Aromatization and 5α-Reduction of Androgens in Discrete Hypothalamic and Limbic Regions of the Male and Female Rat[1]

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ABSTRACT. The *in vitro* aromatization and 5α-reduction of androgens to estrogens and dihydrotestosterone (DHT) were determined in incubations of microdissected brain regions of male and female gonadectomized, adrenalectomized rats. Metabolites formed from [1α,2α-^{3}H]androstenedione or [1α,2α-^{3}H]testosterone were purified by celite liquid-liquid partition chromatography, silica gel chromatography and recrystallization to stable ^{3}H/^{14}C ratios. The medial preoptic nucleus-anterior hypothalamic nucleus exhibited the highest aromatase activity and the second highest conversion to DHT. The lateral preoptic and lateral hypothalamic nuclei showed little aromatase activity yet exhibited high rates of formation of DHT. The medial basal hypothalamus showed the second highest level of aromatase activity but consistently formed the lowest amount of DHT. The discrete anatomical localization of these enzymatic conversions is suggestive of their being involved in the physiological actions of androgens. (*Endocrinology* **101**: 841, 1977)

THE CAPACITY of central nervous system tissue to aromatize androgens to estrogens (1) and to reduce testosterone (T) to dihydrotestosterone (DHT) (2) has been known for some time. In males, aromatization of androgens to estrone (E_1) and estradiol (E_2) has recently been demonstrated in hypothalamic and limbic structures of the rat, rabbit, rhesus monkey and human fetus (3).

Several lines of evidence support the hypothesis that intracerebral aromatization of androgens to estrogens is a requisite step in the central action of sex steroids in males of several mammalian species (4,5). Estrogen and aromatizable androgens maintain male sexual behavior (4), suppress gonadotropin levels in adult castrated males (6) and prevent the feminizing effects of neonatal castration in male rats (7). Dihydrotestosterone, a non-aromatizable androgen (8), does not sustain copulatory behavior in adult castrates (9), although reports to the contrary exist (10,11), nor does it block the feminizing effects of neonatal orchidectomy (12). However, DHT is a potent androgen in the differentiation (13) and maintenance of accessory sex organs (14) and in suppressing gonadotropin levels in castrated adult (15) and neonatal males (12). Finally, neurons taking up labelled T and E_2 in male and female rats exhibit considerable anatomical overlap (16,17) and specific E_2 and DHT binding macromolecules have been demonstrated in several brain regions of the adult male rat (18,19).

In the adult ovariectomized rat, lordosis behavior may be maintained with aromatizable androgens (4) while DHT is not effective in this action (20). Neonatally administered androgens and estrogens (7,21) but not DHT (12,22) will masculinize adult lordosis behavior and the action of tes-

Received October 26, 1976.

[1] Supported in part by NIH grants HD08924 and HD08692 and a center grant from The Rockefeller Foundation.

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Abbreviations used for the nuclear dissections are as follows: MPN—medial preoptic nucleus, AHN—anterior hypothalamic nucleus, LPN—lateral preoptic nucleus, LHN—lateral hypothalamic nucleus, MBH—medial basal hypothalamus and Am—amygdaloid complex.

TABLE 1. Dissection of discrete brain nuclei and regions

Nucleus or region*	Section numbers†	Cannula lumen diameter (μm)	Wet weight (mg)§	Protein content (mg)§
MPN-AHN	1, 2, & 3	2 × 500.0‡	15.0	1.18
			15.6	1.10
			19.8	0.98
			20.4	1.28
LPN	1 & 2	2 × 500.0	11.9	0.94
			—	1.03
			14.2	0.72
			10.6	1.02
MBH	6–10	2,100.0	—	6.00
			107.4	8.44
			94.3	8.55
			132.1	8.28
LHN	6–10	500.0	14.6	0.96
			13.4	1.05
			17.4	0.92
			19.3	1.21
Am	6–10	2,100.0	194.9	19.20
			232.2	22.70
			236.3	21.20
			209.1	22.00

* Abbreviations: MPM = medial preoptic nucleus; AHN = anterior hypothalamic nucleus; LPN = lateral preoptic nucleus; LHN = lateral hypothalamic nucleus; MBH = medial basal hypothalamus including the median eminence, arcuate nucleus, ventromedial nucleus and ventral aspects of the dorsomedial nucleus; Am = amygdaloid complex including the basal, central, lateral and medial amygdaloid nuclei.

† Rostral face of first section corresponded to König and Klippel A6860 (28).

‡ Two punches were taken for these nuclei on each side of the brain.

§ Values for the four Experiments in Table 3 are presented to demonstrate the consistency of the dissection.

tosterone propionate may be antagonized by the anti-estrogen MER-25 (22). Finally, in the female brain, 5α-reductase has been demonstrated in the neonatal rat (23) and aromatization has been observed in the adult rat and rabbit and in fetal and neonatal rats (3).

A large body of evidence in the male suggests that feedback effects of sex steroids occur in the medial basal hypothalamus while effects of these steroids on copulatory behavior involve a larger area including, in particular, the medial preoptic and anterior hypothalamic nuclei (17,24). We report here that these specific hypothalamic regions have a marked differential capacity for the conversion of androstenedione and testosterone to estrogens and dihydrotestosterone. Portions of this work have been previously presented (25,26).

Materials and Methods

Male and female Sprague-Dawley rats (Simonsen), 180–240 g in weight, were housed under a 14:10, L:D lighting schedule with lights on at 0500 h. Three days following gonadectomy and adrenalectomy, animals were decapitated between 1000 and 1600 h and their brains rapidly removed and frozen. Serial, 300 μm frontal sections were cut through the hypothalamus in a cryostat at −9 C and the brain dissected according to the method of Palkovits (27). The initial plane of section corresponded to König and Klippel A6860 (28). Brain nuclei and regions were removed bilaterally, with the exception of the medial basal hypothalamus, as detailed in Table 1. The wet weight and protein content values for four separate experiments demonstrate the consistency of the dissection. Brain fragments were expelled into 0.5 ml of ice-cold, Krebs-Ringer phosphate buffer, pH 7.4, containing 11 mM glucose that was equilibrated with 95% O_2 and 5% CO_2. Tissue from 19–25 rats was pooled for each experiment. Tissues were transferred to incubation tubes containing 1.0 μM [1α,2α-^{3}H]androstenedione or [1α,2α-^{3}H]testosterone (59 Ci/mmol; New England Nuclear). An NADPH-generating system consisting of 1 mM $NADP^+$, 10 mM glucose-6-phosphate and excess glucose-6-phosphate dehydrogenase was added and the final volume adjusted to 1.0 ml. The tubes were then incubated at 37 C for 3.5 h in a Dubnoff Metabolic Shaker. Incubation conditions were chosen on the basis of previous studies of the aromatase enzyme as it occurs in human placenta (29) and the 5α-reductase in human prostate (13). While the low levels of activity in rat brain preclude extensive characterization of the aromatase enzyme, there is no reason to believe that it differs markedly from the human enzyme (3). The reaction was stopped and the steroids extracted by the addition of 6.0 ml of chloroform per tube. The two phases were separated after 10 min of centrifugation at 3000 rpm. Known amounts of ^{14}C-labelled authentic marker steroids

TABLE 2. Confirmation of the identity of incubation products by celite and thin-layer chromatography and crystallization ($^3H/^{14}C$ ratios)*

	Experiment 1: Estrone						Experiment 2: Dihydrotestosterone					
	MPN-AHN	LPN	MBH	LHN	Am	Blank	MPN-AHN	LPN	MBH	LHN	Am	Blank
Column pool	122	34	107	42	187	27	92	110	90	105	430	1.50
TLC-1	130	25	92	37	139	13	93	106	86	103	425	1.48
TLC-2	50	10	61	9	92	6	100	123	83	106	442	1.49
TLC-3	37	9	64	8	96	6	95	115	91	105	418	2.21
Acetylation												
TLC-4	36	8	56	8	87	7	99	126	89	—	430	1.44
TLC-5	38	8	55	9	97	6	—	—	—	—	—	—
Mother liquor 1	39	17	57	12	80	10	87	95	85	—	401	1.68
Mother liquor 2	33	10	51	9	97	9	79	103	83	—	394	1.93
Mother liquor 3	28	7	56	8	77	5	83	95	86	—	379	1.60
Final crystals	34	7	48	6	81	5	92	104	87	—	406	1.20

* Typical purification values presented here are from male Experiment 1 in Table 3 (E_1) and Table 4 (DHT). Solvent systems used were as follows: TLC-1, 100% chloroform; TLC-2, 70:30 ethyl acetate: iso-octane; TLC-3, 13:1 chloroform: ethyl acetate; TLC-4, 70:30 iso-octane; ethyl acetate; TLC-5, 95:5 chloroform: ethyl ether.

were added and the metabolites resolved by liquid-liquid partition chromatography as described by Siiteri (13). Products were collected, pooled and after the addition of approximately 12 mg of carrier steroids, further purified by a series of silica gel thin-layer plates before and after acetylation followed by recrystallizations to stable $^3H/^{14}C$ ratios as described previously (16; Table 2). In preliminary experiments it was shown that dihydrotestosterone, the only 5α-product quantified, represented about 50% of the total 5α-reduced products formed which included androsterone and a mixture of 3α- and 3β-androstanediols. In addition, the relative proportion of DHT to total 5α-products formed did not vary substantially between the five nuclear areas studied.

Following the incubation and steroid extraction the tissue was dissolved in 1N NaOH and the protein content determined by the semimicro method of Lowry *et al.* (30). Versatol® was used as a standard. Radioactivity was counted in a Packard Tri-Carb liquid scintillation spectrometer (Model 3385) to a counting error of less than 1 percent. Counting fluid contained 160 ml Liquifluor® (New England Nuclear) per 3.79 liters toluene. Results are expressed as pmol/100 mg protein/h of product formed. Blank incubations consisting of buffer without tissue were carried through each experiment. Data were analyzed by single-factor analysis of variance followed by the Neuman-Keuls multiple range test (31).

Results

The conversion of androstenedione (A) to E_1 and DHT in male and female rats is shown in Table 3. Estrone formation in the male was somewhat higher than in the female depending on the area; however, due to the limited number of data points, further discussion of possible sex differences appears unwarranted. In contrast, no suggestion of any sex difference was observed for DHT formation. Comparison of the mean values for both conversions (Table 3) revealed the striking finding that the relative distribution of aromatase and 5α-reductase was identical in both male and female rats. Since the relative activity of the various male and female areas is identical for both conversions, we have chosen to group the male and female data for purposes of statistical analysis. Single factor analyses of variance indicate highly significant differences between brain regions in E_1 ($F = 9.03$; $df = 3/16$; $P < .001$) and DHT ($F = 6.02$; $df = 3/16$; $P < .01$) formation. For aromatization of A to E_1, the Newman-

TABLE 3. Conversion of androstenedione to estrone and dihydrotestosterone in brain regions of male and female rats (pmol/100 mg protein/h)

Brain area	Male Exp 1	Male Exp 2	Male $\bar{x}$	Female Exp 1	Female Exp 2	Female $\bar{x}$	Combined $\bar{x}$ ± SEM
			Estrone formation				
MPN-AHN	18.8	41.9	30.4	5.1	20.0	12.6	21.5 ± 8.8
MBH	5.3	4.9	5.1	4.0	3.3	3.6	4.4 ± 0.5
LPN	1.5	3.8	2.7	0.6	1.9	1.3	2.0 ± 0.8
Am	2.9	1.1	2.0	0.8	1.4	1.1	1.6 ± 0.5
LHN	0.9	1.1	1.0	0.4	0.8	0.6	0.8 ± 0.2
			Dihydrotestosterone formation				
LHN	13.5	20.4	17.0	5.5	29.3	17.4	17.2 ± 5.9
MPN-AHN	19.0	10.2	14.6	5.0	17.2	11.1	12.9 ± 3.7
LPN	14.7	11.3	13.0	6.9	14.0	10.5	11.7 ± 2.0
Am	4.9	4.6	4.8	5.7	1.5	3.6	4.2 ± 1.1
MBH	0.9	2.2	1.6	1.0	0.5	0.8	1.2 ± 0.4

See footnote to Table 1 for brain area abbreviations.

Keuls multiple range test indicates the MPN-AHN region to be significantly higher than the other four areas ($P = .01$). Similar analysis of 5α-reduction to DHT indicates the activity in the LHN to be significantly greater than in the Am and MBH ($P = .05$); however, the LHN activity does not differ significantly from the MPN-AHN or LPN.

Estrone was the principal estrogen formed from androstenedione (.001 to .03% converted) whereas estradiol levels were much lower and not markedly different between nuclear areas (Table 4). Estradiol formation was detected at low levels in all five areas in the study using A as precursor (.0005% converted; Table 4) but was not detectable in the two lateral areas in a second incubation using T as substrate (Table 5).

As may be seen in Table 5, DHT formation was substantially greater when T was the substrate (.01 to .2% converted) while aromatization to E_2 remained at the limit of detectability. The relative abilities of brain regions to form DHT from T were nearly identical to the pattern seen in the A incubations (Table 3) with the exception of the LPN which became the highest rather than the third highest area. However, it is difficult to comment on differences in the activity of the LHN, MPN-AHN and LPN since all three areas showed high levels of DHT formation which were not statistically different in the incubations presented in Table 3. Reduction of A to T (Table 4) also showed regional variation. 17β-Oxidoreductase activity roughly correlated with that of the 5α-reductase in that the LHN, MPN-AHN and LPN were high and the MBH and Am substantially lower.

Discussion

Autoradiographic studies in male rats have shown intravenously injected [^{3}H]testosterone to be taken up and the resultant radioactivity to be highly concentrated largely over neuronal perikarya in the medial preoptic nucleus, arcuate nucleus, ventromedial nucleus and amygdala with lighter labelling in the lateral and anterior hypothalamic nuclei (16,32). Recently, E_2 has been identified as a metabolite of T in limbic but not cortical cell nuclear fractions from neonatal (33) and adult (34) male rats. In the present study, the MPN-AHN exhibited substantial aroma-

TABLE 4. Formation of testosterone (T) and estradiol (E_2) from androstenedione in brain regions of male rats (pmol/100 mg protein/h)*

Brain area	T	E_2
LHN	665	0.6
MPN-AHN	733	0.8
LPN	644	0.5
Am	1.7	0.2
MBH	289	0.3

* These data were determined from the male incubation designated Experiment 1 in Table 3.
See footnote to Table 1 for brain area abbreviations.

tase activity while the LPN, a specific nucleus lying in close proximity, was relatively inactive in this conversion. Consonant with this finding are reports that significant aromatization is found in the anterior hypothalamus and not in several other brain areas in the adult male rat (3) and that increased E_2 binding capacity is found only in the anterior hypothalamus following castration (19).

Strong evidence for the involvement of intracerebral aromatization in the MPN-AHN in male sexual behavior comes from recent steroid implant experiments. Implantation of testosterone propionate (35) or T (36) and estradiol benzoate (37) or estradiol (36) but not dihydrotestosterone (35) into the MPN-AHN hypothalamic region maintained mounting behavior in males castrated as adults. Simultaneous implantation of the aromatase inhibitor, androst-1,4,6-triene-3,17-dione, with T, blocked this effect while E_2 implanted with the aromatase inhibitor resulted in normal male sexual behavior (36).

The present findings of high rates of aromatization in the MPN-AHN are consistent with this conversion playing an integral part in the sequence of events leading to the initiation of male sexual behavior. The role of aromatization in the MBH is unknown though it could be involved in sexual behavior or in the feedback control of LH and FSH. Markedly different activities of these two areas in forming DHT were observed. The low rate of formation of DHT by the MBH is not consistent with the notion that intrahypothalamic 5α-reduction plays a role in steroid feedback.

An unexpected finding was that the two lateral areas (LPN and LHN) exhibited substantial 5α-reduction of T to DHT. Since ring-A reduced androgens have been shown to inhibit aromatization (38), areas active in 5α-reduction might be expected to show little aromatase activity. However, the MPN-AHN exhibited substantial levels of both activities. This may suggest heterogeneous neuronal subpopulations in the MPN-AHN, involved, perhaps specifically, in aromatization *or* 5α-reduction.

It is not clear what role intracerebral aromatization might play in the female which presumably may utilize estrogen of ovarian origin. Nevertheless, aromatization has been demonstrated in the female brain of several species (3) and we report here that the brain distribution is identical in the adult male and female rat. Sexual receptivity in the female rhesus monkey may be stimulated by adrenal androgens (39) and numerous reports also exist suggesting that androgens modulate female libido in humans (40). Indeed, postmenopausal women, adrenalectomized for treatment of breast carcinoma, report a decrease in libido (41). This suggests a role for adrenal androgens in sex drive which may involve central or peripheral aromatization. The pharmacological action of neonatally injected aromatizable androgens in masculinizing female reproductive physiology and be-

TABLE 5. Formation of dihydrotestosterone (DHT), estradiol (E_2) and estrone (E_1) from testosterone in brain regions of male rats (pmol/100 mg protein/h)

Brain area	DHT	E_2	E_1
LHN	134.7	*	*
MPN-AHN	96.9	0.5	*
LPN	151.0	*	*
Am	19.8	1.9	0.5
MBH	11.7	0.6	0.2

* Less than twice blank (0.2).
See footnote to Table 1 for brain area abbreviations.

havior has been suggested to involve central aromatization (3,5). However, normal females are not masculinized even though their brain tissue can aromatize androgens to estrogens (42). Whether this protective effect is due to the presence of α-fetoprotein (43,44), to the low neonatal levels of endogenous circulating androgens or estrogens, or both, remains to be determined. The possibility that aromatization plays a role in normal male brain differentiation (3) appears more reasonable.

Recent studies from this laboratory have shown that aromatizable androgens can induce daily LH surges in castrate female rats (45). Of particular interest are results which indicate that androstenedione, at the dose levels used, exerts positive feedback on LH in the absence of the suppressive effect which is found with testosterone or estradiol. These findings suggest that aromatization of androstenedione in the hypothalamus may play a heretofore unsuspected key role in control of gonadotropin secretion. Consistent with this new hypothesis are the observations in both rats (Selmanoff, Weiner, and Siiteri, unpublished data) and humans (46) that the midcycle LH peak coincides with peak androstenedione rather than estradiol secretion by the ovary. Furthermore, the abnormality of polycystic ovarian disease is characterized by high serum levels of androstenedione and LH and relatively normal estradiol and estrone levels (47). Taken together these facts suggest that positive feedback on LH secretion may be mediated by androstenedione aromatization in the hypothalamus and that circulating estrogen or androgen suppresses synthesis and/or release of LH by the pituitary, respectively. This would be consistent with the much greater accessibility of androstenedione to the brain since it is not bound to sex steroid-binding globulin and is more efficiently aromatized than testosterone as observed in the present study and by others (42,48). Finally, it has recently been reported that estrone but not E_2, implanted in the MPN, will induce LH release in castrate E_2-primed rats (49).

It may be concluded that both estrogenic and 5α-reduced metabolites, formed *in cerebro*, may be active in the central actions of circulating androstenedione and testosterone in the male and female rat. The possibility that functionally distinct neuronal subpopulations in the MPN-AHN, MBH and other regions are addressed specifically by one or the other metabolite awaits more refined experiments.

Acknowledgment

We wish to acknowledge the expert assistance of our manuscript typist, Ms. Salmonica Maeth.

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