ACTIVITY OF SOME ENZYMES IN DIFFERENT LAYERS OF THE RAT KIDNEY IN EXPERIMENTAL NEPHRITIS

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UDC 616.61-002-092.9-008.931

In rats with experimental cytotoxic nephritis the lactate dehydrogenase and succinate dehydrogenase activity in the cortex and medulla of the kidneys is reduced. The activity of Mg-, Na-, and K-adenosinetriphosphatase is unchanged.

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The results of investigation of the human and animal nephron have shown considerable enzymic differentiation between its various component parts. The glomeruli contain relatively small quantities of enzymes, while the cells of the proximal and distal tubules have a much higher content of the various enzymes, including enzymes of the Krebs cycle and of oxidative phosphorylation. The descending and ascending parts of the loop of Henle are comparatively poor in enzymes [12, 13].

It is assumed that alkaline phosphatase in the brush border of the proximal tubules plays an essential role in glucose reabsorption [7], succinate dehydrogenase is connected with sodium reabsorption [1-5, 8], and Mg-dependent and Na- and K-activated adenosinetriphosphatases participate in sodium transport through the cell membrane [11, 16, 17].

It was decided to study quantitative changes in the activity of lactate dehydrogenase (LDH), succinate dehydrogenase (SDH), and Mg-, Na-, and K-adenosinetriphosphatases in the cortical, medullary, and papillary layers of the kidney.

EXPERIMENTAL METHOD

Experimental cytotoxic nephritis was produced in albino rats weighing 200-250 g by Mazuga's method (titer of nephrotoxic serum 1:11,000). The presence of nephritis was confirmed by clinical, biochemical, and histological investigations. Evidence of a moderate proliferative intracapillary glomerulonephritis was observed. Enzymes of the kidney tissue were investigated 7 days after the end of immunization.

LDH activity was determined in the supernatant (12,000 g) of a homogenate (1:10) of the cortex and medulla from the decrease in optical density at 340 mμ in the presence of pyruvate [6]. The protein content in the homogenates was determined by Kalkar's method [14]. SDH activity was determined in the mitochondria of the renal cortex and medulla. Protein in suspensions of mitochondria was determined by the biuret method; SDH activity by a modified colorimetric method [9] based on oxidation of succinate in the presence of 2,6-dichlorophenolindophenol (DCPI) in the presence of phenazine metasulfate as catalyst of electron transfer. The course of the reaction was followed from the decrease in intensity of DCPI coloration at 600 mμ. The reaction mixture contained 2.4 ml 0.1 M phosphate buffer, pH 7.4, 0.1 ml of 0.01 M KCl solution, 0.1 ml of 0.1 M succinate solution, 0.2 ml of suspension of mitochondria (0.2-0.3 mg mitochondrial protein of the cortex and medulla), 0.1 ml of $6.7 \cdot 10^{-4}$ M DCPI solution, and 0.1 ml of 0.1% phenazine metasulfate solution. The change in E_{600} against water both for the experimental sample and for the control containing all components except succinate was measured. The change in E_{600} per minute due to the action of SDH was given by the difference between the experimental and control values. Each experiment and control test was repeated twice. To determine the molar absorption of DCPI, E_{600} of the same

Department of Biochemistry, I. P. Pavlov First Leningrad Medical Institute. Laboratory of Experimental and Clinical Hematology, I. P. Pavlov Institute of Physiology, Academy of Sciences of the USSR, Leningrad. (Presented by Academician V. N. Chernigovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 68, No. 9, pp. 52-55, September 1969. Original article submitted February 27, 1969.

TABLE 1. Activity of Mg-, Na-, K-Adenosinetriphosphatase, Lactate Dehydrogenase, and Succinate Dehydrogenase in Kidney of Control Rats and Rats with Experimental Nephritis (M ± m)

Rats	Mg-, Na-, K-ATPAse (in μg P/h/mg protein)			Lactate dehydrogenase		Succinate dehydrog.	
				m μmoles/min/mg protein			
	cortical layer	medullary layer	papillary layer	cortical layer	medullary layer	cortical mitochondria	medullary mitochondria
Control	84,6±2,7 (24)	78,4±4,2 (24)	42±3,2 (24)	630±29,1 (11)	728±30,1 (11)	39,0±4,2 (7)	25,2±2,2 (7)
With experimental nephritis	80,8±7,0 (12)	64,8±7,0 (13)	38,6±4,5 (12)	448±38,4 (12)	471±13,3 (12)	19,8±2,0 (12)	17,0±2,3 (12)

quantity of DCPI as in the experiment with 2.9 ml of buffer solution was measured. The method of quantitative estimation of SDH activity described above has many advantages over the method of its determination using tetrazolium salts in the presence of which formazan is formed without succinic acid, creating a high background. Activity of Mg-, Na-, and K-adenosintriphosphatases was determined in a 10% homogenate of the cortical, medullary, and papillary layers of the kidney. The incubation medium used to determine activity contained 0.1 ml homogenate of the appropriate layer of the kidney (0.5-2 mg protein). 0.9 ml of 0.2 M tris-HCl buffer, pH 7.2, 0.5 ml NaCl (80 μmoles Na^+), 0.5 ml KCl (20 μmoles K^+), 0.5 ml $MgCl_2$ (5 μmoles Mg^{++}), and 1 ml of the sodium salt of ATP (5 μmoles ATP). After incubation for 30 min at 37° the reaction was stopped by the addition of 1.5 ml of 10% TCA. Activity of the enzyme was judged from the increase in inorganic phosphate, determined by the Kjeldahl method.

Activity of the enzymes was investigated over a linear segment of the graph of reaction velocity as a function of protein content.

EXPERIMENTAL RESULTS AND DISCUSSION

Definite protein urea was observed in the rats with experimental nephritis, and leukocytes and erythrocytes appeared in the urine. The serum urea concentration was the same as in the control animals. The serum electrolyte concentrations in the immunized rats also were unchanged. At the same time, the elimination of potassium and, in particular, of sodium in the urine was substantially reduced: from 98.0 ± 1.4 to 66.8 ± 9.9 meq/liter ($P < 0.05$) and from 190.0 ± 1.6 to 49.8 ± 5.8 meq/liter ($P < 0.01$), respectively.

In the renal cortex of healthy rats SDH activity was much higher than in the medulla (Table 1), in agreement with data in the literature [5, 8]. The activity of Mg-, Na-, K-adenosinetriphosphatase fell successively from the cortical layer to the papillary. LDH activity was distributed about equally in the cortical and medullary layers of the rat kidneys, in almost exactly the same way as the activity of this enzyme in whole kidney homogenate [6].

In experimental nephritis a statistically significant ($P < 0.01$) decrease in LDH and SDH activity was observed in both the cortex ($P < 0.01$) and medulla of the kidneys ($P < 0.05$). Although as regards the activity of Mg-, Na-, K-adenosinetriphosphatase a tendency was observed for this to decrease in various parts of the kidney, the change was not statistically significant.

The decrease in LDH activity in the kidney in experimental nephritis is in agreement with data in the literature [10, 12, 15] describing increased LDH activity in the urine of rabbits, dogs, and rats with toxic injury to the nephron, or infarction and ischemia of the kidney, unaccompanied by changes in the activity of this enzyme in the blood serum. It may be postulated that the increase in LDH content in the urine is associated with elimination of the enzyme from the kidney. The decrease in activity of this enzyme in the renal cortex and medulla in experimental nephritis suggests a disturbance of oxidation — reduction in the kidney and changes in metabolism both in the cortex, where it is aerobic in nature, and in the medulla, characterized by a high level of glycolysis.

The decrease in SDH activity in the cortical and medullary mitochondria of the kidney suggests a decrease in the intensity of oxidation in the tissues in nephritis. The absence of changes in Mg-, Na-, K-adenosinetriphosphatase activity indicates that in this particular experimental disease the processes of sodium transport connected with this system remain undisturbed.

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