Lipid Peroxidation in the Brain of Rats Differing in Resistance to Emotional Stress

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It is found for each of the rat brain regions studied (cerebral cortex, subjacent white substance, and brainstem) that both the initial levels of 2-thiobarbituric acid-reactive products and the rates of their increment are highest in rats resistant to emotional stress and lowest in stress-prone rats, and that the rates at which lipid peroxidation products accumulate are highest in the brainstem and lowest in the white substance. A correlation is presumed to exist between individual resistance to cerebral ischemia and the rate of lipid peroxidation in particular brain regions of healthy rats.

Key Words: *lipid peroxidation, rat brain, open-field behavior*

Experimental animals, and rats in particular, exhibit individual differences in the resistance of their cardiovascular functions to emotional stress (ES) [5]. According to the degree of their resistance to ES, rats have been classified into three groups: resistant, ambivalent, and predisposed [8], which are referred to below as highly-resistant (HR), medium-resistant (MR), and low-resistant (LR), respectively. A predictor of resistance to ES may be, for example, the behavior of rats in an open field [4] or their response to administered physiologically active substances.

It has been shown previously for rats that motor activity correlates with levels of biogenic amines in various brain structures [7] and with the content of β-endorphin, substance P, and delta-sleep-inducing peptide in the blood and hypothalamus [6], and that LR animals are particularly resistant to experimental cerebral ischemia while HR animals are least resistant [1].

In the present experiment, lipid peroxidation (LPO) in selected brain regions of rats differing in resistance to ES was studied in an attempt to offer an explanation of their differential sensitivity to cerebral ischemia.

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MATERIALS AND METHODS

The experiment was conducted during the spring and summer months on 24 random-bred white male rats weighing 220-260 g. For predicting their resistance to ES, orienting motor responses of the rats were evaluated quantitatively in an open-field test [4] 2 weeks before biochemical studies of their brain tissues. To obtain these, the animals were decapitated, their brains were removed in the cold and divided into three regions: brainstem (with cerebellar hemispheres), cerebral cortex, and white substance. The whole dissecting procedure was completed within 3-5 min. Thereafter all 72 specimens were placed in liquid nitrogen and stored at -70°C until use.

In each specimen, levels of carbonyl LPO products were estimated by their reaction with 2-thiobarbituric acid (2-TBA). LPO was induced in a medium containing iron and ascorbic acid. Brain tissue homogenates were prepared in the cold using phosphate buffered saline (PBS), pH 7.4, in a 1:8 ratio (w:v). The homogenates were centrifuged for 10 min at 11,000 rpm and assayed for protein

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by Lowry's method [11]. Ascorbate-dependent LPO was induced in a thermostatically controlled cell to which 4.5 ml of the supernatant (so diluted with PBS that the final protein concentration in the oxidation volume was 1 mg/ml) and 1.2 ml of a mixture of $FeSO_4$ (200 μM) and ascorbic acid (4 mM) solutions were added (at 37°C) to a final concentration of 20 μM and 400 μM, respectively.

At predetermined intervals (0, 5, 15, and 60 min), 0.7 ml of the suspension was collected and transferred to a test tube containing 0.7 ml of a mixture of 30% trichloroacetic acid and 0.67% 2-TBA (1:1) cooled to 0°C. The samples so prepared were kept on ice prior to the assay. When the collection of samples was completed, LPO products that had accumulated therein were determined. For the reaction, the samples were placed in a water bath for 30 min at 85°C and then centrifuged for 5 min at 11,000 rpm. Optical densities of the samples were measured with an SF-16 spectrophotometer at 535 nm. The quantities of formed products were estimated using the molar extinction coefficient of one of them, malonic dialdehyde (MDA), which is equal to 1.56×10^5 $M^{-1}\times cm^{-1}$.

The nonparametric Wilcoxon-Mann-Whitney test [3] was used for statistical treatment of the results.

RESULTS

The 24 rats used in the experiment were divided into three groups according to the quantitative evaluation of their orienting motor responses in the open-field test: HR (n=12), MR (n=8), and LR (n=4) groups. For these groups differing in their resistance to ES, MDA levels were then measured in the cerebral cortex, subjacent white substance, and brainstem with the cerebellar hemispheres.

It was found that the mean MDA content in the brainstem samples was higher, albeit insignificantly, than in the samples of white matter (0.63±0.19 *vs.* 0.52±0.10 nmol/mg protein; $p>0.05$). It should be noted here that the reported data on MDA levels in various regions of the rat brain are contradictory; for example, some authors claim the hemispheres to contain approximately twice as much MDA as the brainstem [9], whereas others report only 20% differences at most between different brain regions [12]. Such discrepancies are probably due to the use of different modifications of the method [10] by which 2-TBA-reactive products are measured, as well as of different approaches to topographic separation of the brain into regions where these products were assayed. Moreover, MDA levels in various rat organs are known to show seasonal variations.

When baseline (zero-time) MDA levels were compared in rats differing in susceptibility to ES, the mean level for HR rats was found to be 38% higher than for LR rats (0.71±0.12 *vs.* 0.47±0.05 nmol/mg protein).

Next, LPO reactivity was evaluated by recording the rates of increment of the MDA content 5, 15, and 60 min after LPO induction in the system containing iron and ascorbic acid ([MDA] at minute n/[MDA] at minute 0). Comparison of LPO reactivity in the three different brain regions showed that the mean rate of MDA increment over the indicated intervals was highest in the brainstem and lowest in the white substance in all three groups (Fig. 1) ($p<0.05$ for LR and

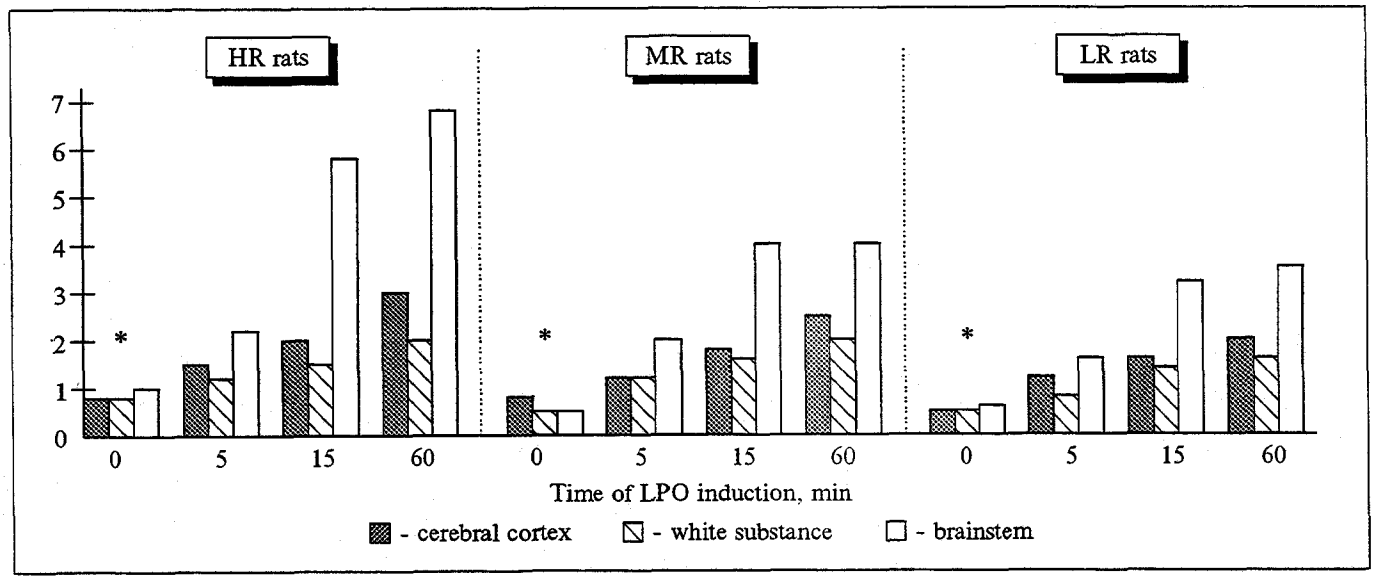

Fig. 1. Differences in LPO reactivity in three different brain regions of HR, MR, and LR rats. Here and in Fig. 2 the ordinate indicates ratios of MDA concentrations (expressed in nmol/mg protein) at minute n and minute 0, and the asterisk marks the baseline MDA level.

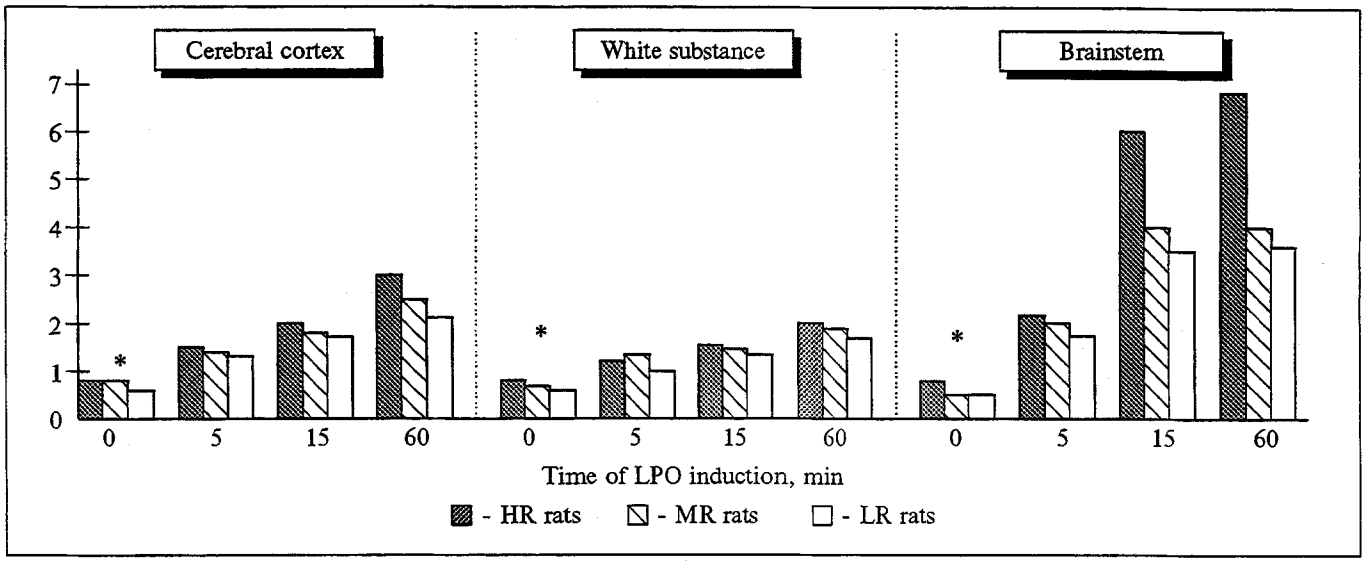

Fig. 2. Differences among the three groups of rats in the LPO reactivity of their cerebral cortex, white substance, and brainstem.

MR rats, $p<0.001$ for HR rats). When the three groups of rats differing in their susceptibility to ES were compared for LPO reactivity in their brain regions, the reactivity in each of the three brain regions proved to be highest for HR and lowest for LR rats (Fig. 2) ($p<0.05$ for the cerebral cortex and brainstem, $p>0.05$ for the white substance). The large scatter of the data, which is associated with individual differences among the samples from different animals, suggests the copresence of various factors limiting LPO reactivity in the tissues tested.

As already noted, it has been reported that in rats there is a link between motor activity and resistance to cerebral ischemia [1] and between motor activity and levels of biogenic amines (norepinephrine, epinephrine, and dopamine) [7]. In the present study, baseline MDA levels in the different brain structures of healthy rats differing in their open-field behavior were different, as were the rates of MDA increment: both the content of preformed LPO products and LPO reactivity were highest in HR rats, particularly in their brainstems.

There is evidence in the literature that differences in the susceptibility to cerebral ischemia among rats differing in behavioral characteristics may be dependent on the preponderance of vascular reactions of a particular type (pressor or depressor) in rats predisposed or resistant to ES [6], on different initial levels of biogenic amines and oligopeptides in the blood and various brain structures, and on differences in the direction in which the levels of these physiologically active substances change in response to stress [6] and, probably, to cerebral ischemia. As the results of this study indicate, the resistance to stress also depends on the initial levels and reactivity of LPO processes (using the model system containing iron and ascorbic acid) in the three rat brain regions examined.

In conclusion it should be added that different initial levels of LPO and different rates of this process in certain brain regions of rats differing in their open-field behavior may be due to differences not only in the initial levels of catecholamines - and, in particular, epinephrine which has been shown to affect the levels of primary and secondary LPO products [2] - but also in the lipid and fatty-acid compositions of the brain [13] and in the initial levels of antioxidants, including α-tocopherol and SH-containing antioxidants.

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