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A NEW DIHYDROTESTOSTERONE-FORMING INDEX (DHT_i)

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Summary—A new index (DHT_i) for the net formation of dihydrotestosterone (DHT) in a specific tissue is presented. This index is based on the main metabolic pathways forming DHT as well as on the main enzymatic activities removing DHT from the tissue. In the rat prostate, the DHT_i is different in the various prostatic lobes. The index is highest in the ventral prostatic lobe, intermediate in the dorsal prostate and coagulating gland, and very low or undetectable in the seminal vesicles and the lateral prostatic lobe. With increasing age of the rats, the DHT_i decreased. Testosterone treatment to old rats leads to an increased index.

INTRODUCTION

Androgens play an essential role in the pathogenesis of prostatic neoplasia [1]. A local accumulation of dihydrotestosterone is a prerequisite for the development of both benign hyperplasia and prostatic cancer [2, 3, 4].

Rats are often used as a model for prostatic cancer as this malignancy may occur spontaneously in senescent rats [5, 6, 7] and may also be induced by sex-hormones [5] or specific carcinogenic agents [8]. The rodent accessory sex organs are composed of structural different prostatic lobes, the seminal vesicles and the coagulating gland which is considered to be a lobe of the rat prostate. In the rat, spontaneous prostatic cancer usually originates from the ventral prostatic lobe [5, 7] and less frequently from the dorsal prostatic lobe [6, 8]. There is only one single report on cancer of the rat coagulating gland [9]. To our knowledge, cancer of the rat lateral prostate or the seminal vesicles has not been described. Benign hyperplasia of the rat prostate is apparently confined to the ventral lobe [7, 10].

As both benign and malignant neoplasia of the rat prostate tend to be restricted to specific lobes, it may be of interest to determine the DHT forming capacity of various anatomic regions of the rat prostate.

EXPERIMENTAL

In previous studies we have measured the activities of most androgen metabolizing enzymes in the different lobes of the rat prostate and seminal vesicles [11, 12]. The conditions of tissue preparation, incubations and assays of androgen metabolites have been described in detail [11, 12, 13], and only a brief review will be presented here. The different tissues were homogenized in Tris–HCl buffer (pH 7.4 at 37°C), and the enzymatic studies were done on the 800 *g* supernatant fraction. The radiolabelled steroids used as substrates were dissolved in Tris–HCl buffer together with one of the cofactors: NAD, NADP, NADH or a NADPH-generating system: 10 μl NADP (2.5 mM in Tris–HCl), 20 μl glucose-6-phosphate (12.5 mM in Tris–HCl) and 10 μl glucose-6-phosphate dehydrogenase (250 μg/ml in Tris–HCl). Incubations were started by the addition of tissue homogenate, and terminated by adding ethylacetate which contained several reference steroids [13]. Separation of steroids were done by TLC [11] or HPLC [14]. V_{max} of the different enzymes was calculated in pmol/min/mg protein by Lineweaver–Burk plots.

The enzymatic activities which are producing DHT are as follows: 5α-reductase (substrate testosterone), 3α-HSOR OX (substrate 3α-A'diol), 3β-HSOR OX (substrate 3β-A'diol), and 17β-HSOR (substrate androstanedione), whereas those removing DHT are (substrate DHT): 3α-HSOR RED, 3β-HSOR RED and 17β-HSOR OX.

Based on our previously published data [11, 12] an index which can be used to compare different tissues with regard to their DHT-forming capacity may be

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Trivial names and abbreviations used: Androstenedione: 4-androstene-3,17-dione; Testosterone: 17β-hydroxy-4-androsten-3-one; Androstanedione: 5α-androstane-3,17-dione; Dihydrotestosterone (DHT): 17β-hydroxy-5α-androstan-3-one; 3α-A'diol: 5α-androstan-3α,17β-diol; 3β-A'diol: 5α-androstane-3β,17β-diol; Androsterone: 3α-hydroxy-5α-androstan-17-one; Epiandrosterone: 3β-hydroxy-5α-androstan-17-one; 17β-Hydroxysteroid oxidoreductase: 17β-HSOR; 3α(3β)-Hydroxysteroid oxidoreductase: 3α(3β)-HSOR; NADPH: Refers in this study to a NADPH-generating system (see Experimental).

Table 1.

Tissue	Enzyme substrate cofactor		Enzymes forming DHT: 5α-Reductase Testosterone NADPH	Enzymes forming DHT: 3α-HSOR OX 3α-A'diol NAD	Enzymes forming DHT: 3β-HSOR OX 3β-A'diol NAD/NADP	Enzymes removing DHT: 3α-HSOR RED DHT NADH	Enzymes removing DHT: 3β-HSOR RED DHT NADH/NADPH	Enzymes removing DHT: 17β-HSOR OX DHT NAD/NADP
		3–6	2.1 ± 0.6	98 ± 5	0	11.0 ± 1.5	0	0
VP	Age	20–22	1.0 ± 0.7	88 ± 16	0	40 ± 0	0	0
	in	28–30	0	16 ± 8	0	15.2 ± 2.7	0	0
	months	30–32*	0.6 ± 0.1	45 ± 32	0	17.3 ± 0.9	0	0
	Cofactor		NADPH	NADP	NADP	NADPH	NADPH	NAD
		3–6	9.3 ± 1.3	7.0 ± 0.8	1.5 ± 0.7	20.3 ± 3.3	20.0 ± 0	2.0 ± 0.5
DP	Age	20–22	1.5 ± 0.7	10.0 ± 0	0.5 ± 0.1	15.0 ± 2.4	5.8 ± 2.4	0
	in	28–30	0.4 ± 0.1	3.8 ± 1.8	0.1 ± 0	16.8 ± 0.2	2.9 ± 0.1	0
	months	30–32*	1.4 ± 0.4	5.75 ± 0.2	0.5 ± 0.3	46 ± 15	6.7 ± 4.6	0
	Cofactor		NADPH	NADP	NAD/NADP	NADPH	NADPH	NADP
		3–6	0	26 ± 5	0	221 ± 25	16.7 ± 5.2	11.0 ± 2.6
LP	Age	20–22	0	10.6 ± 0.8	0	96 ± 6	12.2 ± 3.0	1.6 ± 0.2
	in	28–30	0	12.0 ± 2.1	0	56 ± 4	2.6 ± 1.4	1.1 ± 0.3
	months	30–32*	0	13.6 ± 5.9	0	106 ± 20	7.1 ± 2.3	3.5 ± 0.3
	Cofactor		NADPH	NADP	NAD/NADP	NADPH	NADPH	NAD/NADP
		3–6	1.1 ± 0.1	0.4 ± 0.3	0	6.3 ± 2.2	2.5 ± 1.4	0
SV	Age	20–22	0.5 ± 0.4	0.4 ± 0.1	0	5.9 ± 1.2	0.3 ± 0.2	0
	in	28–30	0	0.4 ± 0.2	0	3.3 ± 2.5	0.3 ± 0.2	0
	months	30–32*	0.05 ± 0	1.0 ± 0	0	6.9 ± 5.9	0.7 ± 0.2	0
	Cofactor		NADPH	NADP	NAD	NADPH	NADPH	NAD/NADP
		3–6	9.4 ± 1.3	2.1 ± 0.3	0.2 ± 0.1	6.3 ± 1.3	1.9 ± 0.3	0
CG	Age	20–22	2.2 ± 1.6	0.2 ± 0.1	0.2 ± 0.1	5.9 ± 1.2	1.5 ± 0.7	0
	in	28–30	1.7 ± 1.0	1.5 ± 0.2	0.1 ± 0	3.1 ± 0.2	0.3 ± 0.2	0
	months	30–32*	2.65 ± 0.4	2.0 ± 0.3	1.1 ± 0.4	3.6 ± 0.3	0.9 ± 0.2	0

Enzymatic activities in incubations of homogenates from different organs in intact young rats (3–6 months old), and in intact middle-aged and old rats (20–30 months old). Rats 30–32 months of age treated with testosterone proprionate (1 mg/kg b.wt/daily) for 10 days before experiments are indicated by an asterisk (*). The tissues were homogenated in Tris–HCl (pH 7.4 at 37°C) and the 800 *g* supernatant fraction was incubated in 15–45 min at 37°C with cofactors and substrates as indicated in the figures. The metabolites were separated by HPLC or TLC. All values are given as V_{max} in pmol/min/mg protein calculated from incubations with different amounts (0.7–8.6 μM) of the substrate.

VP: ventral prostate. DP: dorsal prostate. LP: lateral prostate. SV: seminal vesicle. CG: coagulating gland. NADPH: refers in this study to a NADPH-generating system (see Experimental). 3α/3β-HSOR OX/RED: the oxidative, respectively, the reductive activity of 3α/3β-hydroxysteroid oxidoreductase 17β-HSOR OX: the oxidative activity of 17β-hydroxysteroid oxidoreductase.

calculated from the formula:

$$DHT_i = 5\alpha\text{-Reductase} \times \frac{(3\alpha\text{-HSOR OX} + 3\beta\text{-HSOR OX} + 17\beta\text{-HSOR RED})}{(3\alpha\text{-HSOR RED} + 3\beta\text{-HSOR RED} + 17\beta\text{-HSOR OX})}$$

In this formula, 5α-reductase is a multiplying factor, while the activities of the enzymes added in the numenator of the fraction tend to increase the DHT concentration and the index, and those in the denumenator will lower it.

We have not measured the activity of 17β-HSOR RED using androstanedione as substrate. The 5α-reductase activity is, however, very low compared to the corresponding activity measured with testosterone as substrate [11, 12]. The contribution to the formation of DHT via this route is therefore presumably negligible.

RESULTS

The main enzymatic activities which are involved in the regulation of DHT in the various prostatic lobes and the seminal vesicles are shown in Table 1.

These tissues displayed differences with regard to the cofactor required to obtain maximum enzymatic activity, except the 5α-reductase which could only be measured using NADPH.

The values presented in Table 1 are only those activities measured with the cofactor giving the highest enzymatic activity, based on the assumption that sufficient preferred cofactor is available in the tissues. However, adding the activities obtained for the two cofactors used, both in the oxidative and the reductive enzymatic reactions, approximately the same values for DHT_i were obtained (data not shown).

All activities in Table 1 are calculated from a minimum of 4 different incubation experiments, and are given as mean ± SD.

Calculation of our DHT-forming index based on the mean enzymatic activities presented in Table 1 gives the values of DHT_i presented in Table 2.

DISCUSSION

The DHT_i was highest in the ventral prostate in the young and untreated old rats, mainly due to a very high oxidative 3α-hydroxysteroid oxidoreductase in this lobe. The dorsal prostate and the coagulating gland showed similarities in the various enzymes which balance the DHT-concentration, except that testosterone treatment led to a higher rise in the DHT-producing enzymes in the coagulating gland. In the lateral prostate, the lack of 5α-reductase activity using our assay gave a zero value of DHT_i. In addition, the activities which removed DHT were 10-fold higher than those which produce DHT, indicating a tendency to expel DHT from the lateral prostate. Similar to the lateral prostate, the DHT_i in the seminal vesicle was very low in all age groups.

Pathological processes of the prostate involve changes in enzymatic systems, including androgen metabolizing enzymes. An index introduced by Isaacs *et al.*[15] may be used to predict hormone sensitivity of rat prostatic cancer, based on different enzymatic changes in the tumor.

For benign prostatic hyperplasia, an enzymatic index has also been proposed [2]. This index is made by adding the enzymatic activities which are forming DHT and substracting those removing DHT from the tissues, and shows that in this tissue the enzymes favouring the production of DHT dominate. This is consistent with previous reports that DHT is the major intraprostatic androgen metabolite in human prostatic hyperplasia [3, 4].

In the presented formula for DHT-forming capacity, 5α-reductase activity is a multiplying factor as the 5α-reduction of testosterone or androstenedione is a mandatory and irreversible step in the formation of DHT. Hence, an undetectable level of 5α-reductase will give a zero value of the index and the DHT-concentration in a tissue. This may not be obtained in a formula adding, respectively, subtracting all the enzymatic activities which form and remove DHT in a tissue [2].

In prostatic tissue, androgens are rapidly converted to a variety of metabolites whereas the enzymes involved in these transformations have a much slower turnover rate. Scanty information is available of how definite DHT-concentrations measured in a tissue is influenced by operative procedures and tissue processing. Further studies are necessary to clarify the problem whether steady state *in vivo* concentration of DHT is best expressed by direct measurement or indirect by calculating the enzymes which form and remove DHT in a tissue.

From other studies [1, 5–9], the frequency of androgen dependent diseases in the various regions of

Table 2.

Organ	DHT-forming index (DHT_i). VP	DP	LP	SV	CG
Young rats (3–6 months old)	18.7	1.9	0	0.05	2.6
Old rats (20–22 months old)	2.2	0.8	0	0.03	0.5
Old rats (28–30 months old)	0	0.1	0	0	0.8
Old rats (30–32 months old, testosterone-treated	1.6	0.2	0	0.01	1.8

DHT_i in rat ventral (VP), dorsal (DP) and lateral prostate (LP), seminal vesicle (SV) and coagulating gland (CG). Conditions, see legend to Table 1.

the rat prostate seems to correlate with the presented values of DHT_i. We have also calculated the DHT_i in old rats and observed that testosterone treatment leads to an increase in the index in these animals.

The DHT_i may also represent a tool to examine the influence of other specific compounds, e.g. antineoplastic drugs, on the DHT-forming capacity. This index might also be of value in a systematic comparative study on normal and pathological tissues, including preneoplastic changes.

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