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SHORT COMMUNICATION

CONTENT OF 5α-REDUCED ANDROGENS IN SUBCELLULAR COMPARTMENTS OF DOG PROSTATE

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SUMMARY

The distribution of 5α-dihydrotestosterone (DHT), and 3α-androstanediol (3α-diol), 17β-hydroxy-androgens, and 5α-androstanedione, and androsterone, 17-ketoandrogens was determined by measuring their content by radioimmunoassay in subcellular components of prostate from normal adult dogs. DHT followed by 3α-diol was the most abundant 5α-reduced androgen measured in dog prostate. DHT appears to concentrate particularly in the nucleus whereas the other androgens measured do not show such a selective localization.

Evidence suggests that normal growth of the prostate of man and the dog is stimulated by a 5α-reduced androgen metabolite(s) of testosterone or other pre-hormones [1–5]. The presumption has been that DHT is the principal active androgen in promoting growth of the prostate in man and dog and that testosterone is the principal androgen in some tissues such as muscle [3–8]. Hyperplasia is common with aging in both species [1]. It is, however, uncertain which androgen (or androgens) produces normal and abnormal growth of the prostate of man or the dog.

In 1962, Harding and Samuels[6] were the first to carefully investigate the subcellular distribution of androgens in the rat prostate following administration of radiolabeled steroid. The current study is the first attempt to our knowledge to determine the content of 5α-reduced androgens in the subcellular compartments of dog prostate.

Prostates (8.0 ± 1.1 SEM, g, wet weight) were excised from ten adult mongrel dogs immediately after death and prepared as described previously [8]. Two-4 g of tissue were homogenized and the nuclear, mitochondrial, microsomal and cytosolic fractions were isolated as described by Fang, Anderson and Liao[7]. An aliquot of the nuclear fraction was used for assay of DNA [9] and then each fraction was extracted twice with 20 ml of Desal's solvent (methanol–dimethoxymethane, 4:1, V/V) once for one hour and then overnight [8]. In order to remove excess lipid with a minimal loss of steroids, 15 ml of petroleum ether were added to the dried extract and then extracted 4 times with 4 ml of 70% methanol. After evaporation of the methanol, the volume was adjusted to 2 ml with water. The water was extracted twice with 15 ml of dichloromethane and chromatographed as reported previously [8]. The mitochondrial, microsomal and cytosolic fractions were extracted twice with dichloromethane after addition and mixing of tritiated steroid indicator and chromatographed with the Bush A solvent system. All values were corrected for procedural losses. DHT, 3α-diol and androsterone were assayed [8]. Antisera to 5α-androstanedione were prepared by injecting rabbits with androstenedione-17-hemisuccinate conjugated to bovine serum albumin [8]. The sensitivity of the assays was less than 10 pg.

The antisera were highly specific and blanks processed through the entire procedure were less than 5 pg/tube for each steroid. Mean values for recoveries ($n = 21$) for tritiated steroids through all isolation procedures varied from 40 ± 10%, mean SE to 74 ± 6%. The average accuracy for the 1–2 ng of the four steroids added to water or tissue ($n = 12$) and processed as described for the tissue samples was 94% for androsterone, 96% for 3α-diol, 102% for DHT, and 120% for 5α-androstanedione. The mean interassay coefficient of variation for the tissue samples ranged from 9 to 14% ($n = 12$).

The total concentration of DHT in the four fractions and its level in the nuclear compartment per mg of DNA were both significantly higher than the other three androgens (Table 1). The concentration of DHT per g wet weight of tissue in the crude nuclear component was significantly ($P < 0.005$, 0.005 and 0.05, respectively) higher than in the mitochondrial, microsomal and cytosolic fractions (Table 2). When compared to 5α-androstanedione and androsterone, DHT was also significantly higher in all four fractions. Its content also significantly exceeded that of its 3α-hydroxy metabolite, 3α-diol, in the nuclear and cytosol components (Table 2). 5α-Androstanedione did not exceed significantly the concentration of its 3α-hydroxy metabolite, androsterone, in any fraction. 3α-Diol was not significantly different in the nuclear, microsomal and cytosolic fractions, but the nuclear fraction was elevated when compared to the mitochondrial components ($P < 0.05$).

In skeletal muscle, the concentration of DHT was 0.2 ng/g of wet weight of tissue and it was 0.07 ng/g of tissue in cytosol and less in other fractions. 3α-Diol, androsterone and 5α-androstanedione were undetectable in muscle.

The radioimmunoassays developed for the measurement of the content of steroids in subcellular compartments isolated by conventional techniques are sensitive, specific, accurate and reproducible. The mean total concentration of DHT in all fractions was in good agreement with total values observed by other investigators [2, 10].

The total content of the 17-ketoandrogens, 5α-andros-

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The abbreviations and trivial names used are: 5α-androstanedione; 5α-androstan-3,17-dione; 3α-diol; 3α-androstanediol; 3α,17β-dihydroxy-5α-androstane; androsterone; 3α-hydroxy-5α-androstane-17-one:DHT; 17β-hydroxy-5α-androstane-3-one.

Table 1. Total ng of steroid of the four subcellular compartments/g

	Wet weight of prostate			
	3α-Diol	DHT	Androsterone	5α-Androstanedione
Mean ± SEM	0.95 ± 0.32	2.4 ± 0.29	0.18 ± 0.06	0.24 ± 0.06
Paired *t*-test vs DHT	<0.005	—	<0.001	<0.001
	ng Steroid in Nuclear Fraction/mg DNA			
	3α-Diol	DHT	Androsterone	5α-Androstanedione
Mean ± SEM	0.26 ± 0.09	1.47 ± 0.30	0.22 ± 0.14	0.14 ± 0.04
Paired *t*-test vs DHT	<0.001	—	<0.001	<0.001

Table 2. ng steroid in subcellular fractions/g wet weight of prostate

	3α-Diol	DHT	Androsterone	5α-Androstanedione
		Nuclear		
Mean ± SEM	0.32 ± 0.13	1.1 ± 0.13	0.09 ± 0.04	0.15 ± 0.03
Paired *t*-test vs DHT	<0.005	—	<0.005	<0.001
		Mitochondrial		
Mean ± SEM	0.06 ± 0.019	0.27 ± 0.10	0.017 ± 0.01	0.033 ± 0.015
Paired *t*-test vs DHT	<0.1	—	<0.05	<0.05
		Microsomal		
Mean ± SEM	0.12 ± 0.03	0.46 ± 0.10	0.07 ± 0.03	0.05 ± 0.03
Paired *t*-test vs DHT	<0.1	—	<0.01	<0.01
		Cytosolic		
Mean ± SEM	0.28 ± 0.06	0.78 ± 0.09	0.12 ± 0.09	0.06 ± 0.03
Paired *t*-test vs DHT	<0.05	—	<0.005	<0.005

tanedione, and androsterone were substantially lower than the 17β-hydroxymetabolites, DHT and 3α-diol, in all four subcellular fractions. This was unexpected. The relative plasma level of a major 17-ketoandrogen and precursor, androstenedione is known to be only slightly lower than a major 17β-hydroxy precursor, testosterone [11].

The factors that regulate sex-steroid action are most complex. Considerable information has been obtained about major pathways of androgen metabolism and about binding proteins in the prostate of mammals [1, 2, 7, 12–14]. It has not been possible to predict, however, the cellular or subcellular content of androgens. The content of a steroid in a subcellular compartment appears to have relevance to its biologic effect. The apparent selective localization of DHT in the nucleus as compared to the other androgens measured supports the concept that it may exert critical action on growth of the gland in dogs. The measurement of sex-steroids in the subcellular components appears to be a valuable new technique which may provide additional insight into the physiology and pathophysiology of steroids in the prostate.

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