Opiatergic Mechanisms of Cardioprotective and Antiarrhythmic Effects of Adaptation

Yu. B. Lishmanov, L. A. Maimeskulova, E. V. Uskina, and L. N. Maslov

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Adaptation to hypoxia and short-term immobilization stress, as well as preconditioning with Rhodiolae rosea extract produces pronounced antiarrhythmic and cardioprotective effects in the model of adrenergic damage to the heart. Preliminary blockade of opioid receptor significantly decreases the protective effect of adaptation. Using selective opiate receptor antagonists (naltrindole, ICI 174,864, and norbinaltorphimine) we show that the antiarrhythmic effect of adaptation is mediated predominantly via activation of κ-receptors, and to a lesser extent μ- and δ-receptors.

Key Words: *adaptation; opiatergic system; isoproterenol-induced damage; myocardium*

Adaptation to environmental changes is a fundamental biological property of living organisms. A number of general "cross" effects are involved in this process, in particular, improvement of cardiac tolerance to excessive adrenergic stimulation [2]. The mechanisms of this phenomenon are still unclear. There is a good reason to assume an important role of endogenous opioid peptides in the protective effect of adaptation to extreme conditions in animals adapted to hypoxia or treated with the natural adaptogen Rhodiolae rosea extract (RRE) [1,10]. The μ-, δ- and κ-opiate receptor (OR) agonists possess antiarrhythmic activity [1]. It is unclear, to what degree the endogenous opioid peptides improve tolerance to arrhythmogenic and cardiotoxic effects of catecholamines.

Our aim was to study the role of endogenous opioid system in the realization of antiarrhythmic and cardioprotective effects of adaptation.

MATERIALS AND METHODS

Experiments were carried out on male Wistar rats weighing 150-180 g. Control group comprised intact rats. The following adaptation schemes were used:

- daily short-term immobilization in the supine position during 15 days [3];
- preconditioning with RRE in a dose of 1 ml/kg *per os* during 8 days [4];
- adaptation to high-altitude hypoxia in a pressure chamber (daily 6-hour sessions, 5,000 m over the sea level, for 45 days [2]).

Arrhythmias were modeled with intravenous epinephrine (100 μg/kg) under light ether narcosis. ECG was recorded for 5 min and the incidence of ventricular arrhythmias (paroxysmal tachycardia, extrasystole, and fibrillation) was accessed. To study the contribution of OR in the formation of the antiarrhythmic effect of adaptation, the rats were injected intravenously with the following OR blockers 15 min prior to arrhythmia modeling:

- naloxone (Sigma), which in a dose of 0.2 mg/kg inhibits only μ-OR, and in a dose of 2 mg/kg blocks all types of OR [11];
- naltrindole, a selective δ-OR antagonist (synthesized by Dr. Portoghese, Department of Medical Chemistry, College of Pharmacy, Minneapolis) in a dose of 10 mg/kg sufficient to complete inactivation of central and peripheral OR [5];
- ICI 174,864 (N,N-diallyl-Tyr-Aib-Aib-Phe-Leu-OH([N,N-diallyl-Tyr1,Aib2,3] Leu-enkephalin, Chiron Mimotopes Peptide Systems), a selective δ-OR blocker in a dose of 2.5 mg/kg [6];

Department of Experimental Cardiology, Institute of Cardiology, Tomsk Research Center Siberian Division of the Russian Academy of Medical Sciences

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♦ norbinaltorphimine (Research Triangle Institute), a selective κ-OR blocker in a dose of 10 mg/kg [13].

In special series, the cardioprotective effect of adaptation was studied on the model of isoproterenol-induced necrosis, the most severe experimental adrenergic damage to the heart [7]. The degree of cardiomyocyte damage was accessed by the accumulation of ^{99m}Tc-pyrophosphate (Pyrphotech) in the myocardium [12]. The data are presented in percents of the total dose of this agent per gram tissue×10^{-2}. The isoproterenol-induced damage was produced by subcutaneous injection of 40 mg/kg DL-Isopropylarterenol×HCl (Serva) 24 h prior to the experiment. In this case, the blockade of OR was effected via intravenous injection of naloxone (2 mg/kg, [11]) 15 min prior to injection of the β-adrenomimetic. The results were statistically analyzed using Student's t test and χ^2 test.

RESULTS

All modes of adaptation improved cardiac tolerance to the arrhythmogenic effect of epinephrine (Table 1 and Fig. 1). Adaptation to hypoxia and short-term immobilization stress, as well as preconditioning with RRE significantly increased the number of rats without cardiac rhythm disturbances (by 10, 12.5, and 4.5 times, respectively) and prevented the development of ventricular tachycardia and fibrillation. The antiarrhythmic efficiency of adaptation procedures decreases in the order: short-term immobilization stress>hypoxia> RRE preconditioning.

To study the possible role of endogenous opioids in the antiarrhythmic effect of adaptation, arrhythmia was modeled in adapted rats against the background of pharmacological blockade of various OR. Naloxone in a dose of 2 mg/kg that blocks all types of OR completely abolished the antiarrhythmic effect of repeated short-term immobilization (Table 1). In the rats subjected to hypoxia or treated with the phytoadaptogen, this dose of naloxone only partially reduced the antiarrhythmic effect of adaptation. The percentage of arrhythmia-resistant rats decreased 6- and 4-fold, respectively, but the incidence of severe rhythm disturbances (ventricular tachycardia) caused by epinephrine remained unaffected. The antiarrhythmic effect of naloxone can be surely excluded, because injection of

TABLE 1. Effect of OR Blockade on Antiarrhythmic Action of Adaptation (Induced by Short-Term Stress Stimulation and by Course Treatment with Rhodiolae Rosea Extract) on the Model of Adrenal Arrhythmias

Adaptation type	n	Without ventricular arrhythmias		Multiple ventricular extrasystoles		Ventricular tachycardia		Ventricular fibrillation	
		n	%	n	%	n	%	n	%
Control	25	2	8	18	72	15	60	7	28
Naloxone, 2 mg/kg	17	1	6	11	65	10	59	6	35
Naloxone, 0.2 mg/kg	16	2	13	13	81	8	50	3	19
ICI 174,864, 2.5 mg/kg	20	1	5	13	65	9	45	3	15
Norbinaltorphimine, 10 mg/kg	19	0	0	14	74	12	63	7	37
Naltrindole, 10 mg/kg	16	2	13	10	60	10	60	3	19
Rhodiolae	25	10	40*	10	40*	7	28	3	12
Rhodiolae+naloxone, 2 mg/kg	19	2	11^{+}	10	53	10	53	1	5
Rhodiolae+naloxone, 0.2 mg/kg	18	5	28	10	56	9	50	2	11
Rhodiolae+ICI 174,864, 2.5 mg/kg	16	8	50	9	56	7	44	1	6
Rhodiolae+norbinaltorphimine, 10 mg/kg	20	1	5^{+}	10	50	10	50	4	20
STIS	20	20	100**	0	0**	0	0**	0	0*
STIS+naloxone, 2 mg/kg	18	5	28^{++}	12	67^{++}	10	56^{++}	5	28^{+}
STIS+naloxone, 0.2 mg/kg	16	10	63^{+}	1	6	6	38^{+}	3	19^{+}
STIS+naltrindole, 10 mg/kg	18	16	89	2	11	0	0	0	0
STIS+norbinaltorphimine, 10 mg/kg	16	1	6^{++}	10	63^{++}	9	50^{++}	4	25^{+}

Note. STIS: short-term immobilization series. *p<0.05 compared with the control; ^{+}p<0.05, ^{++}p<0.001 compared with adaptation+epinephrine groups.

all OR blockers to intact rats did not modify the arrhythmogenic effect of exogenous catecholamine.

Under similar conditions, naltrindole (a selective δ-OR blocker) did not affect markedly the enhanced tolerance of stress-adapted rats to arrhythmogenic stimuli. Injection of ICI 174,864 (antagonist of δ-OR) to the rats preconditioned with RRE or adapted to hypoxia also did not modify essentially the antiarrhythmic effect of adaptation. This fact indicates that δ-OR play only a minor role in the formation of cardiac tolerance to arrhythmogenic action of epinephrine. Naloxone in a dose of 0.2 mg/kg that blocks only μ-OR produced similar effect in all types of adaptation. Our findings and published data on the presence of μ-, δ-, and κ-OR in cardiomyocyte sarcolemma [8,9] suggests the involvement of κ-OR in the antiarrhythmic effect of adaptation. Injection of the selective κ-OR antagonist norbinaltorphimine to adapted rats abolished cardiac tolerance to the arrhythmogenic action of epinephrine.

In special experiments we studied the cardioprotective effect of adaptation on the model of isoproterenol-induced necrosis, which is the most severe experimental model of adrenergic damage to the heart [7]. Taking into account previous data on the suppressive effect of enkephalins or their synthetic analogs on catecholamine synthesis in acute ischemia and isoproterenol-induced necrosis [1], we proposed that cardioprotective effect of adaptation in the isoproterenol-damaged heart results from activation of the endogenous opioid system.

Preliminary injection of β-adrenomimetic to intact rats 4-fold increased ^{99m}Tc-pyrophosphate intake by cardiomyocytes. Adaptation to hypoxia, short-term immobilization stress, and preconditioning with phytoadaptogen significantly reduced accumulation of ^{99m}Tc-pyrophosphate in isoproterenol-damaged hearts (by 75, 60, and 71%, respectively), which attests to a pronounced cardioprotective effect of adaptation.

To evaluate the contribution of OR into the cardioprotective effect of adaptation, the degree of adrenergic damage to the heart in adapted rats was assessed after pharmacological blockade of OR. The cardioprotective effect of all types of adaptation was completely abolished by naloxone, a nonselective OR blocker.

Our findings suggest that antiarrhythmic and cardioprotective effects of these types of adaptation are mediated via activation of the opioid system. The antiarrhythmic effect of adaptation is mediated predominantly via activation of κ-OR, and to a lesser extent μ- and δ-OR.

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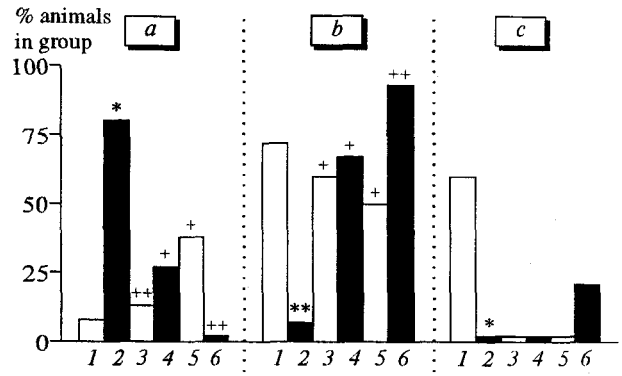

Fig. 1. Antiarrhythmic effect of adaptation to hypoxia under condition of opiate receptor blockade. *1*) control; *2*) hypoxia+epinephrine; *3*) hypoxia+naloxone (2 mg/kg)+epinephrine; *4*) hypoxia+naloxone (0.2 mg/kg)+epinephrine; *5*) hypoxia+ICI 174,864 (2.5 mg/kg)+epinephrine; *6*) hypoxia+norbinaltorphimine (10 mg/kg)+epinephrine. *a*) without ventricular arrhythmias, *b*) multiple ventricular extrasystoles, *c*) ventricular tachycardia. Each series comprised at least 16 rats. $^{*}p<0.05$ compared with the control; $^{+}p<0.05$, $^{++}p<0.001$ compared with *2*.

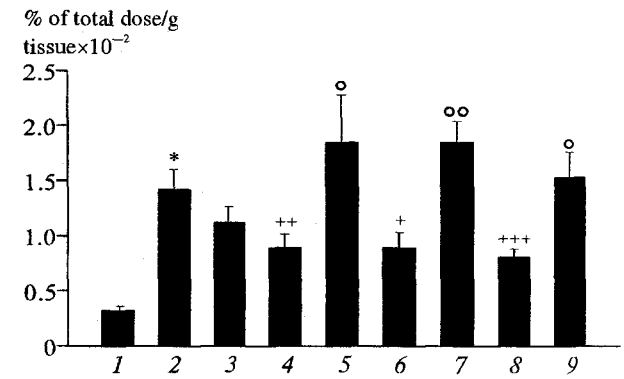

Fig. 2. Effect of different types of adaptation on isoproterenol-induced myocardial damage in rats assessed by accumulation of ^{99m}Tc-pyrophosphate. 1) intact group; *2*) isoproterenol, 40 mg/kg; *3*) naloxone, 2 mg/kg; *4*) preconditioning with Rhodiolae rosea extract+isoproterenol; *5*) Rhodiolae+naloxone (2 mg/kg)+isoproterenol; *6*) short-term immobilization+isoproterenol; *7*) short-term immobilization+naloxone (2 mg/kg)+isoproterenol; 8) hypoxia+isoproterenol; 9) hypoxia+naloxone (2 mg/kg)+isoproterenol. Each series comprised at least 11 rats. $^{*}p<0.001$ compared with 1; $^{+}p<0.05$, $^{++}p<0.01$, and $^{+++}p<0.001$ compared with *2*; $^{\circ}p<0.01$ and $^{\circ\circ}p<0.001$ compared with *8*.

REFERENCES

1. Yu. B. Lishmanov and L. N. Maslov, *Opioid Neuropeptides, Stress, and Adaptive Protection of the Heart* [in Russian], Tomsk (1994).
2. F. Z. Meerson, *Adaptation, Stress, and Prophylaxis* [in Russian], Moscow (1981).
3. F. Z. Meerson, in: *Physiology of Adaptive Processes: Handbook on Physiology* [in Russian], Moscow (1986), pp. 521-631.
4. A. S. Saratikov and E. A. Krasnov, *Rhodiolae Rosea as an Efficient Medicinal Plant* [in Russian], Tomsk (1987).
5. M. Broccardo and G. Improta, *Eur. J. Pharmacol.*, **218**, 69-72 (1992).
6. R. Cotton, M. G. Giles, L. Miller, *et al.*, *Ibid.*, **97**, 331-332 (1984).
7. A. Fleckenstein, J. Janre, H. J. Döring, *et al.*, in: *Recent Advances in Studies on Cardiac Structure and Metabolism*, A. Fleckenstein and G. Rona (Eds.), Baltimore (1975).

8. X. D. Huang and T. M. Wong, *Life Sci.*, **48**, No. 11, 1101-1107 (1991).
9. S. A. Krumius, A. I. Faden, and G. Feuerstein, *Biochem. Biophys. Res. Commun.*, **127**, 120-128 (1985).
10. K. P. Mayfield and L. G. D'Alecy, *Brain Res.*, **582**, 226-231 (1992).
11. M. J. Millan, A. Czlonkowski, A. Lipkowski, *et al.*, *J. Pharmacol. Exp. Ther.*, **251**, No. 1, 342-350 (1989).
12. D. G. Miller and S. Mallov, *Pharmacol. Biochem. Behav.*, **7**, No. 2, 139-145 (1977).
13. P. S. Portoghese, A. W. Lipkowski, and A. E. Takemori, *Life Sci.*, **40**, 1287-1291 (1987).