TISSUE RESPIRATION AND ADENOSINETRIPHOSPHATASE ACTIVITY IN DOGS IN DEEP HYPOTHERMIA

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In connection with the introduction of open heart operations, involving deep hypothermia, into surgical practice, investigations of metabolism in the arrested heart and also in the brain and other organs in hypothermic conditions have become not only of theoretical, but also of great practical importance.

Investigations on hibernating animals and on animals under artificial hypothermia have shown that the metabolic processes take place more slowly in organs at low temperatures, and that their requirements of oxygen and nutrient substances are considerably reduced [1-4, 6, and others]. As a result, in deep hypothermia the organism becomes less sensitive to hypoxia.

The object of the present investigation was to study the tissue respiration and the adenosinetriphosphatase (ATPase) activity in the brain, heart, adrenals, and skeletal muscle during deep hypothermia and their relationship to prolonged arrest of the circulation and subsequent warming of the animals to a normal temperature.

EXPERIMENTAL METHOD

Experiments were carried out on mongrel dogs weighing 17-22 kg. A control group consisted of animals from which the test organs (heart, brain, skeletal muscle, adrenals) were extracted after administration of a general anesthetic at a normal temperature (37°).

Anesthesia was induced as follows (G. A. Ryabov). One hour before the experiment began, premedication was given: morphine was injected subcutaneously in a dose of 5 mg/kg body weight and promedol in a dose of 5 mg/kg body weight was given in the same way. For intravenous anesthesia a 1% solution of hexobarbital was given (to produce sleep) without causing respiratory arrest. Anesthesia was maintained with nitrous oxide and oxygen (3:2) and small doses of ether (3-4%), using a semiopen system, with an apparatus providing automatically controlled respiration. The relaxant lysthenon was injected once or twice in a dose of 1 mg/kg body weight.

TABLE 1. Tissue Respiration of the Brain, Heart, and Skeletal Muscle of Dogs at 37° (Control Group)

Test object	Absorption of O_2 (μg/mg untreated tissue)	CO_2 production	Respiratory quotient CO_2/O_2
Brain	5.26	4.84	0.92
Heart	4.24	3.84	0.90
Skeletal muscle	2.27	2.13	0.96

TABLE 2. Respiratory Quotient during Deep Hypothermia and Subsequent Warming to a Normal Body Temperature

Series of experiments	Brain	Heart	Skeletal muscle
First	0.67	0.67	0.73
Second	0.6	0.6	0.73
Third	0.92	0.8	0.8

Cooling (to 10° in the mediastinum) and subsequent warming were carried out in the course of 10-15 min by an extracorporeal method, using an artificial circulation apparatus filled with blood cooled to 0° or warmed to 37°, respectively.

In the first series of experiments the circulation was stopped completely for 30 min, and in the second series for 60 min. In the third series of experiments, after arrest of the circulation for 30 min the animals were warmed to a normal temperature.

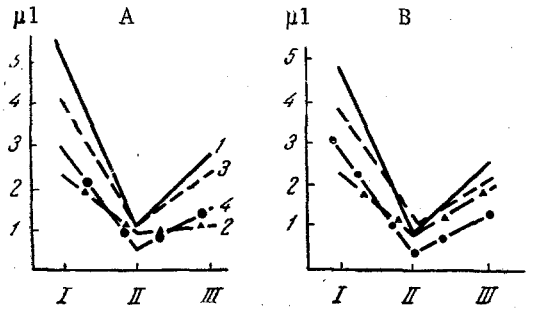

Fig. 1

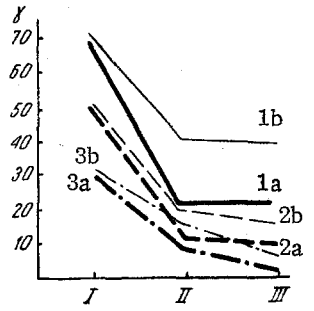

Fig. 2

Fig. 1. Effect of deep hypothermia on tissue respiration. A) O_2 consumption; B) CO_2 production; I) before cooling; II) after arrest of the circulation for 30 min; III) after incubation at 37°; 1) brain; 2) skeletal muscle; 3) heart; 4) adrenals.

Fig. 2. Effect of deep hypthermia on ATPase activity. I) Before cooling; II) after arrest of the circulation for 30 min; III) after arrest of the circulation for 60 min; 1) brain; 2) heart; 3) skeletal muscle; a) after cooling; b) after incubation at 37°.

The respiration of minced pieces of the test organs was investigated in a Warburg's apparatus. The tissues were incubated in an atmosphere of oxygen at 10° in the first and second series of experiments and at 37° in the third. The intensity of the respiration of the minced tissues was expressed as the volume of O_2 absorbed and of CO_2 given off during incubation for 2 h by 1 mg dry weight of tissue (in microliters).

In order to determine the ATPase activity, the tissue was rapidly weighed and ground in porcelain mortars, cooled in ice to homogenization, and borate buffer solution (pH 8.4) was added gradually. Into each of a series of test tubes with incubation mixture 1 ml of the resulting tissue suspension was added, containing 200 mg of tissue. The composition of the incubation mixture was: 1 ml borate buffer solution, 0.25 ml of $1 \cdot 10^{-3}$M $MgCl_2$ solution, 0.25 ml of 0.004M $CaCl_2$ solution, and 1 ml of ATP (2 mg). In the first and second series of experiments the experimental samples were incubated for 30 min at 37° and, in parallel tests, at the tissue temperatures reached when the intraesophageal temperature was 10°. The ATPase activity was judged by the increase in inorganic phosphorus and was expressed as micrograms percent per 100 mg untreated tissue.

EXPERIMENTAL RESULTS

The results of the investigation of the tissue respiration of the various organs from dogs at a normal temperature (37°) are given in Table 1.

In the experimental animals there was a marked decrease in the respiration of the cooled tissues of all the test organs incubated at 10° 30 min after arrest of the circulation (Fig. 1). This was shown by a decrease in the volume of both absorbed and excreted CO_2, the disturbance of the processes of decarboxylation being more marked. The greatest fall in the intensity of respiration was observed in the brain tissue, where the absorption of O_2 fell to 0.88 µl, only 17% of the normal value. A still more marked fall took place in the CO_2 production of the brain, namely to 0.58 µl (12.5% of normal). The greater decrease in CO_2 production than in O_2 absorption led to a considerable decrease in the respiratory quotient in the tissues of all the organs, and especially the brain (Table 2).

After a more prolonged arrest of the circulation (60 min) the intensity of respiration fell by roughly the same degree as after stopping the apparatus for 30 min.

Experiments in which the animals were warmed, and also in which the cooled tissues were incubated at 37°, showed that the changes observed during deep hypothermia were fully reversible. In normal temperature conditions the level of the tissue respiration rose considerably during incubation (2 h), although not to the normal level but to only 50-60% of normal. During warming the processes of decarboxylation were more intensive, as shown by an increase in the volume of CO_2 produced and by a corresponding increase in the respiratory quotient.

When the ATPase activity of the control animals was investigated, its highest level was found in the brain tissue. In deep hypothermia the changes in ATPase activity were mainly parallel to the changes in tissue respiration.

The activity of this enzyme fell considerably during cooling in all the tissues, and especially in the brain tissue (Fig. 2). In contrast to the tissue respiration in the cardiac and skeletal muscles, in the brain the changes in ATPase activity were dependent on the duration of arrest of the circulation; stopping the apparatus for 1 h caused a more severe depression of the activity of the enzyme than stopping it for ½ h. So far as the brain tissue is concerned, the activity of the enzyme in this tissue was at the same level after 60 min as after arrest of the circulation for 30 min. During warming of the animals the ATPase activity, like the tissue respiration, increased in all the tissues although it did not reach the normal level, but reached only 50-60% of its initial value.

It should be noted that the experiments to investigate the respiration in the brain tissue were carried out without adding any substrate to the incubation medium. The level of the tissue respiration of the minced brain from animals in the conditions under study was therefore dependent on the varied initial concentration of substrate in the animals' brain.

These experimental results suggests that the conservation of high-energy phosphorus compounds reported by several workers [5, 7, 8] in deep hypothermia may be to some extent the result of a decrease in ATPase activity.

The results of the experiments showing the recovery of tissue respiration and ATPase activity as the animals were warmed demonstrate that the changes in oxidative energy metabolism during prolonged stoppage of the circulation in deep hypothermia are reversible, and are connected with the temporary inhibition of these processes, but not with their inactivation.

SUMMARY

The authors studied the intensity of tissue respiration (studied monometrically) and the adenosinetriphosphatase activity (by increase of inorganic phosphorus) in the brain, heart, and skeletal musculature of dogs in deep hypothermia depending on the length of circulatory arrest (30 and 60 minutes) and subsequent warming of the animals to normal temperature. Cooling (to 10° in the mediastinum) and subsequent warming was effected in 10-15 minutes by means of extracorporeal circulation. The tissue respiration and adenosinetriphosphatase activity showed a marked change especially in the brain tissue under the effect of deep hypothermia. Respiratory disturbances of all the tissues investigated were manifested by a reduction of the absorbed O_2, and by a drop of the CO_2 discharged; the decarboxylation process was disturbed more acutely as seen by the reduced respiratory quotient. A more marked depression of adenosinetriphosphatase activity was revealed in the tissues of cardiac and skeletal muscles following a 60 minute circulatory arrest. After the animals were warmed the tissue respiration and adenosinetriphosphatase activity augmented in all the tissues, indicating that the oxidative metabolic changes in conditions of deep hypothermia were reversible.

LITERATURE CITED

1. T. A. Achkasova, A. A. Kalikhman, B. M. Kostyuchenok, et al. Khirurgiya, 1, 78 (1956).
2. I. V. Burakovskii and E. V. Gubler. Abstracts of Proceedings of the 26th Congress of Surgeons of the USSR [in Russian], p. 74. Moscow, 1955.
3. E. V. Gubler, G. S. Fenster, and V. M. Pinchuk. In: Artificial Hypothermia [in Russian], No. 3, p. 46. Tashkent, 1961.
4. A. A. Kalikman. In: Problems in Cryopathology [in Russian], p. 61. Moscow, 1953.
5. E. V. Maistrakh. In: Mechanisms of Pathological Reactions [in Russian], No. 21-25, p. 296. Leningrad, 1952.
6. N. P. Meshkova and S. E. Severin. Practical Manual of Animal Biochemistry [in Russian], Moscow, 1950.
7. I. R. Petrov, et al., Fiziol. zh. SSSR, 2, 107 (1957).
8. I. R. Petrov. In: Artificial Hypothermia [in Russian], No. 3, p. 105. Tashkent, 1961.
9. K. Spohn, et al., Thoraxchirurgie, 1960, Bd. 8, S. 228.
10. L. Szekeres, et al., Arch. Int., Pharmacodyn., 1958, v. 115, p. 131.