Receptor Specificity of the Antiarrhythmic Effect Produced by Opioid Peptides Dalargin and DADLE during Myocardial Reperfusion

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Nonselective agonists of μ- and δ-opioid receptors dalargin (D-Ala^2,Leu^5,Arg^6-enkephalin) and DADLE (D-Ala^2,D-Leu^5-enkephalin) administered immediately before coronary reperfusion in a dose of 0.1 mg/kg prevented the development of ventricular arrhythmias. Blockade of μ-opioid receptors abolished the antiarrhythmic effect of these peptides. Hence, antiarrhythmic activity of dalargin and DADLE is primarily associated with activation of μ-opioid receptors.

Key Words: *reperfusion arrhythmias; opioid receptors; dalargin; DADLE*

Our previous studies showed that peptide agonist of opioid receptors (OR) D-Ala^2,Leu^5,Arg^6-enkephalin (dalargin) administered before coronary occlusion prevents ventricular arrhythmias [2,7]. However, preventive treatment with antiarrhythmic preparations before the development of acute myocardial infarction is hardly possible in clinical practice and these drugs are usually administered after coronary occlusion. The type of OR mediating the antiarrhythmic effect of dalargin remains unclear.

Here we studied antiarrhythmic activity of opioid-like peptides dalargin and DADLE administered immediately before reperfusion and evaluated receptor specificity of their effects.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 200-250 g. The rats were narcotized with α-chloralose (100 mg/kg intraperitoneally) and ketamine (5 mg/kg intravenously) and artificially ventilated. Acute myocardial ischemia modeled by 10-min ligation of the left coronary artery was followed by 10-min reperfusion [7].

ECG (lead II) was recorded during reperfusion using an UBF4-03 amplifier and analyzed using original software. Multiple ventricular extrasystoles (MVE) and episodes of ventricular tachycardia (VT) and ventricular fibrillation (VF) were counted in each experimental group. The total duration of VT and VF per rat was calculated.

The agonists of μ- and δ-OR dalargin (D-Ala^2, Leu^5,Arg^6-enkephalin, 0.1 mg/kg) [1] and DADLE (D-Ala^2,D-Leu^5-enkephalin, 0.1 mg/kg) [5] were injected intravenously 8 min after the start of ischemia (2 min before reperfusion). Control rats received 0.2 ml physiological saline 2 min before reperfusion.

The δ-OR antagonist TIPP(ψ) (H-Tyr-TicPψ-[CH_2NH]-Phe-Phe-OH) [10] or μ-OR antagonist CTAP (H-D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH_2, Multiple Peptide Systems) [4] was injected in a dose of 0.5 mg/kg 25 min before agonists. The doses of preparations were chosen taking into account published data on their cardiovascular activity [1-7,10].

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

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RESULTS

During reperfusion after 10-min ischemia the total duration of VT and VF in control animals was comparable with that observed in our previous experiments [2,7] and other studies [8] (Table 1).

Dalargin decreased the incidence of MVE and VT by 2 and 4 times, respectively, compared to the control (Table 1). None of these animals had VF. Arrhythmias were absent in 40% rats of this group. The duration of VT in dalargin-treated rats was much lower than in control animals (Table 1). These results indicate that dalargin not only produces an antiarrhythmic effect after administration before ischemia [2,7], but also selectively prevents ventricular arrhythmias during reperfusion.

Blockade of δ-OR with TIPP(ψ) partially abolished the antiarrhythmic effect of dalargin: the number of rats with MVE and the duration of VT in this group markedly increased (Table 1). However, blockade of δ-OR had no effect on the incidence of VT and VF and number of animals without arrhythmias. These findings confirm minor role of δ-OR in antiarrhythmic activity of dalargin during reperfusion.

The μ-OR antagonist CTAP injected before dalargin increased the incidence of MVE, VT, and VF by 4 and 3 times and 30%, respectively, compared to rats receiving dalargin alone (Table 1). Thus, μ-OR antagonist CTAP completely abolished the antiarrhythmic effect of dalargin.

The μ- and δ-OR agonist DADLE injected 2 min before reperfusion decreased the incidence of MVE and VT by 2 and 1.7 times, respectively, and prevented the development of VF (Table 1). Similarly to experiments with dalargin, μ-OR blockade abolished the antiarrhythmic effect of DADLE. Under these conditions the incidence and duration of VT increased by 2.2 and 2.1 times, respectively. VF was also observed in this group (Table 1).

Our results indicate that dalargin and DADLE administered immediately before coronary reperfusion prevented ventricular arrhythmias. Antiarrhythmic activity of dalargin and DADLE was associated with activation of μ-OR.

Since dalargin does not cross the blood-brain barrier [1], it can be hypothesized that its antiarrhythmic effect is realized via activation of peripheral μ-OR. However, localization of peripheral receptors mediating cardiotropic activity of dalargin is unknown. Our previous experiments on isolated heart demonstrated cardioprotective activity of dalargin [3]. In these studies dalargin was injected intravenously 15 min before isolation of the heart. Therefore, the role of cardiac and other peripheral OR in the antiarrhythmic effect of these peptides remains unclear.

The antiarrhythmic effect of dalargin and DADLE can be mediated by the following intracellular mechanisms. Activation of OR is associated with blockade of slow Ca^{2+} channels [9]. Therefore activation of μ-OR is followed by changes in free Ca^{2+} content in cardiomyocyte cytoplasm. Accumulation of cAMP during acute ischemia promotes calcium overload of cardiomyocytes and arrhythmia development [8]. Our previous studies showed that dalargin reduces cAMP concentration in cells during acute coronary occlusion [7]. These data suggest that the effect of dalargin on $G_{i/o}$ protein-coupled adenylate cyclase underlies the opioid-induced inhibition of Ca^{2+} influx into cardiomyocytes through L-type Ca^{2+} channels and antiarrhythmic activity of these substances.

Our findings can be used in the search for new antiarrhythmic preparations among OR agonists.

TABLE 1. Effects of the Activation of μ- and δ-Opioid Receptors on the Incidence (Number of Animals in Group) and Duration of Reperfusion Ventricular Arrhythmias in Rats (*M*±*m*)

Parameter	Control (*n*=24)	Dalargin			DADLE	
		without blockers (*n*=15)	+TIPP(y) (*n*=13)	+CTAP (*n*=14)	without blockers (*n*=14)	+CTAP (*n*=14)
Without arrhythmias	2 (8)	6 (40)**	3 (23)	3 (21)	5 (35)**	4 (28)
MVE	21 (88)	6 (40)*	10 (77)$^{++}$	11 (79)$^{++}$	6 (43)*	10 (71)
VT	19 (79)	3 (20)*	6 (46)	9 (64)$^{+}$	4 (29)*	9 (64)^{o}
VF	8 (33)	0 (0)**	0 (0)	4 (29)$^{++}$	0 (0)**	4 (29)^{oo}
Duration, sec						
VT	51.4±7.5	13.83±7.50*	45.58±10.55^{++}	44.28±12.55^{++}	23.4±7.3**	49.3±12.8^{oo}
VF	11.07±5.50	0	0	23.47±9.56	0	16.70±5.21

Note. *p<0.01 and **p<0.05 compared to the control; ^{+}p<0.01 and ^{++}p<0.05 compared to dalargin; ^{o}p<0.01 and ^{oo}p<0.05 compared to DADLE. In parentheses: % of the total number of rats in group.

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