

## Conversion of Androgen to Estrogen and Other Steroids in the Vertebrate Brain

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**SYNOPSIS** Aromatase activity has been detected in the central nervous system (CNS) of representatives of each major vertebrate group with the exception of the Agnatha.  $5\alpha$ -reductase and  $17\beta$ -oxidoreductase are also present in brain tissues of many vertebrates and in the cerebral ganglion of the lobster. These comparative studies together with autoradiographic, physiological, and behavioral data in mammals and selected non-mammalian species support the view that metabolism *in situ* is an important component of androgen action and a general characteristic of the vertebrate brain.

### INTRODUCTION

Until recent years, end organ metabolism was not believed to be an essential component of the mechanism of action of steroids. This idea originated from Jensen and Jacobsen's classical studies of estradiol- $17\beta$  in the uterus, a system in which virtually all the hormone was recovered unchanged. While this generalization remains valid for many steroid hormone-target organ systems, androgens are among the notable exceptions. The intracellular formation of metabolites in the prostate and seminal vesicles seems to be an important step for the appearance of the biological activities of testosterone and may also mediate the expression of hormone action in several other androgen target tissues. For a fuller discussion of end organ metabolism, see King and Mainwaring (1974).

In the central nervous system (CNS) of mammals, aromatization of androgen to estrogen may be required for the manifestation of certain behavioral and neuroen-

docrine responses (Naftolin *et al.*, 1975). Direct biochemical evidence for aromatase activity in brain tissues *in vitro* and *in vivo* supports this hypothesis. In addition, a number of other androgen-converting enzymes have been identified in the mammalian CNS, and it is likely that different metabolites of a single precursor have separate and unique biological actions (Perez-Palacios *et al.*, 1975).

Because aromatase in mammals is located in the phylogenetically ancient brain and these same areas concentrate sex steroids and appear to control reproduction and sex behavior, we suggested that steroid metabolism might be a primitive feature of the CNS and a fundamental component of central androgen action characteristic of vertebrates in general. Comparative studies initiated to test this hypothesis are summarized here and discussed in relation to more extensive information available in mammals.

### STEROID-CONVERSIONS IN THE VERTEBRATE BRAIN

#### *Aromatase*

Metabolic pathways characteristic of the vertebrate brain are depicted in Figure 1. Androgens are the immediate precursors of estrogens, and the conversion is regulated by the aromatase complex, one of a

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TABLE 1. *Phylogenetic distribution of androgen converting enzymes in the brain.*

Species	aromatase	5 $\alpha$ -reductase	5 $\beta$ -reductase	17 $\beta$ -oxido-reductase	17 $\alpha$ -oxido-reductase	3 $\alpha$ -hydroxy-steroid dehydrogenase	3 $\beta$ -hydroxy-steroid dehydrogenase	References <sup>a</sup>
Common/Scientific name								
human, <i>Homo sapiens</i>	X	X		X			X	10, 7
monkey, <i>Macaca mulatta</i>	X	X		X				6, 20
cow, <i>Bos taurus</i>	X	X		X				14, 19
cat, <i>Felis catus</i>	X			X				5
dog, <i>Canis familiaris</i>		X					X	15
rabbit, <i>Oryctolagus cuniculus</i>	X			X				16, 17
hamster, <i>Mesocricetus auratus</i>	X	X	X					4
guinea pig, <i>Cavia porcellus</i>		X		X		X	X	21
rat, <i>Rattus rattus</i>	X	X	X	X		X		11, 18, 19, 7
mouse, <i>Mus musculus</i>	X							12
chicken, <i>Gallus domesticus</i>	X	X	X	X	X	X	X	3, 13
starling, <i>Sturnus vulgaris</i>		X	X					9
snake, <i>Natrix taxispilota</i>	X	X			X			3
turtle, <i>Chrysemys picta</i>	X			X	X			1, (Table 3)
<i>Chelonia mydas</i>	X	X		X				3, 8
<i>Podocnemis expansa</i>		X		X				8
frog, <i>Rana catesbeiana</i>	X	X		X				2
mudpuppy, <i>Necturus maculosus</i>	X							3
sculpin, <i>Myoxocephalus octadecimspinosus</i>	X			X				3
shark, <i>Squalus acanthias</i>	X							3
skate, <i>Raja ocellata</i>	X							3
hagfish, <i>Myxine glutinosa</i>		X						3
lobster, <i>Homarus americanus</i>		X		X				3

<sup>a</sup> References: (1) Callard *et al.*, 1977. (2) Callard *et al.*, 1978a. (3) Callard *et al.*, 1979. (4) Callard, unpublished. (5) Ficher, 1976. (6) Flores *et al.*, 1973a. (7) Jaffe, 1969. (8) Lisboa *et al.*, 1978. (9) Massa *et al.*, 1977. (10) Naftolin *et al.*, 1971a. (11) Naftolin *et al.*, 1972. (12) Naftolin *et al.*, 1975. (13) Nakamura and Tanaki, 1975. (14) Osawa, 1975. (15) Perez-Palacios *et al.*, 1970. (16) Reddy *et al.*, 1972. (17) Reddy *et al.*, 1974b. (18) Rommerts and van der Molen, 1971. (19) Sholiton and Werk, 1969. (20) Sholiton *et al.*, 1974. (21) Sholl *et al.*, 1975.

metabolize C-21 steroids (Karavolas and Nuti, 1976).

DISTRIBUTION OF AROMATASE AND OTHER  
ANDROGEN-CONVERTING ENZYMES IN THE BRAIN  
AND NON-NEURAL TISSUES

*Neuroanatomic distribution: Aromatase*

The distribution of aromatase activity in the adult brain of representative species from the major vertebrate groups is shown in Figure 2. Aromatase in adult mammals is restricted to the "limbic" system which includes the preoptic area, hypothalamus, septum, amygdala, and hippocampus (Naftolin *et al.*, 1975). Autoradiographic and biochemical determinations of sex steroid binding in brain cytosol and nuclei indicate that target cells are concentrated in these same areas (McEwen and Pfaff, 1973), suggesting a functional interrelationship between binding and metabolism. Physiological and behavioral experiments provide further evidence that control of reproductive functions is centered in the limbic system.

Aromatization is consistently found in combined preoptic and hypothalamic tissues of non-mammalian species and, with the exception of the shark in which conversions were near the limits of detection, telencephalic structures also synthesize estrogen (Callard *et al.*, 1977; 1978a; 1979). The telencephalon of non-mammalian species is represented in the limbic lobe of mammals; thus it appears that aromatase has been retained in homologous regions of the anterior brain stem and archi- and paleocortex throughout phylogeny. In contrast, the greatly expanded neocortex of adult mammals which has no clearly defined counterpart in other vertebrates is aromatase-negative.

In all groups, highest levels of aromatase activity are found in the forebrain, but in lower vertebrates (skate, sculpin, bullfrog) activity is present in the mid- and hind-brain also (Callard *et al.*, 1978a; 1979). More posterior brain divisions have estrogen synthesizing potential in early developmental stages of animals in which no activity is found in adults. This is true for

the newly hatched chick (Callard *et al.*, 1979) and fetal rabbit (George *et al.*, 1978), and suggests that aromatase activity in certain regions is secondarily lost during development. It may be relevant that the adult turtle cerebellum synthesizes substantial quantities of estrogen after 4 days in culture (Table 3), although activity is absent when homogenates are assayed.

The cerebral cortex of fetal and neonatal mammals (Schindler, 1975; Reddy *et al.*, 1974a; George *et al.*, 1978) has been reported to synthesize small amounts of estrogen despite the absence of activity from this brain region in adults. Cytosol and nuclear estrogen binding likewise is found in the cerebral cortex of newborn rats, disappearing after the first few weeks (McEwen *et al.*, 1975). Adult mammals in general have low brain aromatase activity relative to early developmental stages and compared to some non-mammalian vertebrates (Table 2). The proliferation of newer, aromatase-negative cell types during cortical development may dilute activity below detectable limits. The presence of aromatase activity and estrogen-binding mechanisms in the cerebral cortex during early developmental stages may be related to general growth-stimulating properties of estrogen rather than to reproductive functions *per se*. Dramatic effects of estrogen or aromatizable androgen on neurite outgrowth have been observed in newborn mouse hypothalamic cultures (Toran-Allerand, 1978).

By looking more closely at the areas of the brain in which aromatase activity is concentrated, it can be seen that in the rat, rabbit, and human fetus, preoptic/hypothalamic activity exceeds that in the limbic cortex (Table 2). By contrast, in the turtle (*Chrysemys*) highest estrogen yields are obtained from the segment of the brain homologous to the mammalian amygdala (Fig. 3), and the same is true in the hamster and chick (Table 2). The amygdala is known to be involved in the onset of reproduction maturation in rats (Elwers and Critchlow, 1960) and a peak in limbic aromatase activity corresponds to the steroid-sensitive period of brain sex differentiation in neonatal males (Reddy *et*

ESTROGEN SYNTHESIS: ADULT BRAIN

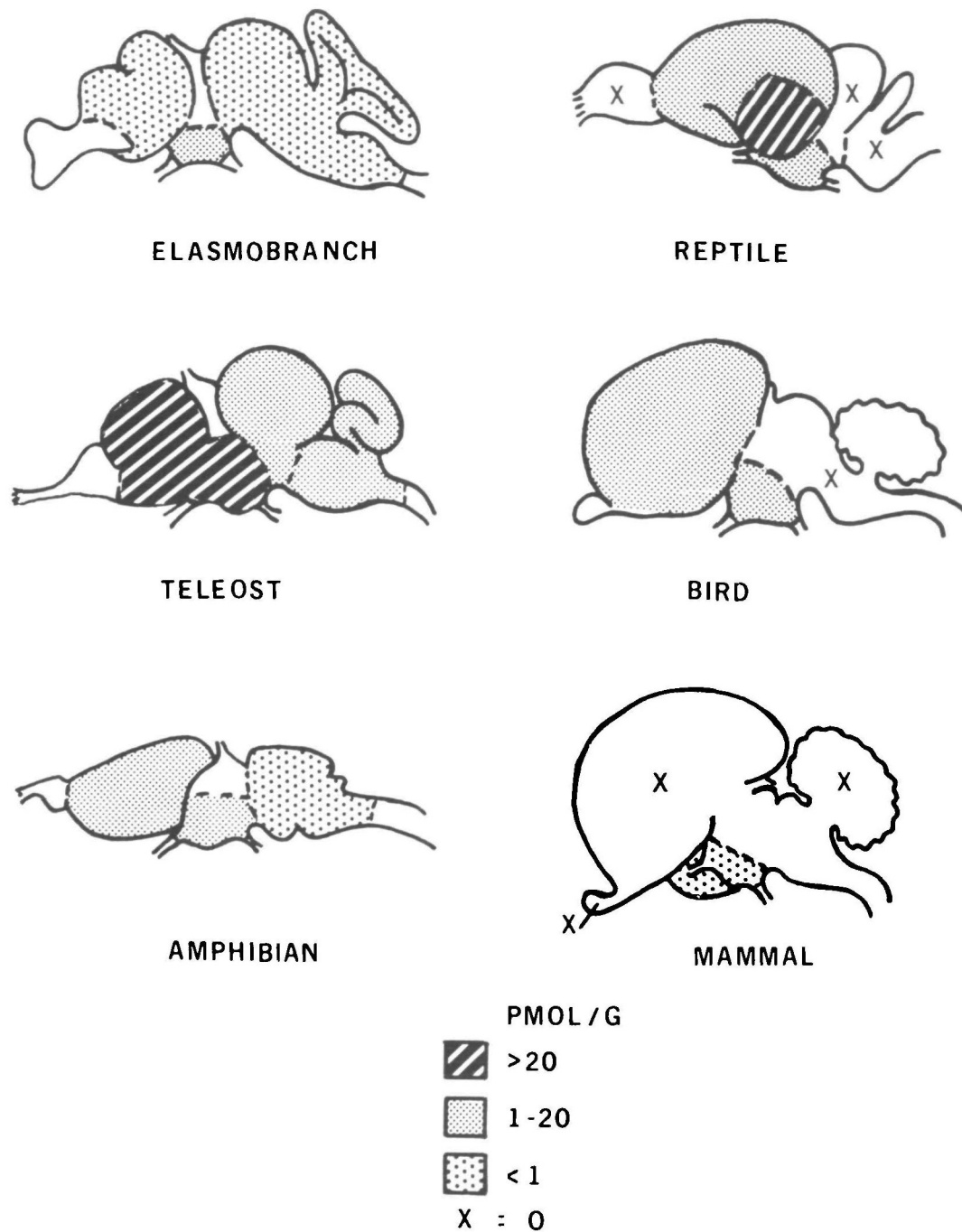


FIG. 2. Neuroanatomic distribution of aromatase activity through the phyletic series. Only values from representative adult vertebrates are depicted. Elasmobranch (*Raja ocellata*); teleost (*Myoxocephalus oc-*

*ladecimspinosus*); amphibian (*Rana catesbeiana*); reptile (*Chrysemys picta*); bird (*Gallus domesticus*); mammal (*Rattus rattus*). For references, see Table 1.

TABLE 2. Aromatase activity in the limbic cortex, hypothalamus/preoptic area, and gonads of selected vertebrates.

	pmol estrogen/g <sup>a</sup>			References <sup>f</sup>
	Limbic cortex	Hypothalamus/ Preoptic area	Gonad	
Rat ♂	0.34	0.71	e	1
fetal ♂	2.77	5.14	e	
♀	0.24	0.53	e	2
fetal ♀	nil	3.65	e	
Rabbit ♂	0.14	0.80	e	3
♀	0.09	0.28	e	
Human fetal ♂	2.97	33.02	e	4
Hamster ♂	4.56	1.14	nil	
♀	2.33	1.32	730.0	5
Chicken 4-day ♂	2.85 <sup>b</sup>	1.77	6.71	
♀	2.50 <sup>b</sup>	1.83	74.43	6
Turtle ♂	14.44 <sup>c</sup>	10.67	nil	
♀	6.56 <sup>c</sup>	3.17	nil	5
Sculpin ♀	122.32 <sup>d</sup>	66.65	4.3	
♂	9.89 <sup>d</sup>	15.84	e	5
Skate ♂	0.42	0.94	nil	
♀	0.52 <sup>d</sup>	1.23	46.62	

<sup>a</sup> Includes total E<sub>1</sub>, E<sub>2</sub>β and E<sub>2</sub>α formed from <sup>3</sup>H-androstenedione. In some instances, yields are calculated from data provided in the references cited.

<sup>b</sup> Segment containing *nucleus taenia*.

<sup>c</sup> Segment containing strioamygdaloid complex.

<sup>d</sup> Forebrain remaining after removal of hypothalamus/preoptic area.

<sup>e</sup> Not measured.

<sup>f</sup> References: (1) Reddy *et al.*, 1974a; (2) Reddy *et al.*, 1972; (3) Naftolin *et al.*, 1971a; (4) Callard, unpublished; (5) Callard *et al.*, 1979; (6) Callard *et al.*, 1977.

TABLE 3. Conversion of androstenedione to estrogen and other metabolites by cultured explants of turtle brain (*Chrysemys picta*).<sup>a</sup>

	pmol/2 ml media/dish <sup>b</sup>		
	AMY	HTH/POA	CB
Estrone	1.370 (1695/1892)	0.890 (1100/1154)	0.610 (756/703)
Estradiol-17β <sup>c</sup>	2.220 (2740/2848)	0.800 (988/1050)	0.690 (856/916)
Testosterone	47.900 (59,104/59,832)	31.800 (39,210/39,060)	43.700 (53,926/55,246)
Testosterone-17α	42.100 (51,946/50,986)	31.900 (39,394/40,324)	21.900 (27,008/24,942)

<sup>a</sup> Explants (<0.5 mm<sup>3</sup>) of one strioamygdaloid complex (AMY, ≈ 17.5 mg) or the entire hypothalamic/preoptic area (HTH/POA, ≈ 18.0 mg) or cerebellum (CB, ≈ 8.2 mg) were incubated in 35 mm plastic culture dishes with 2 ml Eagles' Basal Medium, fetal calf serum (10%), penicillin (200 U/ml), streptomycin (200 μg/ml) and 3H-7α-androstenedione (120 nM) for 4 days at 30°C in 5% CO<sub>2</sub>.

<sup>b</sup> After recrystallization to constant specific activity, steroid content of media only was computed. No estrogens were found in ovarian cultures or in dishes without tissue.

<sup>c</sup> No estradiol-17α was detectable.

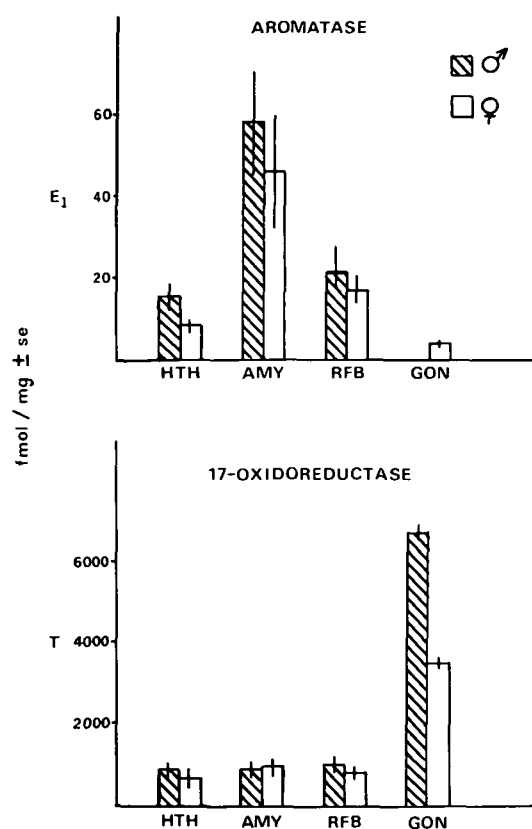


FIG. 3. Sex differences in aromatase and  $17\alpha\beta$ -oxidoreductase activity in turtle (*Chrysemys picta*) brain and gonad. Homogenized tissues (9–20 mg) were incubated with  $^3\text{H}$ - $7\alpha$ -androstenedione (266 nM), unlabelled estrone (5  $\mu\text{g}$ ) and cofactors in a final volume of 500  $\mu\text{l}$  for 60 min at 27°C. Yield was calculated after thin layer chromatography (T/T $\alpha$ ) and additional phenolic partition (E<sub>1</sub>). Procedural details and steroid identification has been reported previously (Callard *et al.*, 1977). Animals were collected in October (n = 3  $\delta$ ; 3  $\text{f}$ ). Preoptic area/hypothalamus (HTH); strioamygdaloid complex (AMY); remaining forebrain (RFB); gonads (GON).

*al.*, 1974a). Throughout phylogeny the amygdala is the site of high concentrations of estrogen- and androgen-binding cells (Morrell and Pfaff, 1978; Kim *et al.*, 1978), but how it functions in the reproduction of non-mammalian species is unknown.

#### Neuroanatomic distribution: $5\alpha$ -Reductase and other enzymes

The distribution of  $5\alpha$ -reductase and  $17\beta$ -oxidoreductase is not as specific as that of aromatase, activity having been

identified in all major brain divisions in mammals and in non-mammalian species (Fig. 3; see Table 1 for references). Somewhat higher yields of  $5\alpha$ -reduced steroids have been obtained in rat hypothalamic incubates compared to other brain areas (Martini, 1976). Assays of individually dissected hypothalamic and limbic nuclei indicate that  $5\alpha$ -reductase and aromatase activity coexist in the same loci, but ratios of the two enzymes differ from region to region (Selmanoff *et al.*, 1977). In these studies, the pattern of  $17\beta$ -oxidoreductase activity corresponded to that of  $5\alpha$ -reductase. Unless these enzymes are compartmented within individual cells *in vivo*, it is unlikely that they function independently. Not only does  $5\alpha$ -reductase compete with aromatase for substrate, but  $5\alpha$ -reduced steroids are known to inhibit aromatization *in vitro* (Siiteri and Thompson, 1975). By converting T to  $\Delta^4\text{A}$  which is the preferred substrate for aromatization in brain (Flores *et al.*, 1973a; Weisz and Gibbs, 1974),  $17\beta$ -oxidoreductase can also influence estrogen synthesis.

#### Non-neural tissues

Although aromatase and  $5\alpha$ -reductase are sometimes found in peripheral, non-endocrine tissues other than the brain, they are by no means ubiquitous, and there is species variation in tissue distribution. Substantial estrogen yields are obtained from the brain of the freshwater turtle, for example, but aromatase activity is not present in muscle, liver, fat body, or in the gonads of either sex under the same assay conditions (Callard *et al.*, 1977), although subsequent studies confirmed minimal ovarian conversions (Fig. 3). The primary sites of aromatization in mammals are the gonads and placenta, but in addition to brain, the adrenal, breast tissue, fat, kidney, liver, bone, lung, thymus, skin, and muscle have also been shown to synthesize estrogen (see Longcope *et al.*, 1978). In non-mammalian species, aromatase activity per unit weight of brain generally exceeds that in the testis, and in the sculpin, turtle and snake, central estrogen yields

also exceed ovarian conversions (Table 2; Fig. 3; Callard *et al.*, 1979). Results of a recent investigation in the fetal rabbit indicate that the CNS is the most important site of estrogen synthesis (George *et al.*, 1978). The contribution of central aromatization to the circulating estrogen pool is not known.

#### Subcellular localization

Because of the high yield of estrogen in the brain of the turtle and sculpin, it has been possible to assay aromatase in subcellular fractions. Results from the turtle are shown in Table 4. In both species, aromatase is concentrated in the microsomal fraction, although activity is found in the mitochondria also. Brain is similar in this respect to endocrine tissues like the gonads and placenta (Engel, 1975). The distribution of 5 $\alpha$ -reductase is like that of aromatase, but 17 $\alpha$ / $\beta$ -oxidoreductase is found in both the microsomal and cytosol fractions of turtle brain. C-17 $\alpha$  vs. -17 $\beta$  metabolites in both fractions are approximately equal. The 5 $\alpha$ -steroid reductase is localized in the microsomal (and possibly nuclear) fraction of the rat brain, but 17 $\beta$ -oxidoreductase is a soluble enzyme

(Rommerts and van der Molen, 1971; Martini, 1976).

The presence of enzymatic activity in cell homogenates is only the first step in proving that conversions occur *in vivo*; however, estrogen synthesis has been detected in the isolated, perfused monkey brain (Flores *et al.*, 1973b) and in fetal rat hypothalamic monolayer cultures (Canick *et al.*, 1977). In addition, aromatase, 17 $\alpha$ - and 17 $\beta$ -oxidoreductase activity is present in explant cultures of turtle brain (Table 3; Fig. 4). There is no direct evidence that aromatization takes place in the neuron itself; however, by inhibiting proliferation of non-neuronal elements in hypothalamic monolayer cultures, growth of neuronal processes and aromatase activity are stimulated (Canick *et al.*, 1977).

#### FUNCTIONAL ROLE OF CENTRAL ANDROGEN TRANSFORMATIONS

##### Mammals

Biochemical, behavioral, and physiological studies in mammals indicate that a single, blood-borne hormone (*e.g.*, T), upon reaching the CNS might be converted *in situ* to one or more neutral and

TABLE 4. Subcellular distribution of aromatase, 5 $\alpha$ -reductase, and 17 $\alpha$ / $\beta$ -oxidoreductase in the brain of the turtle *Chrysemys picta*.<sup>a</sup>

	Total protein (mg)	10 <sup>3</sup> dpm/mg protein <sup>b</sup>		
		E <sub>1</sub>	5 $\alpha$ -A	T/1 $\alpha$
Whole homogenate	121.0	25.2	2.6	360.3
Nuclei	6.9	nil	nil	25.5
Mitochondria	13.2	18.2	4.9	258.5
Microsomes	8.7	61.6	14.9	553.4 <sup>c</sup>
Cytosol	40.8	2.5	2.8	279.0 <sup>c</sup>

<sup>a</sup> Whole forebrains in buffer containing sucrose (250 mM), K-phosphate (50 mM), dithiothreitol (10 mM) and MgCl<sub>2</sub> (3 mM) were homogenized using a Polytron. Fractions were prepared by centrifugation as follows: nuclei, 850  $\times$  g  $\times$  10 min, pellet resuspended and centrifuged in 2.0 M sucrose at 51,000  $\times$  g  $\times$  45 min; mitochondria, 11,500  $\times$  g  $\times$  30 min, washed once; microsomes, 100,000  $\times$  g  $\times$  60 min, pellet; cytosol, 100,000  $\times$  g  $\times$  60 min, supernatant. Aromatase was assayed in fractions containing 30-50  $\mu$ g protein as described in Figure 3 with the addition of dithiothreitol (10 mM) and EDTA (1 mM) to the assay buffer.

<sup>b</sup> Product yield is corrected for procedural losses and computed after thin layer chromatography only (5 $\alpha$ -A, T/T $\alpha$ ) or after additional phenolic partition (E<sub>1</sub>). No E<sub>2</sub> $\beta$  or E<sub>2</sub> $\alpha$  are formed under these conditions. Values represent the mean of quadruplicate assays.

<sup>c</sup> Proportion of T vs. T $\alpha$  in these fractions was determined by reverse isotope dilution and recrystallization to constant specific activity. Microsomes: 236.9 vs. 316.0  $\times$  10<sup>3</sup>dpm for T and T $\alpha$ . Cytosol: 131.4 vs. 147.6  $\times$  10<sup>3</sup>dpm for T and T $\alpha$ .

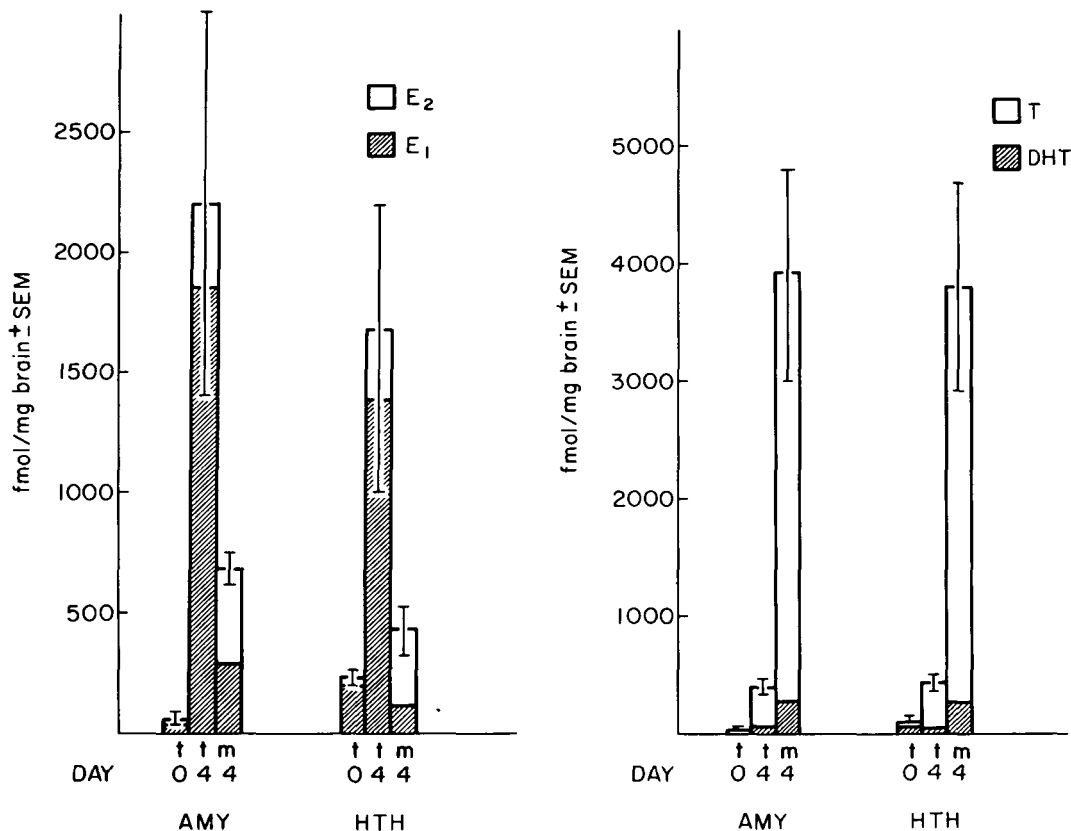


FIG. 4. Aromatization and tissue estrogen retention in explant cultures of turtle (*Chrysemys picta*) brain. Culture conditions are described in Table 4. E<sub>1</sub>, E<sub>2</sub>, T, and DHT were radioimmunoassayed after celite chromatography (Callard *et al.*, 1978a). Strioamygdaloid complex (AMY) or hypothalamic/preoptic area

(HTH) of 6 turtles were bisected. In one half, endogenous steroids were assayed at time 0. The other half was cultured for 4 days with unlabelled androstenedione (233 nM) and steroids assayed separately in tissue (t) and media (m).

phenolic steroids which alone or in combination exert actions on different biological end points. Neonatal sex differentiation, adult sex behavior, the onset of sexual maturation, and the control of gonadotropin secretion have all been attributed to the action of estrogens or catechol estrogens formed in the brain (Naftolin *et al.*, 1975; Parvisi and Ellendorf, 1975). Although DHT and DIDL do not seem to be involved in rodent brain masculinization, secretion of gonadotropin and initiation of sex behavior in certain species are regulated by these androgens (Perez-Palacios *et al.*, 1975). For complex sexual behavior patterns, individual components may be controlled by different steroids, and several steroids acting in concert may be re-

quired. In the rat, for example, small doses of E<sub>2</sub>β in combination with DHT completely restore male sexual behavior after castration, although neither steroid is effective alone (Feder *et al.*, 1974). The concept that metabolites have biological actions in their own right is based on experiments which show the following: 1) the metabolite mimics and may be more potent than the parent steroid; 2) the biological effects of the parent steroid can be blocked by administration of substances which inhibit metabolism or the action of the metabolite; 3) a natural or synthetic steroid not able to undergo conversion to the metabolite in question has no biological activity; and 4) the metabolite binds to cytosol receptors with high affinity and can

be extracted from brain cell nuclei after administration of labelled precursor peripherally (Naftolin *et al.*, 1975; Lieberburg *et al.*, 1978). While the majority of studies have been conducted in rodents, similar observations in non-mammalian vertebrates may be cited in support of a biological role for central steroid transformations as a general vertebrate phenomenon.

#### *Non-mammals*

There are numerous examples in non-mammalian species of "paradoxical" in contrast to "sex specific" effects of androgens and estrogens on sex behavior (Young, 1961) and psychosexual brain differentiation (Adkins, 1978). Some of these may be explained by central conversion of androgen to estrogen, although metabolism at peripheral sites cannot be ruled out. More direct evidence that brain aromatase has a functional role has been obtained in male and female lizards (*Dipsosaurus dorsalis*); either  $E_2\beta$  or T implanted in the hypothalamus will prevent seasonal gonadal recrudescence (Lisk, 1967). In addition, T in the hypothalamus of castrated doves induces courtship, but DHT, a non-aromatizable androgen, is relatively ineffective (Hutchison, 1976). In non-mammalian species, as in mammals, cells which concentrate sex steroids are found in greatest numbers in the areas of the brain in which aromatase activity is concentrated, and to some extent labelled areas after injection of  $E_2\beta$  or T overlap (Morrell and Pfaff, 1978; Kim *et al.*, 1978). Turtle brain explants in culture selectively retain  $E_1$  and  $E_2\beta$  synthesized from  $\Delta^4A$ , whereas T and DHT remain in the media (Fig. 4).

It is unlikely that the mechanism of action of androgen in the brain is based exclusively on conversion to estrogen and there may be species variations to consider. Both T and DHT restore clasping in castrated *Xenopus* (Kelley and Pfaff, 1976), but these same androgens are ineffective in *Rana pipiens* (Palka and Gorbman, 1973). In neither species will

$E_2\beta$  elicit male sexual behavior. Unconverted steroid as well as products of metabolism (DHT or  $E_2\beta$ ) have been isolated from brain cell nuclei after peripheral injections of  $^3H$ -T in birds (Zigmond *et al.*, 1972) and frogs (Kelley *et al.*, 1978). Since cellular steroid retention in the brain, as in other target organs, is thought to be an early step in the expression of hormone action, biochemical data of this kind support physiological and behavioral studies in assigning a function to a metabolite.

Whether endogenous substrate is available for central conversions under physiological conditions in non-mammalian species is a critical question. The gonads of fishes, amphibians, reptiles and birds synthesize androgens from precursors *in vitro*, and in some species substantial quantities of androgen have been measured by radioimmunoassay in the peripheral plasma of both sexes (Ozon, 1972a,b; D'Istria *et al.*, 1974; Shahabi *et al.*, 1975; Callard *et al.*, 1976; I. Callard *et al.*, 1978).

Observed differences in brain enzyme activity in conjunction with naturally occurring reproductive parameters might be supporting evidence for a functional interrelationship, but no consistent patterns emerge from comparative studies. Forebrain areas of mature female sculpin, for example, give markedly greater estrogen yields than comparable tissues from males when tested after spawning (Callard *et al.*, 1979). Generally, the reverse is true in mammals (see below), and in birds there appears to be no distinct difference between 4-day old female chicks and laying hens (Callard *et al.*, 1979). In newborn and mature rats and in adult rabbits, males have greater brain aromatase activity than females (Table 2), but sex differences are not apparent in fetal rabbits (George *et al.*, 1978), newly hatched chicks, or in mature, reproductively active skates or mudpuppies (Callard *et al.*, 1979). Such comparisons must be viewed with caution, since the aromatase assays in the experiments cited are not strictly quantitative and based on pooled samples. Apparent sex differences in turtles (Table 2) are not significant when individual specimens are assayed (Fig. 3).

## CONCLUSIONS

From the species surveyed to date, it appears that the presence of steroid transforming enzymes is a primitive characteristic of the CNS that has been widely conserved through vertebrate phylogeny. Unless these enzymes arose independently many times during the course of evolution, we can assume that the ability of the CNS to transform steroid substrates is an inheritance from a common ancestor. Sterol metabolism is a fundamental property of eukaryotic and prokaryotic cells (Sandor *et al.*, 1975), therefore, steroid-converting enzymes may have been retained by some target cells as a component of steroid hormone action. An increased capacity for the synthesis and secretion of steroid hormones (or prohormones) possibly developed in other tissues like the gonads and adrenals as a parallel specialization. The most primitive vertebrates in which central aromatase has been detected are the elasmobranchs, suggesting that the potential for converting androgens to estrogens originated early in vertebrate evolution, possibly before the adaptive radiation of gnathostomes ( $300-350 \times 10^6$  y.b.p.). Other enzymes ( $5\alpha$ -reductase,  $17\beta$ -oxidoreductase) may have appeared earlier than the first vertebrates, because they are found in invertebrates also.

At what point in the evolutionary series CNS conversions assumed biological importance is still obscure. The general occurrence of androgen metabolism in the vertebrate brain may itself signify a vital role in brain-steroid interactions, but indirect evidence in selected non-mammalian species supports the idea that certain transformations lead to biological activation. The existence of enzymes in brain which regulate the synthesis of steroids believed to be biologically inert ( $5\beta$ -reductase,  $17\alpha$ -oxidoreductase) is an indication that central transformations may be inactivating also.

In species in which there is no central, glandular source of biologically active steroid, target organ conversions of circulating prohormone would play a crucial role in mediating responses. In two species

in which brain aromatization is exceedingly high (the turtle and sculpin), gonadal aromatization is absent or negligible. Ovarian and peripheral aromatization are inversely related in human females also. In post-menopausal women in whom ovarian aromatization is minimal, circulating estrogen, presumably formed in peripheral tissues from adrenal androgen, sometimes reaches levels observed during menstrual cycles (Siiteri and McDonald, 1973).

In those animals in which the gonads secrete estrogen or other biologically active steroid (*e.g.*, DHT), high levels of binding protein in plasma might prevent access to target cells or buffer fluctuations in secreted steroid. Converting enzymes at the level of the target organ would then regulate synthesis of active steroid from a substrate reservoir of bound prohormone. Substantial quantities of sex hormone binding proteins are present in the plasma of many non-mammalian species, especially in fish and amphibians (Ozon, 1972a; 1972b). It has been hypothesized that in fetal and newborn rats and mice, circulating estrogen binding protein protects the conceptus from maternal estrogen (McEwen *et al.*, 1975). It is relevant that in these species, brain sex differentiation (masculinization) is dependent on central aromatization of testicular androgen.

Even in mammals in which the ovary synthesizes and secretes estrogens, androgen may be the most important product quantitatively (Baird, 1978), and could serve as a signal for events in the brain. The hormonal response is exceedingly complex in the CNS and prior metabolism would increase the versatility of the afferent circuit. Synthesis of different metabolites from a single prohormone might elicit multiple responses simultaneously or interact in a single target cell resulting in a spectrum of modulated responses.

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