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BIOLOGICAL SIGNIFICANCE OF THE METABOLISM OF ANDROGENS IN THE CENTRAL NERVOUS SYSTEM*

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SUMMARY

Several testosterone-dependent actions of the mammalian CNS may be mediated by its metabolites. Regional distribution, selective retention, and further *in vivo* and *in vitro* metabolism of androgens in the pituitary and brain of several species are well known. Male sexual behavior and gonadotropin secretion were used as typical neuroendocrine androgen regulated functions. Since testosterone and androstenedione can be biotransformed to at least five neutral metabolites besides phenolic derivatives, a correlation between androgen metabolism in the CNS and the expression of the activity of the enzymatically formed androgen metabolites was made. It was found that non-aromatizable androgens elicited weak, if any, male copulatory behavior, suggesting that estrogens may play an important role in this function, although antiestrogens can not prevent the androgen-induced behavior and estrogens administered in low dosages *per se* are ineffective. From another series of experiments it was concluded that the expression of male sexual behavior may result either from unmetabolized testosterone or by the combined effects of 5α-ring A reduced metabolites [Dihydrotestosterone] and estrogens (E_2), whereas the gonadotropin inhibition mechanism does not require androgen aromatization at least in the rat. These studies strongly suggest that the androgen mechanism of action of the CNS may be the result of simultaneous effect of various metabolites formed either peripherally or at brain level.

INTRODUCTION

Extensive studies in the past years have demonstrated that testicular synthesized androgens are further biotransformed in non-neural tissues to other neutral [1–7] and phenolic [8–12] metabolites. Some of the resulting compounds not only retain the androgenic activity but exhibit a two- or three-fold increase [13, 14] and in some instances may show different biological activities [5, 15, 16]. Presence of the enzyme systems necessary to modify the molecular structure of gonadal androgens in target tissues has been demonstrated and the study of the sequence of molecular processes by which androgens or their metabolites elicit their effects [17, 18] have led to a better understanding of their mode of action.

The actions of gonadal androgens on several brain substrates are well established. Thus testosterone administration is known to stimulate male and female sexual behavior [19, 20], regulate pituitary gonadotropin secretion (negative and positive feedback mechanism) [21, 22], induce anovulatory sterility in female animals when given during perinatal days (hypothalamic virilization) [23, 24] and induce changes in mood and aggressiveness [25] to mention only some of the best analyzed phenomena.

Since central nervous system structures are androgen target organs, the possibility exists that some of the androgen actions in brain are mediated through systemic or local conversion of testosterone to other metabolites. Furthermore, the biological activity patterns of a given metabolite at brain level may differ from that observed in other peripheral organs such as sexual accessories or muscle. In this paper we attempt to establish a preliminary correlation between androgen metabolism in the central nervous system and the expression of the biological activity of the enzyme-formed androgen metabolites.

BRAIN METABOLISM OF ANDROGENS

That mammalian brain is capable of metabolizing androgenic substrates under *in vitro* conditions was demonstrated in 1966 by Sholiton *et al.*[26] who found formation of more polar and less polar metabolites following incubations of rat brain with [4-^{14}C]-testosterone. Since then, a number of laboratories have demonstrated the ability of mammalian brain and pituitary to convert testosterone to 5α-dihydrotestosterone (DHT), androstenedione (4-ene-A], 5α-androstandione (5α-A), androstandiols, and estrogens [27–41], thus demonstrating the presence of 4-ene-5α-3-ketosteroid oxidoreductase, 17β-ol-dehydrogenase, 3α- and 3β-reductases, and aromatases in these tissues.

Figure 1 shows the *in vitro* metabolism of testosterone and androstenedione by dog hypothalamus, hippocampus and pituitary. The pituitary showed the greatest enzymatic conversion of both substrates to DHT, and 5α-A. The hypothalamus gave metabolic conversion to a lesser extent than the pituitary but higher than those effected by the hippocampus. As

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EXP.	INCUBATED SUBSTRATE	INCUBATED TISSUES	TISSUE WEIGHT (mg)	ISOLATED METABOLITES			
				5α-Dihydro A (μmoles/mg)	5α-Dihydro T (μmoles/mg)	T (μmoles/mg)	A (μmoles/mg)
1	Androstenedione -4-^{14}C 0.7 μCi	Pituitary	34.7	12.0×10^{-9}	2.6×10^{-9}	34.1×10^{-9}	-
		Hypothalamus	67.5	4.4×10^{-9}	1.1×10^{-9}	10.7×10^{-9}	-
		Limbic Area	152.0	3.1×10^{-9}	0.8×10^{-9}	3.0×10^{-9}	-
2	Testosterone 7-^{3}H 4 μCi	Pituitary	34.3	31.7×10^{-9}	81.0×10^{-9}	-	52.4×10^{-9}
		Hypothalamus	74.0	7.2×10^{-9}	31.6×10^{-9}	-	17.0×10^{-9}
		Limbic Area	128.0		16.0×10^{-9}	-	

Fig. 1. Biotransformation of testosterone and androstenedione by dog pituitary, hypothalamus and limbic area (hippocampus). (From: Pérez-Palacios *et al.*: *Biol. Reprod.* **3** (1970) 205).

shown, 4-ene-A can also be converted to 5α-reduced metabolites by brain tissues. Similar results have been obtained in our laboratory by incubating ^{3}H-4-ene-A with rat pituitary and brain tissues [42].

DHT is the major T metabolite of the pituitary and the hypothalamus in most species. Denef *et al.*[43] have reported that in other rat brain areas such as the preoptic region, cerebral cortex, pineal gland or cerebellum, the conversion rate of T to DHT was found to be very low, suggesting a regional differentiated distribution of the 5α-reductase system.

Several investigators [37, 43, 44] have reported that castration results in a significative increase of DHT formation from T by the pituitary, suggesting a feedback regulatory mechanism of the NADPH dependent-enzyme. Recently we have found that castration increases significantly the conversion of 4-ene-A to androsterone in male rats. A higher conversion rate of T to DHT and androstanediols in males than in female animals has also been observed. In contrast to the 5-reductase regional distribution, the aromatizing activity in several species is probably present exclusively in limbic tissues. Male animals also showed a higher level of aromatization than the females, and castration increases the enzymatic activity [45].

That testicular androgens are able to pass the blood-brain barrier has been well established by the use of several procedures. Studies of *in vivo* and *in vitro* [46–51] uptake of testosterone by the mammalian brain and pituitary have been reported, demonstrating selective retention of the androgen. We have studied the regional distribution of three natural androgens (T, DHT and 4-ene-A) in the brain and pituitary of castrated male rats [52]. As shown in Fig. 2 the highest uptake of radioactivity was observed in the pituitary. The pituitary/cerebral cortex ratio was 13·1 for DHT, 5·6 for T and 2·9 for 4-ene-A. Only DHT was accumulated significantly by some brain areas (hippocampus) although nearly all neural structures accumulated more androgens or its metabolites than the cerebral cortex. Similar results were obtained in castrated male rabbits after injection of high specific activity ^{3}H-T (Fig. 3). The highest uptake was observed in the seminal vesicles and in the pituitary. The seminal vesicles/cortex and pituitary/cortex ratios were 5·89 and 3·86 respectively at 120 min. The hypothalamus showed a higher although no significant tissue/cortex ratio 120 min after T injection. The relatively low and non significant uptake of radioactive-labeled androgens by the hypothalamus does not mean that this structure is not a target for androgens. Autoradiographic evidence indicates that only a small proportion of hypothalamic neurons retains T in the rat [53]. Recently, Sar and Stumpf[50] have demonstrated by dry autoradiography that, following administration of ^{3}H-T, the radioactivity is found to be selectively concentrated and retained in specific neurons in the nucleus arcuatus and ventromedialis of the hypothalamus besides the hippocampus and the amygdala.

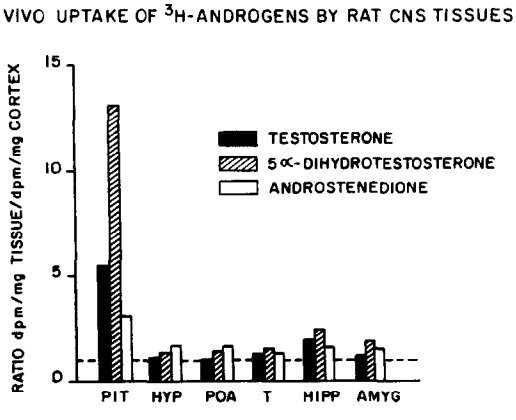

Fig. 2. Regional distribution of isotopically-labeled androgens in the brains of castrated male rats. (From: Pérez-Palacios *et al.*: *Biol. Reprod.* **8** (1973) 395).

Intracellular binding components for testosterone or its metabolites at cytoplasmic or nuclear level appear to be present in brain and pituitary in order to retain testosterone or its metabolites at the cytoplasmic or nuclear level. Samperez *et al.*[54] demonstrated the presence of a macromolecular association of ^{3}H-T in the cytoplasma of anterior pituitary and hypothalamus of normal and castrated male rats. These soluble androphilic molecules were later characterized by Jouan *et al.*[32, 55]. The same investigators have recently reported the presence of two macromolecular associations of T in purified nuclei of

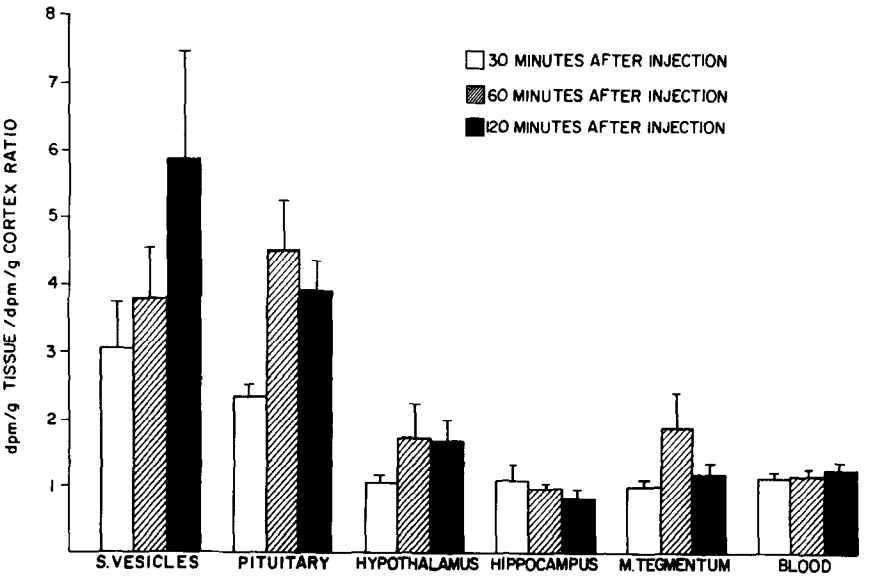

Fig. 3. *In vivo* uptake of ^{3}H-testosterone by male rabbit brain, pituitary and seminal vesicles.

rat pituitary [56]. In addition, Kato and Onouchi[57] have demonstrated a specific DHT-binding component in the rat hypothalamus cytosol. Such androgen receptor molecule has a sedimentation constant of 8·6 S with a dissociation constant of $7{\cdot}4 \times 10^{-10}$ M. The precise role of these so-called "receptors" for androgens in brain remains to be elucidated.

ANDROGEN AND MALE SEXUAL BEHAVIOR

Although a great number of androgens have been studied for their peripheral "androgenic" and "anabolic" potency [58, 17], very few have been tested for stimulation of sexual behavior in the male mammal. In 1970 McDonald *et al.*[59] reported that in contrast to its important peripheral effects, DHT failed to initiate sexual behavior in prepuberally castrated male rats. This finding which questioned the role of a 5α-reduced metabolite of T and suggested that hormone effects in the central nervous system may be different from that observed in several accessories was confirmed in other laboratories [60–62]. In order to establish the characteristics of the androgens required to stimulate sexual behavior in the male rat and to compare them with those required for inducing development of sexual accessories, we examined the effectiveness of 10 of the most important natural androgens [64], including compounds with structures 4-ene-3-keto, 5-ene-3β-OH, 3-keto-5α,3α-5α,3α-5β, 17β-OH, 17-keto and 11β-OH. Androgens were administered (1 mg daily) subcutaneously for 33 days. All animals were tested once for sexual behavior the control day and every third day post treatment. After the last test, the animals were killed and ventral prostate and seminal vesicles were dissected and weighed. None of the rats displayed sexual activity during the control test. Figure 4 shows the cumulative percentage of rats displaying mounting, intromission and ejaculation. The highest proportion of rats that displayed mounting was found in those groups treated with T, 4-ene-A and androstenediol. In addition animals from these three groups responded with mounting significantly earlier than those of remaining

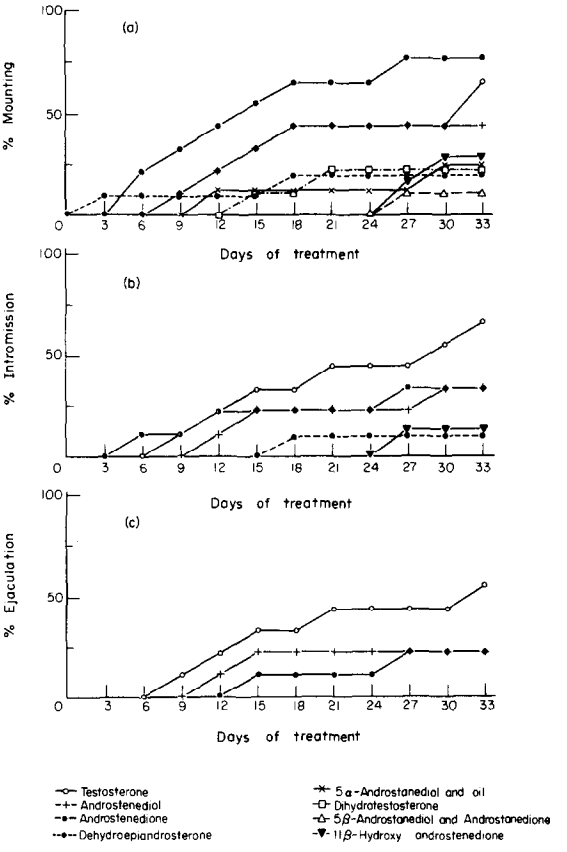

Fig. 4. Effect of several androgens on male sexual behavior. Results are expressed as the cumulative percentage of rats displaying mounting, intromission, and ejaculation. (From: Beyer *et al.*: *Horm. Behav.* 4 (1973) 99).

groups. Furthermore the complete copulatory pattern (including intromission and ejaculation) occurred only in the same three groups. T appeared to be more effective than 4-ene-A and androstenediol although this difference was not statistically significant. On the other hand, the study of the sexual accessories in the diverse groups revealed that the most effective androgens to stimulate growth of the ventral prostate and seminal vesicles were: 3α,5α-androstanediol, DHT and T.

The results clearly demonstrated that at the dose employed, only T, 4-ene-A and androstenediol stimulated copulatory behavior in the male rat. The finding that some androgens with potent androgenic activity at sexual accessories failed to induce sexual behavior support the concept that the mechanisms underlying the behavioral response to androgens differ from those related to growth responses. Since only one dose level of the three androgens was employed, the lack of group differences observed can not be taken as demonstrating that T, 4-ene-A and androstenediol are equally potent for eliciting sexual behavior. Another study using three dose levels (0·3, 1·0 and 3·0 mg daily) of the three androgens was undertaken [65]. Figure 5, depicts the results obtained in each group expressed as cummulative percentage of animals responding each test day. T was more active in initiating male sexual behavior than 4-ene-A or androstenediol, and 4-ene-A was the least potent. This result contrasts with that of Whalen and Lutge[62] who found 4-ene-A as potent as T in maintaining behavior when administered immediately after castration to sexually experienced animals, thus suggesting that the hormonal factors required for maintenance may differ from those required for initiation of male sexual behavior.

The finding that androstenediol at the 3 mg dose level was as effective as T, suggests that either androstenediol can activate sexual behavior *per se* or that a highly efficient conversion of androstenediol to T occurs either peripherally or in brain substrates. Some recent evidences also suggest that androstenediol might be more effective than T in hypothalamic virilization [63]. Preliminary results in our laboratories have shown that androstenediol in castrated male rats is maintained in blood in its free and conjugated forms, long time after injection, thus suggesting that they may serve as precursors for T or other metabolites.

The three androgens which were found to be effective in stimulating full copulatory behavior are unsaturated compounds possessing either the 4–5 or 5–6 double bond. Hydrogenation of the double bond by formation of the 5α (trans A/B ring junction) or the 5β (cis A/B ring junction) isomers results in compounds which failed to stimulate male sexual behavior, although some 5α-reduced metabolites retain strong androgenic activity.

It is important to mention that 11β-hydroxy-androstenedione, while possessing the characteristic 4–5 double bond, do not induce sexual behavior. However, 11β-hydroxy-androstenedione shares with the 5α and 5β saturated androgens the inability to aromatize due to the presence of an axial hydroxyl group at carbon 11 [66]. Therefore it can be concluded that all androgens effective in terms of behavioral activity are stereochemically able to be aromatized while the compounds which lack behavioral activity are unable to be biologically converted to estrogens.

That conversion of androgens to estrogens plays an important role for induction of estrus behavior was suggested by the findings of Beyer and Vidal[67] in the rabbit and Whalen *et al.*[68] in the rat that antiestrogens can block the testosterone-induced sexual behavior in the female. This hypothesis has been extended to the male rat; however the fact that large amounts of estrogen are required to maintain sexual activity in castrated male rats [69]

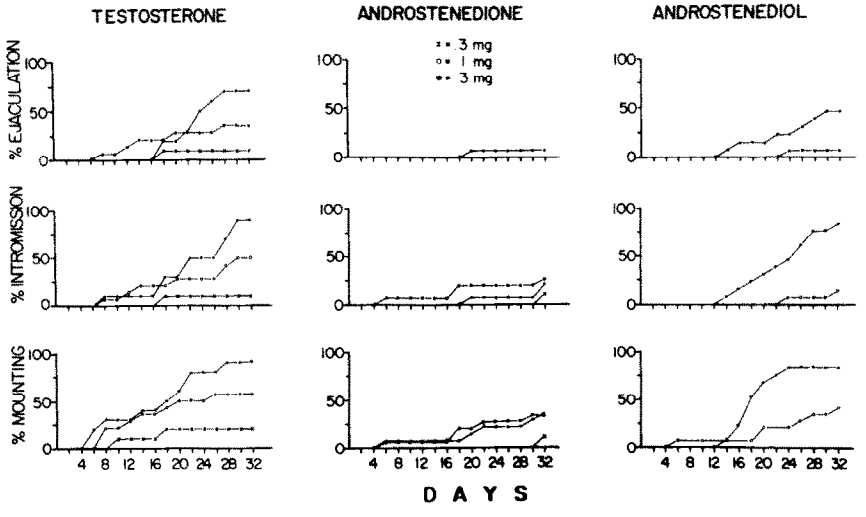

Fig. 5. Comparative effect of testosterone, androstenedione and androstenediol on male sexual behavior at three different dose levels. (From: Morali *et al.*: *Horm. Behav.* **5** (1974) 103).

strongly suggests that estrogens alone are not responsible for the full copulatory behavior but they may regulate one or several of the many steps intervening between the secretion or injection of an androgen and the final behavioral expression.

If the induction of male sexual behavior requires aromatization as an obligatory event, the simultaneous administration of antiestrogenic compounds with testosterone should block the behavioral response. We have studied the effects of MER-25 upon the T-induced male copulatory behavior. At daily doses of 2, 8 and 25 mg of MER-25 simultaneously administered with testosterone, the expression of the behavioral response not only was unblocked but showed a synergistic effect with T. Identical results have been obtained in our laboratories by using cis-clomiphen at several doses. Cis-clomiphen was used because it lacks some of the estrogenic activity of the antiestrogens. It can be concluded that the blockade of the estrophilic molecules (receptors) in brain by using competitive compounds does not prevent the activation of male sexual behavior induced by testosterone.

From a purely endocrine point of view, the results observed in these experiments show a large latency between T administration and the expression of sexual behavior in the castrated animals. Since it has been reported that castration resulted in a significant depletion of the so-called receptors in target tissues [70], we felt it was of interest to pretreat some groups of castrated male animals with two behaviorally ineffective compounds DHT and estradiol prior to testosterone administration. The aim of these experiments was to see if DHT or estradiol benzoate (EB) may build T-receptor molecules in the brain which in turn would facilitate the action of T by reducing the latency period. Daily pretreatment with 1 mg of EB or 1 mg of DHT was given for 15 days and 1 mg/day of T was administered for 20 additional days. The results demonstrated that pretreatment with EB does not modify the latency period nor the extent of the behavioral response and that DHT pretreated animals showed a shorter although significant latency and an increase of sexual behavior activity. These findings may be explained by a significant development of sexual accessories during DHT treatment.

Larsson *et al.*[71] recently reported that the combined administration of EB and DHT to castrated male rats induces full copulatory behavior similar to that observed following T treatment. Similar results have also been reported recently [72, 73]. The fact that DHT and EB, two inffective compounds *per se*, have synergistic behavioral effects when combined, suggest that male sexual behavior may be the result of two intracellular metabolites derived from T and that estrogens may play some role in the regulation of mating behavior of the intact male rat or that androgens and estrogens may act at two neural sites. More recently we have shown that administration of MER-25 (8 mg daily) to the combined treatment of DHT (1 mg) and EB (2·5 μg) to castrated male rats does not interfere with the induction of sexual behavior. These data confirm that the pharmacological combination of DHT and EB is effective in inducing sexual behavior in the male rat and is not impaired by antiestrogen administration, demonstrating that estrogens participate in a still undefined manner in stimulating sexual behavior. In this connection the findings of Zucker[74] and more recently of Whalen and Edwards[75] are interesting. They showed that cyproterone acetate, a potent antiandrogen, does not lead to an inhibition of T-maintained mating behavior in male rats in spite of a strong antiandrogenic activity in sexual accessories observed in these animals. Since antiestrogens and antiandrogens are unable to inhibit the hormonal induced copulatory behavior, it is suggested that androgens and/or estrogens do not necessarily require the classical intracellular receptors in order to induce male behavioral response as occurs for other effects at peripheral target organs.

ANDROGENS AND GONADOTROPINS REGULATION

The role of testosterone in feedback mechanisms regulating pituitary gonadotropin secretion has been well established. However, few data were available about the role of metabolites derived from T in this brain function until recently. Earlier reports suggested that DHT was effective as suppressor of gonadotropic hormones [76, 77]. Beyer *et al.*[78] demonstrated that DHT at relatively low dosages effectively blocked ovarian compensatory hypertrophy in rats, suggesting that this 5α-reduced derivative of T inhibited gonadotropin secretion. Furthermore in a comparative study it was demonstrated that DHT was more effective in inhibiting the serum rise of immunoassayable levels of LH and FSH observed after castration of female rats [79]. Thus daily administration of 100 μg of DHT for 14 days to recently ovariectomized rats resulted in a total LH suppression whereas the same dose of T suppressed LH only partially (50%). Similar data using DHT in male rats have been reported by Naftolin and Feder[80]. In addition these investigators demonstrated that DHT in an acute administration also shows a suppressive LH effect. The effect of 5α-reduced metabolites of T as potent LH inhibitors has been also reported by other laboratories [81] and strongly suggests that aromatization of androgens is not required for this androgen activity. Zanissi *et al.*[82] have also reported that 3α-5α-androstanediol is even more potent than DHT to suppress gonadotropin release.

Although the sites of androgen-induced negative feedback for gonadotropin release under physiological situations are still unclear, there is good experimental evidence which suggests that hypothalamus [83] and pituitary [84] are the anatomical substrates. Both T and DHT have been shown to modulate the pituitary response to the exogenous administration of LH-RH [85, 86]. In addition to the

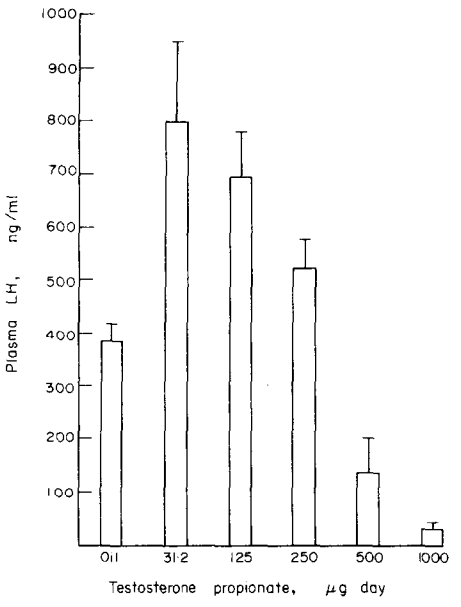

Fig. 6. Dose dependent positive and negative LH feed back mechanisms induced by testosterone propionate in long-term castrated adult rats.

inhibitory activity of gonadotropin release, androgens, under certain conditions, may also stimulate gonadotropin release. Rubinstein and Kurland reported in 1941 [87] that small doses of T increase testicular weight, and very recently, Bloch *et al.*[22] demonstrated that in castrated adult rats, administration of 6 μg of TP/100 g/day for 10 days increased plasma LH concentration while larger doses totally suppressed plasma LH levels. We have recently demonstrated in long-term castrated adult rats (more than 100 weeks) dose-dependent positive and negative feedback mechanisms induced by TP. Figure 6 shows that the lowest doses of TP (31·2 and 125 μg) elicited a statistically significant increase in plasma LH whereas the highest dose levels (500 and 1000 μg) clearly suppressed the plasma LH values. Similar effects have been observed in the intact female rat[88]. In addition it has been reported that 3α-5α-androstanediol may induce LH secretion in the rat, and it is suggested that these compounds may trigger the hypothalamic-pituitary axis for the onset of puberty [89].

In summary, we may conclude that in contrast to male sexual behavior, ring A reduced derivatives of T are effective for gonadotropin regulation in the rat and that aromatization may not be obligatorily required. It must be pointed out that while 5α-reduction of T results in metabolites with a more potent antigonadotropic potency, 5α-reduction of 4-ene-A results in compounds with very little if any antigonadotropic capability.

OTHER BRAIN ACTIVITIES

The role of T in the induction of hypothalamic virilization has been well documented [23, 24]. T is believed to inhibit the differentiation of neural cells which are later involved in gonadotropin control mechanism. Thus administration of T during the "critical" perinatal days to female rats results in a male type of hypothalamic activity in adulthood. Administration of DHT, however, failed to mimic the action of T, thus demonstrating that 5α-reduction of T abolishes this activity [90–92]. Furthermore, administration of an antiestrogen inhibits the T-induced hypothalamic virilization [93], suggesting that estrogens play an important role in this phenomenon in a similar fashion to their role in the induction of estrous behavior. Furthermore support for this concept can be derived from the observation that EB mimics the action of T in inducing anovulatory sterility during perinatal days.

CONCLUDING REMARKS

Major circulating androgens synthesized by the gonads enter into the central nervous system where they are regionally distributed and selectively retained. They may remain at the neuronal level either unchanged as T, 4-ene-A or androstenediol, or they may further be converted to 5α-reduced or to phenolic derivatives. These three groups of compounds may induce the physiological effects traditionally attributed to T in the mammalian male brain. Our present knowledge can be summarized as depicted in Fig. 7.

1. *Gonadotropin secretion regulation.* Negative and positive feedbacks may result from unchanged T or from 5α-reduced T-metabolites in the rat. 5α-reduction in the case of T increases the biological potency while in the case of 4-ene-A decreases the activity of the derivative. Aromatization may not necessarily be required for this activity.

2. *Induction of male sexual behavior.* Results either from unchanged T or from the synergistic effects of 5α-reduced T and estradiol. Both 5α-reduction and aromatization may be required for this activity. It

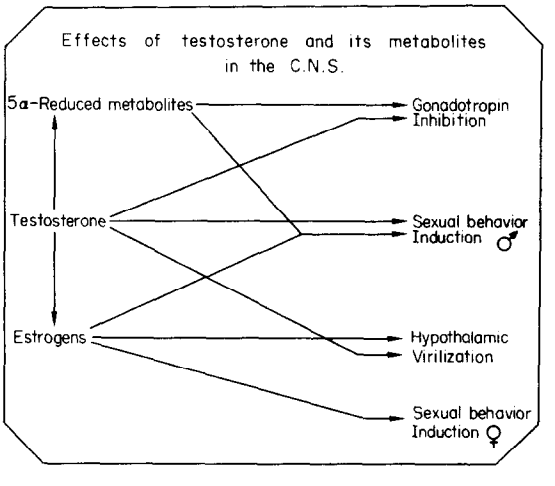

Fig. 7. Role of testosterone and its metabolites on several brain activities.

seems possible that this androgen-induced activity does not necessarily involve hormone-receptor interactions in the way that occurs in peripheral organs.

3. *Induction of female sexual behavior and hypothalamic virilization.* Results from conversion of T to estrogens. Aromatization is obligatory and 5α-reduction is not required.

REFERENCES

1. Bruchovsky N. and Wilson J. D. *J. biol. Chem.* **243** (1968) 2012–2021.
2. Wilson J. D. and Walker J. D.: *J. clin. Invest.* **48** (1969) 371–379.
3. Wilson J. D. and Gloyna R. E.: *Recent Prog. Horm. Res.* **26** (1970) 309–336.
4. Pérez-Palacios G., Morato T., Pérez A. E., Costañeda E. and Gual C.: *Steroids* **17** (1970) 471–492.
5. Baulieu E. E., Lasnitzki I. and Robel P.: *Nature Lond.* **219** (1968) 1155–1156.
6. Morato T., Flores F. and Pérez-Palacios G.: In *Recent Advances in Endocrinology* (Edited by E. Mattar, G. B. Mattar and V. H. T. James). Excerpta Med. Inter. Cong. Ser. **238** (1972) pp. 242–249.
7. Wilson J. D. and Lasnitzki I.: *Endocrinology* **89** (1971) 659–688.
8. West C. D., Damast B. L., Sarro S. D. and Pearson O. H.: *J. biol. Chem.* **218** (1956) 409–418.
9. McDonald P. C., Rombaut R. P. and Siiteri P. K.: *J. clin. Endocr. Metab.* **27** (1967) 1103–1111.
10. Slaunwhite W. R.: *Steroids* **2** (1965) 211–215.
11. Gallegos A. J. and Canales E. S.: 51st Meeting U.S. Endocrine Soc. New York 1969 (Abstract).
12. Grodin J. M., Siiteri P. K. and McDonald P. C.: *J. clin. Endocr. Metab.* **36** (1973) 207–214.
13. Dorfman R. I. and Shipley R. A.: In *Androgens: Biochemistry, Physiology, and Clinical Significance* (Edited by R. I. Dorfman and R. A. Shipley). John Wiley & Sons (1956) New York, p. 116.
14. Liao S. and Fang S.: *Vit. Horm.* **27** (1969) 17–90.
15. Huggins C., Jensen E. V. and Cleveland A. S.: *J. exp. Med.* **100** (1954) 225–229.
16. Mann T., Rowson L. E. A., Baronos S. and Karagiannidis A.: *J. Endocr.* **51** (1971) 707–718.
17. Vida J. A.: *Androgens and Anabolic Agents, Chemistry and Pharmacology.* Academic Press, New York, 1960.
18. Liao S., Tymoczko J. L., Castañeda E. and Shao T. C.: In *Normal and Abnormal Growth of the Prostate* (Edited by L. Axelrod). In press, 1974.
19. Beach F. A.: *Hormones and Behavior.* P. B. Hoeber Inc. New York (1948).
20. Young W. C.: In *Sex and Internal Secretions* (Edited by W. C. Young). Williams & Wilkins Co. (1961), Baltimore pp. 1173–1224.
21. Bogdanove C. M. and Gay V. L.: *Endocrinology* **81** (1967) 930–933.
22. Bloch G. J., Masken J., Kragt C. L. and Ganong W. F.: *Endocrinology* **94** (1974) 947–951.
23. Barraclough C. A.: *Endocrinology* **68** (1961) 68–74.
24. Barraclough C. A.: *Recent Prog. Horm. Res.* **22** (1966) 503–508.
25. Guhl A. M.: In *Sex and Internal Secretion* (Edited by W. C. Young). Williams & Wilkins Co. (1961), Baltimore pp. 1240–1267.
26. Sholiton L. J., Mornell R. T. and Werk E. E.: *Steroids* **8** (1966) 265–275.
27. Jaffe R. B.: *Steroids* **14** (1969) 483–498.
28. Sholiton L. J. and Werk E. E.: *Acta endocr., Copenh.* **61** (1969) 641–648.
29. Pérez-Palacios G., Castañeda E., Gómez-Pérez F., Pérez A. E., and Gual C.: *Biol. Reprod.* **3** (1970) 205–213.
30. Sholiton L. J., Hall I. L. and Werk E. E.: *Acta endocr. Copenh.* **63** (1970) 512–518.
31. Kniewald Z., Massa R. and Martini L.: 3rd Intl. Congr. Hormonal Steroids (Edited by V. H. T. James and L. Martini) Excerpta Med. (1971) 784–791.
32. Jouan P., Samperez S., Thieulant M. L. and Mercier L.: *C.r. hebd. Séanc Acad. Sci., Paris* **272** (1971) 2368–2371.
33. Naftolin F., Ryan K. J. and Petro Z.: *J. clin. Endocr. Metab.* **33** (1971) 368–370.
34. Rommerts F. F. G. and Van der Molen H. J.: *Biochem biophys. Acta* **248** (1971) 489–502.
35. Stahl F., Poppe I. and Dorner G.: *Acta biol. Med. Germ.* **26** (1971) 855–858.
36. Stern J. M. and Eisenfeld A. J.: *Endocrinology* **88** (1971) 1117–1125.
37. Massa R., Stupnicka E., Kniewald Z. and Martini L.: *J. steroid Biochem.* **3** (1972) 385–399.
38. Mikan H.: *Steroids* **19** (1972) 659–665.
39. Naftolin F., Ryan K. J. and Petro Z.: *Endocrinology* **90** (1972) 295–298.
40. Flores F., Naftolin F., Ryan K. J. and White R. J.: *Science* **180** (1973) 1074–1075.
41. Flores F., Naftolin F. and Ryan K. J.: *Neuroendocrinology* **11** (1973) 177–182.
42. Pérez A. E., Ortiz A., Cabeza M., Beyer C. and Pérez-Palacios G.: *Steroids* (1975) 53–62.
43. Denef C., Magnus C. and McEwen B. S.: *J. Endocr.* **59** (1973) 605–621.
44. Thieulant M. L., Congthien N., Samperez S. and Jouan P.: *Biochimie* **55** (1973) 991–992.
45. Reddy V. V., Naftolin F. and Ryan K.: *Endocrinology* **92** (1973) 589–594.
46. Resko J. A., Goy R. W. and Phoenix C. H.: *Endocrinology* **80** (1967) 490–498.
47. Roy S. K. Jr. and Laumas K. R.: *Acta endocr., Copenh.* **61** (1969) 629–640.
48. McEwen B. S., Pfaff D. W. and Zigmond R. E.: *Brain Res.* **21** (1970) 17–28.
49. Monbon M., Loras B., Reboud J. P. and Bertrand J.: *Brain Res.* **53** (1973) 139–150.
50. Sar M. and Stumpf W. E.: *Endocrinology* **92** (1973) 251–256.
51. Leavitt W. W., Kimmel G. L. and Friend J. P.: *Endocrinology* **92** (1974) 94–103.
52. Pérez-Palacios G., Pérez A. E., Cruz M. L. and Beyer C.: *Biol. Reprod.* **8** (1973) 395–399.
53. Tuohimaa P.: In *Basic Actions of Sex Steroids on Target Organs* (Edited by P. O. Hubinot, F. Leroy and P. Galland). Karger (1971), Basel pp. 208–216.
54. Samperez S., Thieulant M. L. and Jouan P.: *C.r. hebd. Séanc. Acad. Sci., Paris* **268** (1969) 2965–2967.
55. Jouan P., Samperez S., Thieulant M. L. and Mercier L.: *J. steroid Biochem.* **2** (1971) 223–236.
56. Jouan P., Samperez S. and Thieulant M. L.: *J. steroid Biochem.* **4** (1973) 65–74.
57. Kato J. and Onouchi T.: *Endocr. Jap.* **20** (1973) 429–432.
58. Kruskemper M. L.: *Anabolic Steroids.* Academic Press, New York (1968).
59. McDonald P., Beyer C., Newton F., Brien B., Baker R., Tan H. S., Sampson C., Kitching P., Greenhill R. and Pritchard D.: *Nature, Lond.* **227** (1970) 964–965.
60. Davidson J. M., Johnston P., Bloch G. L., Smith E. R. and Weick R. F.: In 3rd Intl. Congr. Hormonal Steroids (Edited by V. H. T. James and L. Martini). Excerpta Medica, Amsterdam (1971) pp. 727–730.
61. Feder H. H.: *J. Endocr.* **51** (1971) 241–252.
62. Whalen R. E. and Luttge W.: *Horm. Behav.* **2** (1971) 117–125.
63. Johnson D. C.: *J. Reprod. Fert.* **32** (1973) 159–161.
64. Beyer C., Larsson K., Pérez-Palacios G. and Moralí G.: *Horm. Behav.* **4** (1973) 99–108.

65. Moralí G., Larsson K., Pérez-Palacios G. and Beyer C.: *Horm. Behav.* **5** (1974) 103–110.
66. Gual C., Morato T., Hayano M., Gut M. and Dorfman R. I.: *Endocrinology* **71** (1962) 920–925.
67. Beyer C. and Vidal N.: *J. Endocr.* **51** (1971) 401–402.
68. Whalen R. E., Battie C. and Luttge W.: *Behav. Biol.* **7** (1972) 311–320.
69. Davidson M.: *Endocrinology* **84** (1969) 1365–1372.
70. King R. J. B. and Mainwaring W. I. P.: *Steroid-cell Interactions.* Butterworths (1974) London pp. 190–262.
71. Larsson K., Sodersten P. and Beyer C.: *J. Endocr.* **57** (1973) 563–564.
72. Feder H. H., Naftolin F. and Ryan K. J.: *Endocrinology* **94** (1974) 136–141.
73. Baum M. J. and Ureeburg J. T. M.: *Science* **182** (1973) 283–285.
74. Zucker I.: *J. Endocr.* **35** (1966) 209–210.
75. Whalen R. E. and Edwards D. A.: *Endocrinology* **84** (1969) 155–156.
76. Boofomely A. C. and Foley S. J.: *J. Physiol., Lond.* **94** (1938) 26–39.
77. Dorfman R. I. and Kincl F. A.: In *Methods in Hormone Research* (Edited by R. I. Dorfman). Academic Press, New York V (1966) pp. 147–203.
78. Beyer C., Morali G. and Cruz M. L.: *Endocrinology* **89** (1971) 1158–1161.
79. Beyer C., Gay V. and Jaffe R. B.: *Endocrinology* **91** (1972) 1372–1375.
80. Naftolin F. and Feder H. H.: *J. Endocr.* **56** (1973) 155–156.
81. Swerdloff R. S., Walsh P. C. and Odell W. D.: *Steroids* **20** (1972) 13–22.
82. Zanisi M., Motta M. and Martini L.: In *Hypothalamic Hypophysiotropic hormones: Physiological and Clinical Studies* (Edited by C. Gual and E. Rosemberg). Excerpta Med. Inter. Cong. Ser. **263** (1973) 24–32.
83. Davidson J. M.: In *Frontiers in Neuroendocrinology* (Edited by W. F. Ganong and L. Martini). Oxford University Press, New York (1969) pp. 353–388.
84. Kingsley T. R. and Bogdanove E. M.: *Endocrinology* **93** (1973) 1398–1409.
85. Debeljuk L., Arimura A. and Schally A. V.: *Endocrinology* **90** (1972) 1578–1581.
86. Debeljuk L., Vilchez-Martínez J. and Arimura A.: 55th Meeting U.S. Endocrine Soc. (1973) (Abstract).
87. Rubinstein H. S. and Kurland A. A.: *Endocrinology* **28** (1941) 495–505.
88. Beyer C., Cruz M. L., Gay V. L. and Jaffe R. B.: *Endocrinology* **95** (1974) 722–727.
89. Eckstein B., Golan R. and Shani J.: *Endocrinology* **92** (1973) 941–945.
90. Whalen R. E. and Luttge W. G.: *Endocrinology* **89** (1971) 1320–1322.
91. Luttge W. G. and Whalen R. E.: *Horm. Behav.* **1** (1970) 265–281.
92. Ladowski W., Kesikowski W. and Souza Zeije L. N.: *Acta Physiol. Lat. Amer.* (1974) (In press).
93. McDonald P. and Doughty C.: *J. Endocr.* **55** (1972) 455–456.