EFFECT OF SYNTHETIC ENKEPHALINS ON PROSTAGLANDIN SYNTHESIS AND LIPID PEROXIDATION IN THE ISOLATED HEART DURING ACTIVATION OF FREE RADICAL PROCESSES

Yu. B. Lishmanov, T. Yu. Rebrova, S. A. Afanas'ev, and A. I. Kravchenko

UDC 616.12-008.939.15-39]+616.12-008.94:577.175.859]-02-07

KEY WORDS: opioid neuropeptides; lipid peroxidation; prostaglandins; heart

Research in recent decades has revealed the essential role of lipid peroxidation (LPO) in the pathogenesis of atherosclerosis [2], of ischemic and reperfusion heart damage [14], and also in mechanisms of the response of the organism to various extremal factors [1]. There is reason to regard activation of LPO as a nonspecific component of the response of the body to any factor unusual as regards its strength or the duration of its action [1]. Meanwhile the question of the mechanisms of the effect of stress-limiting systems on the state of LPO during adaptation to extremal factors remains open. In previous studies we showed that preliminary injection of synthetic enkephalin analogs (D-Ala^2-Leu^5-Arg^6-enkephalin and D-Met^2-Pro^5-enkephalinamide) reduces the severity of lesions of heart muscle associated with ischemia and stress [7, 9] and also reduces the degree of activation of LPO in the rat myocardium [3]. This inhibitory effect is realized through activation of peripheral stress-limiting systems: antioxidants and prostaglandins [3, 8]. Nevertheless, the problem of the mechanisms of the cardioprotective and antiradical action of enkephalins is not completely clear. We know that at the whole body level enkephalins can reduce the sensitivity of myocardial adrenoreceptors and also inhibit the release of catecholamines from the adrenals and sympathetic terminals [10], which theoretically can lead to inhibition of stress-induced activation of LPO. However, we could find no convincing experimental proof of such a mechanism of action of enkephalins in the literature. The question of the ability of the enkephalins to exert a direct antioxidant action at the whole body level, and also to exhibit their effects on arachidonic acid metabolism likewise remains open.

The aim of this investigation was to study the effect of synthetic enkephalins on the intensity of LPO and on synthesis of prostacyclin (PC) and thromboxane (Tx) during nonischemic activation of free-radical processes in the muscle of the isolated rat myocardium.

EXPERIMENTAL METHOD

Experiments were carried out on the isolated hearts of noninbred albino female rats weighing 200-250 g, perfused by Langendorff's method, with solution at a temperature of 37 ± 0.5°C. The Krebs–Henseleit physiological saline had the following composition, in mmoles/liter: NaCl – 120, KCl – 4.8, $CaCl_2$ – 2, $MgSO_4$ – 1.2 NaH_2CO_3 – 25, KH_2PO_4 – 1.2, glucose – 10. The hearts contracted at a spontaneous frequency of the order of 4.5 Hz. Synthetic enkephalin analogs D-Ala^2-Leu^5-Arg-^{6}-enkephalin (dalargin, synthesized at the Cardiologic Scientific Center, Russian Academy of Medical Sciences, by Doctor of Chemical Sciences M. I. Titov) and D-Met^2-Pro^6-enkephalinamide GYKI 14.238 (generously provided by Dr. J. I. Szekely, Institute of Pharmacology, Hungarian Academy of Sciences) were used in the experiments. LPO in myocytes of the isolated heart was activated by means of an O_2^-generating

Laboratory of Radionuclide Methods of Investigation, Research Institute of Cardiology, Tomsk Scientific Center, Russian Academy of Medical Sciences. (Presented by Academician of the Russian Academy of Medical Sciences R. S. Karpov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 114, No. 11, pp. 468-470, November, 1992. Original article submitted April 29, 1992.

 0007-4888/92/0010-1596$12.50

TABLE 1. Effect of Preliminary Injection of GYKI 14.238 and Dalargin on Lipid Peroxidation during Activation of Free-Radical Processes (M ± m)

Group of animals	Number of observations	MDA concentration, nmole/mg protein	CD level, OD_{232}/g tissue	AOA, μeq Tx/mg lipids
Control I	5	0,37 ±0,063	1,67±0,10	0,037±0,0009
Control II	6	1,66±0,018	3,72±0,63	0,041±0,012
		p_1<0,001	p_1<0,001	p_1>0,05
Dalargin intraveneously + activation of LPO		0,89±0,037	1,65±0,18	0,031±0,0048
	8	p_1<0,001	p_1>0,05	p_1>0,05
		p_2<0,001	p_2<0,001	p_2>0,05
		1,56±0,017	1,65±0,27	0,030±0,0008
	9	p_1<0,001	p_1>0,05	p_1<0,001
		p_2>0,05	p_2<0,001	p_2>0,05
GYKI 14.238 intravenously + activation of LPO		1,21±0,107	2,29±0,16	0,033±0,0063
	8	p_1<0,001	p_1<0,001	p_1>0,05
		p_2<0,05	p_2<0,05	p_2>0,05
GYKI 14.238 by perfusion + activation of LPO	9	1,42±0,095	2,66±0,21	0,037±0,0022
		p_1>0,05	p_1<0,001	p_1>0,05
		p_2>0,05	p_2<0,05	p_2>0,05

Legend. Here and in Table 2: p_1) significance of difference compared with control I group, p_2) compared with control II group.

TABLE 2. Effect of Synthetic Enkephalin Analogs on Prostaglandin Concentrations in Myocardium during Activation of LPO (M ± m)

Group of animals	Number of observations	Prostacyclin, ng/g tissue	Thromboxane, ng/g tissue	PC/Tx
Control I	5	994,58±120,58	61,71±5,53	16,11±1,77
Control II	6	355,70±57,96	54,19±10,38	6,56±0,92
		p_1<0,001	p_1>0,05	p_1<0,001
Dalargin intravenously + activation of LPO	8	565,51±35,44	33,28±2,44	16,12±1,32
		p_1<0,01	p_1<0,001	p_1>0,05
		p_2<0,01	p_2<0,001	p_2<0,001
Dalargin by perfusion + activation of LPO	9	370,88±36,78	32,91±2,36	10,62±0,98
		p_1<0,01	p_1<0,001	p_1<0,01
		p_2>0,05	p_2<0,001	p_2<0,001
GYKI 14.238 by perfusion + activation of LPO	8	691,25±98,56	60,23±5,80	9,84±1,01
		p_1>0,05	p_1>0,05	p_1<0,01
		p_2<0,05	p_2>0,05	p_2<0,001
GYKI 14.238 by perfusion + activation of LPO	9	315,99±39,70	38,41±3,62	8,68±0,87
		p_1<0,01	p_1<0,001	p_1<0,001
		p_2>0,05	p_2<0,001	p_2>0,05

system Fe^{2+}-ascorbate. It has been shown experimentally that LPO in cardiomyocytes is activated by Fe^{2+} and ascorbate in concentrations of 0.2 and 0.5 mM respectively. The effect of dalargin and of GYKI 14.238 was investigated in the following series: 1) dalargin 100 μg/kg was injected intravenously into the animals. The animals were decapitated under superficial ether anesthesia 5 min after injection of the preparation and their hearts were perfused for 20 min by the method described previously with Krebs–Henseleit solution containing $FeSO_4$ and ascorbate; series 2 – dalargin was added to the Krebs–Henseleit solution in a concentration of 200 μg/100 ml. The isolated hearts of conventionally intact rats were perfused with this solution for 5 min, after which the composition of the perfusion fluid was changed for a solution containing $FeSO_4$-ascorbate, which was passed through the system for 20 min. The series with GYKI 14.283 followed a similar plan. In the control experiments perfusion of the isolated hearts for 25 min was carried out with the Krebs–Henseleit physiological saline (control I) or with Krebs–Henseleit solution containing $FeSO_4$ (0.2 mM) and ascorbate (9.5 mM) (control II). At the end of each experiment the hearts were frozen in liquid nitrogen. To assess the state of LPO processes in the myocardial tissue, the concentrations of primary LPO products (conjugated dienes – CD), as the characteristic absorption at 232 nm [11], and also of an end product – malonic dialdehyde (MDA), by the method in [15], were determined spectrophotometrically. The antioxidative activity (AOA) of the lipids was determined by Glavind's method [12], using the stable radical α-diphenylpicrylhydrazine (DPPH) at a wavelength of 517 nm. To determine the concentrations of PC and of TxA_2,

based on levels of their stable metabolites 6-keto-prostaglandin $F_{1\alpha}$ and TxB_2, enzyme immunoassay kits of Russian manufacture (MP "ASPID," Research and Production Center for Medical Bioengineering, Ministry of Health of Russia) were used.

EXPERIMENTAL RESULTS

Perfusion of the isolated heart with solution containing 0.2 mM $FeSO_4$ and 0.5 mM ascorbate caused an increase in concentrations of MDA and pC by 4.5 and 2.2 times respectively compared with the intact control group (Table 1), evidence of activation of LPO in the heart. No significant changes in AOA were observed.

Preliminary intravenous injection of dalargin led to significant weakening of the stimulating effect of the Fe^{2+}-ascorbate system on LPO. The MDA concentration in heart tissue homogenates from this group was only half of that in control group II. The CD level was the same as in the "intact hearts." In the analogous group, using GYKI 14.238, a significant decrease in assessable parameters of LPO activity also was observed (Table 1), evidence of a decrease in the intensity of LPO.It can thus be concluded that with this method of administration of the preparations dalargin has a stronger inhibitory effect on LPO in the myocardium than GYKI 14.238.

So far as the system of lipid antioxidants is concerned, no significant changes were observed in the groups examined. Considering the multistage nature of free-radical lipid oxidation, it can be regulated at different stages by systems of enzymic and nonenzymic nature. It can be tentatively suggested that the fall in the level of LPO processes in our experiments took place on account of activation of the enzymic component of antiradical defense.

No significant reduction of MDA in the heart was observed in the series in which synthetic enkephalins were injected into the perfusion system, compared with control group II (Table 1). Meanwhile the CD concentration in groups with dalargin or GYKI 14.238 was depressed by 55 and 28% respectively (Table 1). These facts suggest that enkephalins may have an effect on intermediate stages of the LPO process. There were no significant changes in the value of total AOA of lipids in the groups examined.

Stimulation of LPO in isolated heart tissue was accompanied by a significant fall in the PC level in the myocardium by 60% (Table 2). Hence a role for endoperoxides in the mechanism of inhibition of PC biosynthesis after administration of $FeSO_4$-ascorbate can be suggested, for we know that the intensification of LPO is a powerful inhibitory factor of prostacyclin synthetase [13].

The Tx level under these circumstances showed no significant changes (Table 2). The fall of the PC/Tx ratio relative to the intact control is evidence of inhibition of the functional activity of this component of the PG system [5].

Intravenous injection of dalargin, like that of GYKI 14.238, largely prevented the fall in PC level compared with control II. It can be postulated that this effect could have been due to a reduction in the intensity of free-radical processes, for a decrease in the turnover of lipid peroxides is known to promote restoration of the functional activity of the enzymes involved in PC synthesis [13]. A significant decrease in Tx compared with the control groups was observed in the group receiving dalargin (Table 2), which led to normalization of the PC/Tx ratio. Adrenergic activation of LPO is also one of the mechanisms of intensification of Tx synthesis from arachidonic acid during stress [7].

Injection of dalargin or GYKI 14.238 into the perfusion solution caused no significant changes in PC compared with the control group with activation of LPO, but the Tx concentration under these circumstances was lowered by 41 and 30% respectively, leading to an increase in the PC/TX ratio. The simultaneous decrease in synthesis of PC and Tx may evidently be due to an increase in the relative importance of the lipooxygenase pathway of arachidonic acid metabolism.

The results are thus evidence of the complex character of interaction between different representatives of stress-limiting systems. On the basis of the results it can be concluded that the antiperoxide effect of enkephalins may be realized through activation of systems of antioxidants and prostaglandins. The magnitude of the response of the peripheral components of the stress-limiting systems depends on the structure of the synthetic analog and the method of its administration. The protective effects of enkephalins are manifested more especially at the whole body level, so that an important role can be ascribed to neuroendocrine and other regulatory systems in the realization of defensive opiatergic effects. At the same time the possibility that enkephalins may act at the organ level likewise cannot be

ruled out, for there is evidence of a fall in the level of CD and of Tx synthesis in response to addition of enkephalins to the perfusion solution.

REFERENCES

1. V. A. Barabai, Fiziol. Zh, No. 5, 36 (1989)
2. A. N. Klimov, Biochemistry of Lipids and Their Role in Metabolism [in Russian], Moscow (1981).
3. Yu. B. Lishmanov, Yu. A. Travkov, T. V. Fedotova, and T. Yu. Rebrova, Byull. Éksp. Biol. Med., No. 6, 619 (1991).
4. Kh. M. Markov, Patol. Fiziol., No. 5, 3 (1979).
5. Kh. M. Markov, Prostanoids in Heart Diseases [in Russian], Moscow (1989).
6. F. Z. Meerson, Pathogenesis and Prevention of Stress-Induced and Ischemic Heart Damage [in Russian], Moscow (1984).
7. L. V. Maslova and Yu. B. Lishmanov, Byull. Éksp. Biol. Med., No. 6, 662 (1989).
8. E. L. Mikaelyan and M. M. Melkanyan, Zh. Éksp. Klin. Med., **19**, No. 5, 15 (1979).
9. V. S. Pavlenko, V. D. Slepushkin, Yu. B. Lishmanov, et al., Vopr. Med. Khim., No. 6, 64 (1984).
10. V. D. Slepushkin, Yu. B. Lishmanov, and G. K. Zoloev, Usp. Fiziol. Nauk, **16**, No. 4, 106 (1985).
11. J. L. Bolland and U. P. Koch, J. Chem. Soc., **7**, 445 (1945).
12. V. Glavind, Acta Chem. Scand., **17**, No. 6, 1635 (1963).
13. S. Moncada and J. R. Vane, Adv. Prostagland., **13**, 81 (1985).
14. A. D. Romaschin, G. J. Wilson, and H. Thomas, Am. J. Physiol., **259**, No. 1, 116 (1990).
15. K. Yagi, Biochem. Med., **15**, 212 (1976).