Effect of *in Vivo* and *in Vitro* Stimulation of δ_1-Opioid Receptors on Myocardial Resistance to Arrhythmogenic Action of Ischemia and Reperfusion

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Preliminary intravenous injection of peptide agonist of δ_1-opioid receptors DPDPE (0.5 mg/kg) decreased the incidence of occlusion (10 min) and reperfusion (10 min) arrhythmias in rats. By contrast, δ_2-opioid receptor agonist DSLET produced no effect on the incidence of arrhythmias provoked by coronary occlusion and reperfusion. Preliminary injection of selective δ-receptor antagonist ICI 174,864 (2.5 mg/kg) or TIPP[ψ] (0.5 mg/kg) completely abolished the antiarrhythmic effect of DPDPE. Stimulation of cardiac δ_1-opioid receptors with DPDPE added to perfusion saline in concentrations of 0.1 and 0.5 mg/liter decreased the incidence of reperfusion arrhythmias. Addition of DPDPE to perfusion saline in a concentration of 0.1 mg/liter prevented reoxygenation destruction of cardiomyocytes. By contrast, no cardioprotective effect of this peptide was observed at a concentration of 0.5 mg/liter in perfusion saline or when it was injected intravenously. It is concluded that the cardioprotective and antiarrhythmic effects of DPDPE are caused by activation of cardiac δ_1-opioid receptors.

Key Words: *opioid receptors; arrhythmias; heart; ischemia; reperfusion*

We previously showed that endogenous opioid system is involved in the formation of adaptive resistance of the heart to arrhythmogenic stimuli [1]. There is also evidence on cardioprotective effects of μ-opioid receptors (OR) agonists in ischemia and reperfusion *in vivo* and *in vitro* [2]. However, cardiomyocyte membrane possesses primarily δ- and κ-OR [7]. There are data on antiarrhythmic effect of κ-OR agonists [12], although the role of δ-OR in arrhythmogenesis is still unclear. Only few papers described the antiarrhythmic effect of δ_1-agonists *in vivo* [4]. It is unclear, whether the antiarrhythmic effect of δ_1-agonists is related to activation of cardiac OR, or the protective effect of these agonists results from activation of extracardiac receptors.

Our aim was to study possible antiarrhythmic effect of δ_1-OR stimulation *in vivo* and *in vitro* during myocardial ischemia and reperfusion.

MATERIALS AND METHODS

In vivo experiments were carried out on Wistar rats (250-300 g) anesthetized with α-chloralose (Sigma, 100 mg/kg intraperitoneally) and ketamine (Moscow Endocrine Plant, 5 mg/kg intravenously). Acute ischemia (10 min) and reperfusion (10 min) were modeled by ligation of the left anterior descending coronary artery [9]. The rats were artificially ventilated using an RO-2 apparatus. ECG was recorded in thoracic lead I using an UBF4-03 amplifier coupled to PC with Pentium 200 processor. Analysis of ECG was carried out using original software. The incidence of multiple

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ventricular extrasystoles (MVE), ventricular tachycardia (VT), ventricular fibrillation (VF), and the percentage of animals without ventricular arrhythmia (VA) were determined.

The role of cardiac δ_1-OR in the regulation of heart resistance to arrhythmogenic action of reperfusion was studied on isolated rat heart subjected to long-term (45 min) total ischemia followed by 30-min reperfusion. The rats were sacrificed under ether narcosis by cervical dislocation. The heart was immediately isolated and placed in cold (4°C) Krebs—Henseleit solution. After termination of spontaneous beats Langendorff retrograde perfusion with standard Krebs--Henseleit solution was performed at a pressure of 60 mm Hg. After 20-min adaptation to normoxic conditions, perfusion was stopped for 45 min (total myocardial ischemia) and then resumed. Observations were continued for 30 min after reperfusion. ECG was recorded for 10 min with electrodes placed on the right atrium and left ventricle.

Selective δ_1-OR agonis DPDPE (H-Tyr-D-Pen-Gly-Phe-D-Pen-OH) [6], selective δ_2-OR agonist DSLET (H-Tyr-D-Ser-Gly-Phe-Leu-Thr-OH), and δ-OR antagonist ICI 174,864 (N,N-Diallyl-Tyr-Aib-Aib-Phe-Leu-OH) were used in the study (all substances from Multiple Peptide Systems). The ligands were dissolved in 0.9% NaCl. The doses of ligands were chosen on the basis of our previous study of the antiarrhythmic and cardioprotective effects of opiates [1,2].

In *in vivo* experiments, δ_1-OR agonist was injected intravenously in doses of 0.1 and 0.5 mg/kg 15 min before ischemia and 2 min before reperfusion, respectively. ICI 174,864 was injected in a dose of 2.5 mg/kg intravenously 25 min before coronary occlusion. Peripheral OR were blocked with methylnaloxone (RBI), a nonselective OR agonist, which cannot cross the blood-brain barrier [11].

Activation of δ_1-OR *in vitro* was performed by adding DPDPE into the perfusion solution in concentrations of 0.1 and 0.5 mg/liter. To this end, DPDPE was added to Krebs—Henseleit solution in the above concentrations after termination of a 20-min adaptation period (normoxic perfusion), and after 10 min the heart was washed from the ligand for the next 10 min. Then 45-min total ischemia and 30-min reperfusion were performed. In a special experimental series, activation of cardiac δ_1-OR was performed for the first 10 min of reperfusion. To this end, after 45-min ischemia the heart was perfused for 10 min with Krebs-Henseleit solution containing DPDPE (final concentration 0.1 mg/liter). The peptide was dissolved in physiological saline and added to perfusion solution immediately before use.

Rats and isolated hearts subjected to acute ischemia and reperfusion served as the controls in *in vivo* and *in vitro* experiments, respectively.

The data were analyzed statistically using the χ^2 test.

RESULTS

Preliminary injection of δ_1-OR agonist DPDPE (0.1 mg/kg) significantly increased the percentage of rats resistant to the arrhythmogenic effects of ischemia and reperfusion (Table 1). Stimulation of δ_1-OR completely prevented VF and markedly decreased the incidence of VT and MVE during both ischemia and reperfusion (Table 1). The same effect was observed after injection of DPDPE in a dose of 0.5 mg/kg. By contrast, stimulation of δ_2-OR with DSLET did not change the incidence of ischemic and reperfusion VA (Table 1).

The study of receptor specificity of the antiarrhythmic effect of δ_1-OR agonist showed that this effect was completely abolished by preliminary injection of ICI 174,864, a δ-OR antagonist (Table 1). ICI 174,864 alone had no effect on the incidence of arrhythmias (data not shown).

When δ_1-OR stimulation was performed 2 min before removal of the ligature from the coronary artery, the incidence of reperfusion arrhythmias decreased 3-fold compared to the control (Table 1). It should be emphasized that stimulation of δ_1-OR completely prevented VF and significantly (4-fold) decreased the incidence of VT (Table 1). This effect was observed when DPDPE was injected before coronary occlusion or 2 min before reperfusion.

These data indicate that activation of δ_1-OR enhances myocardium tolerance to arrhythmogenic effect of coronary occlusion and reperfusion. It should be noted that DPDPE produced a pronounced antiarrhythmic effect not only when it was injected before coronary occlusion, but also when it was applied immediately before reperfusion. The antiarrhythmic effect of DPDPE is related to activation of δ-OR, since ICI 174,864, a selective antagonist of these receptors, completely prevented the protective effect of DPDPE. It can be assumed that the antiarrhythmic effect of δ_1-OR agonist DPDPE is caused by activation of δ_1-OR, because δ_2-OR agonist DSLET produced no effect on the incidence of occlusion and reperfusion arrhythmias. Our results agree with published data that stimulation of δ_1-OR improves myocardial resistance to the arrhythmogenic effects of coronary occlusion and reperfusion [4].

It is known that opioid peptides poorly cross the blood-brain barrier and in doses below 0.5 mg/kg cannot activate central OR [3]. Therefore, it can be assumed that the antiarrhythmic effect of DPDPE is associated with activation of peripheral δ_1-OR. Indeed, DPDPE-induced improvement of cardiac resistance to occlusion and reperfusion arrhythmias was abolished

TABLE 1. Effects of Agonists of δ_1- and δ_2-OR (DPDPE and DSLET) and OR Blockers (ICI 164,864 and Methylnaloxone) on Incidence of VA during Ischemia and Reperfusion *in Vivo*

Experimental conditions	Ischemia								Reperfusion							
	without VA		MVE		VT		VF		without VA		MVE		VT		VF	
	abs.	%	abs.	%	abs.	%	abs.	%	abs.	%	abs.	%	abs.	%	abs.	%
Control (n=16)	2	13	12	75	7	44	1	6	1	6	14	88	10	63	3	19
DPDPE, 10 min before ischemia, mg/kg																
0.1 (n=14)	12	86*	3	21**	2	14**	0	—	12	86**	2	14**	2	14*	0	—
0.5 (n=14)	11	80*	2	14*	2	14**	0	—	13	93*	1	7*	2	14*	0	—
DPDPE, 2 min before reperfusion, 0.5 mg/kg (n=14)	—	—	—	—	—	—	—	—	9	63**	5	35**	4	28***	1	7
DSLET, 10 min before ischemia (n=14)	3	21	11	79	9	64	1	7	2	14	12	86	11	79	2	14
Methylnaloxone, 25 min before ischemia (n=14)	3	21	11	79	8	57	1	7	2	14	12	86	10	71	2	14
+DPDPE, 10 min before ischemia (n=14)	3	21	10	67	7	47	1	7	2	13	12	86	11	73	2	13
ICI 164,864, 25 min before ischemia (0.5 mg/kg)+DPDPE, 10 min before ischemia (n=14)	2	14	12	86	6	43	1	7	1	7	13	93	11	79	2	14

Note. *p<0.001, **p<0.01, ***p<0.05 compared to the control.

by preliminary injection of methylnaloxone, a blocker of OR, which does not cross the blood-brain barrier [11]. In our experiments methylnaloxone in a dose of 5 mg/kg completely abolished the protective effect of DPDPE during ischemia and reperfusion (Table 1).

Localization of δ_1-OR, whose activation efficiently protected the myocardium against ischemia-reperfusion arrhythmias was studied in experiments on isolated heart. In these experiments, preliminary stimulation of cardiac δ_1-OR with DPDPE also improved heart resistance to reperfusion arrhythmias. In control series, VA during the first 10 min of reperfusion were observed in 75% isolated hearts, of them 25% were MVE (25%), VT (56%), and VF (31%, Fig. 1). Preliminary perfusion of isolated hearts with Krebs—Henseleit solution containing 0.1 mg/liter DPDPE significantly decreased the incidence of reperfusion arrhythmias. Stimulation of cardiac δ_1-OR 3-fold decreased the incidence of VT and completely prevented VF (Fig. 1). Therefore, activation of cardiac δ_1-OR was achieved with the dose of 0.1 mg/liter.

Addition of 0.5 mg/liter DPDPE to perfusion solution also improved cardiac resistance to reperfusion arrhythmias. During the first 10 min of reperfusion, no VF were observed, and the incidence of VT decreased significantly (Fig. 1). Thus, the dose of 0.1 mg/liter is sufficient for attaining the maximum stimulation of cardiac OR.

In the next experimental series we studied the antiarrhythmic effect of DPDPE administration at the start of reperfusion. In this case, the peptide was ineffective. Activation of cardiac δ_1-OR during the first 10 min after resuming coronary circulation had no effect on the number and type of reperfusion arrhythmias: the incidence of VF and VT was the same in experimental and control hearts (Fig. 1).

It can be assumed that the antiarrhythmic effect of DPDPE applied before ischemia is mediated by δ_1-OR-mediated decrease of intracellular cAMP concentration in the myocardium, which is supposed to be an "endogenous arrhythmogenic factor" [8]. This hypothesis is confirmed by the data on decreased cAMP content in the myocardium after preliminary perfusion of the isolated heart with solutions containing δ-OR-agonists [5].

Therefore, our findings suggest that DPDPE-induced improvement of myocardial resistance to arrhythmogenic effects of ischemia and reperfusion is associated with activation of peripheral δ_1-OR partially located in the myocardium. Preliminary activation of cardiac δ_1-OR improves heart resistance to arrhythmogenic effects of ischemia and reperfusion. However, there is another pool of δ_1-OR. Activation of these receptors immediately before removal of the ligature improves resistance of the myocardium to reperfusion

arrhythmia. These δ_1-OR are located not in the myocardium, because their effect cannot be reproduced in isolated heart.

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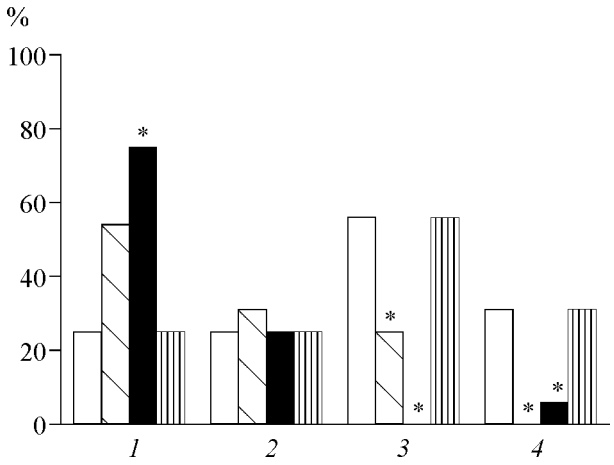

Fig. 1. Effect of activation of cardiac δ_1-opioid receptors on the incidence of reperfusion arrhythmias in isolated heart. Light bars: control values; oblique hatching: DPDPE 0.1 mg/liter before ischemia; solid bars: DPDPE 0.5 mg/liter before ischemia; vertically hatched bars: DPDPE 0.1 mg/liter after ischemia. *1*) without ventricular arrhythmias; *2*) multiple ventricular extrasystoles; *3*) ventricular tachycardia; *4*) ventricular fibrillation. **p*<0.05 compared to the control.

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