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SHORT COMMUNICATION

FAILURE OF HUMAN BENIGN PROSTATIC HYPERPLASIA TO AROMATISE TESTOSTERONE

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SUMMARY

The conversion of androgens to oestrogens in benign human prostatic tissue was examined. Cytosol preparations were incubated separately with radiolabelled androstenedione and testosterone in the presence of a co-factor. The metabolic products were separated by phenolic partition and thin layer chromatography. The results of our investigations suggest that under the present incubation conditions there was no evidence for the aromatisation of androgen in the human prostate.

The susceptibility of the prostate to high doses of oestrogen is well known and forms the basis of widely employed therapy for advanced cancer of the prostate [1]. The effects of oestrogen may be partly mediated through the high affinity, low capacity oestrogen binding sites detected in the prostate by several groups of workers [2–4]. In addition to the possession of receptors the ability of the prostate to metabolise oestrogens has long been recognised [5] and significant levels of oestrone and oestradiol occur in the organ [6, 7].

Although there are reports on the biosynthesis of methoxyoestrone from testosterone [8] and the formation of oestrone in fibroblast cultures from human benign prostatic hypertrophy (BPH) and cancer of the prostate, the production of the active oestrogens, oestrone and oestradiol, from labelled androgen precursors in preparations of whole human prostate has not been reported. Furthermore, in view of the potential interest in the possibility of relating steroid metabolism and hormone levels in the prostate to diagnosis of cancer and to response to therapy [9] an attempt was made to demonstrate oestrogen production from radiolabelled androstenedione and testosterone in cytosol preparations of whole human prostate tissue.

Tissue obtained after retropubic prostatectomy from six untreated patients (with benign prostatic hypertrophy) was transported to the laboratory in ice-cold 0.9% NaCl (w/v). Approximately half of the studies described in this paper were performed using fresh specimens and the remainder using frozen tissue; these were usually transported in liquid nitrogen and stored at −30°C for varying periods up to 3 weeks before analysis. Fragments of every prostatic specimen included in this study were examined histologically. The presence of simple fibromuscular and glandular hyperplasia of the prostate was confirmed in every case. 1 g of tissue was taken from each specimen, finely minced with a razor blade and suspended in 2 vol. of the buffer system used in this study which contained 10 mmol Tris (hydroxymethyl)–methylamine, 1.5 mmol EDTA, 1.0 mmol dithiothreitol and 0.5 mmol NADPH generating system. The pH of the buffer was adjusted to 7.4. The minced suspension was then homogenised with an ILADO laboratory dispenser (Scottish Scientific Instruments Centre Ltd) using three 15-s bursts at setting 7 with 1 min cooling intervals. Aromatization was determined by the procedure previously applied to human mammary tumors [10]. Briefly, separate whole homogenates were incubated in the presence of 1 μCi radiclabelled [1,2,6,7-^{3}H]androstenedione (SA 80 Ci/mmol) and 2 μCi radiolabelled [1,2,6,7-^{3}H]testosterone (SA 85.5 Ci/mmol) for 1½ h at 37°C in a shaking water bath. Under the present incubation conditions, the entire androstenedione was available for aromatization since minimal and insignificant amounts of the precursor are converted to testosterone and dihydrotestosterone [11, 12]. Although 50% of the testosterone was, on the other hand, reduced to other metabolites sufficient quantities of the unconverted substrate remained in solution to enable the aromatase to be detected. Incubations were terminated by the addition of an acetone solution containing 25 μg non-labelled testosterone, androstenedione, oestrone and oestradiol as carriers and tracer amounts 400 d.p.m. of the ^{14}C labelled steroids for recovery purposes. The acetone was evaporated off and the steroids extracted four times with 1 ml diethyl ether. The pooled extracts were evaporated dry and a preliminary separation of oestrogens from androgens achieved by redissolving the residue in 1 ml 1 N-NaOH and extracting twice with 1 ml benzene. The benzene removes about 95% of the androgens. The alkaline oestrogen solution was neutralized with HCl and extracted four times with 1 ml diethyl ether. After evaporating off the ether the oestrogens were redissolved in 100 ml ethanol and further purified by thin layer chromatography.

A two step chromatographic system was developed to isolate oestrone and oestradiol from androstenedione, testosterone and other metabolites. A preliminary run in ether–heptane (3:1, v/v) on polysilicic acid gel impregnated glass fibre sheets 20 × 20 cm (Gelman Sciences Incorporated, Ann Arbor, Michigan, U.S.A.) produced a clear separation of oestradiol (R_F = 0.62) from all other steroids except 5α-dihydrotestosterone (R_F = 0.61). The oestradiol dihydrotestosterone band was consequently rerun twice in benzene–ethanol (96:4, v/v), producing a clear separation of the two components (oestradiol, R_F = 0.39; dihydrotestosterone, R_F = 0.58).

The R_F of oestrone (0.80) in the ether–heptane system, was higher than that of any of the other components. As an additional precautionary step, the oestrone band was reduced to oestradiol with $NaBH_4$ and re-run on the same system to provide a clear separation of oestradiol from all other reduction products. The position of the radioactive steroids was determined by reference to unlabelled compounds run alongside. After chromatography the steroids were localized by spraying the reference steroids with sulphuric acid in ethanol, followed by 10 min in an oven at 100°C. The zones corresponding to the reference steroids

were marked, cut with scissors and directly immersed in a Triton X-100 scintillation cocktail and counted. The percentage recoveries (mean ± SD) were 41.3 ± 2.4% for oestradiol and 35.7 ± 4.1% for oestrone.

Conversion of androstenedione to oestrogen has been demonstrated in fibroblast monolayer cultures derived from normal, BPH and cancerous prostates [13]. The rates of conversion to oestrogen were reported to be in the region of approximately 5.0 pmol/100 mg protein/h. In the present study we were most concerned that the sensitivity of the assay employed should allow for an even lower rate of conversion. High specific activity precursors were therefore used and in spite of the recovery losses and the efficiency of the counter (62%) a conversion of only 1% of that detected by Schweikert in fibroblasts yielded a readily detectable 400 c.p.m. in the oestrogen bands in our experiments.

Our results clearly demonstrate the absence of tracer amounts of oestrogens in the extracts suggesting that under the present experimental conditions, prostatic tissues are not involved in the peripheral conversion of either androstenedione or testosterone to oestrone and oestradiol. The possibility that later intermediates in the aromatization pathway, namely the 19-hydroxy and 19-oxo derivatives, may be converted into oestrogen by prostate tissue has not, however, been excluded.

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REFERENCES

1. Huggins C. and Hodges C. V.: Studies on prostatic cancer; the effect of castration, of oestrogen and of androgen injection on serum phosphatases in metastatic carcinoma of the prostate. *Cancer Res.* **1** (1941) 293–297.
2. Hawkins E. F., Nijs M., Brassine C. and Tagnon H. J.: Steroid receptors in the human prostate 1/oestradiol-17β binding in benign prostatic hypertrophy. *Steroids* **25** (1975) 458–468.
3. Bashirelahi N., Toole J. H. and Young J. F.: A specific 17β-oestradiol receptor in human benign hypertrophic prostate. *Biochem. Med.* **15** (1975) 254–261.
4. Sidh S. M., Young J. D., Karmi S. A., Powsker J. R. and Bashirelahi B.: Adenocarcinoma of prostate: role of 17β-oestradiol and 5α-dihydrotestosterone binding proteins. *Urology* **13** (1979) 597–603.
5. Acevado H. J. and Goldzieher J. W.: The metabolism of [^{14}C]-oestrone by hypertrophic and carcinomatous human prostate tissue. *Biochim. biophys. Acta* **97** (1965) 571–578.
6. Belis J. A.: Methodologic basis for the radioimmunoassay of endogenous steroids in human prostatic tissues. *Invest. Urol.* **17** (1980) 332–336.
7. Ghanadian R. and Puah C. M.: Relationships between oestradiol-17β, testosterone, dihydrotestosterone and 5α-androstane-3α,17β-diol in human benign hypertrophy and carcinoma of the prostate. *J. Endocr.* **88** (1981) 255–262.
8. Farnsworth W. E.: Metabolism of 19-nortestosterone by human prostate. *Steroids* **8** (1966) 825.
9. Habib F. K.: Evaluation of androgen metabolism studies in human prostate cancer. Correlation with zinc levels. *Prev. Med.* **9** (1980) 650–656.
10. Miller W. K. and Forrest A. P. M.: Oestradiol synthesis from C-19 steroids by human breast cancers. *Br. J. Cancer* **33** (1976) 116–118.
11. Habib F. K., Tesdale Ann L., Chisholm G. D. and Busuttil A.: Androgen metabolism in the epithelial and stromal components of the human hyperplastic prostate. *J. Endocr.* **91** (1981) 23–32.
12. Habib F. K., Rafati G., Robinson M. R. G. and Stitch S. R.: Effects of tamoxifen on the binding and metabolism of testosterone by human prostatic tissue and plasma *in vitro*. *J. Endocr.* **83** (1979) 369–378.
13. Schweikert H. U.: Conversion of androstenedione to oestrone in human fibroblasts cultured from prostate, genital and non-genital skin. *Horm. Metab. Res.* **11** (1979) 635–640.