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# TESTOSTERONE METABOLISM IN BRAIN CELLS AND MEMBRANES

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**Summary**—The central nervous system (CNS) is considered a target structure for the action of all the classes of hormonal steroids produced by the organism. Well-characterized genomic and less well-understood membrane mechanisms of action are probably involved in the steroid modulation of brain activities. Moreover, some classes of steroids need to be converted into "active" metabolites before interacting with their effector systems. In particular, testosterone (T) exerts many of its effects after conversion to 5α-dihydrotestosterone (DHT) and estrogens. The CNS possesses both the 5α-reductase, the enzyme which produces DHT and the aromatase which transforms T into estrogens; however, the relative role and distribution of these enzymes in the various structural components of the CNS has not been clarified so far. The 5α-reductase has been found to be present in high concentrations in brain white matter structures because these are particularly rich in myelin membranes, to which the enzymatic activity appears to be associated. This membrane localization might suggest a possible involvement of steroidal 5α-reduced metabolites in membrane-mediated events in the CNS. Moreover, the distribution of 5α-reductase was studied in neurons, astrocytes and oligodendrocytes isolated from the brain of male rats by density gradient ultracentrifugation, as well as in neurons and glial cells grown in culture. The aromatase activity was also evaluated in neurons and glial cells grown in culture and in isolated oligodendrocytes. Among the three cell types isolated, neurons appear to be more active than oligodendrocytes and astrocytes, respectively, in converting T into DHT. Also, in cell culture experiments, neurons are more active in forming DHT than glial cells. Only neurons possess aromatase activity, while glial cells are apparently unable to aromatize T.

## INTRODUCTION

Steroids exert the majority of their actions by genomic activation of hormone responsive genes, but short latency effects have also been reported; these probably involve membrane mechanisms which have not been well-characterized so far [1–3]. Both genomic and membrane effects are probably involved in the modulation of brain functions by steroid hormones. Neurons have been shown to possess intracellular receptors for all the steroid hormones produced by the organism [4]; on the contrary, the actual influence of steroids on the different type of glial cells [the other important cell type present in the central nervous system (CNS)] and the steroid receptor status of these cells have been studied less to date [5, 6]. Nevertheless, there is evidence indicating that glial cells also may be influenced by sex steroids [7] and glucocorticoids [8].

The testicular secretion of androgens exerts an important influence on brain development and functions; apparently, these steroids, to exert the whole spectrum of their action, should be metabolized into the so-called "active" metabolites, namely 5α-reduced androgens and estrogens. However, while the aromatization into estrogens has been clearly identified as the basic mechanism through which testosterone (T) exerts many of its actions on the brain, particularly during the developmental period, the role of 5α-reduced androgens is, at the moment, less well-understood [9]. On the other hand, the process of 5α-reduction can occur, possibly through the action of the same enzyme, also on progesterone and corticosterone [10], and therefore the 5α-reduced metabolites of these steroids might also have some importance in modulating CNS activity. As a matter of fact, the further 3α-hydroxylation of 5α-reduced steroids has attracted a growing interest in the last few years, since it has been shown that this steroid conformation is a structural feature of those pregnane derivatives (mainly progestin and corticosteroids) which are known to be very

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potent ligands for the GABA receptor complex [11, 12]. Consequently, the role of 5α-reduction of steroids in the brain should not only be considered as a necessary step to activate steroids before intracellular receptor binding, but also as a possible mechanism to create steroids with possible membrane sites of action.

In this paper, the experiments which have led to identifying the presence of the 5α-reductase activity on myelin membranes will be briefly reviewed, together with our recent findings on the differential localization of the 5α-reductase and aromatase activity in neurons and glial cells.

## RESULTS AND DISCUSSION

In the last few years, this laboratory has been interested in studying the metabolism of T in different areas of the rat brain. These studies have been mainly conducted utilizing the microdissection procedure known as the "Palkovitz punch technique" [13]. This is based on the dissection of brain areas on frozen sections by calibrated needles. The 5α-reductase activity was evaluated in all these studies by a sensitive radioenzymatic method in which the formation of the 5α-reduced metabolites, 5α-dihydrotestosterone (DHT) and 5α-androstan-3α,17β-diol (3α-diol), is quantified after 2 h of incubation with carbon-labelled T. The method is described in full detail in previous papers [14–16]. In one of these studies, it was decided to investigate also whether the brain regions composed of white matter were able to metabolize T [16].

Figure 1 shows the formation of DHT and 3α-diol in punches obtained from the superficial layer of the cerebral cortex (considered as control tissue since it is mainly composed of gray matter) and from different white matter structures: anterior commissure (CA), fornix (FX), habenulo-interpenduncular tract (HP), corpus callosum (CC), stria medullaris (SM), optic chiasm (CO), fimbria of the hippocampus (FI), cerebral peduncle (PC), pontine fibers (FP), cerebellar medulla (CMD) and corticospinal tract (TCS). The formation of DHT is significantly higher in all the white matter structures considered than in the cerebral cortex. Moreover, in these structures, the amounts of DHT formed appear to increase significantly in the rostro-caudal direction; also the formation of 3α-diol is significantly higher in nearly all the white matter structures examined (except for CA and HP) than in the cerebral cortex, even if the degree of the increment is much lower than that found for DHT. Also, in experiments in

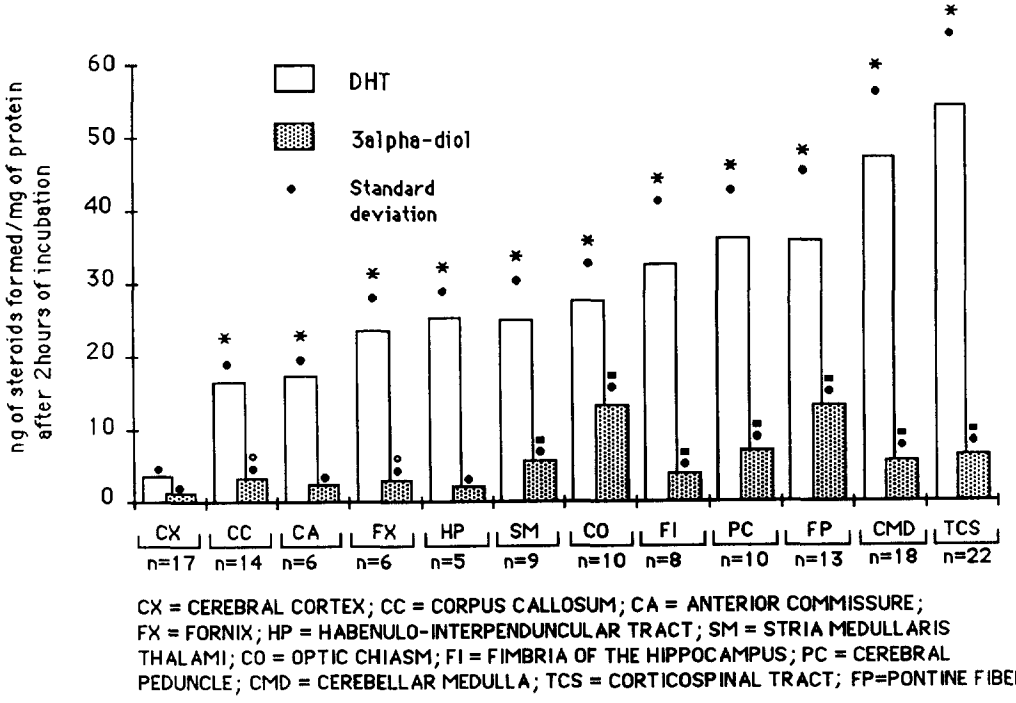


Fig. 1. DHT and 3α-diol formation in different white matter structures of the adult male rat brain, measured *in vitro* after incubation with [4-$^{14}$C]T n = Determinations. *$P < 0.01$ vs DHT formed in the CX; ■ $P < 0.01$ vs 3α-diol formed in the CX; and ○ $P < 0.05$ vs 3α-diol formed in the CX.

which fresh tissue samples (and not frozen sections) were utilized, the amount of DHT formed by the subcortical white matter is at least 3 times higher than that produced by the cerebral cortex [17, 18].

In agreement with these results obtained in rats, Sholl and coworkers [19] recently found a much higher 5α-reductase activity in the cerebral cortex of fetal monkey brain than in the other brain structures considered: medio basal hypothalamus, cerebellum, amygdala, cingulate cortex and cerebral cortex. Therefore, on the basis of these data it appears that the presence of a high 5α-reductase activity in the white matter is not a characteristic peculiar to the rodent brain.

Since white matter structures are mainly composed of myelinated axons, it was decided to verify whether the 5α-reductase activity could be associated with the myelin membranes. Therefore, myelin was purified from the whole brain of adult male rats by ultracentrifugation on sucrose density gradients [20].

The purity of the myelin preparation used was checked both by electron microscopy and by the evaluation of 2′,3′-cyclic nucleotide 3′-phosphohydrolase (CNP), which is generally considered a marker for myelin membranes and cytochromo-*c*-reductase, an enzyme present in the microsomal membranes. At electron microscopy the myelin fraction appears to be composed of typical multilamellar or unilamellar figures, essentially free of cellular debris, nuclei, mitochondria and other organelles.

The cytochromo-*c*-reductase, evaluated by the method of Sottocasa *et al.* [21], was undetectable in the myelin preparation studied, indicating that microsomal membranes contamination is absent or very low. On the contrary, the enrichment of the purified myelin membranes preparation for the CNP activity, evaluated by a colorimetric method [22], was 5 to 6-fold with respect to brain homogenates (the starting material for the isolation of myelin). This result is comparable with or even better than those present in the literature for the best-characterized and purified myelin preparations [20].

Figure 2 shows the formation of the 5α-reduced metabolites of T in brain homogenates and in the purified myelin. It is apparent that the purified myelin preparation possesses an extremely active 5α-reductase. The enzyme activity present in the purified myelin preparation appears to be 8.3 times higher than that of the

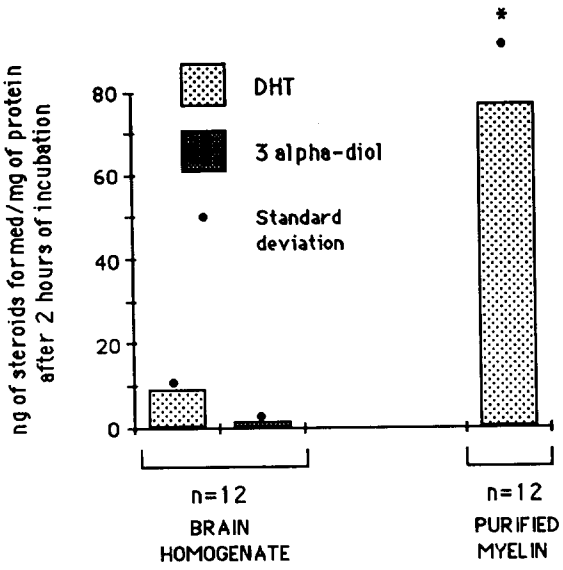


Fig. 2. DHT and 3α-diol formation in whole brain homogenate and in the purified myelin preparation, measured *in vitro* after incubation with [4-$^{14}$C]T. *n* = Determinations. *$P < 0.01$ vs brain homogenate.

brain homogenate. It is interesting to note that 3α-diol does not appear to be formed by myelin incubates, while minute amounts of this steroid are formed by the brain homogenate.

In order to verify whether the differences in the 5α-reductase activity of the various brain components might be due to the presence of different concentrations of the same enzyme or to different isoenzymes, the kinetic properties $K_m$ and $V_{max}$ were measured in the purified myelin as well as in homogenates of the subcortical white matter and the cerebral cortex [23].

The apparent Michaelis–Menten constant ($K_m$) and the maximum velocity ($V_{max}$) were calculated using a Lineweaver–Burk plot. In the experimental conditions adopted, in the presence of an excess of cofactors, the apparent $K_m$ was very similar in the three structures examined, i.e. $1.93 \pm 0.2\ \mu M$ in the purified myelin, $2.72 \pm 0.73\ \mu M$ in the white matter and $3.83 \pm 0.49\ \mu M$ in the cerebral cortex homogenates. On the contrary, the $V_{max}$ values obtained for the various tissues were consistently different, being $34.4 \pm 5.54$, $19.57 \pm 2.36$ and $6.47 \pm 1.03$ ng/h/mg of protein ± SE in the myelin, white matter and cerebral cortex, respectively. This indicates that the myelin possesses a concentration of 5α-reductase which is much higher than that present in the white matter and in the cerebral cortex.

The $K_m$ values obtained for the enzyme in the myelin, white matter and cerebral cortex, are very similar, all in the μM range, indicating that

the enzyme present in the different nervous structures has the same affinity for T, the substrate used in the present experiments. This suggests that the enzyme may be present, in the three types of samples examined, in the same form.

It is therefore apparent that, in the rat brain, much of the 5α-reductase activity is associated with myelin membranes and that the enzyme therefore may be localized on the cell surface also and not only inside the cytoplasm.

At present, the physiological meaning of this finding remains to be elucidated, however some hypotheses may be proposed.

While studying the ontogenetic development of the 5α-reductase activity in the rat brain we have found that the profile of the enzymatic activity in the myelin preparations shows a peak activity at 19–21 days of life; this is followed by a rapid decrease to lower values [24]. The enzymatic content appears to be always much higher than that present in the other cerebral structures considered; these moreover, showed a peak activity earlier in the brain development (4–5 days after birth in the cerebral cortex, and 6–7 days after birth in the hypothalamus). It is noteworthy that the peak of 5α-reductase activity in myelin membranes coincides with the maximal accumulation rate of myelin according to Norton [25]. A possible interpretation of these data is that, at the very beginning of the myelination process (when the actual deposition of myelin membranes has not yet started), the 5α-reductase is synthesized at a high rate by the oligodendrocytes—the cells which form the myelin. In line with this suggestion, it has been shown that the oligodendrocytes of 7-day-old animals (i.e. in a period when the myelination process is not yet initiated) possess a much higher ability to convert T into DHT than the oligodendrocytes of adult animals. The 5α-reductase is therefore present in high concentrations in their cell bodies where the biosynthetic machinery resides. This could probably also explain the peak in the enzymatic activity found in the first few days of life in the cerebral cortex and in the hypothalamus.

Thereafter, the enzyme might start to be transferred from the oligodendrocytes into the myelin membranes; this transport apparently shows a peak in the third week of life, when the process of myelination is particularly active [25].

Since a possible involvement of this enzyme in the myelination process might be put forward, work is presently in progress in this laboratory, in order to verify this hypothesis, utilizing recently synthesized compounds able to block the activity of the enzyme: the 5α-reductase inhibitors.

The cellular distribution of the enzymes metabolizing T between neurons and glial cells was initially studied indirectly, lesioning the brain cortex by the insertion of a fine needle and studying T metabolism punching the scar tissue around the lesion, which is composed mainly of reactive astrocytes [26]. The results indicated that, in the lesioned area, from where neurons had almost disappeared, the formation of DHT was significantly reduced with respect to the control tissue.

A more direct approach was utilized thereafter, and the cellular distribution of T metabolism was studied in freshly isolated cell preparations and in well-characterized cell cultures [27]. In a first group of experiments, brain cells were dissociated with trypsin and by passages on calibrated screens and isolated by ultracentrifugation, utilizing a sucrose density gradient in the case of neurons and astrocytes [28] and an *in situ* generated percoll density gradient in the case of oligodendrocytes [29]. In a second group of experiments, neuronal cell cultures were performed according to the methods of Gensburger *et al.* [30] and Borg *et al.* [31] with small modifications. The glial cell cultures were performed according to the methods of Labourdette *et al.* [32, 33]. The purity of the cellular preparations utilized in the experiments was evaluated by electron microscopy or by indirect immunofluorescence:

*Isolated cells.* Cells were examined by light and transmission electron microscopy. Purity was 80–90% for the neurons, 90–95% for the oligodendrocytes and 60–70% for the astrocytes. Impurities were due to different glial cells (ependymal type in the case of neurons), disrupted cells and rare isolated nuclei.

*Cultured cells.* Neuronal cultures were analyzed at 7 days in culture by indirect immunofluorescence, utilizing monoclonal antibodies anti-160 kDa neurofilament polypeptide and anti-microtubules associated protein (MAP-2). Purity appeared to be >90%. Glial cultures were analyzed at 15 days in culture by indirect immunofluorescence utilizing monoclonal antibodies raised against the glial fibrillary acidic protein (GFAP). Cultures appeared to consist of more than 90% of GFAP-positive cells (i.e. astrocytes).

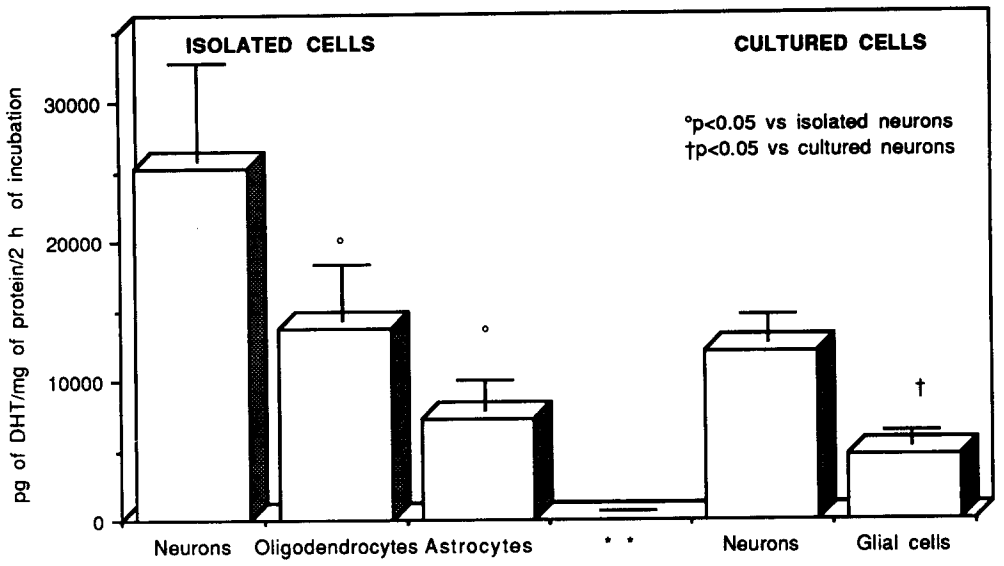


Fig. 3. DHT formation in isolated and cultured brain cells measured *in vitro* after incubation with [4-$^{14}$C]T. For experimental details see Ref. [27].

In Fig. 3 the 5α-reductase activity of neurons, oligodendrocytes and astrocytes isolated by ultracentrifugation from the brain of adult male rats is shown. Neurons convert T into DHT in significantly higher amounts than oligodendrocytes and astrocytes. Astrocytes appear to possess a lower 5α-reductase activity than oligodendrocytes; however, the difference in T converting activity between the two types of glial cells is not statistically significant.

Similar results were obtained (Fig. 3) when cultured neuronal and glial cells we incubated. Neurons possess a significantly higher ability to form DHT from T than cultured glial cells.

It appears, therefore, that all the brain cells are able to form DHT from T and among the three cell types, neurons are more active than oligodendrocytes, which are more active than astrocytes.

On the contrary, preliminary results [34] seem to indicate that the aromatase activity is present only in cultured neurons and is almost completely absent in mixed cultures of glial cells (comprising >90% of astrocytes) and in freshly isolated oligodendrocytes.

In conclusion, these data seem to indicate that the two main T metabolizing activities present in the brain, 5α-reductase and aromatase, have a different cellular distribution. While aromatase is present only in neurons, 5α-reductase is expressed, although in lower amounts, also in glial cells. Moreover, this enzymatic activity is present in large amounts in myelin membranes. The functional significance of these findings is still under investigation, but the association of the 5α-reductase with the cellular membranes might provide the basis to explain the existence of extracellular effects of 5α-reduced steroids (like, for instance, the modulatory effect exerted on GABA receptors) and to postulate their possible involvement in the process of myelination.

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