ACTIVITY OF KIDNEY TISSUE ENZYMES IN PHENYLHYDRAZINE ANEMIA AND POSTTRANSFUSIONAL POLYCYTHEMIA

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Activity of hexokinase, glucose-6-phosphate dehydrogenase, lactate and succinate dehydrogenases, and acid and alkaline phosphatases in kidney tissue was investigated. At the same time the blood supply to the cortex and medulla of the kidneys was studied in rats with phenylhydrazine anemia and posttransfusional polycythemia in order to discover any possible connection between changes in the activity of the above enzymes and the erythropoietin-forming function of the kidneys. The blood supply of the kidneys in rats with phenylhydrazine anemia was sharply reduced, whereas in animals with posttransfusional polycythemia it was considerably increased. No changes were found in enzyme activity in the kidney tissue. The results are evidence that the activity of these enzymes is not a regulating factor in the production of erythropoietin by the kidney.

KEY WORDS: *kidney tissue enzymes; blood supply; anemia; polycythemia.*

The participation of kidneys in the production of a factor stimulating erythropoiesis is no longer in question. However, the mechanism and site of formation of the erythropoietic factor (erythropoietin) by the kidneys remains uncertain [2, 3].

Under the influence of erythropoietic stimulants a decrease in the oxygen uptake and the accumulation of para-aminohippuric acid and of ions in the kidney tissue are observed [4, 7, 14]. A decrease in the ATP concentration and an increase in the concentrations of AMP and inorganic phosphorus [8], an increase in the molecular forms of lactate dehydrogenase (LD) responsible for activation of anaerobic glycolysis, and accumulation of lactate (M forms) have been discovered [9, 10, 13]. In order to discover any possible connection between changes in the activity of various enzymes in the kidneys and their erythropoietin-forming function it was decided to investigate the activity of hexokinase (HK), the key enzyme of glycolysis, of glucose-6-phosphate dehydrogenase (G6PD), one of the principal enzymes of the hexose monophosphate pathway of glucose metabolism, of lactate dehydrogenase (LD) and succinate dehydrogenase (SD), which are oxidoreductases, and of the hydrolytic enzymes acid and alkaline phosphatase (AcP and AlP).

Meanwhile the blood supply of the cortex and medulla of the kidneys was studied in rats with phenylhydrazine anemia and posttransfusional polycythemia.

EXPERIMENTAL METHOD

Anemia was induced in male albino rats weighing 180–200 g by subcutaneous injection of a 2% alcoholic solution of phenylhydrazine sulfate. Polycythemia was induced by two intraperitoneal injections of 5–7 ml of an 80% suspension of homologous erythrocytes washed with physiological saline. The cortex and medulla of the kidney were homogenized in the cold in 0.154 M KCl solution (1:10) and centrifuged in a refrigeration centrifuge at 12,000g. Protein in the supernatant of the homogenate was determined spectrophotometrically. G6PD activ-

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TABLE 1. Blood Supply to Kidneys in Rats with Phenylhydrazine Anemia and Posttransfusional Polycythemia (M ± m)

	Relative volume of capillary system, %		Number of erythrocytes in glomeruli	
	cortex	medulla	cortex	juxtamedullary layer
Healthy rats	0,55±0,13	1,82±0,45	17,0±0,56	7,7±0,82
Rats with anemia	0,13±0,05	0,30±0,04	6,8±0,59	4,5±0,89
Rats with polycythemia	1,98±0,39	3,01±0,40	54,05±0,9	21,1±1,32

TABLE 2. Enzyme Activity (in nmoles/min·mg) of Kidney Tissue in Control Rats and in Rats with Phenylhydrazine Anemia and Posttransfusional Polycythemia (M ± m)

Enzyme	Control ($n=15$)		Anemia ($n=20$)		Polycythemia ($n=20$)
HK:					
cortex	12,1±0,5		11,8±0,68		8,8±0,65
P		>0,05		<0,05	
medulla	30,1±1,4		29,5±1,5		18,6±0.74
P		>0,05		<0,01	
LD:					
cortex	650±25,2		580±26,2		570±28.6
P		>0,05		<0,01	
medulla	750±40,5		676±52,3		637±16.0
P		>0,05		<0,01	
G6PD:					
cortex	13,5±0,9		14,7±0,82		13,3±0,76
P		>0,05		>0,06	
medulla	24,4±1,3		20,2±0,91		19,6±0,65
P		>0,05		>0,05	

ity was determined from the absorption of light (at 340 nm) by the NADPH formed [11], HK activity from the absorption of light (at 340 nm) by NADPH formed by combination of the hexokinase and G6PD reaction [15], and LD activity by the absorption of light by NAD (at 340 nm) formed in the reaction of conversion of pyruvate into lactate. Activity of the enzymes was expressed in nmoles NADPH or NAD formed per minute per milligram protein. Activity of the enzymes in the kidney tissue was determined in parallel experiments by quantitative histochemical methods. The kidney was frozen with liquid nitrogen and sections were cut to a thickness of 6 μ in a cryostat. Activity of LD, SD, G6PD [6], AlP (by the azo-coupling method), and AcP [5] was determined. Photomicrographs of the preparations were obtained with the MUF-6 instrument and these were examined photometrically with the MV-2 apparatus. The blood supply to the kidneys was studied by measuring the relative volume of the capillary system of the renal cortex and medulla by a stereological method [1]. The blood supply to the glomeruli of the cortical and juxtamedullary layers of the kidneys was investigated in the same sections by counting the number of erythrocytes in the capillaries of the glomeruli directly. The enzyme activity and blood supply of the kidneys were determined at the height of development of anemia and polycythemia (hematocrit index 25.6 ± 2.3 and 66.8 ± 3.60%, respectively).

EXPERIMENTAL RESULTS

In posttransfusional polycythemia the relative volume of the capillary system in the cortex was increased by 3.6 times and in the medulla by 1.6 times. The blood supply increased almost equally in the cortical and juxtamedullary glomeruli (by 3.2 and 2.7 times, respectively). In phenylhydrazine anemia the relative volume of the capillary system was reduced in the cortex by 75% and in the medulla by 83%. The blood supply in the cortex was reduced by 60% and in the juxtamedullary layer by 41% (Table 1).

In phenylhydrazine anemia no changes were observed in the activity of the enzymes studied (Table 2). In polycythemia activity of HK and LD in the cortex and medulla of the kid-

TABLE 3. Enzyme Activity (in conventional units) in Proximal Convoluted Tubules of Rats with Phenylhydrazine Anemia and Posttransfusional Polycythemia

Enzyme	Polycythemia	Control for polycythemia	Anemia	Control for anemia
SD	0,61±0,07	0,65±0,06	0,40±0,02	0,42±0,03
LD	0,30±0,02	0,37±0,03	0,29±0,02	0,29±0,02
G6PD	0,15	0,15	0,15	0,12
AcP	0,52±0,03	0,56±0,04	0,55±0,55	0,59±0,08
AlP	0,78±0,03	0,89±0,04	0,60±0,02	0,56±0,07

Legend. Differences between indices in animals with polycythemia and anemia and control animals significant ($P < 0.05$).

neys was reduced. On histochemical investigation of SD, LD, G6PD, AlP, and AcP in ten animals of each group, no changes in their activity were found (Table 3). In rats with phenylhydrazine anemia the blood supply to the kidneys is thus sharply reduced, but the activity of the enzymes of glycolysis and of the hexose monophosphate pathway of glucose metabolism, as well as of oxidoreductases and hydrolases, is unchanged. This is evidence of the high adaptive powers of the enzyme systems. In rats with posttransfusional polycythemia, a considerable increase in the blood supply to the kidneys is accompanied by a decrease in HK and LD activity, especially in the medulla, where anaerobic glycolysis predominates. These changes are evidently the result of the inhibitory effect of oxygen on glycolysis (the Pasteur effect). Similar results were obtained by a study of the effect of oxygen on oxidation of glucose in the various layers of the rabbit kidney [12].

The absence of changes in enzyme activity in the kidney tissue in anemia, accompanied by the production of factors stimulating erythropoietin, and during polycythemia when erythropoietin formation is inhibited, is evidence that the activity of the enzyme studied is not the controlling factor in the production of erythropoietin by the kidney.

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