Peripheral μ-Opiate Receptors and Modulation of Myocardial Tolerance to Arrhythmogenic Stimuli

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The peripheral μ-opiate receptor agonists DALDA and PL017 are highly effective against arrhythmias induced by adrenaline, aconitine, or $CaCl_2$. The effect was abolished by the μ-opiate receptor antagonist CTAP. It is likely that Ca-channel block is involved in antiarrhythmic effect of DALDA.

Key Words: *peripheral μ-opiate receptors; arrhythmias*

The progress in the search for new antiarrhythmics without adverse effects (arrhythmogenic activity or negative inotropic effect [4]), depends on the knowledge about mechanisms affecting myocardial tolerance to arrhythmogenic stimuli. Since endogenous opioid peptide analogs possess antiarrhythmic activity [3,5, 6,10], the opiatergic system is of special interest. However, opiates used in clinical practice produce negative effects, the narcotic dependency being the most harmful. At the same time, the opiate receptor (OR) agonists, which cannot cross the blood-brain barrier and, therefore, lack negative properties of conventional opiates, also display antiarrhythmic activity [1,3,5,6,10]. The intrinsic receptor mechanisms responsible for antiarrhythmic activity of opioid peptides remain unclear.

Our aim was to examine modulating effect of peripheral μ-OR on myocardial tolerance to antiarrhythmic stimuli.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats (body weight 150-200 g). Arrhythmia was induced by adrenaline (100 μg/kg) or $CaCl_2$ (100 μg/kg) injected intravenously under light ether anesthesia. The ECG was recorded in the second standard lead for 5 min after injection. Extrasystoles, episodes of tachycardia, and ventricular fibrillations were counted. A special series of experiments was undertaken with intravenous aconitine (Sigma, 50 μg/kg) as arrhythmogenic agent. Aconitine (1 mg) was suspended in Tween-80 (20 μl) and mixed with 20 ml 0.9% NaCl. This suspension was injected in a dose of 1 ml/kg body weight. For the ECG recording and computer analysis we used an UBF4-03 amplifier and originally designed software.

The following peripheral μ-OR selective agonists (1, 2) and antagonists (3) (Chiron Mimotopes Peptide Systems) were used:

1) H-Tyr-Pro-N-Me-Phe-D-Pro4-NH_2 (PL017) [8,13];
2) NH_2-Tyr-D-Arg-Phe-Lys-NH_2 (DALDA) [12];
3) NH_2-D-Phe-Cys-Tyr-D-Trp-Arg-Thr-L-Pen-Thr-NH_2 (CTAP) [11].

These substances were dissolved in 0.9% NaCl and injected intravenously 15 min before arrhythmogenic substances. CTAP (0.1 mg/kg) was administered 10 min before DALDA. PL017 was administered in a dose of 0.1 mg/kg, DALDA in doses of 0.1 and 0.5 mg/kg.

The results were statistically analyzed by the χ^2 test and Student's t test.

RESULTS

The peripheral μ-OR selective agonist DALDA almost completely prevented ventricular tachycardia

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and extrasystoles induced by adrenaline, which is consistent with our previous findings [5] (Table 1). The percent of rats which were absolutely resistant to arrhythmogenic effect of adrenaline, substantially increased. The peripheral μ-OR excitation by PL017 also enhanced the resistance to adrenaline-induced arrhythmias. Peripheral μ-OR selective blocker CTAP did not affect adrenal arrhythmias (Table 1) and abolished the effect of DALDA.

These data indicate that μ-OR stimulation increases myocardial tolerance to arrhythmogenic effect of adrenaline; no tonic control of this tolerance by endogenous peripheral μ-OR agonists exists. However, a question arises whether antiarrhythmic effect of DALDA and PL017 persist for arrhythmias induced by substances with other than adrenaline-like activity. To answer this question, experiments with $CaCl_2$ and aconitine-induced arrhythmias were performed.

In case of $CaCl_2$ injection, antiarrhythmic effects of DALDA and PL017 were observed (Fig. 1). However, the effects of these OR ligands were weaker than in case of adrenaline arrhythmias. Moreover, with $CaCl_2$ a higher dose of DALDA (0.5 mg/kg) was necessary. In case of aconitine-induced arrhythmia, DALDA and PL017 increased the latency of arrhythmia (Fig. 2).

Our previous findings indicate that antiarrhythmic effects of μ-OR agonists are not related to tonus changes in the autonomic nervous system [5]. Evaluation of the role of peripheral OR in myocardial tolerance to different arrhythmogenic stimuli will shed light on the intrinsic mechanisms of this phenomenon.

The ability of catecholamines to provoke arrhythmias is associated with increased cAMP synthesis and the rise of free Ca^{2+} level in cardiomyocytes [9,14], while arrhythmogenic effect of $CaCl_2$ results directly from excessive entry of Ca^{2+}. Since

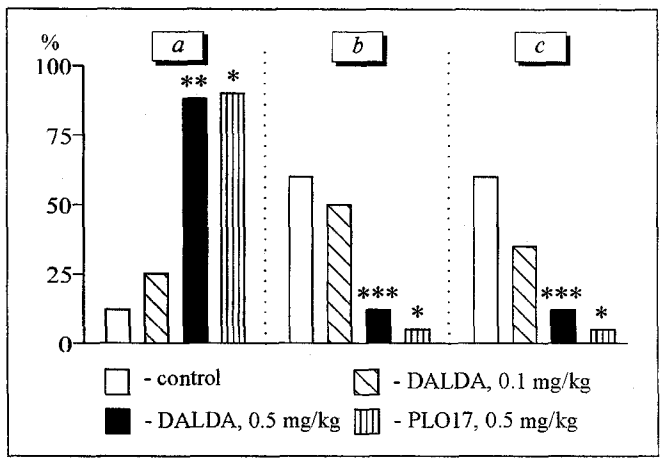

Fig. 1. Effect of μ-opiate receptor agonists on $CaCl_2$-induced arrhythmia. Without ventricular arrhythmia (*a*), multiple ventricular extrasystoles (*b*), ventricular tachycardia (*c*). *$p<0.001$, **$p<0.01$, ***$p<0.05$, compared with control (χ^2-test).

the effects of DALDA and PL017 on adrenal arrhythmias is more profound than that of $CaCl_2$, it is most likely that μ-opiate ligands influence both Ca^{2+} transmembrane transfer and intracellular cAMP level. Indeed, the μ-OR nonselective agonist dalargin inhibits norepinephrine-induced cAMP synthesis in the myocardium [3]. Cyclic AMP is a regulator of transmembrane Ca^{2+} transport, and, suppresses the synthesis of this messenger, which limits Ca^{2+} entry into the myoplasm [14]. We observed a Ca^{2+}-dependent component of OR ligand antiarrhythmic action in experiments with aconitine. Arrhythmogenic effect of aconitine is due to longer Na^+-channel inactivation [2], which increases intracellular Na^+ concentration, leading to intracellular Ca^{2+} rise [7].

Analysis of DALDA-induced ECG changes in intact animals suggests the involvement of myocardial μ-OR in the maintenance of intracellular Ca^{2+} homeostasis. Fifteen minutes after administration of DALDA (0.1 mg/kg) heart rate decreased from 353±9.6 beats/

Table 1. Effects of μ-OR Ligands on Adrenalin-Induced Arrhythmias

Substances	*n*	Without VA	VE		VT	VF
			single	multiple		
		n%				
Control	20	1 (5)	1 (5)	10 (50)	16 (80)	4 (20)
DALDA, 0.1 mg/kg	25	17 (68)*	2 (8)	2 (8)	3 (13)*	2 (8)
PL017, 0.1 mg/kg	19	15 (79)*	0	2 (11)	2 (11)**	0
CTAP, 1 mg/kg	20	3 (15)	1 (5)	9 (45)	16 (80)	3 (15)
CTAP+DALDA	20	4 (20)	1 (5)	10 (50)	10 (50)	1 (5)

Note. *$p<0.01$, **$p<0.001$, compared with control (χ^2-test). VA) ventricular arrhythmia; VE) ventricular extrasystoles; VT) ventricular tachycardia; VF) ventricular fibrillation.

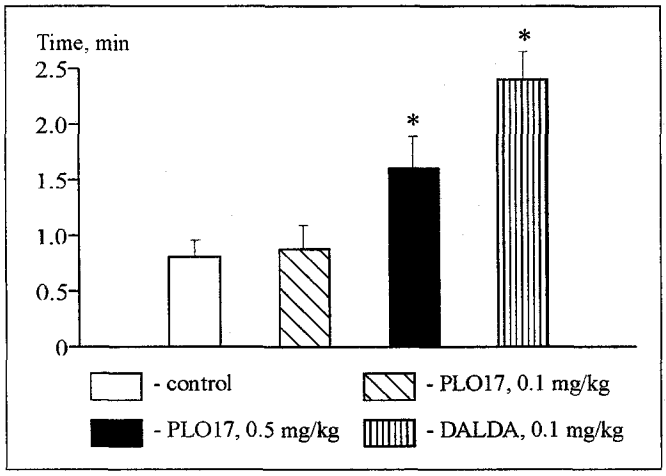

Fig. 2. Effect of μ-opiate receptor agonists on aconitine-induced arrhythmia. PL017 and DALDA were injected 15 min before aconitine. *$p<0.001$, compared with control (Student's t test).

sec (initial value) to 292±12.7 beats/sec ($p< 0.001$), and the *PQ* interval increased from 55±1.1 msec to 66.8±1.6 msec ($p<0.001$). Stimulation of μ-OR had no effect on the *QRS* complex and *QT* interval. Such changes in cardiac automaticity and conductibility are characteristic of Ca-channel blokade [4].

Thus, our results indicate that peripheral μ-OR play an important role in cardiac tolerance to arrhythmogenic stimuli. Opiatergic inhibition of cAMP synthesis and modification of transmembrane Ca^{2+} transport seem to be the most likely mechanisms underlying this phenomenon. Interactions of endogenous opioid peptides with peripheral μ-OR play no essential role in tonic modulation of cardiac tolerance to arrhythmogenic stimuli.

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REFERENCES

1. T. I. Grekova, K. M. Resnikov, O. V. Vinokurova, *et al.*, *Eksper. Klin. Farmakol.*, **57**, No. 2, 24-26 (1994).
2. M. Lazdinskii, G. F. Reno, In: *Physiology and Pathophysiology of the Heart (Ed. N.Sperelakis)* [in Russian], *593-617,* Moscow (1990).
3. Yu. B. Lishmanov, L. N. Maslov, *Opioid Neuropeptides, Stress, and Adaptational Heart Resistance* [in Russian], Tomsk (1994).
4. N. A. Mazur and A. Abdala, *Pharmacotherapy of Arrhythmias* [in Russian], Moscow (1995).
5. L. N. Maslov, A. V. Krylatov, and Yu. B. Lishmanov, *Byull. Eksp. Biol. Med.*, **122**, No. 7, 25-27 (1996).
6. S. D. Mikhailova, T. M. Semushkina, and N. A. Bebyanova, *Kardiologiya*, **31,** No. 1, 13-15 (1991).
7. A. I. Khankoeva, P. A. Galenko-Yaroshevskii, and A. S. Dukhanin, *Byull. Eksp. Biol. Med.*, **122**, No. 7, 45-47 (1996).
8. K. -J. Chang, E. T. Wei, A. Killian, and J. -K. J. Chang, *J. Pharmacol. Exp. Ther.*, **227**, 403-408 (1983).
9. W. F. Lubbe, A. I. Gilchrist, and R. K. Holland, *J. Mol. Cell. Cardiol.*, **19**, Suppl. N 5, 23-33 (1987).
10. L. N. Maslov and Yu. B. Lishmanov, *Clin. Exp. Pharmacol. Physiol.*, **22**, 812-816 (1995).
11. J. T. Pelton, W. Kazmierski, K. Gulya *et al.*, *J. Med. Chem.*, **29**, 2370-2375 (1986).
12. P. W. Schiller, T. M.-D. Nguyen, and N. N. Chang, *Ibid.*, **32**, No. 3, 698-703 (1989).
13. J. E. Shook, P. K. Lemcke, C. A. Gehrig *et al.*, *J. Pharmacol. Exp. Ther.*, **249**, No. 1, 83-90 (1989).
14. N. Sperelakis and G. M. Wahler, *Mol. Cell. Biochem.*, **82**, 19-28 (1988).