Myocardial Resistance to Ischemic and Reperfusion Injuries under Conditions of Chronic Administration of Opioid Receptor Agonists and Antagonists

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Chronic treatment with opioid receptor ligands: nonselective peptide opioid receptor agonist dalargin (intraperitoneally in a dose of 1 mg/kg), selective nonpeptide κ-receptor agonist GR 89696 (subcutaneously in a dose of 0.03 mg/kg), nonselectrive nonpeptide antagonist quadazocine (subcutaneously in a dose of 3 mg/kg) or naltrexone (subcutaneously in a dose of 10 mg/kg) for 20 day had no effect of the incidence of ischemic ventricular arrhythmias and the size of necrotic zone after coronary occlusion and reperfusion in rats *in vivo*.

Key Words: *chronic treatment with opioid receptor agonists; myocardium; ischemia; reperfusion*

Morphine is used in clinical practice for about 150 years, while opioid abuse is a prevalent phenomenon. However, the effects of chronic opioid treatment on the function of the cardiovascular system are poorly studied and the corresponding data are controversial. Some authors believe that course morphine treatment improves the resistance of rat heart to the arrhythmogenic effects of coronary occlusion and reperfusion [7] and delayed the formation of the necrotic zone in local ischemia in mice [11]. Other authorities observed the development of supraventricular and ventricular arrhythmias in opioid abusers [1]. These opposite effects of chronic morphine treatment were not explained.

Receptors mediating the cardiovascular effects of morphine under conditions of chronic administration were not identified. The fact should be also taken into account that morphine crosses the blood-brain barrier and activates all three types of opioid receptors (μ-, δ-, and κ-OR) identified in the brain and at the periphery [9,12,13]. There are no data on the effect of chronic OR blockade on heart resistance to the pathogenic effects of ischemia-reperfusion.

Here we studied the effect of chronic activation and blockade of μ-, δ-, and κ-OR on heart resistance to ischemia and reperfusion.

MATERIALS AND METHODS

Experiments were carried out on male Wistar rats weighing 200-250 g (n=80).

The animals received μ-, δ-, and κ-OR agonist dalargin (D-Ala^2,Leu^5,Arg^6-encephalin) intraperitoneally in a dose of 1 mg/kg (n=15) [2,3]; κ_2-OR agonist GR 89696 subcutaneously in a dose of 0.03 mg/kg (n=16) [6]; or nonselective nonpeptide OR antagonist quadazocine subcutaneously in a dose of 3 mg/kg (n=13) [8,10] for 20 days. The dose of nonselective OR antagonist naltrexone (10 mg/kg, subcutaneously, n=15) was chosen experimentally

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by the capacity of this agent to prevent the chronoropic effect of U-50488 (selective κ-OR agonist). Control animals (n=12) received isotonic NaCl. The last injection of the test drugs was made 24 h before coronary occlusion. The doses of the preparations and administration routes were chosen from published reports and the data of our previous experiments [2,3,5,6,8,10,14].

Dalargin was manufactured in the Laboratory of Peptide Synthesis, Russian Cardiology Research-and-Production Complex; GR 89696 was kindly provided by Dr. Kelly Halliday (GlaxoSmithKline); quadazocine was provided by Dr. Wendy C. Chang (Sanofi-Synthelabo Research). Naltrexone was purchased from Sigma.

Before coronary occlusion, the animals were narcotized with α-chloralose (100 mg/kg intraperitoneally). During all subsequent manipulations, the rats were artificially ventilated with atmospheric air using a RO-2 apparatus. A ligature was applied on the upper third of the left coronary artery for 45 min followed by resumption of the blood flow for 120 min [12,13]. The hypoperfusion area (risk area) in the myocardium was visualized by staining with Patent blue violet (40 mg/kg intravenously) [12, 13]. After the heart was removed from the thorax, the left ventricular myocardium was cut into 5 transverse sections (2-2.5-mm thick). The necrotic zones were visualized by staining with 0.1% p-nitroblue tetrazolium [12,13].

ECG in standard lead II was recorded during ischemia and the first 10 min of reperfusion and the number of animals with ventricular extrasystoles, ventricular tachycardia, and ventricular fibrillation was determined for each experimental group.

The results were processed using Student t test for quantitative signs (weight and volume of the myocardium) and χ^2 test for qualitative signs (incidence of heart rhythm disturbances).

RESULTS

The infarction/risk area ratio (IA/RA) in animals subjected to coronary occlusion and reperfusion after course treatment with dalargin of GR 89696 did not differ from the control (Fig. 1). The incidence of heart rhythm disturbances in these experimental groups was also close to the control (Table 1).

It is known that dalargin cannot cross the blood-brain barrier and exhibits affinity to μ-, δ-, and κ-OR [2,3]. Hence, it can be hypothesized that chronic activation of peripheral μ-, δ-, and κ-OR did not lead to considerable changes in myocardial sensitivity to ischemic and reperfusion injuries and rhythm disturbances.

TABLE 1. Effect of Chronic Administration of Dalargin (1 mg/kg) and GR 89696 (0.03 mg/kg) on the Incidence of Ischemic and Reperfusion Ventricular Arrhythmias

Group	Ischemia, 1-10 min				Ischemia, 10-20 min				Reperfusion, 1-10 min			
	No VA	MVEx	VT	VF	No VA	MVEx	VT	VF	No VA	MVEx	VT	VF
Control (n=17)	3 (17.6)	14 (82.4)	12 (70.6)	9 (53)	7 (41)	10 (59)	7 (41)	4 (23.5)	16 (94)	1 (5.9)	0	0
Dalargin (n=15)	5 (33)	10 (66.6)	10 (66.6)	7 (46.6)	8 (53.3)	7 (46.6)	7 (46.6)	1 (6.6)	14 (93.3)	1 (6.6)	0	0
GR 89696 (n=16)	6 (37.5)	10 (62.5)	10 (62.5)	2 (12.5)	11 (68.8)	5 (31.3)	4 (25)	3 (18.8)	16 (100)	0	0	0

Note. Here and in Table 2: percents are shown in parentheses No VA: without ventricular arrhythmias; VT: ventricular tachycardia; VF: ventricular fibrillation; MVEx: multiple ventricular extrasystoles.

TABLE 2. Effect of Chronic Administration of Quadazocine (3 mg/kg) and Naltrexone (10 mg/kg) on the Incidence of Ischemic and Reperfusion Ventricular Arrhythmias

Group	Ischemia, 1-10 min				Ischemia, 10-20 min				Reperfusion, 1-10 min			
	No VA	MVEx	VT	VF	No VA	MVEx	VT	VF	No VA	MVEx	VT	VF
Control (n=17)	3 (17.6)	14 (82.4)	12 (70.6)	9 (53)	7 (41)	10 (59)	7 (41)	4 (23.5)	16 (94)	1 (5.9)	0	0
Naltrexone (n=15)	3 (20)	11 (73.3)	9 (60)	7 (46.6)	2 (13.3)	10 (66.6)	7 (46.6)	2 (13.3)	14 (93.3)	1 (6.7)	1 (6.7)	0
Quadazocine (n=17)	2 (11.8)	15 (88)	14 (82)	8 (47)	6 (35.3)	10 (59)	7 (41)	5 (29.4)	16 (94)	1 (5.9)	0	0

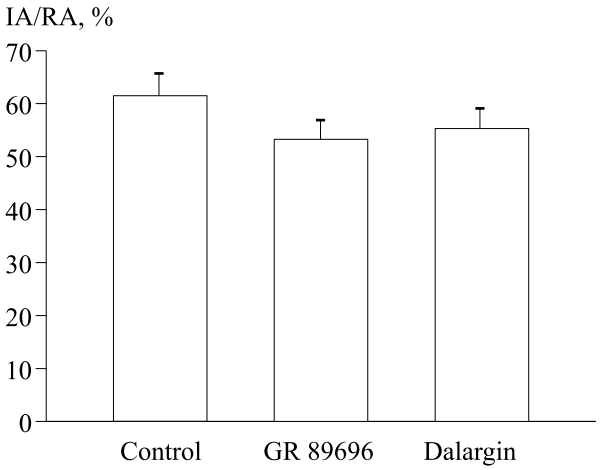

Fig. 1. Effect of chronic administration of dalargin (1 mg/kg) and GR 89696 (0.03 mg/kg) on infarction/risk area ratio after 45 min coronary occlusion and 120-min reperfusion in rats.

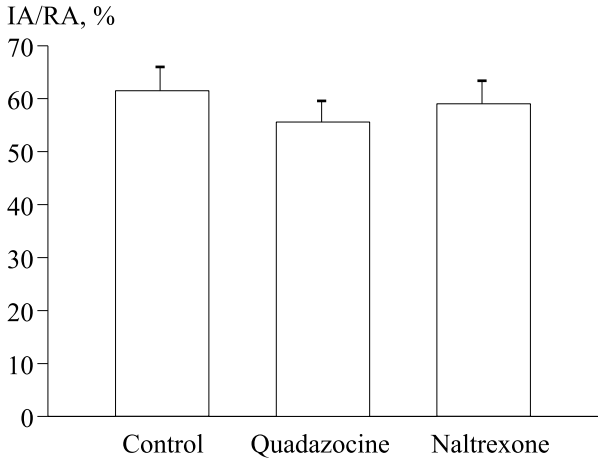

Fig. 2. Effect of chronic administration of quadazocine (3 mg/kg) and naltrexone (10 mg/kg) on infarction/risk area ratio after 45 min coronary occlusion and 120-min reperfusion in rats.

GR 89696 is a selective κ_2-OR agonist and in the dose used in our experiments can produce central effects [6]. Our findings suggest that preliminary chronic stimulation of central κ_2-OR and peripheral μ-, δ-, and κ-OR had no effect of the state of cardiomyocytes during acute coronary occlusion and reperfusion.

Hence, we can conclude that the previously reported cardioprotective and antiarrhythmic effects of chronic morphine administration [7,11] can be mediated by activation of central μ-, δ-, and κ-OR.

The second part of the study evaluated the effect of chronic OR blockade on the resistance of rat myocardium to the damaging and arrhythmogenic effects of ischemia and reperfusion. We used quadazocine in a dose of 3 mg/kg and naltrexone in a dose of 10 mg/kg. We found that none of the studied OR ligands under conditions of their chro-

nic treatment affected the incidence of ischemic ventricular arrhythmias (Table 2) and the IA/RA ratio (Fig. 2). Hence, chronic inhibition of central and peripheral OR did not change heart resistance to ischemia and reperfusion.

Thus, neither long-term activation of peripheral μ-, δ-, and κ-OR and central κ_2-OR, nor chronic blockade of OR modulated the resistance of rat myocardium to damage and rhythm disturbances provoked by acute ischemia and reperfusion.

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