

LIPID PEROXIDATION IN RATS WITH EMOTIONAL STRESS: CORRELATION WITH OPEN FIELD BEHAVIOR

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The initial characteristics of free behavior are such that it is possible to predict the course and outcome of emotional stress (ES) in rats [7], and of damage to the gastric mucosa developing under these circumstances [10]. These characteristics correlate with certain neurochemical processes that determine the resistance of animals to experimental ES [6].

Activation of lipid peroxidation (LPO) is one of the factors disturbing physiological functions during ES [5, 9]. LPO processes in the tissues are modified at different phases of development of stress [5], and they correlate to a certain degree with changes in animal behavior [2].

This paper describes an attempt to compare parameters of open field (OF) behavior before exposure to stress and the content of malonic dialdehyde (MDA), an intermediate product of LPO, in the organs of animals after ES, on simple models of ES in Wistar rats.

EXPERIMENTAL METHOD

Experiments were carried out on 65 male Wistar rats. All the rats were tested in OF after starvation for 18 h, at 8-9 a.m.

Rats of group 1 (control) were killed at this stage. The remainder were divided into two groups. Animals of group 2 were immobilized for 24 h in constriction cages made of steel wire. Rats of group 3 were kept for 24 h in roomy cages without food or water. All the rats were decapitated after the experiment.

The MDA content was studied in homogenates of brain, liver, and heart by the reaction with 2-thiobarbituric acid (TBA) by measuring the concentration of TBA-active products (TBAAP), using a Specol-11 spectrophotometer (East Germany), at 532 nm. The results are given in the form of optical density units $\times 1000$ in the text and in Table 1. To estimate the ability of the liver and heart homogenates to undergo oxidation in the presence of Fe^{2+} ions, the concentration of TBAAP in them was studied before and after 30 min of incubation at 37°C with Fe^{2+} ions (100 μM).

The experimental results were subjected to statistical analysis by the Mann—Whitney and Kendall's nonparametric methods. Intergroup differences were tested by nonparametric single-factor dispersion analysis after Kruskal and Wallis, followed by paired comparison of groups after Konover.

EXPERIMENTAL RESULTS

Behavioral parameters, damage to the gastric mucosa, and accumulation of TBAAP in the organs indicated that 24-hourly immobilization, like deprivation of the animals of food and water for the same period, were powerful stressors.

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TABLE 1. Concentration of TBAAP in Tissues (in optical density units $\times 1000$)

Groups of rats	N	Liver	Heart	Brain
Control	5	5.6 ± 1.3	25.0 ± 5.8	12.6 ± 2.4
Immobilization	23	$34.9 \pm 1.1^*$	26.5 ± 2.7	$59.4 \pm 6.0^*$
Deprivation	28	$36.2 \pm 2.5^*$	20.9 ± 1.7	$54.7 \pm 3.9^*$

Legend. Difference from control level: $*p < 0.001$.

After 24 h of immobilization the horizontal (ambulation) and vertical (rearing) activity of the rats in OF was reduced by 2.6 and 4.1 times respectively, much more than the degree by which these parameters were reduced after starvation for 24 h in group 2 (by 1.76 and 2.4 times). In all groups the decrease in the number of vertical rears was significantly greater ($p < 0.05$).

The content of TBAAP in the liver and brain of the rats of groups 2 and 3 was significantly higher than the control level (Table 1). The content of TBAAP in the ventricles of the heart did not differ in rats of the three groups studied.

Further analysis revealed correlation between some characteristics of open field behavior of the rats and effects of immobilization or deprivation of food and water for 24 h.

Depression of the open field behavior of the rats, which according to many investigators is characteristic of stress [11], induced by immobilization or by prolonged starvation, was most marked in the initially active rats. Reduction of horizontal mobility correlated with its initial values in rats of group 2 ($p < 0.05$) and group 3 ($p < 0.025$).

Among the 23 animals which survived after immobilization 11 showed macroscopic features of injury to the gastric mucosa. After 24 h of starvation these injuries were observed much less frequently ($p < 0.03$) – in six of 28 rats. Gastric lesions caused by immobilization for 24 h correlated positively ($p < 0.05$) with total motor activity (TMA = ambulation + rearing) in the first OF test. Unilateral or bilateral disturbances of limb movements (their true nature was not precisely established) arose after immobilization in only five rats with high initial values for rearing/ambulation (R/A), indicating [7] low resistance of this particular subgroup to emotional stress. However, the TBAAP concentration in the liver of the immobilized rats correlated negatively ($p < 0.025$) with the value of the R/A ratio, i.e., the highest concentrations of TBAAP were observed in the subgroup with behavioral parameters indicating high resistance to emotional stress.

In the rats of group 3 the TBAAP concentration in the brain correlated negatively with the intensity of grooming ($p < 0.025$) and positively with the number of fecal boluses ($p < 0.025$), in agreement with data obtained by comparison of rats of two Tryon lines [8].

The TBAAP concentrations in different organs correlated only weakly. However, after immobilization the amplitude of the increase in TBAAP induced by Fe^{2+} ions in liver homogenates correlated negatively with the corresponding parameter for heart homogenates ($p < 0.05$). The nonhomogeneous reaction of LPO in the different organs to the stress situation is in agreement with data obtained by other workers [4].

In the deprived group the degree of depression of motor activity in OF correlated positively with the TBAAP concentration in the liver, but negatively with the amplitude of the increase in TBAAP under the influence of incubation with Fe^{2+} . With the considerable depression of ambulations, this parameter was significantly lower than in the subgroup in which horizontal activity did not change significantly (3.0 ± 2.1 compared with 25.9 ± 8.2 ; $p < 0.01$). If the reduction of activity in OF is considered to be a manifestation of stress, these data may indicate activation of LPO in the liver in emotional stress with depression of its antioxidative potential. A considerable number of factors linked with LPO parameters in the liver may be determined by its important role in adaptation to the stress situation [1].

Unlike the liver, the increase in the TBAAP concentration in the heart agreed more closely with a decrease in the number of vertical rears in the second OF test. In the subgroup with reduced vertical activity after 24 h of deprivation of food and water, the TBAAP concentration in the heart was significantly higher than in the remaining rats of group 2 (23.4 ± 2.3 and 17.5 ± 2.5 respectively, Mann–Whitney test; $p < 0.05$).

These results are evidence that the original parameters of the rats' behavior in OF, which can serve as characteristics of their stable individual features [1], correlate differently with changes in the animals' behavior and activation of LPO in response to different forms of stress. These differences may be due both to specific features of the procedures themselves and to differences in the speed of the course of particular phases of ES. Further investigations are needed to determine the parameters of free behavior that reliably predict the individual resistance of animals to ES.

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