT alone was detected in cell nuclei. There was no evidence of any [^{3}H]estradiol in cell nuclei, confirming that DHT can also be regarded as a non-aromatizable androgen in the primate brain. Since the nuclear concentrations of androgens were 2–3 times higher in the brain following [^{3}H]DHT than they were in the earlier [^{3}H]T experiments, the relative lack of effectiveness of DHT in restoring the sexual behavior of male castrates cannot be related to an inability of DHT to enter brain cell nuclei.

INTRODUCTION

The distribution of radioactivity in autoradiograms of the male primate brain following the administration of [^{3}H]5α-dihydrotestosterone ([^{3}H]DHT) is both more widespread and more intense than that following the administration of [^{3}H]testosterone ([^{3}H]T) [1–3]. This radioactivity is concentrated in the nuclei of neurons. However, the potency of DHT in restoring the sexual behavior of castrated male rhesus and cynomolgus monkeys appears to be considerably lower than that of T [4–6]. One possible explanation for the difference in the behavioral activity of these androgens may lie in the observation that some areas of the brain contain an enzyme complex (aromatase) capable of converting T, but apparently not DHT, to estradiol [7]. Our own studies on the male rhesus monkey [8] and on the male cynomolgus monkey (unpublished data) have shown that [^{3}H]estradiol is the major form of radioactivity in cell nuclei from the hypothalamus, preoptic area and amygdala after the administration of [^{3}H]T. However, we currently lack data on the distribution and identity of radioactive metabolites in the nuclei of neurons in the primate brain following the administration of [^{3}H]DHT. This information has particular relevance for the behavioral actions of DHT, and the evidence for the non-aromatization of DHT in the primate brain is incomplete since it is based entirely on the irreversibility of the T to DHT conversion and on *in vitro* experiments using human placenta [9]. The aromatization of only a small percentage of the T in brain produces elevated levels of estradiol in nuclei [8]. It follows that a sensitive test of whether or not DHT is aromatized would be to administer [^{3}H]DHT and then measure [^{3}H]estradiol concentrations in brain cell nuclei. Another possible reason for the differences in the behavioral activity of T and DHT might depend on the higher affinity of DHT for sex-hormone binding globulin [10], which would tend to impede its entry into the brain [11] where it is then more rapidly de-activated by conversion to androstanediol [12]. We addressed these issues by administering [^{3}H]DHT to three castrated male rhesus monkeys and studying the brains both by thaw-mount autoradiography and by subcellular fractionation and high performance liquid chromatography. Preliminary autoradiographic data for one of these animals have been published [1].

EXPERIMENTAL

Animals and tissue samples

Three days after castration, three adult male rhesus monkeys were tranquilized with ketamine hydrochloride (Ketaset, Bristol Laboratories, 10 mg/kg, i.m.), and a catheter was placed in each right saphenous vein to obtain blood samples. At zero time, each

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male was rapidly injected via the left saphenous vein with 2.0 mCi [1,2,4,5,6,7,-^{3}H]5α-dihydrotestosterone (Male 1, body weight 10.5 kg, 131 Ci/mmol from New England Nuclear Corp.; Male 2, 9.4 kg, 147 Ci/mmol from Amersham Corp.; Male 3, 8.2 kg, 104 Ci/mmol from Amersham Corp.) in 2 ml 15% ethanol–normal saline. At 30 min, each animal was anesthetized with sodium pentobarbital (Nembutal, Abbott Laboratories, 8 mg/kg, i.v.). At 40 min, the head was placed in a stereotaxic apparatus and the calvarium was removed extradurally in preparation for speedy removal of the brain. At 60 min, a lethal dose of sodium pentobarbital was administered, the heart was incised and the brain was exposed. Using a knife mounted stereotaxically, frontal cuts were made at A18, A8.5 and A0 according to the atlas of Snider and Lee[13]. The blocks of brain were placed in ice as they were removed. Autoradiographic studies utilized the brain and pituitary gland from Male 1; the left half of the brain and samples of ductus deferens, seminal vesicles, prostate, penis, and scrotal skin from Male 2; and samples of pituitary gland and prostate from Male 3. Chromatographic studies in Males 2 and 3 utilized pituitary gland, samples of seminal vesicles, prostate and glans penis, and the right halves of the brains, which were dissected to obtain the following brain samples: hypothalamus, preoptic area and bed nucleus of stria terminals, amygdala, septum, mammillary body area, basal forebrain, hippocampus, caudate, putamen, thalamus, postcentral cortex and cerebellar cortex. The pituitary gland was divided into anterior part (pars distalis and anterior portion of pars intermedia) and posterior part (pars nervosa and posterior portion of pars intermedia). All tissue samples for biochemical analysis were immediately placed in ice-cold isotonic sucrose buffer (0.32 M sucrose, 3 mM $MgCl_2$, 1 mM potassium phosphate, pH 6.8).

Autoradiography

Tissues were frozen on dry ice and stored in liquid nitrogen. Coronal sections were cut at 4 μm in a Harris Wide-range cryostat at −20° to −25°C, and mounted on emulsion-coated (Kodak NTB3) slides at room temperature, using the thaw-mount procedure of Stumpf and Sar[14]. After up to 40 weeks exposure at −19°C the autoradiograms were developed (Kodak D19 diluted 1:1, 15°C, 4 min without agitation), fixed, rinsed and stained with methyl green-pyronin. A cell in the brain was considered labeled if its nucleus had a silver grain density at least twice that of the adjacent neuropil background (counted in 20 fields each of 625 μm^2). The labeling indices (percentage of the total number of neurons in a given region that was labeled) in 10 brain regions (Table 2) were estimated by counting 300 neurons each in Males 1 and 2 (6,000 neurons). In other brain regions, less precise estimates of labeling were based on counts of at least 50 neurons.

Subcellular fractionation

Nuclei were prepared according to the method of McEwen and Zigmond[15] modified by us[8]. This involved homogenization of tissues in isotonic sucrose buffer containing 0.25% Triton X-100. After 1 h, the samples were centrifuged at low speed (500 ***g***) for 10 min to prepare crude nuclear pellets. These were washed once to remove the detergent, resuspended in 2 M sucrose buffer and centrifuged at 6000 ***g*** for 90 min to prepare the purified nuclear pellet.

Extraction and high performance liquid chromatography

The nuclear pellets and aliquots of the first supernatant (crude cytosol) were extracted as follows: 200 μl (500 μl for supernatants) of a standard steroid mixture containing estriol, 17β-estradiol, estrone (all 10 μg/ml), androst-4-ene-3,17-dione (androstenedione) (20 μg/ml), testosterone, 5α-androstane-3,17-dione (androstanedione) (both 50 μg/ml), 5α-androstane-3α,17β-diol (androstanediol), 5α-dihydrotestosterone (both 100 μg/ml) in 50% aqueous acetonitrile were added to each sample, and the mixtures were extracted once with 3 ml freshly distilled ethyl ether. The ether extracts were decanted into silanized glass tubes, blown to dryness under nitrogen and redissolved in 200 μl 50% acetonitrile (500 μl for supernatants). Extracts of supernatants were filtered to remove insoluble material. Aliquots of nuclear or supernatant extracts (100 μl) were injected onto a high performance liquid chromatograph column (reverse-phase, Spherisorb 5-ODS, 4.6 × 250 mm) (Alltech Associates) and eluted with 50% aqueous acetonitrile at 1 ml/min and 32°C. Eluates were monitored at 206 nm (LKB Uvicord) and collected in 1 ml fractions in low background polypropylene scintillation vials. Radioactivity was measured in the fractions at 37.7% efficiency (Packard TriCarb) and results were transmitted to a computer which checked for spurious counts by a double-count, channels ratio method. Counting time was limited for practical reasons to 10 min per fraction giving a theoretical error of ±10% on a total of 100 counts. This was equivalent to a nuclear concentration of about 50 fmol/mg DNA for the smallest sample analyzed in this study. Tissues with less than this minimum of 10 cpm are described as having "insufficient counts". Areas of the u.v. absorbance peaks of the tracer steroids were computed with a Perkin Elmer Sigma 10 data station and compared with an unextracted standard mixture to estimate procedural losses. Radioactivity in the fractions was identified on the basis of the retention times of the carrier steroids. Aliquots of the remaining extract were used to estimate the total extracted radioactivity. Plasma samples (0.1 ml) were extracted and analyzed in the same way as supernatants. Total radioactivity in supernatant and plasma samples was

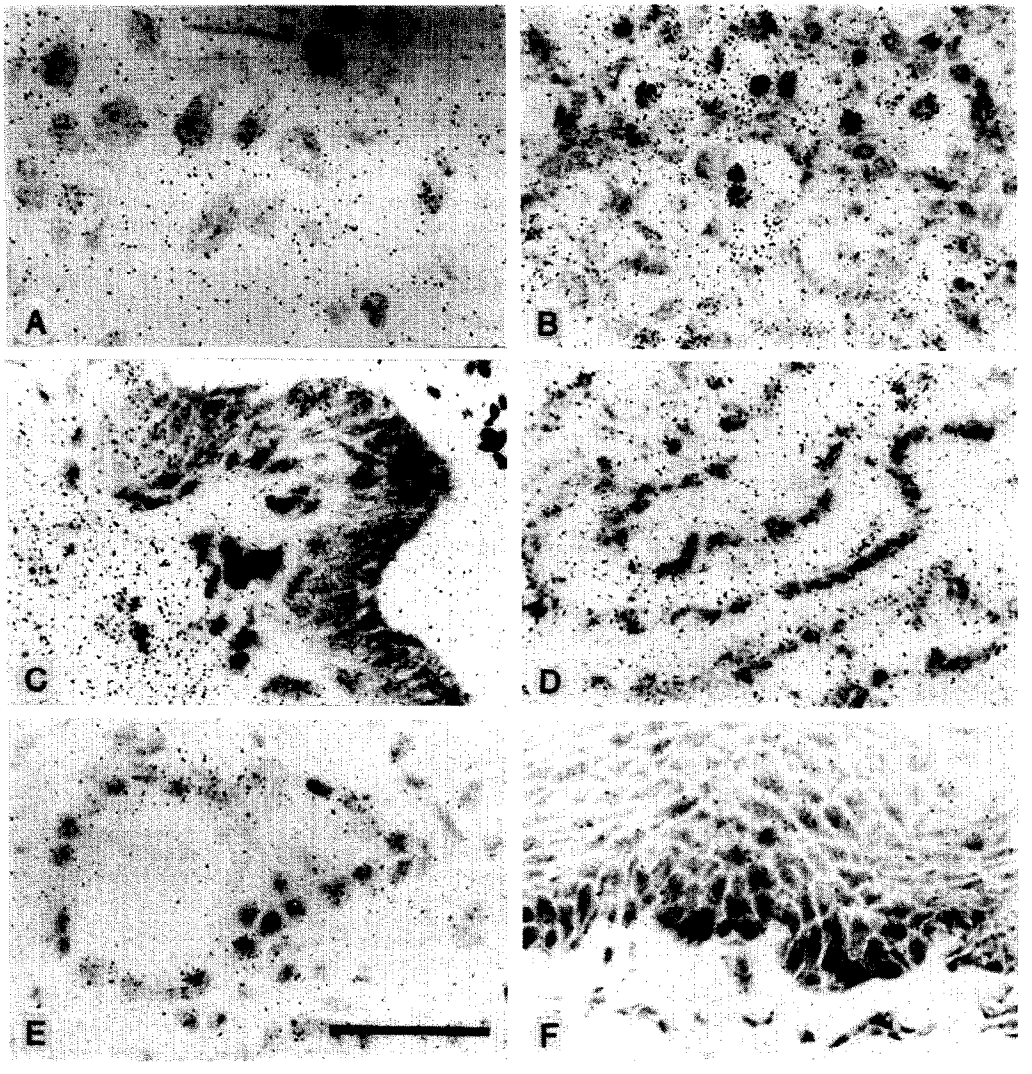

Fig. 1. Thaw-mount autoradiograms illustrating concentration of radioactivity in cells 2 h after [^{3}H]DHT injection. Neurons of the hypothalamic arcuate nucleus (A) and pars distalis cells of the pituitary gland (B) were strongly labeled, as were epithelial and connective tissue cells of the ductus deferens (C). Some epithelial cells were weakly labeled in seminal vesicles (D) and prostate (E). Epidermal cells of the penis (F) were also weakly labeled. Autoradiographic exposure 420 days. Scale = 50 μm.

estimated at about 25% efficiency in supernatant and plasma samples after oxidation (Packard TriCarb Model 306 Oxidizer). DNA was estimated in the extracted nuclear fractions by the method of Burton[16] as modified by Giles and Myers[17], using calf-thymus DNA (Sigma Chemicals, D1501) as standard. Protein was estimated in supernatant samples by the method of Lowry *et al.*[18] using a bovine serum albumin (Sigma Chemicals, A7906) as standard. Results are reported as percentages of the total ether-extracted radioactivity and as fmol per mg DNA (nuclei) or fmol per mg protein (supernatants) after correction for procedural losses.

RESULTS

Autoradiography

In the brain, cellular labeling occurred only over neurons and was confined predominantly to their nuclei (Fig. 1A). The silver grain density of the most heavily labeled neurons was about 20 times that of the neuropil background. Labeled neurons were located in widespread areas of the brain, which are listed in Table 1 and classified on the basis of their mean labeling index. Labeled neurons in Male 2 were observed in all the areas previously described in Male 1 [1], and not in any additional areas. The labeling indices in 10 selected regions (Table 2) generally showed close agreement between the two animals.

In the pituitary gland (Fig. 1B), the intensity of labeling within individual cells was greatest in the pars distalis (up to 12 times background), which had a labeling index of 3% in Male 1 and 7% in Male 3. The pars intermedia and pars nervosa, which were examined only in Male 1, had labeling indices of 6 and 1% respectively.

Table 1. Autoradiographic labeling indices (% of neurons labeled) in brain structures 1 h after the injection of [^{3}H]DHT in male rhesus monkeys (means of Males 1 and 2)

Region	Labeling index		
	1–24%	25–49%	50–100%
Preoptic and septal areas	n. accumbens medial septal n.	lateral septal n. bed n. of stria terminalis	medial preoptic n.
Hypothalamus and mammillary body	paraventricular n. periventricular n. lateral hypoth. area posterior hypoth. area	anterior hypoth. area arcuate n. median eminance dorsomedial n. supramammillary n.	ventromedial n. premammillary n. intercalated n.
Amygdala and hippocampus	lateral n. basal n. entorhinal cortex prorhinal cortex region CA2	medial n. cortical n. accessory basal n.	
Thalamus	ventral posteromedial n. reuniens n. ventral posterolateral n. (oral part) pulvinar n. (lat. part) mediodorsal n.	subfascicular n. centromedian n. lateral dorsal n. pulvinar n. (post inf. and med. parts) reticular n. anterodorsal n.	parafascicular n.
Circumventricular organs	pineal subfornical organ subcommissural organ area postrema		
Midbrain	inferior colliculus zona incerta pretectal area substantia nigra	periaqueductal gray superior colliculus oculomotor n. trochlear n. interpeduncular n. mesencephalic reticular n.	red n.
Pons and cerebellum	spinal trigeminal n. principal trigeminal n. inferior vestibular n. dorsal cochlear n. superior olivary n. inferior olivary n. dentate n.	mesencephalic trigeminal n. abducens n. parabrachial n. locus coeruleus dorsal raphe n. superior central n. pedunculopontine tegmental n.	motor trigeminal n. facial n. lateral vestibular n.
Medulla	dorsal motor n. of vagus solitary n. accessory cuneate n.	hypoglossal n. gracile n. cuneate n.	n. ambiguus

In the ductus deferens more than 50% of epithelial cells were labeled moderately [up to 10 times background] (Fig. 1C). Many glandular epithelial cells of the seminal vesicles (Fig. 1D) and prostate (Fig. 1E) were labeled, but the intensity was weaker (up to 5 times background). Smooth muscle cells were labeled frequently in the ductus deferens, but only rarely in the seminal vesicles and prostate. Very high concentrations of silver grains were found over connective tissue areas of the ductus deferens, penis, and prostate, and a few fibroblasts within these areas were weakly labeled. In the epidermis of the penis (Fig. 1F), a few cells of the stratum Malpighii were weakly labeled.

Table 2. Comparison of autoradiographic labeling indices (% of neurons labeled) in different brain regions of two male rhesus monkeys (means ± SE)

Region	Male 1	Male 2
Medial preoptic n.	52 ± 6.4	55 ± 2.8
Bed n. of stria terminalis	33 ± 3.3	46 ± 1.2
Lateral septal n.	30 ± 6.7	38 ± 1.5
Anterior hypothalamic area	35 ± 1.2	47 ± 6.8
Ventromedial n.	59 ± 3.3	60 ± 10.1
Arcuate n.	41 ± 8.8	51 ± 6.9
Cortical amygdaloid n.	41 ± 8.2	43 ± 5.5
Medial amygdaloid n.	46 ± 4.7	46 ± 6.4
Lateral amygdaloid n.	10 ± 6.8	16 ± 10.6
Hippocampus (region CA2)	8 ± 1.8	7 ± 1.5

Means were obtained by counting 100 neurons in each of 3 sections for each region (6000 neurons).

Table 3. Concentrations of ether-extractable radioactivity and the percentages of radioactive steroids in nuclear and supernatant fractions from brain regions and peripheral tissues analyzed by HPLC

Brain region or tissue	Male no.	Nuclear fraction		Supernatant fraction		
		dpm/mg DNA	% DHT	dpm/mg protein	% A-diol	% DHT
Hypothalamus	2	15093	81.2	684	23.1	45.6
	3	9165	88.1	1098	23.9	67.2
Preoptic area	2	12230	90.8	662	17.8	50.8
and bed nucleus	3	8421	83.2	992	25.9	59.3
Septum	2	I.C.		878	16.0	33.8
	3	7590	92.7	899	16.8	56.8
Basal forebrain	2	I.C.		800	10.7	29.9
	3	6722	73.4	649	30.5	49.6
Amygdala	2	4466	95.1	485	25.4	35.9
	3	4951	88.4	722	25.6	50.5
Mammillary	2	I.C.		906	22.2	39.2
body area	3	3638	103.3	1142	26.4	48.9
Thalamus	2	2668	98.7	575	30.1	40.9
	3	3850	71.2	702	40.3	63.4
Hippocampus	2	I.C.		542	32.8	34.5
	3	2662	92.4	534	33.9	44.7
Inf. temporal	2	1932	108.1	502	34.6	41.8
cortex	3	2130	74.5	674	39.2	40.5
Caudate	2	I.C.		496	34.1	41.5
	3	1818	83.2	622	23.9	53.7
Postcentral	2	1427	60.2	547	24.2	49.4
cortex	3	1434	112.5	761	32.2	52.5
Putamen	2	1226	88.8	485	26.3	44.5
	3	1096	105.2	622	32.3	46.5
Cingulate	2	804	84.9	605	19.9	53.9
cortex	3	1081	77.6	1021	34.2	46.7
Cerebellar	2	395	91.2	394	22.8	38.0
cortex	3	330	86.4	534	20.9	55.3
Anterior	2	10909	90.5	994	4.9	67.5
pituitary	3	15080	97.3	1363	3.3	85.3
Posterior	2	29642	98.8	758	0.0	47.3
pituitary	3	20230	95.3	1204	0.0	72.5
Seminal vesicles	2	28354	92.2	2322	28.0	28.6
	3	32107	102.1	2669	3.5	80.8
Glans penis	2	33344	93.1	1935	32.0	40.9
	3	42031	95.2	1709	6.9	68.3
Prostate	2	31137	93.9	2277	9.1	28.8
	3	52768	97.1	1969	11.2	63.8

% A-diol = Percentage of ether-extractable radioactivity in the HPLC fraction corresponding to 5α-androstane-3α,17β-diol.
% DHT = Percentage of ether-extractable radioactivity in the HPLC fraction corresponding to 5α-dihydrotestosterone.
I.C. = insufficient counts of radioactivity for analysis.
Two male rhesus monkeys 60 min after the administration of 2 mCi [^{3}H]dihydrotestosterone.

Analysis of radioactivity in nuclear fractions

Brain. There was considerable regional variation in the concentrations (dpm/mg DNA) of ether-extractable radioactivity detected in cell nuclei isolated from the brains of Males 2 and 3. Highest concentrations in both males occurred in the hypothalamus (mean: 12,129 dpm/mg DNA) and lowest concentrations in both males occurred in cerebellar cortex (mean: 363 dpm/mg DNA). In Table 3, results are presented for all brain regions in descending order of concentration of radioactivity. Radioactivity in the DHT fraction accounted for 88.3 ± 2.6% of the ether-extractable radioactivity, and no radioactivity was detected in the estradiol fraction of any brain region. Only in cerebellar cortex was any radioactivity detected in the T (Males 2 and 3, mean: 5.9%) or androstanediol (Male 2, 5.1%) fractions. Radioactivity in the androstanedione fraction accounted for a proportion of the ether-extractable radioactivity only in inferior temporal cortex (17.4%) and putamen (19.3%) of Male 2 and in the hypothalamus (6%) and amygdala (3.1%) of Male 3. To facilitate comparisons between animals, concentrations of [^{3}H]DHT in cell nuclei were expressed as fmol/mg DNA using the assumption that all the radioactivity in the DHT fraction was in the form of [^{3}H]DHT (Fig. 2). Nuclear concentrations of [^{3}H]DHT showed the same rank order as the concentrations of ether-extractable radioactivity in Table 3, being highest in the hypothalamus and lowest in the cerebellar cortex. Although absolute concentrations differed somewhat in the two males, they both showed the same rank order of nuclear concentrations.

Pituitary gland and genital tract. The results for these tissues are shown in Table 3. Concentrations of ether-extractable radioactivity in isolated cell nuclei ranged between a mean of 12,995 dpm/mg DNA in the anterior pituitary and a mean of 41,953 dpm/mg DNA in prostate; these values were higher than for any brain region. Radioactivity in the DHT fraction accounted for 95.6 ± 1.1% of the radioactivity injec-

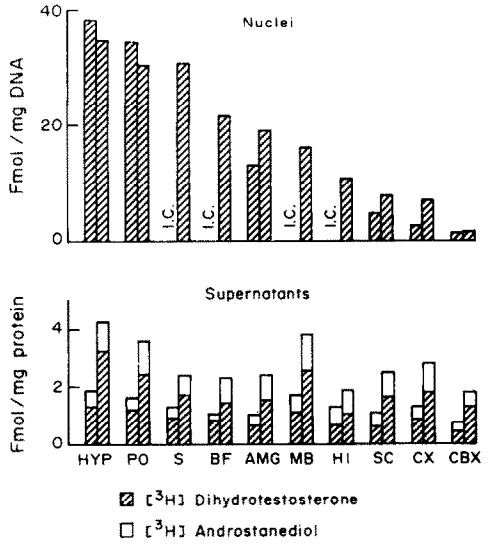

Fig. 2. Concentrations of [^{3}H]dihydrotestosterone (hatched part of histograms) and of [^{3}H]androstanediol (open part of histograms) in cell nuclei and in supernatant fractions of brain regions 60 min after the administration of 2 mCi [^{3}H]dihydrotestosterone to 2 adult castrated male rhesus monkeys. HYP = hypothalamus; PO = preoptic area with bed nucleus of stria terminalis; S = septum; BF = basal forebrain; AMG = amygdala; MB = mammillary body area; HI = hippocampus; SC = subcortical areas; CX = cerebral cortex; CBX = cerebellar cortex. I.C. = insufficient counts. For each region, left histogram = Male 2, right histogram = Male 3.

ted into the HPLC. Only in the posterior pituitary of Male 2 was any radioactivity (9.4%) recovered in the estradiol fraction. Small amounts of radioactivity, less than 4% of the amount injected, were detected in the T, androstanediol or androstanedione fractions from tissues of both males. Nuclear concentrations of [^{3}H]DHT (dpm/mg DNA) in pituitary gland and in genital tract structures were much higher than in brain (Figs 2 and 3). Levels for Male 2 in pituitary gland and genital tract structures were consistently lower than those for Male 3.

Analysis of radioactivity in extranuclear fractions

Brain. Levels of radioactivity in supernatant fractions from brain tissues showed less regional variation than the radioactivity in nuclear fractions (Table 3). Concentrations of total radioactivity ranged between a mean of 1175 dpm/mg protein in the mammillary body area and 675 dpm/mg protein in the hippocampus. Concentrations of ether-extractable radioactivity ranged between 1024 dpm/mg protein in the mammillary body area and 464 dpm/mg protein in cerebellar cortex and represented 85.5 ± 2.0% in Male 2 and 77.1 ± 2.1% in Male 3 of the total radioactivity in all brain samples. Mean concentrations of ether-extractable radioactivity were lower in Male 2 (611 ± 42 dpm/mg protein) than in Male 3 (784 ± 55 dpm/mg protein). The DHT fraction contained more radioactivity than any other fraction, but it represented less than half (47.0 ± 1.7%, $N = 28$) of the total radioactivity injected into the HPLC. Although the identity of the small amounts of radioactivity in the other fractions could not be determined with certainty, radioactivity in the androstanediol fraction represented 26.6 ± 1.4% of the total and was detected in all samples. Concentrations of [^{3}H]DHT and [^{3}H]androstanediol in supernatants are shown in Fig. 2 for comparisons between males. Similarly, radioactivity in the androstanedione fraction represented 4.6 ± 0.9% of the total and was detected in 24 of the 28 samples, while some radioactivity was detected in the estradiol (2.2 ± 0.7%) and T (3.8 ± 0.8%) fractions of some samples.

Pituitary gland and genital tract. Concentrations of ether-extractable radioactivity were generally higher in pituitary gland and genital tract structures than they were in brain tissue (Table 3). However, ether-extractable radioactivity represented a smaller percentage (Male 2: 43.0 ± 5.2%, Male 3: 42.9 ± 3.1%) of the total radioactivity in peripheral organs than in brain, possibly because of greater contamination with conjugated radioactive steroids in blood. The distribution of radioactivity in the androstanediol and DHT fractions differed considerably in different tissues and between the two males, and Fig. 3 gives the concentrations of [^{3}H]DHT and [^{3}H]androstanediol in supernatants for purposes of comparison.

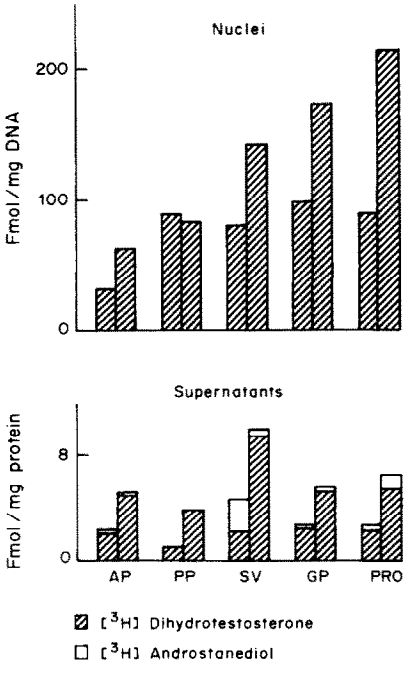

Fig. 3. Concentrations of [^{3}H]dihydrotestosterone (hatched part of histograms) and of [^{3}H]androstanediol (open part of histograms) in cell nuclei and in supernatant fractions of pituitary gland and genital tract tissues 60 min after the administration of 2 mCi [^{3}H]dihydrotestosterone to 2 adult castrated male rhesus monkeys. AP = anterior pituitary; PP = posterior pituitary; SV = seminal vesicles; GP = glans penis; PRO = prostate. For each tissue, left histogram = Male 2, right histogram = Male 3.

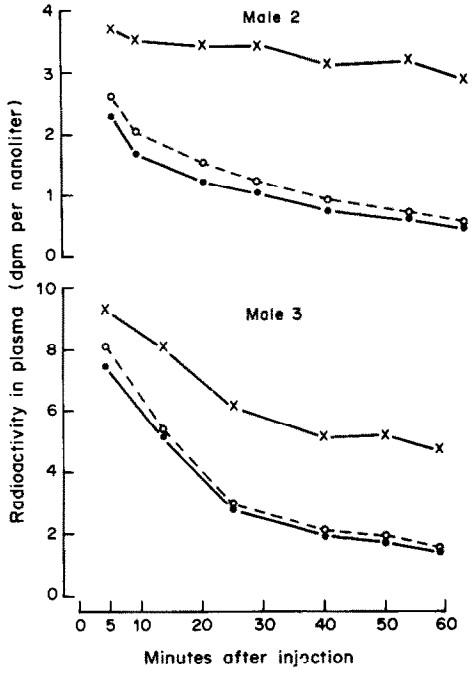

Fig. 4. Concentrations of radioactivity in blood plasma from two castrated male rhesus monkeys after the injection of 2 mCi [3H]dihydrotestosterone: × —— × = total radioactivity; O----O = ether-extractable radioactivity; ●——● = radioactivity in the dihydrotestosterone fraction after HPLC.

Analysis of radioactivity in plasma samples

These results are given in Fig. 4. In Male 2, total radioactivity in plasma declined by 21% during the period betweeen 5 min and 58 min after the injection, and in Male 3, it declined by 51% during the period between 4 min and 59 min after the injection. Radioactivity recovered in the DHT fraction accounted for most of the ether-extractable radioactivity in plasma throughout the survival period in both males, and levels of total radioactivity and of [3H]DHT were considerably lower in Male 2 than in Male 3. In Male 2, concentrations of [3H]DHT declined by 78% during the period between 5 and 58 min after the injection, and in Male 3, it declined by 80% during the period between 4 and 59 min after the injection.

DISCUSSION

The autoradiographic results for the two males were in good general agreement. The labeling index data in Table 2 differed somewhat from those in the preliminary report [1], but the present results were based on counts of 300, rather than 50, neurons for each brain region. On superficial examination the autoradiograms of brain following [3H]DHT and those following [3H]T administration in earlier experiments [3] appeared quite similar, but closer scrutiny revealed important differences. The labeling in Fig. 5 is quantitatively accurate (each dot representing the location of 6 labeled cells), and on the left of the figure are given the results (autoradiography and tissue analyses) following the administration [3H]DHT, while on the right are given the results following [3H]T administration. It is immediately obvious that the distribution of labeled neurons following [3H]DHT injection was much more widespread than that following [3H]T injection. Following [3H]DHT administration, labeling was observed in all of the regions labeled following [3H]T administration, including the medial preoptic n., bed n. of the stria terminalis, anterior hypothalamic area, ventromedial n., posterior hypothalmic area, premammillary n., cortical, medial, and accessory basal amygdaloid n., and periaqueductal gray. However, many additional areas were labeled in the present study following [3H]DHT administration, including the lateral septal n., arcuate n., paraventricular n., dorsomedial n., lateral hypothalamic area, supramammillary n., intercalated mammillary n., anterior amygdaloid area, basal and lateral amygdaloid n., region CA2 of hippocampus, red n., interpeduncular n., parts of the thalamus (particularly the "diffuse" thalamic and association n.), and several motor and sensory n. of cranial nerves. In an attempt to increase the labeling of neurons by [3H]T we administered a larger dose (5 mCi) and reduced the survival time to 30 min (unpublished results). This had no significant effect on the distribution of labeled cells. Increasing the survival time might have allowed the accumulation of metabolites of [3H]T, particularly [3H]DHT, but no autoradiographic data are available for the primate brain at survival periods longer than 60 min. However, after continuous infusion of [3H]T for 4 h, unchanged [3H]T, not [3H]DHT, is still the predominant form of radioactivity recovered from the primate brain [19].

The localization of [3H]DHT-concentrating cells in the pars distalis, pars intermedia, and pars nervosa of the pituitary gland in male rhesus monkeys agreed with previous autoradiographic data in females [20], but labeling indices for all three lobes in the present study were considerably lower than those reported for the male baboon [21]. These differences may reflect procedural as well as species differences. DHT was the major metabolite of T in the male reproductive tract, and between 86 and 99% of the radioactivity in the nuclear fractions from seminal vesicles, prostate, and glans penis was in the form of DHT [8]. In the present study, the pattern of autoradiographic labeling following [3H]DHT was identical with that following [3H]T administration (unpublished results), and labeled cells were seen in the epithelium, smooth muscle, and connective tissue of the ductus deferens, seminal vesicles, and prostate, and in the connective tissue and epidermis of the penis. A similar localization of radioactivity following [3H]DHT administration was described for the male baboon [22].

The biochemical data were quite consistent with

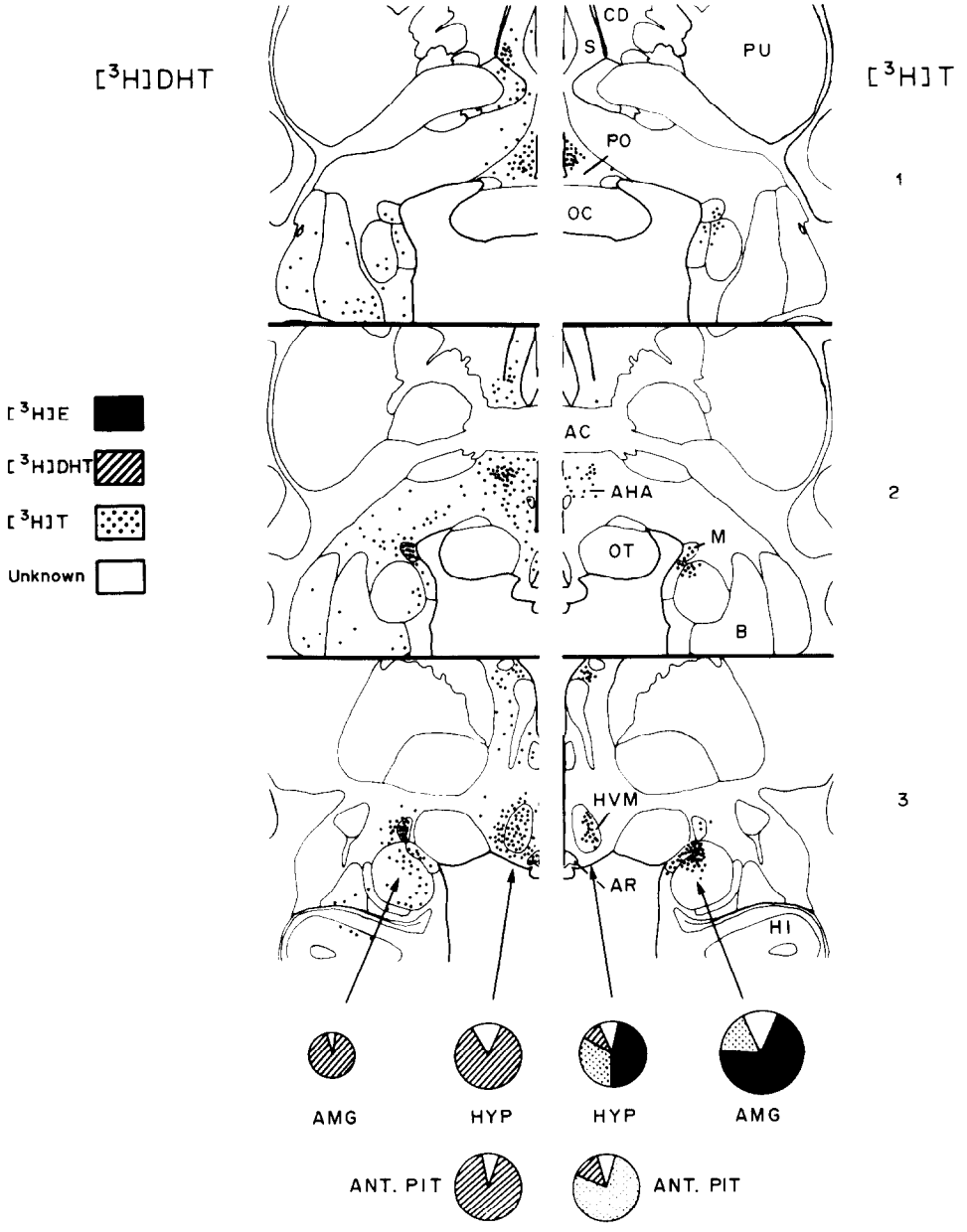

Fig. 5. Autoradiograms at three representative levels of the diencephalon of the male rhesus monkey: 1 preoptic area, 2 anterior hypothalamus, and 3 mid-hypothalamus. Autoradiograms following the administration of [^{3}H]DHT (left side) showed much more widespread labeling of cells than autoradiograms following the administration of [^{3}H]T (right side) [3]. Each dot represents six labeled neurons. The areas of the circles at the bottom of the figure are proportional to the amounts of ether-extractable radioactivity in nuclei from brain regions indicated by the arrows. The steroids identified by HPLC are shown in the key and the percentages of these steroids are shown by the areas of the segments within the circles. Corresponding data for the anterior pituitary gland are given at the bottom of the figure. [^{3}H]DHT was the only form of radioactivity identified in nuclei following [^{3}H]DHT administration, whereas [^{3}H]estradiol and [^{3}H]T predominated in nuclei after the administration of [^{3}H]T. AC, anterior commissure; AHA, anterior hypothalamic area; AMG, amygdala; ANT. PIT, anterior pituitary; B, basal amygdaloid n.; CD, caudate; HI, hippocampus; HVM, ventromedial n.; HYP, hypothalamus; M, medial amygdaloid n.; OC, optic chiasm; OT, optic tract; PO, preoptic area; PU, putamen; S, septum.

the autoradiographic data, and there was agreement between brain areas with high labeling indices and those with high nuclear concentrations of radioactivity, although the anatomical resolution of the biochemical techniques was relatively crude. The very low amounts of radioactivity recovered from brain cell nuclei precluded the rigorous identification of metabolites by mass spectroscopy and, in these experiments, we have relied on high performance liquid chromatography; however, collaborative HPLC–MS

studies using derivatives are planned. The present results strongly suggested that the great majority of the steroid bound in cell nuclei was unchanged [3H]DHT. None of the radioactivity in nuclei (with the exception of cerebellar cortex) was in the form of androstanediol despite its high concentration in supernatant fractions (Table 3). Thus, DHT appears to enter the primate brain and act directly on neuronal nuclei without undergoing any prior metabolic conversion. It also seems clear that DHT, unlike T, was not sufficiently converted to estradiol in the primate brain to act in the cell nucleus as an estrogen. Although a small amount of radioactivity was recovered from supernatants in the estradiol fraction, none was recovered as estradiol from the nuclei. In supernatants, the identity of the small amounts of radioactivity detected in the estradiol and T fractions remains unknown, but it presumably represented polar metabolites of DHT. These studies have established that DHT is a substantially non-aromatizable androgen in the primate brain.

The analysis of blood samples revealed considerable differences between the two males used in the biochemical studies (Fig. 4). These differences could only be partly explained by the heavier body weight of Male 2 and by a slight loss of [3H]DHT on injection. The higher tissue levels of radioactivity reflected in the supernatant fractions in Male 3 were presumably related to the higher blood levels. The concentrations of [3H]DHT in plasma collected between 4 and 6 min after the bolus of isotope was administered were comparable to the concentrations of [3H]T in previous studies. However, blood levels of [3H]DHT declined somewhat more slowly than blood levels of [3H]T, and this was consistent with the finding that DHT has a lower metabolic clearance rate than T in primates [23].

Tissue levels (supernatant values) of radioactivity were 6–10-fold higher for [3H]T than for [3H]DHT, and this agreed with the higher background counts in autoradiograms following [3H]T. In contrast, the nuclear labeling of neurons was both less intense and more restricted following [3H]T administration but, as shown in Fig. 5, much of this radioactivity was in the form of [3H]estradiol. The nuclear concentrations of androgens were 2 to 3 times higher in brain following [3H]DHT than following [3H]T whereas they were quite similar in the pituitary gland. However, following [3H]T, radioactivity in the pituitary gland was mostly in the form of [3H]T, while following [3H]DHT it was in the form of [3H]DHT. In genital tract structures, [3H]DHT was the major nuclear metabolite following the administration of both androgens. In the intact rhesus monkey, the ratio of plasma concentrations of T and DHT is about 4:1 during the day and about 9:1 at night [24], and the results reported here suggest that circulating DHT may be a significant source of androgen in the brain. In primates, the relative lack of effectiveness of DHT in restoring the sexual behavior of male castrates cannot be related to an inability to enter the nuclei of neurons in relevant areas of the brain.

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