Activation of Peripheral Opioid κ1 Receptor Prevents Cardiac Reperfusion Injury

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Short title: Activation of Peripheral κ1-Opioid Receptor

Summary: The role of opioid κ_1 and κ_2 receptors in reperfusion cardiac injury was studied. Male Wistar rats were subjected to a 45-min coronary artery occlusion followed by a 120-min reperfusion. Opioid κ receptor agonists were administered intravenously 5 min before the onset of reperfusion, while opioid receptor antagonists were given 10 min before reperfusion. The average value of the infarct size/area at risk (IS/AAR) ratio was 43 – 48% in untreated rats. Administration of the opioid κ_1 receptor agonist (-)-U-50,488 (1 mg/kg) limited the IS/AAR ratio by 42%. Administration of the opioid κ receptor agonist ICI 199,441 (0.1 mg/kg) limited the IS/AAR ratio by 41%. The non-selective opioid κ receptor agonist (+)-U-50,488 (1 mg/kg) with low affinity for opioid κ receptor, the peripherally acting opioid κ receptor agonist ICI 204,448 (4 mg/kg) and the selective opioid κ_2 receptor agonist GR89696 (0.1 mg/kg) had no effect on the IS/AAR ratio. Pretreatment with naltrexone, the peripherally acting opioid receptor antagonist naloxone methiodide, or the selective opioid κ receptor antagonist nor-binaltorphimine completely abolished the infarct-reducing effect of (-)-U-50,488 and ICI 199,441. Pretreatment with the selective opioid δ receptor antagonist TIPP[ψ] and the selective opioid μ receptor antagonist CTAP did not alter the infarct reducing effect of (-)-U-50,488 and ICI 199,441. Our study is the first to demonstrate the following: (a) the activation of opioid κ_2 receptor has no effect on cardiac tolerance to reperfusion; (b) peripheral opioid κ_1 receptor stimulation prevents reperfusion cardiac injury; (c) ICI 199,441 administration resulted in an infarct-reducing effect at reperfusion; (e) bradycardia induced by opioid κ receptor antagonists is not dependent on the occupancy of opioid κ receptor.

Keywords: heart; ischemia; reperfusion; opioid κ receptors.

Introduction

Despite the widespread use of coronary angioplasty, in-hospital mortality in acute myocardial infarction (AMI) is 3.8% - 7.5% (Hwang *et al.* 2018, Fabris *et al.* 2017). Two main reasons for this state of affairs are: rapid reperfusion injury of the heart and the lack of highly effective drugs that can significantly reduce infarct size after ischemic damage of the heart has already occurred (Maslov and Barbarash 2018). It is obvious that there is an urgent necessity for the creation of fundamentally new drugs which could effectively limit reperfusion cardiac injury. We propose that opioid receptor (OR) agonists could serve as a prototype for the creation of such drugs (Maslov *et al.* 2016). It was reported that pretreatment with the non-selective OR agonist morphine decreased infarct size in rats at a dose of 0.3 mg/kg (Schultz *et al.* 1997). There are clinical recommendations on the use of morphine in patients with AMI at a dose no more than 0.1 mg/kg (Maslov *et al.* 2016). Perhaps for this reason, morphine did not have the infarct limiting effect in patients with AMI (Eitel *et al.* 2020). Consequently, it seems appropriate to search for cardioprotective drugs among other opioids.

Endogenous κ OR agonists are involved in the cardioprotective effect of ischemic postconditioning (Guo *et al.* 2011). Gross's group has previously demonstrated that the κ_1-OR agonist U-50,488 can prevent reperfusion injury of the heart (Peart *et al.* 2008). It has been reported that acute (Maslov and Lishmanov, 1993) or chronic administration (Skrabalova *et al.* 2012) of opioids can prevents the appearance of I/R arrhythmias in rats. However, a comparative analysis of the infarct-reducing activity of κ OR agonists during reperfusion remains to be determined. The opioid κ receptor subtype which is associated with the infarct-limiting effect of κ-OR agonists during reperfusion with the activation has not yet been determined. It has also been documented that there are at least three subtypes of κ-OR: opioid κ_1 receptor, opioid κ_2 receptor, opioid κ_3 receptor (Rothman *et al.* 1992, Horan *et al.* 1993, Cheng *et al.* 1992). At this time, the precise anatomical location(s) of opioid κ receptors which enhance resistance of the heart to reperfusion has not been determined. A study, which was performed utilizing the isolated rat heart model, indicated that these receptors are localized in the heart (Peart *et al.* 2008). The presence of opioid κ receptor in isolated cardiomyocytes has been documented (Ventura *et al.* 1992). κ-ORs were detected in cardiac sarcolemma (Ventura *et al.* 1989). However, one cannot exclude the possibility that the infarct-reducing effect of U-50,488 may also require the activation of central opioid receptors, since intrathecal administration of morphine increases cardiac tolerance to ischemia (Wong *et al.* 2012).

Objective: to evaluate the role of opioid κ_1 and κ_2 receptors in the regulation of cardiac tolerance to reperfusion injury in vivo.

Methods

Study design

The male Wistar rats, weighing 250-300 g, were used in these experiments. The animals were placed at $23 \pm 1°C$ with a relative humidity of 60–70% and a 12-h light/dark cycle and they had free access to both water and standard rat chow. The procedures were governed by 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). The Ethical Committee of Cardiology Research Institute of the Tomsk National Research Medical Center granted the approval for these experiments.

Animals were anesthetized with chloralose (60 mg/kg) intraperitoneally. Coronary artery occlusion lasted 45 min and reperfusion was 120 min. Five min before reperfusion all OR agonists were injected intravenously. Meanwhile, all opioid antagonists were administered 10 min before reperfusion. In 1986, Sander et al. (Sander e*t al.* 1986) demonstrated that the cardiovascular effects of met-enkephalin in pentobarbital anesthetized dogs differ from the cardiovascular effects of met-enkephalin in conscious animals. However, the cardiovascular effects of met-enkephalin in conscious animals and chloralose anesthetized dogs were identical. Therefore, we used chloralose in this study and other investigations.

The selective opioid κ_1 receptor agonist (-)-U-50,488 was used at a dose of 0.1 mg/kg and 1 mg/kg (Peart e*t al.* 2008, Lishmanov e*t al.* 2007). The opioid κ_1 receptor agonist (+)-U-50,488 having low affinity for opioid κ_1 receptor was injected at a dose of 1 mg/kg. The opioid κ_2 receptor agonist GR89696 was administered at a dose of 0.1 mg/kg because it is known that it exhibits neuroprotective properties at a lower dose of 0.03 mg/kg (Birch e*t al.* 1991). The non-selective opioid κ receptor agonist ICI 199,441 was administered at a dose of 0.02 and 0.1 mg/kg. This compound produces an antinociceptive effect (ED_{50}) at a dose of 0.05 mg/kg subcutaneously (Barlow e*t al.* 1991). The peripherally acting opioid κ receptor agonist ICI 204,448 was administered at a dose of 4 mg/kg. It has also been documented that this compound has the infarct reducing effect at a dose of 0.3 mg/kg (Peart e*t al.* 2004).

Naltrexone was administered at a dose of 5 mg/kg (Maslov e*t al.* 2009). The non-selective OR antagonist naloxone methiodide, which does not penetrate through the blood brain barrier, was used at a dose of 5 mg/kg (Maslov e*t al.* 2009). The selective opioid μ receptor agonist CTAP (NH_2-D-Phe-c[Cys-Tyr-D-Trp-Arg-Thr-L-Pen]-Thr-NH_2) was used at a dose of 0.1 mg/kg (Maslov e*t al.* 2013). The selective opioid δ receptor agonist TIPP[ψ] (H-Tyr-Ticψ [CH_2NH]Phe-Phe-OH) was administered at a dose of 0.5 mg/kg (Maslov e*t al.* 2013). The peptides were donated by the National Institute on Drug Abuse NIH Catalog 2016. The selective κ receptor agonist nor-binaltorphimine was used at a dose of 2 mg/kg (Guo e*t al.* 2011). We received TIPP[ψ] and CTAP from the National Institute on Drug Abuse, NIH (Bethesda, USA). Both peptides were synthesized in PolyPeptide Laboratories (San Diego, USA). We purchased ICI 199,441, GR89696, (+)-U-50,488, (-)-U-50,488, ICI 204,448 from Tocris Bioscience (Bristol, UK). We purchased naltrexone and naloxone methiodide from Sigma-Aldrich (St. Louis, MO, USA). We injected all compounds intravenously in the volume of 1 ml/kg. All compounds were dissolved in isotonic saline.

Experimental model of coronary artery occlusion and reperfusion

The rats were anesthetized intraperitoneally with α-chloralose (60 mg/kg). After tracheotomy, lungs were ventilated with room air by means of a SAR-830 Series device (Central Wisconsin Engineers Inc., Schofield, USA). Atelectasis was prevented by maintaining a positive end-expiratory pressure of 5–10 mm H_2O. Throughout an experimental study, arterial pH, PCO_2, and PO_2 were determined utilizing a blood gas analyzer (Stat Profile M, Nova Biomedical Corporation, Waltham, MA, USA) and were kept within a normal physiological range by adjusting the respiratory rate and/or

tidal volume. Body temperature was controlled at 37°C via a PhysioSuite heating pad (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 done with the MP35 apparatus (Biopac Systems, Inc., Goleta, CA, USA) and a computer which used the BSL PRO 3.7.3 software (Biopac Systems Inc., Goleta, CA, USA). The right femoral vein was cannulated for the administration of pharmacological agents or vehicles. Regional myocardial ischemia/reperfusion was induced as described by Neckar et al. (Neckar e*t al.* 2002). Left thoracotomy was carried out and after 10-min stabilization, regional myocardial ischemia was induced by tightening a ligature (6-0 Prolene) that was placed around the left anterior descending coronary artery near its origin. Characteristic changes in the configuration of ECG and a transient decrease in blood pressure verified coronary artery occlusion. After a 45-min occlusion period, the ligature was released and reperfusion of previously ischemic tissue continued.

Infarct size determination

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

Incidence of arrhythmias

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

Statistical analysis

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

RESULTS

Hemodynamics and ventricular arrhythmias

Coronary artery occlusion and reperfusion had no significant effect on hemodynamics (Table 1a). Systolic blood pressure did not differ between the groups and their values at the end of ischemia and reperfusion remained relatively stable. None of OR antagonists had any significant effect on hemodynamic parameters (Table 1a). The opioid κ receptor agonists (-)-U-50,488 and ICI 199.441 induced bradycardia during reperfusion that was not eliminated by pretreatment with naltrexone, naloxone methiodide, nor-binaltorphimine (Table 1b).

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 during reperfusion were observed in some rats. The incidence of reperfusion PVCs was not significantly affected by neither OR agonists nor by administration of OR antagonists (data not shown).

Infarct size

None of OR ligands used in this study had any effect on the AAR/LV ratio (data were not shown). An attempt was made to find out if the opioid κ_1 receptor agonist (-)-U-50,488 would be able to prevent reperfusion injury of the heart. It was injected intravenously at a dose of 0.1 mg/kg 5 min before reperfusion. It did not affect the IS/AAR ratio at this dose. When a dose of (-)-U-50,488 was increased 10-fold to 1 mg/kg, it decreased the IS/AAR ratio by 42% (Fig. 1). The enantiomer of U-50,488, which had low affinity for κ-OR (+)-U-50,488 when administered at a dose of 1 mg/kg had no effect on IS/AAR ratio. Administration of the selective opioid κ_2 receptor agonist GR89696 at a dose of 0.1 mg/kg 5 min before reperfusion did not affect the IS/AAR ratio (Fig. 1). The opioid κ receptor agonist ICI 204,448, which does not cross the blood-brain barrier, did not affect the IS/AAR ratio when used at a dose of 4 mg/kg (Fig. 1). The non-selective opioid κ receptor agonist ICI 199.441 at a dose of 0.02 mg/kg also did not affect the IS/AAR ratio, but reduced the IS/AAR ratio by 41% when used at a dose of 0.1 mg/kg (Fig. 1).

In further studies, an attempt was made to determine a role of κ-ORs in the infarct-reducing effect of (-)-U-50,488 and ICI 199,441. It was also found that naltrexone (5 mg/kg) completely eliminated the cardioprotective effect of (-)-U-50,488 and ICI 199,441 (Fig. 2 and Fig. 3). It was found that naloxone methiodide (5 mg/kg), which did not cross the blood-brain barrier, completely eliminated the infarct-limiting effect of (-)-U-50,488 and ICI 199,441 (Fig. 2 and Fig. 3). The selective δ-OR antagonist TIPP[ψ] did not affect the infarct-sparing effect of (-)-U-50,488 and ICI 199,441 (Fig. 2 and Fig. 3). The selective μ-OR antagonist CTAP also had no affect the infarct-reducing effect of (-)-U-50,488 and ICI 199,441 (Fig. 2 and Fig. 3). The selective opioid κ receptor antagonist nor-binaltorphimine (2 mg/kg) eliminated the infarct-reducing effect of (-)-U-50,488 and ICI 199,441 (Fig. 2 and Fig. 3).

None of OR antagonists that were used alone affected hemodynamic parameters (Table 1a) and the IS/AAR ratio (Fig. 4).

Discussion

Our data indicate that (-)-U-50,488 and ICI 199,441 induced bradycardia which is not mediated via ORs. Consequently, this result indicates that both ligands can interact not only with opioid κ receptor but also with other unknown receptor (s) which have yet to be identified. It should be noted that Pugsley et al had previously documented that U-50,488-induced bradycardia was not mediated via ORs stimulation (Pugsley *et al*. 1992).

The ability of (-)-U-50,488 to limit infarct size and the absence of the infarct-limiting effect of (+)-U-50,488, which has low affinity for κ-OR, suggests that the cardioprotective effect of (-)-U-50,488 is mediated via OR activation. The absence of the infarct-reducing effect of the opioid κ_2 receptor agonist GR89696 indicates that κ_2-OR is not involved in the regulation of cardiac tolerance to reperfusion. We used GR89696 at a dose (0.1 mg/kg), which is 10-fold less than a dose of (-)-U-50,488. However, it is known that GR89696 has a neuroprotective effect at a dose of 0.03 mg/kg (Birch e*t al.* 1991). Consequently, it acts at a dose that is 3-fold less than the dose which was used. We were not able to detect any infarct-limiting effect of ICI 204,448 (4 mg/kg). Evidently this is due to low affinity of this opioid for κ-OR (Shaw e*t al.* 1989). However, ICI 199,441 resulted in an infarct-limiting effect at a dose of 0.1 mg/kg, which, apparently, is associated with its high affinity for κ-OR (Barlow e*t al.* 1991). It is 14-fold superior to U-50,488 in its affinity for κ-OR. Based upon the results of our study, (-)-U-50,488 at a dose of 0.1 mg/kg did not affect infarct size. According to Peart et al. (Peart *et al.* 2008) (-)-U-50,488 at a dose of 0.1 mg/kg reduced the IS/AAR ratio. The reason for this contradiction remains to be determined. The results of our study do not coincide with the data of Peart et al. (Peart *et al.* 2004) who found that ICI 204.448 at a dose of 0.3 mg/kg limits infarct size if it is administered before coronary artery occlusion. Our study indicates that ICI 204,448 does not affect infarct size at a dose of 4 mg/kg if administered before reperfusion. It is possible that this opioid prevents ischemic damage of the heart, but it does not affect reperfusion cardiac injury.

Our study using naloxone methiodide indicates that the infarct-sparing effect of ICI 199,441 and (-)-U-50,488 is associated with the activation of ORs that are localized outside the brain. These appear to be κ-OR which are localized in the heart because, according to Peart et al. (Peart *et al.* 2008)

U-50,488 increases resistance of the isolated heart to reperfusion. Since the selective δ-OR and μ-OR antagonists did not affect the infarct limiting effect of (-)-U-50,488 and ICI 199,441, it is arguable that this effect is independent of the activation of δ-OR and μ-OR. The selective opioid κ receptor antagonist nor-binaltorphimine eliminated the infarct-reducing effect of (-)-U-50,488 and ICI 199,441. This result suggests that the infarct-reducing effect of these opioids was associated with the activation of κ-OR. (-)-U-50,488 is the standard opioid κ_1 receptor agonist. The fact that its infarct reducing-effect disappears after blocking opioid κ receptor with nor-binaltorphimine and after blocking peripheral ORs with naloxone methiodide provided convincing evidence that activation of peripheral opioid κ_1 receptor increased cardiac tolerance to reperfusion.

It has previously been documented that pretreatment with (-)-U-50,488 elicits the antiarrhythmic effect during 10-min coronary artery occlusion and 10-min reperfusion (Lishmanov *et al.* 2007). However, the antiarrhythmic effect of (-)-U-50,488 during reperfusion after a 45-min ischemia could not be found. This may be attributed to the fact that after a 45-min ischemia only single PVCs are observed in some rats. Most of animals did not have reperfusion arrhythmias. Consequently, the antiarrhythmic effect of (-)-U-50,488 could not be detected. After 10-min of ischemia, the incidence of ventricular tachycardia was 90%. Therefore, this shorter ischemic time was the reason that the antiarrhythmic effect could be detected.

Conclusion

As described above, the infarct-limiting effect of the opioid κ_1 receptor agonist (-)-U-50,488 does not occur under κ-OR blockade by nor-binaltorphimine and after peripheral OR blockade by naloxone methiodide. The enantiomer U-50,488 with low affinity for opioid κ receptor (+)-U-50,488 does not affect infarct size. This confirms the κ-OR involvement in the cardioprotective effect of (-)-U-50,488. Our study using GR89696, suggests that opioid κ_2 receptor is not involved in the regulation of cardiac resistance to reperfusion. Therefore, we propose that the activation of peripheral opioid κ_1 receptor increases cardiac tolerance to reperfusion. Moreover, infusion of OR antagonists did not affect the IS/AAR ratio. Consequently, endogenous opioids do not affect cardiac tolerance to reperfusion in naive rats.

Acknowledgment

Conflict of Interest

The authors report no conflicts of interest.

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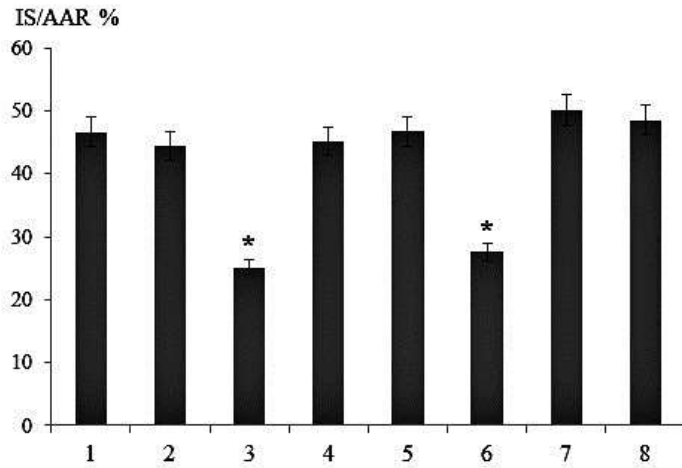
FIGURE LEGENDS

Fig. 1. The effect of κ opioid receptor agonists on infarct size (IS) as percentage of the area at risk (AAR) after a 45-min ischemia and a 120-min reperfusion. 1) control; 2) U-50,488 (0,1 mg/kg); 3) U-50,488 (1 mg/kg); 4) (+)-U-50,488 (1 mg/kg); 5) ICI-199,441 (0,02 mg/kg); 6) ICI-199,441 (0,1 mg/kg); 7) ICI-204,448 (4 mg/kg); 8) GR-89969 (0,1 mg/kg). Number of animals in each group was 12. Mean ± SEM. *P<0.05.
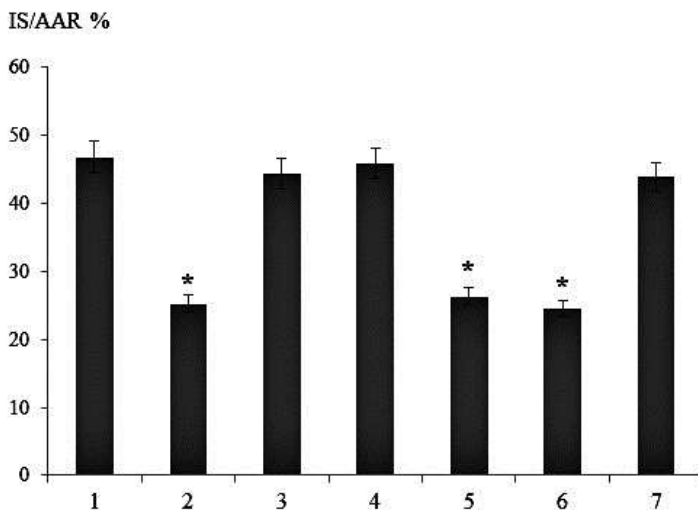

Fig. 2. The involvement of opioid receptors in the cardioprotective effect of κ_1-opioid receptor agonist U-50,488. The data is presented as the infarct size/area at risk ratio (IS/AAR):1) control; 2) U-50,488 (1 mg/kg); 3) Naltrexone (5 mg/kg) + U-50,488 (1 mg/kg); 4) Naloxone methiodide (5 mg/kg) + U-50,488 (1 mg/kg); 5) TIPP[ψ] (1 mg/kg) + U-50,488 (1 mg/kg); 6) CTAP (0.1 mg/kg) + U-50,488 (1 mg/kg); 7) nor-BNI (2 mg/kg) + U-50,488 (1 mg/kg). Number of animals in each group was 12. Mean ± SEM. *P<0.05.
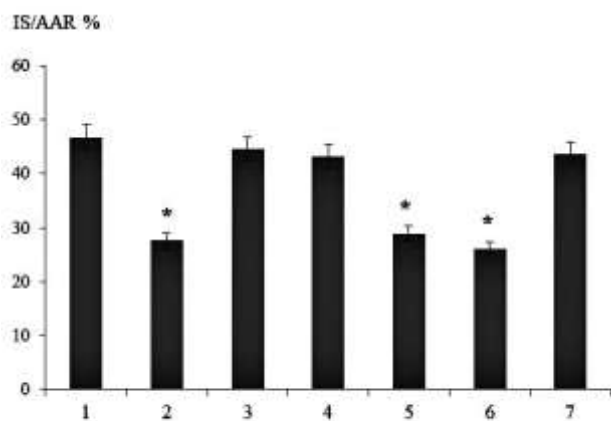
Fig. 3. The involvement of opioid receptors in the cardioprotective effect of κ-opioid receptor agonist ICI-199,441. The data is presented as the infarct size/area at risk ratio (IS/AAR): 1) control; 2) ICI-199,441 (0.1 mg/kg); 3) Naltrexone (5 mg/kg) + ICI-199,441 (0.1 mg/kg); 4) Naloxone methiodide (5 mg/kg) + ICI-199,441 (0.1 mg/kg); 5) TIPP[ψ] (1 mg/kg) + ICI-199,441 (0.1 mg/kg); 6) CTAP (0.1 mg/kg) + ICI-199,441 (0.1 mg/kg); 7) nor-BNI (2 mg/kg) + ICI-199,441 (0.1 mg/kg). Number of animals in each group was 12. Mean ± SEM. *$P<0.05$.
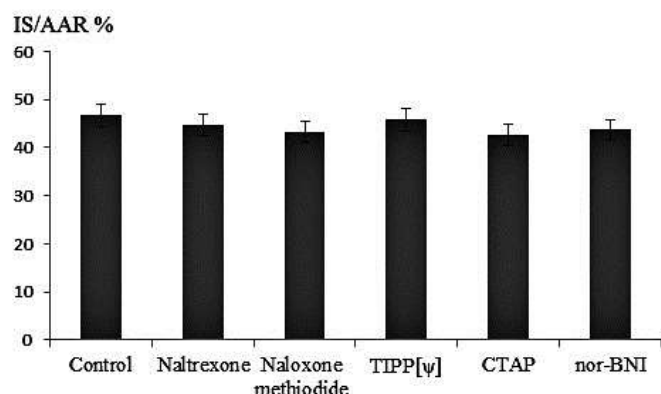

Fig. 4. The effect of opioid receptor antagonists on infarct size (IS) as percentage of the area at risk (AAR) after a 45-min ischemia and a 120-min reperfusion: 1) control; 2) Naltrexone (5 mg/kg); 4) Naloxone methiodide (5 mg/kg); 5) TIPP[ψ] (1 mg/kg); 6) CTAP (0.1 mg/kg); 7) nor-BNI (2 mg/kg). Number of animals in each group was 12. Mean ± SEM.

Table 1a. Hemodynamic Data

Hemodynamics	n	Baseline		45 min Ischemia		After 30 min Reperfusion		After 120 min Reperfusion	
		Heart Rate	SBP	Heart Rate	SBP	Heart Rate	SBP	Heart Rate	SBP
Control	12	364 ± 3	125 ± 2	356 ± 4	122 ± 3	352 ± 5	119 ± 4	342 ± 6	115 ± 4
Naltrexone	12	366 ± 4	123 ± 3	358 ± 3	120 ± 4	354 ± 4	117 ± 5	345 ± 5	113 ± 5
Naloxone methiodide	12	362 ± 4	128 ± 2	357 ± 5	125 ± 3	354 ± 2	123 ± 6	341 ± 7	118 ± 5
CTAP	12	364 ± 3	123 ± 3	358 ± 3	120 ± 4	352 ± 5	119 ± 4	342 ± 6	115 ± 4
TIPP[ψ]	12	361 ± 5	120 ± 4	355 ± 4	117 ± 3	351 ± 4	115 ± 5	339 ± 7	112 ± 5
nor-BNI	12	366 ± 4	126 ± 3	360 ± 3	124 ± 3	354 ± 4	121 ± 5	345 ± 5	118 ± 3
U-50,488 (0.1 mg/kg)	12	363 ± 2	121 ± 4	356 ± 5	118 ± 3	331 ± 4*	115 ± 2	321 ± 4*	112 ± 4
U-50,488 (1 mg/kg)	12	365 ± 4	124 ± 2	357 ± 3	122 ± 5	328 ± 3*	119 ± 4	318 ± 4*	116 ± 5
(+)-U-50,488	12	364 ± 5	121 ± 2	359 ± 4	119 ± 3	351 ± 5	116 ± 4	342 ± 5	111 ± 3
ICI-199,441 (0.02 mg/kg)	12	367 ± 3	126 ± 4	362 ± 3	124 ± 4	265 ± 5*	121 ± 3	250 ± 7*	117 ± 3
ICI-199,441 (0.1 mg/kg)	12	359 ± 4	122 ± 4	355 ± 3	120 ± 3	271 ± 4*	117 ± 3	258 ± 6*	114 ± 4
ICI-204,448	12	364 ± 4	125 ± 4	357 ± 5	122 ± 3	352 ± 4	120 ± 4	344 ± 2	116 ± 5
GR-89696	12	366 ± 5	127 ± 5	360 ± 4	125 ± 3	355 ± 3	123 ± 4	347 ± 4	119 ± 5

Values are means ± SEM; n, number of rats; SBP, systolic blood pressure; * $p < 0.05$. – vs control; It was used following compounds: Naltrexone (5 mg/kg); Naloxone methiodide (5 mg/kg); CTAP (0.1 mg/kg); TIPP[ψ] (0.5 mg/kg); nor-BNI (2 mg/kg); U-50,488 ((0.1 and 1 mg/kg); ICI-199,441 (0.02 and 0.1 mg/kg).

Table 1b. Hemodynamic Data

Hemodynamics	n	Baseline		45 min Ischemia		After 30 min Reperfusion		After 120 min Reperfusion	
		Heart Rate	SBP	Heart Rate	SBP	Heart Rate	SBP	Heart Rate	SBP
Control	12	364 ± 3	125 ± 2	356 ± 4	122 ± 3	352 ± 5	119 ± 4	342 ± 6	115 ± 4
Naltrexone (5 mg/kg) + U-50,488 (1 mg/kg)	12	363 ± 4	122 ± 3	356 ± 4	119 ± 3	321 ± 4*	116 ± 3	312 ± 5*	111 ± 4
Naloxone methiodide (5 mg/kg) + U-50,488 (1 mg/kg)	12	361 ± 4	120 ± 2	355 ± 3	117 ± 3	320 ± 5*	115 ± 3	309 ± 5*	112 ± 5
nor-BNI (2 mg/kg) + U-50,488 (1 mg/kg)	12	365 ± 5	125 ± 4	360 ± 4	122 ± 3	324 ± 4*	118 ± 4	315 ± 5*	113 ± 3
Naltrexone (5 mg/kg) + ICI-199,441 (0.1 mg/kg)	12	363 ± 4	121 ± 3	357 ± 5	118 ± 3	269 ± 4*	115 ± 3	255 ± 4*	111 ± 5
Naloxone methiodide (5 mg/kg)+ ICI-199,441 (0.1 mg/kg)	12	367 ± 3	126 ± 4	362 ± 4	123 ± 3	275 ± 5*	120 ± 3	262 ± 5*	114 ± 3
nor-BNI (2 mg/kg) + ICI-199,441 (0.1 mg/kg)	12	362 ± 4	123 ± 3	356 ± 4	120 ± 3	271 ± 4*	116 ± 3	258 ± 4*	111 ± 4

Values are means ± SEM; n, number of rats; SBP, systolic blood pressure; * $p < 0.05$. – vs control; It was used following compounds: Naltrexone (5 mg/kg); Naloxone methiodide (5 mg/kg); nor-BNI (2 mg/kg); U-50,488 (1 mg/kg); ICI-199,441 (0.1 mg/kg).