CHANGES IN ACTIVITY OF ENZYMES CONVERTING GLUCOSE-6-PHOSPHATE IN THE RAT TESTIS DURING HYPOXIA

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Hexokinase activity in the rat testis was lowered during acute, but raised during chronic hypoxic hypoxia. Activity of glucose-6-phosphate dehydrogenase was raised in both types of hypoxia, but more so in the chronic form.

Male sex hormones are highly sensitive to hypoxia. Even mild degrees disturb spermatogenesis [20]. The biochemical nature of these disturbances is not yet explained. Some workers [13, 18, 19] attribute the increased sensitivity of the testes to hypoxia to the special character of its glucose metabolism. The crucial link of glucose metabolism is its phospholytic conversion into glucose-6-phosphate (G6P), which is catalyzed by hexokinase (HK) [1, 11, 14, 16]. An important place among the subsequent transformations of G6P is occupied by the pentose phosphate cycle, the trigger stage of which is catalyzed by a dehydrogenase (G6P DH) [12], generating the carbohydrate component of nucleic acids consitituting the chemical basis of the spermatozoa. It is important to study the activity of these enzymes during hypoxia. Experimental data on this problem are few in number and are concerned primarily with assessment of enzyme activity of the brain [7], skeletal muscles [7, 8], and erythrocytes [9]. Evidence has recently been obtained [2-4] of the important role of hydrocortisone in the regulation of hexokinase activity in the liver and skeletal muscles, but with respect to the testes this problem has received little study.

This paper describes the results of a comparative investigation of activity of the enzymes HK (2.7.1.1) and G6P DH (1.1.1.49) in the rat testis during different forms of hypoxic hypoxia, and also after administration of hydrocortisone.

EXPERIMENTAL METHOD

Experiments were carried out on Wistar rats weighing 150-200 g. Acute hypoxia was produced by keeping the animals for 1.5 h in a pressure chamber at 200 mm Hg, and chronic hypoxia by keeping the animals for 5 weeks, for 6 h daily, in a chamber with a pressure of 260 mm Hg. Hydrocortisone was injected intramuscularly for 3 days in a daily dose of 5 mg/100 g body weight. The hormone was injected into the "hypoxic" rats during the 3 days when they were kept in the pressure chamber. Because of the large volume of blood in the testes [10], they were washed thoroughly to remove all the blood, freed from membranes, and homogenized in the cold with 5 volumes of 0.25 M sucrose solution and 0.002 M EDTA solution. The homogenate was centrifuged at 4° for 45 min at 45,000 g. Activity of HK and G6P DH was determined spectrophotometrically in the supernatant [15]. The composition of reaction mixture (in mmoles) for determination of HK was: sodium salt of ATP 5, NADP 0.25, $MgCl_2$7, tris buffer (pH 7.6) 100, G6P DH (free from HK) 0.2 unit, glucose 0.5, and supernatant 0.08 ml, total volume 3 ml; for determination of G6P DH the composition was: NADP 0.3, $MgCl_2$7, tris-buffer (pH 7.6) 100, sodium salt of G6P 4.5, supernatant 0.08 ml. Protein was determined by Lowry's method [17].

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TABLE 1. Activity of HK (in μ moles NADP reduced/10 min/10 mg protein) and of G6P DH (in units of change of optical density at 340 mμ/10 min/10 mg protein) in Rat Testes in Hypoxia and after Administration of Hydrocortisone (M ± m)

Experimental conditions	HK activity	G6P DH activity	Weight of organ, g
Control	1,45±0,18 (22)	1,90±0,13 (23)	1,32±0,05 (14)
Acute hypoxia	0,81±0,11 —44%*, $P<0,05$ (11)	3,26±0,39 71%*, $P<0,01$ (11)	1,23±0,02 —7%*, $P>0,05$ (15)
Chronic hypoxia	2,22±0,10 53%*, $P<0,01$ (20)	10,29±0,86 441%*, $P<0,001$ (18)	0,64±0,03 —51%*, $P<0,001$ (16)
Control + hydrocortisone	1,66±0,19 14%, $P>0,05$ (14)	2,26±0,13 19%, $P>0,05$ (25)	1,24±0,01 —9%*, $P>0,05$ (16)
Chronic hypoxia + hydrocortisone	2,55±0,18 74%*, $P<0,001$ (20)	11,64±0,81 512%, $P<0,001$ (18)	0,69±0,02 —48%, $P<0,001$ (16)

Note. Number of experiments shown in parentheses.

*Changes relative to control.

EXPERIMENTAL RESULTS AND DISCUSSION

HK activity in the testis in acute hypoxia was lowered by 44%, and in chronic hypoxia it was raised by 53% (Table 1). Activity of G6P DH was increased in both forms, by 71 and 441%, respectively. The weight of the organ remained unchanged in acute hypoxia, but was reduced by 51% in chronic hypoxia, indicating the occurrence of atrophy. The decrease in hexokinase activity observed in the acute experiments agreed with the results obtained by Pomytkin [7], who observed a decrease in HK activity in the rat testis during hypoxemic hypoxia of short duration, and also with results [8] indicating a decrease in HK activity of the brain and skeletal muscles of animals exposed to various types of acute hypoxia. The initial response of tissues, including those of the testis, to acute hypoxia is evidently uniform in type and consists of slowing of the utilization of glucose. The increase in HK acitivity during chronic hypoxia can probably be regarded as a compensatory phenomenon aimed at increasing the energy potential of the cells through an increase in the rate of phosphorylation of glucose.

In contrast to HK, the G6P DH activity was raised both in the acute and, in particular, in the chronic experiments. Evidently G6P metabolism in the testis is switched over predominantly to the pentose-phosphate pathway, which not only maintains the ATP and NADP-H_2 levels, but also supplies pentoses required for synthesis of nucleic acids, the metabolism of which is disturbed [5]. The specific shift toward the pentose cycle can evidently be regarded as a manifestation of biochemical adaptation of the testis to hypoxia. Changes in the isoenzyme spectrum of G6P DH in the rat testis observed previously [6] in experiments to study chronic hypoxia suggest that the increase in activity of the enzyme protein was due to its more rapid synthesis. Following injection of hydrocortisone, no changes were observed in HK or G6P DH activity whether in intact or in "hypoxic" rats. The enzymes of G_6P metabolism in the testis studied in the present experiments, by contrast to those of the liver [2], are evidently less sensitive to the dose of hydrocortisone used.

The results obtained thus indicate specific changes in the enzyme metabolism of the testis. The intensity and direction of these changes depend on the degree and duration of hypoxia.

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