Morphine Reduces the Threshold of Remote Ischemic Preconditioning Against Myocardial Ischemia and Reperfusion Injury in Rats: The Role of Opioid Receptors

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Objectives: **Opioid receptors mediate the cardioprotection of remote ischemic preconditioning (RIPC). The authors tested the hypothesis that morphine reduces the threshold of cardioprotection produced by RIPC.**

Methods: **A randomized, prospective study.**

Setting: **A university research laboratory.**

Participants: **Forty-five male Sprague-Dawley rats.**

Interventions: **Anesthetized, open-chest, male Sprague-Dawley rats were assigned randomly to 1 of 7 treatment groups. RIPC1 and RIPC3 were, respectively, induced by 1 or 3 cycles of 5 minutes of femoral artery ischemia interspersed with 5 minutes of reperfusion. Morphine (MOR, 0.1 mg/kg) and the opioid receptor antagonist naloxone (NAL, 6 mg/kg) were administered 30 minutes before sustaining ischemia. MOR + RIPC1 and NAL + MOR + RIPC1 groups received the combination of MOR and RIPC1 in the absence or presence of NAL before coronary artery occlusion. Ischemia and reperfusion injury then were induced by 30 minutes of left coronary artery occlusion followed by 120 minutes of reperfusion.**

Measurements and Main Results: **Infarct size, as a percentage of the area at risk, was determined by 2,3,5-triphenyltetrazolium staining. RIPC3 and the combination of MOR and RIPC1 groups significantly reduced the infarct size compared with the control group. RIPC1, MOR, and NAL did not affect infarct size. NAL pretreatment reversed cardioprotection of the combination of MOR and RIPC1 treatments.**

Conclusions: **MOR reduces the threshold of RIPC, and opioid receptors mediate this augmentative effect.**

KEY WORDS: morphine, remote ischemic preconditioning, myocardial ischemia, opioid receptor

PRZYKLENK ET AL[1] found that brief episodes of ischemia in one coronary bed render remote virgin myocardium resistant to infarction; this phenomenon was named "remote ischemic preconditioning (RIPC)." Subsequent studies confirmed the existence of this protective effect of remote preconditioning in other organs, such as the kidney,[2] small intestine,[3] or skeletal muscle.[4] Although the mechanisms of RIPC are incompletely understood, some similarities to ischemic preconditioning have been discovered in the conveyance of external signals to intracellular targets that ultimately lead to protection.[5] RIPC also can be elicited via opioids, bradykinin, and adenosine, which are released from the remote organ during the preconditioning ischemia and carried to the heart in the bloodstream.[5] It was shown that δ1- and κ-opioid receptors are mediated in the cardioprotection of RIPC.[6,7] Morphine, a μ-receptor agonist with δ- and κ-receptor agonist properties,[8] has been shown to mimic ischemic preconditioning in the intact and isolated rat hearts.[9,10] Previous studies indicated that morphine enhances the cardioprotection induced by isoflurane or helium preconditioning.[11-13] The addition of morphine infusion to RIPC during reperfusion could confer a greater percentage of ST-segment resolution and lower peak troponin I levels in patients subjected to primary percutaneous coronary intervention.[14] In this study, the authors hypothesized that morphine reduces the threshold of cardioprotection induced by RIPC. The authors further hypothesized that this protective effect is mediated by the activation of opioid receptors in rats.

METHODS

This study was conducted in accordance with the institutional guidelines on the use of live animals for research, and the experimental protocol was approved by the Animal Care and Use Committee of Anhui Medical University, Hefei, China. Male Sprague-Dawley rats weighing between 280 and 300 g were used for this study. Rats were anesthetized by the intraperitoneal administration of pentobarbitone (50 mg/kg) and maintained by repeat doses of 25 mg/kg every 60 to 90 minutes as necessary. All 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 to 80 breaths/min. Their body temperature was monitored and maintained at 37° ± 1°C (mean ± standard deviation) using a heating pad. The right carotid 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 connected to a PowerLab monitoring system (ML750 PowerLab/4sp with MLT0380 Reusable BP Transducer; AD Instruments, Colorado Springs, CO). Hemodynamic values, including heart rate and mean arterial blood pressure (MAP), were recorded at baseline, at the end of the treatment period, and at the end of the ischemia and reperfusion periods for comparison. The left femoral vein was cannulated for drug administration. A left thoracotomy was performed to expose the heart at the 5th intercostal space. After removing the pericardium, a 6-0 Prolene loop along with a snare occluder were placed at the origin of the left coronary artery in preparation for inducing ischemia-reperfusion injury. 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 MAP, and cardiac cyanosis. Rats were omitted from further data analysis if severe hypotension (arterial mean blood pressure <30 mmHg) or intractable ventricular fibrillation occurred. After surgical preparation, the rat was allowed to stabilize for 15 minutes.

Rats were assigned randomly to receive 1 of 7 treatments (Fig 1). All animals were subjected to 30 minutes of ischemia by occlusion of the left coronary artery followed by 2 hours of reperfusion by release of the occlusion; the RIPC1 and RIPC3 groups were, respectively, induced by 1 or 3 cycles of 5 minutes of right femoral artery ischemia induced by

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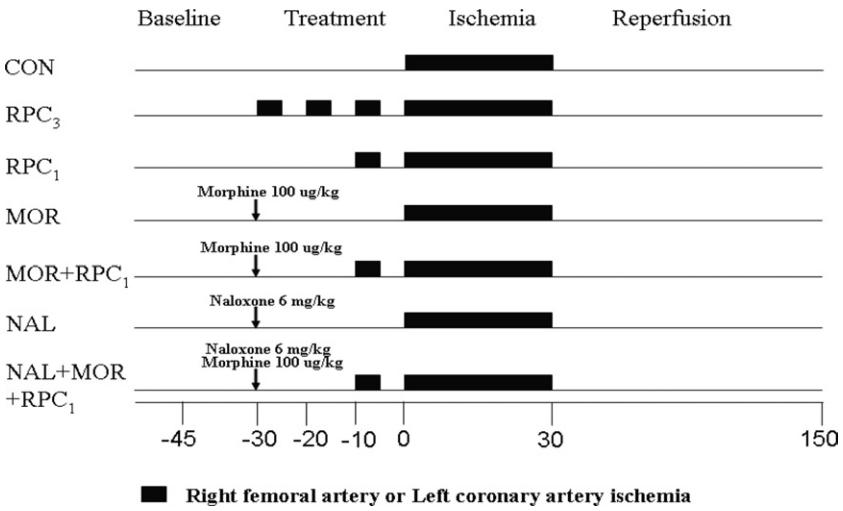

Fig 1. Bar graphs depicting the experimental protocol.

an atraumatic vessel clip interspersed with 5 minutes of reperfusion before coronary occlusion. A nonspecific opioid-receptor antagonist naloxone (NAL) was used to evaluate the involvement of opioid receptors. The intravenous morphine (MOR) and NAL groups were, respectively, induced by MOR (0.1 mg/kg[13]) or NAL (6 mg/kg[13]) administered 30 minutes before sustaining ischemia. MOR + RIPC1 and NAL + MOR + RIPC1 groups of rats received 1 cycle of 5 minutes of right femoral artery ischemia interspersed with 5 minutes of reperfusion plus morphine (0.1 mg/kg) in the absence or presence of NAL (6 mg/kg) before ischemia and reperfusion injury. The negative control (CON) group merely underwent ischemia and reperfusion injury.

The hearts were excised and transferred to a Langendorff apparatus on completion of the reperfusion period and immediately perfused with normal saline for 1 minute at a pressure of 100 cmH_2O to flush out residual blood. The snare was retightened securely, and 0.25% Evan blue dye was injected to stain the normally perfused region of the heart. This procedure allowed visualization of the normal, nonischemia region and the area at risk (AAR). The hearts then were frozen and cut into 2-mm slices. Thereafter, the slices were stained by incubation at 37°C for 20 minutes in 1% 2, 3, 5-triphenyltetrazolium in phosphate buffer at a pH of 7.4. This was followed by immersion in 10% formalin for 20 minutes to enhance the contrast of the stain. The areas of infarct and risk zone for each slice were traced and digitized using a computerized planimetry technique (SigmaScan 4.0; SYSTAT Software, Inc, Richmond, CA). The infarct size (IS) was expressed as a percentage of the AAR.

Data are expressed as mean ± standard deviation, and data analysis was performed with a personal computer statistical software package (Prism v4.0; GraphPad Software, San Diego, California). The hemodynamic data were analyzed using two-way analysis of variance, with the Bonferroni correction applied for multiple comparisons if significant F ratios were obtained. Risk areas and infarct sizes, expressed as a percentage of the area at risk (IS/AAR), were analyzed among groups using 1 way analysis of variance with a Student–Newman–Keuls post hoc test for multiple comparisons. Statistical differences were considered significant if the *p*-value was less than 0.05.

RESULTS

A total of 45 rats were instrumented to obtain 42 rats in the study. Three rats were omitted from further data analysis for severe hypotension or intractable ventricular fibrillation. There was 1 each from those receiving RIPC3, NAL, and MOR + RIPC1 treatments.

Hemodynamic values including heart rate, mean arterial blood pressure, and the rate-pressure product (RPP) at baseline, after treatment, 30 minutes after left coronary artery occlusion, and after 2 hours of reperfusion were collected (Table 1). There were no significant differences among groups at baseline, after treatment, at 30 minutes of occlusion, and at 2 hours of reperfusion. Compared with baseline, MAP and RPP were reduced only after MOR + RIPC1 treatment. During ischemia and reperfusion, MAP and RPP declined 23% to 39% and 34% to 44%, respectively, from baseline ($p < 0.05$ and $p < 0.01$ *v* baseline), confirming the successful induction of ischemia and reperfusion injury model.

The AAR ranged from 0.41 ± 0.04 cm^3 to 0.46 ± 0.06 cm^3, and there was no difference in AAR between the control and treatment groups. As shown in Figure 2, the IS/AAR of the CON group was 54.7% ± 6.0%; RIPC3 and MOR + RIPC1 markedly reduced IS/AAR to 28.3% ± 4.9% and 29.0% ± 7.0%, respectively ($p < 0.01$ *v* CON). RIPC1 and MOR (0.1 mg/kg) or NAL (6 mg/kg) before sustaining ischemia had no effect on infarct size (IS/AAR: RIPC1, 51.1% ± 7.4%; MOR, 53.8% ± 6.7%; NAL, 54.4% ± 6.9%; $p > 0.05$ *v* control). However, NAL could reverse the cardioprotective effect produced by MOR + RIPC1 treatment (IS/AAR: NAL + MOR + RIPC1, 55.2% ± 6.5%; $p < 0.01$ *v* MOR + RIPC1).

DISCUSSION

The results from this study confirmed previous findings[7] showing that 3 cycles of 5 minutes each of femoral artery ischemia interspersed with 5 minutes of reperfusion before coronary occlusion produced a protective effect against myocardial ischemia and reperfusion injury. Then, 1 cycle of 5 minutes of femoral artery ischemia interspersed with 5 minutes of reperfusion or pretreament with MOR (0.1 mg/kg) without ischemia before sustaining ischemia could not show this protection. However, the combination of this low dose of MOR and a single 5-minute cycle of femoral artery ischemia and reperfusion reduced the myocardial infarct size to a similar extent to 3 cycles of femoral artery ischemia and reperfusion, indicating that morphine reduces the threshold of RIPC. NAL abolished the protective effect induced by the combination of