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CONVERSION OF TESTOSTERONE INTO 5α-REDUCED METABOLITES IN THE ANTERIOR PITUITARY AND IN THE BRAIN OF MATURING RATS

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SUMMARY

The anterior pituitary of normal female rats shows a considerable 5α-reductase activity. Such an activity is higher in the very first days of life and decreases from birth until the 28th day of age. The anterior pituitary of normal male rats also shows 5α-reductase activity, but this is smaller than that of the anterior pituitary of female animals. Significant differences between activities present in the two sexes can be observed from birth until the 21st day of age. A slight difference in the 5α-reductase activity of the hypothalami of maturing male and female animals has also been observed.

As previously stated, the 5α-reductase activity of the anterior pituitary of female rats is much higher than that of male animals on day 14 of life. Castration performed on neonatal male rats brings about an increase in the 5α-reductase activity of their anterior pituitaries evaluated on day 14 of age. The levels of the enzyme become very similar to those found in the anterior pituitary of normal females of the same age. Conversely, the neonatal administration of testosterone propionate to female rats induces a decrease of the 5α-reductase activity of their pituitaries measured on day 14 of life. Such an activity becomes very similar to that of normal males of the same age. Analogous variations, although of a smaller magnitude, have been observed at hypothalamic level following neonatal castration in male animals or following neonatal administration of testosterone to female animals.

INTRODUCTION

It is now generally accepted that, in its peripheral target structures, testosterone (T) must be converted into 5α-androstan-17β-ol-3-one (androstanolone or dihydrotestosterone, or DHT), 5α-androstane-3α, 17β-diol (3α-diol) and 5α-androstane-3β, 17β-diol (3β-diol) in order to become fully active [1–4]. It has been recently demonstrated that the conversion of T into DHT and 3α-diol occurs also in those central structures (anterior pituitary, hypothalamus, etc.) on which T exerts its feedback and behavioural effects [5–15]. A previous paper from this laboratory [5] has indicated that the anterior pituitary of normal adult male rats converts T into its 5α-reduced metabolites to a large extent. The hypothalamus of adult male rats is also able to transform T into DHT and 3α-diol, but the 5α-reducing activity of the hypothalamus is lower than that of the pituitary. In adult male rats, some reductive metabolism of T occurs also in the amygdala and in the cerebral cortex.

In another set of experiments [16], it has been demonstrated that the central structures (anterior pituitary, hypothalamus, amygdala and cerebral cortex) of neonatal male rats convert T into its 5α-reduced metabolites at a rate which is much higher than that of adult animals. It has also been shown [16] that, in all these structures, the ability to reduce T in the 5α position diminishes with advancing age. It has consequently been proposed [16] that this fact might provide a biochemical basis for explaining the change in the set-point of the steroid-sensitive gonadotropin controller which occurs at time of puberty [17–21]. This conclusion was reinforced by the finding that DHT and 3α-diol are better suppressors of gonadotropin release than T itself [22–25].

Recent studies have indicated that the 5α-reductase and the 3α-hydroxy-steroid-dehydrogenase (the two enzymes necessary to convert T into DHT and subsequently into 3α-diol) are present also in several structures of the female endocrine system. Using labelled progesterone as the substrate, it has been shown that, in females, these enzymes exist in the uterus [26], in the hypothalamus and in the anterior pituitary [27–30]. It has been postulated that these enzymes might convert progesterone into more effective metabolites [26–27].

The present series of experiments have been designed: (a) to analyze whether the 5α-reductase present in the central structures (anterior pituitary, hypothalamus, etc.) of female animals is able to convert T as well as progesterone; (b) to study whether the activity of this enzyme shows modifications from birth up to 60 days of age similar to those previously found in males [16]; and (c) to clarify some of the factors which control these modifications. Special attention has been paid to a possible role played by androgens during the neonatal period, since it is known that, at this time, androgens exert crucial effects on the sexual differentiation of the hypothalamic-pituitary axis of the rat [31–32].

EXPERIMENTAL

Sprague–Dawley rats were used throughout this study. They were maintained on a constant pellet

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diet and water *ad libitum* and were exposed to artificial light from 6.00 a.m. to 8.30 p.m. in rooms with controlled humidity and temperature.

Surgery on baby rats was performed under hypothermia. Testosterone propionate was injected subcutaneously in sesame oil. The incubation procedure and the analysis of T metabolites was performed following the methods described by Kniewald, Massa and Martini[33]. Only minor modifications have been adopted.

The data presented are the means ± the standard errors of at least three different experiments (usually 5–7). Statistical analysis has been carried out using Student's *t*-test.

RESULTS AND DISCUSSIONS

Figure 1 summarizes the data on the 5α-reductase activity of the anterior pituitary of female animals. Comparison is made with the observations previously made in males [16]. The data are expressed in terms of "total" 5α-reduced metabolites formed (DHT + 3α-diol) per mg of fresh tissue in the 3 h of the incubation period. It is evident, first of all, that the 5α-reducing activity of the female anterior pituitary is higher than that of the male up to 21 days of age. It also appears that the pattern exhibited by the 5α-reducing activity of the anterior pituitary at the different ages is totally different in the two sexes. As previously reported [16], in males the ability of the anterior pituitary to transform T into DHT and 3α-diol progressively decreases from birth to day 60. On the contrary, the 5α-reductase activity of the anterior pituitary of normal females shows a rapid and significant increase ($P < 0.05$) between day 1 and day 3 of life. Subsequently, a drop of such an activity is observed between day 3 and 7. This decrease is followed by a plateau up to day 14, and by a further progressive decline from day 14 to day 28. This final decline brings the 5α-reductase activity of the female anterior pituitary to levels found in the anterior pituitary of male animals of comparable age. The data here reported are similar in several respects to recent observations by Denef, Magnus and McEwen[12].

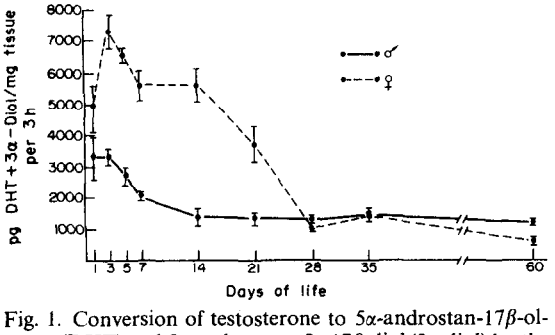

Fig. 1. Conversion of testosterone to 5α-androstan-17β-ol-3-one (DHT) and 5α-androstane-3α,17β-diol (3α-diol) by the anterior pituitary of male and female rats. The data are expressed in terms of picograms of steroids formed (DHT + 3α-diol) per mg of wet tissue, following a 3-h incubation with 160 ng of 4-[^{14}C]-testosterone (specific activity: 59·4 mCi/mM).

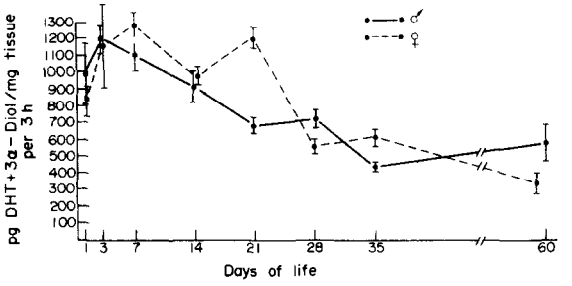

Fig. 2. Conversion of testosterone to 5α-androstan-17β-ol-3-one (DHT) and 5α-androstane-3α,17β-diol (3α-diol) by the basal hypothalamus of male and female rats. The data are expressed in terms of picograms of steroids formed (DHT + 3α-diol) per mg of wet tissue, following a 3-h incubation with 160 ng of 4-[^{14}C]-testosterone (specific activity: 59·4 mCi/mM).

Figure 2 shows the data obtained studying the 5α-reducing activity of the hypothalamus of female and male rats at various intervals after birth. It must be noted, first of all, that at any age considered the 5α-reducing ability of the hypothalami of male and female animals is, in quantitative terms, much lower than that of the anterior pituitary. This is confirmatory of data previously obtained in adult animals [5]. Contrary to what happens in the anterior pituitary, there is an increase of the 5α-reductase activity of the hypothalamus between days 1 and 3 of life in both males and females. Beginning from day 3, the ability to transform T into DHT and 3α-diol progressively declines in male animals. On the contrary, in females, a further increase is observed up to day 7. This increase is followed by a decline between days 7 and 14, by a further elevation around day 21, and by a subsequent drop. This drop brings the 5α-reductase level of the female hypothalamus to become similar to that of the male, beginning from day 28. The observations here reported agree, in general, with data recently published by Denef and co-workers [12]. However, their samples were collected beginning from day 5 of life; this has prevented them from registering the increase of 5α-reductase activity which occurs in the first few days after birth.

The data obtained using the cerebral cortex of female and male animals are shown in Fig. 3. It

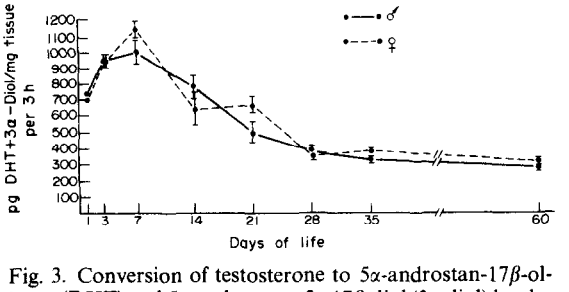

Fig. 3. Conversion of testosterone to 5α-androstan-17β-ol-3-one (DHT) and 5α-androstane-3α,17β-diol (3α-diol) by the cerebral cortex of male and female rats. The data are expressed in terms of picograms of steroids formed (DHT + 3α-diol) per mg of wet tissue, following a 3-h incubation with 160 ng of 4-[^{14}C]-testosterone (specific activity: 59·4 mCi/mM).

is clear that, in agreement with previous findings [5], there is some 5α-reductase activity also in this tissue. In both sexes, the 5α-reductase activity of the cerebral cortex is higher in the first period of life, with a parallel increment between days 1 and 7. Subsequently, there is a progressive decline of such an activity, which brings its levels down to those found in adults beginning from day 28. There are no differences between males and females at any age considered.

The reason and the biological significance of the sex-linked differences observed in the developmental pattern of the 5α-reductase activity in the anterior pituitary and in the hypothalamus of male and female animals are not understood at the present moment. The working hypothesis has been formulated that the sexual dimorphism here reported might be linked to the neonatal exposure to androgens which occurs in male animals, and which does not occur in females. It is known that the administration of exogenous testosterone to the neonatal female rat permanently transforms the activity of its hypothalamic-pituitary axis to a pattern which resembles that normally found in the male [31, 32]. It has also been shown that castration performed in the neonatal male rat brings about a permanent transformation of its hypothalamic-pituitary axis, which becomes more similar to that of the female [31, 32].

In order to study the possible role played by androgenic steroids circulating in the neonatal period in inducing the sexual dimorphism here reported, it has been decided to study the 5α-reductase activity of the anterior pituitary, of the hypothalamus and of the cerebral cortex in the following four groups of animals: (a) normal males; (b) normal females; (c) females androgenized at day 3 of life with a low dose of TP (100 μg/rat); (d) females androgenized at day 3 of life with a high dose of TP (1 mg/rat); (e) females androgenized at day 9 of life with a high dose of testosterone propionate (TP, 1 mg/rat), and (f) males castrated at 1 day of life. The 5α-reductase activity of the three structures considered has been evaluated at the age of 14 days. This particular period was selected for this preliminary study mainly because, at this age, there are clear-cut differences in the 5α-reductase activity of the anterior pituitaries of the two sexes.

Figure 4 shows again that, at the age of 14 days, the anterior pituitary of normal female rats is able to convert T into DHT and 3α-diol to a far greater extent than the anterior pituitary of normal male animals. It is also apparent that the administration of 1 mg of TP on day 3 of life, brings the 5α-reductase activity of the anterior pituitary of female animals to levels as low as those found in the anterior pituitary of normal males of comparable age. The lower dose of TP (100 μg) administered on day 3 of life is also effective in decreasing the 5α-reductase activity of the anterior pituitary of female animals. However, the decrease is lower than that observed following administration of the larger dose of T. This dose-related effect is in accordance with previous evidence

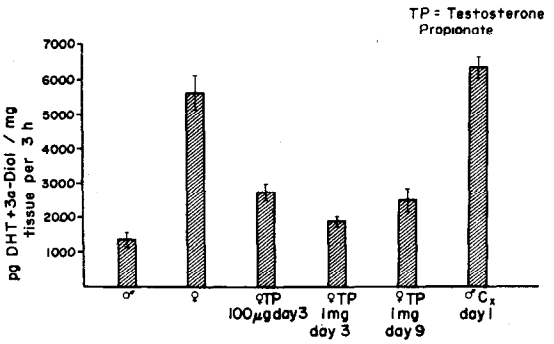

Fig. 4. Conversion of testosterone to 5α-androstan-17β-ol-3-one (DHT) and 5α-androstane-3α,17β-diol (3α-diol) by the anterior pituitary of male and female rats of 14 days of age. The data are expressed in terms of picograms of steroids formed (DHT + 3α-diol) per mg of wet tissue, following a 3-h incubation with 160 ng of 4-[^{14}C]-testosterone (specific activity: 59·4 mCi/mM). Females TP 100 μg day 3 vs normal females, $P < 0{\cdot}0005$; females TP 1 mg day 3 vs normal females, $P < 0{\cdot}0005$; females TP 1 mg day 9 vs normal females, $P < 0{\cdot}0025$; castrated males day 1 vs normal males, $P < 0{\cdot}0005$.

indicating that, when T is given on day 3 of life, higher doses of the steroid provoke a stronger masculinizing effect on the hypothalamic-pituitary axis than lower doses [31, 32]. The data shown in Fig. 4 indicate that female animals treated with 1 mg of TP on day 9 of life still exhibit a decrease of anterior pituitary 5α-reductase activity. However, the decrease is not as big as that obtained with the same dose of TP given on day 3. This is perfectly in line with the observations of Barraclough[31] and Gorski[32], which indicate that the same dose of T is less efficient in masculinizing the hypothalamic-pituitary complex if given on day 10 than on previous days of life. Finally, Fig. 4 indicates that castration of male animals, performed on day 1 of life, brings about a significant increase in the 5α-reductase activity of the anterior pituitary measured at day 14. The 5α-reductase activity of the anterior pituitary of male animals castrated on day 1 of life becomes similar to that of the anterior pituitary of normal females of the same age.

The data obtained evaluating the 5α-reductase activity of the hypothalami of the animals submitted to the different types of neonatal treatments are shown in Fig. 5. As previously indicated, at this particular age there are not significant sex-linked differences in the 5α-reductase activity of this structure. However, neonatal androgenization performed with 1 mg of TP (given either on day 3 or day 9 of life) brings about a decrease in the 5α-converting ability of the hypothalamus of female animals. The decrease observed is much lower than that previously found at anterior pituitary level even if it is significant at the statistical analysis ($P < 0{\cdot}005$). Castration performed in male animals on day 1 of life does not seem to significantly modify the 5α-reductase measured at 14 days.

Figure 6 shows the effects of the different treatments on the 5α-reductase activity of the cerebral cortex.

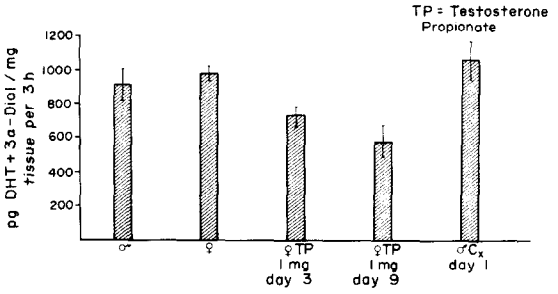

Fig. 5. Conversion of testosterone to 5α-androstan-17β-ol-3-one (DHT) and 5α-androstane-3α,17β-diol (3α-diol) by the basal hypothalamus of male and female rats of 14 days of age. The data are expressed in terms of picograms of steroids formed (DHT + 3α-diol) per mg of wet tissue, following a 3-h incubation with 160 ng of 4-[^{14}C]-testosterone (specific activity: 59·4 mCi/mM). Females TP 1 mg day 3 vs normal females, $P < 0{\cdot}005$; females TP 1 mg day 9 vs normal females, $P < 0{\cdot}0025$.

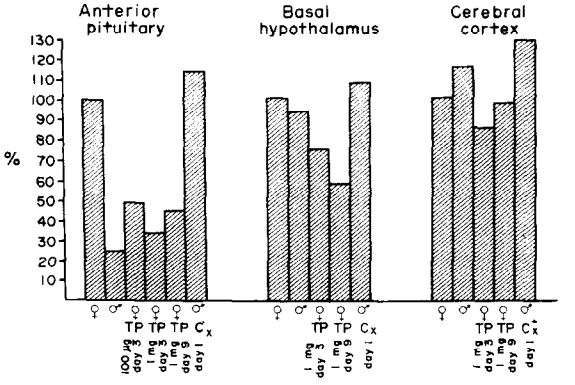

Fig. 7. Per cent variations of the conversion of testosterone to 5α-androstan-17β-ol-3-one (DHT) and 5α-androstane-3α,17β-diol (3α-diol) in the anterior pituitary, the basal hypothalamus and the cerebral cortex of male and female rats of 14 days of age (converting ability of the normal female structure = 100).

No significant differences have been recorded in the various groups of animals.

The data of this series of experiments are summarized in Fig. 7, in which the values have been transformed in terms of per cent variations (converting ability of the female structure = 100%). The reason for presenting the data in this way is the desire to analyze them on the same type of scale. It is obvious from Fig. 7 that neonatal androgenization of female animals, and neonatal castration of male animals, have much more pronounced effects on the 5α-reductase activity of the anterior pituitary than on the enzymes of the hypothalamus.

These results seem to indicate that neonatal exposure to androgens may be one of the factors which create and entertain the sexual dimorphism of the 5α-reductase activity of the anterior pituitary and of the hypothalamus. Unfortunately, it was not possible to make a longitudinal study (at 21, 28 days, etc.) to confirm whether the effect of the neonatal

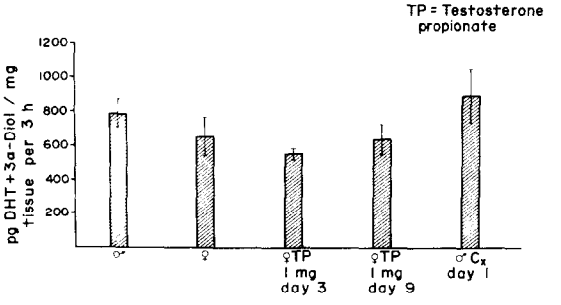

Fig. 6. Conversion of testosterone to 5α-androstan-17β-ol-3-one (DHT) and 5α-androstane-3α,17β-diol (3α-diol) by the cerebral cortex of male and female rats of 14 days of age. The data are expressed in terms of picograms of steroids formed (DHT + 3α-diol) per mg of wet tissue, following a 3-h incubation with 160 ng of 4-[^{14}C]-testosterone (specific activity: 59·4 mCi/mM).

* The fact that castration performed in neonatal animals (5 days old) is followed by a much bigger increase in the 5α-reductase activity at pituitary level, than castration performed in adults, has been recently confirmed by Denef *et al.*[12].

administration of androgens is permanent or transitory. Such a study (which is presently in progress) is made desirable by the observation that, in adult animals, castration enhances and the administration of testosterone diminishes the levels of the 5α-reductase in the anterior pituitary [5, 10, 12]. There are, however, a few reasons for believing that the effect here reported is different from the one which is observed in adults. First of all, when castration is performed in mature animals, the increase of the 5α-reductase of the anterior pituitary observed 14 days following the operation is much smaller (164%) than that found here (452%).* Finally, it is not expected that testosterone administered on day 3 might still be circulating and exerting biological effects on day 14.

The data obtained in the longitudinal study made in the male and female rat from day 1 of life up to day 60 have provided evidence for the existence of sex-linked differences in the developmental patterns of the 5α-reductase in the anterior pituitary and in the hypothalamus. It appears from the results that the sexual dimorphism here reported is more pronounced at anterior pituitary level. The biological significance of such a dimorphism, as well as the role played in the female by the variations in 5α-reductase activity encountered in the central structures at the different ages remain unclear. An hypothesis has already been put forward which relates the changes observed in male animals to the pubertal phenomenon [16]. It is possible that the big variations exhibited by the 5α-reductase of the anterior pituitary of female animals might also be correlated with the occurrence of puberty. Such a possibility is presently under investigation.

The results obtained manipulating testosterone levels in the neonatal period seem to support the view that exposure to androgens in early life might be one of the factors which control the 5α-reductase activity of the central structures. It has been found that castration, performed in male rats during the neonatal period, transforms to a female pattern the

5α-reductase activity of the masculine pituitary gland; and that, vice versa, administration of exogenous testosterone to neonatal females transforms the 5α-reducing capacity of their anterior pituitary towards masculine patterns. At present, it is impossible to decide whether the effect of the neonatal exposure to androgens on the pituitary 5α-reductase is exerted directly on the gland, or whether it is modulated by hypothalamic influences. The fact that similar, although smaller, effects are observed at hypothalamic level might point to a nervous point of attack. However, preliminary evidence obtained in this laboratory (Massa and Martini, unpublished observations) indicates that the Luteinizing Hormone-Releasing Hormone (LH-RH) added to incubation media containing anterior pituitary tissue has no influence on the 5α-reductase activity of the gland. This preliminary finding might suggest that the activation of the 5α-reductase of the anterior pituitary of neonatally castrated males, and conversely the decline of such an activity reported in neonatally androgenized females, are not subject to hypothalamic influences, or, at least, do not depend on LH-RH.

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