EFFECT OF HYPOXIA ON DYNAMICS OF CHANGES IN HEXOKINASE ACTIVITY IN SUBCELLULAR FRACTIONS OF NEONATAL RAT TISSUES

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Hexokinase (HK) activity in the total homogenate and cytoplasmic and mitochondrial fractions of the brain, heart, and liver of newborn rats was studied in relation to the severity of exposure to hypoxia. With a mild form of hypoxic hypoxia an increase in the activity of the mitochondrial-bound form of the enzyme was observed in the brain and liver tissue accompanied by a decrease in cytoplasmic HK activity and, in the brain, by a marked increase in the rate of glucose phosphorylation. Deep hypoxia led to a decrease in HK activity in the total homogenate and in both subcellular fractions in all tissues investigated. The results point to a disturbance of certain mechanisms in the tissues of newborn rats after exposure to a severe degree of hypoxia.

KEY WORDS: hypoxic hypoxia; intracellular distribution of hexokinase; newborn rats.

The increased intensity of glycolysis in newborn animals exposed to hypoxia is regarded as one of the most important mechanisms of adaptation. An essential stage in the maintenance of a high level of glycolysis is hexokinase (HK) activity. Since some HK in most tissues is known to be bound with subcellular particles [3, 7, 14] and also since the enzyme in the form bound with mitochondria is, under certain conditions, more active than the cytoplasmic form [1, 9, 14], it was interesting to study the intracellular distribution of HK in the tissues of newborn rats during exposure to hypoxia.

In this investigation changes in HK activity were studied in fractions of the brain, heart, and liver of newborn rats in different phases of exposure to hypoxia. Considering that the glucose-6-phosphate level is one of the principal factors controlling the intracellular distribution of HK [14], the concentrations of glucose-6-phosphate and glucose in the tissues were determined under the same conditions.

EXPERIMENTAL METHOD

Newborn rats (1-3 days after birth) were used. The animals were kept in a chamber in which all the oxygen of the air was replaced by nitrogen 15 min after the beginning of the experiments. In the course of 8-10 min the young rats developed signs of a mild degree of oxygen deficiency (as shown by the respiration rate, ECG, and color of the skin). Later they developed a severe degree of hypoxia. The young rats could survive under these conditions for 35-40 min. One group of animals was taken from the chamber 8 min and the other group 26 min after the beginning of the experiment. They were quickly decapitated and the tissues removed were quickly cooled and homogenized at 0-4°C in 9 volumes of 0.25 M sucrose with 0.01 M Tris–HCl, pH 7.5. The fraction of mitochondria obtained by differential centrifugation (12,000g, 20 min) was resuspended in 0.25 M sucrose with 0.01 M Tris–HCl and 0.5% Triton X-100, after which the supernatant obtained by centrifugation at 100,000g (30 min) was investigated. The cytoplasmic fraction was isolated by centrifuging the total homogenate at 100,000g (30 min, 0-4°C). The total homogenate was tested after treatment with 0.5% Triton X-100 and centrifugation under the conditions described above. HK activity was determined spectrophotometrically by reduction of NADP [12]. The concentrations of glucose-6-phosphate and glucose were determined by Lowry's method [10].

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TABLE 1. HK Activity and Glucose and Glucose-6-Phosphate Content in Tissues of Newborn Rats Exposed to Hypoxia for Different Times (M ± m)

Tissue tested	Group of animals	HK activity, μmoles substrate/h/g tissue			Glucose content, μmoles/g tissue	Glucose-6-phosphate content, μmoles/kg tissue
		total homogenate	cytoplasmic fraction	mitochondrial fraction		
Brain	Control	182±15,3 (10)	59,4±2,24 (17)	35,3±1,03 (16)	2.40±0,22 (8)	84,0±5.7 (8)
	Hypoxia:					
	8 min	184±17,0 (10)	49,8±2,75* (11)	43,1±1,98* (11)	1,28±0,17* (8)	47,0±3.4* (8)
	26 min	127±12,6* (10)	44,3±3,41* (12)	31,6±1,67 (12)	0,55±0,083* (8)	66,8±12.4 (8)
Liver	Control	67,2±3,06 (14)	56,5±2,30 (16)	2,09±0,086 (15)	5,94±0,39 (8)	272±34 (8)
	Hypoxia:					
	8 min	70,7±4,15 (11)	51,6±2,83 (16)	2,43±0,114* (13)	7,32±0,57* (8)	231±37 (8)
	26 min	57,4±2,52* (12)	44,0±2,05* (12)	1,85±0.062* (11)	4.95±0,55 (8)	146±45* (8)
Heart	Control	299±9,98 (10)	168±9,79 (11)	31.8±1,47 (10)	2,64±0,37 (5)	156±8.3 (5)
	Hypoxia:					
	8 min	299±10,2 (10)	154±8.06 (11)	32,8±1,68 (9)	1,96±0,20 (5)	143±12.0 (5)
	26 min	249±10,2* (8)	122±10,9* (10)	23,6±3,02* (8)	1,10±0,22* (5)	94,0±11.0* (5)

*P < 0.05 compared with control group.
Legend. Number of experiments in parentheses.

EXPERIMENTAL RESULTS AND DISCUSSION

The total HK activity after exposure to hypoxia for 8 min was unchanged in all the tissues tested. An increase in the activity of mitochondrial-bound HK by 22% was found in the brain tissue, whereas the activity of the cytoplasmic form of the enzyme was reduced by 17%. In the liver the decrease in content of cytoplasmic HK was not significant but the activity of the mitochondrial enzyme was increased by 16% (Table 1). In the initial stage of hypoxia part of the enzyme in these tissues was probably converted from the soluble into the bound form. Since only a small proportion of the HK in the liver is bound with the mitochondria, this redistribution must necessarily have a substantial effect on the mean value of the HK activity in the cytoplasmic fraction.

Meanwhile a considerable lowering of the glucose (by 47%) and glucose-6-phosphate (by 44%) levels was observed in the brain tissue. In the liver, on the other hand, the glucose content was increased (by 23%) whereas the glucose-6-phosphate content was unchanged.

The lower level of glucose in the brain of the newborn rats reflected its more rapid utilization. The increase in the rate of phosphorylation of glucose was evidently the result of the fall in the level of glucose-6-phosphate, which inhibits HK, and the increase in the proportion of bound enzyme, which is more active than the cytoplasmic when the substrate is deficient [1]. The intracellular redistribution of HK could in turn be due to the fall in the glucose-6-phosphate level. The decrease in the glucose-6-phosphate content observed on acceleration of the hexokinase reaction indicates an increase in the velocity of the final stages of its utilization and, consequently, an intensification of glycolysis in the brain of the newborn rats.

The increase in the glucose concentration in the liver tissue in stage I of hypoxia was evidently the result of its formation from glycogen, large quantities of which are present in the liver of newborn rats [2, 13]. Evidence of the ability of the liver in young rats to convert glycogen into glucose is given by the fact that their glucose-6-phosphatase activity is high [6, 11]. Conversion of part of the liver glycogen into glucose evidently maintains the glucose level in tissues that do not possess large reserves of this carbohydrate, especially the brain. It can be postulated on the basis of this increase in the activity of the relatively more active form of HK, bound with the mitochondria, that the rate of phosphorylation of glucose in the liver tissue is increased in stage I of hypoxia.

During the further development of hypoxia (26 min) HK activity fell in the total homogenate of the brain, heart, and liver. An even more marked decrease in activity of the cytoplasmic fraction of HK was observed in the brain tissue. Activity of the mitochondrial-bound enzyme fell considerably compared with the initial stage of hypoxia, to a lower value than in the control. The glucose content in the brain was reduced by 77% compared with the control. In the heart and liver a decrease in HK activity was observed in both the subcellular fractions, together with a decrease in the glucose and glucose-6-phosphate content compared with the initial stage of hypoxia.

The lowering of mitochondrial-bound HK activity was probably caused by a disturbance of the structural organization of the mitochondrial membranes taking place in the tissues of the newborn animals during exposure

to severe hypoxia [4, 5], and conversion of the enzyme into the soluble form. The reduction in cytoplasmic HK activity could be due to inactivation of the enzyme molecule during the change in the intracellular pH which takes place in the tissues at a time of severe hypoxia and leads to inhibition of glycolysis [8].

The decrease in HK activity in the total homogenate and, in particular, in the mitochondrial fraction signifies a worsening of the conditions for glucose phosphorylation and can be regarded as a disturbance of adaptation to hypoxia. This applies to a greater degree to the brain tissue, in which glucose is the main substrate for energy reactions during hypoxia. A decrease in the ability of brain tissue to phosphorylate glucose, together with a sharp fall in its level during exposure to a severe degree of hypoxia, could be among the main causes of the great vulnerability of the brain of newborn rats exposed to hypoxia.

The results of these experiments thus showed that mild, brief hypoxia is accompanied by adaptive changes in the HK distribution in the brain tissue of newborn rats, as a result of which the phosphorylation of glucose and its utilization in glycolysis takes place more rapidly. A severe degree of hypoxia leads to disturbance of adaptation in this stage of energy reactions.

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