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DISTRIBUTION OF ANDROGEN TARGET CELLS IN RAT FOREBRAIN AND PITUITARY AFTER [^{3}H]-DIHYDROTESTOSTERONE ADMINISTRATION

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SUMMARY

The distribution of androgen concentrating cells in male rat forebrain and pituitary was studied by autoradiography. One h after injection of a non-aromatizable androgen [^{3}H]-dihydrotestosterone(17β-hydroxy-5α-androstan-3-one) (DHT), nuclear concentration of radioactivity is found in neurons at specific hypothalamic and extrahypothalamic sites as well as in certain anterior pituitary cells. In the forebrain radioactively labeled cells are found in certain nuclei of the septum, preoptic region, anterior and central hypothalamus, mammillary body, epithalamus, amygdala and hippocampus. Furthermore, nuclear labeling is observed in ventricular recess organs, including the pituitary, the pineal and the subfornical organ.

Competition studies with unlabeled estradiol-17β at a dose similar to the labeled DHT has no or little effect on the nuclear uptake of radioactivity in neurons, whereas a dose of estradiol-17β ten times higher than the dose of the labeled DHT reduces the nuclear uptake in certain neuronal groups by 20–25%. Unlabeled DHT inhibits nuclear uptake of radioactivity. The autoradiographic results suggest that DHT is one of the major active metabolites responsible for the central action of testosterone.

INTRODUCTION

Actions of testosterone on the regulation of gonadotropin secretion and sexual behavior in male animals have been thought to be mediated through metabolites rather than the hormone itself. This is supported by the finding that testosterone can be metabolized to androstenedione (4-androstene 3,17-dione) and 5α-dihydrotestosterone or aromatized to estrogen by brain tissue[1–4]. Substantial amounts of DHT, androstenediol and testosterone have been extracted from rat brain following infusion of [^{3}H]-testosterone[5]. After [1,2 ^{3}H]-testosterone injection, uptake of radioactivity into nuclei of neurons in hypothalamic and extrahypothalamic sites, as well as in anterior pituitary cells has been demonstrated[6–8]. Since estrogen can be produced from testosterone in the brain, the notion exists that all or some of the central actions of testosterone are mediated through estrogen rather than androgen[9].

This report provides further evidence of androgen target cells in the forebrain and pituitary after injection of [^{3}H]-dihydrotestosterone (DHT), a non-aromatizable androgen, with or without prior administration of estradiol-17β as potential competitor, using autoradiographic techniques developed in our laboratory for the study of non-covalently bound compounds[10].

MATERIALS AND METHODS

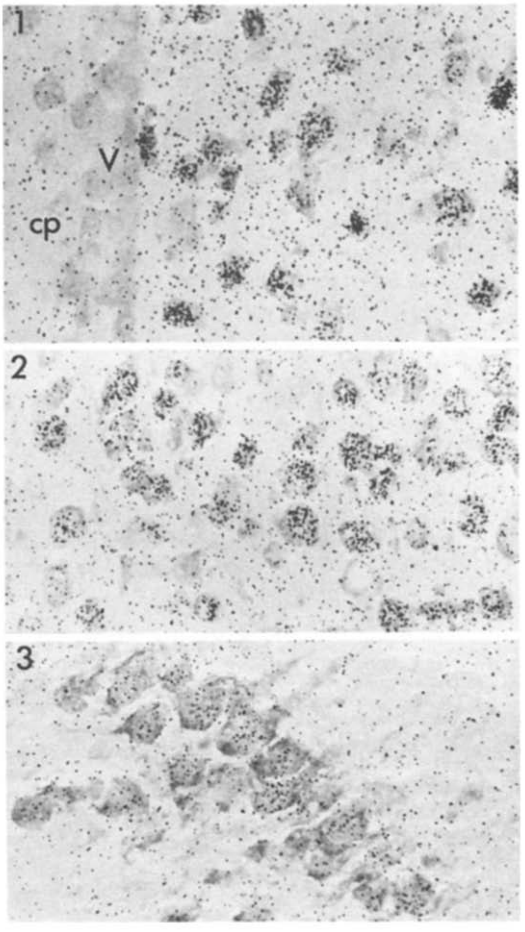
Six 26-day old and three 60-day old male Holtzman rats, orchiectomized and adrenalectomized for 96 h, were each injected intravenously with 1 or 2 μg per 100 g body weight of [1,2α-^{3}H]-dihydrotestosterone, S.A. 44 Ci/m-mol (New England Nuclear), dissolved in 10% ethanol in isotonic saline. The radiochemical purity was established by thin layer chromatography using the solvent system of benzene–ethyl acetate (2:1, v/v) and found to be greater than 98%. In order to examine whether or not estradiol can inhibit the nuclear uptake of radioactivity after [^{3}H]-dihydrotestosterone injection, two immature rats were each injected intravenously 5 min prior to the labeled DHT with 2 μg or 20 μg per 100 g body weight of estradiol-17β, dissolved in ethanol. In addition, two orchiectomized and adrenalectomized immature rats were injected intravenously, each with 100 μg of unlabeled DHT per 100 g body weight 5 min prior to the injection of [^{3}H]-DHT, to show the specificity of androgen localization.

One hour after injection of [^{3}H]-dihydrotestosterone, the rats were killed, the brain and pituitary removed, mounted on tissue holders, and frozen in $-180°$ C liquefied propane. Four μm serial frozen sections were cut in a Wide Range Cryostat (Harris Mfg. Co., North Billerica, MA) and dry- or thaw-mounted on photographic emulsion (Kodak NTB-3) coated slides. The brain tissues and pituitary from an untreated orchiectomized and adrenalectomized rat were also processed as control for chemographic artifacts. After autoradiographic exposure for 4–9 months, the slides were photographically processed and stained with methylgreen pyronin for DNA and RNA[10].

Quantification. The average number of silver grains in nuclei of neurons was determined by counting the number of reduced silver grains in 50–100 labeled neurons of a specific nucleus, using 100 × objective, after correcting for background radioactivity. The background radioactivity was determined by assessing the number of silver grains per square μm in areas not occupied by tissue sections. Assuming that nuclei of neurons are round, the nuclear area was expressed in square μm and the background radioactivity per nucleus was calculated by multiplying the number of background silver grains per unit area with the total nuclear area. The value for nuclear concentration of radioactivity was obtained by subtracting the background radioactivity from total radioactivity. The student *t*-test was utilized to determine the significant level between control and estrogen treatment groups. Each labeled neuron of a particular hypothalamic nucleus was considered as one sample when significance levels were determined between identical hypothalamic nuclei of different groups. A cell was considered labeled when the nucleus contained four or five times the number of silver grains per unit area above background.

RESULTS

Autoradiograms of brain tissue of orchiectomized and adrenalectomized immature and mature male rats show concentration and retention of radioactivity in nuclei of certain neurons and ependymal and subependymal cells in certain regions, as well as cells of the pia mater (Figs 1–3). The topographic distribution of the labeled cells appears similar in immature and mature rats (Figs 4 and 5). In the area of the lamina terminalis radioactively labeled neurons are found ventrally in the nucleus (n.) tractus diagonalis and in the region of the optic recess organ, and dorsally in the n. septi lateralis (Figs 1 and 4) and n. triangularis septi. In the hippocampus neurons in the pyramidal layer show radioactive labeling throughout (Fig. 3), with neurons in CA1 and CA2 displaying stronger accumulation of radioactivity when compared with CA3, CA4 and subiculum, while under the same conditions, neurons of the dentate gyrus are not labeled (Fig. 5). In the amygdala, neurons of the n. amygdaloideus medialis are strongly labeled in contrast to the weakly labeled neurons of the n. amygdaloideus centralis, n. basalis pars medialis, and n. corticalis (Fig. 5). In the preoptic area, labeled neurons are concentrated in the n. preopticus medialis and lateralis (Figs 2 and 4). Accumulations of labeled neurons exist further in the n. interstitialis striae terminalis, n. periventricularis hypothalami, n. paraventricularis, n. ventromedialis, n. arcuatus and n. premammillaris ventralis (Fig. 5). Dispersed labeled neurons are also seen in the n. dorsomedialis, the n. perifornicalis and the zona incerta, the anterior, lateral and posterior hypothalamic areas, further in the n. premammillaris dorsalis, n. prelateralis mammillaris, n. supramammillaris, n. mammillaris posterior, n. parafascicularis and the periventricular gray. Certain cells of the organum subfornicale, n. habenulae medialis and pineal are labeled. Cells of the cerebral cortex do not appear to be labeled under the present experimental condition.

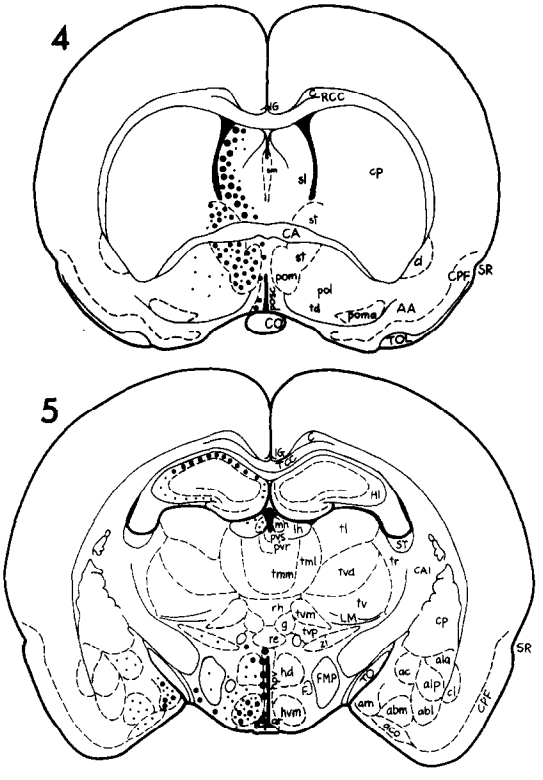


Figs. 1–3. Autoradiograms of rat prepared 1 h after intravenous injection of [^{3}H]-dihydrotestosterone into orchiectomized and adrenalectomized rats showing nuclear concentration of radioactivity in neurons of nucleus septi lateralis (Fig. 1), in neurons of nucleus preopticus medialis (Fig. 2), and in pyramidal cells of hippocampus (Fig. 3). Exposure time 270 days. Magnification × 520. Stained with methyl-green pyronin. cp is nucleus caudatus putamen; v is ventricle.

In the anterior pituitary only a small percentage of cells (approximately 10–15%) are labeled after injection of [^{3}H]-DHT (Fig. 6). These cells are identified as gonadotropes by immunocytochemical staining using antisera against ovine LH or its β-subunit (unpublished observation). The results are comparable to those obtained with [^{3}H]-testosterone[7, 8].

Differences in the intensity of neuronal nuclear labeling exist among different nuclear areas of the brain (Table 1). For instance, the intensity of subcellular nuclear labeling in neurons is highest in the nucleus (n.) septi lateralis, n. premammillaris ventralis, and n. periventricularis hypothalami; it is medium in neurons of the n. interstitialis striae terminalis, n.

preopticus medialis and lateralis, and n. arcuatus hypothalami; and it is medium to low in neurons of the n. ventromedialis hypothalami, n. medialis amygdalae and the pyramidal layer of the hippocampus (Table 1). Nuclear concentration of radioactivity in neurons is inhibited when unlabeled 5α-DHT was injected before the injection of [^{3}H]-DHT. Administration of unlabeled estradiol-17β at a dose similar to the labeled DHT 5 min prior to the injection of labeled DHT has no or little effect on the nuclear uptake of radioactivity in neurons, whereas a dose of estradiol-17β ten times higher than the dose of labeled DHT reduces the nuclear concentration of radioactivity in such areas as n. septi lateralis ($P < 0.05$), n. preopticus medialis ($P < 0.01$), and n. premammalaris ventralis ($P < 0.01$) by 20–25% (Table 1, Figs. 7–9).



Figs. 4 and 5. Schematic drawings showing distribution of androgen concentrating neurons in preoptic region (Fig. 4) and in central hypothalamus (Fig. 5), in frontal plane 1 h after injection of [^{3}H]-dihydrotestosterone. The size and number of dots indicates the intensity of nuclear uptake of radioactivity and the frequency of occurrence of androgen concentrating cells. The schematic drawings were prepared after serial section autoradiograms according to the atlas of König and Klippel[23]. Abbreviations: abm, nucleus (n) amygdaloideus basalis pars medialis; ac, n. amygdaloideus centralis; aco, n. amygdaloideus corticalis; am, n. amygdaloideus medialis; ar, n. arcuatus hypothalami; CA, Commissura anterior; CO, chiasma opticuum; FMP, Fasiculus medialis prosencephali; FO, Fornix; hd, n. dorsomedialis hypothalami; HI, hippocampus; hpv, n. periventricularis hypothalami; hvm, n. ventromedialis hypothalami; mh, n. medialis habenulae; pol, n. preopticus lateralis; pom, n. preopticus medialis; posc, n. preopticus pars suprachiasmatica; sl, n. septi lateralis; st, n. interstitialis striae terminalis; zi, zona incerta. For other abbreviations see König and Klippel[23].

DISCUSSION

The localization of radioactivity as demonstrated here is androgen since unlabeled estradiol-17β in similar doses as labeled DHT injected prior to [^{3}H]-DHT does not inhibit the nuclear uptake of radioactivity and 5α-dihydrotestosterone, an A-ring reduced steroid, is not convertible to estrogen. These results are consistent with the biochemical findings of specific 5α-dihydrotestosterone binding protein in the cytosol fraction of male rat hypothalamus, pineal and pituitary[11–13] and the association of DHT with the nuclear fraction of "hypothalamus" and pituitary[14]. Only a dose of estradiol-17β higher than the dose of [^{3}H]-DHT reduces the nuclear uptake of radioactivity in certain nuclear groups. These data suggest that the DHT receptor has some low affinity for estradiol, although further experiments are required in order to establish more precisely the receptor specificity.

The present autoradiographic results for the first time describe the topographical distribution of androgen target cells obtained with [^{3}H]-DHT. These data in general agree well with our earlier observation on androgen localization in the brain after injection of [^{3}H]-testosterone[6, 15]. However, localization of radioactivity in cells of cerebral cortex was not observed after [^{3}H]-DHT. In contrast, weakly labeled cells in certain regions of cerebral cortex have been found after [^{3}H]-testosterone[15]. The absence of labeling in cortical cells may be attributed to the use of labeled DHT with low S.A. or the cortical cells may have specific affinity for testosterone binding. The concentrated radioactivity in neurons is likely to be DHT, since the major metabolite recovered from brain tissue 30 min after intravenous injection or 3 h after continuous infusion of labeled DHT is DHT[16, 17] and prior injection of unlabeled DHT reduces the nuclear uptake of radioactivity. Whether or not other metabolites of DHT, such as androstenediol and androstenedione have an effect on [^{3}H]-DHT uptake has not been investigated, although androstenedione has been shown to inhibit the nuclear uptake of radioactivity obtained with [^{3}H]-testosterone[15].

The autoradiographic demonstration of androgen-concentration in specific regions and cell types in the brain, as well as in the pituitary, suggests a direct action of DHT on these structures. It further supports the concept that DHT is one of the major active metabolites and important for the central action of "testosterone", as it has been shown for other androgen-responsive tissues such as prostate and seminal vesicles. Dihydrotestosterone has been found to be more potent than testosterone, suppressing serum LH levels while equipotent to testosterone in suppressing

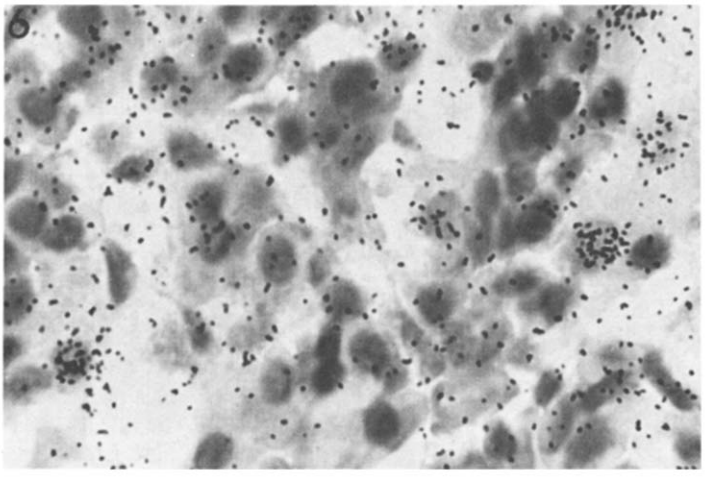


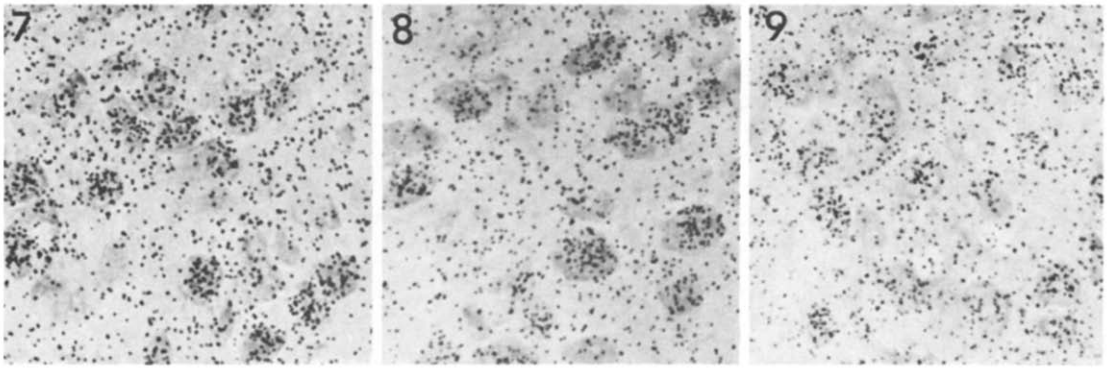
Fig. 6. Autoradiogram of rat anterior pituitary showing nuclear concentration of radioactivity in a few cells, after intravenous injection of [^{3}H]-dihydrotestosterone. Exposure time 110 days. Magnification ×520. Stained with methylgreen pyronin.

FSH[18] and comparable to testosterone in maintaining mating behavior in hypophysectomized rats[19] and in stimulating sexual behavior in guinea pig[20] and Rhesus monkey[21]. Also pretreatment with 5α-DHT has been reported to increase pituitary LH release by LH-RH[22].

Table 1. Effects of estradiol-17β on nuclear concentration of radioactivity in neurons of rat brain one h after injection of [^{3}H]-dihydrotestosterone 2 μg/100 g body weight

Structure	Control (2)* (mean ± S.E.)	Estradiol-17β (2)* 2 μg/100 g b.wt (mean ± S.E.)	Estradiol-17β (2)* 20 μg/100 g b.wt (mean ± S.E.)
n. septi lateralis	21.31 ± 2.22	19.53 ± 1.72	16.12 ± 1.08†
n. interstitialis striae terminalis	17.05 ± 2.03	15.36 ± 1.20	13.20 ± 1.60
n. preopticus medialis	18.09 ± 1.55	15.90 ± 1.11	12.57 ± 0.08‡
n. periventricularis hypothalami	22.00 ± 2.15	21.18 ± 2.12	18.50 ± 1.20
n. arcuatus hypothalam	14.46 ± 1.29	15.00 ± 0.54	13.58 ± 1.67
n. ventromedialis hypothalami	10.82 ± 2.05	10.25 ± 0.44	8.89 ± 0.51
n. premammillaris ventralis	22.90 ± 1.81	19.60 ± 1.32	17.05 ± 1.33‡
n. amygdaloideus medialis	11.14 ± 0.92	11.00 ± 0.85	10.23 ± 0.79
hippocampus (area CA-1)	10.30 ± 0.68	9.20 ± 0.40	8.80 ± 0.91

* The number in parentheses indicates the number of animals. Unlabeled estradiol-17β dissolved in ethanol was injected intravenously 5 min prior to the injection of [^{3}H]-dihydrotestosterone. The mean value for nuclear concentration of radioactivity is based on counting of silver grains in 50–100 randomly selected labeled neurons after correcting for background radioactivity. Exposure time 120 days. S.E.: standard error. Significantly differ from the control, †$P < 0.05$; ‡$P < 0.01$.



Figs 7–9. Autoradiograms of the nucleus preopticus medialis prepared 1 h after injection of [^{3}H]-DHT showing the competitive effects of 2 μg (Fig. 8) or 20 μg (Fig. 9). Estradiol-17β on nuclear uptake of radioactivity. Exposure time 270 days; thickness 4 μm; magnification ×520; stained with methylgreen pyronin. Note the reduction of nuclear concentration of radioactivity after 20 μg of estradiol-17β (Fig. 9), and the lack of reduction after 2 μg of estradiol-17β (Fig. 8) as compared to the control without estradiol-17β pretreatment (Fig. 7).

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