Opiatergic Mechanisms of the Antiarrhythmic Effect of Adaptation

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

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A decrease in the severity and occurrence of epinephrine- and $CaCl_2$-induced cardiac arrhythmias and an increase in the β-endorphin and enkephalin contents in the brain, myocardium, adrenals, and blood plasma are observed in rats adapted to stress. The antiarrhythmic effect of adaptation is abolished by naloxone. A single administration of D-kyotorphin, a liberator of endogenous peptides, to intact animals also increases the resistance to arrhythmogenic factors. Intravenous administration of the enkephalinase inhibitor RB101 produces a significant antiarrhythmic effect in control animals.

Key Words: *opiate receptors; opioid peptides; adaptation; arrhythmia; stress*

Recent studies show that adaptation to periodical stress reduces the arrhythmogenic effect of coronary occlusion [3]. The nature of this phenomenon remains obscure. Previously, we showed that some antagonists of μ- and δ-opiate receptors (OR) exhibit antiarrhythmic activity both after intracerebroventricular (i.c.v.) and the systemic administration [1,9]. In addition, the content of major endogenous opioid peptides (OP) in tissues and organs of adapted rats is higher than in intact animals [1]. These findings suggest that stimulation of endogenous opioid system is crucial for the adaptogenic increase of the myocardial electrical stability. This suggestion requires experimental corroboration.

Our purpose was to study the receptor mechanisms whereby endogenous ligands of OR are involved in the realization of the antiarrhythmic effect of adaptation.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 250-300 g. The rats were adapted by immobilization on the back during a 12-day period: day 1 for 15 min, day 2 for 30 min, day 3 for 45 min, and then every other day for 60 min. Previously, it was shown that this type of adaptation reduces the arrhythmogenic effect of coronary occlusion [3].

Arrhythmia was modeled by intravenous administration of epinephrine (100 μg/kg) or $CaCl_2$ (100 mg/kg) under light ether anesthesia. Electrocardiogram was recorded for 5 min, and ventricular arrhythmias (paroxysmal tachycardia, extrasystole, and fibrillation) were counted in each experimental group.

The following compounds were studied:

1. The liberator of endogenous enkephalins kyotorphin (BioPro, Novosibirsk, Russia). The preparation was dissolved *ex tempore* in 0.9% NaCl and infused i.c.v. in a volume of 10 μl (400 μg) per rat at a flow rate of 5 μl/min 5 min prior to induction of arrhythmia. It was reported that intracerebral administration of D-kyotorphin in this dose produces an antinociceptive effect [13,14].

2. The enkephalinase inhibitor RB101 (N-[(R,S)-2-benzyl-3-[(S)-2-amino-4-methylthiobutyldithio]-1-[oxopropyl]-L-phenylalanine benzyl) (France). The compound was dissolved *ex tempore* in a mixture consisting of 10% ethanol, 10% cremophor EL (Sigma), and 80% H_2O and injected intravenously in a dose of 10 mg/kg 20 min prior to induction of ar-

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

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TABLE 1. Opioid Receptor Effect of Antagonist on Epinephrine- and $CaCl_2$-Induced Arrhythmia in Adapted Rats

Group	Number of animals with epinephrine-induced arrhythmias				Number of animals with $CaCl_2$-induced arrhythmias			
	n	Without VE	VE	VF	*n*	Without VE	VE	VF
Control	19	7 (37)	12 (63)	- (-)	29	9 (31)	12 (41)	6 (21)
Adaptation	21	21 (100)**	- (-)**	- (-)	15	10 (67)*	3 (20)	1 (7)
Adaptation+naloxone (0.2 mg/kg)	16	2 (12)**	7 (43)**	3 (16)*	16	2 (12)**	8 (50)	5 (32)
Adaptation+naltrindole	21	21 (100)**	- (-)**	- (-)	20	14 (70)**	5 (25)	1 (5)
Kyotorphin	20	15 (75)*	4 (20)**	- (0)	-	- (-)	- (-)	- (-)

Note. *n*: number of animals in group (percentage is given in parentheses). *$p<0.025$, **$p<0.001$ compared with the control; *$p<0.05$, **$p<0.001$ compared with adapted rats. VE: ventricular extrasystole; VF: ventricular fibrillation. Results were analyzed using χ^2 test.

rhythmia. The choice of the dose was based on the analgesic activity of the preparation [12].

3. The selective antagonist of δ-OR naltrindole (synthesized by Dr. P. S. Portoghese, Department of Medical Chemistry, College of Pharmacy, Minneapolis, USA). The preparation was administered in a dose of 10 mg/kg, which is sufficient for complete inactivation of peripheral and central receptors [5].

4. The nonselective OR inhibitor naloxone (Sigma). The preparation was administered in a dose of 0.2 mg/kg, which is sufficient for blockade of only μ-OR [4,10].

For i.c.v. administration of the preparations a stainless cannula was implanted in the lateral cerebral ventricle under barbamyl anesthesia (50 mg/kg intraperitoneally). The cannula was fixed on the head with the aid of dental cement. The operation was performed using a SEZH-5 stereotaxic apparatus (Konstruktor, Ukraine). Coordinates relative to the bregma were: AP 1.5 mm, L +2.0 mm, and V 3.5 mm [11].

The OR antagonists or isotonic solution (control) were injected intravenously 15 min prior to the injection of arrhythmogenic preparations.

Results were analyzed using the χ^2 test.

RESULTS

Table 1 shows that adaptation of rats by short-term immobilization reduces the occurrence of epinephrine- or $CaCl_2$-induced arrhythmias. The resistance of adapted animals to the epinephrine-induced arrhythmia increased 3-fold and to the $CaCl_2$-induced arrhythmia 2-fold (by the number of animals without arrhythmias) compared with the control. It is likely that higher resistance to the arrhythmogenic effects of epinephrine and $CaCl_2$ is due both to lowered adrenoreactivity of cardiomyocytes and intensified clearance of excessive Ca^{2+} from the cytoplasm. This hypothesis is based on the fact that both arrhythmogenic agents provoke arrhythmia by the formation of ectopic foci [7,8]. However, it should be noted that $CaCl_2$ induces arrhythmias due to increased Ca^{2+} entry and calcium mobilization from the sarcoplasmic reticulum [7,8], while epinephrine provokes arrhythmia by activation of β-adrenoreceptors followed by an increase in the content of cAMP, an endogenous arrhythmogenic factor [11]. This hypothesis is consistent with the observation that adaptation reduces the affinity of β-adrenoreceptors and enhances the activity of Ca^{2+} pump in the myocardium [3].

To find out whether endogenous opioids are involved in the realization of the antiarrhythmic effect of adaptation we performed a series of experiments in which arrhythmia in adapted animals was induced after selective blockade of OR.

Table 1 shows that naloxone abolished the antiarrhythmic effect of adaptation. Naltrindole, a selective blocker of δ-OR, had no appreciable effect on increased resistance of adapted rats to the arrhythmogenic agents.

These observations suggest that the increase in myocardial resistance after adaptation is associated with stimulation of μ-OR by endogenous opioids, since the effect is abolished by administration of naloxone in a dose blocking μ-OR. Antiarrhythmic activity of naloxone should be ruled out, since its administration to intact rats in the same dose (0.2 mg/kg) had no effect on the arrhythmogenic effects of epinephrine and $CaCl_2$ [1]. Presumably, endogenous agonists of δ-OR are not involved in the realization of the antiarrhythmic effect of adaptation, since this effect is not abolished by the selective blocker naltrindole.

Intracerebroventricular infusion of D-kyotorphin prior to induction of ventricular arrhythmia increased the resistance to arrhythmia 2-fold and decreased the occurrence of ventricular extrasystoles 3-fold (Table 1). This indicates that endogenous OP and central OR are involved in the formation of myocardial resistance to arrhythmogenic agents, since

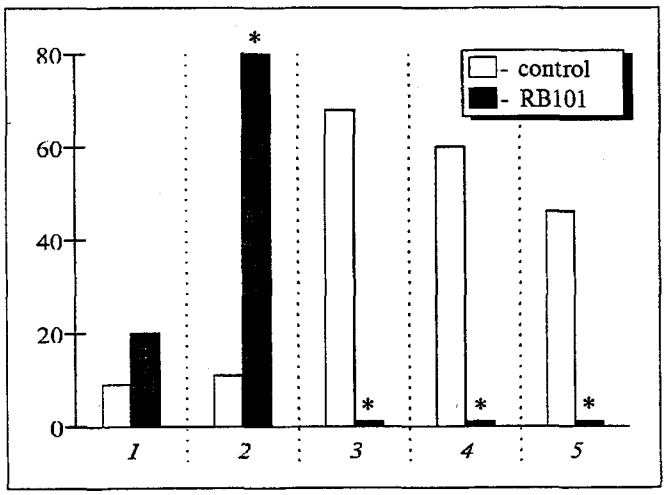

Fig. 1. Effect of intravenous administration of RB101 on epinephrine-induced arrhythmias. *$p<0.001$ compared with the control (χ^2 test). *1)* without ventricular extrasystoles; *2)* occasional ventricular extrasystoles; *3)* numerous ventricular extrasystoles; *4)* ventricular tachycardia; *5)* ventricular fibrillation.

D-kyotorphin is a liberator of endogenous enkephalins [14].

Intravenous administration of RB101 to intact rats also had a pronounced effect on the epinephrine-induced arrhythmia (Fig. 1). The percentage of animals resistant to arrhythmia increased 2.5-fold compared with the control, and none of the rats developed ventricular tachycardia or fibrillation. RB101 inhibits both aminopeptidase N and neutral endopeptidase (enzymes degrading endogenous enkephalins) [12]. This preparation elevates the content of endogenous enkephalins [12], which may increase electrical stability of the heart in response to arrhythmogenic stimuli.

Our results indicate that endogenous opioids are involved in the realization of the antiarrhythmic effect of adaptation. First, this effect disappears after blockade of μ-OR. Second, similarly to adaptation, D-kyotorphin-stimulated release of endogenous enkephalins prevents the development of arrhythmias. Third, blockade of enzymes degrading endogenous enkephalins in intact animals significantly increases heart resistance to induced arrhythmias.

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REFERENCES

1. Yu. B. Lishmanov and L. N. Maslov, *Opioid Neuropeptides, Stress, and Adaptational Protection of the Heart* [in Russian], Tomsk (1994).
2. L. N. Maslov and Yu. B. Lishmanov, *Vopr. Med. Khimii,* **38**, No. 2, 21-23 (1992).
3. F. Z. Meerson and M. G. Pshennikova, *Adaptation to Stress Situations and Physical Exercises* [in Russian], Moscow (1988).
4. H. D. Allescher, S. Ahmad, E. E. Daniel, *et al., Am. J. Physiol.,* **255**, No. 3, Pt. 1, G352-G360 (1988).
5. M. Broccardo and G. Improta, *Eur. J. Pharmacol.,* **218**, 69-73 (1992).
6. T. Howlett, S. Tomlin, L. Ngahfoong, *et al., Br. Med. J.,* **28**, 1950-1952 (1984).
7. Y. Kohama, K. Iwabuchi, T. Shibahara, *et al., J. Pharmacobiodyn.,* **9**, 806-810 (1986).
8. J. J. Lynch, R. G. Rahwan, and D. T. Witiak, *J. Cardiovasc. Pharmacol.,* **3**, 49-60 (1981).
9. L. N. Maslov and Yu. B. Lishmanov, *Int. J. Cardiol.,* **40**, 89-94 (1993).
10. M. J. Millan and F. C. Colpaert, *Neuroscience,* **42**, No. 2, 541-553 (1991).
11. T. Podzuweit, L. H. Opie and W. F. Lubbe, *Lancet,* **1**, 341-342 (1976).
12. M. Ruiz-Gayo, A. Baamonde, S. Turcaud, *et al., Brain Res.,* **571**, 306-312 (1992).
13. S. Sakurada, T. Sakurada, H. Jin, *et al., J. Pharm. Pharmacol.,* **34**, No. 11, 750-751 (1982).
14. H. Takagi, H. Shiomi, H. Ueda, and H. Amano, *Nature,* **282**, 410-412 (1979).