Journal of Steroid Biochemistry, Vol. 9, pp. 411–417

0022-4731/78/0501-0411$02.00/0

STUDIES ON THE MODE OF ACTION OF ANDROGENS IN THE NEUROENDOCRINE TISSUES

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SUMMARY

Experiments were performed to study the factors which might influence the activity of the 5α-reductase-3α-hydroxysteroid dehydrogenase system in the hypothalamus and in the anterior pituitary of male and female rats and to investigate the modulatory effects of a chronic treatment of testosterone (T) and of its 5α-reduced metabolites on LH and FSH secretion. Estradiol benzoate (EB), in a dose of 50 ng/rat/day either for 7 or 14 days, did not have any effect on the enzymatic activities of the anterior pituitary and of the hypothalamus of castrated animals of both sexes and on the hypothalamus of castrated male rats. On the other hand the same dose significantly enhanced the testosterone conversion in the hypothalamus of castrated female rats. Higher dose of EB (5 μg/rat/day) proved able to bring back to precastration levels the 5α-reductase activity of the anterior pituitary of animals of both sexes in the 7-day schedule of administration and to further reduce it in the 14-day schedule. The same dose, while ineffective on the activity of the enzymes of the hypothalamus of the female, was highly active in suppressing it in the males.

The treatment with 800 μg/rat for 5 days of prolactin did not modify the rate of conversion of T in the anterior pituitary and in the hypothalamus of normal male rats and in the gland of castrated males. A significant suppression of the enzymatic activities of the hypothalamus of castrated male rats was observed.

T, dihydrotestosterone (DHT), 5α-androstan-3α, 17β-diol (3α-diol) were given, in a dose of 2 mg/day for 6 days, to castrated male and female rats in order to assess their inhibiting effect on LH and FSH secretion. DHT and 3α-diol were shown to be better suppressors of LH than T itself in both sexes. With regard to FSH no steroid was able to affect FSH release in castrated female and male rats with the exception of DHT which showed some inhibiting effect in males.

The data suggests that EB and prolactin, by modifying the rate of conversion of T into DHT and 3α-diol, may modulate the effects of T at anterior pituitary and at hypothalamic levels. In addition they indicate that T probably exerts its negative feedback effect on gonadotropin secretion following its conversion into DHT and 3α-diol.

INTRODUCTION

It is now generally accepted that, in male mammals, testosterone is converted into 5α-androstan-17β-ol-3-one (dihydrotestosterone, DHT) and 5α-androstan-3α, 17β-diol (3α-diol) in the anterior pituitary and in several CNS structures (e.g. hypothalamus, midbrain, amygdala, etc.) [1–7]. Such conversions occur under the influence of a 5α-reductase-3α-hydroxysteroid dehydrogenase system (see Fig. 1). In the brain and in the anterior pituitary, testosterone may also be converted into 5α-androstan-3, 17-dione, after having been metabolized to Δ_4-androstene-3, 17-dione, through a reversible reaction [8,9] (see Fig. 1). The conversion of testosterone into DHT and 3α-diol seems to be a crucial step for the hormone to exert its negative feedback effect on LH secretion [6,7,10], and may play a role in the control of FSH release, of the sexual organization of the brain during the neonatal or fetal period, and in the expression of male sexual behavior (see [11] for references). Similar enzymatic activities have been found in the anterior pituitary and in the hypothalamus of female mammals where progesterone is probably the physiological substrate. Progesterone is indeed converted into 5α-pregnan-3, 20-dione (dihydroprogesterone, DHP) and 3α-hydroxy-5α-pregnan-20-one (3α-ol) in these structures [1,12–14].

Very little is known about the factors which control the activity of the 5α-reductase-3α-hydroxysteroid dehydrogenase system in the brain and in the anterior pituitary of male and female animals. A crucial role of gonadal hormones is suggested by the fact that, in both sexes, castration enhances the 5α-reductase activity of the anterior pituitary, and that sex steroids, if given in sufficiently large amounts, may counteract such an effect [2–4,6,7,12–16]. Age related changes of the 5α-reductase activity of the central structures have been reported to occur in both sexes [3,16,17]. In the female animals, the activity of these central enzymes shows a cyclic pattern related to the different phases of the estrous cycle [12–14]. Other factors which may be involved in the control of the 5α-reductase activity of the central structures are FSH [3], LH-RH [18], the pineal hormone melatonin [19], and the light–dark schedule [20].

The experiments to be reported here have been planned: (a) to gain a better understanding of some factors which might modulate the activity of the 5α-reductase-3α-hydroxysteroid dehydrogenase system in the hypothalamus and in the anterior pituitary

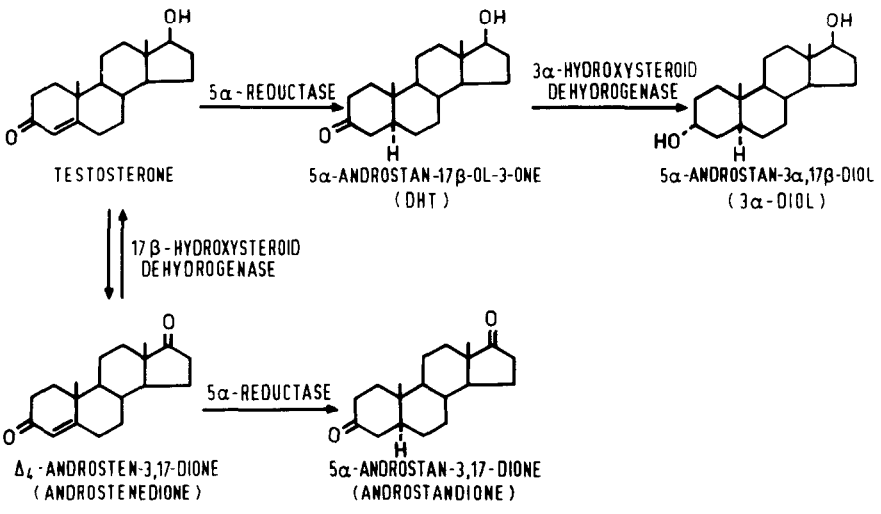

Fig. 1. 5α-Reduction of androgens.

of male and female rats; and (b) to reevaluate the effects of testosterone, and of its 5α-reduced metabolites on gonadotropin secretion.

EFFECTS OF ESTRADIOL ON THE 5α-REDUCTASE ACTIVITY OF THE ANTERIOR PITUITARY AND OF THE HYPOTHALAMUS

In these experiments, hormonal treatments have been performed *in vivo* and the enzymatic activities have been evaluated *in vitro* using labelled testosterone as the substrate. The different metabolites have been identified using techniques previously described [2,21].

In agreement with previous studies of this and other laboratories, DHT and 3α-diol have been found to be the two major metabolites formed from testosterone in the anterior pituitary of adult animals of both sexes. Figures 2 and 3 show that the anterior pituitary of normal male and female animals form practically the same amounts of DHT and 3α-diol when incubated in identical conditions. In both sexes, castration increases very significantly the formation of DHT at pituitary level. The increase in the 5α-reductase activity of the anterior pituitary induced by castration is identical in both sexes. Apparently, the maximum increase of such an activity is already attained 7 days after castration, since the results obtained at 14 days are similar. Gonadectomy also induces a small increase in the formation of 3α-diol (Figs 2 and 3).

Figures 2 and 3 also indicate that the *in vivo* administration of a small dose of estradiol (50 ng/rat/day, for 7 or 14 days beginning the day of castration) does not have any effect on the 5α-reductase activity of the anterior pituitary of castrated animals of both sexes. On the contrary, a higher dose of estradiol (5 μg/rat/day) proved effective in bringing back to normality the 5α-reductase activity of the anterior pituitary of castrated males and females when given for 7 days. Moreover, in both sexes, when the treatment with this dose of estrogen was continued up to 14 days after castration, the 5α-reductase activity of the anterior pituitary dropped well below that present in normal animals.

The results summarized in Figs 4 and 5 also indicate that the hypothalamus of normal males and females is able to convert testosterone into DHT and 3α-diol. However, in both sexes, the total amounts

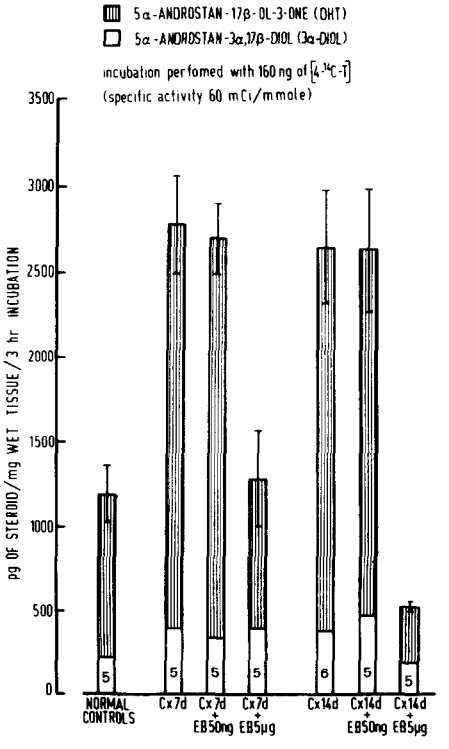

Fig. 2. Effect of castration and of *in vivo* treatment with estradiol benzoate (EB, 50 ng or 5 μg/rat/day s.c., for 7 or 14 days) on testosterone metabolism *in vitro* in the anterior pituitary of adult male rats.

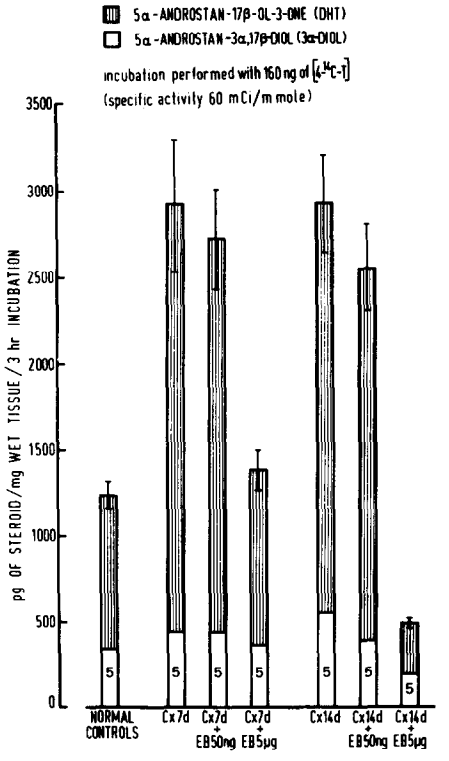

Fig. 3. Effect of castration and of *in vivo* treatment with estradiol benzoate (EB, 50 ng or 5μg/rat/day s.c., for 7 or 14 days) on testosterone metabolism *in vitro* in the anterior pituitary of adult female rats.

of 5α-reduced metabolites formed by the hypothalamus are lower than those formed at anterior pituitary level (see for comparison Figs 2 and 3). It is also apparent from Figs 4 and 5 that the 5α-reductase-3α-hydroxysteroid dehydrogenase system of the hypothalamus is more active in males than in females. An interesting qualitative difference between the results obtained using the anterior pituitary and those obtained using the hypothalamus resides in the fact that the ratio 3α-diol/DHT is higher in the nervous structures than in the pituitary gland.

Castration does not significantly modify, either at 7 or 14 days, the 5α-reductase-3α-hydroxysteroid dehydrogenase activities of the hypothalamus of female animals (Fig. 5). A small but not significant decrease in both activities was observed in males 7 days following orchidectomy (Fig. 4). The small dose of estradiol (50 ng/rat/day) had no effect on the enzymatic activities of the hypothalamus in either sex when given for 7 days (Figs 4 and 5). This same dose of estradiol was also totally ineffective in males when the treatment was continued for 14 days (Fig. 4). On the contrary, in the 14-day schedule of administration, it significantly enhanced the 5α-reductase-3α-hydroxysteroid dehydrogenase activities of the hypothalamus of the female animals (Fig. 5). The higher dose of estradiol (5 μg/rat/day) was totally ineffective in the two groups of animals when given for 7 days. When the treatment with this dose was prolonged for 14 days, a significant suppression of the activity of the two

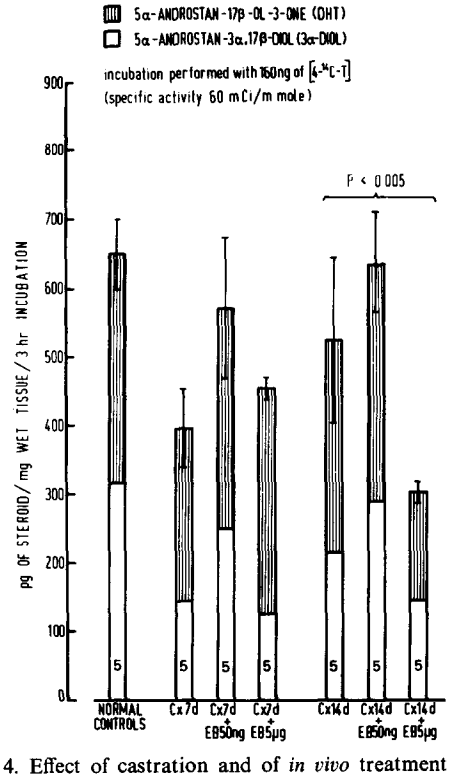

Fig. 4. Effect of castration and of *in vivo* treatment with estradiol benzoate (EB, 50 ng or 5 μg/rat/day s.c., for 7 or 14 days) on testosterone metabolism *in vitro* in the hypothalamus of adult male rats.

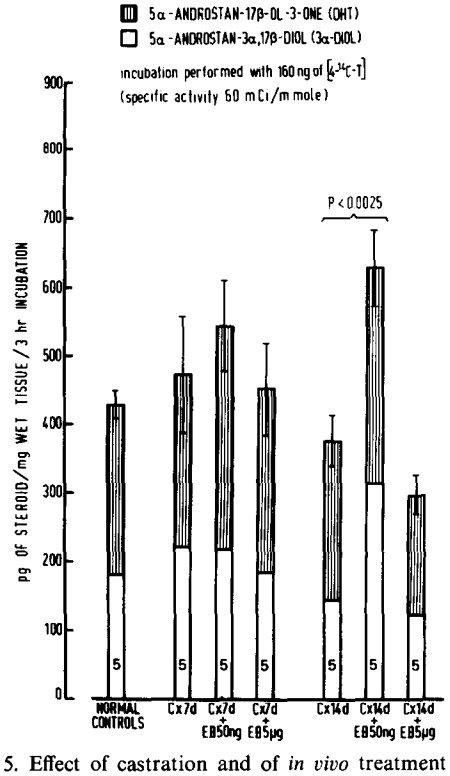

Fig. 5. Effect of castration and of *in vivo* treatment with estradiol benzoate (EB, 50 ng or 5 μg/rat/day s.c., for 7 or 14 days) on testosterone metabolism *in vitro* in the hypothalamus of adult female rats.

enzymes was observed in the hypothalamus of castrated males, while there was no significant effect in females.

EFFECTS OF PROLACTIN ON THE 5α-REDUCTASE ACTIVITY OF THE ANTERIOR PITUITARY AND OF THE HYPOTHALAMUS

In this study, rat prolactin was administered subcutaneously to adult normal or castrated male rats, in the daily dose of 800 μg/rat for 5 days. In addition to DHT and 3α-diol, also the amounts of 5α-androstan-3, 17-dione and of Δ_4-androstene-3, 17-dione formed by the anterior pituitary and by the hypothalamus have been quantitated.

Figure 6 shows that in normal male rats the administration of rat prolactin has no significant effects on the formation of DHT, 3α-diol and 5α-androstan-3, 17-dione at anterior pituitary level. Consequently, the hormone does not appear to modify either the 5α-reductase or the 3α-hydroxysteroid dehydrogenase activities of this structure. The conversion of testosterone to Δ_4-androstene-3,17-dione was also unmodified following treatment. Figure 7 summarizes similar results regarding the hypothalamus of normal male rats. It is apparent that rat prolactin does not modify the amounts of DHT, 3α-diol and 5α-androstan-3,17-dione formed by this structure. A small but significant decrease in the amounts of Δ_4-androstene-3,17-dione produced was observed.

As expected from the findings previously described, castration significantly increased the 5α-reductase activity of the anterior pituitary of male rats (see Figs 6 and 8, noting the differences in scales). Rat prolactin, administered to castrated animals, did not exert any significant effect on the enzymatic activities of the gland. The situation was quite different at hypothalamic level as shown in Fig. 9. Rat prolactin given following castration significantly diminished the total amounts of the 5α-reduced metabolites formed by this structure. DHT, 3α-diol and 5α-androstan-3,17-dione

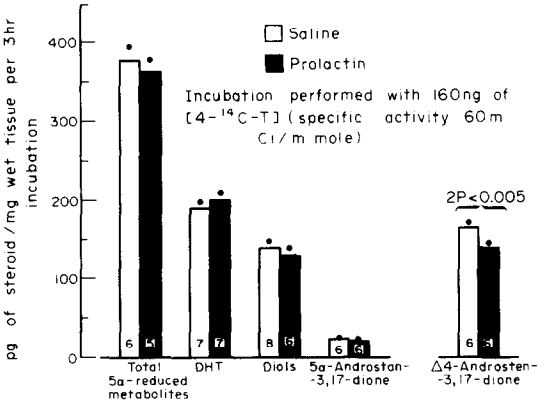

Fig. 7. Effect of *in vivo* treatment with rat prolactin (400 μg/rat twice daily s.c., for 5 days) on testosterone metabolism *in vitro* in the hypothalamus of normal male rats.

were all proportionally decreased by the treatment. The production of Δ_4-androstene-3,17-dione was also decreased.

EFFECTS OF TESTOSTERONE, DHT AND 3α-DIOL ON GONADOTROPIN SECRETION

It has been previously reported that DHT and 3α-diol, when given in one single dose 24 h before sacrifice, are much more effective than testosterone in suppressing LH release in adult castrated male rats. Under the same experimental conditions, testosterone and DHT inhibit to a certain extent, but do not suppress, FSH release, while 3α-diol is totally ineffective on this gonadotropin [10]. This work has now been extended utilizing a chronic schedule of administration of the different steroids. Adult rats of both sexes have been castrated 4 weeks before the initiation of the experiment. Testosterone, DHT and 3α-diol (in the free alcohol form) have been subsequently administered in the daily dose of 2 mg/rat for 6 days. The animals have been sacrificed on the seventh day.

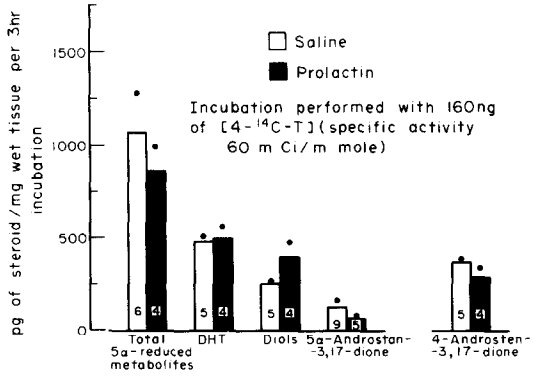

Fig. 6. Effect of *in vivo* treatment with rat prolactin (400 μg/rat twice daily s.c., for 5 days) on testosterone metabolism *in vitro* in the anterior pituitary of normal male rats.

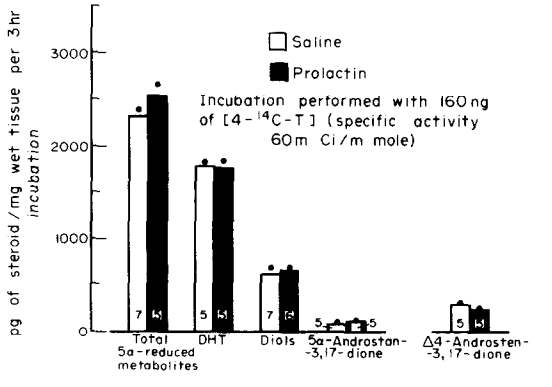

Fig. 8. Effect of *in vivo* treatment with rat prolactin (400 μg/rat twice daily s.c., for 5 days) on testosterone metabolism *in vitro* in the anterior pituitary of castrated male rats.

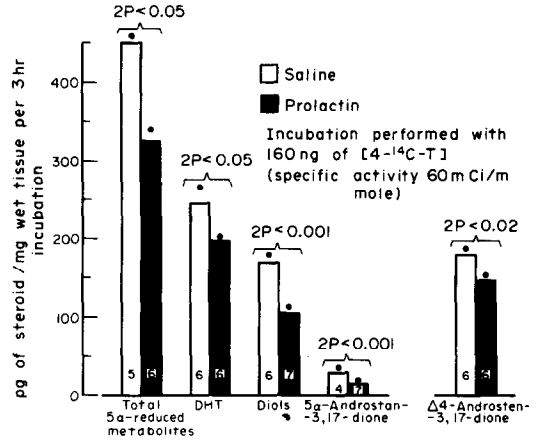

Fig. 9. Effect of *in vivo* treatment with rat prolactin (400 μg/rat twice daily s.c., for 5 days) on testosterone metabolism *in vitro* in the hypothalamus of castrated male rats.

Serum levels of LH and FSH have been quantitated with standard radioimmunoassay procedures [22,23]. It is apparent from Table 1 that, in castrated male rats, the administration of testosterone reduced, although not significantly, the release of LH. DHT and 3α-diol given in equal amounts were both much more effective than testosterone in bringing serum LH down to either undetectable or extremely low levels. In castrated females, testosterone proved to be much more active than in males in inhibiting LH release, DHT was as effective as testosterone, while 3α-diol almost totally suppressed the secretion of this gonadotropin.

As shown in Table 1, testosterone and 3α-diol did not modify the release of FSH in castrated males, while DHT showed some inhibiting effect. None of the steroids tested proved able to affect the release of FSH in castrated females.

DISCUSSION AND CONCLUSIONS

The data summarized in this paper try to enlarge present knowledge about the mode of action of testosterone on the anterior pituitary and on the hypothalamus. Two distinct approaches have been used, namely: (a) the study of some of the factors which may control testosterone metabolism in the central structures; and (b) the feedback effects on gonadotropin secretion of the physiological 5α-reduced metabolites of testosterone.

It has been shown that estradiol, if given in sufficient amounts and for a sufficient period of time, may decrease the 5α-reductase-3α-hydroxysteroid dehydrogenase activities of the anterior pituitary of castrated male and female animals to levels lower than those found in normal animals. Estradiol also influences the two enzymatic activities at hypothalamic level. However, in this structure, the response to estradiol is dose- and sex-dependent. Small doses of estrogens increase the 5α-reductase-3α-hydroxysteroid dehydrogenase activities of the hypothalamus of castrated females but leave unchanged both enzymatic activities in the male. On the contrary, a higher dose of estradiol diminishes the 5α-reductase-3α-hydroxysteroid dehydrogenase activities of the male hypothalamus, but is totally ineffective in females.

Prolactin has been shown not to influence the enzymatic activities of the anterior pituitary and of the hypothalamus of normal male rats. Following orchidectomy, the hormone remains without effect on the two enzymes at anterior pituitary level, but significantly decreases the activities of the 5α-reductase and of the 3α-hydroxysteroid dehydrogenase at hypothalamic level. Such an effect of rat prolactin on the hypothalamus is believed to be a direct one, since it occurs exclusively in castrated animals, in which it is not expected that prolactin might act via the release of hormones from the peripheral target glands. Evidence indicating a direct effect of prolactin on the hypothalamus is already available. Prolactin, when implanted into the median eminence, has been shown to decrease the release of endogenous prolactin via a "short" feedback effect [24–26]. Systematically administered prolactin has been reported to modify the firing rate of hypothalamic neurons [27,28]. Finally, prolactin has been recently identified in the hypo-

Table 1. Effects of testosterone (T), dihydrotesterone (DHT), and 5α-androstan-3α, 17β-diol (3α-diol) on the secretion of LH and of FSH in castrated male and female rats

Treatment and dose (2 mg/rat/day/6 days)		LH (NIAMDD-rat LH-RP-1) (ng/ml serum)		FSH (NIAMDD-rat FSH-RP-1) (ng/ml serum)
Castrated males				
OIL	(5)†	374.54 ± 92.47‡	(5)	3974.20 ± 63.31
T	(3)	165.59 ± 45.36	(2)	4020.50 ± 21.50
DHT	(5)	N.D.	(5)	1090.00 ± 728.12**
3α-DIOL	(4)	23.28 ± 20.76*	(4)	3712.50 ± 147.44
Castrated females				
OIL	(29)	648.88 ± 46.56	(18)	2919.19 ± 174.17
T	(14)	114.49 ± 29.72***	(8)	2534.50 ± 163.61
DHT	(18)	91.80 ± 18.41***	(9)	3180.11 ± 227.10
3α-DIOL	(15)	19.92 ± 6.89***	(8)	2600.50 ± 181.64

† Number of rats in parentheses. ‡ Mean ± S.E. * $2P < 0.01$ *vs* OIL. ** $2P < 0.005$ *vs* OIL. *** $2P < 0.001$ *vs* OIL.

thalamus [29]. It remains for further experiments to clarify why prolactin does not change the 5α-reductase-3α-hydroxysteroid dehydrogenase activities in the hypothalamus of normal animals. Probably, when the gonads are present, sex hormones interfere with this effect of prolactin.

The data taken as a whole suggest that estradiol and prolactin may probably modulate the effects of testosterone at anterior pituitary and at hypothalamic level, by modifying the rates of the conversion of the hormone into DHT and 3α-diol.

The study devoted to analyze the effects of testosterone and of its principal 5α-reduced metabolites on gonadotropin secretion in castrated animals has shown that, in both sexes, DHT and 3α-diol, when chronically administered, are better suppressors of LH secretion than testosterone. The present observations are in agreement with the data previously obtained using an acute approach [10] and support once more the view that testosterone probably exerts its negative feedback effect on LH release following the intrapituitary and/or intrahypothalamic conversion into DHT and 3α-diol [6,7,10]. With regard to FSH, a minor discrepancy has been observed between the acute and the chronic experiment. At variance with the data reported by Zanisi *et al.* [10], testosterone has been found not to affect FSH release in castrated male rats. In both types of experiments, however, DHT showed some inhibitory effect and 3α-diol was inactive.

The data reported here underline some interesting sexual differences in the responses of pituitary gonadotropins to the administration of the various androgens. Testosterone seems to be a better suppressor of LH in females than in males; on the contrary, DHT appears to be more potent in inhibiting this gonadotropin in males than in females; finally, DHT does not modify FSH release in females while inhibiting this hormone in males. The reasons for these sex differences remains to be ascertained.

The data of the present study cannot be directly compared with those of previous authors, because of major methodological differences. In the present experiments the various steroids have been administered to chronically castrated animals (treatment was initiated 4 weeks after castration), while the majority of the other investigators have initiated their treatments immediately after gonadectomy. However, it must be pointed out that no major discrepancies exist between the data reported here and those of Beyer *et al.* [30] and Eckstein *et al.* [31] in females, and of Swerdloff *et al.* [32], Eik-Nes [33], and Verjans and Eik-Nes [34,35] in males.

Acknowledgements—The experiments described here were supported by the Ford Foundation, New York and by the Consiglio Nazionale delle Ricerche, Rome, through the program "Biology of Reproduction". All such support is gratefully acknowledged.

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