RESISTANCE OF RATS WITH DIFFERENT BEHAVIOR TYPES TO CIRCULATORY CEREBRAL HYPOXIA

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Prediction of resistance to circulatory cerebral hypoxia still remains a virtually unresolved problem. The particular features of energy metabolism of the brain predetermining death of animals after cerebral ischemia were first discovered by the technique of intravital NMR-spectroscopy in relation to phosphorus-containing metabolites. This raised the question of the search for other parameters, for example, those characterizing animal behavior, which would provide an easier method of determining their resistance to circulatory cerebral hypoxia.

In the investigation described below the resistance of rats with different types of behavior to circulatory cerebral hypoxia was studied.

EXPERIMENTAL METHOD

Experiments were carried out on 103 noninbred male rats. The type of the animals' behavior was determined by means of two tests: open field [4] and enforced swimming [6]. Motor activity of the rats was assessed by keeping them for 10 min in an open field and recording the number of squares crossed, the number or rearings, the number of visits to the center of the field, the number of groomings, and the time taken for extinction of motor activity. To assess the tendency toward the development of a depressivelike state the rats were placed for 10 min in a basin of water and the time during which the rat remained stationary (passive swimming) was recorded. Depending on the parameters of motor activity and the level of depression (the duration of passive swimming) the rats were divided appropriately into the following groups: inactive, moderately active, and highly active, and with low, average, and high levels of depression. The distribution among groups within each of the tests was determined by dividing the rats in accordance with the value of each parameter into three sets of equal size (the boundaries of the groups were the 33 and 67% quantiles). Different combinations of the three gradations (low, average, and high) of the two features (level of motor activity and level of depression) enabled nine groups of animals to be distinguished: two extreme groups — with an active type of behavior (with high motor activity and a low level of depression) and with a passive type of behavior (a low level of motor activity and a high level of depression), one middle group (with average levels of motor activity and depression), and six mixed groups. After determination of the rats' behavior, they were kept under standard conditions in the animal house for 1 month. Next, under pentobarbital anesthesia (50 mg/kg body weight) the common carotid arteries were ligated in the neck (circulatory hypoxia), and the number of animals dying and surviving after 48 h of ischemia was recorded. The significance of differences between mortality levels in the different groups of rats was tested by the chi-square test or by Fisher's accurate test. To determine the significance of

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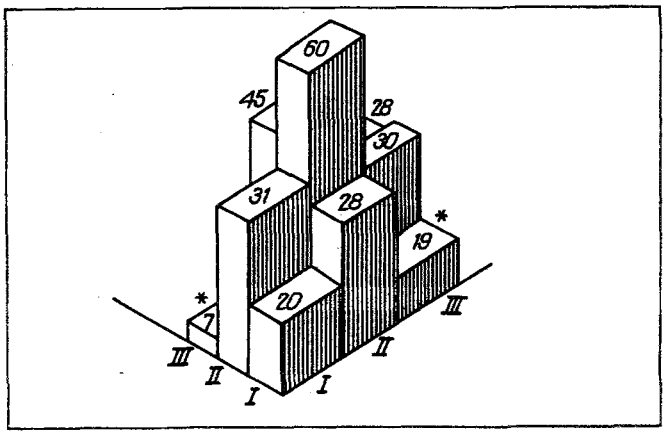

Fig. 1. Mortality in different groups of rats. I) Low, II) average, III) high level of motor activity (on left) and level of depression (on right); numbers show mortality (in %). *p < 0.05 Compared with average group.

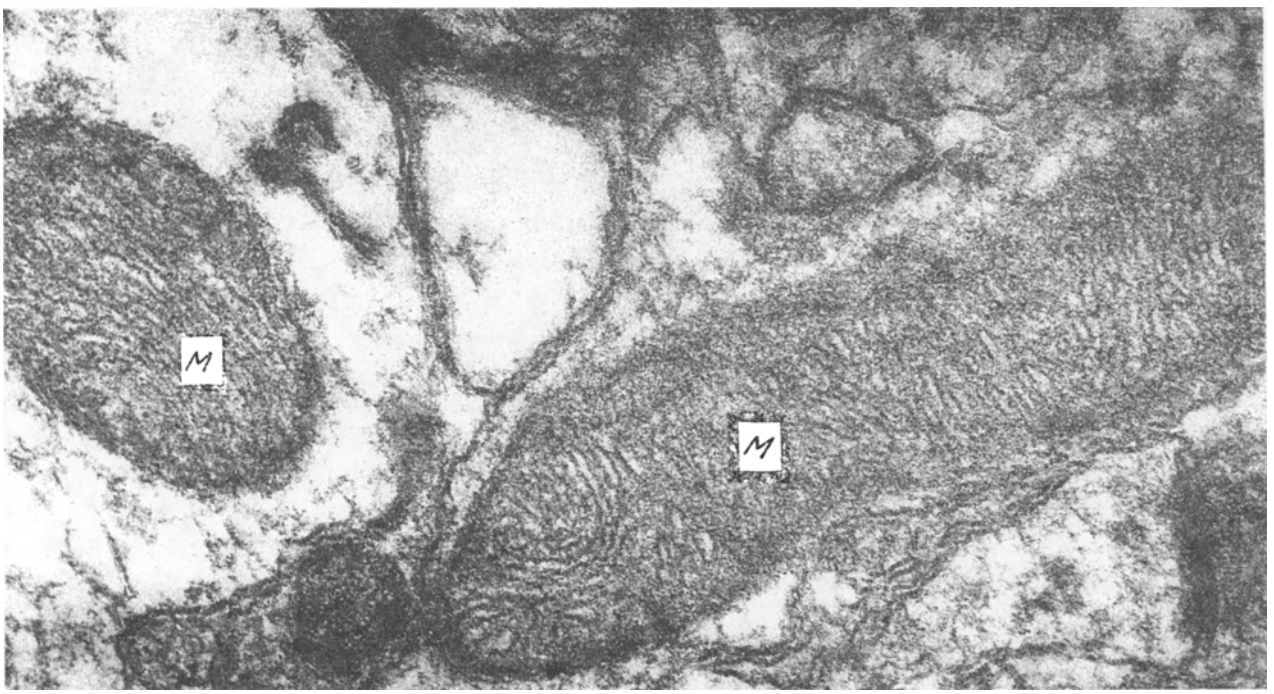

Fig. 2. Hypertrophy of mitochondria in neuropil of rat sensomotor cortex 1 h after ligation of common carotid arteries. Enlarged mitochondria and high packing density of cristae can be seen. M) Mitochondria; 46,000×.

the differences between the behavioral parameters in animals surviving and dying from ischemia, the distributions of values of the features in these groups of animals were determined by the chi-square test. Light and electron microscopic investigations were undertaken on the dying and surviving rats with active and passive types of behavior, and activity of succinate dehydrogenase (SDH), an enzyme of energy metabolism, was studied by a quantitative histochemical method [5]. SDH activity was expressed in conventional units – micromoles formazan/mole protein nitrogen/min

EXPERIMENTAL RESULTS

Of 103 rats 30 (29%) died from ischemia. Significant differences in mortality were found only between three groups of rats: between the two extreme groups and the middle group (Fig. 1). Minimal mortality (7%) was characteristic of rats

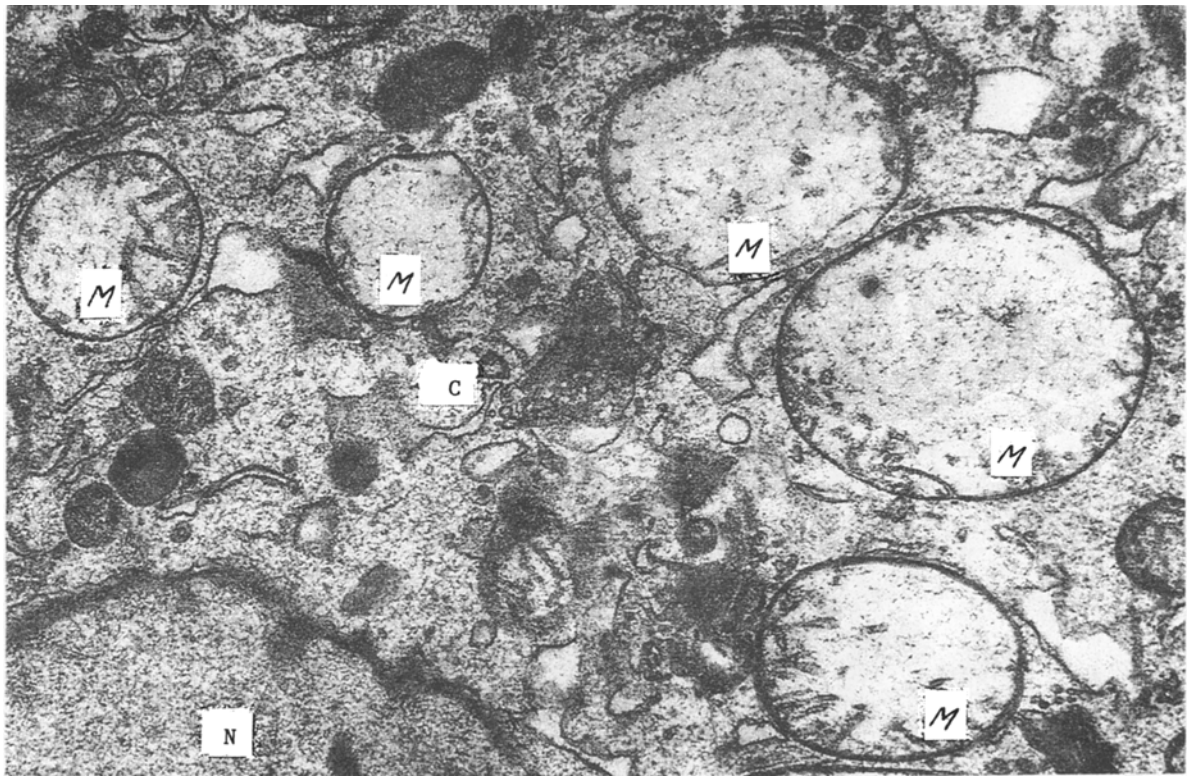

Fig. 3. Almost total destruction of cristae in hypertrophied mitochondria of a nerve cell from the rat sensomotor cortex 48 h after ligation of common carotid arteries. N) Nucleus, C) cytoplasm, M) mitochondria; 46,000×.

with an active type of behavior (14 animals), maximal (60%) of rats of the middle group (10 animals). Mortality among rats with a passive type of behavior (16 animals) was only a little higher (19%) than in rats with an active type of behavior, and significantly lower than in rats of the middle group. Analysis of differences between the behavioral parameters in rats surviving and dying showed that the most informative feature for predicting survival of rats after circulatory cerebral hypoxia is the level of depression ($\chi_4^2 = 9.82$, $p = 0.04$) and the number of rearings ($\chi_4^2 = 6.23$, $p = 0.18$). There is a certain "critical" value of the level of depression below which the probability of death is negligibly small. For instance, of rats whose passive swimming time was within the limits of the lower 20% of the values in the group (18 animals), none died.

In the dying rats, irrespective of the type of behavior, common morphological changes were found: cerebral anemia, emptying of the majority of cerebral vessels, and congestion of a few vessels with blood, petechial hemorrhages, in some cases proliferation of glial cells, and an increase in the number of hypochromic cells in the cerebral cortex with signs of chromatolysis and cells of ischemic type.

Changes in mitochondrial ultrastructure and SDH activity were observed in rats which survived after ischemia. Many elongated and enlarged mitochondria with very dense packing of their cristae, which is not characteristic of intact animals (Fig. 2), and also mitochondria in a stage of incomplete division, were observed 1 h after ischemia. These changes in the mitochondria, which are a compensatory reaction of the brain to oxygen deficiency, were observed in rats of both groups. Together with hypertrophied mitochondria, mitochondria with destruction of their cristae also were found, more often in rats with a passive type of behavior. There was a marked increase in the intensity of energy metabolism of the brain 1 h after ischemia, but this was more marked in rats with the active type of behavior [an increase in SDH activity up to 140.8 ± 2.4 conventional units (c.u.), 100.0 ± 0.7 c.u. in the control] than in rats with a passive type of behavior (an increase in SDH activity up to 120.3 ± 8 c.u. compared with 90.0 ± 0.6 c.u. in the control). Processes of destruction of the mitochondria became less marked 24 h after cerebral ischemia and extended to all structural elements of the brain – neurons, glia, and processes of neurons. SDH activity fell and became virtually identical in rats with the active (79.2 ± 2.4 c.u.) and passive (72.2 ± 1.7 c.u.) types of behavior. Almost total destruction of the mitochondria was observed 48 h after

ischemia in the majority of neurons and glia, with destruction of the rough reticulum and other pathological changes (Fig. 3). These ultrastructural disturbances were more marked in rats with an active type of behavior and were combined with reduction of SDH activity to 69.1 ± 0.9 c.u. in rats with an active type and to 63.2 ± 0.6 c.u. in rats with a passive type of behavior.

The investigations thus showed that survival rate of rats after circulatory cerebral hypoxia depends on the severity of the structural and metabolic changes taking place at different times after cerebral ischemia. The high resistance of the brain of rats with an active type of behavior to circulatory cerebral hypoxia is evidently linked with the more marked compensatory processes in the brain and, above all, with increased reactivity (well-marked hypertrophy) and with the greater resistance of brain mitochondria to oxygen deficiency, and also with the stronger activation of the succinate dehydrogenase pathway of substrate oxidation in the mitochondria. The switch to mainly oxidation of succinate, caused by increased SDH activity, is known to play an adaptive role under the conditions of oxygen insufficiency [3]. Since catecholamines are SDH activators [2] it can be tentatively suggested that the different degrees of increase of succinate dehydrogenase activity of the mitochondria in rats with cerebral ischemia and with different types of behavior is due to differences in the level of activity of the catecholaminergic systems. Hence it becomes clear why the level of depression, which correlates negatively with the brain catecholamine level [6], is the most informative behavioral parameter for predicting the resistance of rats to circulatory cerebral hypoxia. The results obtained are evidence that resistance to circulatory cerebral hypoxia (ischemia) can be predicted on the basis of behavioral characteristics.

LITERATURE CITED

1. I. V. Gannushkina, U. V. Baranchikova, L. A. Sibel'dina, et al., Zh. Nevropatol. Psikhiat., No. 1, 27 (1989).
2. M. N. Kondrashova, Metabolic Regulation of the Physiological State [in Russian], Pushchino (1984), pp. 3-6.
3. M. N. Kondrashova, Pharmacologic Correction of Hypoxic States [in Russian], Moscow (1989), pp. 51-70.
4. D. A. Kulagin and V. K. Fedorov, Genetics of Behavior [in Russian], Leningrad (1969), pp. 35-42.
5. R. P. Nartsissov, I. I. Dyukova, and I. S. Peterson, Arkh. Anat., **57**, 112 (1969).
6. R. D. Porsolt, A. Bertin, N. Blavet, et al., Eur. J. Pharmacol., **57**, 201 (1979).