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THE METABOLISM OF ANDROGENS IN CENTRAL NEUROENDOCRINE TISSUES*

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SUMMARY

Although prehormone androgens such as testosterone and androstenedione are converted to ring A reduced products such as dihydrotestosterone and androstanedione in central tissues (brain and pituitary gland), there is ample evidence suggesting that many central reproductive functions of these prehormone androgens may require their conversion to estrogens. Because of this, we have sought and demonstrated the local *in vitro* and *in vivo* conversion of androstenedione and testosterone to estrone and estradiol in central tissues from humans, rhesus monkeys, rabbits, rats and mice. Central aromatizing activity is greater in males than in females and is affected by castration and sex steroid and antiandrogen pretreatment. The anterior hypothalamus is the most actively aromatizing central tissue. However, because of the importance of the limbic system in neuroendocrine rhythms, the control of gonadotrophins and the onset of puberty, it has also been investigated, and has been found to convert androgens to estrogens. We believe that the classical concept of androgen action must be enlarged to encompass central effects that require the aromatization of androgens as well as those requiring ring A reduction.

INTRODUCTION

The gonad, regardless of whether it is an ovary or a testis, secretes androgens and estrogens (Fig. 1). The adrenal gland also furnishes androgens to the systemic circulation. Figure 2 depicts the metabolism of androgens which may occur once these androgens reach the central tissues (brain and anterior pituitary gland). Several laboratories have shown that ring A reduction occurs in central tissues [1, 2] and is a major pathway of androgen metabolism. If the estrogenic pathway is followed, there are intermediates formed; for example, the 19-hydroxylated compounds [3] which may themselves have central effects [4, 5]. After estrone or estradiol are formed the next step might be the central hydroxylation of estrogens [6]. In addition because of the use of 19-nor testosterone derivatives in oral contraceptives, it behooves us to consider the possibility of their central metabolism to estrogens; e.g. 17α-ethynyl 19-nor testosterone becoming ethynyl estradiol [7].

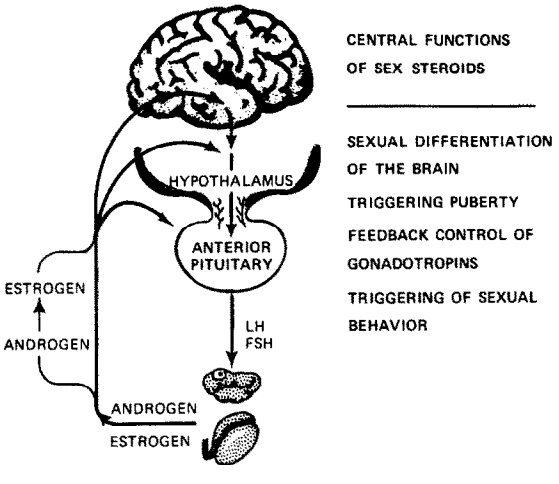

Fig. 1. The central-gonadal axis responsible for reproductive function (from Naftolin *et al.*[32]).

PATHWAYS OF ANDROGEN METABOLISM

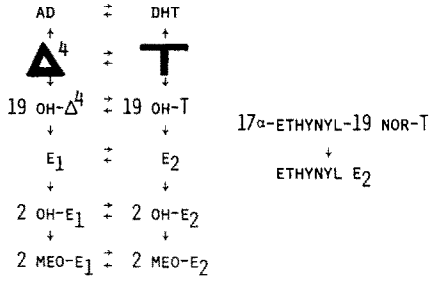

Fig. 2. A schematic of possible androgen metabolism by central tissues. AD, Androstanedione; DHT, dihydrotestosterone; Δ^4, androstenedione; T, testosterone; E_1, estrone; E_2, estradiol; MEO, methoxy.

That extragonadal conversion of androgens to estrogens occurs in the body cannot be doubted. Figure 3 demonstrates a summary of several studies [8, 9] showing that in the normal course of a 24-h day, both men and women form considerable portions of their estrone from androstenedione in an extragonadal site(s). The conversion rate of androstenedione to estrone is in the range of 1·5%. However, when one takes into account that androgens are made in mg quantities during the course of a day and

* The unpublished work alluded to in this paper was done in collaboration with Mrs. M. Kuhn, Mr. Z. Petro, and Dr. H. Morishita.

Δ^4 ANDROSTENEDIONE AS A PREHORMONE

	♀	♂
PRODUCTION RATE Δ^4A/24°	3400 μg	1400 μg
CONVERSION RATE $\Delta^4 \rightarrow E_1$	1.3 %	1.3 %
PRODUCTION OF E_1 from Δ^4A/24°	44 μg	18 μg
PORTION OF E_1 derived from Δ^4A/24°	25–50%	10–25%

Fig. 3. (From Naftolin *et al.*[32]).

that the estrogens made from them are active in μg quantities, the importance of even the smallest conversion of androgen to estrogen is apparent. In this case 1·3% peripheral conversion of androstenedione in both men and women furnishes something between a quarter and a half of the estrone produced during the course of a day in women and a lesser but still significant portion in men.

Figure 4 lists the central functions of sex steroids and reviews some of what is known about the roles of androgens and estrogens in each case. The review is selective, limited and necessarily reflects the reviewers' bias.

Brain differentiation

Estrogens are by far the most potent compounds in differentiating the newborn rodent's brain [10–12]. Interestingly, McDonald and Doughty[4] have recently shown that in controlling brain differentiation, the intermediate 19-hydroxytesterone is of intermediate potency between estradiol and testosterone while dihydrotestosterone is ineffective [4, 11, 13, 14]. The anti-estrogen, MER-25, has been shown to block the testosterone effect in rat brain differentiation [15].

Gonadotrophin control

In the feedback control of gonadotrophins by administered steroids, estrogens are the most potent in controlling LH and FSH [16]. Testosterone is effective in all species but requires considerably larger doses than estrogen, while dihydrotestosterone is inconsistent, not being effective in the human [17, 18] and in the neonatal rat (unpublished), while it is equally effective as testosterone in the weaned immature and mature rat [19, 20]. The anti-estrogen, chlomiphene, has been shown to block the effect of testosterone in controlling gonadotrophins in men [21].

Sexual behavior

Estrogens are again far more potent than androgens in causing their effects. While testosterone is generally effective in all species, dihydrotestosterone is not effective in certain species or requires very long periods of time to act [5, 22]. Administered estrogen, in proper animal models, can produce male or female sex behavior [23]. Furthermore, anti-estrogens have been shown to block the sexual behavior provoking effect of testosterone [24].

Timing of puberty

Study of this complex phenomenon is only just beginning. While some ring A reduced compounds have been shown to hasten puberty in rats, estrogens are far more potent in this action [25].

RAT BRAIN DIFFERENTIATION
E > 19 OH-T > T
ANTI-E BLOCKS T EFFECT

GONADOTROPIN CONTROL
E > T/DHT (DHT NOT EFFECTIVE IN ALL SPECIES)
ANTI-E BLOCKS T EFFECT

SEX BEHAVIOR
E > T/DHT (DHT NOT EFFECTIVE IN ALL SPECIES)
E → ♂ OR ♀ SEX BEHAVIOR
ANTI-E BLOCKS T EFFECT

TIMING OF PUBERTY
E > T/RING A REDUCED ANDROGENS
DHT NO EFFECT ON PUBERTY

Fig. 4. Summarizing the relationship of various androgen metabolites to central reproductive functions.

UPTAKE STUDIES

Autoradiographic studies depicting the localization of radiolabel in central tissues after administration of tritiated androgen or estrogen have been very helpful in mapping the central areas of interest in investigations of brain metabolism. In addition, "competition" studies using pretreatment with unlabeled steroids have been very useful in interpreting these uptake studies and their possible physiologic implications.

In reviewing the autoradiographic uptake studies (Fig. 5) it seems clear that in rats after [^{3}H]-testosterone administration, radiolabel localizes in the central neuroendocrine tissues [26, 27]. However, the identity of the localized steroid is not known because of the minute quantities present in any single cell. A similar pattern of labeling is found after administration of [^{3}H]-estradiol, except that there is more cell nuclear labeling than after [^{3}H]-testosterone administration [27]. Finally, if the [^{3}H]-testosterone injection is preceded by treatment with unlabeled estradiol or testosterone the nuclear labeling is diminished while pretreatment with dihydrotestosterone has no effect [28].

RADIOLABELLING OF RAT BRAIN AFTER ^{3}H-T ADMINISTRATION

1) LOCALIZES IN NEUROENDOCRINE TISSUES
2) CHEMICAL IDENTITY OF LOCALIZED COMPOUND UNKNOWN
3) SAME PATTERN AS AFTER ^{3}H-E_2 ADMINISTRATION
4) AFTER ^{3}H-E_2, NUCLEAR LABELLING GREATER THAN AFTER ^{3}H-T
5) AFTER ^{3}H-T, NUCLEAR LABELLING LESS IF ANIMAL PRETREATED WITH E_2,T BUT NO CHANGE IF PRETREATED WITH DHT.

Fig. 5. Summarizing various uptake and competition studies on central androgen mechanisms.

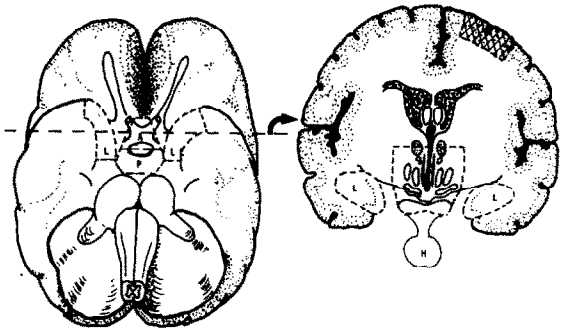

Fig. 6. Central areas which concentrate androgens and estrogens. These areas were studied for the presence of aromatizing enzymes. L, Limbic tissues (amygdala and hippocampus); A and P, anterior and posterior hypothalamus; H, anterior hypophysis; Crosshatched, parietal cortex (from Naftolin *et al.*[32]).

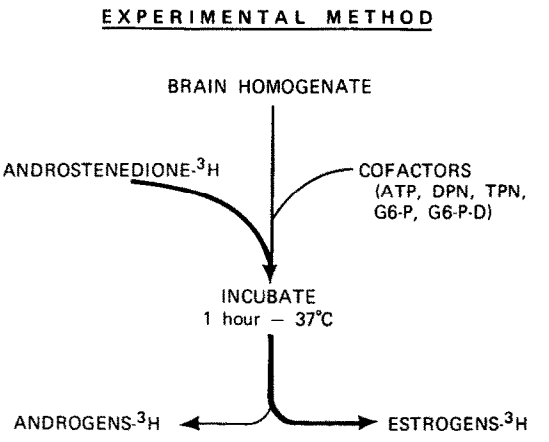

Fig. 7. (From Naftolin *et al.*[32]).

CENTRAL AROMATIZATION

In light of the above, it should be clear why one might consider central neuroendocrine tissues as a site for the production of estrogens. The areas investigated were those identified as taking up radiolabel after injection of radiolabeled sex steroids [26, 27] (Fig. 6). The method used to investigate this metabolism is one that has been long in use in our laboratory [29] (Fig. 7); the tissue homogenate is incubated with substrate (in the illustration, androstenedione) and cofactors for one hour, the estrogens formed are separated from the androgens and are purified by stringent techniques (Fig. 8). Identification of the newly formed estrogen relies on organic partitioning, phenolic separations, paper chromatography, derivative formation, rechromatography and co-crystallization to constant S.A.

When we apply this method to central tissues of the human fetus [30, 31, 32], rhesus monkeys [33, 34], rabbits [32, 35], rats [36, 37] and mice (unpublished), we can find this metabolism in each species (Table 1). Generally, using [^{3}H]-androstenedione, we have been able to demonstrate new estrone formed in each case. In a number of *in vitro* and *in vivo* experiments, estradiol was also formed from androstenedione. Testosterone, when tested, has shown the formation of estradiol, and on occasion, estrone [38]. 17α-Ethynyl 19-nor testosterone has also been shown to form ethynyl estradiol in very low yields. This is problematic because of the possibility of artifactual formation of

Table 1. Substrates and products in central steroid aromatization (from Naftolin *et al.*[38])

	Substrates			
	Δ^4	T	17α-ethynyl-19-nor-T	E_1
Human Fetus	E_1,E_2	-	EE_2	-
Rhesus	E_1 E_2*	E_2 E_1*	-	-
Rabbit	E_1,T	E_2,Δ^4	-	E_2
Rat	E_1	-	-	-
Mouse	E_1	-	-	-

* During *in vivo* experiments both E_1 and E_2 were formed from androstanedione (Δ^4) or testosterone (T).

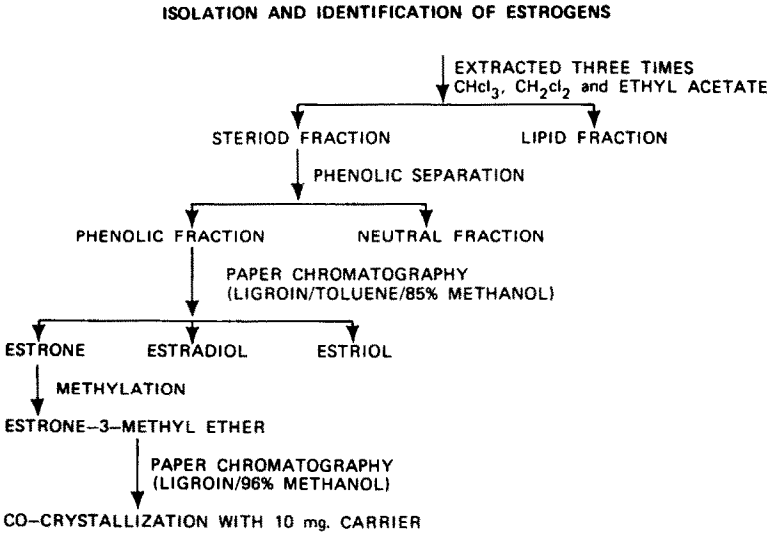

Fig. 8. (From Naftolin *et al.*[32]).

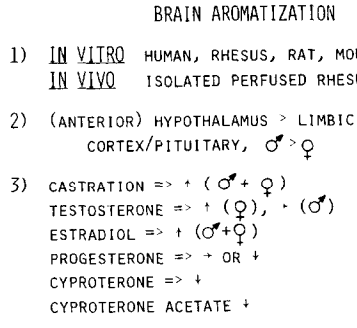

Fig. 9. Summarizing much of what is known of central steroid aromatizing activity.

estrogen during the exposure to alkali [39]. In our work the method employed no acid or alkali. Ethynyl estradiol was identified using the same stringent criteria as for other estrogens (unpublished).

With this general background we should like to discuss a few of the newer aspects of estrogen formation by central tissues (Fig. 9). *In vitro* aromatization, generally by the hypothalamus and limbic system, has been found in all species thus far studied [38, 40]. This has recently been confirmed by Weisz and Gibbs[41] in the neonatal female rat and was initially indicated by Knapstein *et al.* in the rhesus [42]. *In vivo* aromatization of androgens by the immature and mature rhesus has also been proven by the use of the isolated perfused rhesus brain [34]. When one divides the hypothalamus into anterior and posterior fragments by an arbitrary cut across the median eminence (Fig. 6), the major portion of the activity is found in the anterior areas [32, 36]. The limbic system is active—far more active than the occasional activity seen in cortex or pituitary. In addition, we regularly find that male brains have more activity than female. In rabbits, castration results in an elevation of the activity, particularly in the hypothalamus of both males and females. Testosterone pretreatment elevates the activity in females more than in males, and so the sex difference disappears. Estradiol elevates the activity in both males and females. Progesterone's effect is variable; pretreatment of rabbits has resulted in a decrease or no change in *in vitro* aromatization in our hands [37, 38]. All of the above studies have recently been reviewed in some detail [38].

Rather than expanding upon any certain technical or metabolic aspect we have tried here to draw a perspective from which one might view the emerging picture of the metabolism of androgens in central neuroendocrine tissues. Ring A reduction not only occurs in these tissues; it apparently accounts for a far greater percentage of androgen metabolism than does ring A aromatization. However, the list of inconsistencies of action of ring A reduced compounds, the evidence that anti-estrogens so strongly interfere with central "androgen actions" and the many-fold greater potency of estrogens in all central actions (even those previously identified as effects of testosterone) have focused attention upon the potential effects of even the smallest *in situ* estrogen formation in central tissues. Interestingly, both ring A reduction and aromatization appear to be sensitive to changes in the hormonal milieu which may be found in life. We should like to extend the meaning of "central androgen action" to include the formation and effects of estrogens as well as ring A reduction. This is in contradistinction to "peripheral androgen action" which has come to be almost synonymous with ring A reduction and its products. In making this distinction we may be anticipating the ultimate relating of the cellular and synaptic, and nervous and anterior pituitary actions that control reproduction.

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REFERENCES

1. Jaffe R. B.: *Steroids* **114** (1969) 483–498.
2. Kniewald Z., Massa R. and Martini L.: In *Hormonal Steroids*, Exerpta Med. Inter. Cong. Ser. **219** (1970) 784–790.
3. Engel L.: In *Handbook of Physiology* (Edited by R. O. Greep). American Physiological Society, Washington, D.C., Vol. II (1973) Part 1, pp. 467–489.
4. McDonald P. G. and Doughty C.: *J. Endocrinol.* **61** (1974) 95–104.
5. Parrott R. F.: *J. Endocr.* **61** (1974) 105–115.
6. Fishman J.: Proceedings of the 55th Annual Meeting of the Endocrine Society (1973) Abstract 39.
7. Heller C. G., Moore D. J., Paulsen C. A., Nelson W. O. and Laidlaw W. M.: *Fedn Proc.* **18** (1959) 1057–1063.
8. MacDonald P. C., Rombaut R. P. and Siiteri P. K.: *J. clin. Endocr. Metab.* **27** (1967) 1103–1114.
9. Baird D., Horton R., Longcope C. and Tait J. F.: *Persp. biol. Med.* **11** (1968) 384–402.
10. Kincl F. A., Folch Pi, A., Maqueo M., Lasso L. H., Oriol A. and Dorfman R. I.: *Acta endocr., Copenh.* **49** (1965) 193–214.
11. Gorski R. A.: *Am. J. Physiol.* **205** (1963) 842–849.
12. Gorski R. A. and Barraclough C. A.: *Endocrinology* **73** (1963) 210–219.
13. Luttge W. G. and Whalen R. E.: *Horm. Behav.* **1** (1970) 265–280.
14. Brown-Grant K., Munck A., Naftolin F. and Sherwood M. R.: *Horm. Behav.* **2** (1971) 173–200.
15. McDonald P. G. and Doughty C. J.: *Endocrinology* **55** (1972) 455–470.
16. McCann S. M. and Ramirez V. D.: *Rec. Prog. Horm. Res.* **20** (1964) 131–170.
17. Sherins R. J., Loriaux D. L.: *J. clin. Endocr. Metab.* **36** (1973) 886–904.
18. Stewart-Bentley M., Odell W. and Horton R.: *J. clin. Endocr. Metab.* **38** (1974) 545–560.
19. Swerdloff R. S., Walsh P. C. and Odell W. D.: *Steroids* **20** (1972) 13–20.
20. Naftolin F. and Feder H. H.: *J. Endocr.* **56** (1973) 155–156.
21. Naftolin F., Judd H. L. and Yen S. S. C.: *J. clin. Endocr. Metab.* **36** (1973) 285–293.
22 Beyer C., Vidal N. and Mijares A.: *Endocrinology* **87** (1970) 1386–1396.
23. Feder H. H., Naftolin F. and Ryan K. J.: *Endocrinology* **94** (1974) 136–150.
24. Beyer C. and Vidal N.: *J. Endocr.* **51** (1971) 401–414.
25. Eckstein B., Golan R. and Shani J.: *Endocrinology* **92** (1973) 941–957.
26. Pfaff D. W.: *Science* **161** (1968) 1355–1356.

27. Stumpf W. E., Baerwaldt C. and Sar M.: In *Basic Actions of Sex Steroids on Target Organs* (Edited by P. O. Hubinont and F. Leroy). Karger, Basel (1971) pp. 3–27.
28. Sheridan P. J., Sar M. and Stumpf W. E.: *Endocrinology* (in press).
29. Ainsworth L. and Ryan K. J.: *Endocrinology* **79** (1966) 875–894.
30. Naftolin F., Ryan K. J. and Petro Z.: *J. clin. Endocr. Metab.* **33** (1971) 368–370.
31. Naftolin F., Ryan K. J. and Petro Z.: *J. Endocr.* **51** (1971) 795–796.
32. Naftolin F., Ryan K. J., Davies I. J., Petro Z. and Kuhn M.: In *Advances in the Biosciences* (Edited by G. Raspé). Pergamon/Vieweg, Oxford. Vol. 14 (in press).
33. Flores F., Naftolin F. and Ryan K. J.: *Neuroendocrinology* **11** (1973) 177–183.
34. Flores F., Naftolin F., Ryan K. J. and White R. J.: *Science* **180** (1973b) 1074–1076.
35. Reddy V. V. R., Naftolin F. and Ryan K. J.: *Endocrinology* **94** (1974) 117–132.
36. Naftolin F., Ryan K. J. and Petro Z.: *Endocrinology* **90** (1972) 295–298.
37. Reddy V. V. R., Naftolin F. and Ryan K. J.: *Endocrinology* **92** (1973) 589–602.
38. Naftolin F., Ryan K. J., Davies I. J., Reddy V. V., Flores F., Petro Z., Kuhn M., White R. J., Takaoka Y. and Wolin L.: *Recent Prog. Horm. Res.*, Vol. 31 (in press).
39. Breuer H.: *Lancet* **11** (1970) 615.
40. Ryan K. J., Naftolin F., Reddy V., Flores F. and Petro Z.: *Am. J. Obstet. Gynecol.* **114** (1972) 454–462.
41. Weisz J. and Gibbs C.: *Endocrinology* **94** (1974) 616–624.
42. Knapstein P., David A., Wu C. H., Archer D. F., Flickinger G. L. and Touchstone J. C.: *Steroids* **11** (1968) 884–893.