INFLUENCE OF CORTISONE ON THE HEXOKINASE ACTIVITY IN THE TISSUES OF WHITE RATS UNDER CONDITIONS OF ACUTE HYPOXIA

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The administration of cortisone exerts a favorable effect upon the carbohydrate-phosphate metabolism in various diseases of man [1] and increases the period of survival of normal and adrenalectomized animals subjected to the influence of hypoxia [8, 11]. According to the results of the investigations of a number of authors [6, 13], acute oxygen insufficiency of the organism is accompanied by a reduction of the hexokinase activity in the tissues—an enzyme which is the "weakest" glycolytic link and determines the maximum rate of glycolysis [5]. It is known that the hexokinase reaction is the enzymatic step whose rate is determined by the influence of factors of hormonal regulation [4]. And yet, the question of the influence of hormones on the hexokinase activity under conditions of hypoxia has been insufficiently studied.

This work presents the results of an investigation of the influence of single and multiple injections of cortisone acetate on the hexokinase reaction in the skeletal muscles and brain of rats subjected to the influence of acute heminic hypoxia.

PROCEDURE

The experiments were conducted on white male rats, 150-250 g in weight. The hexokinase activity was determined in tissue extracts according to the decrease in glucose [14] after precipitation of the proteins with $CdSO_4$ in weakly alkaline medium [12] and was expressed in milliunits (in millimicromoles of glucose per mg of protein in the samples in one minute of incubation). The amount of protein in the muscle and brain extracts was determined by the biuret method [10] (in the brain after preliminary extraction of the lipids with an alcohol-ether mixture for 1.5 h). Acute oxygen starvation of the organism was induced by subcutaneous injection of sodium nitrate in a dose of 10 mg per 100 g of weight of the animal. Methemoglobin was determined according to the method of Gorn [3], using a photoelectrocolorimeter after decapitation of the animal 40-45 min from the moment of injection of the $NaNO_2$.

Two series of experiments were conducted. In the first series of experiments we investigated the hexokinase activity in the tissues of the control rats and animals that had received single and repeated injections of cortisone. In the second series of experiments, we studied the rate of the hexokinase reaction in the tissues of rats subjected to hypoxia and those of rats in which acute oxygen insufficiency was induced against a background of single or repeated injections of cortisone. Single injections of cortisone, in amounts of 10 mg per rat, were performed 12 h before the experiment, and the repeated injections—in a dose of 2.5 mg during a 6 day period, the last injection being administered 24 h before the experiment.

RESULTS

The results of the experiments on the determination of the hexokinase activity in the tissues of the control rats show that the rate of phosphorylation of glucose in extracts of the brain is almost six times as high as in extracts of the skeletal muscles. This agrees with the generally known data on the dominant role of glucose as the source of

Variations of the Hexokinase Acitivity (in milliunits) in the Tissues of the Control Rats and Rats with Hypoxia Under the Influence of Single and Repeated Injections of Cortisone

Investigated tissue	Statistical index	Experimental conditions					
		First series			Second series		
		Control	Cortisone, single	Cortisone, repeated	Hypoxia	Cortisone, single + hypoxia	Cortisone, repeated + hypoxia
Skeletal muscles	n	30	8	9	22	10	10
	M±m	9.13±0.52	3.52±0.54*	9.02±0.82†	5.51±0.50*	3.30±0.77*	9.13±0.99‡
	% of activity with respect to control	–	38.6	98.8	60.4	36.1	100.0
Brain	n	31	9	10	21	11	10
	M±m	53.80±2.35	55.97±1.87‡	59.17±1.79‡	43.51±3.11†	57.01±2.98‡	56.68±1.97‡
	% of activity with respect to control	–	104.0	110.0	80.3	106.0	105.4

* $p < 0.01$.
† $p < 0.02$.
‡ $p > 0.05$.

nutrition in the energy exchange of the central nervous system and with data [15] on the great affinity of the brain hexokinase for glucose. Long [14], studying the optimum conditions under which the activity of hexokinase is manifested, showed that the activity of the enzyme in the brain tissue is far higher than in 14 other tissues that he studied.

As can be seen from the table, repeated injection of cortisone in amounts of 10 mg leads to substantial depression of the hexokinase reaction in the muscles and does not influence the hexokinase activity in the brain. Data analogous to those cited were obtained [9] upon the addition of cortisone in vitro to extracts of these tissues. In an investigation of the hexokinase activity in the hyaloplasm and mitochondria of normal and regenerating liver after the introduction of large doses of cortisone [7], a reduction of the activity of the enzyme was found in the fractions both of the normal and of the regenerating livers.

Repeated injections of cortisones into rats did not exert a statistically reliable influence on the hexokinase activity in the investigated tissues. This, it might seem, is in contradiction with the inhibition of the hexokinase activity in the muscles after a single injection of cortisone. In all probability, repeated injections of cortisone, which increases the insulin requirement of the organism [2], leads to the establishment of a dynamic equilibrium between the factors determining the activity of the enzyme – glucocorticosteroids, on the one hand, and insulin, on the other. This evidently also corresponds to maintenance of the rate of the hexokinase reaction in the muscles of these animals, equal to that for the control rats.

In the second series of experiments, the amount of methemoglobin in the blood was preliminarily investigated 40-45 min after the injection of $NaNO_2$ in intact rats and in rats in which hypoxia had been induced against a background of single and repeated injections of cortisone. In the animals of all three-groups, the amount of methomoglobin fluctuated from 43 to 68%, without any significant difference among the individual groups.

Acute oxygen starvation, induced by the injection of $NaNO_2$, is accompanied, just as in the experiments conducted by other authors [6, 13], in the case of hypoxic and arterial hypoxia, by an inhibition of the rate of the hexokinase reaction, both in the skeletal muscles and in the brain. Oxygen insufficiency of the organism, induced against a background of the action of cortisone, exerts no influence on the hexokinase activity in the muscles, inhibited as a result of its action. At the same time, a single injection of cortisone prevents the decrease in the rate of phosphorylation of glucose in the brain, observed under conditions of hypoxia.

The hexokinase activity in the skeletal muscles and brain of rats that received injections of $NaNO_2$ against a background of repeated injections of cortisone proved the same as in the corresponding tissues of the control animals,

which did not receive cortisone. Consequently, repeated injections of cortisone normalize the incorporation of glucose into the metabolic processes in the tissues during hypoxia. This agrees with the indications in the literature of a favorable influence of repeated injections of ACTH, which, as is well known, stimulates the production of corticosteroids, on the hexokinase activity of the skeletal muscles and heart of rats during hypoxic hypoxia [6].

This, a single administration of cortisone to rats in large doses substantially inhibits the hexokinase reaction in the skeletal muscles, while acute hypoxia induced against this background by the injection of $NaNO_2$ does not affect the enzyme, although it itself inhibits its activity. A single injection of cortisone prevents the depression of the hexokinase reaction in the brain, observed during hypoxia, and does not affect the enzymatic activity in the brains of the control animals. Repeated injection of cortisone does not influence the hexokinase activity in the tissues of the control rats and normalizes the rate of the hexokinase reaction under conditions of acute heminic hypoxia.

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