Glycolysis and Oxidtion Enzyme Activity in Rat Brain During Insulin-Induced Hypoglycemia against the Background of Alloxan-Induced Diabetes Mellitus

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Insulin-induced hypoglycemic coma in animals with alloxan diabetes was observed at a higher basal glucose level in the blood compared to healthy animals. It was associated with inhibition of glycolysis and glycogenolysis and decreased activities of succinate dehydrogenase and glutamate dehydrogenase in the cerebral hemispheres and brainstem.

Key Words: *brain; insulin-induced hypoglycemia; alloxan diabetes; glycolysis; energy metabolism*

Glucose is a major plastic and energy substrate in the nervous tissue. Glycolytic energy production provides function of nerve cells [5,8,10]. Hypoglycemia induces neuronal death and development of posthypoglycemic encephalopathy [4,6]. Pathochemistry of this state requires further investigations. Hypoglycemia is often observed in insulin overdosage during therapy for diabetes mellitus. These changes determine the development of brain dysfunction [1].

Here we studied the intensity of glycolysis and glycogenolysis, measured activity of oxidative enzymes in the brain, and estimated the concentration of energy substrates in the blood from rats with alloxan diabetes accompanied by insulin-induced hypoglycemia.

MATERIALS AND METHODS

Experiments were performed on outbred albino male rats weighing 200-240 g. The animals fed a standard diet. They were deprived of food 18-24 h before the experiment, but had free access to water.

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The animals were divided into 4 groups. Group 1 included intact rats (control). Group 2 consisted of rats with hypoglycemic coma (HGC). Group 3 rats were examined 14-15 days after alloxan administration. Rats with 15-day alloxan diabetes accompanied by HGC entered group 4. Alloxan was injected intraperitoneally in a single dose of 125 mg/kg. HGC (development of seizures and loss of postural reflexes) was induced by intramuscular injection of insulin in a dose of 40 U/kg.

The intensity of glycolysis and glycogenolysis in the cerebral hemispheres and brainstem was determined by the rate of lactate accumulation in the incubation medium. Glucose, glucose-6-phosphate, and glycogen were used as the substrates [3]. Activities of glutamate dehydrogenase (EC 1.4.1.2), succinate dehydrogenase (SDH, EC 1.3.99.1), lactate dehydrogenase (EC 1.1.1.27), and glucose-6-phosphate dehydrogenase (EC 1.1.1.49) were measured spectrophotometrically [2]. The rate of a glutamate dehydrogenase-catalyzed reaction was determined in 10% nervous tissue homogenate. The intensity of lactate accumulation and activities of lactate dehydrogenase and glucose-6-phosphate dehydrogenase were estimated in the supernatant obtained after centrifugation of 10% homogenate at

14,000*g* for 15 min. Blood glucose concentration in the blood obtained during killing was measured by the enzymatic method [2]. Protein concentration was estimated by the method of Lowry. The results were analyzed by Student's *t* test.

RESULTS

Blood glucose level in healthy animals, rats with alloxan diabetes, and rats of groups 2 and 4 was 5.12±0.27, 6.64±0.45 ($p<0.05$ compared to the control), 1.52±0.14, and 2.21±0.17 mmol/liter, respectively. The differences between animals of groups 2 and 4 were statistically significant (45%, $p<0.001$). Therefore, insulin-induced HGC in rats with alloxan diabetes was observed at a higher basal level of glucose in the blood compared to healthy animals. These data suggest that alloxan diabetes is accompanied by an increase in the sensitivity to hypoglycemia. It probably results from changes in activity of enzyme systems responsible for energy supply to the brain.

The intensity of glycolysis and glycogenolysis and activity of oxidation enzymes remained unchanged in rats of groups 2 and 3 (Table 1).

The rate of lactate accumulation in the cerebral hemispheres decreased in rats with HGC on day 15 of alloxan diabetes. This effect was observed in experiments with the initial substrates of glucose (by 39%, $p<0.001$), glucose-6-phosphate (by 37%, $p<0.01$), and glycogen (by 15%, $p<0.05$). Experiments with glucose as the initial substrate showed that the decrease in lactate accumulation in the brainstem of these animals (by 12%, $p<0.02$) was accompanied by a reduction of SDH and lactate dehydrogenase activities (by 23 and 18%, respectively, $p<0.01$).

Posthyperglycemic hypoglycemia considerably modulated energy production in the brain (glycolysis and glycogenolysis). The observed changes can be associated with a decrease in activity of allosterically regulated brain phosphofructokinase. Acetyl CoA and ketone bodies serve as inhibitors of phosphofructokinase, hexokinase, and phosphorylase [9].

During HGC glucose consumption in the brain decreases by $^1/_3$-$^1/_2$ from the normal, while oxygen consumption remains unchanged or moderately decreases under these conditions [4,6]. These changes reflect oxidation of non-glucose substrates in the nervous tissue (higher fatty acids, ketone bodies, and lactate). Free fatty acids in the blood, as well as fatty acids formed during degradation of membrane lipids in the nervous tissue, can serve as the source of higher fatty acids. The concentration of

TABLE 1. Rate of Lactate Accumulation and Activities of Glutamate Dehydrogenase, SDH, Lactate Dehydrogenase, and Glucose-6-Phosphate Dehydrogenase in the Cerebral Hemispheres and Brainstem of Rats with Alloxan Diabetes and Insulin-Induced Hypoglycemia ($M\pm m$, nmo×min^{-1}×mg protein^{-1})

Parameter, brain area		Group			
		1 (control)	2	3	4
Rate of glycolysis					
glucose	cerebral hemispheres	53.6±1.1	55.1±1.0	50.2±4.4	32.9±2.2***
	brainstem	57.6±1.0	55.7±1.1	59.4±2.5	50.5±2.0**
glucose-6-phosphate	cerebral hemispheres	66.1±4.0	63.6±2.0	56.1±8.3	41.9±3.7***
	brainstem	73.7±5.5	67.4±4.0	74.0±5.5	68.3±1.3
glycogen	cerebral hemispheres	19.8±1.1	20.1±1.6	21.8±0.5	16.8±0.3*
	brainstem	18.7±1.5	21.3±1.2	19.4±1.0	16.9±0.2
Glutamate dehydrogenase	cerebral hemispheres	474±30	485±23	420±16	529±37
	brainstem	501±20	492±26	484±34	411±20**
SDH	cerebral hemispheres	833±35	849±87	769±43	915±83
	brainstem	693±30	671±90	593±38	532±29**
Lactate dehydrogenase	cerebral hemispheres	304±7	295±8	320±8	299±10
	brainstem	279±11	283±13	284±5	271±12
Glucose-6-phosphate dehydrogenase					
	cerebral hemispheres	11.9±0.3	12.2±0.5	12.2±0.3	11.7±0.4
	brainstem	16.1±0.4	15.8±0.6	16.1±0.3	16.0±0.3

Note. Each group consists of 6-8 animals. *$p<0.05$, **$p<0.01$, and ***$p<0.001$ compared to the control.

higher fatty acids in the brain increases in rats with HGC [4,6]. The decrease in the intensity of glycolysis and glycogenolysis in the brain of rats with alloxan diabetes and HGC is probably associated with inhibition of enzymes with acetyl CoA and ketone bodies. The increase in the concentration of non-glucose substrates involved in energy metabolism in the brain can be followed by inhibition of glycolytic energy production and development of adverse consequences.

The adverse effect of hypoglycemia is related to excitotoxicity of glutamate and particularly of aspartate in high concentrations found in the intercellular fluid of the brain [4,6]. These changes are probably associated with inhibition of glycolysis and glycogenolysis during hypoglycemia. Brain dysfunction is mainly related to the decrease in glycolytic energy production, but not to changes in the energy state of the nervous tissue [5,8,10]. The observed changes are accompanied by an increase in the sensitivity to excitotoxic damage and high risk of cell death in response to excitatory amino acids in normal concentrations [7]. The decrease in activities of SDH and glutamate dehydrogenase in the brain of rats with alloxan diabetes and hypoglycemia can be associated with enzyme dysfunction due to activation of lipid peroxidation [4], which attests to serious disturbances in energy metabolism and contributes to the impairment of glutamate utilization in the brain and progression of glutamate-induced toxicity.

We conclude that posthyperglycemic hypoglycemia is accompanied by inhibition of glycolysis and glycogenolysis and decrease in activities of oxidative enzymes in the nervous tissue. Episodes of hypoglycemia observed during therapy for diabetes mellitus increase the risk of irreversible damage to neurons and play a role in the pathogenesis of brain dysfunction.

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