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TESTOSTERONE METABOLISM IN PERIPHERAL NERVES: PRESENCE OF THE 5α-REDUCTASE-3α-HYDROXYSTEROID-DEHYDROGENASE ENZYMATIC SYSTEM IN THE SCIATIC NERVE OF ADULT AND AGED RATS

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Summary—Previous reports from this laboratory indicate that the 5α-reductase, the enzyme which converts testosterone into its "active" metabolite 5α-androstan-17β-ol-3-one (dihydrotestosterone, DHT) is highly concentrated in the white matter structures of the CNS, which are mainly composed of myelinated fibers. No studies have been performed up to now, in order to evaluate the possible presence of the 5α-reductase activity in peripheral myelinated nerves. To this purpose the 5α-reductase activity has been evaluated in the sciatic nerve of the rat and compared to that present in the cerebral cortex and in the subcortical white matter, a central structure mainly composed of myelinated fibers. The study has been performed in normal adult male rats (60–90-day-old) and in aged (20-month-old) animals. The data obtained in 60–90-day-old animals indicate the presence of an active metabolism of testosterone at the level of the sciatic nerve. In this structure, testosterone is actively transformed into DHT and 5α-androstan-3α, 17β-diol (3α-diol); in the sciatic nerve, the formation of DHT is equal to that found in the subcortical white matter and higher than that found in the cerebral cortex. Moreover, at variance with what happens in CNS structures, where 3α-diol is produced only in small amounts, in the sciatic nerve this metabolite is produced in amounts similar to those of DHT. The study in aged rats has shown that in the sciatic nerve, the formation of DHT and particularly that of 3α-diol are much lower than in younger animals. No age-related variations in the 5α-reductase activity in the cerebral cortex and in the subcortical white matter have been observed.

INTRODUCTION

Recent data from this laboratory indicate that in the brain of the rat and of the mouse, the 5α-reductase, the enzyme which converts testosterone into its "active" metabolite 5α-androstan-17β-ol-3-one (dihydrotestosterone, DHT) is preferentially localized in the white matter structures [1–3]. Other studies have shown that the enzyme is also present in purified myelin membranes; myelin membranes do not seem to possess [3, 4] the 3α-hydroxysteroid-dehydrogenase, the enzyme which in the central nervous system (CNS) further transforms DHT into 5α-androstan-3α,17β-diol (3α-diol) and which is normally present, even if in small amounts, both in white and gray matter components of the brain [3].

To the authors' knowledge, no studies have been performed up to now, in order to evaluate the possible presence of the 5α-reductase complex in myelinated nerves of the peripheral nervous system (PNS). The purpose of the present investigation was to study *in vitro* the metabolism of testosterone into its 5α-reduced metabolites (DHT and 3α-diol) in the sciatic nerve of the male rat; this is a myelinated peripheral sensomotor nerve which can be obtained in sufficient amounts to perform metabolic studies even from a small animal like the rat. The cerebral cortex and the subcortical white matter (a central structure mainly composed of myelinated axons) have been used as control tissues.

Old animals have also been included in this study in order to explore the possibility that testosterone metabolism in the peripheral nervous structures might be influenced by the process of aging.

EXPERIMENTAL

Animals

Adult (60–90-day-old) and aged (20-month-old) male Sprague–Dawley rats (Charles River, Calco, Italy) were used in these experiments. The animals were maintained in animal quarters with controlled temperature and humidity. The light schedule was 14 h light and 10 h dark (lights on at 6.30 am). Animals were fed a standard pellet diet and water was provided *ad libitum*.

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Sample preparation

Samples of cerebral cortex, subcortical white matter and sciatic nerve were dissected macroscopically.

Incubation procedures

Two samples (each composed of tissue fragments weighing about 5 mg) of cerebral cortex, subcortical white matter and sciatic nerve were obtained from each animal.

They were separately incubated in 250 μl of Krebs–Ringer buffer solution in the presence of a NADPH generating system (NADP, disodium salt, Boehringer Mannheim, 9.32×10^{-3} M; glucose 6-phosphate, disodium salt, Boehringer Mannheim, 11.76×10^{-2} M and glucose 6-phosfate dehydrogenase from yeast grade 1, Boehringer Mannheim, 3.5×10^{-2} IU) and [^{14}C]testosterone 3.16×10^{-6} M, (SA ~56.9 μCi/mmol, Amersham, England) as the labeled substrate. Vials without tissue provided the blanks. The incubation was carried out at 37°C for 2 h in a Dubnoff metabolic shaker under a stream of O_2/CO_2 (98:2).

Detection of metabolites

At the end of incubation, the reaction was stopped by deep freezing the samples. Tritium labeled DHT and 3α-diol (about 5000 dpm each) were added to each sample in order to evaluate the recoveries. The metabolites formed were extracted twice with diethylether and separated on a thin layer silica gel chromatography plate (Merck 60 F_{254}, DC) eluting three times with a mixture of dichloromethane-diethylether (11:1 v/v). The DHT and 3α-diol spots were identified with iodine vapors, scraped off and the radioactivity counted in a Packard 300C liquid scintillation spectrometer. Quench corrected dpm of the two isotopes were obtained by a calibration standard curve. The identification of the two metabolites formed in the sciatic nerve was performed by recrystallization to constant ^{3}H/^{14}C ratio (Table 1).

Table 1. Recrystallization of DHT and 3α-diol to constant ^{3}H/^{14}C ratio

	^{3}H/^{14}C ratio	
	DHT	3α-diol
Starting solution	1.47	2.29
First crystallization (Absolute ethanol/water)	1.29	2.26
Second crystallization (Acetone/water)	1.28	2.27
Third crystallization (Diethyl ether/*n*-hexane)	1.29	2.25

The identification of the metabolites formed in the cerebral cortex and in the subcortical white matter has been previously described [3]. The metabolites were expressed as pg of steroid formed per mg of tissue after 2 h of incubation.

Statistical analysis

The data were analyzed by one-way analysis of variance. To determine the levels of significance of the responses, the *t*-values were compared with the values of Dunnett's table for multiple comparison [5].

RESULTS

Figure 1 shows the formation of DHT and 3α-diol in the two central structures considered (cerebral cortex and white matter) as well as in the sciatic nerve of 60–90-day-old male rats. It is apparent that the

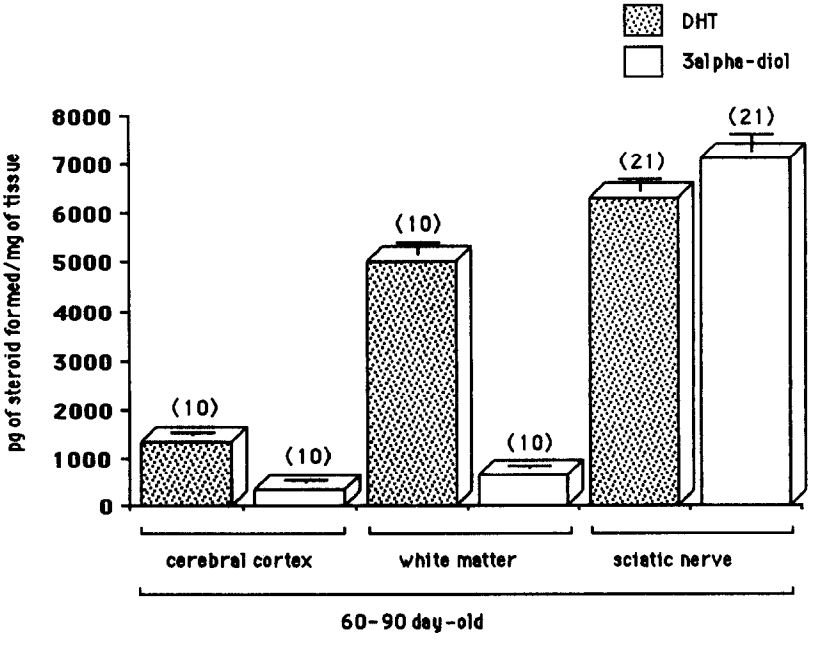

Fig. 1. Formation of DHT and 3α-diol in the cerebral cortex, subcortical white matter and sciatic nerve of adult rats. The values are expressed as mean ± SEM.

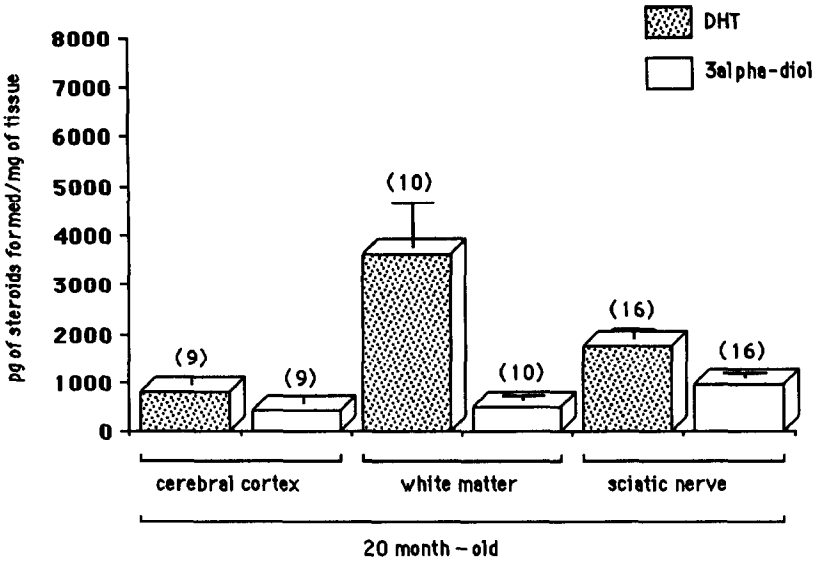

Fig. 2. Formation of DHT and 3α-diol in the cerebral cortex, subcortical white matter and sciatic nerve of aged rats. The values are expressed as mean ± SEM.

white matter possesses a much higher 5α-reductase activity than the cerebral cortex. The production of 3α-diol in the white matter is slightly but significantly higher than in the cerebral cortex. The pattern of testosterone metabolism in the sciatic nerve is substantially different from that found in the two central component studied. First of all, in the sciatic nerve, testosterone is metabolized into DHT in amounts comparable to those found in the white matter. Secondly, in this peripheral structure the formation of 3α-diol is even higher than that of DHT; consequently, the amounts of 3α-diol formed by the sciatic nerve largely exceed those formed in the two central structures. The ratio 3α-diol/DHT is about 1.12 in the sciatic nerve while it is respectively 0.29 and 0.13 in the cerebral cortex and in the white matter.

Figure 2 shows the formation of DHT and 3α-diol in the cerebral cortex, in the white matter and in the sciatic nerve of aged (20-month-old) rats. The pattern of the metabolism of testosterone in the central structures appears to be similar to that found in younger animals. Both the cerebral cortex and the subcortical white matter form DHT and 3α-diol, and the amounts of DHT formed by the white matter are higher than those produced by the cerebral cortex. A comparison of the data shown in Figs 1 and 2 indicates that age does not induce statistically significant modifications in the formation of the two testosterone metabolites in the two central components considered. At variance with what occurs at CNS level, age deeply modifies testosterone metabolism in the sciatic nerve; in this nerve, the production of DHT and 3α-diol is much smaller in old rats than in the 60–90-day-old animals. Moreover, in the sciatic nerve during the process of aging, the formation of 3α-diol decreases even more than that of DHT; consequently, the ratio 3α-diol/DHT changes from 1.12 in 60–90-day-old rats to 0.54 in the old ones.

DISCUSSION

The present study shows the presence of a conspicuous metabolism of testosterone at the level of the sciatic nerve of adult male rats. In this structure, testosterone is actively transformed into DHT and 3α-diol. In the sciatic nerve the formation of DHT is at least equal to that found in the central white matter and higher than that found in the cerebral cortex. Moreover, at variance with what happens in the two CNS structures studied, in which 3α-diol is produced only in small amounts, in the sciatic nerve 3α-diol is produced in amounts similar to those of DHT. The study in old animals has shown that in the sciatic nerve, the formation of DHT and even more that of 3α-diol are significantly decreased during aging. No age-related variations in the 5α-reductase activity have been found in the cerebral cortex and in the white matter.

To the authors' knowledge, no data are available on the uptake and metabolism of testosterone in peripheral nerves, which have never been considered so far as possible target structures for androgenic steroids. The data presented here, however, demonstrate that the sciatic nerve is able to transform testosterone into its "active" 5α-reduced metabolites with significant yields. On the basis of this preliminary observation it would be tempting to suggest that also the peripheral nervous system (PNS) might be included among the androgen-sensitive target structures; however, as prevously mentioned, the presence of androgen receptors has not been demonstrated so far in the PNS. It is also necessary to underline that in peripheral steroid-dependent structures (prostate, uterus, etc.), in the anterior pituitary and in the CNS, the 5α-reductase system is able to metabolize also progesterone [6–8] and corticosteroids [6]; it remains to be ascertained which one of these three families of steroids represents the physiological substrate for

the 5α-reductase-3α-hydroxysteroid-dehydrogenase complex in the sciatic nerve. The fact that, using the synthetic glucocorticoid dexamethasone, a week labeling has been shown to occur in the Schwann cells prepared from the sciatic nerve [9, 10] might imply that corticoids might also be involved.

The data of the present work have shown that the patterns of testosterone metabolism are different in the central and in the peripheral myelinated structures. In the white matter, 3α-diol is formed only in low amounts, while this metabolite is produced with considerable yields by the sciatic nerve. This is particularly surprising on the basis of the observations previously reported [3, 4] that α-diol is completely absent, as a testosterone metabolite, in purified myelin obtained from the central structures. The high peripheral production of 3α-diol might be explained by the fact that the peripheral myelin differs metabolically from that of the CNS; this would be supported by the finding that the peripheral myelin has a biochemical composition slightly different from that present in the brain [11, 12]. Moreover, it should be underlined that the present data do not clarify whether in the sciatic nerve the enzymes involved in the metabolism of testosterone are localized in the myelin, in the axon or in the Schwann cells. In order to clarify these issues work is actively in progress, in this laboratory.

No apparent explanation may be offered for the conspicuous age-related decrease in the formation of both DHT and 3α-diol occurring in the sciatic nerve; however, the fact that aging does not significantly modify the activity of the 5α-reductase complex in the CNS structures studied might be indicative of the existence of different mechanisms controlling testosterone metabolism in the peripheral and in the central nervous structures.

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