

Role of central and peripheral opioid receptors in the cardioprotection of intravenous morphine preconditioning

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Abstract

Background Both central and peripheral opioid receptors activation produce cardioprotection. This study investigates the role of central and peripheral opioid receptors in intravenous morphine preconditioning (MPC) and ischemic preconditioning (IPC).

Methods Sixty-five anesthetized, open chests, male Sprague–Dawley rats were assigned to one of nine groups after intrathecal catheter placement. IPC was induced by three cycles of intermittent occlusion of left anterior descending artery (5 min occlusion interspersed with 5 min of reperfusion). MPC was induced by three consecutive intravenous infusions of 100 µg/kg morphine over five minutes. The opioid receptors antagonist naloxone methiodide (NM), at a dose of 20 µg/kg, was intravenously or intrathecally given 10 min before IPC or MPC (IVNM + IPC, ITNM + IPC, IVNM + MPC, ITNM + MPC). Control group (CON) and intravenously or intrathecally administered NM (IVNM, ITNM) were used as negative controls, respectively. All hearts were subjected to 30 min of ischemia follow by 2 h of reperfusion. Infarct size, as a percentage of the area at risk, was determined by 2, 3, 5-triphenyltetrazolium staining. Heart rate and mean arterial blood pressure were monitored.

Results The infarct size was significantly reduced in the IPC and MPC groups compared with control. The additional of intravenous or intrathecal NM both reversed the cardioprotective effects of MPC. In comparison only intravenous administration of NM before IPC could attenuate the cardioprotection.

Conclusions MPC could mimic IPC, produce a similar cardioprotective effect. Both central and peripheral opioid receptors mediate in the cardioprotection of MPC, however, only peripheral opioid receptors in IPC.

Keywords Opioid receptors · Morphine · Preconditioning · Spinal cord

Introduction

Opioid receptors (ORs) regulate cardiovascular function in both normal and diseased myocardium. These receptors have been localized to the central nervous system and peripherally to autonomic presynaptic nerve endings and on cardiac myocytes. Schultz et al. [1] first reported that the protective effect of ischemic preconditioning (IPC) is mediated by ORs. Intravenous morphine preconditioning (MPC) has been shown to mimic IPC by reducing infarct size (IS) in anesthetized open-chest rats or rabbits, when administered before ischemia [2–4] and reperfusion [5]. In a previous study, it was demonstrated that not only cardiac ORs mediated the cardioprotection of intravenous remifentanyl preconditioning, but also it is possible that ORs, that are located outside the myocardium may also mediate this effect [6]. One possible location for these extracardiac ORs is in the central nervous system and activation of the ORs by morphine administered intrathecally reduces IS in a rat model of myocardial ischemia reperfusion injury [7, 8]. Although studies revealed that central nervous system (CNS) participation is probably required for the effect intravenous opioid preconditioning [6] and not for IPC [9], there is no direct evidence to demonstrate central ORs mediates the protective effect of MPC and IPC.

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In this study, we intrathecally or intravenously administered naloxone methiodide (NM), a quaternary non-selective ORs antagonist, which dose not cross the blood brain barrier [10], to investigate the role of central and peripheral ORs in the cardioprotective effects of MPC and IPC.

Materials and methods

This study was conducted in accordance with our institutional guidelines on the use of live animals for research, and experimental protocol was approved by Animal Care and Use Committee of Anhui Medical University.

Surgical preparation

Intrathecal catheter placement

Intrathecal (IT) catheters were placed in Male Sprague–Dawley rats anesthetized by an intraperitoneal injection of pentobarbitone (50 mg/kg body weight). After sterile preparation of the posterior neck, a small polyethylene-10 catheter (4 cm) (Smiths Medical International Ltd, UK) was inserted through an opening in the atlanto–occipital membrane to the thoracic spinal cord according to the method of Yaksh and Rudy [11]. The wound was closed with interrupted sutures. After recovery, these animals were examined for any gross motor or sensory deficits. Animals demonstrating any deficits were excluded from further experimentation. Preconditioning experiments were conducted at a minimum of 3 days following IT catheter placement. In addition after finishing the experiment, Evan blue dye was injected through the IT catheter to determine the catheter location and any damage to the spinal cord.

Ischemia and reperfusion injury

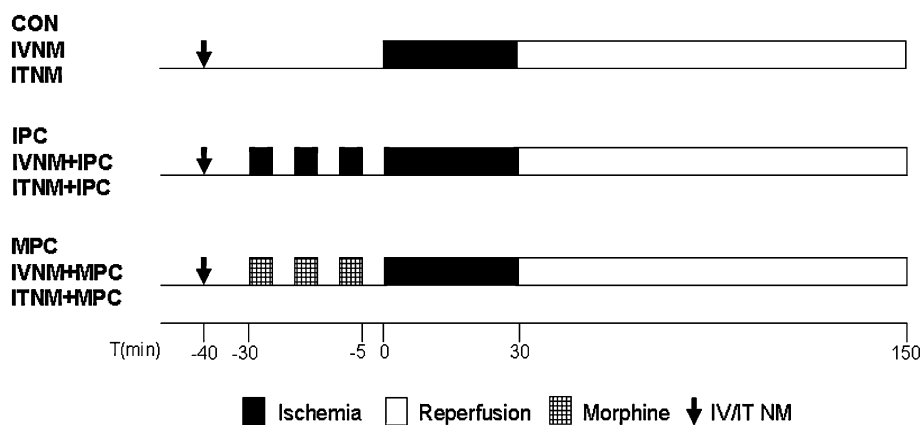
Male Sprague–Dawley rats with IT catheters were anesthetized by intraperitoneal administration of pentobarbitone

(50 mg/kg body weight) and maintained by repeat doses of 25 mg/kg every 60–90 min as necessary. All of the animals underwent tracheotomy and tracheal intubation. Mechanical ventilation was provided with a Harvard Apparatus Rodent Respirator (Harvard Apparatus, Boston, MA), and the rats were ventilated with room air at 70–80 breaths/min. Body temperature was monitored and maintained at $37 \pm 1^\circ\text{C}$ (mean \pm SD) using a heating pad. The femoral artery was cannulated for direct blood pressure monitoring via a pressure transducer and a lead-II electrocardiogram monitored heart rate via subcutaneous stainless steel electrodes. These electrodes were in turn connected to a PowerLab monitoring system (ML750 PowerLab/4sp with MLT0380 Reusable BP Transducer; AD Instruments, Colorado Springs, CO). The right femoral vein was cannulated for saline infusion. A left thoracotomy was performed to expose the heart at the fifth intercostal space. After removing the pericardium, a 6–0 Prolene loop, along with a snare occluder, was placed at the origin of the left anterior descending artery. Regional ischemia was induced by pulling the snare and securing the threads with a mosquito hemostat. Ischemia was confirmed by electrocardiographic changes, a substantial decrease in mean arterial pressure and cardiac cyanosis. Rats were omitted from further data analysis if severe hypotension (arterial mean blood pressure less than 30 mmHg) or intractable ventricular fibrillation occurred. After surgical preparation, the rat was allowed to stabilize for 15 min.

Study groups and experiments protocol

Rats were randomly assigned to receive one of nine treatments (Fig. 1). All animals were subjected to 30 min of ischemia by occlusion of the left anterior descending artery followed by 2 h of reperfusion by release of the occlusion: 1. CON, rats were induced by 30 min ischemia and 2 h reperfusion; 2. IPC, before the 30 min ischemia of the left anterior descending artery, rats were subjected to preconditioning by ischemia (IPC, 3 cycles of 5 min ischemia and

Fig. 1 Bar graphs depicting the experimental protocol. CON control, IVNM intravenous naloxone methiodide, ITNM intrathecal naloxone methiodide, IPC ischemic preconditioning, MPC intravenous morphine preconditioning



5 min reperfusion); 3. MPC, MPC hearts were subjected to three 5 min cycles of intravenous morphine at 100 µg/kg interspersed with 5 min drug-free periods prior to sustained ischemia; 4. IVNM + IPC, naloxone methiodide (NM) 20 µg/kg i.v. 10 min before IPC; 5. ITNM + IPC, naloxone methiodide (NM) 20 µg/kg i.t. 10 min before IPC; 6. IVNM + MPC naloxone methiodide (NM) 20 µg/kg i.v. 10 min before MPC; 7. ITNM + MPC, naloxone methiodide (NM) 20 µg/kg i.t. 10 min before MPC; 8. IVNM, naloxone methiodide (NM) 20 µg/kg i.v. 40 min before ischemia; 9. ITNM, naloxone methiodide (NM) 20 µg/kg i.t. 40 min before ischemia.

Determination of infarct size

The hearts were excised and transferred to a Langendorff apparatus on completion of the reperfusion period and immediately perfused with normal saline for 1 min at a pressure of 100 cm H₂O to flush out residual blood. The snare was securely retightened and 0.25% Evan blue dye was injected to stain the normally perfused region of the heart. This procedure allowed visualization of the normal, nonischemic region and the area at risk (AAR). The hearts were then frozen, and cut into 2 mm slices. Thereafter, the slices were stained by incubation at 37°C for 20 min in 1% 2, 3, 5-triphenyltetrazolium (Sigma Chemical Co.) in phosphate buffer at pH 7.4. This was followed by immersion in 10% formalin for 20 min to enhance the contrast of the stain. The areas of infarct and risk zone for each slice was traced and digitized using a computerized planimetry technique (SigmaScan 4.0, Systat Software Inc., Richmond, CA). The volumes of the left ventricles, IS, and AAR were calculated by multiplying each area with slice thickness and summing the product. The IS was expressed

as a percentage of the AAR (IS/AAR) and this ratio was used to compare the differences among the groups.

Statistical analysis

Data were expressed as mean ± SD and data analysis was performed with a personal computer statistical software package (Prism v4.0; GraphPad Software, San Diego, CA). Hemodynamic data were analyzed using two-way analysis of variance, with the Bonferroni correction applied for multiple comparisons if significant *F* ratios were obtained. The IS expressed as percentage of the area at risk (IS/AAR) were analyzed between the groups using analysis of variance with a Student–Newman–Keuls posthoc test for multiple comparisons. Statistical differences were considered significant if the *P* value was <0.05.

Results

A total of 65 rats were used in the study. Four rats were excluded because of motor or sensory deficits following intrathecal catheter placement and seven rats were omitted from further data analysis for severe hypotension (mean arterial blood pressure less than 30 mmHg) or intractable ventricular fibrillation. A total of 54 rats completed the study.

Hemodynamics

Hemodynamic values including heart rate, mean arterial blood pressure and rate-pressure product at baseline, 30 min after left anterior descending artery occlusion, and after 2 h of reperfusion, were collected (Table 1). There

Table 1 Hemodynamic parameters ($\bar{x} \pm s$) hemodynamic parameters for different groups

Group	<i>n</i>	Baseline			30 min occlusion			2 h reperfusion		
		HR	MAP	RPP	HR	MAP	RPP	HR	MAP	RPP
CON	6	382 ± 20	113 ± 15	43 ± 6	373 ± 15	99 ± 11	37 ± 3	367 ± 17	112 ± 18	41 ± 6
IPC	6	389 ± 21	110 ± 16	43 ± 6	382 ± 13	94 ± 19	36 ± 7	384 ± 29	109 ± 16	42 ± 7
MPC	6	391 ± 20	115 ± 16	45 ± 5	385 ± 11	104 ± 13	40 ± 5	375 ± 14	105 ± 19	39 ± 7
IVNM + IPC	6	392 ± 25	109 ± 20	43 ± 8	389 ± 22	100 ± 7	39 ± 3	383 ± 18	99 ± 16	38 ± 6
ITNM + IPC	6	404 ± 24	109 ± 11	44 ± 4	398 ± 18	101 ± 17	40 ± 6	399 ± 28	107 ± 19	42 ± 7
IVNM + MPC	6	401 ± 25	106 ± 18	43 ± 8	396 ± 19	98 ± 17	39 ± 8	394 ± 13	101 ± 20	40 ± 8
ITNM + MPC	6	397 ± 19	107 ± 16	43 ± 5	391 ± 10	98 ± 11	38 ± 5	387 ± 17	98 ± 13	38 ± 6
IVNM	6	391 ± 27	106 ± 17	42 ± 7	384 ± 22	98 ± 11	37 ± 5	385 ± 12	101 ± 8	39 ± 3
ITNM	6	397 ± 22	104 ± 18	41 ± 7	390 ± 18	97 ± 15	38 ± 7	395 ± 20	103 ± 21	40 ± 8

Values are presented as mean ± SD. Baseline = 15 min after surgery; 30 min occlusion = 30 min after regional ischemia; 2 h reperfusion = 2 h after reperfusion; HR heart rate (beats per min), MAP mean arterial blood pressure (mmHg), RPP rate-pressure product (mmHg/min per 1,000), CON control, IVNM intravenous naloxone methiodide, ITNM intrathecal naloxone methiodide, IPC ischemic preconditioning, MPC intravenous morphine preconditioning

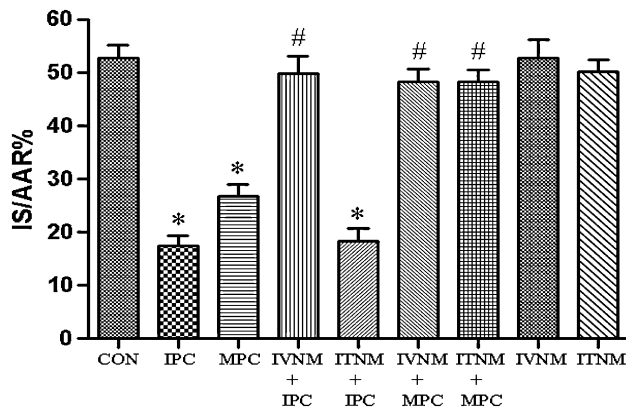


Fig. 2 Myocardial infarct size (IS) as a percentage of the area at risk (AAR) for the different groups. *CON* control, *IVNM* intravenous naloxone methiodide, *ITNM* intrathecal naloxone methiodide, *IPC* ischemic preconditioning, *MPC* intravenous morphine preconditioning. Values are presented as mean \pm SD. * $P < 0.01$ versus *CON*; # $P < 0.01$ versus *IPC* or *MPC*

were no significant differences between the groups at baseline, 30 min of occlusion, and at 2 h of reperfusion. There was a slight, but nonsignificant drop of mean blood pressure at 30 min of occlusion, expected from the successful induction of ischemia.

Infarct size

There were no differences in the AAR between groups and they ranged from $0.40 \pm 0.03 \text{ cm}^3$ to $0.45 \pm 0.05 \text{ cm}^3$. The IS/AAR of the control group was $52.8 \pm 8.9\%$. The IS/AAR were significantly reduced in the *IPC* ($17.6 \pm 4.3\%$) and *MPC* ($26.9 \pm 5.3\%$) groups ($P < 0.01$) (Fig. 2). The addition of intravenous naloxone methiodide reversed the cardioprotective effects of *IPC* ($50.0 \pm 7.8\%$) whereas intrathecal administration of the drug did not have any effect on *IPC* ($18.4 \pm 5.8\%$). Intrathecal or intravenous administration of naloxone methiodide both abolished the cardioprotection of *MPC* ($48.3 \pm 5.6\%$ and $48.3 \pm 6.0\%$, respectively). The sole administration of naloxone methiodide either intrathecally or intravenously did not affect the infarct size ($50.2 \pm 5.5\%$ and $52.8 \pm 8.5\%$, respectively).

Discussion

The present data demonstrate that both intrathecal and intravenous administration of naloxone methiodide (NM) block the infarct sparing effect of intravenous morphine preconditioning, while only intravenous administration of naloxone methiodide (NM) blocked that of ischemic preconditioning. Since naloxone methiodide (NM) does not cross the blood brain barrier, these data suggest that central and peripheral ORs both mediate the cardioprotective effect

of intravenous morphine preconditioning, but only peripheral ORs mediate the effect of ischemic preconditioning.

ORs are present both in the central nervous system and peripheral organs, including in the heart [12]. Naloxone hydrochloride blockade of the protective effect induced by ischemic preconditioning was first reported in 1995, indicating that ORs mediate in this cardioprotection [1]. Since naloxone hydrochloride could cross the blood brain barrier, the site at which ORs act could not be ascertained. Through intravenous administration of naloxone methiodide, Schultz et al. [3] and Chien et al. [9] found that the cardioprotective effect of ischemic preconditioning is probably mediated by peripheral ORs mechanism in the intact rat and rabbit hearts, respectively. In the current study, we found that only intravenous but not intrathecal administration of naloxone methiodide before ischemic preconditioning, could abolish the cardioprotective effects. This confirmed that central ORs are not involved with the protective effect of ischemic preconditioning.

Previous studies have shown that a brief infusion of morphine can produce a preconditioning effect [2–4]. All three subtypes of ORs, namely μ -, δ - and κ -OR, mediate the action of remifentanyl preconditioning [6]. But since μ -ORs are not present in the heart [13], the μ -ORs must therefore reside in an extra cardiac location, most likely in the central nervous system [6]. Since intravenous administered morphine can cross the blood brain barrier to produce analgesia, it remains to be elucidated whether central ORs mechanism contributes to intravenous morphine preconditioning. Groban et al. [7] first demonstrated that morphine administered intrathecally reduces IS in a rat model of myocardial ischemia reperfusion injury. Later, Li et al. [8] demonstrated that μ -, δ - and κ -OR mediated the cardioprotective effect of intrathecal morphine preconditioning. Wong et al. [14] then demonstrated that these ORs were restricted to the CNS. The results from this study indicate that central ORs are also involved in intravenous morphine preconditioning. Intrathecal and intravenous administered naloxone methiodide both reversed the cardioprotective effect of intravenous morphine preconditioning, whereas only intravenous administered naloxone methiodide abolished the effect of ischemic preconditioning. These indicate that central and peripheral ORs both mediate in intravenous morphine preconditioning, but only peripheral ORs mediate in ischemic preconditioning.

It is well known that peripheral ORs activation leads to a complex downstream signaling pathway to produce cardioprotective, such as protein kinase C (PKC) [4], the mitochondrial ATP-sensitive potassium (K_{ATP}) channel [2], glycogen synthase kinase beta [5] and so on. But the specific mechanism of cardioprotective effect induced by central ORs activation is still unknown. Experimental [15, 16] and clinical [17, 18] evidence suggest that the

cardiovascular actions of opioid receptor agonists can be related to alterations of the sympathetic-parasympathetic balance, favoring the inhibition of the central sympathetic activity.

There were no significant differences between the groups at baseline, 30 min of occlusion, and at 2 h of reperfusion, showing all treatments in this study have the same effect in haemodynamic values. There was a slight, but nonsignificant drop of mean blood pressure at 30 min of occlusion, defining the successful induction of myocardial ischemia.

In summary, our findings have found that intravenous morphine preconditioning could mimic ischemic preconditioning, produce a similar cardioprotective effect, but their ORs mediated mechanism is different. Both central and peripheral ORs mediate in the cardioprotection of MPC, however, only peripheral ORs in IPC.

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