EFFECT OF OPIOID NEUROPEPTIDES ON THE PROSTAGLANDIN SYSTEM AND ON LIPID PEROXIDATION IN THE MYOCARDIUM DAMAGED BY STRESS

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An important role in the mechanisms of protection of the heart against stress-induced damage is played by activation of the "stress-limiting systems" [7], which can be divided into central (GABA- and serotoninergic, dopaminergic, and opiatergic systems) and peripheral [systems of prostaglandins (PG), adenine nucleotides, and antioxidants]. Meanwhile the problem of interaction between these systems during adaptation to stress remains unsolved. The effect of opioid peptides on the PG system and on lipid peroxidation (LPO) in the myocardium are the least studied aspects of this problem.

We give below data on the effect of the synthetic opioid peptide D-ala^{2}leu^5-arg^6-enkephalin (dalargin) or of preliminary adaptation, during which the concentration of endogenous opioids rises [3, 4], on concentrations of thromboxane (Tx) and prostacycline (Pc) in the myocardium in the course of its stress-induced damage, and also on the possibility of regulation of LPO processes during stress by dalargin.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 180-200 g. Stress damage was induced in the myocardium by the model described in [11]. States of adaptation were produced by a series of short immobilizations (10 times) [6] or a course of oral administration, daily for 8 days, of rhodiola extract — a preparation obtained from the plant *Rhodiola rosea* and possessing marked adaptogenic properties [8], in a sessional dose of 1 ml/kg. The rats were decapitated at the end of the course of rhodiola extract or series of immobilizations, 8 h after the last adaptogenic procedure. The enzyme resistant Leu-enkephalin analog dalargin was injected in a dose of 100 μg/kg before the beginning of exposure for 6 h to emotional-painful stress (EPS). Animals of the control groups received isotonic saline in equivalent volumes. The rats were killed 8 h after the end of stress. The degree of stress-induced myocardial damage was estimated as the percentage of uptake of the injected dose ($5.6 \cdot 10^{-3}$ MBq/100 g body weight, intravenously) of radioactive technetium pyrophosphate (^{99m}Tc-PP) by the cardiomyoctyes. The concentrations of Pc and Tx of the A_2 type in the myocardium of the experimental animals were determined from the levels of their stable metabolites 6-keto-prostaglandin $E_{1\alpha}$ and Tx of the B_2 type, by a radioimmunochemical method using standard kits from the Institute of Isotopes, Hungarian Academy of Sciences. Radioactivity was measured on a Gamma-spectrometer ("Tracor Analytic," USA). Lipids were isolated from the heart by the method in [12]. Accumulation of primary LPO products (conjugated dienes — CD) was estimated by their characteristic absorption at 232 nm [9]. End products of LPO, namely Schiff bases, were determined as fluorescence of lipids in chloro

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TABLE 1. Effect of Dalargin and Preliminary Adaptation on Concentrations of Pc and Tx (in pg/mg) in Myocardium of Rats under the Influence of Stress (M ± m)

Experimental conditions	Number of observations	PC	TX	PC/TX
Control	10	30,29±1,86	6,31±0,40	4,79±0,76
EPS for 6 h	10	24,97±3,32	9,83±0,78	2,54±0,89
p_1		>0,05	<0,001	<0,01
Dalargin + 6 h EPS	12	56,45±2,08	6,93±0,31	8,14±0,92
p_1		<0,01	>0,05	<0,01
p_2		<0,01	<0,01	<0,001
Series of immobilizations	9	45,86±3,68	5,22±0,67	8,79±0,68
p_1		<0,01	>0,05	<0,01
Series of immobilizations + 6 h of EPS	8	35,11±3,10	7,11±0,53	4,94±0,98
p_1		>0,05	>0,05	>0,05
p_2		<0,01	<0,01	<0,05
p_3		<0,05	<0,05	<0,01
Course of rhodiola extract	9	34,71±1,85	4,12±0,46	8,42±1,02
p_1		>0,05	<0,05	<0,01
Course of rhodiola extract + 6 h	10	31,87±2,06	6,56±0,76	4,86±0,56
p_1		>0,05	>0,05	>0,05
p_2		>0,05	<0,05	<0,01
p_4		>0,05	<0,01	<0,01

Legend. p_1) Significance relative to intact animals; p_2) significance compared with stress control; p_3) significance relative to animals receiving course of immobilizations; p_4) significance relative to animals adapted by rhodiola extract.

TABLE 2. Effect of Dalargin on LPO in Rat Cardiomyocytes During Stress (M ± m)

Group of animals experimental conditions	Number of observations	MDA, nmoles/mg protein	Schiff bases, relative units/mg lipids	CD, nmoles/mg lipids
Control	10	0,65±0,03	19,60±1,21	0,41±0,02
EPS for 6 h	9	1,64±0,12***	43,12±1,43*	0,68±0,05*
Dalargin + 6 h EPS	9	0,56±0,10	19,63±1,36	0,49±0,05

Legend. *$p < 0.05$ compared with group of intact animals.

form [10]. Measurements were made on "Hitachi" fluorometer (Japan), which was calibrated before each series of measurements with a 1% solution of quinine sulfate in 0.1 N H_2SO_4. Malonic dialdehyde (MDA) in the myocardium was determined by the method in [15] and estimated on the basis of their characteristic absorption at 535 nm on an SF-26 spectrophotometer. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

The development of EPS was accompanied by a significant increase in ^{99m}Tc-PP accumulation in the cardiomyocytes from 0.00704 ± 0.00031 in the group of intact rats to 0.0251 ± 0.00212% cpm/g in the group of stressed rats. The level of radioactivity of the heart of rats undergoing a course of training immobilizations or receiving rhodiola extract was increased after exposure to stress to 0.0140 ± 0.00051 and 0.0101 ± 0.00165% cpm/g respectively. In rats receiving dalargin once before stress, no significant increase in ^{99m}Tc-PP accumulation by the myocardium took place. The intensity of accumulation of indicator in the myocardium in all cases when adapted animals were exposed to stress was 2-3.5 times less than in animals of the corresponding stress-control groups. This result was in agreement with data obtained by the writers previously [1, 4].

It follows from Table 1 that stress increased the concentration of TxA_2 in the heart tissue by comparison with the group of intact rats by 1.56 times. One of the main mechanisms of intensification of Tx formation from arachidonic acid is considered to be the adrenergic activation of LPO characteristic of stress [5]. Meanwhile, intensification of LPO is a powerful inhibitor of prostacycline synthetase [14]. This evidently explains the absence of significant changes in the Tx antagonist, Pc, during stress and the reduction by 1.9 times of the ratio Pc/Tx, characterizing functional activity of the PG

system, compared with the intact control. It will be clear from Table 2 that after stress accumulation of end products of LPO was observed: CD by 1.6 times, Schiff bases by 2.2 times. The concentration of MDA, an intermediate product of LPO, in the heart rose by 2.5 times. The reason for LPO activation in the heart muscle could be stress-induced excitation of the sympathoadrenal system [5].

Injection of dalargin led to limitation of the rise of the Tx level in the myocardium of the stressed rats by 2.1 times compared with the stress control (Table 1). Meanwhile the stimulating action of the peptide was observed on Pc production, as shown by elevation of the Pc level in the heart muscle by 2.2 times. The Pc/Tx index was increased from 2.54 during stress to 8.14 in the experiment. This effect of dalargin is evidently connected with its antiadrenergic properties [13], one result of which could be weakening of the signal from the receptor to the enzyme and contractile systems of the cell. Furthermore, the action of enkephalin on the system of antioxidants could play a role in the mechanism of this phenomenon, for administration of the peptide inhibited activation of LPO processes during stress, as was shown by limitation of the rise of MDA, Schiff bases, and CD compared with the stress control (Table 2).

It can be postulated that besides their action on the PG system [2], enkephalins can also realize their cardioprotective action in stress through the system of endogenous antioxidants.

It was shown previously that preliminary adaptation as a result of 5-8 doses of rhodiola extract or 5-10 sessions of training immobilizations causes elevation of the β-endorphin and Leu-enkephalin levels in the blood plasma, hypothalamus, and mesencephalon, and as a result, an increase in the resistance of the heart to damage by stress [3, 4].

Table 1 shows that the Tx concentration in the myocardium after a series of immobilizations did not differ significantly from that in intact rats, whereas the Pc level was increased by 1.5 times, evidently a definite compensatory reaction. The above changes led to activation of the PG system (the Pc/Tx index was 7.9).

Changes in activity of the myocardial PG system during adaptation by a course of rhodiola extract were rather different. In this case, for instance, the Pc level did not change appreciably, but the concentration of Tx in the cardiomyocytes was reduced by 1.5 times compared with that in intact animals, possibly on account of inhibition of the enzyme systems responsible for Tx formation.

The ratio between Pc and Tx, just as after a course of training immobilizations, rose to 8.42, but this time it was determined to a greater degree by Tx.

Exposure of animals subjected to adaptation by a series of short immobilizations to stress was accompanied by a decrease in the Pc concentration in the heart muscle to that observed in intact rats, and a small increase in the Tx concentration in the myocardium. However, the latter, under these circumstances, did not differ from its values in the control animals. As will be clear from Table 1, activation of the PG system, characteristic of EPS, did not take place after adaptation by a series of short immobilizations, as was shown by the absence of any changes in the Pc/Tx index. EPS after adaptation by a course of rhodiola extract led to an increase in Tx in the heart tissue compared with adapted animals, up to the level of intact animals, but elevation of the Tx level to values found in the stress control group was no longer observed. The Pc concentration in the myocardium of the adapted animals was unchanged by stress. The ratio between Pc and Tx fell to that observed in intact animals.

Thus preliminary adaptation of rats by means of a course of rhodiola extract or of immobilization training sessions induces a significant increase in the Pc/Tx ratio in the heart tissue, and this is accompanied by reduction of the severity of heart damage during subsequent exposure to EPS. The ability of opioid neuropeptides to diminish stress-induced heart damage may be linked with their direct and (or) indirect modulating effect on the peripheral stress-limiting systems (in our investigations the PG and antioxidant systems).

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EFFECT OF DALARGIN ON THE HEMODYNAMICS OF ANESTHETIZED RATS AFTER TRUNCAL VAGOTOMY

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Dalargin, a synthetic Leu-enkephalin analog, has been successfully used in combination with measures of anesthesiologic protection during various types of surgical operations [1, 5, 6, 8]. Dalargin effectively stabilizes the hyperdynamic response of the circulation to a supramaximal nociceptive stimulus [9], and the addition of an opioid peptide to the program of combined general anesthesia has led to a substantial decrease in the amounts of general anesthetics used during operations on the abdominal organs without any detrimental effect on the quality of the anesthesia [2, 7]. General anesthesia with dalargin also was characterized by lower values of total peripheral resistance at the traumatic stages of the operation compared with patients undergoing surgery under neuroleptanalgesia. This phenomenon may probably be the result of the depressant effect of dalargin on the autonomic nervous system. However, we have noted that atropine, if given as a component of premedication, leads to some impairment of the course of combined general anesthesia with dalargin, manifested sometimes as the development of moderate arterial hypertension, frequently unconnected with traumatic stages of the operation.

The aim of this investigation was to study the effect of dalargin on the hemodynamics of anesthetized rats after bilateral frontal vagotomy and in response to nociceptive stimulation.

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