EFFECT OF INSULIN AND HYDROCORTISONE ON HEXOKINASE AND GLUCOSE-6-PHOSPHATE DEHYDROGENASE ACTIVITY IN THE TESTES OF RATS EXPOSED TO ACUTE HYPOXIA

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Administration of insulin to intact animals did not alter the hexokinase activity but increased glucose-6-phosphate dehydrogenase (G6PD) activity whereas administration of hydrocortisone inhibited the activity of both enzymes. If the hormones were injected simultaneously the inhibitory effect of the glucocorticoid was not manifested. In acute hypoxia injection of insulin restored to normal the reduced hexokinase activity and sharply increased G6PD activity, whereas injection of hydrocortisone reduced the activity of hexokinase alone. When both hormones were injected simultaneously, G6PD activity was increased while hexokinase activity remained inhibited.

The participation of insulin and glucocorticoids in the regulation of enzymes of glucose-6-phosphate metabolism in the tissues of the testes during exposure to chronic hypoxia was demonstrated previously [3].

In the investigation described below the effect of insulin and hydrocortisone on the activity of hexokinase and glucose-6-phosphate dehydrogenase (G6PD) in the rat testes was studied during exposure to acute hypoxia.

EXPERIMENTAL METHOD

Wistar rats weighing 150-210 g were used. Acute hypoxia was produced by keeping the animals in a ventilated pressure chamber for 1.5 h (P=200 mm Hg). The hormones were injected before the animals were placed in the pressure chamber, intramuscularly, for a period of three days: insulin in a dose of 0.5 units twice a day, hydrocortisone (Gedeon Richter, Hungary) in a dose of 10 mg/100 g body weight once daily. Intact rats served as the control. The testes were washed to remove all the blood, the membranes were removed, and 0.5 g of the material was homogenized for 2 min in the cold with five volumes of 0.5 M sucrose and 0.002 M EDTA. The homogenate was centrifuged at 4°C for 45 min at 45,000 g on a TsVR-1 centrifuge. Hexokinase [4] and G6PD [5] activity was determined spectrophotometrically in the supernatant. The composition of the reaction mixture (in mM) for hexokinase determination was: 5 Na-ATP, 0.25 NADP, 7 $MgCl_2$, 100 tris-HCl, pH 7.6, with 0.2 unit G6PD (Fluka), 0.5 glucose, and 0.08 ml supernatant, with a total volume of 3 ml; for estimation of G6PD: 0.3 NADP, 8 $MgCl_2$, 100 tris-HCl, pH 7.6, 4.5 Na-G6P, and 0.08 ml supernatant, total volume 3 ml. Protein was determined by Lowry's method. The protein concentration was 0.7-1.2 mg in 0.08 ml supernatant.

EXPERIMENTAL RESULTS AND DISCUSSION

In intact animals (Table 1) injection of insulin did not change the hexokinase activity (the slight shift toward an increase is not statistically significant), but increased the G6PD activity by 43%; injection of

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TABLE 1. Hexokinase Activity (in μmoles NADP·H_2 in 10 min per 10 mg protein) and G6PD Activity (in ΔE_{340} in 10 min per 10 mg protein) in Testes of Rats Exposed to Hypoxia after Injection of Hydrocortisone and Insulin

Treatment	Hexokinase activity	Change in activity (%) rel. to control	G6PD activity	Change in activity (%) rel. to control
Control	1,47±0,07 (64)	--	1,90±0,09 (64)	--
Insulin	1,73±0,09 (21)	+18	2,71±0,12 (21)	+43
P	>0,05		<0,05	
Hydrocortisone	1,02±0,08 (21)	—31	1,21±0,11 (21)	—36
P	<0,05		<0,05	
Hydrocortisone + Insulin	1,61±0,12 (15)	+10	2,91±0,2 (15)	+53
P	>0,05		<0,05	
Hypoxia	0,81±0,08 (26)	—45	3,20±0,23 (26)	+68
P	<0,05		<0,001	
Hypoxia + Insulin	1,62±0,09 (18)	+10	4,41±0,20 (15)	+132
P	>0,05		<0,001	
Hypoxia + Hydrocortisone	0,50±0,02 (11)	—66	2,94±0,11 (11)	+55
P	<0,01		<0,01	
Hypoxia + Hydrocortisone + Insulin	1,11±0,09 (15)	—25	4,69±0,09 (15)	+147
P	<0,05		<0,001	

Note. Experiments in parentheses.

hydrocortisone lowered the activity of both enzymes by approximately one-third. After simultaneous injection of both hormones the hexokinase activity remained within normal limits while G6PD activity rose by 53%. The hexokinase activity is substantially lowered normally in acute hypoxia; after injection of insulin it was within normal limits, but it was reduced by two-thirds after injection of hydrocortisone; activity of G6PD, which is substantially increased in hypoxia, continued to rise after the injection of both hormones, and particularly sharply after injection of insulin (2.3 times above the control level). After simultaneous injection of insulin and hydrocortisone into the "hypoxic" rats a significant decrease in hexokinase activity and a marked increase (by 2.5 times) in G6PD activity were observed.

As the results show, the effect of insulin in inducing hexokinase synthesis, which under normal conditions is hardly perceptible, is clearly visible not only in chronic hypoxia [3], but also in the acute form. The tissue of the testes of the "hypoxic" animals proved to be twice as sensitive as the tissues of the intact animals to the repressor action of hydrocortisone [1] on hexokinase. After administration of insulin, the inhibitory effect of hydrocortisone was completely abolished in the control animals and sharply reduced in the "hypoxic" rats. The glucose phosphorylation reaction catalyzed by hexokinase is thus controlled by insulin in the testes as in other organs [1, 6]. Under hypoxic conditions prophylactic administration of insulin has a distinct normalizing action. Its mechanism remains unknown. Insulin had an even stronger stimulating action on G6PD, the activity of which, unlike hexokinase, was increased not only in the hypoxic but also in the intact animals. These results agree with those obtained by Rudack et al. [7] showing that insulin strongly induces G6PD synthesis in liver tissue. The reaction of stimulation of the pentose-phosphate pathway under the influence of insulin is evidently similar in type in functionally different tissues. Meanwhile, the inhibitory effect of hydrocortisone continued to be manifested only in the intact rats and it was absent in the "hypoxic" rats. The switching of glucose-6-phosphate metabolism from glycolysis to predominantly the pentose-phosphate pathway in chronic and acute forms of hypoxia [2] was appreciably increased by insulin, and it can evidently be regarded as a manifestation of a specific form of adaptation of the testicular tissue to hypoxia.

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