ACTION OF ENZYME-RESISTANT LEU-ENKEPHALIN ANALOG ON MYOCARDIAL PROSTANOID LEVELS IN STRESS- AND ADRENALIN-INDUCED DAMAGE

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Data on the ability of Leu-enkephalin analogs to reduce ^{99m}Tc-pyrophosphate (TcPP) accumulation in the myocardium and to lower blood enzyme levels in stress-induced heart damage were obtained by the writers previously [4, 7]. These cardioprotective effects of opiate-like peptides are linked in many respects with their ability to modulate the hormonal mechanisms of stress [5, 6], but the question of the point of application of peptidergic influences in the system of neurohumoral regulation of tissue metabolism in stress-induced damage to the heart muscle remains open. It has been claimed that prostaglandins and cyclic nucleotides can play an indirect role in this process. The effect of enkephalins on the cyclic nucleotide system was studied by the writers previously [9, 12].

This paper gives data on the effect of D-Ala$_2$-Leu$_5$-Arg$_6$-enkephalin (dalargin) on thromboxane (TX) and prostacycline (PC) levels in the myocardium when subjected to stress- or adrenalin-induced damage.

EXPERIMENTAL METHOD

Experiments were carried out on 129 noninbred albino rats weighing 180-250 g. Stress-induced myocardial damage was simulated by the method in [11], using a conflict between unconditioned pulsed electrical stimulation and a conditioned pain avoidance reflex. Adrenalin-induced myocardial damage was caused by a single subcutaneous injection of isoprenoline sulfate ("Germed," East Germany) in a dose of 40 mg/kg. Control animals received isotonic saline. The degree of stress-induced myocardial damage was assessed on the basis of the percentage uptake of the injected dose ($5.6 \cdot 10^{-3}$ MBq/100 g body weight intravenously) of labeled radioactive TcPP, by the cardiomyocytes, and the release of the marker enzyme creatine phosphokinase (CPK), and of myoglobin, into the blood stream. Plasma CPK activity was determined spectrophotometrically using a kit of reagents from "Lachema" (Czechoslovakia), and myoglobin was determined by means of Soviet kits from the "Izotop" Combine. At the end of the 6th hour of emotional-painful stress (EPS) the rats were given an intraperitoneal injection of the synthetic enzyme-resistant Leu-enkephalin analog dalargin, in a dose of 100 μg/kg. Animals of the control groups received isotonic saline in equivalent volumes. The rats were killed 2, 8, and 12 h after the end of stress. Concentrations of PC and of TX A_2 in the myocardium of the experimental animals were determined by measuring levels of their stable metabolites 6-keto-PGF and TX B_2 by radioimmunoassay using standard kits from "Izinta" (Hungary). The final determination of radioactivity of the samples was made on a gamma-spectrometer ("Tracor Analytic," USA) and a "Mark III" beta-scintillation counter (USA). The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

The model with EPS or adrenalin-induced myocardial damage was accompanied by intensification of TcPP accumulation in the cardiomyocytes and by an increase in the blood levels of enzymes and myoglobin, which were most marked 8 h after the beginning of the investigation. This result agreed with those obtained previously [4, 7]. Injection of dalargin significantly limited these manifestations of necrobiosis in the myocardium.

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TABLE 1. Effect of Dalargin on Concentrations of PC (in pg/mg) and TX (in pg/mg) in Myocardium after Damage Induced by Stress and Adrenalin ($\bar{X} \pm x$)

Experimental conditions		PC	TX
EPS			
Intact		29,54±1,40	6,34±0,39
2 h	C	25,72±1,85	10,68±1,51
	p_1	>0,05	<0,05
	E	46,35±2,2	4,265±0,33
	p_1	<0,01	<0,05
	p_2	<0,01	<0,001
8 h	C	25,44±1,09	14,58±0,63
	p_1	>0,05	<0,01
	E	56,45±2,08	6,93±0,31
	p_1	<0,01	>0,05
	p_2	<0,01	<0,01
12 h	C	17,90±1,31	13,46±0,73
	p_1	<0,01	<0,01
	E	48,22±2,21	3,07±0,13
	p_1	<0,01	<0,001
	p_2	<0,01	<0,001
Adrenalin necrosis			
Intact		28,81±1,30	7,63±0,36
2 h	C	25,15±0,39	11,71±0,52
	p_1	<0,05	<0,001
	E	45,17±2,33	8,74±0,68
	p_1	<0,001	<0,05
	p_2	<0,001	<0,001
8 h	C	18,25±1,28	11,79±0,36
	p_1	<0,001	<0,001
	E	39,67±1,72	8,58±0,40
	p_1	<0,001	>0,05
	p_2	<0,001	<0,001
12 h	C	26,23±0,59	13,42±0,53
	p_1	>0,05	<0,001
	E	43,09±2,42	10,17±0,54
	p_1	<0,001	<0,001
	p_2	<0,001	<0,001

Legend. C) Stress-control, E) experiment. p_1) Significance relative to intact rats, p_2) significance relative to stress-control.

Table 1 shows that the TX A_2 concentration in the heart tissue 2 h after the end of EPS was increased by 1.7 times compared with the basal level, after 8 h by 2.3 times, and after 12 h by 2.1 times. The immediate cause of this rise of the TX level, it can be agreed, was potentiation of its biosynthesis by catecholamines both in platelets [2] and in cardiomyocytes [13]. Quantitative changes in PC, a TX A_2 antagonist, under these circumstances was evidence of inhibition of its formation during EPS. For instance, the PC concentration in the myocardium at the corresponding times was reduced by 1.15, 1.16, and 1.65 times compared with that in the group of intact rats. Inhibition of PC synthesis could be the result of inhibition of PC-synthetase under the influence of endoperoxides of fatty acids and low-density lipoproteins, formed in excess when the cardiomyocyte membranes are damaged [10, 14]. Intensification of TX A_2 production, noted above [3], also evidently plays not the least important role in this process.

Injection of dalargin helped to restrict the rise of the TX level compared with the stress-control by 2.5 times after 2 h, by 2.1 times after 8 h, and by 4.4 times after 12 h. Meanwhile the peptide had a stimulating action of PC biosynthesis, as shown by elevation of the PC level in the heart muscle by 1.8, 2.2, and 2.7 times, respectively, compared with the stress-control.

The results suggest that a role in the mechanism of the cardioprotective action of enkephalins in stress-induced heart damage may be played by their action on the intracardiac TX A_2:PC ratio in favor of PC, because TX A_2 is a powerful vasoconstrictor and proaggregant,

whereas PC possesses alternative properties. A positive action of enkephalin on the microcirculation of the heart muscle can accordingly be postulated in stress.

A leading role in the pathogenesis of stress-induced heart damage is played by the cardiotoxic action of high catecholamine concentrations [8]. Our experiments with isoprenoline-induced myocardial necrosis (Table 1) showed that injection of enkephalin, just as during EPS, inhibits the adrenergic rise of the TX concentration by 1.3 times after 2 h, by 1.4 times after 8 h, and by 1.3 times after 12 h and prevents the fall of AP by 1.8, 2.2, and 1.6, times, respectively, compared with the stress-control. These data confirm our previous view that the antiadrenergic properties of opioid peptides are based on their ability to modulate catecholamine formation [1] and to cause desensitization of the cardiomyocytes to the action of sympathicomimetics [9, 12]. The direct or indirect action of enkephalins on activity of the principal prostanoids may therefore be an essential factor in the cardioprotective action of the former against myocardial damage induced by stress and by adrenalin.

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