The Role of Central and Peripheral μ- and δ-Opiate Receptors in Mediating the Antiarrhythmic Effect of Adaptation

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 122, No. 10, pp. 378-381, October, 1996
Original article submitted September 20, 1995

Adaptation prevents epinephrine-induced arrhythmias in rats, the effect being abolished by naloxone, a μ-receptor antagonist, but not by the δ-receptor antagonist naltrindole. Methylnaltrexone, a blocker of peripheral μ-receptors, weakens the effect of adaptation without eliminating it completely. Central and peripheral μ-receptors may play a key role in the antiarrhythmic effect of adaptation.

Key Words: *adaptation; arrhythmias; μ- and δ-opiate receptors*

Protection of the heart by adaptation was originally proposed by H. Selye, who found that the hearts of animals exposed to severe stress became more resistant to repeated stress exposures [4]. In Russia, this concept was expanded by F. Z. Meerson and led to the conclusion that the hearts of adapted animals are resistant to the arrhythmogenic effect of ischemia and reperfusion [3].

Based on these findings and our observations, we suggested [1] that endogenous opioid peptides are involved in the mechanism(s) underlying the arrhythmogenic effect of adaptation, since adaptation increases the tissue and organ content of opioid peptides (OP) [1], and the synthetic OP dalargin ([D-Ala^2, Leu^5,Arg^6]-enkephalin) exhibits antiarrhythmic, cardioprotective, and stress-limiting activities [1,2,8]. However, this hypothesis required experimental support.

The objective of the present study was to assess the role of peripheral and central μ- and δ-opiate receptors (OR) in the mechanisms of the antiarrhythmic effect of adaptation.

MATERIALS AND METHODS

The study was performed on male Wistar rats weighing 250-300 g. They were adapted to stress by immobilization in the supine position over 12 days as follows: 15 min on day 1, 30 min on day 2, 45 min on day 3, and 60 min subsequently every other day. Previously, it was shown that such an adaptation decreases the arrhythmogenic effect of coronary occlusion [3].

Arrhythmias were produced by injection of epinephrine (100 μg/kg intravenously) under light ether anesthesia. Electrocardiogram was recorded for 5 min, and ventricular arrhythmias were counted. In the control group, epinephrine administration was preceded by intravenous or intraperitoneal injection of isotonic NaCl solution or an OR ligand.

In order to block μ-OR some rats were injected with naloxone (Sigma) in an i.v. dose of 0.2 mg/kg [6,9]; central and peripheral δ-OR were blocked with naltrindole (synthesized by Prof. P. S. Portoghese, College of Pharmacy, Minneapolis, USA) injected intravenously in a dose of 10 mg/kg [5,10]. Peripheral OR were inactivated by naltrexone methylbromide (NxMB) (kindly provided by Boehringer Ingelheim KG) administered intraperitoneally in a dose of 10 mg/kg [6]. With the exception of NxMB, which was injected 30 min before epinephrine, all drugs were dissolved *ex tempore* in 0.9% NaCl and injected intravenously 15 min before epinephrine.

In order to confirm the opiatergic nature of the antiarrhythmic effect of adaptation and to establish

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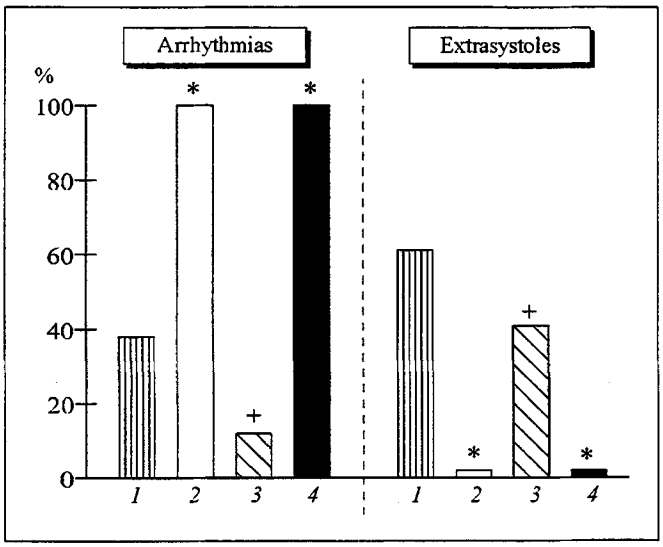

Fig. 1. Effects of antagonists of opiate receptors on epinephrine-induced arrhythmias in adapted rats. *1*) control; *2*) adaptation; *3*) adaptation+naloxone; *4*) adaptation+naltrindole. $p<0.001$: *compared with the control group; +compared with adapted group.

whether central and/or peripheral OR are involved in this process, a separate series of experiments on unadapted rats was performed using two μ-OR agonists: [D-Arg^2,Lys^4]-dermorphin-(1-4)-amide (DALDA, synthesized by Prof. P. W. Schiller (Clinical Research Institute of Montreal, Canada) [11], and [D-Ala^2,N-Me-Phe^4, Gly^5-ol]-enkephalin (DAGO, Bio-Pro Company, Novosibirsk) [7]. DALDA was injected intravenously at 0.1 mg/kg 15 min before epinephrine, while DAGO (20 nM) was infused intracerebroventricularly as described elsewhere [2]. The DALDA dose was chosen on the basis of the antiarrhythmic activity of dalargin [1,8].

The results were analyzed by the χ^2 test.

RESULTS

Adaptation prevented the epinephrine-induced arrhythmias (Fig. 1). In the adapted group, the occurrence of ventricular extrasystoles was only one-third of that in the control (unadapted) group, and none of the rats developed ventricular tachycardia. Blockade of μ-OR with naloxone abolished the antiarrhythmic effect of adaptation. Naloxone injected into unadapted rats had no effect on the epinephrine-induced arrhythmias. The δ-OR antagonist naltrindole did not affect the increased resistance of adapted rats to arrhythmogenic interventions.

Thus, the antiarrhythmic effect of adaptation was associated with activation of μ-OR, since at 0.2 mg/kg naloxone blocks only these receptors [6,9], completely eliminating the antiarrhythmic effect of adaptation. Since naloxone passes through the blood-brain barrier [6], it was unclear whether the μ-OR mediating the antiarrhythmic effect of adaptation are located centrally or peripherally. The effect of adaptation was weakened by NxMB, an antagonist of peripheral OR [6] (Table 1). The occurrence of ventricular fibrillation and extrasystoles developed by adapted rats in response to epinephrine were the same as in control rats. It should be noted that in control rats NxMB had no effect on the epinephrine-induced arrhythmias.

Consequently, both peripheral and central μ-OR may be involved in the mechanisms whereby the electrical stability of the heart increases in adapted animals. In an attempt to confirm this hypothesis we employed systemic and central administration of μ-OR agonists to unadapted rats. Intracerebroventricular administration of the μ-OR agonist DAGO prevented the development of the epinephrine-induced ventricular tachycardias and decreased the occurrence of ventricular extrasystoles 7-fold (Fig. 2). A similar reduction in the occurrence of epinephrine-induced arrhythmias was produced by DALDA, an agonist of peripheral μ-OR [11] (Fig. 3).

Since intracerebral and systemic administration of μ-OR agonists increased electrical stability of the heart, both central and peripheral μ-OR may be involved in the mechanisms underlying the antiarrhythmic effect of adaptation.

What is the mechanism responsible for the antiarrhythmic effect elicited by the centrally administered ligands of μ-OR? Our preliminary findings

TABLE 1. Effect of Naltrexone Methylbromide (NxMB) on the Occurrence of Epinephrine-Induced Arrhythmias in Adapted Rats

Group	Number of rats	Without VA		VE		VT		VF	
		n	%	*n*	%	*n*	%	*n*	%
Control	30	4	13	23	76	20	66	15	50
NxMB	15	5	33	10	66	10	66	5	33
Adapted	14	7**	50	6***	43	1***	7	0**	0
Adapted+NxMB	10	2	20	7	70	2*	20	3+	30

Note. VA: ventricular arrhythmias, VE: ventricular extrasystoles, VT: ventricular tachycardia, and VF: ventricular fibrillation. *$p<0.025$, **$p<0.01$, ***$p<0.001$ compared with the control group; +$p<0.005$ compared with the adapted group (χ^2 test).

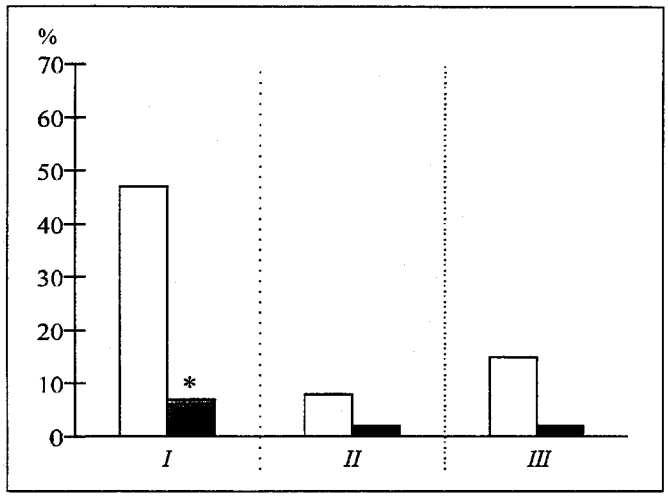

Fig. 2. Effect of intracerebroventricular administration of DAGO on the occurrence of epinephrine-induced arrhythmias. *I*) ventricular extrasystoles; *II*) ventricular tachycardia; *III*) ventricular fibrillation. White bars: control group; black bars: DAGO-injected rats. *$p<0.01$ compared with the control group.

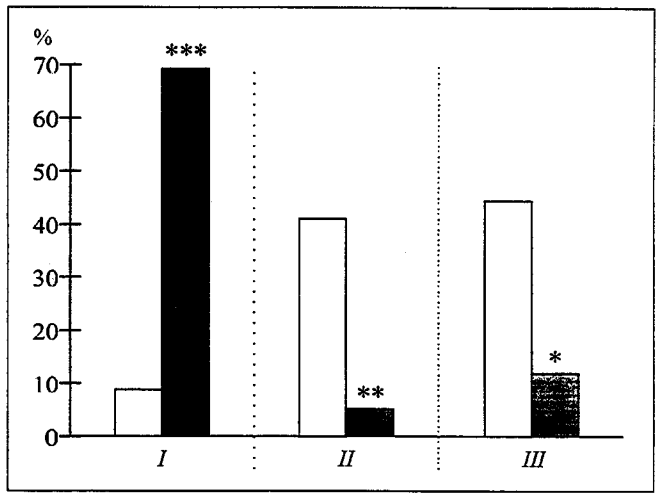

Fig. 3. Effect of intravenous administration of DALDA on epinephrine-induced arrhythmias. *I*) no arrhythmias; *II*) ventricular extrasystoles; *III*) tachycardia. White bars: control group; black bars: DALDA-injected rats. *$p<0.05$, **$p<0.01$, ***$p<0.001$ compared with the control group.

indicate that the antiarrhythmic effect of intracerebroventricularly infused DAGO is associated with increased activity of the nuclei of the vagus nerve, since this effect is abolished by atropine [2]. It is more difficult to explain the antiarrhythmic effect of systemic administration of the μ-OR agonists. It can be suggested that the influence of the vagus on the myocardium is reflexively enhanced in response to the stimulation of peripheral OR located on its afferent fibers [13]. However, the possibility that systemically injected OR agonists directly act on the myocardium cannot be ruled out. This possibility is supported by the presence of OR on the sarcolemma of cardiomyocytes [12] and by the ability of dalargin to prevent isoprenaline-induced damage to the heart and to inhibit isoprenaline-dependent cAMP synthesis in the myocardium [1].

Our results indicate that activation of central and peripheral μ-OR is one of the mechanisms by which the electrical stability of the heart is increased during adaptation. Presumably, the contribution of δ-OR to the antiarrhythmic effect of adaptation is small.

This study was supported by the Russian Foundation for Basic Research, Dr. P. Hillery (National Institute on Drug Abuse, USA), Prof. P. S. Portoghese (College of Pharmacy, Minneapolis, USA), Prof. P. W. Schiller (Clinical Research Institute of Montreal, Canada), and Boehringer Ingelheim KG (Germany).

REFERENCES

1. Yu. B. Lishmanov and L. N. Maslov, *Opioid Neuropeptides, Stress, and Adaptive Protection of the Heart* [in Russian], Tomsk (1994).
2. Yu. B. Lishmanov, L. N. Maslov, and D. S. Ugdyzhekova, *Vestn. Aritmol.*, No. 3, 48-50 (1994).
3. F. Z. Meerson and M. G. Pshennikova, *Adaptation to Stressful Situations and Physical Exercise* [in Russian], Moscow (1988).
4. H. Selye, *At the Level of the Whole Organism* [Russian translation], Moscow (1972).
5. M. Broccardo and G. Improta, *Eur. J. Pharmacol.*, **218**, 69-73 (1992).
6. D. R. Browen, M. J. Robertson, and L. I. Goldberg, *Neuropharmacology*, **22**, No. 3A, 317-321 (1983).
7. B. K. Handa, A. C. Lane, J. A. H. Lord, *et al.*, *Eur. J. Pharmacol.*, **70**, 531-540 (1981).
8. L. N. Maslov and Yu. B. Lishmanov, *Int. J. Cardiol.*, **40**, 89-94 (1993).
9. M. J. Millan and F. C. Colpaert, *Neuroscience*, **42**, No. 2, 541-553 (1991).
10. P. S. Portoghese, M. Sultana, and A. E. Takemori, *Eur. J. Pharmacol.*, **146**, 185-186 (1988).
11. P. W. Schiller, T. M.-D. Nguyen, N. N. Chung, and C. Lemieux, *J. Med. Chem.*, **32**, No. 3, 698-703 (1989).
12. C. Ventura, L. Bastagli, P. Bernardi, *et al.*, *Biochem. Biophys. Acta*, **987**, 69-74 (1989).
13. E. T. Wei, A. Lee, and J. K. Chang, *Life Sci.*, **26**, No. 18, 1517-1522 (1980).