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THE TRANSFORMATION OF TESTOSTERONE INTO DIHYDROTESTOSTERONE BY THE BRAIN AND THE ANTERIOR PITUITARY

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SUMMARY

Slices of rat pituitary gland, hypothalamus, amygdala, cerebral cortex and prostate have been incubated *in vitro* with labelled testosterone; the metabolites formed have been identified. Testosterone is converted into 17β-hydroxy-5α-androstan-3-one (androstanolone, dihydrotestosterone, DHT) by all tissues examined. The prostate is the structure which effects such a conversion to the greatest extent; the pituitary gland and the hypothalamus are also very active; the cerebral cortex and the amygdala are also able to transform testosterone into DHT, but the rate of conversion is not as great as that found in the tissues previously mentioned. Androstenedione, 5α-androstan-3,17-dione and 5α-androstan-3α,17β-diol are also formed by some of the tissues. Castration increases and treatment with exogenous testosterone decreases the transformation of testosterone into DHT at the pituitary and the hypothalamic level. Both at the hypothalamic and the pituitary level, the addition *in vitro* of progesterone, 11-deoxycorticosterone, 11-deoxycortisol and corticosterone reduces the transformation of testosterone into DHT; on the contrary, the addition of pituitary FSH increases the conversion of testosterone into its 'active' metabolite. The ability to transform testosterone into DHT is much higher in all structures examined (with the exception of the prostate) in prepuberal than in adult rats.

INTRODUCTION

RECENT evidence suggests that, in the cells of androgen-sensitive structures (prostate, seminal vesicles, etc.), testosterone is transformed into 17β-hydroxy-5α-androstan-3-one (androstanolone, dihydrotestosterone, DHT) and 5α-androstan-3α,17β-diol [1]; the intracellular formation of these metabolites seems to be essential for the appearance of the androgenic activity [1, 2].

If the formation of DHT and of 5α-androstan-3α,17β-diol is a general feature of androgenic action, one might expect these derivatives to be formed also in the sites in which testosterone exerts its feedback effect on gonadotrophin secretion and influences sexual behaviour. This hypothesis was recently submitted to experimental verification in our laboratory. [^{14}C]-labelled testosterone was incubated *in vitro* with slices of hypothalamus (basal part, including the median eminence region), pituitary gland, amygdala, cerebral cortex, prostate and seminal vesicles, taken from different groups of male rats of the Sprague–Dawley strain. After three hours of incubation the metabolites formed were extracted from the incubation medium, purified and identified using procedures which have been described previously [3].

RESULTS AND DISCUSSION

Experiments in normal adult animals

The results obtained indicate that testosterone is converted into DHT by all tissues examined; however, the rate of conversion appears to vary considerably from tissue to tissue (Table 1). The formation of DHT occurred to a very large extent in the two peripheral androgen-dependent structures (prostate and seminal

Table 1. Conversion of testosterone to 17β-hydroxy-5α-androstan-3-one (DHT) by the basal hypothalamus, the anterior pituitary, the cerebral cortex, the amygdala, the prostate and the seminal vesicles of normal adult male rats[a]

Tissue[b]	DHT pg/mg[c]
Basal hypothalamus (14)	265·0 ± 22·2[d]
Anterior pituitary (18)	914·5 ± 49·0[d,e]
Cerebral cortex (5)	138·2 ± 7·9
Amygdala (5)	128·0 ± 19·8
Prostate (5)	5098·4 ± 965·2
Seminal vesicles (4)	1536·0 ± 199·0

[a]Values are means ± S.E.

[b]Number of experiments performed in parentheses.

[c]Picograms of steroid formed per mg of wet tissue following a 3-h incubation with 160 ng of [4-^{14}C]-testosterone (S.A.: 56,6 mCi/mmol).

[d]$P < 0{\cdot}001$ vs. cerebral cortex, amygdala, prostate and seminal vesicles.

[e]$P < 0{\cdot}005$ vs. basal hypothalamus.

vesicles); this confirms previous findings [1, 2, 4]. Very little DHT was formed by the cerebral cortex or by the amygdala; these cerebral zones were included in this study as 'control' tissues, since they are supposed not to be androgen sensitive. Testosterone was converted into DHT by the anterior pituitary; the amounts of this metabolite formed by the gland were significantly higher than those formed by the two control tissues; however, they did not reach the very elevated levels found in the media in which the peripheral androgen-sensitive organs were incubated. The hypothalamus was also able to reduce testosterone in the 5α position to an extent significantly higher than that of the control tissues; however, the reducing capacity of the hypothalamus appears significantly lower than that of the anterior pituitary. DHT was not the only metabolite formed by the different tissues examined so far: 5α-androstan-3α,17β-diol, 4-androstene-3,17-dione and 5α-androstane-3,17-dione were identified in practically all incubation media. Results similar to the ones here described have been reported by Jaffe [5] and by Sholiton and Werk [6]; however, these authors did not detect any quantitative difference between the amounts of DHT formed by the hypothalamus and those formed by other cerebral structures.

The data presented here suggest then that the transformation of testosterone into DHT is probably also necessary for initiating androgen-induced feedback and behavioural responses. The data also suggest that this androgen may exert its feedback effect both on the hypothalamus and on the anterior pituitary. From the data, one would probably be inclined to assign a prominent role in this process to the anterior pituitary.

Experiments in castrated adult animals

Having shown that the anterior pituitary and the hypothalamus are able to convert testosterone into DHT and other 'active' metabolites, it was deemed of interest to investigate whether the conversion processes might be modified by

experimental manipulations which activate or inhibit the hypothalamic-pituitary axis. First of all, the conversion by the anterior pituitary and by the hypothalamus of gonadectomized, adult, male rats was studied *in vitro*. Animals were killed 2, 7, 14, 21 and 90 days following castration.

Castration considerably activated the transformation of testosterone into DHT at the pituitary level. The activation was evident 2 days after the operation and reached its maximum 2–3 weeks after gonadectomy. The rate of conversion of testosterone was still significantly increased in pituitaries of animals which had been castrated 90 days before (Table 2).

Table 2. Conversion of testosterone to 17β-hydroxy-5α-androstan-3-one (DHT) by the anterior pituitary (AP) of normal and castrated adult male rats[a]

Tissue[b]	DHT pg/mg[c]
AP normal (18)	914·5 ± 49·0
AP castrated	
2 days (6)	2024·0 ± 212·0[d]
7 days (5)	1970·6 ± 309·8[d]
14 days (8)	2420·8 ± 456·3[d]
21 days (6)	2674·0 ± 123·5[d]
90 days (3)	1963·8 ± 210·1[d]

[a]Values are means ± S.E.

[b]Number of experiments performed in parentheses.

[c]Picograms of steroid formed per mg of wet tissue following a 3-h incubation with 160 ng of [4-^{14}C]-testosterone (S.A.: 56·6 mCi/mmol).

[d]$P < 0{\cdot}0005$ vs. AP normal.

The conversion process also seems to be activated by castration at the hypothalamic level; however, the increase in activity of the 5α-reductase in this tissue was not as great as that found at the pituitary level; in addition, it was not constant at all times examined (Table 3). Castration did not modify the conversion capacity of the cerebral cortex (Table 4).

The data obtained following castration may be taken as additional evidence in support of the hypothesis that testosterone must be converted into one or more 'active' metabolite(s) before initiating feedback responses; they also reinforce the conclusion that both the anterior pituitary and the hypothalamus are the loci on which androgen exerts its feedback effect on gonadotropin secretion. It appears again from the data that the pituitary probably plays a more important role than the hypothalamus in this process. It is interesting that following castration a higher uptake of radioactive testosterone has been reported to occur both in the anterior pituitary and in the hypothalamus [7].

Experiments in adult castrated animals treated with testosterone propionate

The next step was that of studying whether the *in vivo* administration of testosterone to castrated animals might bring back to normal the ability of the anterior pituitary and of the hypothalamus to convert testosterone into DHT. Testo-

Table 3. Conversion of testosterone to 17β-hydroxy-5α-androstan-3-one (DHT) by the basal hypothalamus (BH) of normal and castrated adult male rats[a]

Tissue[b]	DHT pg/mg[c]
BH normal (14)	265·0 ± 22·2
BH castrated	
2 days (9)	329·1 ± 16·9[d]
7 days (7)	269·6 ± 10·2
14 days (7)	375·2 ± 33·1[d]
21 days (6)	289·7 ± 18·5
90 days (3)	242·0 ± 18·9

[a]Values are means ± S.E.

[b]Number of experiments performed in parentheses.

[c]Picograms of steroid formed per mg of wet tissue following a 3-h incubation with 160 ng of [4-^{14}C]-testosterone (S.A.: 56·6 mCi/mmol).

[d]$P < 0{\cdot}025$ vs. BH normal.

Table 4. Conversion of testosterone to 17β-hydroxy-5α-androstan-3-one (DHT) by the cerebral cortex (CC) of normal and castrated adult male rats[a]

Tissue[b]	DHT pg/mg[c]
CC normal (5)	138·2 ± 7·9
CC castrated	
2 days (3)	137·2 ± 13·8
7 days (3)	121·7 ± 11·9
14 days (3)	123·6 ± 16·0
21 days (3)	133·6 ± 19·1
90 days (3)	149·1 ± 6·1

[a]Values are means ± S.E.

[b]Number of experiments performed in parentheses.

[c]Picograms of steroid formed per mg of wet tissue following a 3-h incubation with 160 ng of [4-^{14}C]-testosterone (S.A.: 56·6 mCi/mmol).

sterone propionate (TP) was injected subcutaneously, in a daily dose of 2 mg/rat; treatment was initiated immediately after castration. Tissues to be examined were taken 2, 7, 14 and 21 days after the operation; their ability to form DHT *in vitro* was compared with that of corresponding tissues taken from animals which had been castrated but which did not receive any substitution therapy.

Table 5 shows that two days after castration the anterior pituitary of animals treated with testosterone formed much less DHT than the anterior pituitary of the untreated castrated controls. Prolongation of treatment with testosterone was followed by an additional reduction of the ability of the anterior pituitary of

Table 5. Conversion of testosterone to 17β-hydroxy-5α-androstan-3-one (DHT) by the anterior pituitary (AP) of castrated adult male rats pretreated with testosterone propionate (TP, 2 mg/rat/day)[a]

Tissue[b]	Pretreatment	DHT pg/mg[c]
AP castrated		
2 days (6)	–	2024·0 ± 212·0
(3)	TP	1054·0 ± 203·9[d]
7 days (5)	–	1970·6 ± 309·8
(3)	TP	530·6 ± 163·7[d]
14 days (8)	–	2420·8 ± 456·3
(3)	TP	621·1 ± 31·9[e]
21 days (6)	–	2674·0 ± 123·5
(3)	TP	561·5 ± 41·1[f]

[a]Values are means ± S.E.

[b]Number of experiments performed in paretheses.

[c]Picograms of steroid formed per mg of wet tissue following a 3-h incubation with 160 ng of [4-^{14}C]-testosterone (S.A.: 56·6 mCi/mmol).

[d]$P < 0{\cdot}0125$ vs. AP castrated.

[e]$P < 0{\cdot}025$ vs. AP castrated.

[f]$P < 0{\cdot}0005$ vs. AP castrated.

castrated animals to convert testosterone into DHT. However, a plateau was reached after seven days of treatment.

The depressing effect of the substitution therapy with testosterone on the formation of DHT may be noticed also at the hypothalamic level (Table 6).

Table 6. Conversion of testosterone to 17β-hydroxy-5α-androstan-3-one (DHT) by the basal hypothalamus (BH) of castrated adult male rats pretreated with testosterone propionate (TP, 2 mg/rat/day)[a]

Tissue[b]	Pretreatment	DHT pg/mg[c]
BH castrated		
2 days (9)	–	329·1 ± 16·9
(3)	TP	370·6 ± 1·0[d]
7 days (7)	–	269·6 ± 10·2
(3)	TP	271·3 ± 37·1
14 days (7)	–	375·2 ± 33·1
(3)	TP	251·1 ± 12·1[e]
21 days (6)	–	289·7 ± 18·5
(3)	TP	215·7 ± 7·7

[a]Values are means ± S.E.

[b]Number of experiments performed in parentheses.

[c]Picograms of steroid formed per mg of wet tissue following a 3-h incubation with 160 ng of [4-^{14}C]-testosterone (S.A.: 56·6 mCi/mmol).

[d]$P < 0{\cdot}05$ vs. BH castrated.

[e]$P < 0{\cdot}025$ vs. BH castrated.

The effects are less dramatic than those reported for the anterior pituitary gland, and take more time to develop; a significant reduction was observed only after two weeks. It is interesting to note that a short-term (two days) treatment with testosterone seems to increase rather than to decrease the 5α-reductase activity of the hypothalamus. The reasons why the hypothalamus should be different from the anterior pituitary also with regard to the effects of exogenous testosterone are not evident at the present moment.

Table 7 shows that treatment of castrated animals with testosterone propionate does not influence the conversion of labelled testosterone into its 'active' metabolite at the level of the cerebral cortex.

Table 7. Conversion of testosterone to 17β-hydroxy-5α-androstan-3-one (DHT) by the cerebral cortex (CC) of castrated adult male rats pretreated with testosterone propionate (TP, 2 mg/rat/day)[a]

Tissue[b]	Pretreatment	DHT pg/mg[c]
CC castrated		
2 days (3)	–	137·2 ± 13·8
(3)	TP	145·7 ± 15·8
7 days (3)	–	121·7 ± 11·9
(3)	TP	91·6 ± 12·6
14 days (3)	–	123·6 ± 16·0
(3)	TP	132·5 ± 14·5
21 days (3)	–	133·6 ± 19·1
(3)	TP	126·9 ± 29·7

[a]Values are means ± S.E.

[b]Number of experiments performed in parentheses.

[c]Picograms of steroid formed per mg of wet tissue following a 3-h incubation with 160 ng of [4-^{14}C]-testosterone (S.A.: 56·6 mCi/mmol).

In vitro effect of anterior pituitary hormones

The experiments to be described now were performed in order to clarify why castration and the *in vivo* administration of testosterone should modify in opposite directions the activity of the enzyme which, in the anterior pituitary, converts testosterone into DHT. One may hypothesize either that the enzyme is sensitive to changing levels of testosterone, or that it is sensitive to some endogenous compound whose production depends on the levels of testosterone in the general circulation. It is known that castration is followed by a very dramatic increase of plasma levels of FSH and LH, and that treatment of castrated animals with testosterone brings back to normal the secretion of pituitary gonadotrophins [8–11]. Consequently one might assume that the enzyme transforming testosterone into DHT might be influenced by the levels of gonadotrophins in the general circulation rather than by testosterone itself. In order to establish which of these two hypotheses is correct, anterior pituitary tissue from normal male rats was incubated in the presence of different pituitary hormones and of labelled testosterone. As shown in Table 8, anterior pituitary tissue exhibited a very significant increase of its 5α-reducing capacity when incubated

Table 8. Conversion of testosterone to 17β-hydroxy-5α-androstan-3-one (DHT) by the anterior pituitary (AP) of normal adult male rats in the presence of anterior pituitary hormones[a]

Tissue[b]	Hormone added *in vitro*	DHT pg/mg[c]
AP (18)	–	914·5 ± 49·0
AP (8)	FSH 10 μg	1334·9 ± 91·0[d]
AP (2)	ACTH 10 μg	827·5 ± 60·5
AP (3)	LH 10 μg	754·0 ± 61·8

[a]Values are means ± S.E.

[b]Number of experiments performed in parentheses.

[c]Picograms of steroid formed per mg of wet tissue following a 3-h incubation with 160 ng of [4-^{14}C]-testosterone (S.A.: 56·6 mCi/mmol).

[d]$P < 0{\cdot}0005$ vs. AP normal.

in the presence of FSH. It is interesting that LH and ACTH added *in vitro* did not have any effect on the DHT-forming enzyme of the anterior pituitary of normal animals.

If one is allowed to extrapolate from the results obtained in these *in vitro* experiments, the suggestion might be put forward that castration increases the activity of the DHT-forming enzyme via the elevation of plasma levels of FSH produced by the operation. It might also be argued that exogenous testosterone does not inhibit the enzyme in a direct fashion, but operates through suppression of the gonadotrophin secretion which it causes. It is interesting to recall here the observations of Moguilevsky and his associates. They have reported that the oxygen consumption of the hypothalamus is depressed by castration, and that testosterone is able to prevent this effect of castration following administration *in vivo*, but not after having been added to the incubation media *in vitro* [12]. These data were interpreted as indicating that the decreased metabolism of the hypothalamus of castrated rats is not directly due to the lack of testosterone; a prominent role of the increased release of FSH found following castration was supposed. This hypothesis was subsequently confirmed by the demonstration that hypophysectomy increases the oxygen uptake of the hypothalamus [13], and that the addition of FSH *in vitro* is able to mimic the effects of castration and to decrease hypothalamic oxygen consumption [12].

In vitro effect of hormonal steroids

Testosterone is not the only hormonal steroid present in the general circulation. Consequently, it was deemed necessary to acquire some information on the influence other steroids might exert on the DHT-forming enzyme. The ability of anterior pituitary tissue of normal animals to convert testosterone into DHT was studied after addition *in vitro* of progesterone, 11-deoxycorticosterone, 11-deoxycortisol (compound *S*) and corticosterone.

A significant inhibition of the converting activity was found following incubation of the anterior pituitary tissue in the presence of progesterone (Table 9).

Table 9. Conversion of testosterone to 17β-hydroxy-5α-androstan-3-one (DHT) by the anterior pituitary (AP) of normal adult male rats in the presence of other steroids[a]

Tissue[b]	Steroid added *in vitro*	DHT pg/mg[c]
AP (18)	–	914·5 ± 49·0
AP (4)	progesterone 0·1 μg	368·3 ± 36·7[d]
AP (3)	progesterone 10·0 μg	52·0 ± 9·5[d]
AP (3)	progesterone 50·0 μg	37·3 ± 7·6[d]

[a]Values are means ± S.E.
[b]Number of experiments performed in parentheses.
[c]Picograms of steroid formed per mg of wet tissue following a 3-h incubation with 160 ng of [4-^{14}C]-testosterone (S.A.: 56·6 mCi/mmol).
[d]$P < 0{\cdot}0005$ vs. AP normal.

A significant decrease was obtained adding *in vitro* as low a dose of progesterone as 0·1 μg. A much larger inhibition was obtained when higher amounts of progesterone were added. The ability of the anterior pituitary enzyme to convert testosterone into DHT was completely obliterated when 50 μg of progesterone were added *in vitro*. Progesterone added *in vitro* exerted the same inhibitory effect on the hypothalamus, in spite of the reduced converting ability originally present in this tissue (Table 10). How can this inhibiting activity of progesterone be explained? The most obvious explanation is that progesterone might act as a substrate for the enzyme, and be reduced in the 5α position. In order to test this hypothesis, anterior pituitary tissue was incubated simultaneously with labelled progesterone and labelled testosterone; the major metabolites formed were subsequently identified. Table 11 shows that the anterior pituitary is able to convert progesterone into the corresponding 5α-dihydro derivative. These data certainly support the hypothesis that the competition for the enzyme is probably

Table 10. Conversion of testosterone to 17β-hydroxy-5α-androstan-3-one (DHT) by the basal hypothalamus (BH) of normal adult male rats in the presence of other steroids[a]

Tissue[b]	Steroid added *in vitro*	DHT pg/mg[c]
BH (14)	–	265·0 ± 22·2
BH (5)	progesterone 0·1 μg	135·6 ± 12·8[d]
BH (3)	progesterone 50·0 μg	35·7 ± 1·5[e]

[a]Values are means ± S.E.
[b]Number of experiments performed in parentheses.
[c]Picograms of steroid formed per mg of wet tissue following a 3-h incubation with 160 ng of [4-^{14}C]-testosterone (S.A.: 56·6 mCi/mmol).
[d]$P < 0{\cdot}0025$ vs. BH normal.
[e]$P < 0{\cdot}0005$ vs. BH normal.

Table 11. Conversion of testosterone and of progesterone to 17β-hydroxy-5α-androstan-3-one (DHT) and to 5α-pregnane-3,20-dione (DHP) by the anterior pituitary (AP) of normal adult male rats[a]

Tissue[b]	DHT pg/mg[c]	DHP pg/mg[c]
AP (5)	507·7 ± 35·1	1684·6 ± 152·8

[a]Values are means ± S.E.

[b]Number of experiments performed in parentheses.

[c]Picograms of steroid formed per mg of wet tissue following a 3-h incubation with 160 ng of [4-^{14}C]-testosterone (S.A. 56·6 mCi/mmol) and with 150 ng of [4-^{14}C] progesterone (S.A.: 60·0 mCi/mmol).

the way through which progesterone inhibits the transformation of testosterone into DHT.

11-Deoxycorticosterone, 11-deoxycortisol and corticosterone were also able to reduce the formation of DHT when added *in vitro* to the incubation media containing anterior pituitary tissue and labelled testosterone (Table 12). However, the inhibitory activity of these steroids on the conversion of testosterone into its 'active' metabolite was significantly lower than that of progesterone. The inhibiting activity of the four steroids studied so far may be graded as follows: progesterone > 11-deoxycorticosterone > 11-deoxycortisol > corticosterone. It is believed that, as in the case of progesterone, the three corticoids prevent the

Table 12. Conversion of testosterone to 17β-hydroxy-5α-androstan-3-one (DHT) by the anterior pituitary of normal adult male rats in the presence of other steroids[a]

Tissue[b]	Steroid added *in vitro*	DHT pg/mg[c]
AP (18)	–	914·5 ± 49·0
AP (3)	progesterone 10 μg	52·0 ± 9·5[d]
AP (3)	11-deoxycorticosterone 10 μg	187·4 ± 18·3[d,e]
AP (2)	11-deoxycortisol 10 μg	306·1 ± 25·9[f,g]
AP (4)	corticosterone 20 μg	567·8 ± 37·3[h,i]

[a]Values are means ± S.E.

[b]Number of experiments performed in parentheses.

[c]Picograms of steroid formed per mg of wet tissue following a 3-h incubation with 160 ng of [4-^{14}C]-testosterone (S.A.: 56·6 mCi/mmol).

[d]$P < 0·0005$ vs. AP.

[e]$P < 0·0025$ vs. AP + progesterone.

[f]$P < 0·0025$ vs. AP.

[g]$P < 0·025$ vs. AP + 11-deoxycorticosterone.

[h]$P < 0·005$ vs. AP.

[i]$P < 0·01$ vs. AP + 11-deoxycortisol.

formation of DHT from testosterone because they are optional substrates for the 5α-reductase system present in the anterior pituitary. If it is so, it appears that the presence of a hydroxy group in the 21 position (11-deoxycorticosterone) still permits a rather efficient transformation of the steroid into its corresponding 5α-reduced metabolite; consequently, only a small amount of labelled testosterone can be converted into DHT. The introduction of a second hydroxy group in position 17 (11-deoxycortisol), and particularly in position 11 (corticosterone), gives origin to compounds which are not easily transformed by the pituitary enzyme into 5α-reduced metabolites; it follows that, in the presence of steroids of this type, labelled testosterone can still be converted into its 'active' form in rather large amounts.

Experiments in maturing animals

The current consensus is that the centres regulating gonadotrophin secretion in the prepuberal animal are markedly more sensitive to the negative feedback influences of androgen than those of the adult[14–16]. This hypersensitivity to androgen of the immature animal might be due to the fact that before puberty, central structures convert testosterone into its "active" form more efficiently than after adulthood has been reached. This hypothesis was verified by incubating *in vitro* the basal hypothalamus, the amygdala, the cerebral cortex, the anterior pituitary and the prostate of immature rats in the presence of labelled testosterone; in this experiment animals were killed at 7, 14, 21, 28, 35 and 60 days of age. It appears from Fig. 1 that the ability to convert testosterone into DHT of all

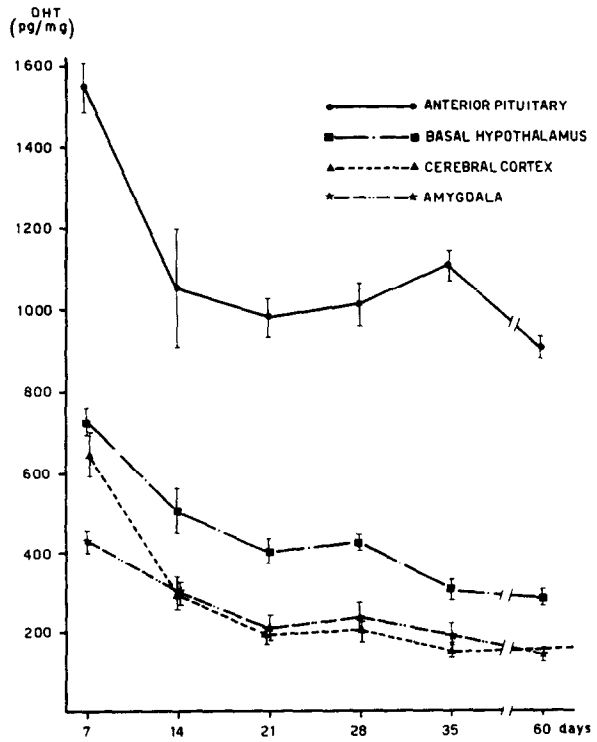

Fig. 1. Conversion of testosterone to 17β-hydroxy-5α-androstan-3-one (DHT) by the basal hypothalamus, the amygdala, the cerebral cortex, and the anterior pituitary of maturing male rats.

brain structures considered and of the anterior pituitary is inversely related to the age of the animal. On the contrary, the activity of the 5α reductase of the prostate is not linked to the age of the animal[17]. These data certainly support the idea that the hypersensitivity to androgen of the prepuberal animal is due to its ability to utilize testosterone better than the adult. They also provide a biochemical basis for explaining the change in the sensitivity of the cerebral "gonadostat" which occurs at the time of sexual maturation, and which seems to be crucial for initiating puberty in male animals[14].

CONCLUSIONS

The results presented here indicate that the anterior pituitary and the basal hypothalamus of the rat are able to convert testosterone into 17β-hydroxy-5α-androstan-3-one (androstanolone, dihydrotestosterone, DHT), the metabolite which is believed to be the 'active' form of the hormone on androgen-sensitive peripheral target structures. It has also been demonstrated that the converting activity of these two tissues is increased following castration and diminished by the *in vivo* administration of testosterone. The addition of FSH *in vitro* apparently stimulates the formation of DHT. The opposite effect has been obtained by adding progesterone, 11-deoxycorticosterone, 11-deoxycortisol or corticosterone to the incubation media. The converting ability of the central structures is much higher in young (prepuberal) than in adult animals.

These results are compatible with the hypothesis that the transformation of testosterone into DHT is probably necessary for initiating androgen-induced feedback and behavioral responses.

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DISCUSSION

Van der Molen: I would like to make a few comments and ask two questions to Dr. Martini, but with the chairman's permission, I would like to show some slides of work that Dr. Rommerts has done in our department, which he presented in Hamburg last year, and which has been published in *Biochim. Biophys. Acta.* **248** (1971) 489. The data in the first slide (Fig. D.1) are in agreement with the data which Dr. Martini showed. The conversion of testosterone to dihydrotestosterone occurs

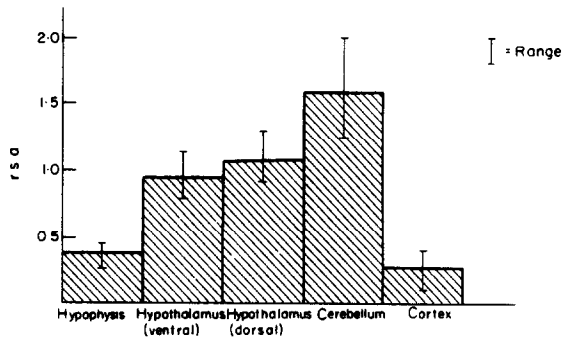

Fig. D1. Distribution of 5α-steroid reductase (5αR) in hypophysis (HF), ventral and dorsal part of hypothalamus (resp. HT_V and HT_D), cerebellum (cereb) and cortex.
The enzyme concentrations in the different tissues have been expressed as relative specific activity (r.s.a. = the ratio of specific activity in a special tissue and the specific activity in total brain homogenate).
Values are given as mean values of three experiments.
Experimental errors have been indicated by the range.

in most of the tissues studied. I think in this slide you can clearly see the specific activities of the steroid reductase. Rommerts actually studied the conversion of testosterone, androstenedione and progesterone, but I have specifically taken the results of the 5α-reductase. The specific activity in the different areas (the hypophysis, hypothalamus, etc.) is expressed relative to the activity of homogenate. I think there may be some difference with the conclusions of Dr. Martini, that if you compare the activities relative to the activity of the total brain tissue, then the cerebellum may be quite high indeed. The specific localization in subcellular fractions of brain tissue was studied in light of the ideas about the formation of dihydrotestosterone and the uptake by nuclei. The results in Fig. D.2 might give the impression that the 900 g pellet, which was considered to contain the nuclei, still contains some activity for reduction of androstenedione and progesterone as well as testosterone. After further purification of the 900 g pellet using centrifugation through several gradients the 5α-steroid reductase activity relative to DNA gradually disappears (Table 1D). This is not reflected in the steroid reducing activity per mg of protein. If we consider DNA as the more important marker for the nuclear fraction then there is hardly any proof in our work that there is 5α-reductase activity in nuclei.

I should also like to ask one or two questions about Dr. Martini's results. What I was most interested in was your effect of FSH on the *in vitro* conversion of testosterone to dihydrotestosterone. I wonder if you have any indication what it might reflect. As far as I understand, if this observation is reproducible, this would constitute one of the very few *in vitro* systems where FSH has a specific

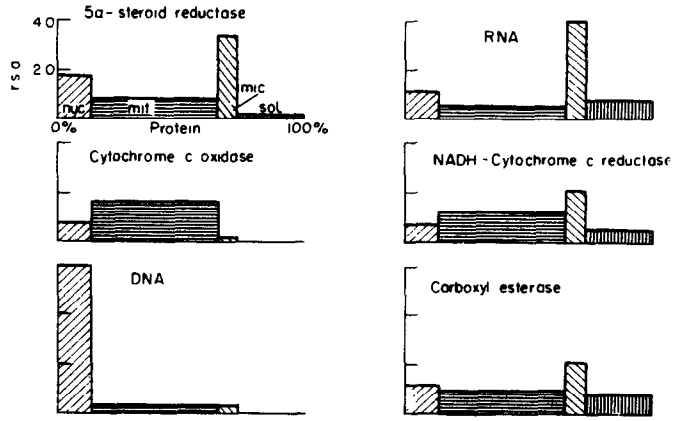

Fig. D2. Distribution of 5α-steroid reductase (5αR), marker enzymes, RNA and DNA in subcellular fractions of brain tissue. Nuc. = nuclear fraction; mit. = mitochondrial fraction; mic. = microsomal fraction; sol = 105·000 g supernatant fraction.
On the ordinate enzyme concentrations are expressed as relative enzyme activity (R.S.A.) as the ratio of percent recovered activity to percent recovered protein. On the abcissa the percentages of recovered protein in the subcellular fractions are indicated.

Table D1. 5α-Steroid reductase activity* (5αR) in nuclear fractions of brain

Fraction	A $\frac{\mu g\ DNA}{mg\ protein}$	B = 5αR $\frac{nmol\ steroid}{mg\ protein}$	$\frac{B}{A} = \frac{5\alpha R}{DNA}$ $\frac{nmol\ steroid}{\mu g\ DNA}$
Total homogenate	12	3·0	0·2
P_{1a} (900 g pellet)	111	7·0	0·06
P_{1b} (gradient I)	123	5·0	0·041
P_{1c} (gradient II)	239	9·0	0·038

*Expressed as nmol converted testosterone by a fraction with 1 mg protein.

Specific activities of 5α-steroid reductase in various nuclear fractions of brain tissue. The 5α-steroid reductase activities (5αR) are expressed as the amount of nmole steroid (dihydrotestosterone) formed per mg protein or μg DNA.

Total homogenate and a washed 900 g pellet (P_{1a}) were used as two nuclear fractions. Further purified fractions (P_{1b}, P_{1c}) were obtained by density gradient centrifugation with different sucrose gradients. The DNA to protein ratio has been used as a characteristic parameter for the purity of the fractions.

effect on an enzyme activity. And then, directly related to your last conclusion, how can you maintain that the dihydrotestosterone formation in itself is the cause, or possible cause, I should say, rather than the result of the maturation of cyclic gonadotrophic activity.

Martini: I'm not sure I've got your second question; could you formulate it again?

Van der Molen: When you conclude that the conversion of testosterone to dihydrotestosterone correlates rather well with the physiological situation of your animals in terms of presumed FSH or LH activity, I am wondering whether the dihydrotestosterone formation, in your opinion, is either the cause or the result of the gonadotrophic activity. On the basis of your *in vitro* results, I would expect

that an increased dihydrotestosterone formation might also *result* of the gonadotrophin appearance in these animals. I also wonder whether you have any other confirmation that the *in vitro* effect of FSH results in either the conversion of an active enzyme to an inactive, or whether it is an effect on protein synthesis, etc.

Martini: I think that there is one additional piece of evidence which indicates that FSH may act directly on hypothalamic enzymes. This comes from the work of Dr. Schiaffini, from Argentina, who has been working in my laboratory. He has found that the oxygen consumption of hypothalamic tissue is directly influenced *in vitro* by the addition of gonadotrophins, and particularly of FSH. So there is some evidence that pituitary hormones added *in vitro* might modify the biochemistry of the tissue which is incubated. With regard to your second question, I do not think that, in the immature animal, the gonadotrophins play a role in modifying the activity of the 5α reductase. Actually, there is evidence that, in the male, FSH secretion does not begin until around the time of puberty, which occurs between 30 and 40 days of age. I think, then, that at least in prepuberal animals, the first trigger is the decrease of the 5α-reductase activity.

De Moor: I want to report some similar studies done by Dr. Verhoeven in my lab. Verhoeven G. and De Moor P.: *Endocrinology* (U.S.A.) **89** (1971) 842. Verhoeven G. and De Moor P.: Submitted for publication in *Endocrinology* (U.S.A.). As far as nuclear metabolism is concerned in the rat, I can confirm most of the results of Dr. Martini. Progesterone inhibits 5α-reduction of testosterone in the nuclei. My second point is a question: what happens if you castrate your animals during the first days of life? That may help in settling the question between the chicken and the egg.

Martini: We have not done it yet.

De Moor: In a similar system, as far as enzymes in the liver were concerned, we could show that castrating the animals during the first days of life (this is work done by C. Denef) abolished the specific male activity in the liver, and I wonder if similar things will happen to your system.

Martini: I know the reason why you were asking your question. We have a project of studying what happens in the late foetal period and in the newborn animals, but we have not done it yet.

Munck: First, have you looked for the same FSH type of effect in the female pituitary? And second, have you tried it in young female pituitaries and neonatals?

Martini: The answer to both questions is no.

Munck: Does dihydrotestosterone suppress the conversion ability in the castrate as testosterone does?

Martini: It hasn't been done yet.

O'Malley: I have two questions. One: would you review for my edification the evidence that DHT is a better physiologic neurosuppressor than testosterone? Secondly, how do you control for changes (during the life of a given species) in endogenous steroid contents in these tissues? Could this influence amount of DHT conversion noted, since a number of other steroids also will compete for this enzyme and could lower the activity in an *in vitro* assay?

Martini: There is some recent evidence obtained in our own laboratory by Dr. M. R. Zanisi and in the United States by Dr. Mahesh, working in Atlanta, Georgia, which indicates that DHT is a much better suppressor of gonadotrophin secretion than testosterone. This is contrary to previous evidence indicating that DHT is not active centrally. I use purposely the word "centrally" because the previous

studies indicating that DHT is not effective were all behavioral studies. Now behaviour means the brain, while feedback means both the brain and the pituitary. Our data do indicate that the pituitary is probably more important than the brain with regard to the feedback effect of androgens. Now with regard to your second question, we do believe that the context in which the testosterone is converted into dihydrotestosterone plays a significant role in modulating the effects of testosterone. It is quite possible that when you have high levels of corticosteroids or of progesterone in blood, testosterone is less effective because it is converted into dihydrotestosterone to a lesser extent.

Grant: On that score, Dr. Martini, Dr. Roger Short in Cambridge tells me that he cannot find the same effect of dihydrotestosterone on the very young female rat brain as you do with testosterone.

Martini: Here again, I believe Dr. Short was looking for behavioural phenomena.

Exley: Dr. Martini, did I understand you to say that you had actually done the work where you've actually looked at the conversion of testosterone to DHT in foetal rats?

Martini: No, we are doing it.

Exley: You're doing it, you haven't got any results yet?

Martini: It's too early.

De Moor: One small question: in your abstract you spoke about traces of tetrahydro derivatives. Are these 3α or 3β?

Martini: They are 3α.

Fazekas (A. G.): What was the amount of tissue you used per incubation? How many mg?

Martini: 15–25 mg.

Siiteri: I'd like to rephrase Dr. O'Malley's question: it seems that whenever you had more testosterone around, you had less enzyme, and if you removed a source of testosterone, you had more enzyme. Are you certain that your enzyme is operating at substrate saturation levels, and that you're not seeing changes because of the exogenous testosterone that you're adding?

Martini: That's a good point. We collected the tissue several hours after the injection of testosterone, so that exogenous testosterone was already gone at the time in which the conversion was studied *in vitro*.

Fazekas (A. T. A.): Could you tell me something about the extra-testicular effect of FSH; more specifically, did you check the effect of FSH on the testosterone-dihydrotestosterone conversion after castration?

Martini: No, FSH has been added only to pituitaries and to the hypothalamus of normal animals. It would be interesting to do what you suggested.

Fazekas (A. T. A.): I asked this because it would be exactly one of the extra-testicular effects of FSH.

Martini: Yes. Thank you for your suggestion.

Crabbé: Dr. Martini, I wonder whether you've had a chance to see what happened to the animals (mainly rats) you've studied when hypothalamic control of the pituitary secretion is altered? In other words, would you be able to prevent this increase in conversion rate of testosterone to dihydrotestosterone resulting from castration if hypothalamic lesions are induced in those animals?

Martini: We have not done it yet.