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TESTOSTERONE METABOLISM BY HOMOGENATES OF HUMAN PROSTATES WITH BENIGN HYPERPLASIA: EFFECT OF TISSULAR CONCENTRATIONS OF ZINC, MAGNESIUM AND COPPER

GÉRARD SINQUIN*, ROBERT MORFIN*†, JEAN-FRANÇOIS CHARLES‡ and HERVÉ H. FLOCH§
*Laboratoire de Biochimie, ERA CNRS No. 784, Faculté des Sciences, 29283 Brest Cedex,
‡Service de Chirurgie générale "Bichat", CHU, 29279 Brest Cedex, and
§Laboratoire de Biochimie, ERA CNRS No. 784, Faculté de Médecine, 29279 Brest Cedex, France

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SUMMARY

Zinc, magnesium and copper concentrations were measured in homogenates of 20 human prostates with benign hyperplasia and a significant direct relationship between zinc and magnesium contents was found. Activities of the testosterone-5α-reductase in the same homogenates were not significantly changed according to the concentrations of the three metals but activities of the 3α- and 3β-hydroxysteroid dehydrogenases were inversely related with the zinc concentrations of the tissue preparations. This inverse relationship may explain the known increased 5α-dihydrotestosterone content in prostatic tissues with high zinc concentrations.

INTRODUCTION

Testosterone transformations by minces and homogenates of human hyperplastic prostate glands have been thoroughly reported by several authors [1–5]. The main metabolic pathway is NADPH-dependent and mimics the *in vivo* metabolism in the gland [6]. Thus, 5α-reduction of testosterone leads to the formation of 5α-dihydrotestosterone which is in turn reduced into epimeric 5α-androstanediols by the 3α- and 3β-hydroxysteroid dehydrogenases.

Both zinc and magnesium were found in large quantities in the human hyperplastic prostate [7–11]. Plasma zinc and copper levels were also studied in patients with diseased prostates [12]. Interrelationships between metals and C_{19}-steroid hormone concentrations in hyperplastic prostates were studied [13] and 5α-dihydrotestosterone concentrations were found to be inversely proportional with the levels of zinc.

Since prostatic levels of 5α-dihydrotestosterone depend both on the rates of testosterone 5α-reduction and of 5α-dihydrotestosterone 3α- and 3β-reductions, it was of interest to investigate the relations which may exist between prostatic metal levels and such steroid-metabolizing activities. The results of these studies are described in this report.

MATERIALS AND METHODS

Radiolabelled steroids

[4-^{14}C]-Testosterone (45.6 mCi/mmol) and [4-^{14}C]-5α-dihydrotestosterone (45.6 mCi/mmol) were purchased from CEA (Saclay, France). The radiosteroids were purified by thin layer chromatography on silica gel $HF_{254+366}$ from Merck (Darmstadt, FRG) in benzene–ethanol (9:1, v/v). The radiochemical purity of the recovered labelled steroids was checked by crystallization to constant specific activity of a carrier-diluted portion [14]. Purity was found at 97% for the [4-^{14}C]-testosterone and at 96% for the [4-^{14}C]-5α-dihydrotestosterone. Both steroids were kept at 2°C in benzene–ethanol (9:1, v/v).

Steroids and reagents

Non radioactive testosterone, 5α-dihydrotestosterone, 5α-androstane-3α,17β-diol and 5α-androstane-3β,17β-diol were purchased from Merck (Darmstadt, FRG). The 5α-androstane-3β,7α,17β-triol was prepared as previously reported [15]. Purity of the steroids was checked by gas chromatography and purifications by recrystallizations carried out when necessary. Glucose-6-phosphate, NADP and glucose-6-phosphate dehydrogenase (EC 1.1.1.49) were from Boehringer (Mannheim GmbH, FRG). Tris–HCl, sodium chloride, zinc chloride, magnesium chloride, copper chloride, dichloromethane and ethyl acetate were of reagent grade and obtained from Merck (Darmstadt, FRG).

Human prostate specimens

Prostate glands with benign hyperplasia were obtained from patients at the moment of transvesical extirpation. Tissues were immediately placed on crused ice and transported into a cold room (4°C).

† To whom correspondence should be addressed.

Histological examinations for confirmation of the diagnosis were carried out on representative portions left in the Pathology department.

Preparation of homogenates

At 4°C, slices of the glands were minced first with scissors then forced through the 1 mm holes of a disc with an arbor tissue press (Harvard Apparatus, Mass., USA). The minced tissue (2 g) was dispersed in 0.01 M tris buffer (pH 7.4) containing 0.05 M sodium chloride, and homogenized with a Polytron unit (Kinematica, Luzern, Switzerland) equipped with a PT-10 probe. Homogenization was carried out three times for 15 s at a setting of half of the maximal power. Homogenates were cooled for 30 s intervals between each homogenization. The homogenate was filtered through five layers of gauze and the filtrate manually homogenized in a Dounce apparatus with 10 strokes of the loosely-fitting pestle and then 10 strokes of the tightly-fitting pestle.

Incubation procedure

Either [4-^{14}C]-testosterone or [4-^{14}C]-5α-dihydrotestosterone (0.29 μg μg) was dried under nitrogen at the bottom of silanized 30 ml incubation tubes. Incubation was started by adding successively 0.1 ml of tris–HCl buffer (pH 7.4) containing 0.05 M sodium chloride, 0.3 ml of the NADPH-generating system in the same buffer (33.3 mM-NADPH, 33.3 mM-Glucose-6-phosphate and 0.5 U of glucose-6-phosphate dehydrogenase) and 1.6 ml of the homogenate. Incubations were carried out at 37°C in a shaking water bath for 10 min with the testosterone substrate and for 30 min with the 5α-dihydrotestosterone substrate. Enzymic actions were stopped by vigorous shaking with 2 ml of dichloromethane and placing the tubes at −20°C.

Extraction and separation of the radiometabolites

After thawing, the dichloromethane layer was removed and the digests were further extracted three times with 5 ml ethyl acetate. Dichloromethane and ethyl acetate extracts were pooled and a portion was counted for computation of the recoveries. After concentration under nitrogen, the radiometabolites of each extract were separated by thin layer chromatography on silica gel $HF_{254+366}$ (Merck, Darmstadt, FRG) developed twice in benzene–95% ethanol (9:1, v/v). Testosterone, 5α-dihydrotestosterone, 5α-androstane-3α,17β-diol, 5α-androstane-3β,17β-diol and 5α-androstane-3β,7α,17β-triol were separated in this system. Radiometabolites were located on the chromatograms by autoradiography with Kodak BB-54 X-ray films, and their identification based upon comparison of their R_F values with those of authentic standards. Radioactive zones were entirely collected in glass vials for counting.

Liquid scintillation spectrometry

The radioactive silica gel collected in scintillation vials was deactivated with three drops of methanol before addition of 10 ml of the scintillation fluid. Counting was carried out with an Intertechnique (France) model SL-4000 liquid scintillation spectrometer. The quenching was corrected on the basis of external standard ratios and all counts expressed in d.p.m. In all cases, sufficient counts were allowed to accumulate so that the counting error did not exceed 1% at the 99.3% confidence level.

Measurement of zinc, magnesium, copper and protein concentrations in homogenates

Measurement of metal concentrations were carried out with a Perkin Elmer model 360 atomic absorption spectrometer. Measurements of zinc were at 213.7 nm on homogenates diluted 50 times with distilled water. A standard solution of zinc chloride (0.4 mg/l) was used. Homogenates were diluted 5–10 times in 0.25% strontium chloride for the measurements of magnesium at 285.2 nm. A standard solution of magnesium chloride (1 g/l) was used. Crude, or 3 times diluted homogenates were used for measurement of copper concentrations at 324.7 nm. Calibration at 50% absorption was carried out with a 0.4 mg/l solution of copper chloride. All cation concentrations in homogenates were obtained in μg/ml. Concentrations could also be computed in μg/g since one ml of homogenate corresponded with 0.066 g of fresh tissue. Protein concentrations in homogenates were measured by the Biuret method.

Calculation of prostatic enzyme activities

The percent conversion of the radiolabelled steroid substrates was based on the radioactivity associated with the metabolites separated by thin layer chromatography. Estimation of the 5α-reductase activity involved the percent formation of 5α-dihydrotestosterone, 5α-androstane-3α,17β-biol, 5α-androstane-3β,17β-diol and 5α-androstane-3β,7α,17β-triol from the [4-^{14}C]-testosterone substrate. Activity of the 3β-hydroxysteroid dehydrogenase was based on the percent formation of 5α-androstane-3β,17β-diol and 5α-androstane-3β,7α, 17β-triol from the [4-^{14}C]-5α-dihydrotestosterone substrate.

Activity of the 3α-hydroxysteroid dehydrogenase was estimated from the percent formation of 5α-androstane-3α,17β-diol from the [4-^{14}C]5α-dihydrotestosterone substrate. Each incubation contained 10^3 pmol of the radiolabelled substrate and from 6.5 to 12.9 mg proteins of the final prostatic homogenate. 5α-Reductase activities were expressed in terms of pmol. $10\,min^{-1}\cdot mg\ protein^{-1}$ and the 3α- and 3β-hydroxysteroid dehydrogenase activities in terms of $pmol\cdot 30\,min^{-1}\cdot mg\ protein^{-1}$.

RESULTS

Zinc, magnesium and copper concentrations in prostatic tissues

The concentration of zinc in homogenates of 17 prostate glands with benign hyperplasia was 0.1 ± 0.01 mM (0.03–0.19 mM) corresponding with 99.2 ± 11.6 μg/g of fresh tissue (33.0–181.5 μg/g).

Magnesium concentrations were measured in 16 of the homogenates and averaged 0.45 ± 0.02 mM (0.30–0.60 mM) or 163.6 ± 8.0 μg/g of fresh tissue (111.3–217.5 μg/g).

Copper concentrations in 17 homogenates were found at $3.3 \pm 0.4 \cdot 10^{-3}$ mM ($0.8–7.4 \cdot 10^{-3}$ mM) or 3.18 ± 0.4 μg/g of fresh tissue (0.78–7.02 μg/g).

Concentrations of zinc, magnesium, copper and proteins in each prostatic homogenate are reported in Figs 2, 3 and 5. If copper and protein concentrations vary independently from those of magnesium or zinc, it appears that, in each homogenate, there is a relation between zinc and magnesium concentrations. The linear regression analysis reported in Fig. 1 shows this relation to be significant with 16 prostatic homogenates ($r = 0.63$; $P < 0.01$).

Prostatic 5α-reductase

The activity of the 5α-reductase was tested with homogenates from seven prostate glands. The 5α-reduction of the testosterone substrate averaged at 29.6 ± 8.4 pmol · 10 min^{-1} · mg protein^{-1} (17.2–39.8) (Fig. 2).

When the activity of the 5α-reductase and the concentrations of zinc, magnesium and copper were compared in each homogenate (Fig. 2), no significant relation could be found. Thus, linear regression analysis of the 5α-reductase activities with zinc concentrations ($r = 0.085$; $P > 0.5$) or with magnesium concentrations ($r = 0.037$; $P > 0.5$) or with copper concentrations ($r = -0.09$; $P > 0.5$) proved that the activity of the prostatic 5α-reductase was not significantly changed by the zinc, magnesium and copper naturally contained in the homogenates.

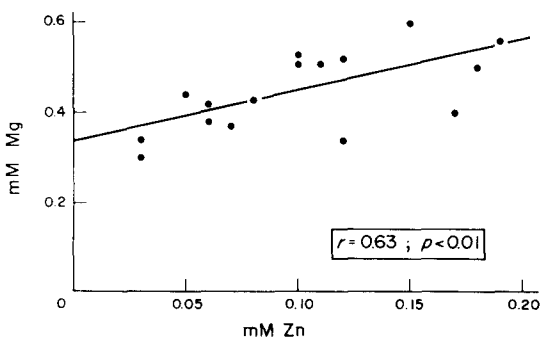

Fig. 1. Relationship between concentration of zinc (Zn) and that of magnesium (Mn) in homogenates of 16 human hyperplastic prostates.

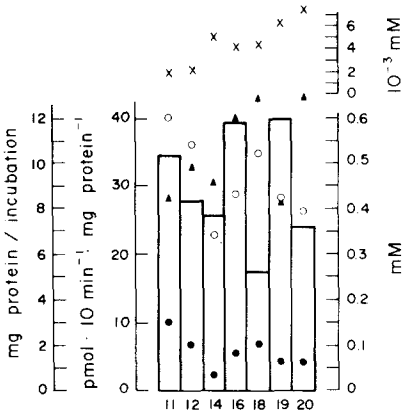

Fig. 2. Concentrations of zinc (●), magnesium (○), copper (×) and proteins (▲) and activity of the 5α-reductase (bars) in homogenates of 7 human hyperplastic prostates.

Prostatic 3α-hydroxysteroid dehydrogenase

Activity of the 3α-hydroxysteroid dehydrogenase was tested with homogenates derived from 14 hyperplastic prostate glands. The NADPH-dependent 3α-reduction of the 5α-dihydrotestosterone substrate ranged from 15.5 to 55.0 pmol · 30 min^{-1} · mg protein^{-1} (Fig. 3) with a mean at 28.6 ± 3.2. In each homogenate, the 3α-reduction was compared with the concentrations of zinc, magnesium and copper (Fig. 3). No significant relation was found between the 3α-reductions and copper concentrations ($r = 0.30$; $P < 0.2$) nor between 3α-reductions and magnesium concentrations ($r = 0.25$; $P > 0.4$). In contrast, a significant inverse relationship was found between zinc concentrations and the 3α-reductions (Fig. 4), ($r = -0.60$; $0.025 > P > 0.01$).

Prostatic 3β-hydroxysteroid dehydrogenase

Homogenates derived from 14 hyperplastic prostate glands were tested for their 3β-hydroxysteroid de-

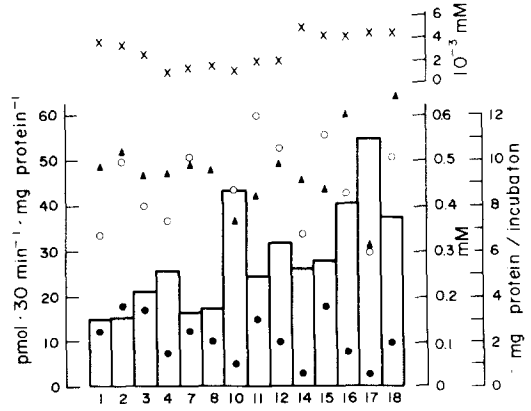

Fig. 3. Concentrations of zinc (●), magnesium (○), copper (×) and proteins (▲) and activity of the 3α-hydroxysteroid dehydrogenase (bars) in homogenates of 14 human hyperplastic prostates.

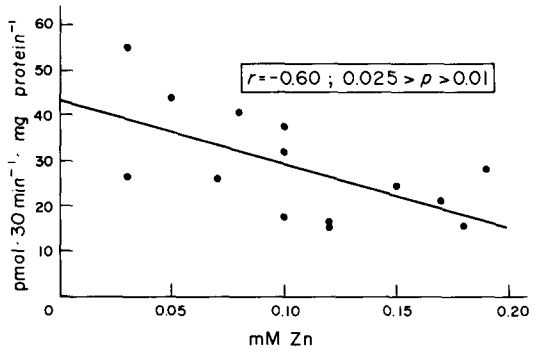

Fig. 4. Relationship between concentrations of zinc (Zn) and activity of the 3α-hydroxysteroid dehydrogenase in homogenates of 14 human hyperplastic prostates.

hydrogenase activity. The NADPH-dependent 3β-reduction of the 5α-dihydrotestosterone substrate ranged from 5.5 to 21.2 pmol · 30 min^{-1} · mg protein^{-1} (Fig. 5) with a mean at 11.2 ± 1.3.

Relations between concentrations of zinc, magnesium or copper and the 3β-reduction in each homogenate were investigated (Fig. 5). No significant relation was found between copper concentrations and

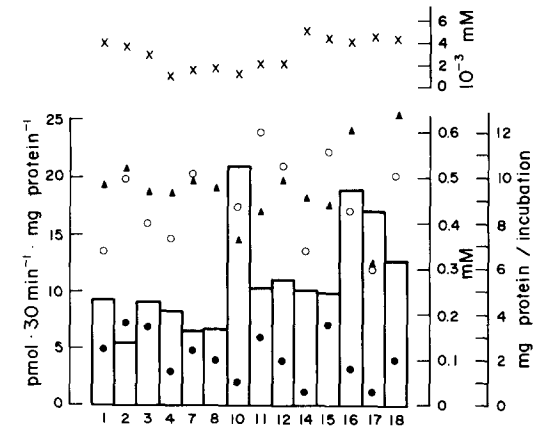

Fig. 5. Concentrations of zinc (●), magnesium (○), copper (×) and proteins (▲) and activity of the 3β-hydroxysteroid dehydrogenase (bars) in homogenates of 14 human hyperplastic prostates.

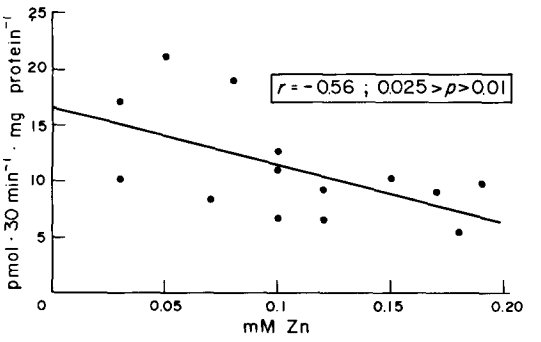

Fig. 6. Relationship between concentrations of zinc (Zn) and activity of the 3β-hydroxysteroid dehydrogenase of 14 human hyperplastic prostates.

the 3β-reductions ($r = 0.19$; $P > 0.5$) nor between magnesium concentrations and 3β-reductions ($r = -0.24$; $P > 0.4$). In contrast, a significant inverse relationship was evidenced between zinc concentrations and 3β-reductions ($r = -0.56$; $0.025 > P > 0.01$) (Fig. 6).

DISCUSSION

The concentration of zinc that we measured in 17 homogenates of hyperplastic prostate glands averaged 99.2 ± 11.6 μg/g of fresh tissue. This level approximates that reported by Habib *et al.* in the same tissue [7, 10, 13], but is lower than the figures published by several other authors [9, 11, 16]. Nevertheless, wide variations of concentrations were reported within any one gland [16] or within different parts of the prostate [7].

For magnesium, the average level in hyperplastic prostate glands was found at 163.6 ± 8.0 μg/g of fresh tissue. Mean concentration reported by Györkey *et al.*[9] was 312.0 ± 10.8 μg/g of fresh tissue for hyperplasia but ranged close of 190 μg/g for normal glands. These authors found significant differences in magnesium and zinc levels between normal and hyperplastic human prostates, but it is now generally admitted that similar levels of zinc occur in the normal and hyperplastic glands since such differences could not be confirmed [10, 13, 17]. Application of our finding of a direct relationship between zinc and magnesium concentrations could justify the fact that with high levels of zinc, the concentrations of magnesium reported by Györkey *et al.*[9] had to be elevated.

The concentrations of copper that we found in the hyperplastic prostate are low (3.18 ± 0.43 μg/g of fresh tissue). We could not find other reports on copper natural levels in the same tissue but one on copper concentrations ranging from 10–14 μg/ml in plasma from patients with prostatic hyperplasia [12].

The prostatic homogenates used in this study were prepared according to Bruchovsky and Lieskowsky[2] and were previously tested to give satisfactory results with optimal substrate and NADPH concentrations for steroid-metabolizing enzymes [18]. Nevertheless, the possible intact stroma and epithelium content of the gauze-retained tissue pulp was neglected. Recent works showed that prostatic stroma and epithelium differed in 5α-reductase and 3α- and 3β-hydroxysteroid dehydrogenase contents [19, 20] and that zinc was mainly located in the epithelial secretory vacuoles [21]. These facts may explain some of the differences in metal contents and enzyme activities that we measured in the various prostate glands. Nevertheless, tissues were always carefully homogenized in the same conditions and the measured differences may be due to other factors.

Our study has shown that neither magnesium nor copper prostatic concentrations could be related with the activities of the testosterone-metabolizing

enzymes of the gland. A previous report indicated that prostatic 5α-dihydrotestosterone levels were inversely related with zinc concentrations [13] but could not determine whether zinc modified the activity of testosterone and 5α-dihydrotestosterone-metabolizing enzymes or influenced the binding of 5α-dihydrotestosterone in the cells. Support for the latter was given in a recent work [22] which showed that zinc was a very effective competitive inhibitor of the binding of 5α-dihydrotestosterone to the prostatic receptor protein in the mouse. Support for a decreased activity of the 5α-reductase was found in the works of Wallace and Grant[16] who showed an inverse relationship between zinc concentrations and 5α-reductase in homogenates of human prostate. We were not able to confirm this relationship. Even when using the zinc concentration units computed by Wallace and Grant [16] (μg zinc/mg protein) we found an inverse relationship less significant than with the mM concentration units. This major difference of our results with those of Wallace and Grant may be explained. Since larger quantities of the testosterone and NADPH substrates were available for the 5α reductase in our cases and since the free prostatic zinc ions contained in the homogenates bind to NADPH [23] in addition to their binding with a specific protein [24], the reported inhibitory action of free prostatic zinc ions [10, 16] may not occur in our incubation system.

In contrast, large 5α-dihydrotestosterone and NADPH concentrations did not prevent the inverse relationship between prostatic zinc concentrations and activities of the 3α- and 3β-hydroxysteroid dehydrogenases. This inverse relationship was found to remain significant when zinc concentration units in μg/ml protein are used. Our evidences for low 5α-dihydrotestosterone metabolizing-enzyme activities related with high zinc concentrations should result into high levels of prostatic 5α-dihydrotestosterone. This does not agree with the inverse relationship reported for prostatic zinc and 5α-dihydrotestosterone levels [13]. Nevertheless, in our incubation system the prostatic zinc must exist mostly in the bound form because of NADPH [23], 5α-dihydrotestosterone receptor [22] and zinc-binding protein [24]. We may now suggest that one of these bound forms inhibits the prostatic 3α- and 3β-hydroxysteroid dehydrogenases.

Such effect of bound zinc on the 5α-dihydrotestosterone-transforming enzymes could favour the known accumulation of 5α-dihydrotestosterone in hyperplastic prostate glands [25, 26] and explain the inverse relationship between zinc and 5α-dihydrotestosterone concentrations in the same tissues [13]. It remains to be seen if this finding is exclusive to the diseased glands or is found in normal prostates. Furthermore, in the event of free zinc ions available in the prostatic cytosol, effects on the testosterone and 5α-dihydrotestosterone-metabolizing enzymes are to be thoroughly investigated.

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