REVIEWS

Metabolism of Androgens in Rat Pituitary Gland and Hypothalamus: Catabolism of Dihydrotestosterone or Transformation of Androgen Signal?

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The role of androgen metabolism and individual androgen metabolites in rat pituitary gland and hypothalamus is discussed on the basis of published data and results of our experiments. It is proposed that metabolism of androgens in these structures is aimed at the formation of not only true sex steroids estradiol-17β and 5α-dihydrotestosterone, but also of androgens contributing to the regulation of cell processes, *i.e.* represents modification (transformation) of androgen signal (testosterone) in target pituitary and hypothalamic cells.

Key Words: *rats; pituitary gland; androgens; metabolism; role of androgen metabolites*

Steroid hormones are natural biological regulators involved in many processes in animals and humans, including reactions to exogenous signals and maintenance of homeostasis. Regulation of reproductive processes is the primary function of steroid hormones.

Mammalian gonads produce both androgens and estrogens, but their ratio considerably varies during ontogeny. Under normal conditions, testosterone is the major androgen secreted by the gonads in postnatal ontogeny, in particular, during puberty and at some stages of prepuberty. Most other plasma androgens are testosterone derivatives (metabolites) produced not only in the gonads, but also in other tissues due to biotransformation (metabolism and steroidogenesis). The amount of metabolites formed in various tissues and their ratio considerably vary and depend on the age, sex, and physiological state of the organism. These variations are determined by activities of enzymes involved in steroidogenesis or metabolism.

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Androgens and estrogens regulate feedback mechanisms between the gonads and central nervous system (primarily, the pituitary gland and hypothalamus). The discovery of androgen metabolism in rat brain in the late 1960s [58] has attracted much attention to this problem. The metabolism of testosterone was found in many brain structures [19,23,45,47,51,55,57], in particular in rat pituitary gland. The intensity of testosterone metabolism in the pituitary gland of male rats is $^1/_5$ of that in the prostate, the main androgen target tissue [22].

Most *in vivo* and *in vitro* studies of androgen metabolism in rat brain were conducted on the pituitary gland, and only some experiments were performed on the hypothalamus. The purpose of these studies was to reveal the relationship between androgen metabolism in the pituitary gland and hypothalamus and the involvement of these hormones in the regulation of gonadotropic functions of the hypothalamic-pituitary complex (HPC).

In the early 1970s, the scheme of androgen metabolism was established by studying testosterone metabolism in rat prostate. The metabolism of 5α-andro-

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stane-3β,17β-diol (3β-D) in rat prostate and pituitary gland was described later (Fig. 1). In respect to androgen metabolism in the pituitary gland, the metabolism of not only testosterone, but also of its natural derivatives (metabolites) attracted much attention. It remained unclear, whether or not the scheme of androgen metabolism in the prostate is applicable to this process in rat pituitary gland. We first studied *in vitro* metabolism of not only testosterone, but also of 5α-dihydrotestosterone (DHT), 5α-androstane-3α, 17β-diol (3α-D), and 3β-D in rat pituitary gland and hypothalamus [5,20]. We revealed some peculiarities of androgen metabolism in these structures of rat brain. In the hypothalamus of male and female rats, testosterone is converted into DHT and 3α-D (Fig. 1), but DHT is then converted into 3α-D, but not into 3β-D. Furthermore, neither DHT, nor 3α-D, but only polar compounds, are *in vitro* formed from 3β-D in both brain structures [5,20]. Our previous experiments demonstrated the formation of polar compounds from DHT and 3α-D in the pituitary gland and hypothalamus of male and female rats, but in this case polar compounds were not the only metabolites [5,20]. We demonstrated that metabolites produced from 3β-D are more chromatographically polar compounds than 3β-D. It was proposed that these compounds possess an additional hydroxyl group [5]. This assumption was confirmed by the data showing that metabolism of 3β-D in rat pituitary gland yields 5α-androstane-3β,6α, 17β-triol, 5α-androstane-3β,7β,17β-triol, and 5α-androstane-3β,7α,17β-triol [33]. Hydroxylation of 3β-D was also demonstrated in the prostate of rats, dogs, and humans [30]. These triols are formed with the involvement of the cytochrome P-450 complex [35]. Recent experiments on rat prostate showed that hydroxylation at all positions of the 3β-D molecule is performed by the same complex of enzymes [30].

The possibility for 3β-D formation from DHT in rat brain, or *vice versa*, is still not established. Some authors believe that 3β-D in rat pituitary gland is produced from DHT only in trace concentrations (or is not formed) [4,18,22,24,41,55], while others leave room for this possibility [12,16]. These contradictory results are due to several reasons. First, androgen metabolism in rat pituitary gland and hypothalamus was studied using testosterone as the only substrate. Second, it is difficult to compare results of different researchers. And third, the most important reason is that after chromatography of formed metabolites some authors detected the sum of 3β-D and 3α-D, because these stereoisomers are poorly separable. At the same time, 3β-D in rat pituitary gland was detected after *in vivo* injection of ^{3}H-testosterone [54]. We assume that 3β-D can be *in vivo* formed from DHT in rat pituitary gland [65], while *in vitro* formation of this androgen can be detected only under certain conditions (in homogenates of the pituitary gland [3] or in the suspension of rat pituitary cell [12]). This peculiarity of 3β-D formation is probably due to extremely high activity of the cytochrome P-450 system in relation to 3β-D in rat pituitary gland and, probably, in the hypothalamus. The physiological role of this phenomenon is discussed below.

In the mid-1970s it became apparent that androgen metabolism in rat brain, including the hypothalamus and pituitary gland, is interesting by itself, but not exceptional and cannot be an argument for its physiological necessity in HPC, because these processes take place in the majority of target tissues and differ only in quantitative ratio between androgen metabolites, but not in their qualitative composition. The only exception is hydroxylation of 3β-D by the cytochrome P-450 system: different hydroxylated metabolites are formed in rat pituitary gland and the prostate in rats, humans, and dogs [30,33].

Physiological importance of androgen metabolism in rat HPC is confirmed by the fact that activity of enzymes responsible for androgen metabolism in rat pituitary gland and hypothalamus changes during ontogeny [4,19,21,31,48,66]. Our experiments showed that the activity of enzymes involved in androgen biotransformation in the pituitary gland and hypothalamus of male and female rats depends on the postnatal age: the intensity of androgen metabolism and, therefore, activity of enzymes involved in this process are tens times higher in male and female rats during the first 23 days of life [4,19]. Sex-related differences in the activity of enzymes responsible for androgen metabolism in the hypothalamus and pituitary gland were also most pronounced until day 23, although during ontogeny activity of these enzymes in the hypothalamus changes to a lesser degree than in the pituitary gland. These variations can play an important role in the postnatal ontogeny of rat HPC. It should be emphasized that gonads in immature males and females secrete mainly 5α-reduced androgens [26,56,59].

Regulation of biosynthesis and secretion of gonadotropins in the brain is the most important aspect of studies devoted to androgen metabolism in HPC and the effects of natural androgen metabolites on this process and, probably, on other pituitary hormones in rats [14]. There is a great body of data showing that the formation of 5α-reduced androgens in the pituitary gland is aimed at the regulation of gonadotropin secretion [27,34,40,47]. However, this problem is still poorly understood. Some authors reported that 5α-reduction of testosterone is not necessary for the regulation of gonadotropin secretion in rats [43,68].

Studies of the influence of androgens on gonadotropin secretion revealed sexual dimorphism in their

effects on the production of luteinizing hormone in castrated rats [7,8,46]. 3α-D and 3β-D produced different inhibitory effects on this process that differed also from the effect of testosterone. Furthermore, the effects of androgens on the secretion of luteinizing hormone were transient and differed between males and females. In castrated females, single injection of androgens inhibited secretion of luteinizing hormone only for 6 h postinjection or shorter (for 3β-D). In castrated male rats, the effect of androgens persisted longer than in females, but did not exceed 24 h [7,8,46].

Some authors hypothesized that 3α-D and 3β-D affect DHT binding to rat HPC cells (i.e., androgen receptors). If so, this influence of androgens is probably indirect, because the affinity of both diols for androgen receptors in rat pituitary gland is much lower than that of testosterone and DHT [10,62]. A more realistic explanation is that 3α-D and, especially, 3β-D modulate estrogen receptors, *i.e.* estradiol-17β (E_2) binding in rat pituitary gland [36,44,67]. *In vivo* experiments confirmed this assumption. Androgens, including 3α-D and 3β-D, can affect the estrogen receptor—E_2 complex in rat pituitary gland. This influence is more pronounced in castrated males than in castrated females [50]. The mechanism of this effect of 3α-D is unclear, but there are experimental data explaining the effect of 3β-D. It was found that this natural androgen possess the highest affinity for estrogen receptors in some organs of humans and animals [13,37, 42,50]. 3β-D markedly inhibits E_2 binding to estrogen receptors in cultured rat pituitary cells [36]. Unlike DHT and 3α-D, a 5-10-fold excess of 3β-D causes a 40-50% inhibition of E_2 binding to estrogen receptors in the cytosol of rat pituitary gland [67]. Despite ambiguous data on the effects of both diols on androgen and estrogen receptors in rat HPC, there are some grounds for these assumptions. Blood concentrations of E_2 and diols in different periods of postnatal ontogeny differ by 1-2 orders of magnitude [17,65]. In females, these parameters differ by 3 orders of magnitude during prepuberty and immediately before the onset of estrous cycles [49,53]. Taking into account that the concentration of 3β-D in rat pituitary gland 4-fold surpasses that in the blood [61] and that the hormone has relatively high affinity for estrogen receptors, we conclude that this androgen plays a specific role in the mechanisms of effects of sex steroids in HPC.

Our previous experiments demonstrated the possibility for the involvement of 3β-D in the regulatory processes in rat pituitary gland. It was shown that in the pituitary gland of male and female rats, 3β-D binds to a specific protein with a sedimentation coefficient of 4.5 S in linear sucrose density gradient and 2 binding sites [2,18]. Parameters of one binding site were similar to those reported by French researchers for 3β-D binding in the pituitary gland of male rats [64]. However, they reported also two different sedimentation coefficients for 3β-D-binding protein in the pituitary gland of male rats (3.0 and 4.5 S) [57,63]. It was also reported that binding of 3β-D in the pituitary gland of male rats varies during postnatal ontogeny [60].

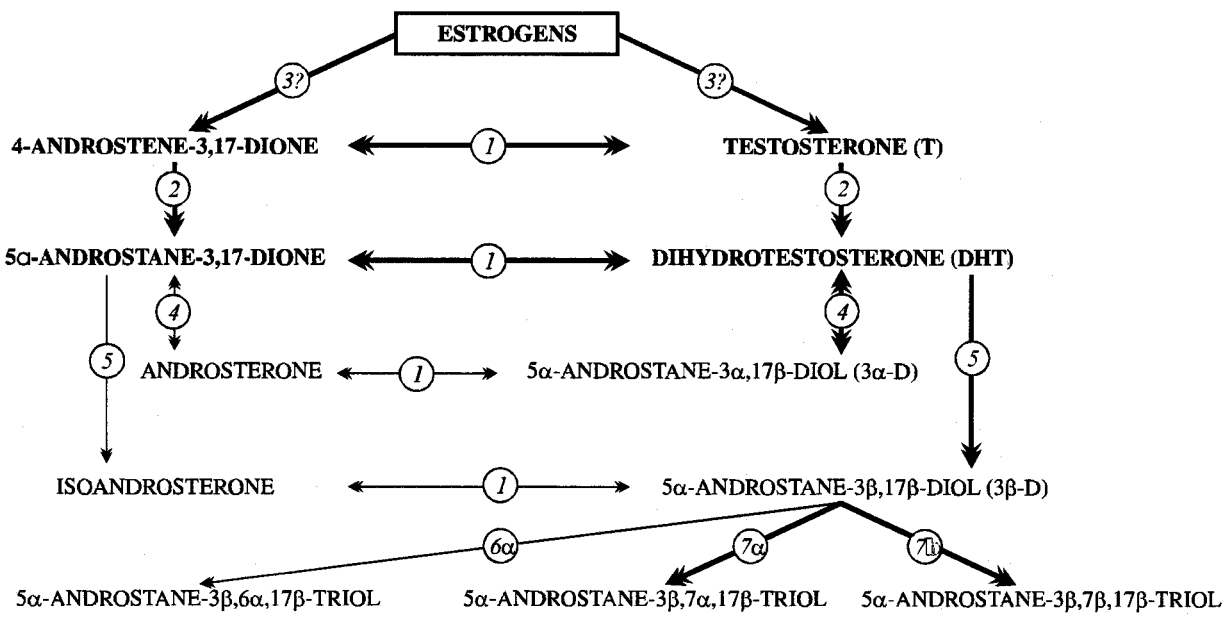

Fig. 1. Metabolism of androgens in animal and human target tissues (except the liver) [15]. Enzymes involved in the metabolism: 17β-hydroxysteroid oxidoreductase (17β-HSOR, *1*), 5α-reductase (*2*), aromatase (*3*), 3α-HSOR (*4*), 3β-HSOR (*5*), 6α-HSOR (6α), 7α-HSOR (7α), and 7β-HSOR (7β); enzymes of the cytochrome P-450 complex (6α, 7α, and 7β). Terms set in bold type refer to androgen metabolism in rat pituitary gland; ?: ambiguous data on the presence of aromatase in rat pituitary gland.

French researchers proposed that 4.5 S (3.0 S) protein binding 3β-D in the pituitary gland of male rats is a subunit of estrogen receptors [64]. However, our experiments showed that the affinity of this protein for E_2 and 3β-D differs from that of 8.5 S estrogen receptors in rat pituitary gland [2]. E_2 and 3β-D produce reciprocal effects on their specific and nonspecific binding in the cytosol of intact male and female rats [2]. These data suggest that 3β-D modulating specific and nonspecific binding of E_2 regulates its action in the pituitary gland of male and female rats and, therefore, is involved in the mechanisms of estrogen effects. Furthermore, 3β-D changes the bound-to-free E_2 ratio in the pituitary gland and indirectly modulates its hypothalamic content [6,32]. 3β-D can induce dissociation of estrogen receptors into subunits and their conformational changes altering their properties (affinity for E_2 or specificity of 3β-D binding, or both in the case of various binding sites on the protein molecule). Therefore, the reciprocal regulation of E_2 and 3β-D binding to the corresponding proteins is probably a mechanism of interaction between estrogens and androgens in rat HPC. It cannot be excluded that the role of 3β-D as a stimulator of sexual maturation of female rats [25,52] results from such interactions in rat HPC. At the same time, 3α-D inhibits sexual maturation of female rats [25,39,49,56].

Our findings and published data allow us to come up with speculations on the role of androgen metabolism and some metabolites, in particular 3α-D and 3β-D, in HPC as the central part of the regulation of reproductive function in rats. Sex-related differences in androgen metabolism in the hypothalamus and pituitary gland suggest that the ratio between androgen metabolites formed in rat HPC during prepuberty contributes to the formation of masculine or feminine regulatory mechanisms of gonadotropic functions. This is probably responsible for different effects of natural androgen metabolites on gonadotropin secretion in adult male and female rats. It cannot be excluded that sexual dimorphism in androgen metabolism in rat HPC results from different processes in HPC of males and females during sexual maturation. At the same time, it can be assumed that androgen metabolism in the pituitary gland plays an important physiological role, especially during prepuberty, and that the formation of 3α-D and 3β-D probably reflects neither catabolism of testosterone and DHT, nor the mechanism, by which pituitary cells affect (decrease, increase, or inactivate) the concentration of true androgen DHT (however, this possibility can not be excluded). Androgen metabolism results in the production of androgens, whose physiological activity during ontogeny (at least their effects on gonadotropic functions of the pituitary gland and hypothalamus) differs from that of testosterone and DHT. However, the mechanism of these effects remains unclear. The formation of DHT from 3α-D in rat hypothalamus and pituitary gland suggests that 3α-D is a store of DHT precursor (as in other target tissues) [11]. Obviously, it is important whether or not androgen metabolites act at the level of rat pituitary gland or hypothalamus, or they affect both these structures. Some authors assume that androgens exert their effects at the level of the pituitary gland [35,68], while others believe that these hormones affect the hypothalamus [28,29]. It was also reported that androgens regulate gonadotropic functions by acting simultaneously on the pituitary gland and hypothalamus [27,34]. In the hypothalamus, androgens produce their effects in lower concentrations than in the pituitary gland [38]. This probably explains the changes in activity of enzymes involved in androgen metabolism in the pituitary gland and hypothalamus during postnatal ontogeny. It should be emphasized that during some age periods, the activity of these enzymes in the hypothalamus is much lower than in the pituitary gland [4,19]. Furthermore, it is assumed that there are qualitative differences in the involvement of androgens in regulatory processes in the pituitary gland and hypothalamus. For example, specific binding of 3β-D was observed in the pituitary gland, but not in the hypothalamus [1,18,60].

3β-D acts on rat HPC by affecting estrogen receptors. Specific binding of 3β-D in rat pituitary gland and its conversion into some metabolites, but not into DHT (despite the presence of 3β-hydroxysteroid oxidoreductase), confirm physiological importance of this androgen in HPC. The metabolism of 3β-D in rat pituitary gland proceeds so that this androgen is rapidly inactivated in cells. A higher activity of the cytochrome P-450 system (compared to that of 3β-hydroxysteroid oxidoreductase) and subcellular localization of these enzyme complexes probably contribute to rapid inactivation of 3β-D in cells, because in homogenates of the pituitary gland of male and female rats 3β-D is transformed into DHT [3].

Recent data indicate that the biological importance of androgens in humans, rats, and other animals requires further studies [9,15,28,29,34,43]. It is clear that androgen metabolism in rat brain not only provides the possibility of regulating androgens-estrogens interrelations at the level of the formation of true sex hormones DHT and E_2, but also yields androgen metabolites, whose activity differs from that of testosterone and DHT and provides the conditions for modification of biological effects of testosterone.

It is difficult to explain or even propose possible mechanisms underlying the effects of androgen metabolites. We assume that all or most reactions associated with the specific effects of various natural andro-

gen metabolites are due to their involvement in allosteric regulation of some enzymes or receptors (not necessarily androgen and estrogen receptors). First, there is the possibility of regulating activities of the most important enzymes (with respect to mechanism of the effects of sex steroids) involved in androgen metabolism in the pituitary gland and hypothalamus, aromatase (Fig. 1, *3*) and 5α-reductase (Fig. 1, *2*). This mechanism allows to regulate the formation of true sex steroids in cells (E_2 and DHT). Second, some metabolites, including 3α-D, probably act as allosteric regulators and affect cell membranes [28] or GABA receptors in the hypothalamus [29]. Other mechanisms cannot be excluded.

Irrespective of the mechanisms underlying the regulatory effect of natural androgen metabolites on HPC, androgen metabolism is not only inactivation of DHT in cells, but also an extranuclear apparatus responsible for the regulation of cell response to true androgen DHT in the nucleus. We assume that metabolism of androgens in rat brain and in other target tissues is qualitative and quantitative modulation of the regulatory androgen signals (testosterone) in HPC cells. These changes are manifested in amplification of the androgen signal: 5α-reduction of testosterone to DHT by 5α-reductase, the key enzyme of androgen metabolism (Fig. 1, *2*) [15]. This assumption probably refers to all steroid-target cells.

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