BIOPHYSICS AND BIOCHEMISTRY

Formation of 5α-Androstane-3β,17β-Diol in the Rat Hypophysis

V. G. Degtyar', T. V. Babkina, and N. E. Kushlinskii

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Quantitative determination of 5α-androstane-3β,17β-diol (3β-D) during its synthesis from dihydrotestosterone in the hypophysis of intact and castrated rats *in vitro* can be performed only in homogenate. Synthesis of 3β-D is enhanced after castration in rats of both sexes. Rapid conversion of 3β-D into other metabolites in integral hypophysis *in vitro* is supposed to underlie nonadequate determination of 3β-hydroxysteroidoxide reductase in this gland.

Key Words: *rats; hypophysis; androgens; metabolism*

The metabolism of androgens in rat hypophysis, one of the most important targets for gonadal steroids, has been extensively studied [12]. Although it was shown that a number of metabolites can be synthesized in the rat hypophysis from the basic androgen testosterone [2,4,6,7,15], there is much controversy on some related questions. For example, it is unclear whether pituitary synthesis of 5α-androstane-3β,17β-diol (3β-D) is possible, the role of which in humans and animals is not strictly determined, although it is supposed to regulate the gonadotropic function in rats [1]. There are controversial data on activity of 3β-hydroxysteroidoxyde reductase (3β-HSOR) that participates in conversion of dihydrotestosterone (DHT) into 3β-D in the rat hypophysis. Some researchers detect no synthesis of 3β-D in rat hypophysis *in vitro* [2,7,8], while others claim that although synthesis of 3β-D in this gland is possible, the metabolite DHT is produced in negligible amounts [9,14]. Our aim was to find adequate conditions for quantitative estimation of 3β-D by its synthesis from DHT in the hypophysis of male and female rats prior to sexual maturity.

N. N. Blokhin Oncology Research Center, Russian Academy of Sciences, Moscow

MATERIALS AND METHODS

The study was carried out on Wistar rats. The animals were maintained on unrestricted food and water diet under controlled illumination (14 h light and 10 h darkness). At days 24-25, some rats were castrated under ether anesthesia; control rats were only anesthetized with ether. At day 7 postcastration, test and control rats were decapitated under a weak ether anesthesia. Hypophyses were extracted, washed in ice-cold physiological saline, and placed in ice-cold buffer.

Inorganic salts and hydrochloric acid were from Merck. α-D-Glucose, D-glucose-6-phosphate, $NADH_2$-, $NADPH_2$-, glucose-6-phosphate dehydrogenase, DHT, 3β-D, 5α-androstane-3α,17β-diol (3α-D), and Folin reagent were from Sigma. All organic solvents were of analytical grade. Specific radioactivity of 5α-dihydro[1,2,4,5,6,7-^{3}H]testosterone (Amersham) was 11 Ci/mmol. ^{3}H-DHT was purified as described previously [4]. For chromatographic separation of androgens, the preparations were applied on 15×15 cm Silufol slides (Kavalier) covered with 0.1 mm silicagel, and developed three times in a benzene:acetone:absolute ethanol system (9:1:0.5, v/v).

The total protein content was determined by the method of Lowry [13].

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Incubation of integral organs with substrate, separation, and qualitative estimation of androgens were described elsewhere [4]. The tracer was ^{3}H-DHT. The hypophyses were homogenized in sodium phosphate buffer (0.05 mmol/l, pH 7.4 at 20°C) by placing 7-9 organs into 750 μl ice-cold buffer in a Teflon-glass homogenizer and then making 10-12 up and down strokes of the pestle. The homogenate was centrifuged at 2500*g* for 20 min with cooling, and the supernatant was used for incubation. The tubes (12×70 mm) were filled with ^{3}H-DHT dissolved in benzene: absolute ethanol mixture (9:1, v/v) on the basis of 3-5×10^4 cpm in a sample, and with ethanol solution of DHT to adjust the concentration to 10^{-7} mol/liter. The solvent was evaporated to dryness under a nitrogen stream or in a hot water bath at no more than 60°C. The homogenate (50 μl) or an equal volume of buffer solution in the control samples was added to ice-cold tubes with the substrate together with 50 μl buffer solution containing α-D-glucose (100 μg), D-glucose-6-phosphate (100 μg), $NADH_2$ (25 μg), $NADPH_2$ (25 μg), and glucose-6-phosphate dehydrogenase (50 mU). The samples were incubated for 1 h in a thermostat at 37°C. After incubation, 25 μl hydrochloric acid (1 N) and 20 μl ethanol solution of DHT, 3β-D, and 3α-D (1 mg/ml of each ingredient) were added. After lyophilization, 100 μl absolute ethanol: benzene (9:1, v/v) mixture was added. Chromatography and radioactivity measurements were described previously [3]. The androgen spots were revealed with iodine vapor. The corresponding metabolite was quantitated in accordance with the total protein content, substrate concentration, and the ratio of radioactivity in the spots of metabolites in the sample. In some samples, the resulting ^{3}H-3α-D and ^{3}H-3β-D metabolites were identified by crystallization [4]. The tracer was ^{3}H-DHT added to each sample to attain activity of no less than 100,000 cpm.

The results were analyzed by Student's *t* test [5].

RESULTS

Testosterone has been generally used as a substrate in the *in vitro* investigations of androgen metabolism in rat hypophysis. In intact rats, the major metabolite in this case is DHT, although some other metabolites are also produced. However, to determine 3α-HSOR and 3β-HSOR activity, the use of DHT as a substrate seems more reasonable.

Table 1 shows that 3α-D was produced in greater amounts in the pituitary homogenates in comparison with integrate hypophyses of both control and castrated male and female rats. We could not detect 3β-D among the metabolites after incubation of DHT with integrate hypophyses of intact rats of both sexes. Only trace amounts of 3β-D were found after incubation of DHT with integrate hypophyses of castrated rats of both sexes (Table 1). At the same time, 3β-D was found after incubation of DHT with pituitary homogenates of intact and castrated rats of both sexes, although its content was smaller in comparison with other metabolites (Table 1).

In all cases, "polar" metabolites were produced from DHT in addition to 3α-D and 3β-D, and their content was comparable to that of 3α-D. It should be noted that more "polar" metabolites were revealed in the pituitary homogenates than in the case when DHT was incubated with integrate organs. Castration did not affect formation of these metabolites neither in integrate hypophyses, nor in homogenates in rats of both sexes, although sex-dependent differences were revealed in the pituitary homogenates of intact and castrated rats (Table 1).

The physiological role of androgen metabolism in the target cells of gonadal steroids is still unclear. In addition to DHT as a "truly" androgen hormone, formation of 4-androsten 3,17-dione from testosterone can be considered as exception, because it may serve as a substrate for aromataze.

Our findings indicate that 3β-D is formed in the hypophyses of prepubertal rats of both sexes, but its quantitative determination can be made only in the homogenates of these organs, although trace amounts of this androgen were found upon incubation of integrate hypophyses of the castrated rats (Table 1). Previously, we showed that 3β-D is not formed from DHT *in vitro* in the hypophysis and in the hypothalamus of intact rats of different ages [2,7].

Thus, a question arises whether 3β-HSOR demonstrates its dehydrogenase activity only upon incubation of integral organs *in vitro*. Our *in vitro* experiments show that only "polar" metabolites of 3β-D are formed but not DHT in integral hypophysis and hypothalamus of rats of different ages [2,7]. "Polar" metabolites that are formed from 3b-D in integrate rat hypophysis *in vitro*, are hydroxylated derivatives of 3β-D — 5α-androstane-3β,6ε (or 7ε), 17β-triol [11]. It can be suggested that these androgens ("polar" metabolites) are also formed in pituitary homogenates, because their mobility on chromatograms is similar to that of metabolites produced in integrate rat hypophysis.

The data obtained can be generalized in the following way. First, it is impossible to detect 3β-D among DHT metabolites in integrate hypophysis of intact rats *in vitro*. Second, there is 3β-HSOR in the rat hypophysis, and the content of 3β-D formed from DHT can be quantitatively determined provided pituitary homogenates of either intact or castrated rats of

TABLE 1. Metabolism of DHT in Integral Hypophyses and Pituitary Homogenates of Prepubertal Intact and Castrated Male and Female rats (*M±m*)

Animals and source of enzymes		Metabolites, pmol/mg protein/h		
		3α-D	3β-D	Polar
Intact males	integrate organs	14.5±2.1*	not detected	7.5±0.9
	homogenate	92.0±20.0	2.0±0.5*	77.0±20.0**
Castrated males	integrate organs	17.7±2.3*	<0.1	8.3±1.4
	homogenate	91.3±13.1***	4.8±0.9	82.7±14.3*
Intact females	integrate organs	22.3±3.7	not detected	7.6±1.3
	homogenate	126.8±18.4*	3.0±1.2**	26.0±10.3
Castrated males	inteqrate organs	28.4±2.9	<0.1	9.1±1.7
	homogenate	163.1±17.6	5.9±1.1	38.2±6.7

Note. *$p<0.05$, **$p<0.02$, ***$p<0.001$ relative to the corresponding index in females; *$p<0.05$, **$p<0.01$ relative to pituitary homogenate of castrated rats of the same sex.

both sexes are used for incubation. Third, by incubation of the integrate hypophyses of castrated animals, one can find only trace amounts of 3β-D. Fourth, even if 3β-HSOR exhibits its activity in rat hypophysis, it does so only in the form of reductase in respect to DHT.

The reason why 3β-D cannot be detected among metabolites by incubation of integral hypophyses of intact rats *in vitro* with DHT can be rapid and complete conversion of 3β-D into the abovementioned triols ("polar" metabolites) with the cytochrome P-450 complex in the cells of integrate hypophysis under the chosen experimental conditions [10]. Importantly, when the total amount of formed 3β-D and of "polar" metabolites as the product of 3β-HSOR activity is taken into account, the activity of the latter will be smaller, but comparable to that of 3α-HSOR in all the cases independent of whether or not 3β-D is determined among the reaction products (Table 1). This is probably the reason why the activity of 3β-HSOR in rat hypophysis is more adequately measured according to the sum of 3β-D and its daughter products, "polar metabolites": the absence of 3β-D among their metabolic products can lead to controversial results in determining the activity of 3β-HSOR in this gland, irrespective of the substrate-precursor used (testosterone or DHT) and the method of incubation.

We belive that the pituitary enzyme 3β-HSOR in rats of both sexes is a masked enzyme which participates in the reaction yielding 3β-D and which can be detected only under specific conditions. At present, it is difficult to explain the revealed phenomenon by the specific property of 3β-D as a substrate for the cytochrome P-450 complex or by some other reasons (for example, by "compartmentation" of 3β-HSOR and P-450 complex in the rat pituitary cells). Nevertheless, it can be suggested that conversion of 3β-D into triols ("polar" metabolites) is an important stage of androgen metabolism in the hypophysis, related presumably to "inactivation" of 3β-D. Probably, this phenomenon has physiological implications for the rats from the viewpoint of sexual differentiation of the hypophysis [1], because there are distinctions in the formation of the products of "inactivation" of 3β-D triols in males and females, at least in pituitary homogenates.

REFERENCES

1. V. G. Degtyar', *Usp. Sovr. Biol.*, **53**, 422-436 (1992).
2. V. G. Degtyar', L. A. Loseva, and V. A. Isatchenkov, *Ontogenez*, **13**, 62-70 (1982).
3. V. G. Degtyar', Yu. V. Miloserdov, and N. E. Kyshlinskii, *Bioorg. Khim.*, **9**, 1416-1421 (1983).
4. V. G. Degtyar', S. A. Pavlinov, L. A. Loseva, and V. A. Isachenkov, *Probl. Endokrinol.*, No. 4, 70-76 (1977).
5. K. A. Brownlee, *Statistical Theory and Methodology in Sciences and Engineering*, London (1965).
6. F. Celotti, R. C. Melcangi, and L. Martini, *Front. Neuroendocrinol.*, **13**, 163-215 (1992).
7. W. G. Degtiar, L. A. Loseva, and V. A. Isachenkov, *Endocrinol. Exp. (Bratisl.)*, **15**, 181-190 (1981).
8. C. Denef, *J. Steroid Biochem.*, **19**, 235-239 (1983).
9. D. Ducouret and Y. Voltaire, *Steroids*, **53**, 763-774 (1989).
10. B. Gemzik, S. Jacob, S. Jennigs, *et al.*, *Arch. Biochem. Biophys*, **296**, 374-383 (1992).
11. J.-M. Guiraud, S. Samperez, and P. Jouan, *Steroids*, **40**, 625-639 (1982).
12. R. B. Jaffe, *Ibid*, **14**, 483-498 (1969).
13. O. H. Lowry, N. J. Rosebrough, A. L. Farr, and K. J. Randall, *J. Biol. Chem.*, **193**, 265-275 (1951).
14. F. F. G. Rommerts and H. J. van der Molen, *Biochim. Biophys. Acta.*, **248**, 489-502 (1971).
15. S. Samperez and P. Joun, *J. Steroid Biochem.*, **11**, 819-831 (1979).