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Review Article

Role of opioids in acute and delayed preconditioning ☆

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Abstract

A number of endogenous mediators, including opioids, adenosine and bradykinin, which act on cardiac cell membrane receptors have been demonstrated to trigger the phenomenon termed ischemic preconditioning (IPC). IPC is an endogenous protective mechanism, whereby a brief period of ischemia or hypoxia protects a cell or organ, in this review the heart, against injury from a subsequent more prolonged stressful insult. Recent data suggest that opioid receptors are important triggers and/or mediators of this protective response. Selective pharmacological antagonists of the δ-or κ-opioid receptor have been shown to block IPC, and agonists of these same receptors have been shown to mimic IPC in intact animals, isolated hearts or isolated cardiomyocytes. This review will summarize the current state of knowledge, which exists defining the role and cellular signaling pathways by which endogenously or exogenously administered opioids produce their cardioprotective response. The potential clinical application and evidence to suggest that opioids produce a similar protective effect in man will also be discussed.

1. Introduction

Ischemic preconditioning (IPC) is a phenomenon in which single or multiple brief periods of coronary artery occlusion result in an increased resistance to a subsequent more prolonged period of occlusion [1]. In most species, this phenomenon has two phases, an acute or early phase in which the cardioprotective effect lasts for 1–3 h and a delayed phase or second window of protection (SWOP), which reappears approximately 24 h after the acute phase and may last for up to 72 h [2]. There has been considerable interest in the triggers and/or mediators of this phenomenon since identification of a mechanism responsible might lead to a powerful new therapeutic approach to treating patients with ischemic heart disease at risk of an acute myocardial infarction or during acute coronary interventions, such as angioplasty, thrombolysis or coronary artery bypass surgery. Adenosine, bradykinin and opioid receptors have all been identified as potential targets for drug development since occupation and activation of all three of these G-protein-coupled receptors have been shown to trigger both acute and delayed IPC in a number of animal models [2]. The purpose of this review then will be to focus on the evidence which suggests that opioid receptor activation is an important component of both early and delayed IPC and potential mechanisms by which opioid receptor agonists produce direct or indirect cardioprotective effects on the cardiac myocyte. Drugs, which have been used to study the role of opioid receptors in cardioprotection, are summarized in Table 1. Finally, we will also discuss possible clinical implications of this approach to cardioprotection.

2. Endogenous opioid peptides and receptors in the heart

Opioid receptors have been shown to mediate and regulate cardiovascular function in normal and disease states and these receptors have been localized to the central nervous system and peripherally to autonomic presynaptic nerve endings and on cardiac myocytes. For a comprehensive summary of the cardiovascular actions of opioids, see the recent review by Pugsley [3]. Binding studies in cardiac myocytes have identified the presence of δ and κ receptors on ventricular myocytes obtained from rats [4,5]. In agreement, Ventura et al. [6] also demonstrated that δ and κ receptors, but not μ receptors, are present on sarcolemmal membranes isolated from rat hearts. These particular receptors appear to be involved in the regulation of myocardial contractile force. Similarly, Krumins et al. [7] demonstrated δ and κ receptors

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Table 1
Relative affinities of opioid agonists and antagonists used in cardioprotection studies (Ki values in cloned mouse receptors)

	δ (nM)	μ (nM)	κ (nM)
Opioid agonists			
ME	1.7	0.65	>1000
LE	4.0	3.40	>1000
β-Endorphin	1.0	1.00	>1000
Dynorphin A	>1000	32	0.50
DADLE	0.74	16	>1000
DPDPE	14	>1000	>1000
Morphine	>1000	14	538
Deltorphin II	3.3	>1000	>1000
U50,448H	>1000	>1000	0.12
Bremazocine	2.3	0.75	0.089
TAN-67	0.70	>1000	>1000
BW 373U86	1.80	15	34
Opioid antagonists			
β-FNA	48	0.33	2.8
Nor-BNI	65	2.20	0.027
Naltriben	0.013	12	13
BNTX	0.66	18	55
Naltrindole	0.02	64	66
Naloxone	17	0.93	2.3

Most values are taken from Ref. [86].

in adult rat hearts but no evidence for μ receptors. Finally, Wittert et al. [8] did not detect any μ-opioid receptor gene expression in rat hearts; however, the δ-opioid receptor was the predominant subtype found. Based on these studies, the effects of IPC and opioid agonists to produce cardioprotection are most likely the effect of δ or κ receptor stimulation, and the studies performed up to the present time appear to support this premise with the majority of evidence supporting a major role for the δ-opioid receptor as the primary receptor responsible for IPC.

Large stores of endogenous opioid peptide precursors also reside in myocardial tissue [9,10]. In this regard, the heart has the ability to synthesize all three major opioid peptides including the enkephalins, dynorphins and endorphins [11,12]. In fact, Howells et al. [13] demonstrated that preproenkephalin mRNA was the highest in rat ventricular tissue as compared to any other organ in the body, including the brain. Low et al. [14] demonstrated that proenkephalin, the precursor to the endogenous enkephalins, was associated with polyribosomes in the myocardium. More importantly, a number of investigators have demonstrated that certain opioid peptides are released during stressful situations into the peripheral circulation [15,16] and that these peptides can result in the modulation of the autonomic nervous system [17]. Myocardial ischemia has been shown to result in the synthesis and release of opioid peptides including Met- and Leu-enkephalin [18,19], and Jackson et al. [20] have demonstrated that four transient brief periods (10 min) of ischemia followed by 10 min of reperfusion in the sinus node of the canine myocardium resulted in an increased release of methionine–enkephalin–arginine–phenylalanine (MEAP) during each ischemic period followed by a return to baseline during the reperfusion periods (Fig. 1). Interestingly, there was a sustained increase in MEAP during a more prolonged period of ischemia. These changes in MEAP resulted in an enhanced vagally mediated bradycardia during occlusion, an effect which was blocked by naltrindole, a selective δ-opioid receptor antagonist or by glibenclamide, a selective K_{ATP}-channel antagonist. This protocol is analogous to a classical IPC protocol and supports the hypothesis first presented by our laboratory, which suggested that δ-opioid receptors and

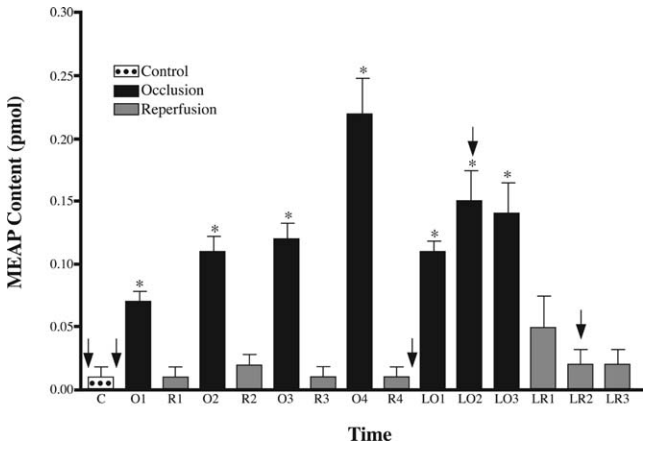

Fig. 1. Nodal MEAP content (pmol) recovered in the dialysate perfusing the sinoatrial (SA) node during a multiple nodal artery occlusion/reperfusion protocol. During the control period the probe was equilibrated with saline (CS) and then switched to vehicle composed of saline and the peptidase inhibitor leucine–arginine (LA). The dialysate was collected at 10-min intervals designated as saline (CS), LA, 10-min occlusions (01, 02, 03, 04), 10-min reperfusion (R1, R2, R3, R4), 30-min (long) occlusions (L01, L02, L03) and 30-min (long) reperfusions (LR1, LR2, LR3). Data are expressed as mean ± S.E.M., $n = 16$ and the arrows indicate the times when the vagus nerve was stimulated. * Statistically different from control ($P < 0.05$). Reproduced from Jackson et al. [20] with permission of Elsevier Science.

K_{ATP} channels mediate the cardioprotective effect of IPC in rat hearts [21].

In support of the above findings, Paradis et al. [22] suggested that increasing concentrations of enkephalins, such as MEAP, in rat ventricular tissue during ischemia may be a compensatory mechanism to help minimize the development of a large infarct. These observations and those of Jackson et al. [20] and our group [21], which implicate endogenous opioids as being triggers and/or mediators of IPC, all suggest that endogenous opioids may serve as an autocrine function possibly through their release from myocytes during ischemia and via an interaction with opioid receptors to limit cell injury.

3. Opioids and cytoprotection in noncardiac tissues

Several papers have suggested that endogenous opioid release results in protection of several organs, including the brain and heart, from hypoxic or ischemic injury [23,24]. Most notably, Mayfield and D'Alecy [25,26] found that several brief periods of whole-body hypoxia produced a phenotype in which mice exposed to a subsequent more prolonged period of hypoxia survived longer than a control group not subjected to the previous hypoxic preconditioning stimulus. In addition, these same investigators discovered that this adaptive response was mediated via the δ-opioid receptor by mimicking the effect with δ receptor agonists and blocking the effect of conditioning hypoxia with a δ-opioid antagonist. There is also intriguing evidence which suggests that hibernating animals possess a hibernation-inducing trigger (HIT) that shares many characteristics with stimulation of the δ-opioid receptor in protecting organs from ischemic insults [27]. Both this HIT factor and the δ-opioid agonist, [D-Ala^2–D-Leu^5]-enkephalin (DADLE), have been shown to increase organ survival time and prolong tissue viability prior to organ transplantation [28]. Taken together, this evidence supports an important role for the δ-opioid system to serve as an endogenous protective mechanism that is activated during stressful stimuli, such as hypoxia or ischemia.

4. Importance of δ-opioid receptors in early preconditioning

The first experimental evidence to demonstrate a role for opioids in early IPC was published by Schultz et al. [29] in the intact blood-perfused rat heart. These investigators demonstrated that the nonselective opioid receptor antagonist, naloxone, completely antagonized the ability of IPC to reduce infarct size whether administered before the IPC stimulus or after the IPC stimulus just prior to the index ischemia. These results suggested that endogenous opioids serve as both a trigger and end effector of IPC in rat hearts (Fig. 2). Similarly, Chien and Van Winkle [30] found that the active enantiomer, (–) naloxone, blocked IPC in rabbit hearts, but, the inactive enantiomer, (+) naloxone, did not block IPC.

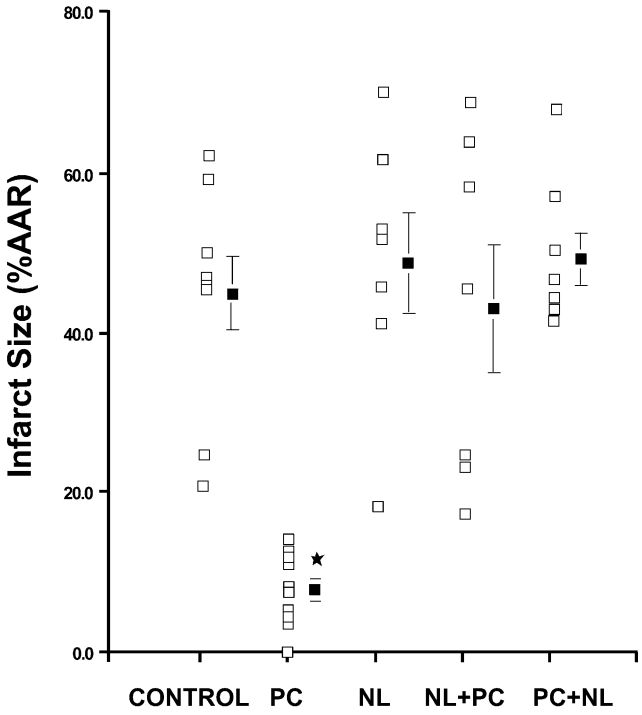

Fig. 2. Infarct size (IS) expressed as a percentage of the area at risk (AAR) in intact rat hearts subjected to vehicle (control), ischemic preconditioning (PC), naloxone (NL) in the absence of PC, NL treatment prior to PC (NL + PC) and NL treatment after PC (PC + NL) before the index ischemic period. The filled squares are the mean ± S.E.M. of each group. * $P < 0.05$ vs. the control group. Reproduced from Schultz et al. [29] with permission of the American Physiological Society.

Furthermore, Schulz et al. [31] determined the role of endogenous opioids in mediating IPC and myocardial hibernation in pig hearts and observed that naloxone blocked IPC but not the effects of short-term hibernation. These data obtained in rats, rabbits and pigs clearly suggest that an opioid receptor is mediating the effect of endogenous opioids to elicit IPC in these three species.

In this regard, Takashi et al. [32] performed a study in isolated adult rabbit cardiomyocytes to determine which endogenous opioid peptides are responsible for the cardioprotection observed during and following IPC. These authors used an isolated rabbit myocyte model in which the cells were subjected to simulated ischemia and/or hypoxia in the presence or absence of IPC or following the administration of certain endogenous opioid peptides known to be released during ischemia. IPC was produced by exposing the cells to 15 min of simulated ischemia and 15 min of reoxygenation prior to a more prolonged 180-min period of simulated ischemia. IPC produced a reduction in cell death and this effect was blocked by naloxone. Similarly, Met^5-enkephalin (ME) and Leu^5-enkephalin (LE) and MEAP produced a reduction in the incidence of cell death, whereas β-endorphin did not protect the cells from injury. These results suggest that the enkephalins are the most likely candidates, which serve as triggers and distal effectors of IPC in the rabbit heart. Recent preliminary data obtained from our laboratory [33] in intact rat hearts demonstrated that when thiorphan, an enkephali-

nase inhibitor, was administered to a nonpreconditioned heart, it produced a reduction in infarct size, an effect that was blocked by pretreatment with naloxone. Taken together, these data suggest that the enkephalins are the most likely triggers and effectors of IPC in both rat, rabbit and swine hearts.

The findings that the enkephalins are the endogenous opioid peptides that are responsible for the reduction in infarct size following IPC is not surprising, since several investigators have previously shown that large amounts of endogenous enkephalins are released from the heart during ischemia [34,35]. In fact, Weil et al. [36] hypothesized that the left ventricle of the adult rat may behave as an endocrine organ that supplies the body with endogenously released enkephalins. In agreement, recent data obtained from our laboratory [37] and those of Dickson et al. [38] suggest that endogenous opioid peptides released from the heart or intestine following coronary or mesenteric occlusion or during reperfusion can produce cardioprotection at a distance in the heart and intestine, a phenomenon termed remote preconditioning. In both of these studies, the cardioprotective effect was blocked by naloxone. Thus, this opioid system appears to be uniquely poised to serve as a major source of opioid peptides during a variety of stress-induced situations.

5. Cardioprotective effects of exogenous opioid agonists

The first study to demonstrate that a nonpeptide opioid agonist mimics IPC was reported by Schultz et al. [39] in our laboratory in the intact rat heart. The rats were exposed to three brief infusions of morphine (100 μg/kg i.v.), an opioid agonist, interspersed with 5 min periods of drug washout prior to the 30-min period of index ischemia and 2 h of reperfusion. This drug protocol was associated with a nearly similar reduction in infarct size as that seen after IPC (Fig. 3). The effect of both IPC and morphine was blocked by naloxone, which suggests that their effects were opioid-receptor mediated. These cardioprotective effects were also blocked by glibenclamide, a nonselective K_{ATP} channel antagonist [39].

Morphine is classically thought to be a μ-opioid receptor agonist, when considering its analgesic effects, however, there is evidence that morphine also possesses important effects on the δ- and κ-opioid receptors and that cross-talk between μ and δ receptors can occur [40]. Therefore, to test the hypothesis that the cardioprotective effect of IPC and morphine were the result of δ receptor stimulation, we administered the selective δ receptor antagonist, naltrindole, to rats prior to IPC or morphine administration. In both cases, their cardioprotective effects were completely abrogated by a dose of naltrindole, which had no effect by itself in nonpreconditioned rats. These data suggest that both IPC and morphine infusions are exerting their beneficial effects by activating the δ-opioid receptor in the in vivo rat heart. In further support of this hypothesis, Sigg et al. [41] recently showed that the general δ-opioid receptor agonist, DPDPE (D-

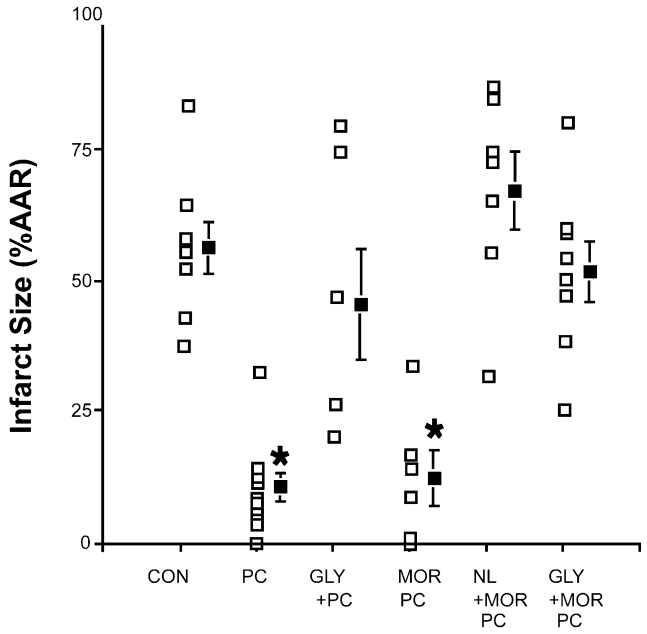

Fig. 3. Infarct size (IS) expressed as a percentage of the area at risk (AAR) in rat hearts subjected to vehicle (CON group), ischemic preconditioning (PC group), glibenclamide (0.3 mg/kg i.v.) given 30 min prior to ischemic PC (GLY + PC group), morphine-induced PC (3 × 100 μg/kg, 5-min i.v. infusion, MOR PC group), naloxone (3 mg/kg i.v.) given 10 min before MOR-induced PC (NL + MOR PC group) and glibenclamide (0.3 mg/kg i.v.) given 30 min before MOR-induced PC (GLY + MOR PC group). Open boxes represent IS/AAR (%) from individual hearts. Closed boxes represent mean IS/AAR (%) in each group. Values are the mean ± S.E.M. * $P < 0.05$ vs. CON. Reproduced from Schultz et al. [39] with the permission of Lippincott, Williams and Wilkins.

Pen^{2,5}-enkephalin) or deltorphan-D, a selective δ_2 agonist, both reduced infarct size in the swine heart. Interestingly, another δ-opioid agonist, DADLE, had no effect on infarct size in this model.

Although the results of these studies all suggest that morphine and endogenous opioid peptides are producing their effects directly on the cardiac myocyte, it is still possible that these compounds are producing some of their beneficial effects on other noncardiac cells, such as leukocytes or macrophages or autonomic nerves innervating the intact heart. With this in mind, Liang and Gross [42] addressed this possibility by performing studies on an isolated embryonic chick myocyte model of hypoxia/reoxygenation injury. These investigators found that a 5-min exposure to different concentrations of morphine (0.1–10 μM) produced a concentration-dependent cardioprotective effect that was maximal at 1 μM and was equivalent to that of hypoxic preconditioning in this model. This effect of morphine was blocked by naloxone and 7-benzylidenenaltrexone (BNTX), a selective δ_1-opioid receptor antagonist. These data agree with those of Schultz et al. [39] and provide direct evidence that morphine appears to be reducing infarct size by activating a δ_1-opioid receptor and not a μ receptor in these cardiac myocytes. Similar results were obtained by McPherson and Yao [43] in a similar chick myocyte model subjected to hypoxia/reoxygenation. These authors incubated the myo-

cytes with morphine or BW 373U86, a selective nonpeptide δ opioid agonist 10 min prior to hypoxia and found that both compounds reduced the incidence of cell death as quantitated by propidium iodide staining. These beneficial effects were blocked by naloxone, BNTX and the mitochondrial K_{ATP} (mito-K_{ATP}) channel blocker 5-hydroxydecanoic acid (5-HD). These results agree with our earlier studies in the intact rat heart and suggest that opioids produce cardioprotection via activation of a δ-opioid receptor and ultimately the mito-K_{ATP} channel. McPherson and Yao [43] also suggested an important role for oxygen-derived free radicals (ODFRs) in triggering this protective response.

6. Evidence that a δ_1-opioid receptor subtype is responsible for acute opioid-induced cardioprotection

Although our initial studies with morphine and IPC suggested that the δ-opioid receptor was responsible for their beneficial effects, both κ and δ receptors have been shown to be present on cardiac myocytes [4–6]. Therefore, Schultz et al. [44] performed a detailed study in the rat heart to determine the opioid receptor responsible for the protection afforded the heart by IPC. Two doses of BNTX, a selective δ_1 antagonist, or naltriben, a selective δ_2-opioid antagonist were administered just prior to IPC. Naltriben had no effect on the protective effect of IPC, however, BNTX produced a dose-related decrease in the infarct size reduction produced by IPC, although the blockade was not complete. However, the protective effect of IPC was not blocked to any extent by pretreatment with the μ or κ selective antagonists, β-funaltrexamine (β-FNA) or nor-binaltorphine (nor-BNI), respectively. These data suggest that the δ_1-opioid receptor appears to be the primary opioid receptor involved in IPC in intact rat hearts. In agreement with these results obtained in the intact rat heart, Huh et al. [45] found that the δ_1 selective opioid agonist, TAN-67 produced a potent cardioprotective effect in chick embryonic myocytes, an effect which was blocked by BNTX but not naltriben or nor-BNI, the δ_2 selective antagonist or κ antagonist, respectively. Similar results have been reported in human and rat myocardium by Aitchison et al. [46] and Tsuchida et al. [47]. In contrast, Wang et al. [48] studied the importance of δ and κ receptors in the mediation of IPC in an isolated perfused rat heart preparation and demonstrated that both receptors mediated IPC as indicated by infarct size reduction, however, only κ receptor activation elicited a pronounced anti-arrhythmic effect. Yu et al. [49] also found that the κ agonist U50,448H possessed an anti-arrhythmic effect in isolated perfused rat hearts which was mediated via a reduction in cyclic AMP concentrations. In contrast, Xia et al. [50] found that IPC increased the ventricular fibrillation threshold (VFT) in rat hearts and that this beneficial effect was associated with a decrease in the affinity of κ receptor binding which would suggest that κ receptor activation may be pro-arrhythmic, a finding at odds with the results of Wang et al. [48] and Yu et al. [49]. Further studies are needed to address the role of κ receptors in arrhythmias.

7. Role of κ-opioid receptors in acute myocardial protection

There is less evidence to support a role for the κ-opioid receptor as opposed to the δ-opioid receptor in mediating the beneficial effects of acute IPC to reduce infarct size. In fact, Aitchison et al. [46] found that a κ-opioid agonist, bremazocine, actually increased infarct size in isolated rat hearts, an effect which was blocked by nor-BNI. On the other hand, as mentioned above, Wang et al. [48] preconditioned the isolated rat heart with two 5-min periods of ischemia interspersed with 5 min of reperfusion prior to a 30-min period of ischemia and determined infarct size. IPC produced a marked reduction in infarct size, an effect, which was attenuated with either the selective κ-opioid antagonist, nor-BNI or by the selective δ-opioid antagonist, naltrindole. U50,448H, a selective κ-opioid receptor agonist or DADLE, a selective δ-opioid agonist, both produced marked reductions in infarct size. Based on these findings, these authors concluded that both δ- and κ-opioid receptors are involved in IPC in the isolated rat heart. Based upon the conflicting results of Aitchison et al. [46] and Wang et al. [48], further studies are needed to clarify the relative importance of these two receptors in acute cardioprotection and IPC in different animal species. One possibility for the differences in the results of these two studies may be related to the use of different κ agonists, U50,448H and bremazocine. Although U50,448H is a selective κ agonist, it is also known to inhibit L-type calcium channels in ventricular myocytes independent of its opioid receptor binding properties [51]. This effect might be partially responsible for at least a portion of its cardioprotective effect, an action bremazocine has not been shown to possess. A comparative study of these two agonists is needed to resolve this possibility.

8. Signaling pathways mediating acute δ-opioid receptor-induced cardioprotection

The results of initial studies performed by Schultz et al. [39,44], Liang and Gross [42], Huh et al. [45] and McPherson and Yao [43] suggested that IPC and δ-receptor-induced cardioprotection were acting via the δ_1-opioid receptor and the mito-K_{ATP} channel to produce their potent infarct size-reducing effects. Additional studies with pertussis toxin in intact rats showed that the cardioprotective effect of IPC and δ_1-opioid receptor activation with TAN-67 were both mediated via a G_i-protein-coupled receptor [52]. Another early event in the signaling pathway produced by IPC and several agonists of G_i-protein-coupled receptors, including the δ_1-opioid receptor, appears to be the generation of a small burst

of ODFRs. Concerning opioids, McPherson and Yao [43] demonstrated in their chick myocyte model that the cardioprotective effect of morphine was blocked by pretreatment with 2-mercaptopropionyl glycine (2-MPG), a thiol reductant and ODFR scavenger. Similar results have been obtained with morphine, acetylcholine and bradykinin but not adenosine in isolated rabbit hearts by Pain et al. [53]. Taken together, these data strongly support the hypothesis that ODFRs are an integral part of the trigger phase of hypoxic or ischemic PC or δ-opioid-induced myocardial protection.

To further address potential signaling pathways involved in opioid-induced protection, Miki et al. [54] observed that morphine produced a cardioprotective effect in isolated rabbit hearts that was blocked by pretreatment with chelerythrine, a nonselective protein kinase C (PKC) inhibitor at a concentration which had no effect on infarct size in nondrug treated hearts.

Subsequently, Fryer et al. [55] extended these findings to the intact rat heart and found that the effect of TAN-67 to reduce infarct size was abolished by chelerythrine and GF 109203X, two PKC inhibitors that act on different binding sites on PKC to produce an inhibitory effect. TAN-67 was also shown by immunohistochemistry to produce a selective translocation of the PKC-δ isoform to rat heart mitochondria. In addition, Fryer et al. [55] showed that the cardioprotective effect of TAN-67 and the translocation of PKC-δ were blocked by a selective inhibitor of PKC-δ, rottlerin. Fryer et al. [56,57] further demonstrated the importance of tyrosine kinase (TK) and the mitogen-activated protein kinase (MAPK) family in opioid-induced cardioprotection. Fryer et al. [58] found that the TK inhibitor, *genistein*, but not lavendustin A or PP2, two *Src TK* inhibitors, and the MEK-1 inhibitor, PD 098059 [57], which attenuates the phosphorylation of the extracellular-regulated kinase (ERK-1/2) and its activation, blocked opioid-induced cardioprotection elicited by TAN-67. All of these data clearly indicate that a *nonSrc-dependent* TK, ERK-1/2 and PKC-δ are integral components of the signaling pathway involved in δ-opioid-induced infarct size reduction.

Finally, a series of experiments were performed in our laboratory to determine the role of the sarcolemmal K_{ATP} (sarc-K_{ATP}) channel and the mito-K_{ATP} channel in mediating the cardioprotection produced by TAN-67, a δ_1-opioid receptor agonist [59] or IPC in rats. Administration of the selective sarc-K_{ATP} antagonist, HMR 1098 did not block the beneficial effects of TAN-67 or IPC to reduce infarct size or preserve mitochondrial ATP synthesis rates, however, treatment with 5-HD, the selective mito-K_{ATP} blocker, abrogated TAN-67 and IPC-induced cardioprotection. These data clearly suggest that δ_1-opioid receptor-induced preconditioning and IPC are both mediated via the mito-K_{ATP} channel in rat hearts. A schematic summary of the similarities and differences we have observed in acute δ-opioid and IPC-induced cardioprotection in intact rat hearts are shown in Fig. 4.

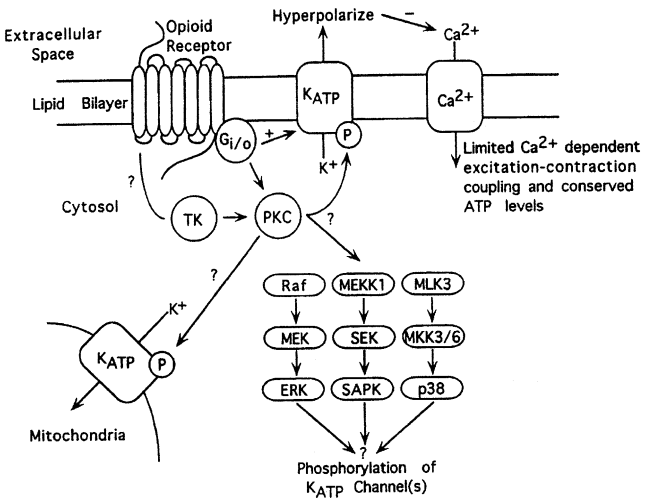

Fig. 4. A schematic diagram of some of the major pathways thought to be involved in acute opioid-induced cardioprotection.

9. Signaling pathways mediating acute κ-opioid receptor-induced cardioprotection

The signaling pathways, which mediate κ-opioid receptor-induced cardioprotection have been less well studied as compared to the pathways involved in δ-opioid receptor-induced protection. Ventura et al. [60] initially showed that U50,448H increased both inositol 1,4,5-triphosphate (IP_3) and cytosolic-free calcium in rat ventricular myocytes and that stimulation of phospholipase C (PLC) was also involved. Subsequently, Sheng et al. [61] observed that pertussis toxin abolished these effects of U50,448H, which suggests the involvement of a $G_{i/o}$ protein in transducing these effects of κ receptor stimulation. Zhang and Wong [62] also showed that U50,448H suppressed the accumulation of cyclic-AMP via the phosphoinositol/calcium pathway in rat ventricular myocytes. Wenzlaff et al. [63] demonstrated that κ receptor stimulation with either U50,448H or the endogenous peptide dynorphin A reduced cell shortening in electrically driven rat ventricular myocytes and that these effects were blocked by pretreatment with pertussis toxin, naloxone and nor-BNI. DADLE, a μ-and δ-opioid agonist had no demonstrable effect on contractility in these myocytes. These investigators suggested that the endogenous release of dynorphins from the heart during hypoxia or ischemia may protect the heart similar to that already described for another endogenous mediator adenosine. Two recent papers by Pyle et al. [64,65] support this hypothesis presented by Wenzlaff et al. [63]. Pyle et al. [64,65] showed in isolated rat hearts that U50,448H decreased the actin–myosin cycling rate in myocytes, which would be expected to lead to a conservation of ATP and a cardioprotective effect during ischemia. These investigators [65] also showed that this effect was partially mediated via a PKC-activated pathway, which led to a reduction of actomyosin-induced ATP consumption. The importance of these specific pathways as well as others already demonstrated to be involved in acute IPC and δ-opioid receptor-induced cardioprotection remain

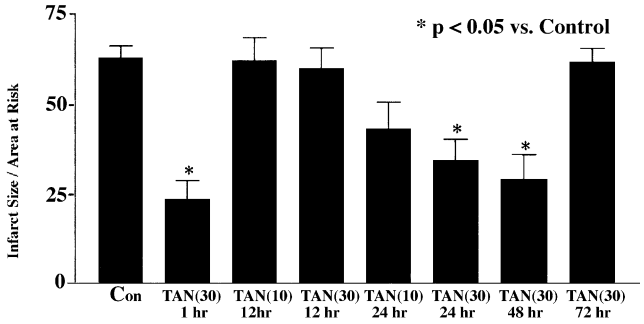

Fig. 5. Infarct size (IS) expressed as a percentage of the area at risk (AAR) in rats pretreated with 10 or 30 mg/kg of TAN-67, either 1, 12, 24, 48 or 72 h before subjecting the hearts to 30 min of ischemia and 2 h of reperfusion. A 1-h pretreatment with TAN-67 produced a significant reduction of IS/AAR. Pretreatment with both doses of TAN-67 12 h prior to ischemia/reperfusion or low-dose TAN-67 24–48 h prior to ischemia had no significant effect on IS/AAR. However, pretreatment with the large dose of TAN-67 24–48 h prior to ischemia/reperfusion significantly reduced IS/AAR. This cardioprotective effect was lost following 72 h of pretreatment. All values are the mean ± S.E.M. * $P < 0.05$ vs. control. Reproduced from Fryer et al. [71] by permission of Lippincott, Williams and Wilkins.

to be determined in acute κ-opioid receptor-induced cardioprotection.

10. Opioid-induced delayed cardioprotection via activation of δ- or κ-opioid receptors

Baxter et al. [66] first demonstrated delayed preconditioning or the SWOP in rabbit hearts 24 h following an IPC protocol or the administration of the selective adenosine A_1 receptor agonist, 2-chloro-N^6-cyclopentyladenosine (CCPA), respectively. Subsequently, this same group and others demonstrated [67,68] that the reduction in infarct size produced by IPC and CCPA was associated with a translocation of PKC to the nucleus and the transcription of cytoprotective proteins, such as certain heat-shock proteins (HSPs) and the inducible isoform of nitric oxide synthase (iNOS). Regarding opioids, Ventura et al. [69] first showed that κ-opioid receptor stimulation resulted in a translocation of PKC to the nucleus of cardiac myocytes and a resultant increased synthesis of opioid peptides. Gustein et al. [70] demonstrated that opioids produced the activation of ERK and p38 MAPK, two kinases thought to be involved in delayed IPC. Based on these findings, Fryer et al. [71] was the first to demonstrate delayed IPC with opioids when he administered TAN-67 to rats and noted a significant reduction in infarct size 24 and 48 h after drug injection (Fig. 5). The cardioprotective effects of TAN-67 were blocked by pretreatment with BNTX, the selective δ_1-opioid receptor antagonist and by glibenclamide and 5-HD. These results suggest that opioids can produce both an acute and delayed cardioprotective response in rats that is produced by a δ_1-mito-K_{ATP} channel linked signaling pathway. Bolli's group has recently confirmed the importance of the δ-opioid receptor in a conscious rabbit model of delayed cardioprotection [72]. Similarly, Wong's group [73–75] also demonstrated that κ-opioid receptor stimulation produced by metabolic inhibition with cyanide and 2-deoxy-D-glucose or direct receptor stimulation with U50,448H produces a delayed cardioprotective effect in rat myocytes 16–20 h following the metabolic insult or drug administration. This delayed protection was shown to be partially dependent on the PKC-ϵ isoform [74] and on the inducible HSP, HSP70 [75]. Thus, these results suggest that activation of both δ- and κ-opioid receptors are capable of resulting in both acute and delayed cardioprotection, however, further studies are necessary to determine if these receptors use similar signaling pathways to produce their beneficial effects in different species and models of ischemia/reperfusion injury.

More recently, Patel et al. [76] investigated the signaling pathways responsible for the delayed reduction in infarct size following the injection of several nonpeptide δ-opioid agonists in our rat infarct model. Patel et al. [76] discovered that 0.1 mg/kg (i.v.) of TAN-67 and BW373U86 both produced a delayed reduction in infarct size and surprisingly, these effects were only partially antagonized by the selective δ-opioid antagonists, naltrindole or BNTX. Similarly, naloxone, which blocks all the three opioid receptors, did not totally antagonize the cardioprotective effects of BW373U86 or TAN-67. On the other hand, pretreatment with the ODFR scavenger, 2-MPG, completely abolished the protective effects of both δ agonists. These data suggest that ODFRs serve as triggers of this delayed cardioprotective response similar to data previously published by several other groups studying both acute and delayed IPC produced by either ischemia or pharmacological agents [44,53].

These results additionally suggest that nonpeptide opioid agonists, such as TAN-67 or BW373U86, may produce part of their beneficial cardiac effects independently of their effects on classical opioid receptors as suggested by the earlier work of Zhu et al. [77] in opioid receptor knockout mice.

Patel et al. [78] further characterized the signaling pathways involved in delayed opioid-induced infarct size reduction. Surprisingly, these investigators observed that the sarc-K_{ATP} channel is important as a trigger and that the mito-K_{ATP} channel serves as a distal effector in opioid-induced cardiac protection. Evidence is also accumulating to suggest that iNOS, cyclooxygenase 2 (COX-2) and 12-lipoxygenase (12-LO) are three additional downstream effectors involved in opioid-induced delayed protection [72,79]. A schematic diagram depicting some of the key signaling events thought to occur in delayed cardiac protection following opioid administration is shown in Fig. 6 .

11. Opioid receptors and cardioprotection in man

In addition to these intriguing results which suggest that opioids produce potent cardioprotective effects in various animal models, there is also some interesting data obtained in human cardiac tissue and results obtained in clinical studies to suggest that opioid receptors may play an important role to protect the human myocardium during times of stress. Bell et

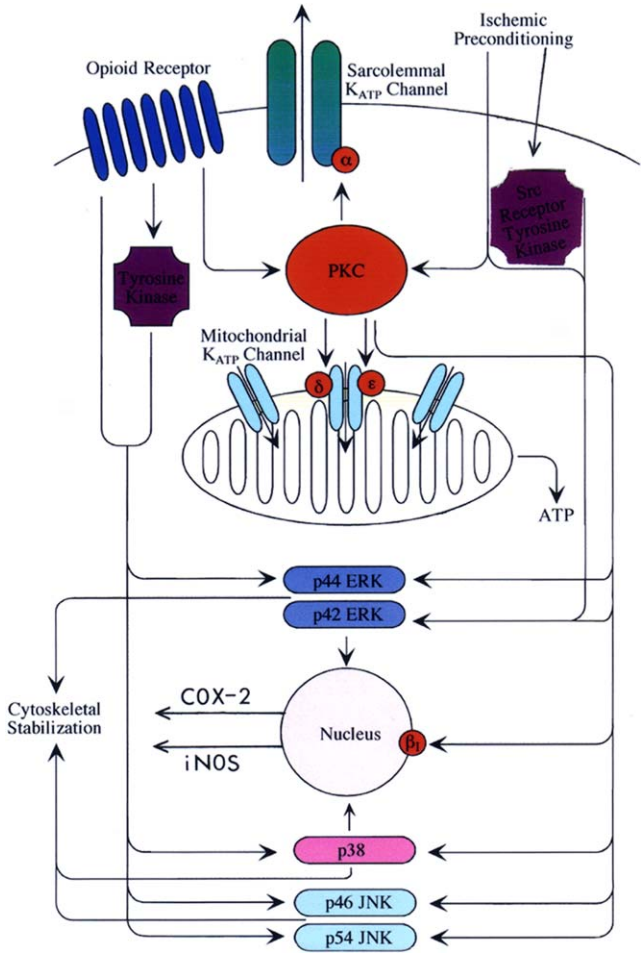

Fig. 6. A schematic diagram of some of the key pathways thought to be involved in delayed opioid-induced cardioprotection.

al. [80] recently showed that messenger RNA encoding the δ-opioid receptor was found in atrial and ventricular biopsies obtained from patients undergoing coronary artery bypass surgery. Using isolated atrial trabeculae obtained from these same patients, these investigators were able to demonstrate that hypoxic preconditioning and the δ-opioid agonist, DADLE, were able to protect atrial tissue from the damage incurred when the tissue was exposed to a simulated ischemia and reoxygenation protocol. The effects of the two interventions were of a similar magnitude and were both blocked by the δ-opioid antagonist, naltrindole, and by the mito-K_{ATP} channel blocker, 5-HD. These data clearly suggest that similar signaling pathways are involved in the human myocardium to those that exist in animal models that have been previously used to study opioid-induced cardiac protection. Similarly, Tomai et al. [81] found that naloxone blocked the adaptation to ischemia, which has been shown to occur in patients undergoing repeated periods of coronary balloon angioplasty (PTCA). Similarly, Xenopoulos et al. [82] demonstrated that intracoronary administration of morphine mimics IPC in man, as assessed by changes in the ST segment of the electrocardiogram. These results, although preliminary, suggest that opioids may have the potential for treating acute or chronic myocardial ischemia in man.

Another area of cardiovascular medicine where opioids may be useful is in the arena of organ transplantation. Bolling et al. [83,84] have demonstrated that DADLE protects hearts from damage following 18 h of cold storage at 4 °C or from global ischemia in the presence of a standard cardioplegic solution. Furthermore, Kevelaitus et al. [85] demonstrated that activation of δ-opioid receptors resulted in an improved recovery of function in cold-stored rat hearts similar to the protection provided by IPC. This group also showed that this cardioprotective effect was mediated by the K_{ATP} channel.

12. Conclusions

In conclusion, the results obtained from numerous animal and human studies suggest that stimulation of either the δ- or κ-opioid receptor produces an acute as well as a delayed cardioprotective effect and that this effect is mediated via signaling pathways similar to those thought to be responsible for acute or delayed IPC. Based upon these intriguing findings, additional animal and human studies are warranted using more selective and perhaps more peripherally acting opioid receptor agonists to further determine the clinical potential of these agents for treating the ischemic myocardium. Since several of these opioid agonists are already being used clinically as analgesics, a prolonged period of time may not be necessary before bringing this novel approach from the laboratory setting to the bedside.

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