cially against the background of calcium antagonists and prostacycline, PMNL induce, not vasoconstriction, but relaxation of the vessel and inhibition of platelet aggregation. This may perhaps be the result of the action of antiaggregating and vasoconstrictor factors formed in PMNL together with substances with the opposite kind of effects on blood vessels and platelets. The possibility cannot be ruled out that under different conditions of stimulation, the action of these substances may be stronger.

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# DEPENDENCE OF THE DEGREE OF STRESS-INDUCED HEART DAMAGE IN ENDOGENOUS β-ENDORPHIN LEVELS DURING PRELIMINARY ADAPTATION


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Emotional-painful stress (EPS) can cause myocardial damage and can disturb the contractile function of the heart [1, 4]. Much attention has recently been paid to the study of the mechanisms of function of stress-limiting systems [5] and, in particular, of opioid neuropeptides [2].

The aim of this investigation was to study the time course of β-endorphin levels in the blood plasma and brain structures during adaptation of an animal to extremal factors and to compare the data with the degree of resistance of the heart of the adapted animals to stress.

## EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 160-180 g. A state of adaptation was produced by a series of short-term immobilizations [6] or a course of Rhodiola extracts (a preparation with marked adaptogenic properties [7]), obtained from the stonecrop Rhodiola rosea, periodically in a dose of 1 mg/kg daily for 8 days. The action of this substance in the intact animal is realized through its effect on brain energy metabolism and its modulating action on the state of the pituitary-adrenal system. The rats were decapitated after 1, 3, and 5 sessions of adaptation by each of the above methods, and also at the end of the course of Rhodiola extract (8th day) or series of immobilizations (10th day),


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TABLE 1. Effect of Preliminary Adaptation of Varied Duration on Degree of Stress-Induced Myocardial Damage (M ± m)

| Experimental conditions | Number of observations | Percent of adsorbed dose of $^{99m}$Tc-PP/g myocardial tissues |
|---|---|---|
| Intact rats | 8 | 0.00704 ± 0.00031 |
| EPS for 6 h | 9 | 0.0251 ± 0.00212*** |
| Course of administration of Rhodiola extract | 12 | 0.0075 ± 0.00101 |
| Rhodiola extract once + 6 h EPS | 12 | 0.0219 ± 0.00073*** |
| Rhodiola extract three times + 6 h EPS | 7 | 0.0126 ± 0.00087*** |
| Rhodiola extract five times + 6 h EPS | 8 | 0.00906 ± 0.00041* |
| Rhodiola extract eight times + 6 h EPS | 8 | 0.0101 ± 0.00165 |
| Immobilization once | 7 | 0.0102 ± 0.00114* |
| Immobilization once + 6 h EPS | 7 | 0.0168 ± 0.00068*** |
| Immobilization three times | 8 | 0.0117 ± 0.00076** |
| Immobilization three times + 6 h EPS | 8 | 0.0210 ± 0.00235*** |
| Immobilization five times | 9 | 0.0121 ± 0.00115** |
| Immobilization five times + 6 h EPS | 7 | 0.0171 ± 0.00101*** |
| Immobilization ten times | 10 | 0.0118 ± 0.00213* |
| Immobilization ten times + 6 h EPS | 11 | 0.0140 ± 0.00051** |

Legend. Here and Table 2, asterisk indicates significance of differences from group of intact animals: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

6-8 h after the last session of adaptation. A model of EPS was created by the method in [8] both in intact rats and in rats previously undergoing one or more sessions of adaptation. The degree of myocardial damage due to EPS was assessed by determining the percentage uptake of an injected dose of $^{99m}$Tc-labeled pyrophosphate ($^{99m}$Tc-PP) per gram of tissue by the heart muscle. The radionuclide $^{99m}$Tc-PP was prepared when required from $^{99m}$Tc-pertechnetate, obtained from a molybdenum generator, and the nonradioactive component TCK-7, from the firm "Sorin" (France). The concentration of immunoreactive β-endorphin in the blood plasma was determined by means of kits from "Immuno Nuclear Corporation" (USA), after preliminary isolation of the fraction of this peptide by affinity chromatography on sepharose. The content of β-endorphin in brain structures was determined after extraction by the method in [10], with the aid of kits from "New England Nuclear" (USA). Radioactivity was counted on a gamma-spectrometer ("Tracor Analytic" USA). The experimental data were subjected to statistical analysis by Student's t test.

## EXPERIMENTAL RESULTS

The results in Table 1 and Fig. 1 show that stress caused marked accumulation of $^{99m}$Tc-PP in heart muscle compared with the group of intact rats. Accumulation of $^{99m}$Tc-PP in the myocardial cells reflects the degree of stress-induced heart damage [3].

Under the influence of stress, animals previously undergoing a course of adaptative treatment, $^{99m}$Tc-PP uptake into the cardiomyocytes exceeded that in the intact animals, but by a much lesser degree than in rats of the stress-control group. For instance, the level of radioactivity of the heart of the "trained" rats after stress rose to 0.0140 ± 0.00051% cpm/g, whereas in animals undergoing a course of Rhodiola extract, it rose to 0.0101 ± 0.00165% cpm/g. However, the intensity of accumulation of $^{99m}$Tc-PP in the myocardium in these cases was almost 2 and 2.5 times lower than in animals of the stress-control group.

Opioid peptides (enkephalins and endorphins) are known to have the property of reducing $^{99m}$Tc-PP accumulation in the myocardium in EPS [1] and stress-induced disturbances of contractility of nonischemic zones of the heart in infarction [6].

It will be clear from Table 2 that after a single does of Rhodiola extract the β-endorphin concentration in the brain structures of the animals did not differ significantly from that in intact rats, whereas the level of this peptide in the blood plasma was increased by 1.6 times, evidently due to a redistribution of endogenous reserves of the neuropeptide.

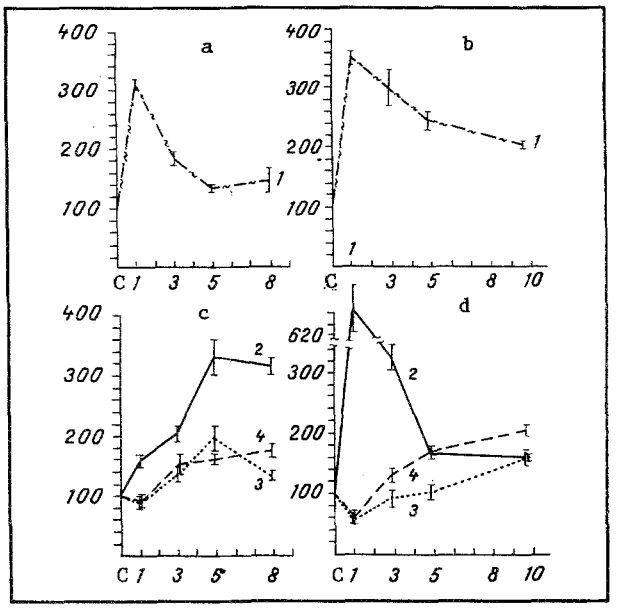


Fig. 1. Degree of heart damage during stress (a, b) and endogenous β-endorphin level (c, d) during preliminary adaptation (a, c – administration of Rhodiola extract; b, d – short periods of immobilization). Abscissa, time, days; ordinate, β-endorphin content, percent of intact control. 1) Accumulation of $^{99m}$Tc-PP in myocardium; 2, 3, 4) β-endorphin in blood plasma, hypothalamus, and midbrain, respectively. C) Control.

By the 3rd day of the experiment the β-endorphin content was increased both in the plasma and in the brain structures, and it reached maximal values by the 5th day of the experiment; this was probably evidence of intensification of the synthesis and secretion of this peptide under the influence of adaptation.

Changes in activity of the endogenous opioid system during short periods of immobilization were rather different. For instance, a single immobilization was accompanied by marked elevation of the plasma β-endorphin level, which is characteristic of the stress reaction [9, 11], with a simultaneous decrease in the β-endorphin concentration in the hypothalamus and midbrain. The reason was evidently that, unlike in "mild" adaptation by administration of Rhodiola extract, immobilization is a stronger stimulus, and leads to the development of a moderately severe stress reaction. This was confirmed by the moderate (compared with the stress-control) increase in $^{99m}$Tc-PP accumulation in the heart muscle cells of the rats under the influence of training immobilization procedures. Lowering of the β-endorphin level observed under these circumstances in the brain structures was evidently connected with redistribution of the peptide in the media of the body in response to stress. Support for this hypothesis is given by the fact that similar, although rather more marked, changes in the level of this peptide were observed in the tissues during simulation of EPS.

During subsequent training procedures the β-endorphin content in the hypothalamus and midbrain increased gradually and, by the 10th day of the experiment, it was 1.5-2 times higher than that in rats of the intact control group. The plasma β-endorphin level in "trained" rats at these times showed a significant fall compared with the first day of the experiment, although it still remained twice as high as in the group of intact rats.

The next experiments showed that a single dose of the adaptogenic agent Rhodiola extract, like a single session of immobilization, had no evident protective action on the myocardium, for $^{99m}$Tc-PP accumulation in the heart muscle of the rats of the above group during subsequent simulation of EPS was virtually identical with that in the stress-control (0.0251 ± 0.00212% cpm/g), and amounted to 0.0219 ± 0.00073 and 0.0168 ± 0.00068% cpm/g, respectively.

When animals receiving several sessions of adaptation were exposed to stress, the degree of $^{99m}$Tc-PP accumulation in the heart muscle fell progressively with an increase in

TABLE 2. β-Endorphin Concentration in Blood Plasma (in pmoles/liter), Hypothalamus (in ng/g tissues), and Midbrain (in ng/g tissue) in Course of Adaptation (M ± m)

| Experimental conditions | β-Endorphin concentration in | | |
|---|---|---|---|
| | blood plasma | hypothalamus | midbrain |
| Intact rats | 4,95±0,60 | 9,78±0,76 | 4,74±0,43 |
| EPS for 6 h | 30,25±4,38*** | 4,59±0,35*** | 1,82±0,40*** |
| Immobilization once | 33,02±2,18*** | 5,29±0,66*** | 2,38±0,42** |
| Immobilization three times | 15,90±1,22*** | 8,97±1,49 | 5,83±0,61 |
| Immobilization five times | 8,03±0,72** | 9,09±1,30 | 7,98±0,23*** |
| Immobilization ten times | 7,48±0,31** | 15,56±1,21** | 9,99±0,64*** |
| Series of immobilizations + 6 h EPS | 6,37±3,25 | 14,36±0,51*** | 6,37±0,86 |
| Rhodiola extract once | 7,89±0,51** | 8,31±1,12 | 4,12±0,80 |
| Rhodiola extract three times | 10,09±0,72*** | 13,55±1,13* | 7,14±0,70* |
| Rhodiola extract five times | 16,45±1,80*** | 19,13±2,03*** | 7,52±0,33*** |
| Rhodiola extract eight times | 15,34±0,89*** | 13,28±0,97* | 8,02±0,48*** |
| Course of Rhodiola + 6 h EPS | 16,14±3,27** | 8,87±0,56 | 5,52±1,08 |

the number of sessions of adaptation (Table 1). This pointed to a protective action of the latter against stress-induced myocardial damage. One sign of factor of increased resistance of the adapated animals could evidently be the "smoothing" of the response of the β-endorphin component of the opioid system which we observed in response to the action of stress factors (Table 2).

Preliminary adaptation of rats by 5-8 injections of Rhodiola extract or 5-10 sessions of training immobilizations thus causes a moderate rise of the plasma β-endorphin level and a significant rise in its level in the brain structures tested, accompanied by the formation of increased resistance of the heart to the damaging action of pathological stress. It can be tentatively suggested that the changes in β-endorphin activity which we found in the body tissues play an essential role in the mechanism of the protective effect of adaptation.

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