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EFFECT OF SURAMIN (BAYER 205) ON RENAL ORNITHINE DECARBOXYLASE ACTIVITY AND POLYAMINE CONCENTRATIONS IN RATS

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Summary: After a single high dose (20 mg/100 g i.v.) of suramin ornithine decarboxylase activity was increased rapidly in the rat kidney. Enzyme kinetic measurements indicate that on the basis of K_m values renal ornithine decarboxylases from control or suramin treated rats are indistinguishable. Renal nucleic acid and polyamine levels were also enhanced in response to suramin. Changes observed in this study are considered as biochemical signs of induced renal growth.

Introduction

It has recently been shown that a single high dose (20 mg/100 g i.v.) of suramin (Bayer 205, Germanin), a trypanocidal drug used in the human therapy and prophylaxis of sleeping sickness induced renal hypertrophy in rats (1). Histological findings indicated that regenerative processes were involved in suramin-induced renal growth which predominantly localized in the proximal tubules, whereas no changes were seen in the glomeruli or medulla. Several lines of evidence suggest that the concentration of polyamines (spermidine and spermine) and their biosynthetic enzymes is high in actively proliferating animal tissues and increases rapidly when growth or differentiation is induced in resting cells (2). The present experiments were undertaken to study renal ornithine decarboxylase (the first enzyme in the pathway for polyamine synthesis) activity and polyamine levels at various times following a single i.v. injection of suramin.

Materials and Methods

Chemicals were purchased from the following sources: L-ornithine monohydrochloride (Reanal, Hungary), DL-[1-14 C]

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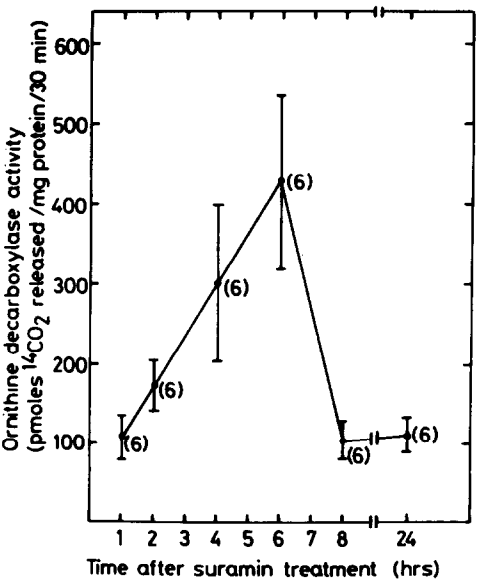

Fig. 1. Ornithine decarboxylase activity in rat kidney after suramin treatment. Rats were injected with a single dose (20 mg/100 g i.v.) of suramin. At various intervals after treatment as indicated on the abscissa the animals were sacrificed and kidney ornithine decarboxylase activities assayed as described in Materials and Methods. Values are given as means ± S.E.M. In parentheses number of rats.

ornithine monohydrochloride, specific activity 1.57 mCi per mmole (Isotope Institute of the Hungarian Academy of Sciences), dithiothreitol (Serva), pyridoxal-5-phosphate (Merck), spermidine. 3HCl (Serva), spermine. 4HCl (Serva), ribonucleic acid from chicken erythrocytes (Reanal, Hungary), hyamine hydroxide (New England Nuclear), suramin (sodium salt of 8-[3-benzamido--4-methylbenzamido] naphtalene-1,3,5-trisulphonic acid, mol wt 1492) was generously provided by Bayer (Leverkusen, Federal Republic of Germany).

Male albino rats of inbred strain (CFY) ranging in weight from 130 to 160 were used. Suramin dissolved in distilled water as a 10 % solution was injected intravenously into the tail vein at a single dose of 20 mg/100 g body weight. Controls received physiologic saline (0.2 ml/100 g i.v.). Food and water were available ad libitum. At different intervals after treatment rats were bled under light ether anaesthesia at the same period of day (between 9 and 10 a.m.), kidneys were promptly removed, decapsulated, blotted dry on filter paper, weighed and immediately processed for RNA (3), DNA(4) and polyamine determination (5). In enzyme studies the animals were sacrificed at times as indicated in Fig 1.

Conditions for the electrophoretic separation of polyamines. Macherey-Nagel (MN-214) chromatography paper (25x4cm), citrate buffer (0.1 M, pH 3.6), 115 V for 80-90 min. The spots developed with the ninhydrine reagent were cut, eluted and read in a spectrophotometer at 505 nm. The intensity of color was found to be

linear with the quantity of polyamines analysed between 5-50 nmoles as checked by standards. Unknown polyamine concentrations were calculated from calibration curves contructed using six different concentration points and expressed as nmoles per g fresh tissue.

Ornithine decarboxylase (L-ornithine carboxy-lyase, EC 4.1.1.17) assay involved the slight modifications of procedure described (6). Kidney was homogenized in a glass-teflon tube in ice cold KH_2PO_4 buffer (0.05 M, pH 7.2) medium containing dithiothreitol (5 mM), The homogenate (5 ml/g wet weight) was centrifuged at 20,000 x g for 20 min at 5°C. The supernatant fraction was used as source of enzyme. Composition of the incubation mixture: KH_2PO_4 (0.05 M, pH 7.2), dithiothreitol (2.5 mM) pyridoxal-5-phosphate (0.1 mM), L-ornithine (1.0 mM), DL[1-^{14}C] ornithine, 0.1 uCi/vial, kidney extract (0.1 ml) in a total volume of 0.5 ml. All components of the system except substrate were agitated for 10 min at 37°C prior addition of substrate, after which the vials were sealed air tight with a rubber stopper from which was suspended a strip of Whatman 1 filter paper impregnated with 50 ul of hyamine hydroxide (1 M). The incubation was continued for 30 min at 37°C. The reaction was terminated with 0.2 ml of trichloroacetic acid (50 %) injected through the self sealing rubber stopper. The mixture was agitated for an additional 60 min at 37°C to allow complete absorption of the $^{14}CO_2$ evolved. The filter papers were then transferred into scintillation vials and radioactivities counted in the presence of 10 ml Bray solution using a liquid scintillation counter (Tracerlab), with an efficiency of 50-60 %. Assays were run in duplicate, blanks were lacking the enzyme. Enzyme activity is given as pmole CO_2 released/mg protein/30 min. Protein was determined by the method of Lowry et al. (7) using bovine albumin as standard. Results were analysed for significace by Student's t test.

Results and Discussion

A single high dose (20 mg/100 g i.v.) of suramin resulted in a rapid approximately four-fold increase of renal ornithine decarboxylase activity reaching its peak at 6 h following treatment and returning to normal level by 8 h (Fig 1.). We compared the kinetic properties of ornithine decarboxylase from kidneys of control and suramin treated rats and found apparent Km values for the substrate 0.51 mM and 0.56 mM (Fig 2.), respectively. This finding appears to indicate that on the basis of Km values the renal ornithine decarboxylases from control or suramin treated rats are indistinguishable.

Renal nucleic acid and polyamine levels were also altered in response to suramin. RNA content was increased significantly at day 2, whereas no significant differences in RNA concentrations

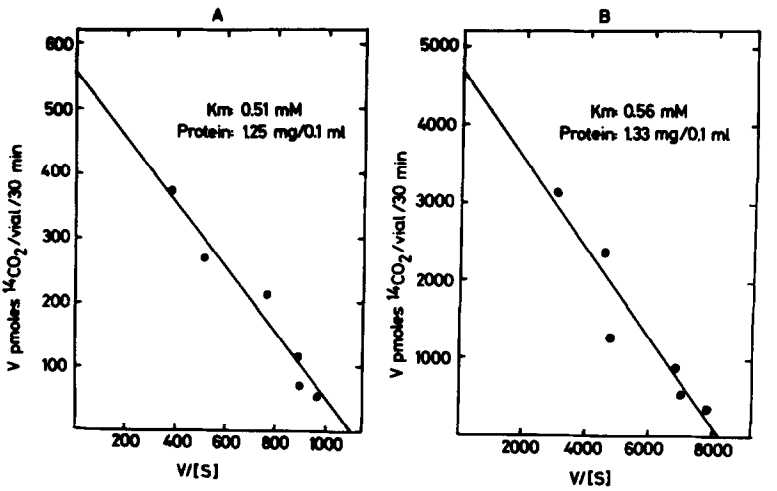

Fig. 2. Comparison of kinetic properties of ornithine decarboxylases from intact (A) and suramin treated (20 mg/100 g i.v.) rat kidney (B). Augustinson-Hofstee plot. The ornithine decarboxylase activity was assayed in 0.1 ml of kidney extract as described in Materials and Methods with variatious in the concentrations [S] of L-ornithine between 0.057-1.032 mM, Ordinate: velocity (V), abscissa: V/[S]. Km values calculated by changing the sign of the negative slopes of the lines are indicated. Protein contents in 0.1 ml of kidney extract are also presented.

were seen at other days tested. DNA decreased during the first days being lower significantly than control at days 2 and 3. This was followed by an accumulation of DNA that reached significance at day 7 when maximal mitotic response by proximal tubule cells has been shown to occur under the effect of suramin (1). Renal spermidine concentration started to rise simultaneously with that of RNA at day 2, thereafter increased further until day 7, while at day 11 (last day of study) did not differ significantly from control. Spermine level was elevated significantly only at day 7 coinciding with the maximal DNA response.

Following the i.v. administration of suramin a high concentration is achieved in the plasma. This falls fairly rapidly for a few hours then more slowly for a few days after which a low concentration is maintained for as long as 3 months. The persis-

Table I.

Effect of suramin on renal nucleic acid and polyamine level in rats

Days after suramin (20 mg/100 g i.v.) treatment	RNA mg/g fresh kidney	DNA mg/g fresh kidney	Spermidine nmole/g fresh kidney	Spermine nmole/g fresh kidney
Control[a]	± 4.274 ± 0.811 (8)	± 8.065 ± 1.023 (8)	± 471.3 ± 85.2 (7)	± 563.1 ± 137.9 (7)
1	± 4.116 ± 0.483 (7)	± 7.467 ± 0.966 (7)	± 493.9 ± 98.0 (7)	± 550.5 ± 183.5 (7)
2	± 5.379** ± 0.864 (8)	± 6.264[x] ± 0.638 (8)	± 588.1* ± 113.5 (8)	± 673.8 ± 90.0 (8)
3	± 4.398 ± 0.490 (8)	± 6.596*** ± 0.728 (8)	± 591.9* ± 107.1 (8)	± 555.0 ± 70.4 (8)
5	± 3.822 ± 0.183 (10)	± 8.887 ± 0.697 (10)	± 639.1*** ± 105.8 (10)	± 685.1 ± 132.4 (10)
7	± 4.104 ± 0.274 (9)	± 12.792[x] ± 1.257 (9)	± 678.7*** ± 120.7 (9)	± 746.6** ± 87.6 (9)
11	± 3.414 ± 0.374 (11)	± 8.938 ± 1.328 (11)	± 566.8 ± 103.7 (12)	± 603.3 ± 46.8 (12)

[a]studied 48 hrs after physiologic saline injection. Values represent means ± S.D. Number of observations are in parentheses. Statistically different from control (Student's t test).

*p<0.05; **p<0.02; ***p<0.01; [x]p<0.001.

tence of suramin in the circulation is due to its firm binding to plasma proteins (8). High concentrations of suramin might even provoke an unfolding of the albumin molecules (9). In experimental animals suramin appeared in the course of the first few hours in the epithelial cells of the proximal tubules of the kidney, being visible even after one or two months where it is stored in combination with a protein carrier (10). Renal ornithine decarboxylase activity has been shown to increase under the effect of various stimuli such as after unilateral nephrectomy (11), adrenal cortical hormones, ACTH, growth hormone (11-15), serum growth factor (12) or other hormonal treatments (16). Folic acid capable of inducing renal hypertrophy in rats (17) has also been reported to elicit an early increase of renal ornithine decarboxylase activity and changes in polyamine levels (18). Fol-

lowing gonadectomy ornithine decarboxylase activity was reduced in kidneys of male mice (19). In the intact animal adapted to a light/dark cycle, the level of renal ornithine decarboxylase activity was rhytmical and paralleled the diurnal rhytm in plasma corticosteroid concentration (14). Other data suggest a synergism between insulin and pituitary hormones in the control of ornithine decarboxylase over a prolonged period of time (20). In view of these facts we suggest that the effect on the enzyme by suramin may not be a specific one but is rather mediated by local and/or hormonal factors. Attempts to detect augmented ornithine decarboxylase activity in the kidney at further days of suramin treatment failed. This does not necessarily rule out the occurrence of further enhancement(s) in activity since the extremely short half life (10 min) of the enzyme (21) constitutes a major obstacle in demonstrating such changes. It is not possible to decide unequivocally whether renal ornithine decarboxylase was induced or only stabilized in response to suramin. Stabilization of ornithine decarboxylase in rat liver has recently been published as a result of treatments with thioacetamide and carbon-tetrachloride (22). The sequence of the biochemical events observed in this study in terms of polyamine and nucleic acid metabolism is considered as characteristic of regeneration. Therefore it is suggested that the suramin-induced renal hypertrophy might be a good model to study such changes in the kidney.

References

1. Selmeci, L., Szepeshazi, K., Posch, E., and Harsing, L. (1978) Experientia 34, 219-220.
2. Tabor, C.W., and Tabor, H. (1976) Ann.Rev.Biochem. 45, 285-306.
3. Fleck, A., and Munro, H.N. (1962) Biochim.Biophys.Acta 55, 571-583.
4. Schneider, N.C. (1957) Methods of Enzymology pp 680-684, Academic Press, New York
5. Inoue, H., and Mizutani, A. (1973) Anal.Biochem. 56, 408-416.

6. Russel, D., and Snyder, S.H. (1968) Proc.Nat.Acad.Sci. 60, 1420-1427.
7. Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951) J.Biol.Chem. 193, 265-275.
8. Rollo, J.M. (1975) The Pharmacological Basis of Therapeutics (L.S.Goodman and A.Gilman, eds.) Macmillan, New York, pp 1081-1089.
9. Muller, W.E., and Wollert, U. (1976) Biochim.Biophys.Acta 427, 465-480.
10. Jancso, N., and Jancso-Gabor, A. (1952) Nature 170, 567-568.
11. Brandt, J.T., Pierce, D.A., and Fausto, N. (1972) Biochim. Biophys.Acta 279, 184-193.
12. Sogani, R., Matsushita, S., Muller, J., and Ruben, M. (1972) Biochim. Biophys. Acta 279, 377-386.
13. Holladay, L.A., Levine, J.H., Nicholson, W.E., Orth, D.N., Salmon, W.D.Jr., and Puett, D. (1975) Biochim.Biophys.Acta 381, 47-60.
14. Nicholson, W.E., Levine, J.H., and Orth, D.N. (1976) Endocrinology 98, 123-128.
15. Andersson, A.C., Henningsson, S., and Rosengren, E. (1975) Experientia 31, 1101-1103.
16. Scalabrino, G., and Ferioli, M.E. (1976) Endocrinology 99, 1085-1090.
17. Taylor, D.M., Threlfall, G., and Buck, A.T. (1966) Nature 212, 472-474.
18. Raina, A., and Jänne, J. (1970) Fed.Proc. 29, 1568-1574.
19. Henningsson, S., and Rosengren, E. (1975) J.Physiol.Lond. 245, 467-479.
20. Sochor, M., Baquer, N.Z., Hothersall, J.S., and McLean, P. (1978) Biochem. Biophys. Res. Commun. 80, 533-539.
21. Russel, D.H., and Snyder, S.H. (1969) Mol.Pharmacol. 5, 253-262.
22. Pösö, H., Guha, S.K., and Jänne, J. (1978) Biochim.Biophys. Acta 524, 466-473.