Opioid Peptide Deltorphin II Simulates the Cardioprotective Effect of Ischemic Preconditioning: Role of δ_2-Opioid Receptors, Protein Kinase C, and K_{ATP} Channels

L. N. Maslov[1,2], E. I. Barzakh[3], A. V. Krylatov[1,2], G. A. Chernysheva[4], T. Krieg[5], N. V. Solenkova[6], A. Yu. Lishmanov[1], S. Yu. Cybulnikov[1,2], and Y. Zhang[7]

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The cardioprotective properties of a δ_2-opioid receptor agonist deltorphin II were studied in rats with coronary occlusion and reperfusion. Opioid receptor ligands and inhibitors (glybenclamide, chelerythrine, and 5-hydroxydecanoate) were injected intravenously before ischemia and reperfusion. A δ_2-opioid receptor agonist deltorphin II significantly decreased the infarction zone/risk zone index. This effect was abolished by naltrexone, naloxone methiodide, and δ_2-opioid receptor antagonist naltriben, but not by a δ_1-opioid receptor antagonist BNTX. The infarct-limiting effect of deltorphin II was not observed after inhibition of protein kinase C or blockade of mitochondrial K_{ATP} channels.

Key Words: *heart; ischemia/reperfusion; opioid receptors; protein kinase C; K_{ATP} channels*

δ_1- and κ-opioid receptors (OR) are involved in the cardioprotective effect of ischemic preconditioning. This phenomenon suggests an increase in cardiac resistance to prolonged ischemia after short-term ischemia session. The regulatory role of δ_2-OR in myocardial tolerance to ischemia/reperfusion injury was evaluated only in one study [10]. Intravenous injection of the opioid peptide deltorphin-D (tentative agonist of δ_2-OR) 45 min before coronary occlusion and subsequent reperfusion in pigs was shown to reduce the infarction zone/risk zone ratio (IZ/RZ; risk zone, myocardial region with blood supply through the infarct-related artery). However, these experiments did not involve the selective antagonists of OR [10]. It remains unclear which type of OR is responsible for the infarct-limiting effect of deltorphin-D. Moreover, there is no evidence that this peptide serves as a selective agonist of δ_2-OR.

This work was designed to evaluate whether selective δ_2-OR agonist deltorphin II can simulate the phenomenon of ischemic preconditioning. We also studied the signal and receptor nature of the protective action of deltorphin II.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 250-300 g. The animals were anesthetized by an intraperitoneal injection of α-chloralose in a dose of 50 mg/kg. Artificial pulmonary ventilation was maintained using a RO-6 device. The left femoral artery was cannulated to measure the mean blood pressure. Pharmacological agents, solution of cyclodextrin, and patent blue violet dye were administered into the right femoral vein. ECG was recorded during coro-

[1]Institute of Cardiology, Siberian Division of the Russian Academy of Medical Sciences, Tomsk; [2]Tomsk State Pedagogical University, Russia; [3]Jewish University, Jerusalem, Israel; [4]Institute of Pharmacology, Siberian Division of the Russian Academy of Medical Sciences, Tomsk, Russia; [5]University of Greifswald, Germany; [6]Vanderbilt University, Nashville, USA; [7]Anhui Medical University, Hefei, China. ***Address for correspondence:*** maslov@cardio.tsu.ru. L. N. Maslov

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nary occlusion. ECG recording in intact animals was conducted before injection of deltorphin II and 5, 10, or 15 min after treatment with the peptide. HR was estimated. *PQ*, *QRS*, *QT*, and *RR* intervals were measured. The *QT* interval corrected for HR (QT_c) was calculated as follows: $QT_c=QT-0.87\times(RR-1000)=QT-87\times(80/HR-1)$ [12].

Coronary occlusion, reperfusion, and staining of the myocardium were performed as described elsewhere [8]. Elevation of the *ST* segment was considered as a sign of myocardial ischemia and effective coronary occlusion. The duration of occlusion was 45 min. The ligature was removed during reperfusion. The duration of reperfusion was 2 h. By the end of this study, the coronary artery was repeatedly ligated. Patent blue violet (150 μl, 10% solution) was injected intravenously. Injection of this dye was followed by myocardial staining in the zone of normal perfusion (blue color). RZ remained unstained under these conditions. The heart was excised. The left ventricle was separated from other tissues and cut into 5 transverse sections. This procedure allowed us to evaluate the myocardial region with normal perfusion (blue-colored area). Unstained RZ was verified. RZ was separated from the nonischemic myocardium, maintained in 0.1% nitro blue tetrazolium in 100 mM phosphate buffered saline (pH 7.4), and incubated at 37°C. The zone of myocardial infarction was separated from other regions of RZ. The size of IZ and RZ was estimated gravimetrically. The size of IZ was expressed as a percentage of RZ (IZ/RZ ratio).

The animals with coronary occlusion were divided into the following four groups: group 1, administration of cyclodextrin (control animals); group 2, administration of deltorphin II; group 3, administration of OR antagonists or inhibitors (chelerythrine, glybenclamide, and 5-hydroxydecanoate); and group 4, administration of OR antagonists or inhibitors before injection of deltorphin II. Selective δ_2-OR agonist deltorphin II [6] was injected intravenously 15 min before ischemia. OR antagonists were administered 25 min before coronary occlusion. The doses of opioid peptides were selected from the results of our previous experiments with OR agonist dalargin. Dalargin in a dose of 150 nmol/kg prevented the development of fibrillation under conditions of coronary occlusion [4]. Nonselective OR antagonist naltrexone was used in a dose of 5 mg/kg. The dose of nonselective OR antagonist naloxone methiodide was 5 mg/kg. Published data show that this agent does not cross the blood-brain barrier [5]. Selective δ_1-opioid receptor antagonist BNTX was administered in a dose of 0.7 mg/kg [11]. The dose of selective δ_2-OR antagonist naltriben was 0.3 mg/kg [3]. The inhibitor of sarcolemmal and mitochondrial ATP-sensitive potassium channels (K_{ATP} channels), glybenclamide, in a dose of 0.3 mg/kg was injected intravenously 45 min before coronary occlusion [8]. The selective inhibitor of mitochondrial K_{ATP} channels, 5-hydroxydecanoate, in a dose of 5 mg/kg was administered 5 min before ischemia [1]. Protein kinase C inhibitor in a dose of 5 mg/kg was administered 25 min before coronary occlusion [2].

Deltorphin II, naltrexone, naloxone methiodide, chelerythrine, and 5-hydroxydecanoate were dissolved in 0.9% NaCl. Chelerythrine is poorly soluble in water at room temperature. Hence, this compound was dissolved in hot physiological saline (55°C) and then cooled to 37°C. Glybenclamide, naltriben, and BNTX were subsequently dissolved in 0.1 ml DMSO and 1 ml 20% hydroxypropyl-β-cyclodextrin. The solutions were prepared *ex tempore*.

Deltorphin II was synthesized at the Multiple Peptide Systems Company. Naltrexone, naloxone methiodide, glybenclamide, 5-hydroxydecanoate, patent blue violet, nitro blue tetrazolium, and α-chloralose were manufactured by Sigma-Aldrich Corporation. Naltriben, BNTX, and hydroxypropyl-β-cyclodextrin were manufactured by Tocris Cookson. Chelerythrine was manufactured by LC Laboratories.

The significance of between-group differences was evaluated by Student's *t* test and Mann–Whitney *U* test.

RESULTS

The mean blood pressure under control conditions (before coronary occlusion) was 106±2 mm Hg. The mean blood pressure decreased 45 min after ischemia (87 mm Hg) and remained low by the end of 2-h perfusion. The basal level of HR was 345±11 bpm. Coronary occlusion and reperfusion had little effect on HR. Intravenous injection of deltorphin II to intact rats had no effect on blood pressure and *PQ*, *QRS*, and *RR* intervals. However, *QT* and QT_c intervals were reduced 5 min after injection of this opioid (by 11 and 9%, respectively; $p<0.001$). Other pharmacological agents had little effect on HR and blood pressure. The decrease in the QT_c interval serves as a sign for accelerated cardiomyocyte repolarization, which results from activation of K^+ fluxes.

No between-group differences were found in the RZ/left ventricular weight ratio. Selective δ_2-OR agonist deltorphin II (0.12 mg/kg) possessed cardioprotective properties. Pretreatment with this peptide was followed by a 27% decrease in the area of infarction (Fig. 1, *a*). Naltrexone (5 mg/kg), naloxone methiodide (5 mg/kg), naltriben (0.3 mg/kg), and BNTX (0.7 mg/kg) did not modify the IZ/RZ ratio (data not shown in Fig. 1). Preliminary administration of naltrexone or inhibition of peripheral OR with naloxone methiodide complete-

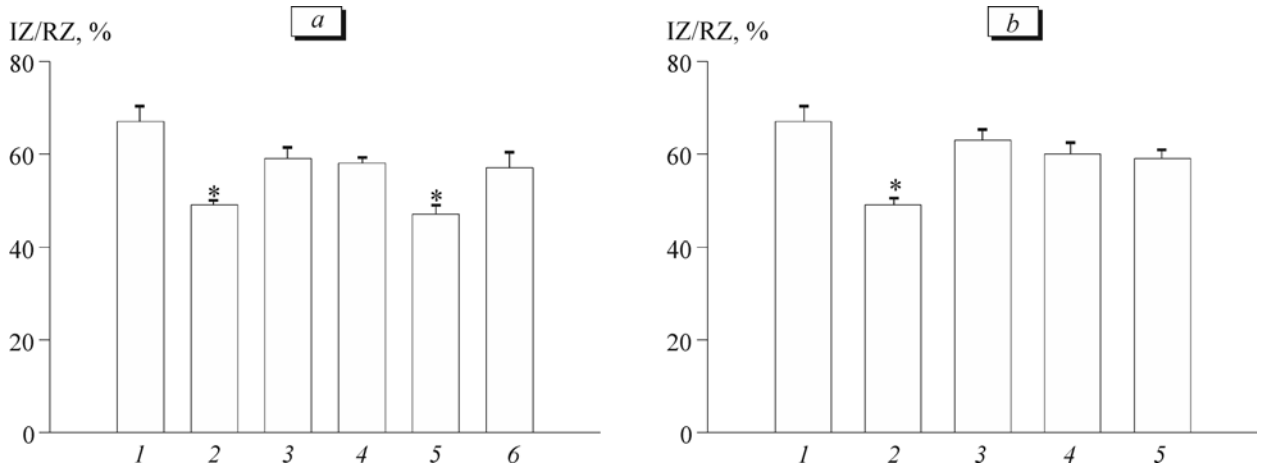

Fig. 1. Effect of OR antagonists (*a*) and inhibitors (*b*) on the cardioprotective effect of deltorphin II. (*a*) Control (*1*); deltorphin II (*2*); naltrexone+deltorphin II (*3*); naloxone methiodide+deltorphin II (*4*); BNTX+deltorphin II (*5*); naltriben+deltorphin II (*6*). (*b*) Control (*1*); deltorphin II (*2*); chelerythrine+deltorphin II (*3*); glybenclamide+deltorphin II (*4*); 5-hydroxydecanoate+deltorphin II (*5*). *$p<0.01$ compared to the control.

ly abolished the cardioprotective effect of deltorphin II. Pretreatment with δ_2-OR antagonist also abolished the infarct-limiting effect of δ_2-OR agonist (Fig. 1, *b*). Blockade of δ_1-OR with BNTX did not modify the cardioprotective effect of deltorphin II. These data indicate that the cardioprotective effect of deltorphin II is associated with activation of peripheral δ_2-OR.

Selective protein kinase C inhibitor chelerythrine completely abolished the cardioprotective effect of deltorphin II (Fig. 1, *b*). The cardioprotective effect of deltorphin II was not observed after administration of glybenclamide (nonselective inhibitor of K_{ATP} channels). Selective inhibitor of mitochondrial K_{ATP} channels, 5-hydroxydecanoate, also abolished the infarct-limiting effect of δ_2-OR agonist. Individual treatment with chelerythrine, glybenclamide, or 5-hydroxydecanoate had no effect on the IZ/RZ ratio (data not shown in Fig. 1). Therefore, protein kinase C and mitochondrial K_{ATP} channels play an important role in the infarct-limiting effect of deltorphin II. These data are consistent with the results of previous experiments. The cardioprotective effect of opioid peptides was shown to be associated with activation of protein kinase C and opening of mitochondrial K_{ATP} channels [1,7,9]. Moreover, our previous studies revealed an increase in K^+ flux. This conclusion was derived from shortening of the QT_c interval.

We conclude that deltorphin II simulates the cardioprotective effect of ischemic preconditioning due to activation of peripheral δ_2-OR. This agent *in vivo* induces an increase in cardiac resistance to the pathogenic influence of ischemia/reperfusion. This effect depends on activation of protein kinase C and mitochondrial K_{ATP} channels. Endogenous agonists of OR are not involved in the regulation of cardiac tolerance to the pathogenic influence of *in vivo* ischemia/reperfusion in non-adapted rats.

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