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Activation of peripheral δ2-opioid receptor prevents reperfusion heart injury

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Abstract

Coronary artery occlusion (45 min) and reperfusion (2 h) was performed in rats anesthetized with α-chloralose. Opioid receptor agonists were administered intravenously 5 minutes before reperfusion, while opioid receptor antagonists were administered 10 minutes before reperfusion. The non-selective opioid δ-receptor agonist DADLE at a dose of 0.088 mg/kg had no effect the infarct size/area at risk ratio. The selective opioid δ-receptor agonist BW373 was administered at a dose of 1 mg/kg. This opioid at a dose of 1 mg/kg reduced infarct size. The selective opioid δ₁-receptor agonist DPDPE at a dose of 0.1 mg/kg and 0.969 mg/kg did not affect infarct size. The selective opioid δ₂-receptor agonist deltorphin II at a dose of 0.12 mg/kg reduced infarct size by one half. The opioid δ-receptor agonist p-Cl-Phe-DPDPE was administered at a dose of 0.105 mg/kg and 1.02 mg/kg. This opioid at a dose of 1.02 mg/kg reduced infarct size. The universal opioid receptor antagonists, naltrexone and naloxone methiodide acting on peripheral opioid receptor, as well as the selective opioid δ-receptor antagonist TIIP[ψ], the selective opioid δ₂-receptor antagonist naltriben eliminated the infarct limiting effect of deltorphin II. The selective opioid κ receptor antagonist nor-binaltorphimine, the selective opioid μ receptor antagonist CTAP, and the selective opioid δ₁-receptor antagonist BNTX did not abolish the protective effect of deltorphin II. Deltorphin II exhibited the most pronounced cardioprotective effect during reperfusion. These studies clearly indicate that the activation of opioid δ₂-receptor located in cardiomyocytes increases the resistance of the heart to reperfusion injury.

Keywords: heart; reperfusion; opioids; opioid δ-receptor

1. Introduction
Over the past 30 years, the mortality from acute myocardial infarction (AMI) has been steadily declining in the world due to the widespread use of thrombolysis and coronary angioplasty (Ottani et al., 2018). Currently, the mortality from AMI is 3.8% - 7.5% (Hwang et al., 2018; Fabris et al., 2017). The restoration of coronary blood flow leads to reperfusion damage of the heart, which, could contribute up to 50% of the total infarct size (Ndrepepa et al., 2017).

The primary reason for the high mortality in AMI can be attributed to the lack of drugs that can prevent reperfusion damage of the heart with high efficiency (Maslov and Barbarash, 2018). Therefore, there is an urgent need to develop drugs that could selectively increase the heart’s tolerance to reperfusion. This is particularly important if the drugs can work when added during reperfusion phase. Opioid δ-receptors agonists can serve as a prototype for the creation of such drugs. In 2004, it was documented that the non-selective opioid receptor agonist morphine and the selective opioid δ-receptor agonist BW373 could reduce reperfusion injury of the heart when ischemic damage had already occurred (Gross et al., 2004). Gross’s group had previously shown that activation of opioid δ1-receptor before ischemia increases cardiac tolerance to ischemia/reperfusion (Huh et al., 2001). In 2009, we found that stimulation of opioid δ2-receptor by deltorphin II before ischemia contributes to a decrease in infarct size during coronary occlusion and reperfusion (Maslov et al., 2009). However, it still remained unclear which opioid δ-receptor subtype(s) can provide cardiac resistance to reperfusion. It has not been clarified whether these receptors are located in the heart or other organs, in the central nervous system, or in peripheral circulation. It is known that stimulation of central opioid receptor by morphine increases the resistance of the heart to ischemia/reperfusion (Zhang et al., 2011). Consequently, one cannot rule out that activation of central opioid δ-receptors provides an increase in cardiac resistance to reperfusion.
Objective: to conduct a comparative analysis of infarct reducing activity of opioid δ-receptor agonists and evaluate the role of opioid δ2-receptor in the regulation of the heart’s tolerance to reperfusion.

2. Methods

2.1. Study Design

The study was carried out on male Wistar rats weighing 250–300 g. The rats were housed at 23 ± 1°C with a relative humidity of 60–70% and a 12-h light/dark cycle with free access to water and standard rat chow. All procedures conformed to the Directive 2010/63/EU of the European Parliament and the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Ethical approval was granted by the Ethical Committee of Cardiology Research Institute, Tomsk National Research Medical Center.

Animals were anesthetized with chloralose (60 mg/kg) intraperitoneally. The duration of coronary artery occlusion was 45 min and the duration of reperfusion was 120 min. All opioid δ-receptor agonists were administered intravenously 5 min before reperfusion. All opioid receptor antagonists were injected 10 min before reperfusion. The selective opioid δ2 receptor agonist deltorphin II (H-Tyr-D-Ala-Phe-Glu-Val-Val-Gly-NH2) was administered at a dose of 0.120 mg/kg (153 nmol/kg) (Maslov et al., 2009). The non-selective opioid δ-receptor agonist DADLE (H-Tyr-D-Ala-Gly-Phe-D-Leu-OH) was used at a dose of 0.088 mg/kg (154 nmol/kg). The selective opioid δ-receptor agonist BW373U86 was injected at a dose of 1 mg/kg (2293 nmol/kg) (Gross et al., 2004) and at a dose of 0.1 mg/kg (229 nmol/kg). The selective opioid δ-receptor agonist p-Cl-Phe-DPDPE (H-Tyr-c[Pen-Gly-4-Cl-Phe-D-Pen]-OH) was administered at a dose of 0.105 mg/kg (154 nmol/kg) and 1.02 mg/kg (1500 nmol/kg). The selective opioid δ1-receptor agonist DPDPE (H-Tyr-c[Pen-Gly-Phe-D-Pen]-OH) was administered at a dose of 0.1 mg/kg (154 nmol/kg) and 0.969 mg/kg (1500 nmol/kg).
Naltrexone was administered at a dose of 5 mg/kg (Maslov et al., 2009). The non-selective opioid receptor antagonist naloxone methiodide which does not penetrate through the blood brain barrier was used at a dose of 5 mg/kg (Maslov et al., 2009). The selective opioid µ-receptor antagonist CTAP (NH$_2$-D-Phe-c[Thr-Tyr-D-Trp-Arg-Thr-L-Pen]-Thr-NH$_2$) was used at a dose of 0.1 mg/kg (Maslov et al., 2013). The selective opioid δ-receptor antagonist TIPP[ψ] (H-Tyr-Ticψ[CH$_2$NH]Phe-Phe-OH) was administered at a dose of 0.5 mg/kg (Maslov et al., 2013). The selective opioid κ-receptor antagonist nor-binaltorphimine was used at a dose of 2 mg/kg (Guo et al., 2011). The selective opioid δ$_1$-receptor antagonist BNTX was injected at a dose of 0.7 mg/kg (Maslov et al., 2009). The selective opioid δ$_2$-receptor antagonist naltriben mesylate was used at a dose of 0.3 mg/kg (Maslov et al., 2009).

We received all peptide opioid receptor ligands from the National Institute on Drug Abuse, NIH (Bethesda, USA). All peptides were synthesized in PolyPeptide Laboratories (San Diego, USA). We purchased BW373U86 from Tocris Bioscience (Bristol, UK). We purchased naltrexone and naloxone methiodide from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Experimental model of coronary artery occlusion and reperfusion

The rats were anesthetized intraperitoneally with α-chloralose (60 mg/kg). After tracheotomy, lungs were ventilated by SAR-830 Series device (Central Wisconsin Engineers Inc., Schofeld, USA) with room air. Atelectasis was prevented by maintaining a positive end-expiratory pressure of 5–10 mm H$_2$O. Arterial pH, PCO$_2$, and PO$_2$ were monitored throughout the experiment by a blood gas analyzer (Stat Profile M, Nova Biomedical Corporation, Waltham, MA, USA) and maintained within a normal physiological range by adjusting the ribespiratory rate and/or tidal volume. Body temperature was maintained at 37°C by a heating pad PhysioSuite (non-invasive monitoring system for mice and rats, Kent Scientific Corporation, Torrington, USA). The left femoral artery was cannulated for blood pressure, heart rate, and blood gases measurements. Blood
pressure and standard peripheral lead electrocardiogram (ECG) recordings were performed with a MP35 apparatus (Biopac Systems, Inc., Goleta, CA, USA) and a computer using the BSL PRO 3.7.3 software (Biopac Systems Inc., Goleta, CA, USA). The right femoral vein was cannulated for administration of pharmacological agents or vehicles. Regional myocardial ischemia/reperfusion was induced as described by Neckar et al. (2002). Left thoracotomy was performed after 10-min stabilization, regional myocardial ischemia was induced by tightening a ligature (6-0 Prolene) placed around the left anterior descending coronary artery near its origin. Characteristic changes in the configuration of the ECG and transient decrease in blood pressure verified the coronary artery occlusion. After a 45-min occlusion period, the ligature was released and reperfusion of previously ischemic tissue continued.

2.3. Infarct size determination

At the end of a 2-h reperfusion, the hearts were excised and perfused with saline through the cannulated aorta. The area at risk and the infarct size were delineated by staining with 5% potassium permanganate and 1% 2,3,5-triphenyltetrazolium chloride, respectively (Neckar et al., 2002). The right ventricle was separated and the left ventricle was cut perpendicularly to its long axis into slices of 1 mm thick and stored overnight in 10% neutral formaldehyde solution. The infarct size, the size of the area at risk, and the size of the left ventricle were determined by a planimetric method using Scanjet G4050 scanner (Hewlett-Packard, Palo Alto, USA). The IS was normalized to the area at risk and the size of the area at risk was normalized to the left ventricle (area at risk/left ventricle).

2.4. Incidence of arrhythmias

Ventricular arrhythmias were recorded from the ECG signal during a 45-min coronary artery occlusion and during the first 10 min of reperfusion. The incidences of single premature ventricular complexes including salvos, ventricular tachycardia and ventricular fibrillation were evaluated separately.
2.5. The protocol of a study of isolated cardiomyocytes

Isolation of ventricular cardiomyocytes and induction of anoxia/reoxygenation were carried out as described earlier (Naryzhnaya et al., 2019). The animals were heparinized (1500 IU, intraperitoneally) and sacrificed by cervical dislocation. After sternotomy, hearts were quickly excised and placed in the Tyrode buffer (4°C) until stopped. The aorta was cannulated and fixed for retrograde (Langendorff) perfusion. The perfusion rate was 10 ml/min, constant temperature was 37°C, and all solutions were pre-saturated with 100 % O₂. Hearts were perfused for 3 min with Tyrode buffer (mmol/l): 140 NaCl, 5.4 KCl, 1 Na₂HPO₄, 1 MgCl₂.6H₂O, 10 glucose, 5 HEPES, 1 CaCl₂, pH 7.4. This was followed by perfusion with calcium-free Tyrode buffer for 3 min. Subsequent perfusion was performed with solution containing (mM/l): 140 NaCl, 5.4 KCl, 1 Na₂HPO₄, 1 MgCl₂.6H₂O, 10 glucose, 5 HEPES, 1.6 g/l fatty acid free BSA, collagenase type II 335 U/ml (Worthington) and XIV protease 0.230 g/l (Sigma) for 15-25 min until myocardial softening. Hearts were perfused with calcium-free Tyrode solution for 4 min for collagenase washout. The ventricular myocardium was excised from the aorta and dispersed by stirring in 10 ml of calcium-free Tyrode buffer containing 10 mg/ml fatty acid free BSA. Resulting cell suspension was filtered through cheesecloth and precipitated at room temperature for 5 min. The supernatant was removed, settled cardiomyocytes were diluted with calcium-free Tyrode buffer to 350-400 thousand cells in 1 ml. The isolated cells were incubated for 1 h at a temperature of 28°C under the 5% CO₂ flow in the MCO-5AC CO₂ incubator (SANYO, Japan) for stabilization.

After incubation, cell survival was monitored by staining with trypan blue. A percentage of the dead (stained) cells and viable rod-shaped cardiomyocytes (with the ratio of length-to-width not less than 3:1) were counted. In each sample, about 200 cells in total were analyzed in non-overlapping visual fields using light microscopy at × 100 magnification (Axio Observer Z1 microscope, Carl Zeiss Surgical GmbH, Germany). The initial survival rate of 50 % or more of rod-shaped cardiomyocytes was considered suitable for a study. In the incubation medium of the
cells, activity of the marker for cardiomyocyte necrosis, lactate dehydrogenase, was determined after anoxia and after reoxygenation using Fluitest lactate dehydrogenase-L kit (Analytical biotechnologies AG, Germany) and a spectrophotometer Infinite 200M (Tekan, Austria). The yield of lactate dehydrogenase was calculated as a percentage of total lactate dehydrogenase activity, which was measured by lysis of cardiomyocytes. For lysis, the cells were incubated for 45 min at 37°C with Triton X-100 at a concentration of 12 μl/ml, centrifuged for 1 min at 10,000 g, and total lactate dehydrogenase activity was measured in the supernatant as described above.

The cells of each group were divided into two sub-groups prior to induction of anoxia. One of them was incubated in a modified Krebs buffer (anoxic buffer) containing (in mmol/l): 118 NaCl, 25 NaHCO₃, 4.7 KCl, 1.2 MgSO₄, 1.2 KH₂PO₄, 5 2-deoxyglucose, pH 7.4. To prevent access of oxygen, the surface of suspension was layered with 5 - 6 drops of mineral oil (Naryzhnaya et al., 2019). The cells were subjected to anoxia for 20 min at room temperature. After termination of anoxia, the cells were carefully pipetted through oil and centrifuged for 1 min at 800 g. The supernatant was carefully removed and used to determine the lactate dehydrogenase concentration. Reoxygenation of cardiomyocytes was carried out by placing them in calcium-free Tyrode buffer for 30 min. At the end of reoxygenation, cell survival was monitored and the lactate dehydrogenase release was measured as described above. Corresponding sub-groups of the cells were resuspended in calcium-free Krebs buffer containing (mmol/l): 118 NaCl, 25 NaHCO₃, 11 glucose, 4.7 KCl, 1.2 MgSO₄, 1.2 KH₂PO₄; pH 7.4 for 20 min at room temperature, then cells were centrifuged for 1 min at 800 g, the supernatant was removed and used to determine lactate dehydrogenase, and the cells were incubated in calcium-free Tyrode buffer for 30 min. These sub-groups were used as controls for each group of cells subjected to anoxia/reoxygenation. Cell death during anoxia/reoxygenation was expressed as a percentage of controls not exposed to anoxia/reoxygenation.

2.6. Opioid receptor ligands in vitro
One of following opioid receptor ligands was added to the medium 10 min before the onset of reoxygenation and at the beginning of reoxygenation, opioid receptor agonist was added to the medium 5 min before the onset of reoxygenation and after 5 min of reoxygenation in the final concentration: the selective opioid δ2-receptor agonist deltorphin-II (64 nM) (Tang et al., 1994), the non-selective opioid receptor antagonist naloxone (300 nM) (Naryzhnaya et al., 2019). Another groups of cells that were not treated with any opioid receptor ligands were used as controls.

2.7. Statistical analysis

Results were expressed as means ± SEM from indicated number of experiments. One-way analysis of variance with Newman Keuls post hoc test was used to detect differences in parametric variables among groups. The Chi squared test was used to detect differences in the incidence of arrhythmias among groups. Differences were considered significant at p < 0.05.

3. Results

3.1. Hemodynamic data and ventricular arrhythmias

The coronary artery occlusion and reperfusion had no significant effect on the hemodynamic data (Table 1). Systolic blood pressure did not differ between the groups and their values at the end of ischemia and reperfusion remained relatively stable. None of the used opioid receptor ligands had any significant effect on the hemodynamic parameters (Table 1).

All rats exhibited premature ventricular complexes (PVCs) during a 45-min coronary artery occlusion. The incidence of ventricular tachycardia was 91% in untreated rats. The incidence of ventricular fibrillation was 33% in control rats. Only single PVCs were observed in some rats during reperfusion. The incidence of reperfusion PVCs was not significantly affected by neither opioid receptor agonists nor by the administration of opioid receptor antagonists (data not shown).

3.2. Infarct size
None of the opioid receptor ligands utilized in these studies had any effect on the area at risk/left ventricle ratio (data were not shown). None of the used opioid receptor antagonists had any effect on the infarct size/area at risk ratio (data were not shown). We found that the selective opioid δ2-receptor agonist deltorphin II when administered at a dose of 0.120 mg/kg (153 nmol/kg) decreased infarct size by 50% (Fig. 1 and Fig. 5). The non-selective opioid δ-receptor agonist DADLE administered at a dose of 0.088 mg/kg (154 nmol/kg) had no effect on infarct size. The opioid δ-receptor agonist BW373U86 at a dose of 1 mg/kg (2293 nmol/kg) decreased infarct size by 50% but had no effect at dose of 0.1 mg/kg (229 nmol/kg) (Fig. 1 and Fig. 5). The selective opioid δ-receptor agonist p-Cl-Phe-DPDPE administered at a dose of 0.105 mg/kg (154 nmol/kg) had no effect on infarct size. However, it reduced infarct size by 40% at a dose of 1.02 mg/kg (1500 nmol/kg) (Fig. 2 and Fig. 5). The selective opioid δ1-receptor agonist DPDPE did not elicit any cardioprotective properties at the doses of 0.1 mg/kg (154 nmol/kg) and 0.969 mg/kg (1500 nmol/kg). Our studies indicate that deltorphin II was most effective in preventing infarction during reperfusion. Consequently, further studies were performed utilizing this peptide.

Our studies also indicate that naltrexone completely abolished the cardioprotective effect of deltorphin II (Fig. 3). Naloxone methiodide which blocks only peripheral opioid receptors also eliminated the infarct reducing effect of deltorphin II (Fig. 3). The selective opioid δ-receptor antagonist TIPP[ψ] completely abolished the infarct limiting effect of deltorphin II (Fig. 4). The selective opioid δ2-receptor antagonist naltriben also eliminated the cardioprotective effect of deltorphin II (Fig. 4). The selective opioid μ-receptor antagonist CTAP, the selective opioid κ receptor antagonist nor-binaltorphimine, and the selective opioid δ1-receptor antagonist BNTX did not alter the infarct limiting effect of deltorphin II (Fig. 4).

3.3. Isolated cardiomyocytes
Deltorphin-II increased survival of isolated cardiomyocytes at concentration of 64 nmol/L and decreased the lactate dehydrogenase release from cardiomyocytes after anoxia/reoxygenation (Table 2). Naloxone completely abolished the cytoprotective effect of deltorphin-II.

4. Discussion

Our studies demonstrate that the opioid δ-receptor agonists had no effect on hemodynamic data. None of the tested opioid receptor antagonists had any effect on infarct size. This indicated that endogenous opioid receptor agonists are not involved in the regulation of cardiac tolerance to reperfusion in non-adapted rats. Moreover, these findings are consistent with the results of many other investigators who have shown that the opioid receptor antagonists do not affect hemodynamic parameters and infarct size during ischemia/reperfusion (Gross et al., 2004; Peart et al., 2004, 2008).

We also demonstrated that BW373U86 provides cardioprotection at a dose of 1 mg/kg (2290 nmol/kg). This result coincides with the data by Gross et al. (Gross et al., 2004), who showed that this opioid at a dose of 0.1 mg/kg prevents reperfusion injury of the heart. However, we found that this opioid had no effect at a dose of 0.1 mg/kg (229 nmol/kg).

The absence of any infarct limiting effect of the selective opioid δ1-receptor agonist DPDPE surprised us, since earlier studies by Gross’s group had shown that the selective non-peptide opioid δ1-receptor agonist TAN-67 at a dose of 10 mg/kg could elicit the the infarct reducing effect associated with opioid δ1-receptor activation (Fryer et al., 2001). These data were confirmed by other investigators (Zeng et al., 2011), who found that TAN-67 at dose of 10 mg/kg prevents ischemia/reperfusion injury of the heart via opioid δ1-receptor stimulation. The use of such a high dose by both groups of investigators was surprising because TAN-67 exhibits a high affinity for opioid δ-receptor (Knapp et al., 1995; Gross, 2003). We hypothesize that the infarct sparing effect of TAN-67 may involve the activation of central opioid δ1-receptor(s) because of the limited passage through the blood-brain barrier, therefore, such a high dose of the drug was required.
Moreover, we could not demonstrate any infarct reducing effect of the selective opioid δ₁-receptor agonist DPDPE even at high dose of 0.969 mg/kg (1500 nmol/kg). It has been previously documented that this opioid exhibited high affinity for δ-opioid receptor (Schiller et al., 1993). In this regard, it should be noted that most opioid peptides poorly penetrate the blood-brain barrier (Maslov, Lishmanov, 2017). We hypothesized that this peptide does not elicit any cardioprotective effect because it does not cross the blood-brain barrier. The selective opioid δ-receptor agonist p-Cl-Phe-DPDPE at a dose of 1.02 mg/kg increased cardiac tolerance to reperfusion. In contrast, the selective opioid δ₂-receptor agonist deltorphin II exhibited the infarct reducing effect at a dose of 0.120 mg/kg (153 nmol/kg). Our studies indicate that all other opioids tested required 10-fold higher doses to produce an infarct sparing effect. Our studies clearly indicate that peripheral opioid δ₂-receptor stimulation was required to initiate the cardioprotective effect of deltorphin II.

It has been previously documented that the affinity of deltorphin II for opioid δ-receptor is only 2-fold greater than DPDPE (Schiller et al., 1993). Deltorphin II has Kᵢ 6.43 nmol for opioid δ-receptor, DPDPE has Kᵢ 16.40 nmol for opioid δ-receptor (Schiller et al., 1993). Therefore, the difference between the cardioprotective effects of these two opioids cannot be explained solely by differences in receptor affinity. The opioid δ-receptor agonist BW373U8 has been documented to have an even greater affinity for opioid δ-receptors than deltorphin II (Gross, 2003). BW373U8 has Kᵢ 1.80 nmol for opioid δ-receptor and DPDPE has Kᵢ 14 nmol for opioid δ-receptor (Gross, 2003). However, a much lower dose of deltorphin II is required to limit infarct size. Affinity of p-Cl-Phe-DPDPE for opioid δ-receptor was 3-fold higher than that of the DPDPE (Toth et al., 1990). p-Cl-Phe-DPDPE has IC₅₀ 1.57 nmol for opioid δ-receptor and DPDPE has IC₅₀ 5.25 nmol for opioid δ-receptor (Toth et al., 1990). Accordingly, the p-Cl-Phe-DPDPE is superior to deltorphin II in receptor affinity. However, to provide infarct size reduction, the dose of p-Cl-Phe-DPDPE must be 10-fold higher than the dose of deltorphin II. We hypothesize that the main difference between these opioids may be in their different selectivity for the opioid δ₁-receptor and opioid δ₂-
receptor. While it is quite is possible that p-Cl-Phe-DPDPE and BW373U8 can interact with opioid δ2-receptor however their affinity for the opioid δ2-receptor was much less than that affinity of deltorphin II. To date no studies have been conducted to clarify which opioid δ-receptor subtypes are present on the sarcolemma of cardiomyocytes (See et al., 2018). We hypothesize cardiomyocytes have a predominance of opioid δ2-receptor, which accounts for our findings that deltorphin II has the most pronounced cardioprotective effect. Our data indicate that this opioid δ2-receptor is located in cardiomyocytes. We have previously demonstrated that intravenous administration of deltorphin-II before coronary artery occlusion promoted a decrease in infarct size via the activation of peripheral opioid δ2-receptor and stimulation of NO-synthase, protein kinase C and ATP-sensitive K\(^+\) channel opening (Maslov et al., 2009). Our preliminary data indicate protein kinase C and ATP-sensitive K\(^+\) channel were involved in the cardioprotective effect of deltorphin-II at reperfusion (unpublished date).

5. Conclusion

A comparative analysis of the infarct limiting activity of opioid agonists indicated that of the 5 opioid δ-receptor agonists (deltorphin II, DADLE, BW373U86, p-Cl-Phe-DPDPE, DPDPE) deltorphin II demonstrated the highest infarct reducing activity during reperfusion. These studies also indicate that the cardioprotective effect of deltorphin II was mediated by activation of peripheral opioid δ2-receptors which are localized in cardiomyocytes. It is our hypothesis that deltorphin II may be considered as a prototypical compound which could ultimately lead to creation of drug(s) which can effectively prevent or reduce reperfusion injury to the heart.

Declaration of competing interest

Authors declare that there is no conflict of interest.

Acknowledgment
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**Authors Contributions**

Leonid N. Maslov: Writing - Original Draft, Supervision, Project administration.

Alexander V. Mukhomedzyanov: Investigation, Visualization, Writing - Original Draft.

Sergey Y. Tsibulnikov: Investigation.

M.-Saadeh Suleiman - Original Draft.

Igor Khaliulin: Writing - Original Draft.

Peter R. Oeltgen: Writing - Original Draft.

**References**


Table 1: Hemodynamic data

<table>
<thead>
<tr>
<th>Hemodynamics</th>
<th>Dose</th>
<th>n</th>
<th>Baseline</th>
<th>45 min Ischemia</th>
<th>After 30 min Reperfusion</th>
<th>After 120 min Reperfusion</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Heart Rate</td>
<td>SBP</td>
<td>Heart Rate</td>
<td>SBP</td>
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<tr>
<td>Control</td>
<td></td>
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<td>365 ± 3</td>
<td>126 ± 3</td>
<td>361 ± 5</td>
<td>122 ± 4</td>
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<tr>
<td>Naltrexone</td>
<td>5 mg/kg</td>
<td>12</td>
<td>366 ± 4</td>
<td>123 ± 3</td>
<td>358 ± 3</td>
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<td>Naloxone methiodide</td>
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<td>362 ± 4</td>
<td>128 ± 2</td>
<td>357 ± 5</td>
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<td>CTAP</td>
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<td>123 ± 3</td>
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<td>TIPP[ψ]</td>
<td>0.5 mg/kg</td>
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<td>361 ± 5</td>
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<td>nor-BNI</td>
<td>2 mg/kg</td>
<td>12</td>
<td>366 ± 4</td>
<td>126 ± 3</td>
<td>360 ± 3</td>
<td>124 ± 3</td>
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<tr>
<td>Deltorphin II</td>
<td>0.12 mg/kg</td>
<td>12</td>
<td>365 ± 5</td>
<td>125 ± 4</td>
<td>361 ± 5</td>
<td>122 ± 4</td>
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<tr>
<td>DADLE</td>
<td>0.088 mg/kg</td>
<td>12</td>
<td>368 ± 6</td>
<td>121 ± 3</td>
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<td>BW373U86</td>
<td>0.1 mg/kg</td>
<td>12</td>
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<td>124 ± 2</td>
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<td>122 ± 5</td>
<td>361 ± 5</td>
<td>120 ± 3</td>
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<tr>
<td>DPDPE</td>
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<td>12</td>
<td>364 ± 3</td>
<td>127 ± 5</td>
<td>361 ± 4</td>
<td>125 ± 3</td>
</tr>
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Abbreviations: SBP, systolic blood pressure; n, number of rats; no significant differences were observed between groups at p<0.05.

<table>
<thead>
<tr>
<th>Group</th>
<th>Survival, %</th>
<th>LDH (U/l)</th>
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<tr>
<td>Control</td>
<td>37.4 ± 1.8</td>
<td>0.072 ± 0.006</td>
</tr>
<tr>
<td>Deltorphine II (64 nM)</td>
<td>47.3 ± 1.9*</td>
<td>0.045 ± 0.006*</td>
</tr>
<tr>
<td>Deltorphine II (64 nM) + Naloxone (300 nM)</td>
<td>29.7 ± 3.2#</td>
<td>0.068 ± 0.004#</td>
</tr>
</tbody>
</table>

Table 2: Survival of isolated cardiomyocytes and the LDH release after anoxia/reoxygenation

Abbreviations: *significantly (p<0.05) different from Control; # significantly (p<0.05) different from Deltorphine II (64nM).
**Figure 1.** Effect of opioid δ-receptor agonists on infarct size as percentage of the area at risk following 45 occlusion and 120 minutes reperfusion. Mean ± SEM. *P<0.05.

**Figure 2.** Effect of opioid δ-receptor agonists on infarct size as percentage of the area at risk following 45 occlusion and 120 minutes reperfusion. Mean ± SEM. *P<0.05.

**Figure 3.** An involvement of opioid receptors in cardioprotective effect of opioid δ2-receptor agonists Deltorphin II. Mean ± SEM. *P<0.05.
Figure 4. An involvement of opioid receptors in cardioprotective effect of opioid δ2-receptor agonists Deltorphin II. 1) control; 2) TIPP[ψ] (0,5 mg/kg) + Deltorphin (0,12 mg/kg); 3) Naltriben (0,3 mg/kg) + Deltorphin (0,12 mg/kg); 4) BNTX (0,7 mg/kg) + Deltorphin (0,12 mg/kg); 5) CTAP (0,1 mg/kg) + Deltorphin (0,12 mg/kg); 6) nor-binaltorphimine (nor-BNI, 2 mg/kg) + Deltorphin (0,12 mg/kg). Mean ± SEM. *P<0.05.

Figure 5. Effect of opioid δ-receptor agonists on infarct size following 45 occlusion and 120 minutes reperfusion.