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Role of Endogenous Agonists of Opioid Receptors in the Regulation of Heart Resistance to Postischemic Reperfusion Injury

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Intravenous injection of nonselective antagonists of opioid receptors (OR) naltrexone (5 mg/kg) and naloxone methiodide (5 mg/kg), selective δ_1-OR antagonist BNTX (0.7 mg/kg), selective δ_2-OR blocker naltriben (0.3 mg/kg), selective κ-OR antagonist norbinaltorphimine (2 mg/kg), and selective blocker of ORL1 opioid receptors JTC-801 (0.1 mg/kg) produced no effect on reperfusion injury to the heart in rats narcotized with α-chloralose. In contrast, selective μ-OR antagonist CTAP (1 mg/kg) limited the infarct size, although this effect was not observed at a lower CTAP concentration of 0.1 mg/kg. Probably, the myocardial infarct size-limiting effect of CTAP was associated with activation of the non-opioid receptors. It was hypothesized that endogenous OR agonists did not affect heart resistance to reperfusion injury in unadapted rats.

Key Words: *opioid receptors; opioids; heart; reperfusion; rat*

Changes in heart contractility in response to stimulation of *n. vagus* or sympathetic nerves are related to the release of acetylcholine or norepinephrine, respectively. Epinephrine also can regulate myocardial contractility. In 1930s, it was a widely accepted view that acetylcholine and catecholamines were the only substances regulating the functional state of the heart. However, in 1970s and 1980s, findings of endogenous opioids [5] were followed by discovery of opioid receptors (OR) on the sarcolemma of the cardiomyocytes. In the isolated heart, blockade of μ-OR prior to ischemia prevented the development of reperfusion contracture [3]. In contrast, pre-ischemic blockade of δ- and κ-OR aggravated reperfusion-provoked contractile dysfunction of the isolated heart [12]. There are data that blockade of the entire pool of peripheral ORs increases heart resistance to the arrhythmogenic effect of coronary occlusion [8,10]. These facts suggest that endogenous OR agonists can regulate heart resistance to the pathological effects of ischemia. However, it is unclear whether endogenous opioids can modulate heart resistance to reperfusion-provoked effects *in vivo*.

Our aim was to elucidate the role of endogenous agonists of ORs in the control of heart resistance to pathogenic action of reperfusion *in vivo*.

MATERIALS AND METHODS

The experiments were carried out on male Wistar rats (*n*=192) weighing 250-300 g. The animals were narcotized intraperitoneally with α-chloralose (Sigma, 60 mg/kg) and artificially ventilated with an SAR-830 Small Animal Ventilator (CWE). Coronary artery occlusion and reperfusion were performed as described elsewhere [11]. BP was measured in the cannulated right carotid artery via an SS13L pressure transducer

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and an MP35 Data Acquisition Unit (Biopac System) controlled by PC. This unit was also used to record ECG.

After 45-min ischemia, the ligature was removed, and resumption of the blood flow was established visually by the appearance of epicardial hyperemia. Duration of reperfusion was 2 h. The infarct size (IS) and hypoperfused area at risk (AAR) were determined as described previously [9]. After reperfusion, the heart was isolated and retrogradely washed through the aorta with physiological saline. To determine AAR, the ligature was tightened again; thereupon the myocardium had been stained by aortal injection of 5% potassium permanganate. After washout, 1-mm coronal sections oriented perpendicular to the long axis of the heart were prepared on an HSRA001-1 Slicer (Zivic Instruments). To determine IS, the sections were stained with 1% 2,3,5-triphenyltetrazolium chloride for 30 min at 37°C. This method is based on the potency of this dye to acquire a stable color after transition from oxidized to reduced state under the action of dehydrogenases. Since the dead cardiomyocytes had no dehydrogenases, the necrotized part of the myocardium was not colored. After staining, the sections were placed in 10% formaldehyde solution for 24 h. Both sides of the sections were scanned with an HP Scanjet G4050 Scanner. IS and AAR were determined by computerized planimetry and IS/AAR ratio was calculated (in %).

All OR antagonists were injected intravenously 10 min prior to reperfusion. To block entire pool of ORs, we used naltrexone (5 mg/kg), whereas peripheral ORs were blocked with naloxone methiodide (5 mg/kg, both reagents from Sigma-Aldrich). μ-OR were blocked with 0.1 mg/kg CTAP (NH_2-D-Phe-c[Cys-Tyr-D-Trp-Arg-Thr-L-Pen]-Thr-NH_2, Multiple Peptide Systems). Inhibition of δ_1- and δ_2-OR was performed with 7-benzylidenenaltrexone (BNTX, 0.7 mg/kg) and naltriben (0.3 mg/kg), respectively (both from Tocris Bioscience). Norbinaltorphimine (2 mg/kg, Sigma-Aldrich) was employed to block κ-OR. Antagonist of type 4 opioid ORL1 receptors JTC-801 [N-(4-amino-2-methylquinolin-6-yl)-2-(4-ethylphenoxymethyl) benzamide monohydrochloride] (Tocris Bioscience) was injected in a dose of 0.1 mg/kg. The control rats received the physiological saline or 20% hydroxypropyl-β-cyclodextrin (1 ml/kg). The dosage of OR antagonists were selected according to [1,6,13].

Naltrexone, naloxone methiodide, CTAP, and norbinaltorphimine were dissolved in 0.9% NaCl. Naltriben, JTC-801, and BNTX were dissolved in 0.1 ml DMSO and then in 0.9 ml 20% hydroxypropyl-β-cyclodextrin. The data were analyzed statistically using Statistica 6.0 software and Mann—Whitney test at $p<0.05$. The results are summarized as $m \pm SEM$. The antiarrhythmic effect of OR antagonists was analyzed statistically by χ^2 test.

RESULTS

In control group, IS/AAR ratio was 47% (Fig. 1). During reperfusion, some rats demonstrated isolated ventricular extrasystoles, whereas most rats had no reperfusion-provoked arrhythmias, so these experiments could not reveal the antiarrhythmic or proarrhythmic effects of examined agents.

Intravenous injection of naltrexone, a blocker of entire OR pool, or naloxone methiodide, a blocker of peripheral OR, produced no significant effect on IS/AAR ratio. It should be stressed that these agents have different affinity to various types of OR. Logically, we examined the effects of antagonists of individual types of OR. CTAP, a selective antagonist of μ-OR, in a dose of 1 mg/kg, significantly decreased IS/AAR by 15% in comparison with the control (Fig. 1). This is a somewhat unexpected result, because the cardioprotective effects of OR blockers were not reported previously. The studies of our team showed that OR agonists did not protect the heart against ischemia/reperfusion injury. However, it should be noted that the above dosage is rather high, so we tested CTAP in a lower dose as well. In contrast, the lower dose of 0.1 mg/kg did not significantly decrease IS/AAR ratio. It can be hypothesized that this low dose is insufficient to block μ-OR despite it eliminates the infarct size-limiting adaptation to hypoxia [7]. Probably, the myocardial infarct size-limiting effect of CTAP in a higher dose (1 mg/kg) resulted from interaction of this peptide with non-opioid receptors. It is noteworthy that CTAP has moderate affinity to somatostatin receptors expressed in the heart [4]. It seems logical that the myocardial

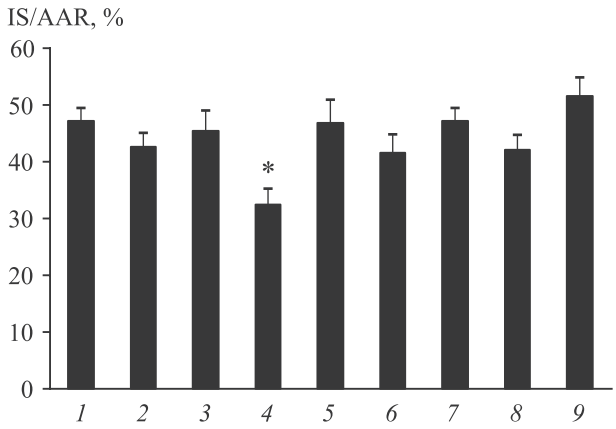

Fig. 1. Effect of 45-min coronary occlusion and 120-min reperfusion on IS/AAR ratio in rats. *1*) Control; *2*) CTAP, 1 mg/kg; *3*) norbinaltorphimine; *4*) naltrexone; *5*) CTAP, 0.1 mg/kg; *6*) naltriben; *7*) naloxone methiodide; *8*) BNTX; *9*) JTC-801. The antagonists were injected 10 min prior to coronary occlusion. $p<0.05$ in comparison with control.

TABLE 1. Effect of 45-min Coronary Occlusion and 120-min Reperfusion on HR and BP in Control Rats (*m*±*SEM*, *n*=12)

Observation period	HR, bpm^{-1}	BP, mm Hg
Prior to coronary occlusion	365±9	134±5
1 min prior to reperfusion	361±6	131±3
Postreperfusion minute 30	355±8	129±5
Postreperfusion hour 2	341±10	125±4

infarct size-limiting effect of CTAP could be associated with action on somatostatin receptors or some unknown targets. Intravenous injection of BNTX or naltriben, the corresponding selective antagonists of δ_1- and δ_2-OR, produced no significant effect on IS/AAR ratio (Fig. 1). Similarly, norbinaltorphimine, a selective antagonists of κ-OR, exerted no significant effect on this ratio. These data agree with the results of other researchers, who did not observe the changes in IS/AAR ratio under the action of OR antagonist applied during reperfusion [2,12].

Blockade of ORL1 receptors with JTC-801 produced no significant effect on IS/AAR ratio (Fig. 1).

In the control group, no significant changes in HR and BP were observed during coronary occlusion and reperfusion (Table 1). All examined OR blockers produced no effect on these parameters (data not shown).

Thus, endogenous agonists of OR are not implicated in the control of heart resistance to the pathogenic action of reperfusion in unadapted rats. However, these data do not exclude possible involvement of endogenous opioids in the regulation of heart resistance to the ischemia/reperfusion injury in the adapted animals.

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