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ERK and p38 MAP kinase activation are components of opioid-induced delayed cardioprotection

Abstract  Opioids have been previously shown to confer acute and delayed cardioprotection against a prolonged ischemic insult. We have extensively characterized the signal transduction pathway mediating acute cardioprotection and have suggested a role for extracellular signal regulated kinase (ERK) in this cardioprotection. Therefore, we attempted to determine a role for ERK and the stress activated MAP kinase, p38, in opioid-induced delayed cardioprotection by using selective inhibitors of these pathways. All rats were subjected to 30 min of ischemia and 2 h of reperfusion (I/R). Control animals, injected with saline 48 h prior to I/R, had an infarct size/area at risk (IS/AAR) of 61.6 ± 1.6. 48-h pretreatment with TAN-67 (30 mg/kg), a δ1-opioid receptor agonist, maximally reduced IS/AAR (31.2 ± 6.5). The involvement of ERK was examined with PD 098059, a selective pharmacological antagonist which inhibits the upstream kinase, MEK-1, that phosphorylates and activates ERK. PD 098059 (0.3 mg/kg) did not alter IS/AAR when administered alone (60.7 ± 4.9). However, PD 098059 (0.3 mg/kg) administration 30 min prior to TAN-67 (30 mg/kg) completely abolished cardioprotection (61.0 ± 7.6). The selective p38 inhibitor, SB 203580 (1.0 mg/kg), had no effect on IS/AAR in the absence of TAN-67 (53.1 ± 2.3). Additionally, SB 203580 (1.0 mg/kg) when administered prior to TAN-67 (30 mg/kg) partially abolished cardioprotection (51.3 ± 6.4). These results suggest that both ERK and p38 are integral components of opioid-induced delayed cardioprotection and may act via parallel pathways.

Key words  Preconditioning – ERK – p38 – delayed cardioprotection – opioid

Introduction

We have previously demonstrated that stimulation of the δ-opioid receptor induces cardioprotection to the in vivo ischemic rat myocardium (15, 37). We have further demonstrated that this is dependent on the δ1, but not the μ or κ opioid receptor and is mediated by G_{i/o} protein activation (36, 38). Activation of this cascade may be an important component of ischemic preconditioning (IPC), whereby brief episodes of ischemia and reperfusion prior to a sustained ischemic insult induce cardioprotection (39).

IPC has been shown to induce a “second window” of cardioprotection, whereby a preconditioning stimulus can induce cardioprotection 24 to 72 hours later (4, 22, 25). This phenomenon has also been shown with other pharmacological agents, including CGPA, an adenosine agonist (6), monophosphoryl lipid A, a non-toxic lipid A derivative of endotoxin (28, 44), and nitric oxide (8, 34). Additionally, we have recently shown that stimulation of the δ1-opioid receptor induces a “second window” of
cardioprotection. We demonstrated that injection of an opioid agonist 24 or 48 hours, but not 72 hours, prior to ischemia is cardioprotective and is dependent on the activation of the mitochondrial ATP-sensitive potassium (K$_{\text{ATP}}$) channel (14).

The delayed phase of preconditioning is cardioprotective against myocardial infarction and stunning. These effects are likely mediated by protein kinase C (5) and tyrosine kinase (13, 19). Additionally, members of the mitogen activated protein (MAP) kinase superfamily may be important mediators of opioid-induced delayed cardioprotection since it has been demonstrated that opioids can stimulate these kinases. Gutstein et al. (16) demonstrated that $\mu$ and $\delta$-opioid receptor stimulation can potently activate ERK in COS cells. Similarly, evidence from our laboratory suggests an important role for ERK in acute cardioprotection induced by opioids (unpublished observation). Therefore, we postulate that ERK activation is a likely signaling pathway by which opioid agonists induce delayed cardioprotection. Indeed, it has been demonstrated that delayed cardioprotection against stunning is dependent on protein synthesis (35) and delayed cardioprotection against lethal injury is thought to require elevated levels of heat shock protein (HSP) 70 and 90, known to be involved in protein folding within the cell (30). Furthermore, Carroll and Yellon (10) have recently demonstrated that delayed cardioprotection in a human cardiomyocyte-derived cell line is dependent on the activation of p38 MAP kinase. Therefore, since recent evidence suggests that opioids may potently activate a MAP kinase signaling pathway and since some MAP kinases have been demonstrated to be an important component of ischemic- or pharmacologically-induced delayed cardioprotection, we examined the likelihood that opioids induce delayed cardioprotection via the activation of specific MAP kinase pathways.

Materials and methods

This study was performed in accordance with the guidelines of the Animal Care Committee of the Medical College of Wisconsin, which is accredited by the American Association of Laboratory Animal Care.

General surgical preparation

Male Wistar rats, 350–450 g, were used for all phases of this study. Rats were administered drug or saline 48-hours prior to the surgical protocol via intraperitoneal (ip) injection. For the surgical protocol, rats were anesthetized via ip administration of Inactin (100 mg/kg). A tracheotomy was performed, and the trachea was intubated with a cannula connected to a rodent ventilator (model 683, Harvard Apparatus, South Natick, MA). Rats were ventilated with room air supplemented with O$_2$ at 60 to 65 breaths per minute. Atelectasis was prevented by maintaining a positive end-expiratory pressure of 5 to 10 mm H$_2$O. Arterial pH, PCO$_2$, and PO$_2$ were monitored at control, 15 minutes of occlusion, and 60 and 120 minutes of reperfusion by a blood gas system (AVL 995 pH/Blood Gas Analyzer). These values were maintained within a normal physiological range (pH 7.35–7.45; PCO$_2$ 25–40 mm Hg; and PO$_2$ 80–110 mm Hg) by adjusting the respiratory rate and/or tidal volume. Body temperature was maintained at 38 °C by the use of a heating pad.

The right carotid artery was cannulated to measure blood pressure and heart rate via a Gould PE50 pressure transducer connected to a Grass (model 7) polygraph. The right jugular vein was cannulated for saline and drug infusion. A left thoracotomy was performed at the fifth intercostal space followed by a pericardiotomy and adjustment of the left atrial appendage to reveal the location of the left coronary artery. A ligature (6-0 prolene) was passed below the coronary artery from the area immediately below the left atrial appendage to the right portion of the left ventricle. The ends of the suture were threaded through a propylene tube to form a snare. The coronary artery was occluded by pulling the ends of the suture taut and clamping the snare onto the epicardial surface with a hemostat. Coronary artery occlusion was verified by epicardial cyanosis and subsequent decrease in blood pressure. Reperfusion of the heart was initiated via unclamping the hemostat and loosening the snare and was confirmed by visualizing an epicardial hyperemic response. Heart rate and blood pressure were allowed to stabilize before the following protocols were initiated.

Study groups and experimental protocols

Rats were randomly divided among 6 groups (Fig. 1). Control rats were administered saline 48-hours prior to 30-minutes of regional ischemia and 2-hours of reperfusion (I/R). TAN-67, a selective $\delta$-opioid receptor agonist, was administered ip, 30 mg/kg, 48-hours prior to I/R. We have previously demonstrated that this dose is selective for the $\delta_1$ receptor and have demonstrated that this timing of opioid administration induces a maximal reduction in IS (14). To determine a role for ERK in opioid-induced delayed cardioprotection, PD 098059 was utilized. PD 098059 inhibits the upstream kinase, MEK-1, that phosphorylates and activates ERK. PD 098059, 0.3 mg/kg, was administered ip in the absence of TAN-67 (30 mg/kg) 48-hours prior to I/R or administered ip 30-minutes prior to TAN-67 (30 mg/kg) 48-hour pretreatment. Similarly, SB 203580, 1.0 mg/kg, was administered during either the control or TAN-67 (30 mg/kg) protocol during the same timing sequence.