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Aromatization of Androstenedione to Estrogen by Benign Prostatic Hyperplasia, Prostate Cancer and Expressed Prostatic Secretions

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Summary. Human prostatic tissue and expressed prostatic secretions (EPS) from patients with benign prostatic hyperplasia (BPH) and prostate cancer were incubated with (1 β ^{3}H) androstenedione. The extent of aromatization was determined by measuring the transfer of ^{3}H from the 1 β position into water. The amount of ^3H_2O recovered corresponds to the estrogens formed. Tissue from 5 patients with BPH yielded 2.13 (± 1.05) pmol/mg protein/h while the EPS from the same patients yielded 727 fmol/mg protein/h. In patients with prostate cancer the mean formation of estrogens was 388 fmol/mg protein/h (± 75). 4-hydroxyandrostenedione, an aromatase inhibitor, successfully inhibited aromatization in BPH and prostate cancer 53–98%.

Key words: Aromatization, BPH, Prostate cancer, Prostatic secretions.

Introduction

Human prostatic tissues are capable of converting androstenedione to estradiol and estrone [13, 14]. Preliminary data suggest that there is an increased production of estrogens in the prostates of patients with BPH. Evidence for aromatization in the peripheral region of the prostate suggests that such may be also linked to prostate cancer [14].

Additional studies now confirm this earlier work. Estrogen formation can be detected in tissue from patients with prostate cancer and in the expressed prostatic secretions (EPS) of patients with BPH. Inhibition of aromatization can be achieved in vitro with 4-hydroxyandrostenedione in both BPH and prostate cancer.

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Materials and Methods

Prostate Tissue and Expressed Prostate Secretions

Prostatic tissue was obtained from patients undergoing either open prostatectomy or transurethral resection. Just prior to the procedure expressed prostatic secretions (EPS) were obtained by digital massage. Specimens were immediately frozen (−70 °C) until utilized. The presence of BPH or prostate cancer was confirmed microscopically.

Aromatase Assay

Glucose-6-phosphate, glucose-6-phosphate dehydrogenase and NADPH were purchased from Sigma Chemical Company (St. Louis, Mo.). (1,2^{3}H) testosterone (41.6 Ci/mmole) was obtained from New England Nuclear (Boston, Ma.). 4-hydroxyandrostenedione (4-OHAD) was obtained from Dr. A. M. H. Brodie, University of Maryland. (1β^3H) androstenedione (29.9 Ci/mM) was prepared from (1,2^{3}H) testosterone by treatment with base [11].

Prostatic tissue and EPS were homogenized on ice (Tekkmar and glass on glass) in 50 mM phosphate buffer (pH 7.5) with 0.5 mM EDTA and 1 mM dithiothreitol (DTT).

The final homogenate (1 cc) was incubated with approximately 10^6 dpm of (1β^3H) AD and 1 μM cold androstenedione in the presence of glucose-6-phosphate (3.3 mM), glucose-6-phosphate dehydrogenase (3.1 units) and NADPH (1.2 mM) for 1 h at 37 °C. The inhibitor 4-OHAD was added to some of the incubations in concentrations equimolar to the substrate and up to 100 fold excess (10^{-9}–10^{-7} moles). Heated prostatic tissue and buffer were used for control incubations. Residual counts contained within the controls were substracted from the products of the incubations.

At the end of the incubation the homogenates were mixed with 1% charcoal, left on ice for 10 min and centrifuged at 2,000 x g x 10 min. The supernatant was lyophilized and counted. The amount of ^3H_2O corresponds to the estrogen formed [11, 14]. Proteins were determined by the method of Lowry [9].

Results

Five patients had either open prostatectomy or TURP for obstructive BPH. Estrogen formation in the hyperplastic tissue ranged from 370 fmols/mg protein/h to 4.8 pmoles/

Table 1. Estrogen formation in benign prostatic hyperplasia and expressed prostatic secretions

Patient	BPH	EPS	Ratio
1	4,610	1,280	3.6
2	4,800	1,540	3.1
3	371	71	5.2
4	538	–	
5	370	18	20.0

Incubation of benign prostatic hyperplasia (BPH) and expressed prostatic secretions (EPS) with (1β ^{3}H) androstenedione. The extent of aromatization was measured by the transfer of ^{3}H from the 1β position into water. Results are expressed in fmol/mg protein/h. Note no EPS obtained from patient #4

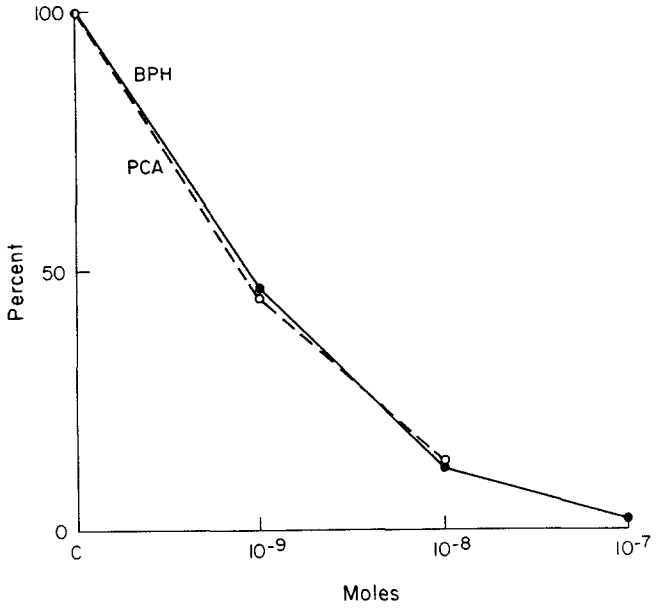

Fig. 1. Incubation of (1β ^{3}H) androstenedione with BPH and prostate cancer homogenates and increasing amounts of 4-hydroxyandrostenedione. Control incubations (*c*) without inhibitor are expressed as 100%. 4-OHAD expressed in moles

Table 2. Estrogen formation in human prostate cancer

Patient	Prostate cancer
1	203
2	327
3	502
4	520

Results are in fmol/mg proteine/h and were obtained as described in legend to Table 1

mg protein/h with a mean of 2.13 pmoles (± 1.05). Expressed prostatic secretions from these same patients demonstrated estrogen formation of 18 fmols/mg protein/h to 1.28 pmoles mg/h with a mean of 727 (± 398). The ratio of aromatization in the BPH to that in the EPS ranged from 3.1–20.0 with a mean of 8.0 (Table 1).

Tissue from patients (4) with prostate cancer yielded 203–520 fmols/mg protein/h with a mean of 388 (± 75) (Table 2).

Incubation of BPH and prostate cancer with 4-OHAD yielded dose dependent suppression of aromatization from 53–98% (Fig. 1).

Discussion

Previous work in this laboratory has demonstrated estrogen formation from androgens in human prostatic tissue. Comparison of periurethral tissue from patients with and without BPH suggested a greater amount of estrogen formation in the former. In addition, the presence of substantial aromatization in the peripheral zone raised questions relative to the role of estrogen formation in the initiation or promotion of prostate cancer.

Estrogen formation in the group of patients with BPH averaged 2.16 pmol/mg protein/h. This is considerably higher than the 223 fmol/mg protein/h reported previously [14]. There are several explanations for this "enhanced" activity. The tissue in the previous report was obtained as a result of cystoprostatectomy for bladder disease. The present group of patients was operated on for obstructive BPH. The buffer system presently utilized contained phosphate as well as EDTA and DTT. Previous experiments utilized a Tris buffer system and there is some evidence that the former system improves yield (unpublished data). Finally, the results obtained in the previous reports were achieved by using the stably labeled (1,2,6,7 ^{3}H) androstenedione at 100 nM with recovery of ^{3}H estrone and ^{3}H estradiol. The present experiment utilized 1 μM (1β ^{3}H) androstenedione with estrogen formation determined by recovery of ^3H_2O.

Experimental evidence is consistent with the probability that estrogen formation may be important in initiating and sustaining BPH. Finding high estrogen levels in the stroma as compared to the sera in patients with BPH [7, 8] may therefore not reflect a "concentrating" effect as has been theorized but rather an accumulation of estrogens via in situ aromatization. Furthermore, reports of low LH [6, 10] found in some patients with BPH might be a result of feedback from increased estrogen production.

Previous work noting the existence of estrogen formation in the peripheral zone of the benign prostate prompted us to investigate its presence in prostate cancer. The levels found are threefold greater than those reported for the normal peripheral zone (388 fmol vs 140). Although some of this increase may be attributed to the same factors mentioned previously in patients with BPH, other experimental evidence indirectly supports the possible role of estrogen involvement in prostate cancer genesis [4]. The presence of estrogen receptor binding sites, the increase in androgen receptor content with the addition of estrogen, and the growth stimulating affects of estrogen on cultured human

prostate cancer cells further support such a hypothesis [2, 7, 8]. The reports of low testosterone levels in patients with prostate cancer or in their brothers [6, 10] may be related to estrogen production by the peripheral zone of the prostate with subsequent negative feedback.

This is the first demonstration of the presence of aromatase in expressed prostatic secretions. The EPS contains many elements from the ductal and glandular epithelial cells [1, 5, 12] and therefore, it is not surprising that aromatase should be present, the implication being that the enzyme is of prostate glandular or ductal origin. The amount of estrogen formed in the EPS appears to parallel that of the BPH tissue (Table 1). However, the sample size is too small to assess whether there is a correlation with prostate size. Rojas [12] reported increased estradiol in the EPS from patients with prostate cancer. Such a finding might result from either increased production of estradiol within the epithelial cells of the peripheral zone or from in situ production in the EPS itself from the aromatase enzyme.

Estrogen formation was inhibited by 4-OHAD in a dose dependent manner in both BPH and prostate cancer. Should aromatase prove to be significant to the pathogenesis of these two diseases, the potential for a novel form of treatment for BPH and prostate cancer may become available. An analogous possibility is presently being studied in patients with breast cancer [3]. The finding of aromatization in the EPS would make such determinations of this enzyme a possible means for patient screening and assessment of treatment response.

References

1. Anderson RU, Fair WR (1976) Physical and chemical determinations of prostatic secretion in benign hyperplasia, prostatitis, and adenocarcinoma. Invest Urol 14:137–139
2. Bouton MM, Porin C, Grandadan JA (1981) Estrogen regulation of rat prostate androgen receptor. J Steroid Biochem 15:403–408
3. Cooms RC, Dowsett M, Goss P, Gazet JC, Brodie A (1984) 4–hydroxyandrostenedione in treatment of postmenopausal patients with advanced breast cancer. Lancet 1237–1239
4. Fishman J, Goto J (1981) Mechanisms of estrogen biosynthesis. J Biol Chem 256:4466–4471
5. Grayhack JT, Lee C, Oliver L, Schaeffer AJ, Wendel EF (1980) Bichemical profiles of prostatic fluid from normal and diseased prostate glands. Prostate 1:227–237
6. Harper ME, Pierrepoint CG, Griffiths K (1984) Carcinoma of the prostate: relationship of pretreatment hormone levels to survival. Eur J Cancer Clin Oncol 20:477–482
7. Kozak I, Bartsch W, Krieg M, Voigt KD (1982) Nuclei of stroma: site of highest estrogen concentration in human prostatic hyperplasia. Prostate 3:433–438
8. Krieg M, Bartsch W, Thompson M, Voigt KD (1983) Androgens and estrogens: their interaction with stroma and epithelium of human benign prostatic hyperplasia and normal prostate. J Steroid Biochem 19:155–161
9. Lowry OH, Rosenburgh NJ, Farr LA, Randall RJ (1951) Protein measurement with the folin penol reagent. J Biol Chem 193:265–269
10. Meikle AW, Stanish WM (1982) Familial prostatic cancer risk and low testosterone. J Clin Endocrinol Metab 54:1104–1107
11. Miyairi S, Fishman J (1985) Radiometric analysis of oxidative reactions in aromatization by placental microsomes. J Biol Chem 260:320–325
12. Rose DR, Laakso K, Sotarauta M, Wynder EL (1984) Hormone levels in prostatic fluid from healthy finns and prostate cancer patients. Eur J Cancer Clin Oncol 10:1317–1319
13. Schweikert HU (1979) Conversion of androstenedione to estrone in human fibroblasts cultured from prostate, genitals and nongenital skin. Hormone Metab Res 11:635–640
14. Stone NN, Fair WR, Fishman J (1986) Estrogen formation in human prostatic tissue from patients with and without benign prostatic hyperplasia. Prostate 9:311–318

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