THE EFFECT OF ACTH ON THE RATE OF THE GLUCOKINASE REACTION IN ALBINO RAT TISSUES UNDER CONDITIONS OF ACUTE OXYGEN LACK IN THE ORGANISM

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According to he results of investigations of many authors [2, 7, 9], acute oxygen lack of the organism is accompanied by a sharp disturbance of the carbohydrate-phosphorus metabolism in the tissues: the reserves of glycogen and ATP are reduced (with the exception of the kidneys, where the amount of ATP increases during hypoxia), and the assimilation of glucose is decreased. It is known that the administration of ACTH to animals substantially increases their resistance to oxygen starvation [16, 18] and, to a considerable degree, prevents the changes in the carbohydrate-phosphorus metabolism produced by acute hypoxia [7, 11].

Most of the studies on the problem of oxygen lack have been devoted to hypoxic hypoxia, while the disturbance of metabolism during the hemic type of hypoxia has received insufficient treatment in the literature.

On the basis of this, a study of the influence of ACTH during hemic hypoxia on the activity of the "key" enzyme of glycolysis—glucokinase—is of undoubted interest.* The latter enzyme catalyzes the reaction of primary phosphorylation of glucose and, as is well known, is the least "powerful" link of glycolysis, which determines its maximum rate [5].

In this work we investigated the glucokinase activity in control and "hypoxic" rats under the influence of ACTH and without it in the skeletal muscles, testes, and brain. The investigation of the testes was undertaken in view of the fact that we found no data in the literature, either with respect to changes in metabolism in this organ during hypoxia, although the testes are extremely sensitive to oxygen lack, or with respect to the influence of various hormones on the activity of the testicular glucokinase.

EXPERIMENTAL PROCEDURE

The investigations were conducted on white male rats 190-250 g in weight. Acute oxygen lack was induced by subcutaneous injection of sodium nitrite in amounts of 10 mg per 100 g of weight of the animal. Forty to 45 min after the injection of $NaNO_2$, when 45-50% of the hemoglobin had been converted to methemoglobin, the animals were killed under light ether narcosis. Methemoglobin was determined according to our modification of Gorn's method [3]. We used a smaller dilution of the blood, which gives a larger difference between the extinction of hemoglobin and methemoglobin and substantially reduces the error in the determination of the amount of the latter. Colorimetry was performed on the FÉK with a red filter. The investigated tissues were frozen and ground in liquid oxygen. The glucokinase activity was determined in extracts of the tissues according to the loss of glucose [17] and were expressed in milliunits (millimicromoles of glucose per mg of protein in the sample in 1 min of incubation). Glucose was determined according to the method of Dumazert [15], protein by the biuret method [14]. Since the lipids of the brain

* According to the systematic nomenclature adopted by the Commission on Enzymes of the International Biochemical Congress (1961), the enzyme is called ATP:D-glucose-6-phosphotransferase.

Glucokinase Activity (in Milliunits) in Tissues of White Rats under the Influence of Hypoxia, Single and Repeated Injections of ACTH

Group of animals	Skeletal muscles		Testes		Brain	
	No. of determinations	M±m	No. of determinations	M±m	No. of determinations	M±m
1	30	9.13±0.52	23	15.80±0.52	31	53.80±2.35
2	22	5.51±0.50*	16	12.05±0.95‡	21	42.51±3.11†
3	7	3.84±0.66*	6	15.48±1.42	7	47.21±3.25
4	6	3.43±0.62*	5	10.92±1.38‡	6	52.35±2.94
5	10	7.74±0.60	12	11.88±1.06†	10	51.40±2.20
6	8	8.45±0.73	14	13.11±0.76‡	13	44.23±2.69†

Note. The asterisks indicate statistically reliable reductions of the glucokinase activity (* —$P<0.01$, † $P<0.02$, ‡ —$P<0.05$) in comparison with the control animals.

and testes, changing the light absorption, interfere with the determination of protein by this method, their extraction after precipitation of the protein was performed with an alcohol-ether mixture (2:1) in a period of 1.5 h.

Six groups of animals were investigated: the 1st contained the control rats, not subjected to any influences, the 2nd the rats that received injections of $NaNO_2$, the 3rd the rats that received a single injection of ACTH, the 4th the rats in which methemoglobinemia was induced against a background of ACTH action; the 5th the rats that received repeated injections of ACTH, and the 6th the rats in which acute oxygen lack was induced after repeated preliminary injections of ACTH.

The animals of the 3rd and 4th groups received subcutaneous injections of 2 units of ACTH, 2 h before the experiment. The animals of the 5th and 6th groups received ACTH for a period of 6 days in amounts of 2 units per day. The rats were taken for the experiment 24 h after the last injection.

The data obtained were treated statistically.

EXPERIMENTAL RESULTS

As can be seen from the data presented in the table, acute oxygen lack of the organism, induced by the injection of $NaNO_2$, leads to a reduction of the glucokinase activity in all the investigated tissues. This reduction of the enzyme activity is statistically reliable and is the most pronounced in the muscles (39.6%), to a lesser degree in the testes (23.7%) and brain (19.7%). The results of the experiments agree with the literature data of most authors [6, 8], which give evidence of an inhibition of the glucokinase activity in the tissues in other forms of hypoxia. The inhibition of the rate of the glucokinase reaction detected in our investigations indicates a reduction of the incorporation of glucose into the metabolic processes [19] and is one of the causes of the deceleration of glycogen synthesis from glucose in the tissues during hypoxia [9]. The mechanism of the inhibition of the glucokinase activity may differ in different tissues. Apparently the inhibition of the glucokinase activity in the muscle tissue occurs under the action of the hormones of the adrenal cortex, the intensified production of which during hypoxia has been established by certain authors [12].

In contrast to the muscle glucokinase, the brain glucokinase is not inhibited in vitro by preparations of the steroid hormones [13]. In view of this, the reduction of the rate of the glucokinase reaction in the brain can be explained by the increase in the amount of glucose-6-phosphate observed in it during hypoxia [10], since, even in small concentrations, it strongly inhibits the glucokinase activity of the brain in experiments in vitro [20].

The mechanism of the inhibition of glucokinase of the testes remains unclear. Apparently, the cause of the inhibition is not intensified production of corticosteroids, since 2-2.5 h after subcutaneous injection of 2 units of ACTH in rats (3rd group of animals), the glucokinase activity in the testes does not differ from its activity in the control animals, while the muscle glucokinase is inhibited an average of 57.9% during this period. In the brain, just as in the testes, the glucokinase activity is unchanged 2-2.5 h after the injection of ACTH.

From the table it can also be seen that acute hypoxia does not influence muscle glucokinase inhibited as a

result of preliminary injection of ACTH. This may be explained by the fact that the influence of hypoxia does not give rise to any essential new increase in the manufacture of corticosteroids, which might lead to a further reduction of the glucokinase activity in the muscle tissue, against a background of inhibition of the glucokinase activity by the corticosteroids, which sets in after the injection of ACTH. Moreover, a single preliminary injection of ACTH does not influence the rate of glucose phosphorylation in the testes, inhibited under conditions of hypoxia. It is interesting to note that a single injection of ACTH in rats entirely prevents the reduction of the glucokinase activity in the brain, observed under conditions of hypoxia. The mechanism of the normalizing influence of a single injection of ACTH remains unclear.

In a study of the influence of repeated preliminary injections of ACTH on the glucokinase activity in the tissues of the control rats (5th group), it was found that they exert no statistically reliable influence on the rate of the glucokinase reaction in the muscles and brain. The glucokinase activity in the testes is distinctly reduced in the rats of this group.

Acute oxygen starvation of the organism in rats that have received preliminary repeated injections of ACTH is not accompanied by any decrease in the glucokinase activity in the muscles, but produces the same decrease in the glucokinase activity in the brain and testes as in "hypoxic" rats that have not received ACTH. The explanation of the normalizing influence of preliminary repeated injections of ACTH on the glucokinase activity in the muscles during hypoxia should evidently be sought in the fact that repeated injections of ACTH, which increase the need of the organism for insulin [1], evidently promote an intensified manufacture of insulin, which counteracts the inhibiting influence of the corticosteroids on the glucokinase activity [4].

Our investigations show that acute hypoxia, induced by the injection of sodium nitrate, leads to a reduction of the rate of the glucokinase reaction in all the investigated tissues. Repeated administration of ACTH to rats inhibits the glucokinase activity in the muscles and does not influence the activity of this enzyme in the brain and testes. At the same time, repeated injection of ACTH exerts a normalizing influence on the glucokinase activity in the brain under conditions of acute oxygen lack. Repeated preliminary injections of ACTH do not influence the glucokinase activity in the muscles and brain, but inhibit the activity of this enzyme in the testes. Repeated preliminary injections of ACTH prevent the reduction of the glucokinase activity in the muscles that arises during hypoxia.

SUMMARY

Acute hypoxia induced by the administration of sodium nitrite was found to result in the reduction of glucokinase activity in the tissue. Single ACTH administration in a dose of 2 units depressed the glucokinase activity in the muscles, without affecting the activity of the enzyme in the brain and the testes. Preliminary single ACTH administration prevented the inhibition of glucokinase activity in the brain as observed in hypoxia. Repeated preliminary ACTH injections (2 units daily for 6 days) did not affect the rate of glucokinase reaction in the muscles and the brain, and reduced the glucokinase activity in the testes; the injections also normalized the muscle glucokinase activity inhibited in conditions of hypoxia.

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