# Role of Endogenous Opioid Receptor Agonists in Regulation of Heart Resistence to the Arrhythmogenic Action of Short-Term Ischemia and Reperfusion

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Preliminary selective block of μ-, $\delta_1$-, $\delta_2$-, and κ-opioid receptors had no effect on the incidence of ventricular arrhythmias during 10-min coronary occlusion-reperfusion in ketamine-narcotized rats. Repetitive short-term immobilization of rats for 2 weeks improved heart resistance to the arrhythmogenic action of coronary occlusion and reperfusion. Selective μ-opioid receptor antagonist CTAP completely abolished, while selective δ- and κ-opioid receptor antagonists did not modulate the antiarrhythmic effect of adaptation. Probably, endogenous agonists of μ-opioid receptors play an important role in the adaptive improvement of heart resistance to arrhythmogenic factors, but are insignificant for the modulation of heart resistance to the arrhythmogenic action of short-term local ischemia-reperfusion in non-adapted animals.

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

Opioid peptides and opioid receptors (OR) are present in the heart. OR are located on cardiomyocyte sarcolemma and are probably involved in the regulation of heart resistance to arrhythmogenic factors. The data on OR and their role in arrhythmogenesis are summarized in our recent reviews [1-3].

Published data are contradictory. Some papers report antiarrhythmic action of OR agonists [8,10], while others show that blockade of OR improves heart resistance to the arrhythmogenic effect of ischemia-reperfusion [1]. All authors reporting improvement of heart resistance to the arrhythmogenic effect of ischemia-reperfusion after OR blockade used non-selective OR agonists or agents with low selective binding to κ-OR or δ-OR [1]. Therefore, OR subtypes promoting arrhythmia upon activation during ischemia-reperfusion were not identified. The effect of initial (preischemic) state of the organism on modulation of arrhythmogenesis by endogenous OR was never examined.

We studied the role of endogenous OR in the regulation of heart resistance to the arrhythmogenic action of short-term ischemia-reperfusion.

## MATERIALS AND METHODS

Experiments were carried out on narcotized Wistar rats weighing 200-250 g. Acute ischemia was modeled by ligation of the left coronary artery for 10 min [8]. This procedure was performed under artificial ventilation with a modernized PO-2 apparatus. During 10-min ischemia followed by 10-min reperfusion, ECG was recorded in standard lead I using an UBF4-03 amplifier. The signals were fed into PC and processed using original software. The endpoints of ECG ana-

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lysis were the incidence of grouped ventricular extrasystoles, ventricular tachycardia, and ventricular fibrillation. Adaptation of the animals to immobilization stress was performed as described elsewhere [11].

The following chemicals were used in the study: non-selective OR antagonist naloxone [9]; non-selective μ- and κ-OR antagonist quadazocine; non-selective OR antagonist naloxone methiodide (cannot cross the blood-brain barrier) [12]; selective μ-OR antagonist β-funaltrexamine (β-FNA) [9]; selective μ-OR antagonist $NH_2$-D-Phe-Cys-Tyr-D-Trp-Arg-Thr-L-Pen-Thr-$NH_2$ (CTAP); selective δ-OR antagonists N,N-Diallyl-Tyr-Aib-Aib-Phe-Leu-OH (ICI 174,864) and H-Tyr-Tic$\psi$ [$CH_2$NH]Phe-Phe-OH (TIPP[ψ]; Tic=ψ[1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid)] [9]; selective $\delta_2$-OR antagonist naltriben (NTB) [9]; $\delta_1$-OR antagonist 7-benzylidenenaltrexone maleate (BNTX) [15], and selective κ-OR antagonist nor-binaltorphimine [5]. All antagonists except for nor-binaltorphimine were injected intravenously 25 min before coronary occlusion. Nor-binaltorphimine was injected intravenously 90 min before coronary occlusion. The chemicals were used in the following doses: naloxone 2 mg/kg; naloxone methiodide 2 mg/kg; β-FNA 5 mg/kg; CTAP 0.5 mg/kg; ICI 174,864 0.5 mg/kg; NTB 0.3 mg/kg; BNTX 0.7 mg/kg; and nor-binaltorphimine 9 mg/kg. BNTX was dissolved (1:9) in a mixture of dimethyl sulfoxide and 20% 2-hydroxypropyl-β-cyclodextrin water solution. All other chemicals were dissolved in 0.9% NaCl. The dosages were chosen based on previous reports [5,12,15] and our studies [10].

The peptide ligands CTAP, TIPP[ψ], and ICI 174,864 were synthesized in Multiple Peptide Systems. The non-peptide OR antagonists β-FNA, BNTX, NTB, and naloxone were from Tocris Cookson Ltd. Naloxone methiodide was from Sigma. Nor-binaltorphimine was synthesized in Research Triangle Institute.

In control rats, the coronary occlusion and reperfusion were performed, but no preparations were administered. The results were statistically analyzed using $\chi^2$ test. The differences were significant at $p<0.025$.

## RESULTS

Coronary occlusion for10-min induced ventricular arrhythmias in 17 of 21 rats. Ventricular extrasystoles were observed in 81% rats. The incidence of ventricular tachycardia was also 81%, while ventricular fibrillation was observed in only 14% rats (Fig. 1). These rhythm abnormalities were reversible and eventuated in recovery of normal sinus rhythm or another arrhythmia (some rats demonstrated several types of ventricular arrhythmias). During reperfusion, all rats except one demonstrated cardiac rhythm disturbances (Fig. 2). During resumption of coronary blood flow, ventricular extrasystoles, ventricular tachycardia, and ventricular fibrillation were observed in 95%, 76%, and 24% rats, respectively. The examined pharmacological agents had no effect on the incidence of ventricular fibrillation.

Blockade of all types of OR with naloxone did not change the incidence of ventricular arrhythmias during coronary occlusion and reperfusion (Figs. 1, 2). Selective δ-OR blockade with ICI 174,864 or TIPP[ψ] produced no significant effect on the incidence of ventricular arrhythmias (Figs. 1, 2). Under conditions of selective blockade of μ- and δ-OR, the incidence of arrhythmias was somewhat higher than in the control, but these differences were insignificant. During selec-

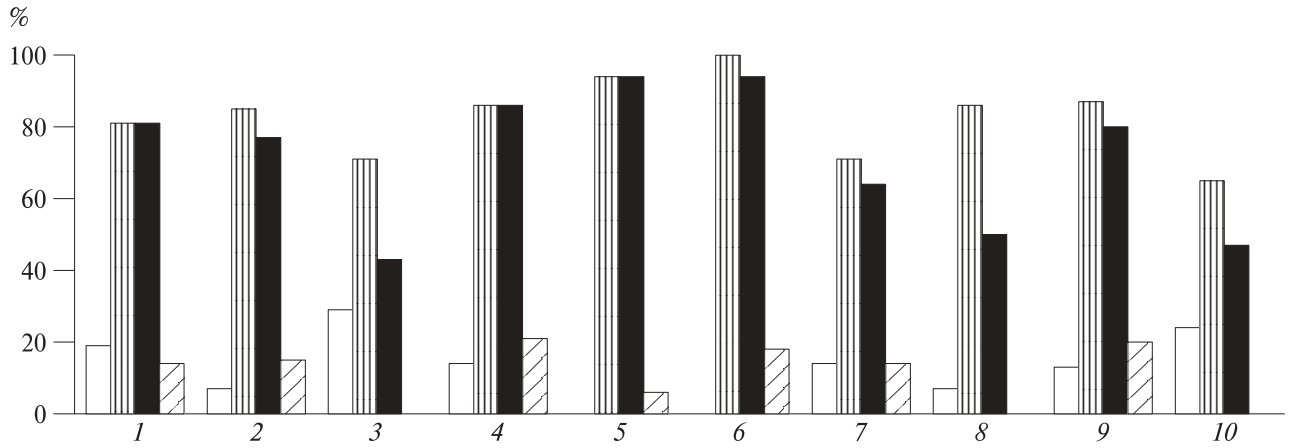


**Fig. 1**. Effect of opioid receptor blockers on the incidence of ischemic arrhythmias. Here and in Fig. 2: *1*) control; *2*) naloxone; *3*) naloxone methiodide; *4*) β-FNA; *5*) CTAP; *6*) ICI-174,864; *7*) BNTX; *8*) naltriben; *9*) nor-binaltorphimine; *10*) quadazocine. Light bars show cases without ventricular arrhythmia. Vertical hatching, solid bars, and skew hatching show cases with multiple ventricular extrasystoles, ventricular tachycardia, and ventricular fibrillation, respectively. The numbers of rats in the control group was 24; experimental groups comprised no less than 15 rats.

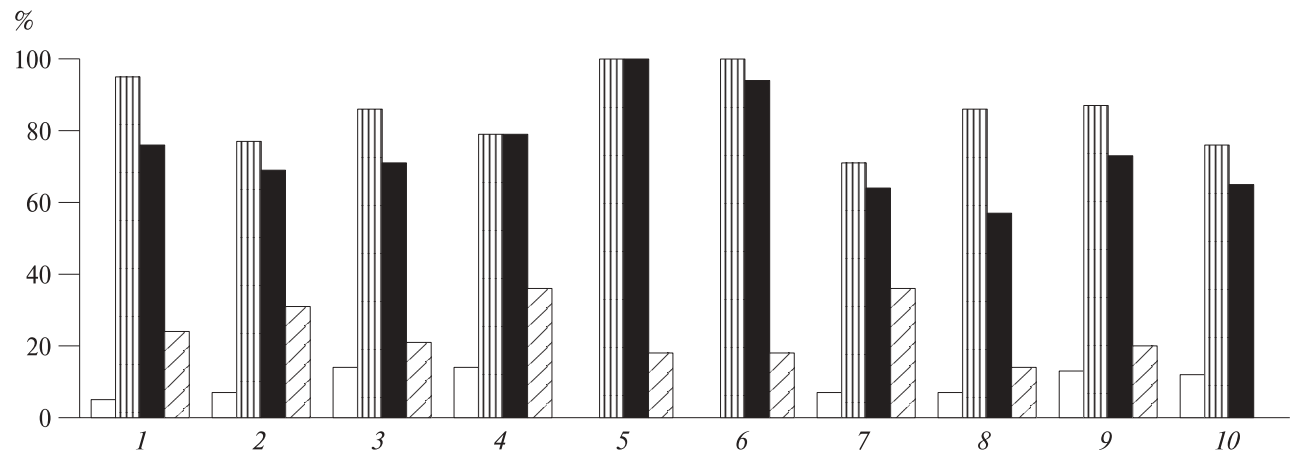


**Fig. 2**. Effect of opioid receptor blockers on the incidence of reperfusion arrhythmias.

tive blockade of peripheral and central $\delta_1$-OR with BNTX, the incidence of ventricular rhythm disturbances was the same as in the control (Figs. 1, 2). Naltriben also produced no effect on the incidence of ischemic and reperfusion arrhythmias. Blockade of κ-OR with nor-binaltorphimine did not modify heart resistance to the arrhythmogenic action of coronary occlusion and reperfusion.

Blockade of μ-, δ-, and κ-OR produced no significant effect on heart resistance to the arrhythmogenic action of 10-min coronary occlusion and 10-min reperfusion. However, some authors reported that blockade of OR improved heart resistance to the arrhythmogenic effect of ischemia and reperfusion [1]. By contrast to this work which used OR blockers in minimum doses, the antiarrhythmic effect of OR blockers were observed at very high doses [1], where the possibility of unspecific membrane-stabilizing action was very high. The antiarrhythmic effects were observed with L-naloxone, which inhibits OR, and its D-isomer, which cannot bind to these receptors [14]. The principal distinctive feature of our experiments is the use of short-term (10 min) coronary occlusion, which produced no irreversible damage to cardiomyocytes, while other researchers [1] used longer ischemia, which produced irreversible damage. Probably, the endogenous opioid peptides are involved in arrhythmogenesis at the later stages of ischemia (>20 min). Long-term (30-45 min) ischemia and the following reperfusion not only induce irreversible changes in ischemized cardiomyocytes, but also form the necrotic focus [8]. Published data corroborate our hypothesis. It is noteworthy that intravenous naloxone demonstrated no antiarrhythmic effects when used in doses of 1, 2, and 10 mg/kg before 20-min coronary occlusion in narcotized pigs [4]. Nalmefene, a non-selective OR antagonist, produced no antiarrhythmic effect in dogs subjected to 20-min-long coronary occlusion, but this effect was observed in experiments with longer ischemia [6]. In narcotized rats, the antiarrhythmic effect of naloxone was observed only during 30-min ischemia [7]. Naloxone exerts the antiarrhythmic effect only during so-called "1b phase", which corresponds to 11-30 min after coronary occlusion [7]. In this study, we used short-term ischemia corresponding to 1a phase [13]. According to previous reports [13], the key role in arrhythmogenesis during 1a phase is played by ischemic zone, where re-entry phenomenon is triggered. By contrast, rhythm abnormalities observed during 1b phase (10-30 min after the onset of coronary occlusion) have other electrophysiological origin [13]. In this case, the key role in arrhythmogenesis is played by peri-infarction zone, while arrhythmias appear as ectopic automaticity [13]. It can be hypothesized that the endogenous opioid system plays no significant role in arrhythmogenesis in 1a phase, but can be involved in the pathogenesis of arrhythmias during 1b phase.

The experiments carried out on adapted rats showed that adaptation significantly increased heart resistance to the arrhythmogenic effects of ischemia and reperfusion (Table 1). No adapted rat demonstrated the ischemia- or reperfusion-induced ventricular fibrillation. Only 3 of 13 rats had ventricular tachycardia, while the incidence of ventricular extrasystoles was almost 2-fold lower than in the control. In addition, 69% adapted rats were resistant to the arrhythmogenic effect of ischemia. Selective blockade of δ- and κ-OR did not significantly affect the antiarrhythmic effect of adaptation, although preliminary blockade of μ-OR completely abolished it. Thus, endogenous μ-OR agonists play an important role in the realization of this adaptation effect.

In conclusion, the endogenous opioid system does not affect arrhythmogenesis caused by short-term coronary occlusion and reperfusion in intact animals. However, endogenous μ-OR agonists can play an important role in the regulation of heart resistance to the arrhythmogenic effect of short-term

**TABLE 1**. Effect of Blockade of OR and ATP-Operated Mitochondrial $K^+$ Channels on Incidence of Ischemic and Reperfusion Arrhythmias in Stress-Adapted Rats

| Group | Ischemia | | | | | Reperfusion | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *n* | NVA | MVE | VT | VF | *n* | NVA | MVE | VT | VF |
| Control | 21 | 4 | 16 | 16 | 8 | 19 | 5 | 14 | 14 | 5 |
| | | 19% | 76% | 76% | 38% | | 26% | 74% | 74% | 26% |
| Adaptation | 13 | 9** | 4** | 1** | 0* | 17 | 4 | 6 | 3** | 0* |
| | | 69% | 30% | 8% | | | 23% | 46% | 18% | |
| Adaptation+CTAP | 17 | $3^+$ | $11^+$ | $7^+$ | 3 | 17 | 3 | $14^+$ | $11^+$ | $5^+$ |
| | | 18% | 76% | 53% | 18% | | 18% | 82% | 65% | 29% |
| Adaptation+TIPP[ψ] | 17 | 10 | 5 | 6 | 1 | 17 | 6 | 6 | $9^+$ | 1 |
| | | 59% | 29% | 35% | 6% | | 35% | 35% | 52% | 6% |
| Adaptation+nor-binaltorphimine | 16 | 7 | 7 | 2 | 1 | 16 | 4 | 12 | 5 | 2 |
| | | 44% | 44% | 13% | 6% | | 25% | 75% | 31% | 13% |

**Note.** NVA, no ventricular arrhythmia; MVE, multiple ventricular extrasystoles; VT, ventricular tachycardia; VF, ventricular fibrillation. *$p<0.05$, **$p<0.01$ compared to the control. $^+p<0.05$ compared to adapted rats.

ischemia and reperfusion in animals adapted to immobilizing stress.

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