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Relation of Cerebral Blood Flow to Neuronal Activity in Rats with Different Susceptibilities to Emotional Stress as a Predictor of Cerebral Ischemia Severity

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According to the results of 5-minute testing of free behavior in an open field carried out in different seasons, white laboratory rats were separated into three groups with high, medium, and low resistance to emotional stress, respectively (HR, MR, and LR rats), and it was found that the proportions of HR and MR rats, but not that of LR animals, varied with the season. Laser fluorimetry showed that local cerebral blood flow rates were highest in the HR group and lowest in the LR group. Conversely, wave amplitudes on the electroencephalogram (EEG) were lowest in the former group and highest in the latter. More significant differences among the three groups in levels of blood supply to the brain were revealed with a new method proposed to estimate blood flow per unit of EEG activity by calculating the ratio between the cerebral blood flow rate (expressed in ml/min/100 g brain tissue) and EEG activity (expressed in μV). HR rats proved to be more susceptible than their MR and LR counterparts to cerebral ischemia produced by occlusion of the common carotid arteries.

Key Words: *cerebral blood flow; neuronal activity; cerebral ischemia*

After occlusion of the same artery or arteries, different individuals may develop cerebral ischemia of differing severity because of individual variations in collateral circulation, nerve tissue metabolism, and immunoreactivity toward brain antigens after the occlusion of a particular artery [1,2,7]. Our previous studies using intravital ^{31}P-NMR spectroscopy demonstrated individual variations in the organization of energy metabolism in the nerve tissue of normal animals and in particular differences between animals with high and low susceptibility to cerebral ischemia [3,4]. A high NAD$^+$+NADH/ATP or NAD$^+$+NADH/CP ratio and a high coefficient of correlation between parameters of ^{31}P-NMR spectra are predictors of a poor prognosis for the cerebral ischemia developing in rats following compression of both common carotid arteries in the neck.

On the other hand, it is well known that rats differing in their resistance to emotional stress also differ in levels of catecholamines in the brain tissue and as well as in levels of certain neuropeptides [5,6,9]. This suggests that there is some concordance between the resistance of animals to

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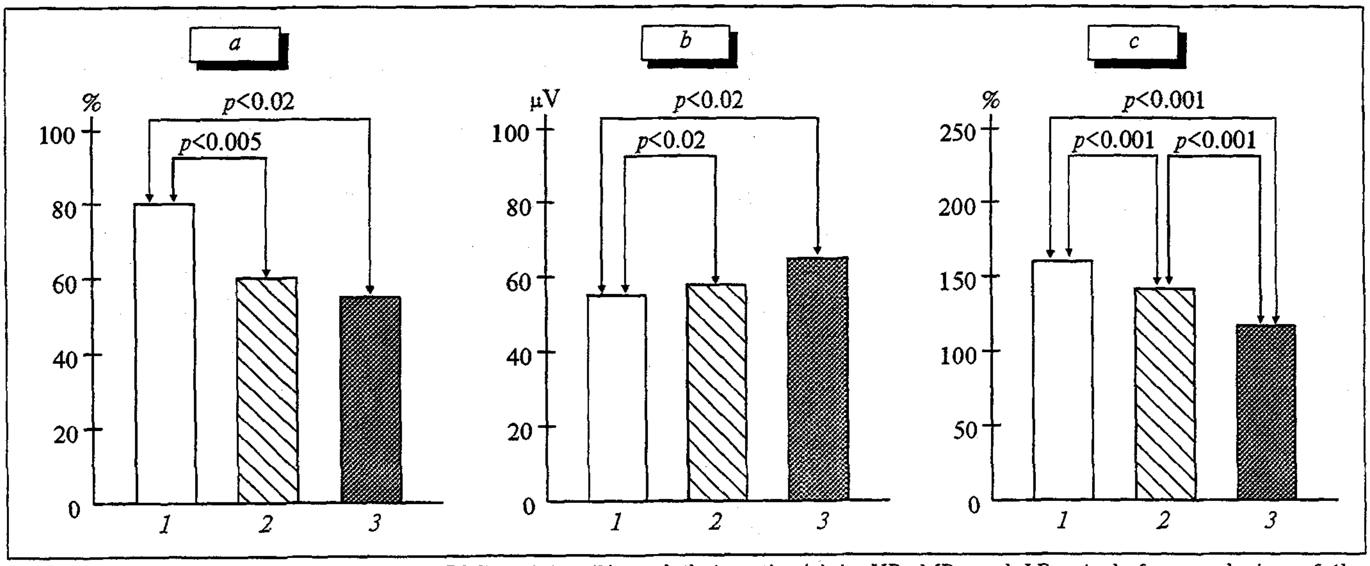

Fig. 1. Local cerebral blood flow (*a*), EEG activity (*b*), and their ratio (*c*) in HR, MR, and LR rats before occlusion of the common carotid arteries. *1*) HR rats ($n=178$); *2*) MR rats ($n=103$); *3*) LR rats ($n=64$).

emotional stress and their susceptibility to cerebral ischemia.

The present study was aimed at finding a method simpler than ^{31}P-NMR spectroscopy for separating animals into subgroups according to susceptibility to cerebral ischemia.

MATERIALS AND METHODS

A total of 385 white laboratory male rats weighing 250-280 g were used. To separate the rats into three groups with high, medium, or low resistance to emotional stress (HR, MR, and LR animals), their free behavior was assayed in an open field for 5 min [5-7,9] in different seasons of the year. In these tests, a circular arena 90 cm in diameter divided into 37 sectors with 8 columns (14 cm high and 4 cm in diameter) was used to determine the latencies of the first movement and of the movement toward to the center of the arena, the number of crossed peripheral and central sectors, the number of upright postures in the peripheral and central sectors, the number of columns explored, grooming time, and the intensity of defecation (number of "boluses" per 5 min).

Local cerebral blood flow was measured in Nembutal-anesthetized rats (40 mg/kg intraperitoneally) using a PF-3 laser fluorimeter and a needle sensor positioned at two symmetrical sites of the parietal bone in the areas where the bone had been thinned out with an electric drill 1 mm in diameter without penetrating into its inner compact lamella. The results were expressed in PeriFluxes.

These sites of the skull were also used to record an electroencephalogram (EEG) simultaneously with cerebral blood flow measurements. A feature of the EEG in small anesthetized animals is the strong dominance of theta waves. With the PM-6000 polygraph (Nikon Kohden) employed in our studies, it is possible not only to record these waves but also to monitor, using the integrating unit of the polygraph, variations in electrical activity of the brain at fixed time intervals chosen by the investigator. In this study, mean amplitudes of waves (mainly theta waves, which have a frequency of 4-8 per second) were determined in μV every 5 sec.

Cerebral ischemia was produced by ligation of both common carotid arteries in the neck of rats anesthetized with Nembutal in the indicated dose. The response of the local cerebral blood flow and EEG activity were recorded 10-15 min and 24 h after occlusion of the arteries. At 24 h postocclusion, the proportions of rats with different degrees of neurological deficit and of dead animals were recorded. The degree of neurological deficit was scored on McGraw's scale. The results were treated statistically using Student's *t* test.

RESULTS

The tests of open-field behavior confirmed the reported need [6,7,9] to distinguish between HR, MR, and LR rats in terms of resistance to emotional stress. It was found that whereas the proportion of LR rats (a total of 90 rats were tested) did not depend on the season in which the tests were performed, the ratios of HR and MR animals varied with the season. Thus, the proportion of HR rats (164 rats were tested) was

34% in winter and 51% in autumn, while that of MR (131 rats were tested) was 25% and 44%, respectively.

Before the common carotid arteries were occluded, the three groups showed significant differences in local cerebral blood flow rates and EEG amplitudes: flow rates were highest in the HR group and lowest in the LR group, whereas EEG amplitudes were, on the contrary, highest in the latter group and lowest in the former; MR rats were intermediate in these respects (Fig. 1, *a* and *b*). More significant differences among these three groups ($p<0.001$) were revealed using a coefficient we have proposed for estimating the extent to which each unit of EEG activity expressed in μV is provided with blood flow (Fig. 1, *c*). This coefficient, which represents the ratio between local cerebral blood flow and EEG activity takes into account not only the cerebral blood flow rate (ml/min/100 g brain tissue) but also the electrical activity of the brain tissue concerned. It indicates that cerebral blood flow per unit of EEG activity in HR rats must be greater than in MR rats and still greater than in LR rats to ensure normal brain functioning. Hence, just from these data, HR animals could be predicted to be more sensitive to cerebral ischemia (in which cerebral blood flow is diminished) than MR and LR animals.

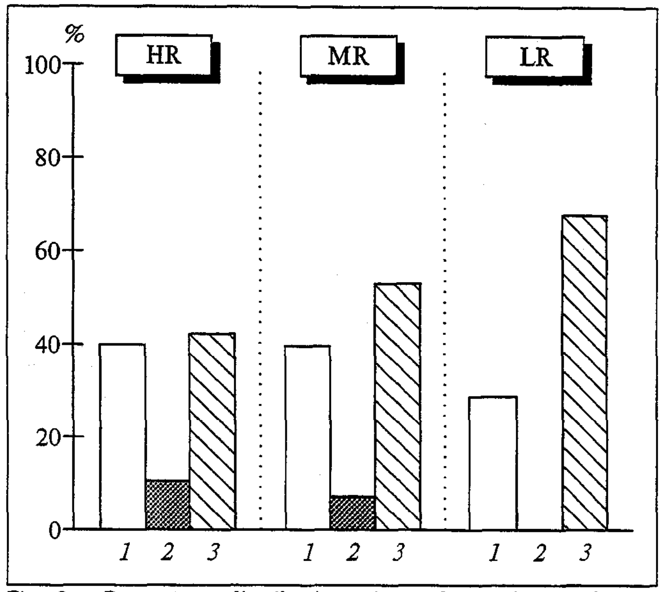

Fig. 2. Percentage distribution of rats 24 h after occlusion of the common carotid arteries. *1*) dead rats; *2*) rats with neurological deficit; *3*) symptom–free rats or animals with minor neurological deficit.

The experiments on rats with cerebral ischemia showed this prediction to be correct. Among

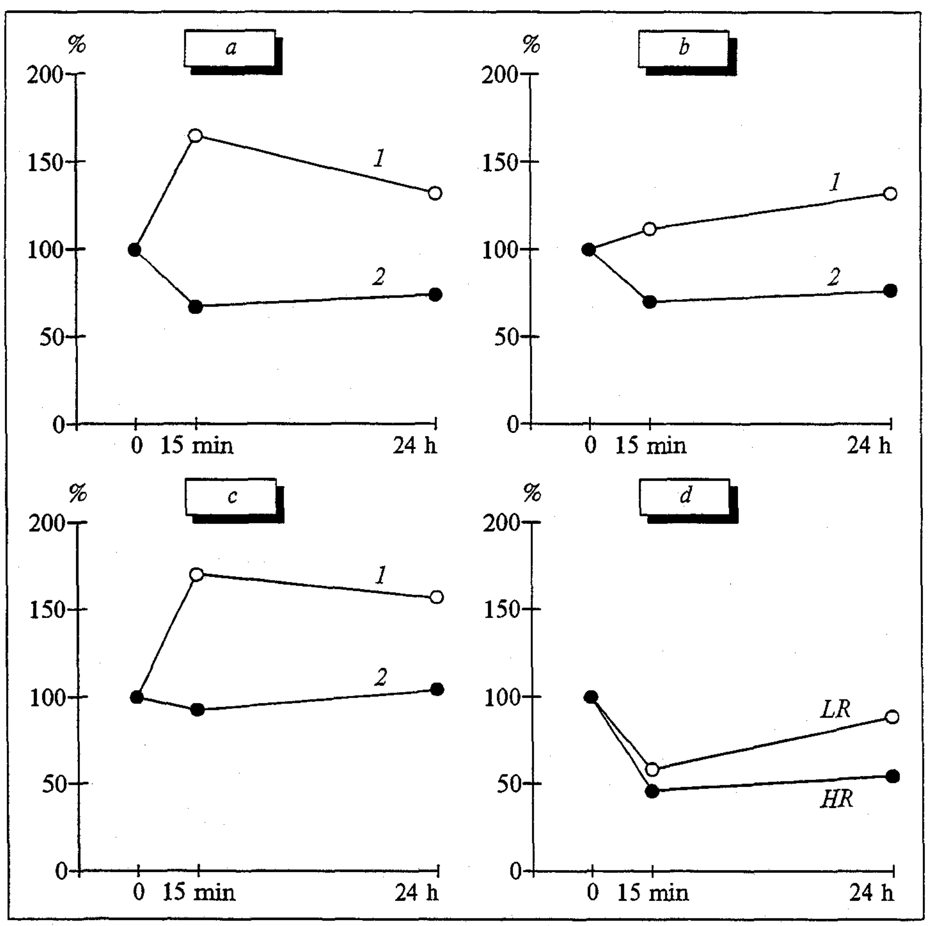

Fig. 3. Time course of EEG activity (*1*) and local cerebral blood flow (*2*) relative to their baseline values in HR (*a*), MR (*b*), and LR (*c*) rats in comparison with the time course of the ratio between local cerebral flow and EEG activity in HR and LR rats (*d*).

HR rats the proportion of dead animals as well as that of animals with severe neurological deficit were higher than among LR rats (Fig. 2).

Intergroup differences were also recorded in local cerebral blood flow and EEG activity 10-15 min and 24 h after arterial occlusion. In the HR and MR groups, the local blood flow rate at 10-15 min postocclusion was reduced to 67-65% of its baseline (preocclusion) level and remained low at 24 h (Fig. 3, *a* and *b*); in the LR group, it remained virtually unchanged at 10-15 min (about 91% of the baseline) and was even increased at 24 h (118% of the baseline) (Fig. 3, *c*). EEG activity rose in all three groups (Fig. 3, *a*, *b*, and *c*). In this animal model of moderate cerebral ischemia, therefore, blockage of the common carotid arteries resulted in elevation of EEG activity by the decreasing local cerebral blood flow. This can best be seen by looking at Fig. 3, *d*, which depicts variations in the local cerebral blood flow/EEG activity ratio in HR *vs.* LR rats.

In the present study, separation of the rats into three groups on the basis of their open-field behavior enabled us to identify some features of blood supply to brain tissue in each group under normal conditions. The brain was best supplied with blood in the HR group and worst in the LR group. Results from other studies [5-7,9] indicate that animals highly resistant to emotional stress have higher neural activity and also higher nerve tissue levels of catecholamines and oligopeptides than less resistant animals. The high level of blood supply to brain tissues we recorded in the HR group is apparently necessary to maintain the synthesis of all essential substances at normal levels. And it is this group that should suffer most as a result of the decrease in cerebral blood flow following occlusion of cerebral arteries. Indeed, HR and MR rats showed a greater imbalance between the change in EEG activity and the reduction in cerebral blood flow than did LR animals.

Open-field testing can therefore identify animals differing in their susceptibility to cerebral ischemia and in the relation between cerebral blood flow and neuronal activity. In our view, this relation may prove useful as a predictor of cerebral ischemia severity, although - and this needs to be stressed - it is less informative than the characteristics of brain energy metabolism as determined with intravital ^{31}P-NMR spectroscopy. Further, comparison of the present results with those obtained in an earlier study [6] indicates that specific features of brain susceptibility to ischemia may be revealed in various rat strains and with various methods of testing behavioral responses.

In conclusion, it is important to emphasize that the proposed method of evaluating cerebral blood flow takes account not only of the amount of blood as measured in ml per min per 100 g brain tissue but also the functional state of this tissue by relating the measured blood flow to the unit of EEG activity expressed in μV. This approach has proved to be considerably more informative than separate measurements of EEG activity and cerebral blood flow because it enables the investigator not only to quantify blood supply to a particular volume of nerve tissue but also to take into account the electrical activity of that volume of tissue.

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