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ANDROGEN METABOLISM IN THE BRAIN

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Summary—The paper summarizes the most recent views on androgen metabolism in the brain. In particular it will be shown that: (1) the enzyme 5α-reductase is particularly concentrated in the white matter; (2) 5α-reductase is also present in the myelin; 5α-reductase is present in higher concentrations in neurons (isolated or cultured) that in glial cells (astrocytes and oligodendrocytes); (4) only neurons possess the capability of aromatizing androgens to estrogens; and (5) a possible role of steroid metabolism in the control of the process of myelinogenesis is suggested.

INTRODUCTION

It has been known for several years that the central nervous system (CNS) is the target for practically all steroid hormones (androgens, estrogens, progesterone, corticoids, etc.), for which specific receptors have been demonstrated in several brain zones [1–12]. It is also known that the CNS represents an area of intense metabolism for steroid hormones. Relatively few data are available on the brain metabolism of estrogens [13], progesterone [14–17] and corticoids [18]; in contrast, the metabolism of testosterone and other androgens has been extensively investigated. In the brain, androgens may be metabolized according to two different major pathways. The first one, known as the aromatase pathway, transforms testosterone into estradiol and androstenedione into estrone (Fig. 1) [19]. The second one, which is similar to that present in the majority of the peripheral androgen-depending structures (prostate, seminal vesicles, etc.) [20, 21], transforms testosterone into 5α-androstane-17β-ol-3-one (dihydrotestosterone, DHT) through the action of 5α-reductase, an enzyme which has recently been cloned [22, 23] (Fig. 2). This has permitted to follow the developmental patterns of the enzyme in the CNS studying the corresponding message [24]. DHT may be further metabolized to yield 5α-androstane-3α,17β-diol (3α-diol) as well as minute amounts of 5α-androstane-3β,17β-diol (3β-diol); the enzymes involved in these processes are 2 (3α and 3β) hydroxysteroid dehydrogenases [19]. In the brain, testosterone may also be converted into 4-androstene-3,17-dione (androstenedione), through a reversible process catalized by 17β-hydroxysteroid dehydrogenase. Androstenedione may be subsequently 5α-reduced to yield 5α-androstane-3,17-dione and androsterone, the two 17 keto derivatives corresponding to DHT and 3α-diol (Fig. 2) [19].

The two metabolic pathways (aromatase and 5α-reductase) probably subserve different functions in the brain. It has been known for some time that the aromatization of androgens to estrogens occurring in the fetal or neonatal brain is a crucial process for the "organization" of male patterns of the brain centers which control sexual behavior and gonadotropin secretion [19]. In adult males, the process of aromatization is important for androgens to exert their typical male behavioral effects [19, 25–27]. This is true for the majority of the species studied so far; there are, however, relevant exceptions since, in some species, DHT, a non-aromatizable androgen, is also effective [28, 29]. The mechanism of action of estrogens derived from androgens in the "organizational" processes is probably linked to the capability of estrogens to influence neuronal sprouting, dendritic spine density [30] and the development and remodeling of synapses in particular brain areas [31–34]; obviously, androgen effects exerted after transformation into estrogens involve the binding of the resulting molecules to the estrogenic receptor [35, 36].

The role of the 5α-reductase pathway has become more mysterious than ever. On the basis of the fact that 5α-reduced androgens are more potent than testosterone in suppressing LH secretion in a variety of animal models, it has been suggested that 5α-reduction of testosterone

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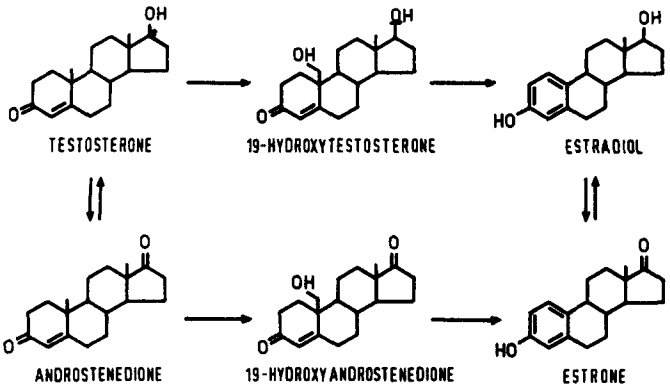

Fig. 1. The aromatase pathway

might play an important role in androgen-dominated feedback mechanisms [19, 37, 38]. In support to such a theory, it has been shown that the administration of testosterone to castrated male rats, in conjunction with a specific inhibitor of 5α-reductase, is less inhibitory on LH secretion than testosterone given alone [39]. Whether this concept may also be applied to humans is still open to controversy. One may recall that serum levels of LH are usually elevated in subjects with the syndrome of Imperato–McGinley (5α-reductase deficiency) [40, 41]; however, recent data have shown that in subjects treated with finasteride (MK 906) (an inhibitor of 5α-reductase used for the treatment of benign prostatic hyperplasia) serum LH levels remain unaltered, in spite of a very significant decrease in serum DHT levels [42].

In the brain, the two processes of aromatization and 5α-reduction of androgens appear to predominate in different regions. First of all, the aromatase system seems to be confined to the hypothalamus and to the limbic system [19]. Moreover, Selmanoff *et al.* [43] have found a significantly higher aromatase activity in the medial preoptic nucleus than in the lateral hypothalamic nucleus and in the mediobasal hypothalamus. On the contrary, 5α-reductase activity appears to be more elevated in the lateral hypothalamic nucleus and in the lateral preoptic nucleus than in the medial preoptic nucleus, the anterior hypothalamic nucleus and the mediobasal hypothalamus. This typical lateral localization of 5α-reductase in the hypothalamus has been confirmed by Melcangi *et al.* [44]. A link probably exists between the two systems; in this context it is interesting to underline that in the brain of some species, but not of others, aromatase activity seems to be under androgenic control [45–48].

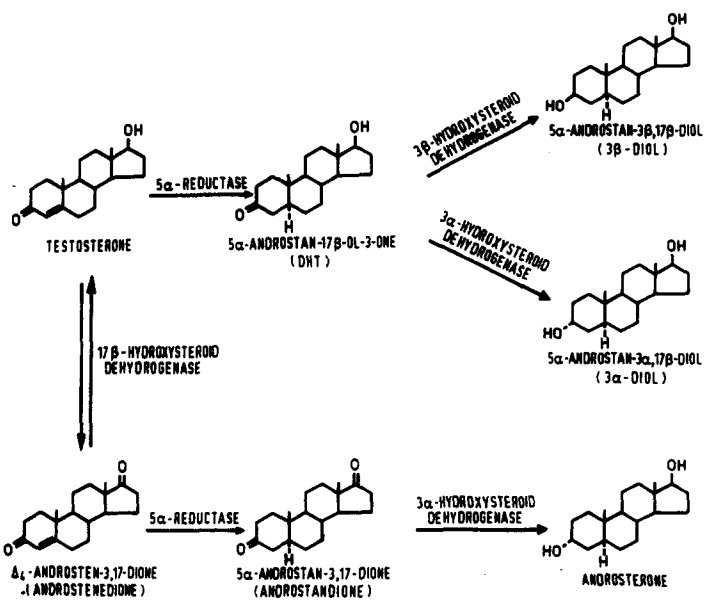

Fig. 2. The 5α-reductase pathway.

The 5α-reductase and the 3α-3β-hydroxysteroid dehydrogenase systems are also present in the anterior pituitary; this appears to be the only system metabolizing the androgens present in the gland, since aromatase is not present [19]. In the pituitary, the activity of the enzyme is clearly influenced by the endocrine status; castration has been reported to significantly increase 5α-reductase activity of the gland, while the administration of either testosterone or estrogens is able to reestablish the original situation [19]. Also LHRH seems to affect 5α-reductase activity of the anterior pituitary [49]. From these and other observations, two general conclusions seem to emerge; namely that: (1) the anterior pituitary might be the most important site for the feedback effect exerted by androgens on LH secretion; and (2) 5α-reductase is probably present almost, if not exclusively, in the gonadotrophs [19]. Contrary to what happens in the pituitary, the 5α-reductase system of the brain, and in particular of the hypothalamus, does not seem to be sensitive to humoral signals. Castration and steroid administration do not influence this activity either in the whole hypothalamus [50], or in those hypothalamic nuclei where the enzyme is particularly concentrated [44] (see above). Also neural inputs seem to be totally ineffective in modifying 5α-reductase activity of the hypothalamus. The pharmacological interruption of cholinergic, andrenergic, serotoninergic and opiatergic inputs to the hypothalamus does not interfere with the 5α-reductase activity of this structure [51]. Also the total surgical deafferentiation of the hypothalamus, performed according to the technique of Halasz, has no effect on this enzymatic activity [51].

It is important to underline that, in numerous studies comparing the 5α-reductase activity of the brain of male and female animals, no sexual differences have emerged [19, 50]. Moreover, previous work performed in this and other laboratories, has shown that 5α-reductase of the brain and of the pituitary is able to convert testosterone [19], progesterone [14–17] and corticosterone [18] into the corresponding 5α-reduced metabolites independently of the sex of the animals. These observations may suggest that, physiologically, the enzyme might utilize different substrates in the two sexes (testosterone in males? progesterone in females? corticosteroids in both sexes?). Only further studies may be able to clarify this issue.

Before describing some of the recent data obtained in the authors laboratory, it is important to recall that, in a series of studies, Baulieu and Robel [52] have shown that the brain is able to synthesize dehydroepiandrosterone, as well as Δ5-pregnenolone and their respective sulfates [52]. These observations, as well as the others which will be described below, seem to underline that, at present, the brain might be viewed not only as an important target for steroid effects, but also as a structure which possesses the machinery for synthesizing hormone precursors and for metabolizing these precursors as well as steroid hormones originating in the peripheral target glands (and transported to the CNS via the general circulation), in order to face the specific functional requirements of given brain structures at any time (for instance, neonatal vs adult).

PRESENCE OF 5α-REDUCTASE IN THE WHITE MATTER AND THE MYELIN

In previous studies from this laboratory, it has been shown that, in the rat brain, the subcortical white matter dissected macroscopically proved able to 5α-reduce testosterone with much higher yields than the cerebral cortex [53, 54]; also the amounts of DHT formed by tissue punches microdissected from frozen sections of the corpus callosum and the optic chiasm (two white matter structures) have been found to be 3 and 6 times higher, respectively, than those formed by punches of the superficial layer of the cerebral cortex [55]. It was consequently decided to analyze in more detail this phenomenon [56]. For this purpose, 5α-reductase activity has been evaluated in different white matter structures microdissected using the punch technique of Palkovits. In these experiments (and in subsequent ones) the formation of 5α-reduced metabolites was measured *in vitro* using labeled testosterone as the substrate.

Figure 3 shows the formation of DHT and 3α-diol in punches obtained from the cerebral cortex and from the different white matter structures examined. It is clear that the formation of DHT is significantly higher in all the white matter structures than in the cerebral cortex. Moreover, in these structures, the amounts of DHT formed appear to increase significantly in the rostro–caudal direction; the metabolic conversion of testosterone appears to be positively correlated ($r = 0.916$) with the distance of each particular white matter structure from the first

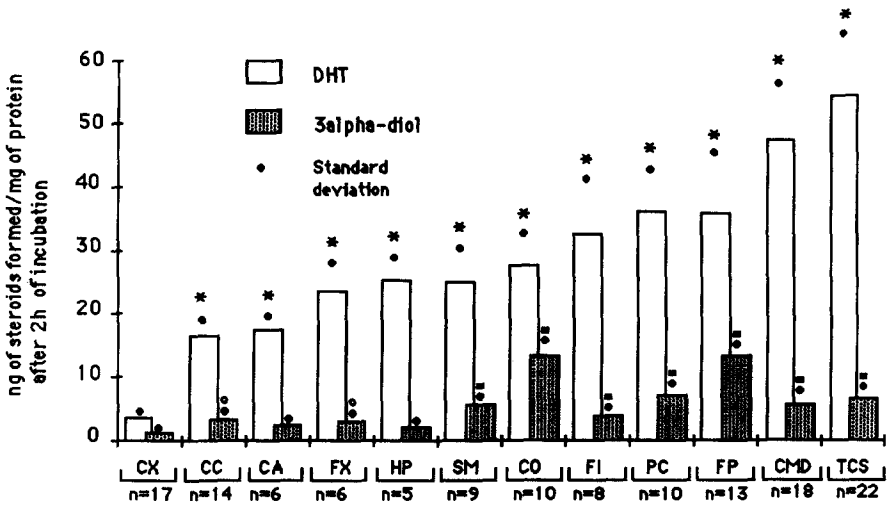

Fig. 3. DHT and 3α-diol formation in different white matter structures of the adult male rat brain, measured *in vitro* after incubation with [4-^{14}C]testosterone (3.16×10^{-6}M). CX = Cerebral cortex; CC = Corpus callosum; CA = Anterior commissure; FX = Fornix; HP = Habenulo–interpenduncular tract; SM = Stria medullaris thalami; CO = Optic chiasm; FI = Fimbria of the hippocampus; PC = Cerebral peduncle; CMD = Cerebellar medulla; TCS = Corticospinal tract; FP = Pontine fiber. n = determinations; $^{*}P < 0.01$ vs DHT formed in the CX; $^{\blacksquare}P < 0.01$ vs 3α-diol formed in the CX; $^{\circ}P < 0.05$ vs 3α-diol formed in the CX.

section plane (a frontal plane passing 2 mm in front of the bregma).

Also the formation of 3α-diol is significantly higher in all the white matter structures examined than in the cerebral cortex, except for CA (anterior commissure) and HP (habenulo–interpeduncular tract). However, at variance with the pattern of the formation of DHT, there was no progressive rostro–caudal increase in the formation of this metabolite. It should also be noted that the DHT/3α-diol ratio (which is 2.76 in the cerebral cortex) is consistently higher (between 4.3 and 11.2) in all the white matter structures examined, except in CO (optic chiasm) and FP (pontine fibers).

Since the white matter is very rich in myelin, it was thought interesting to investigate whether the myelin might possess 5α-reductase activity. The formation of 5α-reduced metabolites of testosterone in brain homogenates (the starting material for the isolation of myelin) and in purified myelin was consequently evaluated. The results are provided in Fig. 4. It is apparent that the purified myelin preparation possesses an extremely active 5α-reductase, which is 8.3 times higher than that of the 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. The purity of the myelin preparation used was investigated by electron microscopy (see [56] for details).

Two new findings emerge from the present studies: (a) the existence of higher 5α-reductase activity in the white matter structures especially in those located more caudally; and (b) the association of the enzyme with the myelin sheaths. Both these findings are difficult to interpret.

To the authors' knowledge, excepting a single report by Hanukoglu *et al.* [57], in which high activity of the progesterone 5α-reductase was shown in the corpus callosum, no data are available in the literature, other than those

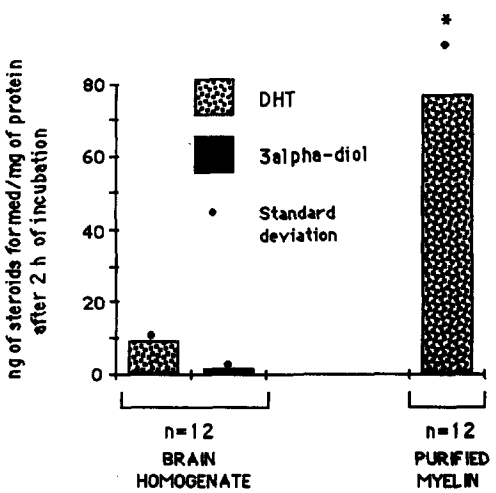

Fig. 4. DHT and 3α-diol formation in whole brain homogenate and in the purified myelin, measured *in vitro* after incubation with [4-^{14}C]testosterone (3.16×10^{-6}M). n = determinations; $^{*}P < 0.01$ vs brain homogenate.

originating from this laboratory [53–55] on 5α-reductase activity of the white matter. Moreover, autoradiographic studies have been unable so far to show the presence, in the white matter, of significant amounts of receptors for androgen [58, 59], corticosterone [60, 61] or progesterone [59], which, as previously mentioned, represent three possible substrates for 5α-reductase. A tentative explanation may be provided for the presence of a cranio–caudal gradient in 5α-reductase activity in the white matter tracts; this is linked to the ontogenesis of the myelination process. The formation of myelin sheaths for isolation of the nerve axons proceeds in a well regulated and ordered fashion; the process of myelination appears first in the spinal cord and hindbrain and then advances rostrally to the forebrain [62]. Taking into account: (a) the continuous increase in the formation of myelin during brain maturation in the rat (from 20 days to 20 months of life); and(b)thefactthatthepurifiedmyelinfromthe fore brain areas possesses characteristics of biochemical immaturity for a longer time than the myelin from the hindbrain [62], it is possible to suggest that the rostro–caudal differences in 5α-reductase activity reported here reflect an intrinsic difference in the process of myelin deposition and maturation in the different brain areas.

It is certainly premature to predict a role for 5α-reductase and for the steroids originating from this process on myelination. However, it may be pointed out that a few studies have underlined the presence of a peak of 5α-reductase activity in the rat brain [63] and in the purified myelin [64] in the early postnatal period, i.e. at the time at which the myelination process is beginning [65].

It is interesting to note that the purified myelin, at variance with the white matter structures and the brain homogenate, does not seem to produce 3α-diol. It is therefore possible to suppose that the 3α-hydroxysteroid dehydrogenase, which converts DHT into 3α-diol, has a different cellular or subcellular localization in the brain.

PRESENCE OF 5α-REDUCTASE AND AROMATASE IN NEURONS AND THE GLIA

The present work has been performed in order to verify, on the basis of direct comparative measurements, whether 5α-reductase is present in the two major cellular components of the brain: neurons and glia (oligodendrocytes and astrocytes) [66]. The selection of these cell types has been made on the basis of the following considerations. The neurons are classically considered to be the cells in which androgens exert their more important biological and endocrinological effects, possibly due to the presence in these cells of the receptors for testosterone and DHT [58]. On the other side, glial cells (and in particular the oligodendrocytes) are involved in the formation of myelin [67, 68], a structure in which the presence of 5α-reductase has been demonstrated in the previous section of this paper.

In the present work the cellular distribution of 5α-reductase has been studied *in vitro* using two different types of nervous tissue preparations: (a) neurons, oligodendrocytes and astrocytes isolated from the whole brain of normal adult male rats by sucrose or percoll density gradients; and (b) cultured neurons and glial cells obtained from fetal or neonatal rat brain.

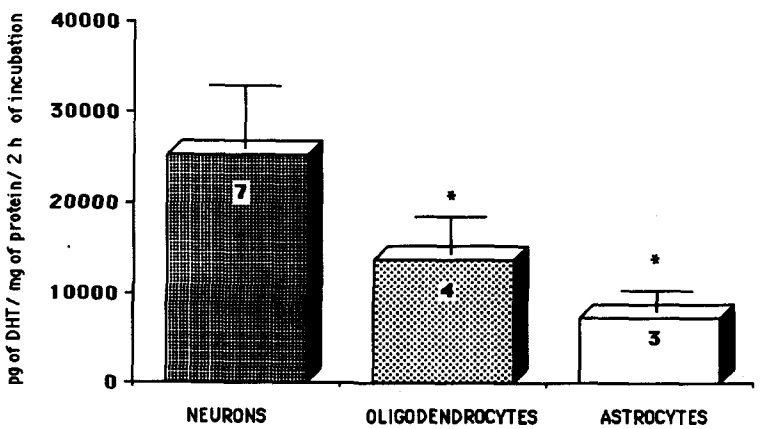

Fig. 5. DHT formation in isolated cells from male rat brain. Numbers inside columns represent the numbers of experiments. Data are expressed as mean ± SD of the mean. $^*P < 0.05$ vs neurons.

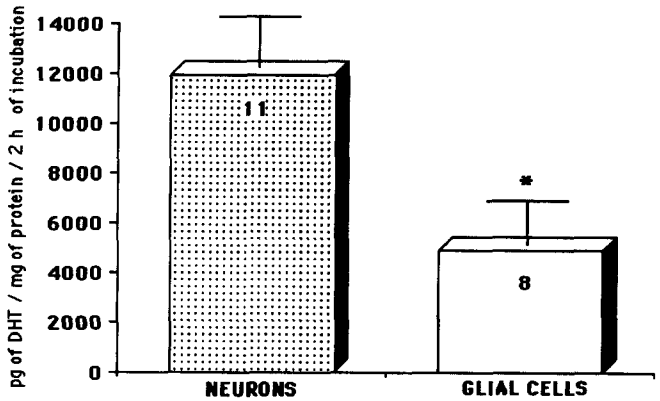

Fig. 6. DHT formation in cultured cells of rat brain. Numbers inside columns represent the number of Petri dishes analyzed. Data are expressed as mean ± SD of the mean. $*P < 0.01$ vs neurons.

Isolated cells

Figure 5 shows 5α-reductase activity of neurons, oligodendrocytes and astrocytes isolated by ultracentrifugation from the brain of adult male rats. It is clear that testosterone is converted into DHT in significantly higher amounts in the neurons than in the oligodendrocytes and in the astrocytes. The astrocytes appear to possess lower 5α-reductase activity than the oligodendrocytes; however, the difference in the testosterone converting activity between the two types of glial cells is not statistically significant. The purity of the different preparations used has been assessed by standard methods, whose details are given in the original paper [66].

Cultured cells

In agreement with the results obtained in the isolated cell study, Fig. 6 shows that the formation of DHT in cultured neuronal cells is significantly higher than in cultured glial cells.

In additional experiments (data not shown), it has been demonstrated that the aromatase activity (as evaluated using androstenedione as the labeled substrate) is present only in cultured neurons and is almost completely absent in mixed cultures of glial cells and in freshly isolated oligodendrocytes. The purity of cultures of the different cell preparations has obviously been evaluated (see [66] for details).

The present data show that purified cell preparations (neurons, oligodendrocytes and astrocytes) isolated from the brain of adult male rats are able to form DHT from testosterone. Neurons appear to be more active than the glial cells in this respect. Moreover, the data indicate that between the two populations of glial cells, the oligodendrocytes seem to possess a slightly higher enzymatic activity than the astrocytes. Neurons appear more active than glial cells in metabolizing testosterone to DHT also when a cell culture system is used; finally, neurons appear to be the only cell type able to aromatize androgens.

The present findings, which indicate that neurons are the cell population in which both 5α-reductase and aromatase are present in high quantities, underline the fact that probably the most important effects of androgens take place in these cells. This is also confirmed by the fact that the majority of the uptake and receptor studies performed in the CNS, using either testosterone or DHT as the ligand, have shown that neurons are a more important target for these androgens [58]. The prevalent localization of 5α-reductase in neurons described in this study apparently disagrees with a previous finding of Canick *et al.* [69]. These authors suggested that, in the brain, 5α-reductase activity is confined primarily to non-neuronal cells. The conclusion of Canick *et al.* [69] has been reached, however, using an indirect approach, i.e. by measuring 5α-reductase in a mixed primary culture of fetal rat hypothalamus, in which the neuronal component had been disrupted by means of kainic acid. However, it must be noted, first of all, that in the study by Canick *et al.* [69] there was only a decrease of the neuronal component, but not its total elimination. This was probably due to the fact that kainic acid neurotoxicity is not equally effective on all neuronal types, but shows some specificity for cerebellar neurons [70–72]. With regard to the presence of 5α-reductase in glial components, shown in this laboratory and confirmed in two recent short reports [73, 74],

it may be pointed out that testosterone uptake has been recently shown to occur in isolated oligodendrocytes grown on an extracellular matrix [75]; similarly, receptors for corticosteroids (another possible substrate of 5α-reductase) have been shown to be present in the oligodendrocytes [75–77]. The low 5α-reductase activity of the astrocytes found in the present study is confirmatory of previous indirect data [55], which have shown that the formation of DHT is significantly decreased in nervous tissues in which a glial proliferation (prevalently astroglial) has been induced by mechanical lesions.

The demonstration that the oligodendrocytes, the cells responsible for the process of myelination, possess significant 5α-reductase activity, may probably explain the presence of the enzyme in the myelin (see above). In this connection, it is interesting to recall that in the oligodendrocytes of developing animals 5α-reductase activity appears to peak just before the beginning of the process of myelination [64], suggesting a possible correlation between this enzymatic activity and the formation of myelin.

CONCLUSIONS

The data presented here indicates that the CNS is the site of intensive metabolism of androgens. In the brain, 5α-reductase and aromatase pathways are both present; the distribution of the enzymes which characterize each pathway is different not only with regard to the brain structures in which each pathway predominates (limbic system and medial hypothalamus for the aromatase; lateral hypothalamus for 5α-reductase), but also with regard to the type of cells in which the corresponding enzymes are present (neurones: 5α-reductase and aromatase; glia: only 5α-reductase). The metabolism of androgens occurring in the brain may subserve different physiological purposes at different times of life. The aromatization of androgens may be crucial for the "organization" of the brain towards a male pattern of gonadotropin secretion and sexual behavior, but may be also involved more in general, in the process of synaptogenesis and of neuronal sprouting. On the other hand, the process of 5α-reduction may be important for the feedback regulation of LH secretion, as well as for the control of some aspects of myelinogenesis. Only further work will clarify how the enzyme is delivered from the oligodendrocytes to the myelin, and the physiological significance of its presence in this structure.

As mentioned throughout the paper, the majority of the data has been obtained using testosterone as the substrate. Since 5α-reductase is active on all steroids possessing a Δ-4 3-keto configuration (e.g. testosterone, progesterone, corticosterone, cortisol, etc.) [19] the possibility exists that, in addition to androgens, progestagens and corticoids might also be physiological substrates for the enzyme. This is an important area for future work, since very little is known about the physiological effects of 5α-reduced progestagens and/or corticoids. With regard to progestagens, it has been shown that not only are their 5α-reduced metabolites more effective than progesterone itself in stimulating gonadotropin secretion in several animal models [15, 78], but also that these metabolites may bind to the GABA A receptor [79]; this obviously opens the field for the possible interrelationships between hormonal steroids on one side and cerebral neurotransmitters on the other. It is also possible that steroid metabolites formed in the brain might not act through the classical intracellular receptorial system; only further study will clarify their effects on possible membrane receptors, ion transport, etc. These phenomena may help us understand some of the neurophysiological data, in which steroids have been shown to be able to elicit responses in such a short time as to exclude the possibility of a genomic effect.

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