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# Tissue Levels of Free Monosubstituted Guanidines in Human Benign Prostatic Hypertrophy

Marc van Sande and Koenraad Van Camp

Urology Unit, University of Antwerp, Antwerp, Belgium

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Summary. Homogenates of tissue of benign prostatic hypertrophy have been analysed by means of ion exchange chromatography for their content of free monosubstituted guanidines. The results obtained showed very low concentrations. The relationship with arginine metabolism is discussed.

Key words: Prostate, Free monosubstituted guanidines, Arginine metabolism.

## INTRODUCTION

To the best of our knowledge, the levels of free monosubstituted guanidines (FMG) have never been studied in prostatic tissue.

In an attempt to obtain some insight into arginine metabolism, we investigated the presence and concentration of FMG in 11 prostates obtained by open surgery from patients suffering from benign prostatic hypertrophy (BPH).

The results obtained show a very low concentration of FMG in the tissues, giving evidence that the Krebs-Henseleit cycle is operative in human prostate.

## MATERIAL AND METHODS

The prostates from 11 patients with BPH, obtained by open surgery, were brought to the laboratory in liquid nitrogen. The samples were treated immediately or stored at -76°C.

Homogenates of the tissues were made in 2 different ways: a. two of the specimens were homogenized in the presence of solid sulphosalicylic acid, with a final concentration of 50 mg sulphosalicylic acid per ml of homogenate; b. the remaining nine specimens were homogenized following the technique of Van Camp (6). Total protein was measured by the Biuret method (1) on homogenates before deproteinisation. The prostate extracts were centrifuged at 50, 000 g at 4°C for 30 min, and an aliquot of the clear supernatant submitted to column chromatography. Results for the FMG detected are expressed in μmol/g protein.

FMG and amino acids were separated simultaneously by means of ion exchange chromatography (Technicon Amino Acid Analyzer). The column effluent was split by a stream divider and 1 part submitted to the ninhydrin reaction, the other to the Sakaguchi reaction as described by Durzan (2).

The following minor modifications were made to Durzan's technique:

a) the sodium citrate buffers and elution gradient were made as described by Efron (3) (pH of the buffer solutions: 2.875 and 4.740); both buffers contained thiodiglycol, a substance which inhibited the Sakaguchi reaction. The N-bromosuccinimide concentration in the Sakaguchi reaction was kept constant at 0.8% throughout the analysis. This concentration was sufficient to compensate for the presence of thiodiglycol.

b) analyses were carried out with a one column system (dimensions: 140 x 0.6 cm), filled with a spherical resin, Chromobeads B, a sulphonated styrene copolymer, 8% crosslinked, with a particle diameter of 18 μ.

Ninhydrin positive substances were detected at 410 nm, Sakaguchi positive compounds at 495 nm.

For calibration purposes, the following standard substances of pure FMG were submitted to column chromatography and are listed in their order of elution: guanidinosuccinic acid, octopine, guanidinoacetic acid, N-α-acetyl-

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arginine, α-hydroxy-δ-guanidinovaleric acid, phosphoarginine, L-α-amino-β-guanidinopropionic acid, γ-guanidinobutyric acid, L-α-amino-γ-guanidinobutyric acid and arginine. L-arginine-methyl ester, L-arginine aspartic acid, homo-arginine and very basic guanidines e.g. agmatine and various diguanidino derivates were not eluted from the resin with the buffer solutions used.

L-α-amino-β-guanidinopropionic acid (AGPA) is a substance which is also ninhydrin positive but could never be detected in prostate extracts. It has been used as an internal standard, and all detectable identified FMG were calculated as AGPA-equivalents in analogy with the internal standard norleucine in amino acid analysis.

## RESULTS

The chromatogram of prostatic tissue for the FMG revealed 10 to 12 very small peaks, excluding arginine; 3 of these peaks could be superimposed in all cases with the elution pattern of the standard solutions, and the addition of known solutions of FMG to the prostate homogenates were eluted at the same place as the unknowns. The 3 peaks were: octopine, guadinoacetic acid and N-α-acetyl-arginine.

There were 2 dominant peaks in the elution pattern. One appeared before the amino acid phosphorylserine and the other, quantitatively less important than the first, appeared after the N-α-acetyl-arginine position. No attempt was made to identify these peaks.

The arginine peak was not taken into account as the value of this amino acid had already been estimated in BPH by means of the ninhydrin reaction (5).

The elution pattern was the same whichever homogenisation technique was used.

The mean values for the concentrations of octopine, guanidinoacetic acid and N-α-acetyl-arginine, together with their range observed, are given in Table 1.

## DISCUSSION

The presence of significant quantities of urea and arginine (5), together with an arginase activity in BPH, strongly suggests the possibility that the Krebs-Henseleit cycle is operative in human prostate.

Table 1. Levels of free monosubstituted guanidines in 11 human BPH. Results in μmol/g protein

| | Octopine | Guanidino-acetic acid | N-α-acetyl-arginine |
|---|---|---|---|
| Mean value | 0.150 | 0.380 | 0.127 |
| Range | 0.087-0.181 | 0.205-0.420 | 0.070-0.165 |

As far as we know, FMG have not yet been determined in prostatic tissues. In urine from control subjects, guanidinosuccinic acid, guanidinoacetic acid, N-α-acetyl arginine and γ-guanidinobutyric acid are always present (personal observation); guanidinoacetic acid is the predominant fraction.

Kochakian (4) suggested that argininase in animal prostate may participate in the transfer of the amidine group of glycine to form guadinoacetic acid in the kidney, but this role suggested for arginase in the cellular metabolism has not been proven.

Our finding of a mean arginine level of 8.57 μ mol/g protein in BPH, a concentration which is about 7 times higher than the plasma value of normal controls, together with a urea level of 560 g/l (5) gives evidence that the prostate arginase level is not suppressed and that the Krebs-Henseleit cycle is operating.

The following explanation could be proposed to account for the FMG found in prostate tissue.

It is known that arginine is involved in muscle biochemistry through its contribution to the synthesis of guanidine bases (7). Octopine stems from a reductive condensation of puryvic acid with arginine and Van Thoai (7) argues for biological similarity between octopine and lactate.

Guanidinoacetic acid could be formed by the action of an amidinotransferase on arginine and glycine. Hypotaurine is the physiological formamidine acceptor, because its sulphinic group is much more closely akin to the carboxyl group of the substrates of arginine: glycine amidinotransferase than is the sulfonic group of taurine (8).

The presence of N-α-acetyl arginine in prostatic tissue is not clear at yet. Probably, arginine is transacetylated in a similar way to ornithine, in a route to the citrate cycle.

Our aim was to look for arginine metabolism in prostatic tissue and to give normal values for non-treated BPH's. These values can serve as references for comparison with values found in adenocarcinoma or serve as markers for the possible influence of treatment with hormones or anti-androgens of BPH.

In summary, our finding of small quantities of FMG in human prostate provides evidence that the Krebs-Henseleit cycle is operating and it seems likely that arginine links up with the Krebs cycle in the classical matter.

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Marc van Sande
Department of Medicine
Urology Unit
Building Gl - Room 4.51
University of Antwerp
B-2610 Wilrijk, Antwerp
Belgium