Vol. 108, No. 1, 1982 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
September 16, 1982 Pages 89-94

ELEVATED NUCLEAR ANDROGEN RECEPTORS IN NUCLEASE DIGESTS OF HYPERPLASTIC PROSTATIC TISSUE OF AGING DOGS

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Received July 23, 1982

Androgen receptors were measured in the cytosol, whole nuclei, nuclear matrix and in the nuclease (DNAase I) solubilized fraction of nuclei from normal and hyperplastic prostates of aging Beagle dogs. Androgen-receptor content was significantly elevated in whole nuclei and particularly in the nuclease solubilized fraction of nuclei from hyperplastic glands of aging dogs. The nuclear matrix of hyperplastic tissue had marginally elevated levels of the androgen receptor, and values in the cytosol were not abnormal. This is the first documentation suggesting that a selective defect in nuclear chromatin androgen-receptor content might be critical in the development of benign prostatic hyperplasia with age.

Introduction

Benign prostatic hyperplasia is a common neoplasia of both aging dogs and men (1). Whole tissue content of 5α-dihydrotestosterone (DHT) is elevated in naturally occurring prostatic hyperplasia of both humans (2-3) and dogs (5-7) and also in androgen-induced prostatic hyperplasia of dogs (6). We observed recently that the nuclear content of DHT was supranormal in hyperplastic prostatic tissue of both species (4,7). In this investigation, we report for the first time that nuclear-androgen receptor solubilized from chromatin by nuclease I is elevated in hyperplastic prostate of aging Beagle dogs. This observation supports the postulate that the nuclear androgen receptor may have a critical role in the pathogenesis of benign prostatic hyperplasia.

Abbreviations

DHT = 5α-dihydrotestosterone, DNAase I = deoxyribonuclease.

BPH = benign prostatic hyperplasia

0006-291X/82/170089-06$01.00/0

Materials and Methods

Prostates were excised from adult male Beagle dogs and kept on ice. All procedures during the preparation of the tissue and binding assays were performed at 0°-4° C. The urethra and capsule were removed. Normal glands (9.6 ± 2.4 g wet weight, mean ± SD, n=7) and hyperplastic glands (21.4 ± 3.5 g wet weight, mean ± SD, n=10) were assessed by the criteria of Berg (8).

Minced prostate was homogenized in 5 vol Buffer A (10 mM TRIS-HCL, .25 M sucrose, 1 mM $MgCl_2$, pH 7.4) for 1.5 min with a Tekmar SDT and a glass/glass tissue grinder using a motorized unit. The homogenate was filtered through two layers of gauze and centrifuged at 900 xg for 10 minutes. The supernatant was adjusted to 10 mM Na_2MoO4 and recentrifuged at 104,000 xg for 60 min to yield the cytosol fraction.

All buffers used in purification of nuclei contained 1 mM pheynlmethyl sulfonyl fluoride (Sigma). After the initial spin, the nuclei were washed twice with Buffer A, layered over 2.2 M sucrose in Buffer A, and centrifuged at 40,000 xg for 60 min in a Beckman SW 25.2 rotor. The purified nuclei were washed twice in Buffer A, treated with Triton-x-100 and washed again.

The nuclear matrix was prepared by treatment of purified nuclei with pancreatic DNAase I, low salt and then high salt buffer systems (9). This removed greater than 90% of DNA and protein. The DNAase I fraction consisted of the combined washes of DNAase I treated nuclei. The reaction mixture contained purified nuclei in Buffer A, 500 units DNAase I/mg DNA/ml, and incubated for 20 min at 4°C. The reaction was stopped by the addition of 10 mM EDTA. Nuclei were centrifuged, washed once, and the supernatants combined and saved.

Binding assays were done in triplicate on all fractions. Aliquots were incubated in total volume of 0.5 ml in Buffer B (Buffer A plus 1 mM dithiothreitol), 10 nM ^{3}H-methyltrienolone (New England Nuclear Corp., 50-80 Ci/mmole) with and without 100 fold excess dihydrotestosterone and incubated at 4°C for 20-24 hrs. Hydroxyl appetite (250 µl of 70% slurry in Buffer A) was added to each soluble fraction. The precipitates in all tubes were washed three times with Tris EDTA buffer and extracted with ethanol. After counting, receptor binding was calculated by the method of Chamness and McGuire (10).

DNA was determined using calf thymus DNA as a standard with the method of Burton (11). Protein was measured by the method of Lowry using bovine serum albumin as a standard (12).

Results

Androgen receptor content in whole nuclei was elevated in BPH tissue when values were normalized for DNA content but not when adjusted for protein levels (Table 1).

Androgen receptor content was significantly higher in nuclease digests of nuclei from hyperplastic prostatic tissue than from normal glands (Fig. 1) This abnormality was observed when the receptor levels were normalized for DNA or protein content of the nuclei.

The nuclear matrix contained approximately 25 to 50% of the total androg receptor content (Table 1). Androgen receptor values were similar in BPH and

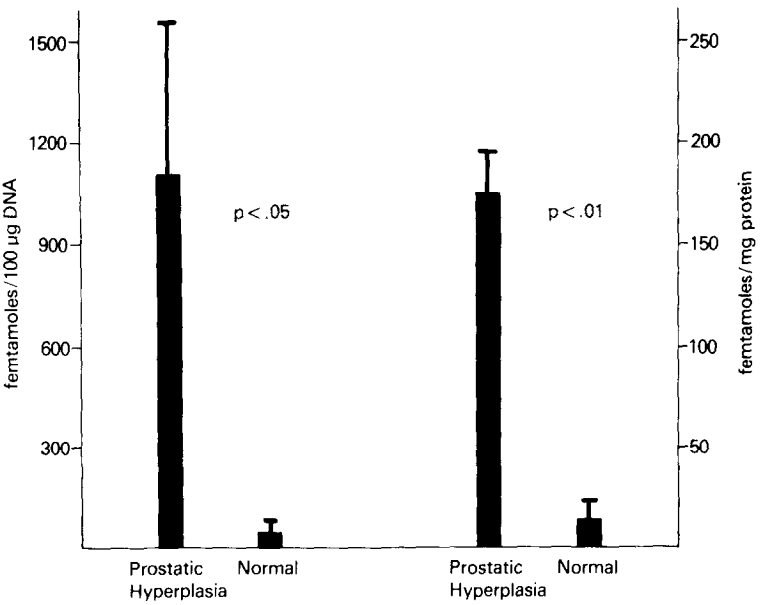

Figure 1: Nuclear androgen receptors in DNAase 1 fraction of prostatic hyperplasia of aging dogs (N=10) and normal prostate (N=7). Values are mean ± SEM. Values normalized for DNA are shown on the left and for protein on the right. Nuclear androgen receptor content was measured after purified prostatic nuclei were incubated for 20 min with DNAase 1, 500 units, at 4°C.

normal tissue when values were corrected for protein content of whole nuclei. Marginally elevated levels of androgen receptor were observed in the nuclear matrix when content was normalized for whole cell DNA. The difference in cytosol androgen content was not significant between normal and hyperplastic prostate (Table 1).

Table 1: Androgen receptor content in whole nuclei, nuclear matrix and cytosol in normal (N=7) and hyperplastic prostatic (N=7) tissue of Beagle dogs. The values are mean ± SEM. Levels for whole nuclei and the nuclear matrix were normalized for protein and DNA content of the whole nuclei and values for cytosol were normalized for protein content. The nuclear matrix was isolated by sequential treatment of purified nuclei DNAase 1, low, and then high salt buffer systems (9).

	Normal	Prostatic Hyperplasia	p Value	Normal	Prostatic Hyperplasia	p Value
	fmoles/mg protein			fmoles/100 µg DNA		
Whole Nuclei	27.1 ± 8.2*	38 ± 12	NS	51 ± 23	435 ± 133	<.02
Nuclear Matrix**	13 ± 3.8	13.1 ± 37	NS	27.6 ± 12.5	101 ± 36	<.1
Cytosol	17 ± 9.8	33.6 ± 43	NS			

*mean ± SEM

**Normalized to DNA and protein in whole nuclei

Discussion

The following observations on androgen receptor content were made in hyperplastic prostate of aging dogs: 1) nuclear androgen receptor content per cell was elevated, 2) nuclease solubilized nuclear androgen receptor level was supranormal, 3) androgen receptor content of the nuclear matrix was marginally elevated, and 4) the androgen receptor content of the cytosol was not abnormal.

The binding of the cytosolic androgen-receptor complex to the nucleus is believed to be critical for modulation of nuclear events that control proliferation and growth of androgen target tissues (13). The precise site of the interaction that produces the hormonal action(s) has been unresolved. Steroid hormone receptors have been observed in various components of the nucleus and include the nuclear membrane (14), DNA (15), various portions of chromatin (16-21) and ribonucleoproteins (22). The role of each of these in the interaction with the androgen-receptor complex remains to be elucidated.

In previous studies of nuclear receptors in prostate tissue of humans and dogs, hypertonic salt solutions were generally used to extract the androgen receptors (24-27). It is well recognized that salt-resistant binding sites for sex-steroids comprise a substantial proportion of the total binding sites (28,29). We have, therefore, observed that nuclear androgen receptor solubilized by nuclease digestion is supranormal in hyperplastic tissue of aging dogs Previous data on androgen receptor content of nuclei and cytosol in prostatic hyperplasia of humans (24,25) and dogs (23,27,29) have indicated that values are either normal or elevated.

Our results suggest that selective nuclear androgen-receptor accumulation in chromatin of prostate may be causally related to the development of benign prostatic hyperplasia. Observations made in whole nuclei alone do not suggest an abnormality of nuclear androgen receptor as reported in the disorder of humans (24). A selective elevation of specific androgen receptors in the nucleus could exist in human tissue as reported here in hyperplastic prostatic tissue of dogs. Activation of specific loci of nuclear chromatin by the binding of the androgen-receptor-complex could result in hyperplasia of the gland.

The location of these sites on chromatin are unknown, but their identification appears critical to furthering our understanding of this neoplastic process (30).

When purified nuclei were isolated from normal and hyperplastic prostates and digested with DNAase 1, receptor solubilization occurred more rapidly than the hydrolysis of DNA. The quantity of nuclease solubilized receptor was much greater in the hyperplastic than in the normal prostate. The fact that the receptor was solubilized much more rapidly than hydrolysis of DNA suggests that the transcriptionally active component contains the predominant quantity (17). Further studies are essential to establish this possibility.

The nuclear matrix may be involved in many functions of the cell that are stimulated by steroid hormones, newly synthesized and heterogenous nuclear RNA, and processing and transport of RNA (9). The nuclear matrix contained 25-50% of the androgen receptor found in the nucleus, but hyperplastic prostatic tissue contained only a marginally higher content of androgen receptor in this component than normal tissue. In conclusion, it therefore seems unlikely that an androgen receptor defect in the nuclear matrix results in hyperplasia of the prostate of aging dogs.

Acknowledgments

This work was supported by the Veterans Administration Hospital and the Clinical Research Center, grant No. RR00064, and an Institutional grant No. 52144. We wish to express thanks to Mrs. Barbara Samuels for technical assistance and Mrs. Miriam Leonard for secretarial help.

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