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INTERCONVERSION BETWEEN 17β-HYDROXY-5α-ANDROSTAN-3-ONE (5α-DIHYDROTESTOSTERONE) AND 5α-ANDROSTANE-3α,17β-DIOL: TISSUE SPECIFICITY AND ROLE OF THE MICROSOMAL NAD: 3α-HYDROXYSTEROID OXIDOREDUCTASE

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SUMMARY

The tissue specificity of the microsomal NAD: 3α-hydroxysteroid oxidoreductase has been investigated. A striking correlation is observed between the responsiveness of the investigated tissues to 3α-androstanediol on the one hand and the activity of the NAD: 3α-hydroxysteroid oxidoreductase as well as their ability to convert 3α-androstanediol into 5α-dihydrotestosterone on the other hand.

INTRODUCTION

Steroid metabolism in target organs may serve a dual role. On the one hand it can inactivate a hormone and limit its effect, on the other hand it can transform a circulating hormone in a biologically more active compound. The steroid 5α-reductase catalyzing the interconversion between the circulating androgen testosterone and 17β-hydroxy-5α-androstan-3-one (5α-dihydrotestosterone) serves as a protoype of an enzyme responsible for the formation of "active metabolites"[1]. The epithet "active metabolite", however, has been assigned to several other androgen metabolites and particularly to 5α-androstane-3α,17β-diol (3α-androstanediol) and 5α-androstane-3β,17β-diol[2]. The activity of 3α-androstanediol e.g. equals or surpasses that of testosterone in rat kidney [3, 4], exorbital lacrimal gland[5], prostate and seminal vesicles[6–9]. Since 3α-androstanediol itself binds poorly to androgen receptor proteins[10] and since this compound is converted into 5α-dihydrotestosterone *in vivo*[11] we considered the possibility that a particular ability to convert the 3α-diol into 5α-dihydrotestosterone might explain the high activity of this diol in some androgen target tissues.

Recently we described and partly identified three 3α-hydroxysteroid oxidoreductases able to interconvert 5α-dihydrotestosterone and 3α-androstanediol in rat kidney[12]. Only one of these enzymes—the microsomal NAD: 3α-hydroxysteroid oxidoreductase—displays favorable characteristics to catalyze the back-conversion between 3α-androstanediol and 5α-dihydrotestosterone. The present paper demonstrates a striking relationship between the presence and activity of this microsomal enzyme in different rat tissues and the ability of slices of the same tissues to use 3α-androstanediol as a precursor for the formation of 5α-dihydrotestosterone.

EXPERIMENTAL

Wistar rats were used in all experiments. 5α-[1,2-^{3}H]-Dihydrotestosterone (48 Ci/mmol) was purchased from New England Nuclear. 3α-[1,2-^{3}H]-Androstanediol was prepared enzymatically from the preceding compound[12]. Subcellular fractionation procedures, routine incubation conditions and chromatographic methods have been reported in detail[12]. For the selective measurement of the microsomal NAD:3α-hydroxysteroid oxidoreductase, advantage was taken from the fact that this enzyme requires NAD and has a remarkable stability at acidic pH. Measurements were performed in acetate buffer ($I = 0.05$) at pH 4.5 unless stated otherwise. The concentration of NADH was 0.64 mM. The incubation mixture contained 5.2 μM 5α-[1,2-^{3}H]-dihydrotestosterone. The final vol. was made up to 2 ml. The amount of microsomes added corresponded to 0.4 mg of original tissue (wet weight) for liver, 4 mg for exorbital lacrimal gland, 10 mg for kidney, 20 mg for prostate and 40 mg for submaxillary gland, seminal vesicles, lung and muscle. The reaction was allowed to proceed for 15 minutes at 37°. Extraction and thin layer chromatography were performed as described previously[12]. The zones corresponding to the origin, 3α-androstanediol and 5α-dihydrotestosterone were scraped off and transferred to counting vials. Ninety three (seminal vesicles) up to 100% (exorbital lacrimal gland, muscle) of the incubated radioactivity was recovered in these regions. Tissue slices weighing about 100 mg were incubated in 1 ml of Eagle's medium in the presence of 3α-[^{3}H]-androstanediol, 10 nM for 1 h at 37°. The medium was supplemented with 3% $NaHCO_3$ and was gassed with 95% O_2 and 5% CO_2. After incubation, tissue and medium were homogenized in an all glass homogenizer and extracted with chloroform–methanol (2:1, v/v). The

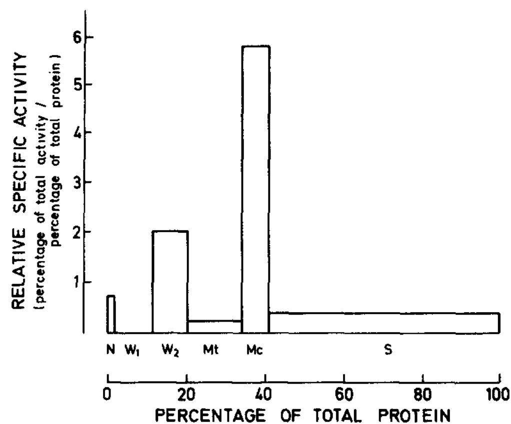

Fig. 1. Subcellular localization of NADH: 3α-hydroxysteroid oxidoreductase activity in rat prostate. Abbreviations: N, nuclei; W_1, 800 *g* nuclear washing fluid; W_2, discontinuous sucrose gradient overlaying the purified nuclei; M_t, mitochondria; M_c, microsomes; S, 108,000 *g* supernatant.

extract was washed twice with chloroform–methanol–water (3:48:47, by vol.). The aqueous phase of the extract and the successive washings were combined and quantitated for radioactivity (conjugated metabolites). The chloroform phase was evaporated to dryness and steroid metabolites were separated by t.l.c. and quantitated by liquid scintillation counting. Results are expressed as the percentage of the recovered radioactivity present in the combined watery extracts or the indicated regions of the chromatogram. The recovery of radioactivity approximated 100% for all tissues studied.

RESULTS

First we compared the subcellular localization and kinetic characteristics of the NADH:3α-hydroxysteroid oxidoreductase activity in rat prostate with that described in kidney. A typical cell fractionation experiment is shown in Fig. 1. It can be concluded from this figure that the highest activity in prostate is localized in the microsomal fraction exactly as in kidney[12]. Kinetic studies on prostate microsomes using 5α-dihydrotestosterone as substrate in an incubation mixture at pH 7.4 reveal an apparent K_M of 3.5 μM, a value that is again very close to that reported for the analogous kidney enzyme (2.8 μM). A similar enzyme—able to catalyze the conversion of 5α-dihydrotestosterone into 3α-androstanediol at pH 4.5—was detected in liver and exorbital lacrimal gland. In all other tissues investigated microsomal NADH-linked activity was low (Table 1).

Table 1. Microsomal NADH-3α-hydroxysteroid oxidoreductase activity in various rat tissues

Origin of microsomes	*n*	NADH:3α-hydroxysteroid oxidoreductase activity
		$\mu mol \times g\ protein^{-1} \times h^{-1}$
Male kidney	7	135 ± 8
Female kidney	9	53 ± 4
Prostate	4	156 ± 7
Seminal vesicles	5	3 ± 1
Submaxillary gland	5	8 ± 3
Exorbital lacrimal gland	5	137 ± 13
Lung	5	16 ± 5
Muscle	5	6 ± 4
Liver	5	1029 ± 49

Values represent the mean ± S.E. of the indicated number of determinations (*n*).

In a next series of experiments we compared the ability of slices from the same tissues to convert 3α-androstanediol into 5α-dihydrotestosterone or the corresponding 17-ketoderivative 3, 17-androstanedione. The results are summarized in Table 2. It can be concluded from these data that the ability to dehydrogenate the 3α-hydroxy function of 3α-androstanediol is considerably higher in tissues endowed with high microsomal NAD:3α-hydroxysteroid dehydrogenase activity (kidney, prostate, exorbital lacrimal gland) than in the other tissues. Only in the liver an apparent discrepancy is observed. In this tissue, however, the majority of reaction products was either conjugated or very polar and the exact nature of these metabolites was not further investigated.

DISCUSSION

The data presented stress the importance of target cell metabolism for the biological activity of

Table 2. Metabolism of [^{3}H]-3α-androstanediol by rat tissue slices

Tissue	Metabolites					
	Conjugated	Polar	3α-androstanediol	(epi) androsterone	5α-dihydro testosterone	3,17-androstane dione
	% ± SE					
Male kidney	9.3 ± 2.8	5.6 ± 0.7	23.5 ± 1.3	20.6 ± 1.9	25.3 ± 1.6	15.0 ± 1.2
Female kidney	7.6 ± 0.5	2.7 ± 0.3	16.7 ± 3.9	28.7 ± 2.9	16.0 ± 2.3	27.3 ± 3.1
Prostate	3.9 ± 0.6	5.4 ± 0.7	11.8 ± 2.4	3.8 ± 1.2	68.7 ± 2.3	5.1 ± 0.6
Seminal vesicles	4.0 ± 0.5	1.6 ± 0.3	74.0 ± 8.0	2.4 ± 0.9	17.1 ± 6.8	0.5 ± 0.5
Submaxillary gland	3.3 ± 0.4	1.6 ± 0.1	57.7 ± 1.7	18.3 ± 1.4	16.1 ± 0.9	2.3 ± 9.6
Exorbital lacrimal gland	4.1 ± 1.0	3.2 ± 0.3	28.1 ± 3.6	8.1 ± 0.7	47.9 ± 6.4	8.0 ± 2.1
Lung	3.9 ± 0.6	5.8 ± 0.5	84.1 ± 1.2	1.5 ± 0.0	4.0 ± 0.3	0.1 ± 0.0
Muscle	2.4 ± 0.2	0.8 ± 0.0	94.0 ± 0.2	0.7 ± 0.1	1.6 ± 0.1	0.1 ± 0.0
Liver	31.0 ± 5.1	55.2 ± 4.4	7.2 ± 0.7	3.9 ± 1.0	1.8 ± 0.5	0.5 ± 0.1

Values represent the mean ± S.E. of 4 determinations.

3α-androstanediol. The latter compound is an active androgen in the rat[4–9], the mouse[13] and the dog[14]. This high biological activity contrasts sharply with the low affinity of 3α-androstanediol for the classical dihydrotestosterone receptor protein[10]. Since no specific 3α-androstanediol receptor has been detected up to now the possibility has to be considered that conversion into 5α-dihydrotestosterone accounts for the high activity of 3α-androstanediol. Several authors demonstrated that after *in vivo* administration of 3α-androstanediol 5α-dihydrotestosterone is the major metabolite in various androgen target tissues[11, 15, 16]. Our tissue slice experiments support the contention that this conversion takes place in the target tissues themselves. Several 3α-hydroxysteroid oxidoreductases can be identified in androgen target tissues[12]: a soluble NADPH-dependent oxidoreductase present in all tissues investigated, a microsomal NADPH-dependent oxidoreductase present in liver and male rat kidney and a microsomal NADH-dependent oxidoreductase. The relative concentration of 5α-dihydrotestosterone and 3α-androstanediol in the steady state will be the result not only of the presence and relative activity of all these oxidoreductases but also of the concentration of the appropriate cofactors. *In vitro* studies of the kinetic characteristics of three 3α-hydroxysteroid dehydrogenases in rat kidney suggest that only one of these enzymes—the microsomal NAD:3α-hydroxysteroid oxidoreductase—has favorable kinetic characteristics and cofactor requirement to catalyse the dehydrogenation of 3α-androstanediol. The striking correlation between the ability of a series of tissues to convert the 3α-diol into 5α-dihydrotestosterone and the activity of the microsomal-NAD-linked dehydrogenase in the same tissues adds further support to the contention that it is the latter enzyme that enables these tissues to use 3α-androstanediol as a precursor for the formation of 5α-dihydrotestosterone.

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REFERENCES

1. Wilson J. D. and Gloyna R. E.: *Recent Prog. Horm. Res.* **26** (1970) 309–336.
2. Robel P., Lasmitzki I. and Baulieu E. E.: *Biochimie* **53** (1971) 81–96.
3. Grossman S. H., Kimm K. H. and Axelrod B.: *Life Sci.* **14** (1974) 567–573.
4. Verhoeven G.: *A Comparative Study of the Androgen Receptor Apparatus in Rodents.* Acco, Leuven (1974) pp. 137–154.
5. Cavallero C. and Ofner P.: *Acta endocr., Copenh.* **55** (1967) 131–135.
6. Dorfman R. I. and Dorfman A. S.: *Acta endocr., Copenh.* Suppl. **42** (1963) 245–253.
7. Cekan Z. and Pelc B.: *Steroids* **8** (1966) 209–218.
8. Moore R. J. and Wilson J. D.: *Endocrinology* **93** (1973) 581–592.
9. Schmidt H., Giba-Tziampiri O., Rotteck G. and Voigt K. D.: *Acta endocr.* Suppl. **73** (1973) 699–611.
10. Verhoeven G., Heyns W. and De Moor P.: *Steroids* **26** (1975) 149–167.
11. Bruchovsky N.: *Endocrinology* **89** (1971) 1212–1222.
12. Verhoeven G., Heyns W. and De Moor P.: *Eur. J. Biochem.* **65** (1976) 565–576.
13. Ohno S., Dofuku R. and Tetternborn U.: *Clin. Genet.* **2** (1971) 128–140.
14. Walsh P. C. and Wilson J. D.: *J. clin. Invest.* **57** (1976) 1093–1097.
15. Bardin C. W., Bullock L. P., Sherins R. J., Moswzowickz T. and Blackburn W. R.: *Recent Prog. Horm. Res.* **29** (1973) 65–109.
16. Becker H., Grabosch E., Hoffmann C. and Voigt K. D.: *Acta endocr., Copenh.* **73** (1973) 407–416.