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Opioids and cardioprotection

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Abstract

Opioid peptides and exogenous opioids such as morphine are known to exert important cardiovascular effects. However, until recently, it was not appreciated that activation of specific receptors results in a potent cardioprotective effect to reduce infarct size in experimental animals and to reduce cell death in isolated cardiomyocytes. In intact rat and rabbit hearts, nonselective opioid receptor antagonists such as naloxone and a selective δ_1-opioid receptor antagonist, 7-benzylidenenaltrexone, have been shown to inhibit the cardioprotective effect of ischemic preconditioning, a phenomenon in which brief periods of ischemia protect the heart against a more prolonged period of ischemia. Selective δ_1 specific agonists such as 2-methyl-4a-α-(3-hydroxyphenyl)-1,2,3,4,4a,5,12,12a-α-octahydroquinolino[2,3,3-g]isoquinoline have been shown to exert potent cardioprotective effects in intact animals and cardiac myocytes via activation of $G_{i/o}$ proteins, protein kinase C, and ultimately, the mitochondrial K_{ATP} channel. These protective effects occur immediately following drug administration, and reappear 24–48 hr post treatment. Although further studies are needed to more clearly define the mechanisms by which opioids exert their cardioprotective effects, the data accumulated and summarized in this review suggest that this class of drugs may not only be useful in alleviating the pain associated with a myocardial infarction, but may also be simultaneously reducing the size of the ultimate infarct. Since many of these drugs are already clinically available, a long period of drug development may not be necessary before the use of these drugs reaches the patient with signs of myocardial ischemia.

Keywords: Ischemia; Opioid peptides; Preconditioning; Infarct size; K_{ATP} channels; Protein kinase

Abbreviations: BNTX, 7-benzylidenenaltrexone; DADLE, [D-Ala^2-D-Leu^5]-enkephalin; DPDPE, [D-Pen(2),D-Pen(5)]-enkephalin; 5-HD, 5-hydroxydecanoic acid; HIT, hibernation induction trigger; HRF, hibernation-related factor; IPC, ischemic preconditioning; MAPK, mitogen-activated protein kinase; ME, Met^5-enkephalin; PC, preconditioning; TAN-67, 2-methyl-4a-α-(3-hydroxyphenyl)-1,2,3,4,4a,5,12,12a-α-octahydroquinolino[2,3,3-g]isoquinoline; PKC, protein kinase C; ppENK, preproenkephalin; PTX, pertussis toxin; TK, tyrosine kinase.

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1. Introduction

In 1986, Murry and colleagues described a cardioprotective phenomenon termed ischemic preconditioning (IPC), in which brief periods of coronary artery occlusion prior to a prolonged ischemic insult prevented irreversible tissue damage and slowed the rate of ATP depletion. This phenomenon has been observed in many species, including pigs (Schott et al., 1990), rabbits (Liu et al., 1991), dogs (Gross & Auchampach, 1992), rats (Liu & Downey, 1992), ferrets (Gomoll, 1996), and possibly humans (Tomai et al., 1994; Ottani et al., 1995). This cardioprotective effect can be separated into a classical, acute phase that lasts for only 30 min to 2 hr, depending on the species, and a delayed phase that disappears after the acute phase, but reappears 12–24 hr later, and may persist for several days (Yellon & Baxter, 1995).

In recent years, a great deal of interest has focused on the phenomenon of IPC and the mechanisms by which its potent cardioprotective effect occurs. This fascinating observation has stimulated numerous studies to determine potential mediators and/or modulators of this myocardial protection. Initially, Liu and co-workers (1991) showed that IPC protected against myocardial infarction and that this effect was mediated by adenosine Type 1 receptors in the rabbit. Gross and Auchampach (1992) were the first to demonstrate that PC was mediated through the K_{ATP} channel in the canine heart. Since those initial discoveries into the mechanism of IPC, a number of other important mediators or modulators, including nitric oxide (Yao & Gross, 1993; Richard et al., 1995; Bolli et al., 1997; Qiu et al., 1997), protein and tyrosine kinases (TKs) (Ytrehus et al., 1994; Speechly-Dick et al., 1994; Maulik et al., 1996; Imagawa et al., 1997; Fryer et al., 1999b), G-proteins (Lasley & Mentzer, 1993; Thornton et al., 1993; Schultz et al., 1998a), mitochondrial K_{ATP} channels (Garlid et al., 1997), and opioids (Schultz et al., 1995, 1996, 1997b, 1998b; Chien & Van Winkle, 1996), have been implicated in the mechanism(s) of IPC and cardioprotection.

2. Opioid receptors

The idea of multiple opioid receptors is an accepted concept, and a number of subtypes for each class of opioid receptors has been identified (Lord et al., 1977; Miller, 1982; Paterson et al., 1983; Goldstein & James, 1984). Through biochemical and pharmacological methods, the μ-, δ-, and κ-opioid receptors have been characterized (Martin, 1983; Paterson et al., 1983; Dhawan et al., 1996). Pharmacologically, it is well known that δ-opioid receptors consist of two subtypes, δ_1- and δ_2-opioid receptors (Jiang et al., 1991; Mattia et al., 1991; Sofuoglu et al., 1991). Recently, the μ-, κ-, and δ-opioid receptors have been cloned, and experimental results indicate that this class belongs to the family of G-protein-coupled receptors (Evans et al., 1992; Kieffer et al., 1992; Chen et al., 1993; Minami et al., 1993; Yasuda et al., 1993).

Opioid receptors involved in regulating the cardiovascular system have been localized centrally to the cardiovascular and respiratory centers of the hypothalamus (Hokfelt et al., 1977; Goodman et al., 1980; May et al., 1989; Mansour & Watson, 1993) and brainstem (Atweh & Kuhar, 1977; Hokfelt et al., 1977; Goodman et al., 1980; Ramirez-Gonzalez et al., 1983; May et al., 1989; Mansour & Watson, 1993) and peripherally to cardiac myocytes (Krumins et al., 1985; Ventura et al., 1989; Tai et al., 1991; Zhang et al., 1996; Zimlichman et al., 1996; Wittert et al., 1996), blood vessels (Peroutka et al., 1980), nerve terminals (Zunkin & Zunkin, 1981), and adrenal medulla (Quirion et al., 1983; Wittert et al., 1996). Myocardial binding studies have shown that κ- and δ-opioid receptors are present on adult ventricular myocytes of the rat (Krumins et al., 1985; Ventura et al., 1989; Tai et al., 1991; Zhang et al., 1996; Zimlichman et al., 1996). Ventura and colleagues (1989) demonstrated that κ- and δ-opioid receptors were located on the ventricular cardiac sarcolemma of the rat. Similarly, Krumins and colleagues (1985) revealed that κ- and δ-opioid receptors, but not μ-opioid receptors, were found on rat atrial and ventricular tissue. Developmental studies of Zimlichman and colleagues (1996) showed the presence of κ- and δ-opioid receptors in adult rat heart, whereas, only μ- and κ-opioid receptors were present in neonatal rat hearts. In addition, functional contractile studies on adult rat ventricular cardiac myocytes demonstrated the presence of κ- and δ-opioid receptors, but not μ-opioid receptors (Ventura et al., 1992). In support of the functional studies, Wittert and colleagues (1996) failed to detect μ-opioid receptor gene expression; however, the δ-opioid receptor transcript was the predominant form detected in the rat heart.

In neuronal tissue, μ- and δ-opioid receptors have been shown to be linked via a G-protein to several K^+ channels (North et al., 1987; Childers, 1991; Cox, 1993; Ikeda et al., 1995), whereas, the κ-opioid receptor has been shown to interact with Ca^{2+} channels (Werz & Macdonald, 1984, 1985; North, 1986, 1993). Evidence has suggested that μ- and δ-opioid receptors differentially couple to G_i and G_o protein subtypes in human neuroblastoma SH-SY5Y cells (Laugwitz et al., 1993). Chen and Yu (1994) demonstrated that co-expression of δ-opioid receptors and a G-protein-activated K^+ channel isolated from atrial cells stimulated the induction of an inward-rectifying K^+ current, which was regulated differently by protein kinase C (PKC) and protein kinase A. Mura and Niroomand (1996) provided evidence that μ- and δ-opioid receptors are present on canine cardiac sarcolemma and inhibit adenylate cyclase activity via activation of G_i proteins. However, this group (Niroomand et al., 1996) suggested that κ-opioid receptors may not be present on canine cardiac sarcolemma, since the κ receptor agonist U50488H did not inhibit adenylate cyclase activity nor stimulate high-affinity GTPase at any concentration studied.

The signaling pathway most characterized and studied is opioid receptor inhibition of adenylate cyclase via $G_{i/o}$ proteins in which all three receptor subtypes have been shown to decrease cyclic AMP production (Sharma et al., 1977; Attali et al., 1989; McKenzie & Milligan, 1990; Niroomand et al., 1996). There is evidence for another second messenger system involving phosphoinositol turnover and κ- and δ-opioid receptors (Periyasamy & Hoss, 1990; Ventura et al., 1991, 1992; Jin et al., 1994; Sheng et al., 1996; Bian et al., 1998). For example, κ-opioid receptors, but not μ- or δ-opioid receptors, have been shown to mediate phosphoinositide turnover in rat brain (Periyasamy & Hoss, 1990). Ventura and co-workers (1992) showed that κ- and δ-opioid receptors, but not μ-opioid receptors, have a negative inotropic action that is mediated by phosphatidylinositol turnover and depletion of Ca^{2+} from intracellular pools in rat ventricular myocytes. Subsequently, Sheng and colleagues (1996) demonstrated in rat ventricular myocytes that δ-opioid receptor stimulation produces a mobilization of intracellular Ca^{2+} secondary to the increase in inositol 1,4,5-triphosphate. However, many electrophysiological studies have demonstrated that opioid receptor stimulation directly regulates ion channels via interaction with G-proteins (i.e., μ- and δ-opioid receptor agonists in opening K^+ channels and κ-opioid receptor agonists in closing Ca^{2+} channels) and not through second messengers (North et al., 1987; Gross et al., 1990).

There is accumulating evidence to support the existence of an intrinsic opioid receptor system in the heart that may contribute to functional changes in normal and diseased myocardium. A number of physiological and pathological cardiovascular responses of δ-opioid receptor stimulation include attenuation of cardiac adrenergic responses (Ruth & Eiden, 1984; Mantelli et al., 1987; Xiao et al., 1997), inhibition of vagal bradycardia induced by acetylcholine release (Caffrey et al., 1995), suppression of the baroreceptor mechanism (Giles et al., 1987), reduction in cardiac performance (Clo et al., 1985; Vargish & Beamer, 1989), and increases in inotropy, chronotropy, and blood pressure (Schaz et al., 1980; Sander et al., 1981; Laurent et al., 1985). κ-Opioid receptor activation has been implicated in arrhythmogenesis in normal and ischemic hearts (Sitsapesan & Parratt, 1989; Wong et al., 1990; Wu et al., 1993; Lishmanov et al., 1997, 1999), in influencing cardiac function (Mantelli et al., 1987; Ventura et al., 1992; Ela et al., 1997; Sheng et al., 1997; Wenzlaff et al., 1998), and in inhibiting norepinephrine release (Gu et al., 1992). The lack of μ-opioid receptor activity in the heart has been supported by a number of receptor-binding studies in ventricular myocytes (Krumins et al., 1985; Ventura et al., 1989).

3. Interactions of opioids and K_{ATP} channels

Opioid receptors have been demonstrated to be linked to K^+ channels via G_i proteins (North et al., 1987; Childers, 1991; Cox, 1993; Chen & Yu, 1994; Ikeda et al., 1995). Neuronal K_{ATP} channels have been shown to mediate antinociception induced by μ- and δ-opioid receptor stimulation (Ocana et al., 1990, 1993, 1995; Wild et al., 1991; Welch & Dunlow, 1993; Robles et al., 1994; Raffa & Martinez, 1995; Kang et al., 1997). Wild and co-workers (1991) demonstrated that the antinociceptive effect of δ-opioid receptors was mediated via K^+ channels, and the subtypes of this receptor were linked to different K^+ channels. Their results demonstrated that the analgesia produced by the δ_1-opioid receptor agonist [D-Pen(2),D-Pen(5)]-enkephalin (DPDPE) could be antagonized by glibenclamide, the K_{ATP}-channel blocker, indicating that the δ_1-receptor subtype was linked to neuronal K_{ATP} channels (Wild et al., 1991). However, the antinociceptive effect of deltorphin II, a δ_2-opioid receptor agonist, was not blocked by glibenclamide, but was antagonized by tetraethylammonium bromide, a voltage-gated K^+-channel blocker, which demonstrates that the δ_2-receptor subtype was linked to K^+ channels other than the K_{ATP} channel (Wild et al., 1991).

Similarly, opioid effects on gall bladder emptying (Patil & Thakker, 1996) and hypoxia-induced pial artery vasodilation (Shankar & Armstead, 1995; Armstead, 1998, 1999) have been demonstrated to involve K_{ATP} channels. Patil and Thakker (1996) showed that glibenclamide antagonized the inhibitory effect of morphine on gall bladder motility. In addition, Shankar and Armstead (1995) provided evidence that δ opioids (methionine- and leucine-enkephalins and the synthetic μ- and δ-opioid receptor agonists [D-Ala2,N-Me-Phe4,Gly-ol5]-enkephalin and DPDPE, respectively) mediated pial artery vasodilation induced by hypoxia, and this opioid action was blocked by glibenclamide. Recent work from Armstead (1999) indicates that the endogenous ligand nociceptin/orphanin FQ, for the opioid-like receptor-1, elicits pial artery dilation via cyclic AMP, K_{ATP}, and $K_{ca}{}^{2+}$-channel activation.

The involvement of cardiac opioid receptors and K_{ATP} channels will be discussed in more detail in the following sections. However, there is accumulating evidence that the cardioprotective effects of opioid receptors in limiting ischemic damage (Schultz et al., 1996, 1998c; Fryer et al., 1999a; Kevelaitis et al., 1999) and arrhythmias (Kita et al., 1998) are mediated via activation of sarcolemmal and/or mitochondrial K_{ATP} channels.

4. Opioid peptides in the heart

As with the opioid receptors, opioid peptides have been found in peripheral cardiovascular organs, such as heart (Lang et al., 1983; Barron et al., 1992; Boluyt et al., 1993; Caffrey et al., 1994), sympathetic nerves (Schultzberg et al., 1979; Yang et al., 1980; Mantelli et al., 1987), and the adrenal medulla (Yang et al., 1980; Lewis et al., 1981; Fleminger et al., 1984; Viveros et al., 1987). The capacity for the heart to synthesize all three types of opioid peptides (enkephalins, endorphins, and dynorphins) has been verified

by demonstrating that the precursors, preproenkephalin (ppEnk), prodynorphin, and proopiomelanocortin genes, are expressed in atrial (Pintus et al., 1994; McLaughlin & Wu, 1998; Millington et al., 1999) and ventricular (Springhorn & Claycomb, 1989, 1992; Forman & Bagasra, 1992; Boluyt et al., 1993; Caffrey et al., 1994; Pintus et al., 1994; Ventura et al., 1994; McLaughlin & Allar, 1998; Weil et al., 1998) cardiac myocytes. In addition, Howells and colleagues (1986) demonstrated that ppEnk mRNA was also found in rat ventricular tissue, and that this type of tissue contained the highest amount of ppEnk mRNA of any other tissue in the rat, including brain. Low and colleagues (1990) showed that proenkephalin, the precursor to enkephalins, was associated with polyribosomes in the heart, suggesting that there was translational capability.

It is well known that opioid peptide levels increase and are ultimately released into the peripheral circulation during situations of stress (Guillemin et al., 1977; Clement-Jones et al., 1980; Lewis et al., 1982; Holaday, 1983; Akil et al., 1984; Howlett et al., 1984). In the heart, opioid peptides (leu- and met-enkephalins), along with their message, have been shown to increase with age (Boluyt et al., 1993; Caffrey et al., 1994; McLaughlin & Allar, 1998; McLaughlin & Wu, 1998), as well as disease (Dumont & Lemaire, 1988; Ouellette & Brakier-Gingras, 1988; Paradis et al., 1992; Forman et al., 1994). Myocardial ischemia has been shown to induce synthesis and release of opioid peptides (Oldroyd et al., 1992; Paradis et al., 1992; Falcone et al., 1993; Miller et al., 1993; Maslov & Lishmanov, 1995; Eliasson et al., 1998). In fact, several studies in humans have demonstrated that levels of circulating β-endorphin are higher in patients with acute myocardial ischemia or those undergoing angioplasty (Oldroyd et al., 1992; Falcone et al., 1993; Miller et al., 1993; Eliasson et al., 1998). It has been speculated that the increased levels of enkephalins in infarcted rat ventricular tissue may be part of a negative feedback to counteract the high amount of catecholamines released during ischemia and may be a compensatory mechanism to minimize the size of an infarcted area (Paradis et al., 1992). In addition, inhibition of the cardiac sympathetic nervous system via opioid receptor stimulation, as demonstrated by Mantelli and co-workers (1987) as well as Xiao and colleagues (1997), may be another potential mechanism of cardioprotection. Therefore, the cardiac effects that are observed under stress (i.e., ischemia) may involve an autocrine process in which opioid peptides may be released from myocytes and interact directly with the myocardial opioid receptor to limit cellular injury by protecting the heart from Ca^{2+} overload.

5. Opioid receptor system in cytoprotection

The endogenous opioid system has been shown to participate in cardiovascular pathophysiology. It is known that opioids are involved in the depressant effects of circulatory shock on baroreceptors (hypotension and hypoventilation) (Holaday, 1983), in congestive heart failure (Liang et al., 1987), and in myocardial ischemia/reperfusion-induced arrhythmias (Zhan et al., 1985; Wong et al., 1990; Lee et al., 1992).

Recent evidence has implicated opioids to be intimately involved in protecting several organs from hypoxic or ischemic insults (Meerson et al., 1987; Mayfield & D'Alecy, 1992, 1994a, 1994b; Chien et al., 1994; Maslov et al., 1996) and other stressors, such as cold or acidic environments (Arrigo-Reina & Ferri, 1980; Ferri et al., 1983, 1988). Furthermore, the δ-opioid receptor has been shown to be involved in protecting tissue from certain stressors, including hypoxia and ischemia (Chien et al., 1994; Mayfield & D'Alecy, 1994a, 1994b).

Ferri and colleagues (Arrigo-Reina & Ferri, 1980; Ferri et al., 1983, 1988) provided evidence that morphine and the synthetic opioid agonist [D-Ala2,MePhe4Met(O)5-ol]-enkephalin had a protective effect against gastric damage in the rat when exposed to cold, restraint, or acidic and basic environments. The opioid receptor system has been implicated in the antiarrhythmic effect of hypoxic or stress adaptation (Meerson et al., 1987; Maslov et al., 1996). Meerson et al. (1987) revealed that adaptation to hypoxia resulted in a significant increase in adrenal gland β-endorphin levels. Mayfield and D'Alecy (1992, 1994a, 1994b) showed that several intermittent hypoxic periods induced an acute adaptation to a subsequent hypoxic challenge in mice, and that the δ_1-opioid receptor mediated this increased tolerance to hypoxia. Maslov et al. (1996) provided evidence that stress adaptation activates δ-opioid receptors and protects the heart from arrhythmias. Lastly, there is accumulating evidence concerning a hibernation induction trigger (HIT) molecule and hibernation-related factor (HRF), a plasma factor found in hibernating animals and identified as a δ-opiate in nature (Oeltgen et al., 1988; Horton et al., 1998), and its potential role in organ protection (Chien et al., 1991, 1994; Oeltgen et al., 1996; Bolling et al., 1997a; Horton et al., 1998). Both HIT and [D-Ala^2-D-Leu^5]-enkephalin (DADLE), a δ-opioid agonist, have been shown to be equally efficacious in extending multi-organ survival time and tissue preservation prior to organ transplantation (Chien et al., 1991, 1994; Oeltgen et al., 1996). Recent evidence demonstrated that HIT, HRF, and DADLE improved left ventricular recovery of function (Bolling et al., 1997b; Horton et al., 1998; Benedict et al., 1999).

6. Opioids and early preconditioning

As previously discussed in Section 5, there are several reports that clearly show that opioids produce a protective effect in the heart and other organs by activating specific opioid receptors (Arrigo-Reina & Ferri, 1980; Ferri et al., 1983, 1988; Meerson et al., 1987; Mayfield & D'Alecy, 1992, 1994a, 1994b; Chien et al., 1994; Maslov et al., 1996).

However, it was not recognized that opioids might be involved in the phenomenon of PC until 1995, when we (Schultz et al., 1995) demonstrated in anesthetized rats that the cardioprotective effect of PC was blocked by the non-selective opioid receptor antagonist naloxone at doses that had no effect on infarct size in nonpreconditioned animals (see Fig. 1). Interestingly, naloxone blocked PC, whether it was administered before or after PC, which suggests that opioid receptor activation serves not only as a trigger of the PC response, but also as a mediator of the memory phase of PC in the rat myocardium. Subsequently, Chien and Van Winkle (1996) showed that the active stereoisomer of naloxone [(−)naloxone] blocked PC in anesthetized rabbit hearts, whereas the inactive stereoisomer [(+)naloxone] had no effect on PC. These observations are consistent with those of Chen and co-workers (1995), who also showed that the (+) isomer of naloxone was without effect in modulating the actions of endogenous opioids in a model of myocardial infarction, whereas the (−) isomer had significant effects.

Since opioid receptors involved in cardiovascular regulation are found in both the CNS (May et al., 1989) and the periphery (Zhang et al., 1996; Zimlichman et al., 1996), the question remained as to whether the PC effect produced by opioids was mediated by central or peripheral mechanisms. To distinguish between these two sites of action, Schultz and colleagues (1997a) selected naloxone, an opioid antagonist that crosses the blood–brain barrier, and its quaternary derivative naloxone methiodide, which does not penetrate the blood–brain barrier, and administered each drug prior to PC, and then subjected the rats to no PC or to a PC protocol that consisted of three 5-min occlusions, separated by 5 min of reperfusion, prior to a prolonged 30-min occlusion period plus 2 hr of reperfusion. Several doses of each antagonist were used. The results showed that a low dose of quaternary naloxone partially blocked the protective effect of PC, whereas a larger dose completely blocked PC. Similar results were found with naloxone. More recently, Chien and co-workers (1999) found similar results in isolated rabbit hearts with a quaternary salt of naloxone, which had time-dependent effects. These results clearly indicate that the cardioprotective effect of PC is mediated by a peripheral opioid receptor mechanism in the intact rat and rabbit heart.

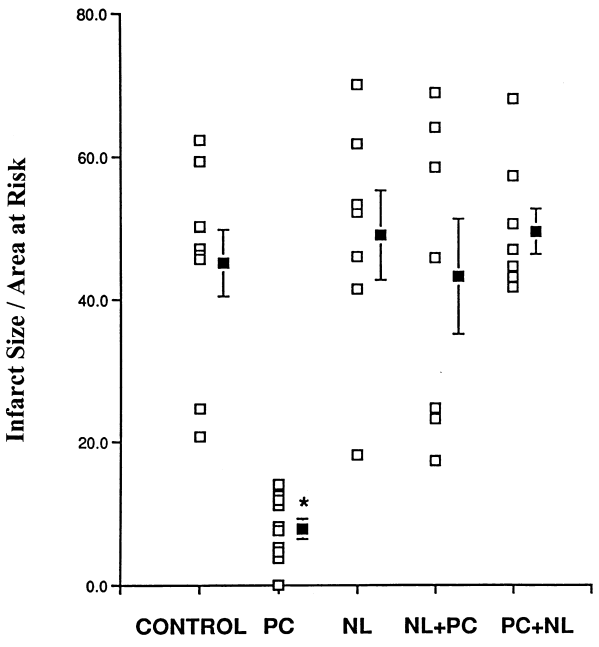

Fig. 1. Infarct size in rat hearts subjected to vehicle (saline) (control), IPC (PC), naloxone without PC (NL), naloxone treatment prior to PC (NL + PC), and naloxone treatment after PC (PC + NL). Filled squares are the mean ± SE of each group. * $P < 0.05$ vs. control group. Open squares are individual points from each heart. AAR, area at risk. Reproduced from Schultz et al. (1995), with permission of the copyright holder, The American Physiological Society, Bethesda.

As mentioned in Section 4, it has been clearly shown that endogenous opioids are released in large amounts in the heart in response to various stimuli, including ischemia (Guillemin et al., 1977; Clement-Jones et al., 1980; Lewis et al., 1982; Holaday, 1983; Akil et al., 1984; Howlett et al., 1984; Oldroyd et al., 1992; Paradis et al., 1992; Falcone et al., 1993; Miller et al., 1993; Maslov & Lishmanov, 1995; Weil et al., 1998; Eliasson et al., 1998), and one group even suggested that the left ventricle of the rat heart behaved as an endocrine organ that supplied the body with endogenously released enkephalins (Weil et al., 1998). Also, as previously discussed in Section 4, all three families of endogenous opioid peptides (enkephalins, endorphins, dynorphins) can be produced in ventricular tissue and isolated myocytes (Howells et al., 1986; Ventura et al., 1994), and two of the three major opiate receptors (κ and δ) are found in high abundance in the adult rodent heart. Based on these findings, Takasaki et al. (1999) recently undertook a study in isolated rabbit myocytes to determine which endogenous opioid peptides are involved in PC. These authors studied the effect of PC and opioid-induced cardioprotection to limit cell death in isolated adult rabbit cardiomyocytes subjected to simulated ischemia and normothermic hypoxia. PC was produced by subjecting the cells to 15 min of simulated ischemia, followed by 15 min of reoxygenation. All cells were then subjected to 180 min of simulated ischemia. These investigators found that both morphine and PC protected the cells, and that these effects were blocked by naloxone. Exogenous Met^5-enkephalin (ME) produced protection; however, β-endorphin did not. Two other proenkephalin products, Leu^5-enkephalin and ME-Arg–Phe, produced cardioprotection similar to that elicited by ME. These results suggested that proenkephalin products are most likely the endogenous mediators of PC, at least in the rabbit heart.

7. Studies with morphine

An initial study was performed by Markiewicz and colleagues (1982) with morphine in rats in which 3 mg/kg

was administered subcutaneously 10 min prior to a permanent coronary artery occlusion. Infarct size was determined 48 hr later by histological techniques. Their results showed that morphine produced a statistically significant increase in infarct size (35.3%–45.8% of the left ventricle). These results suggested that administration of morphine to patients suffering an acute myocardial infarction might lead to a detrimental effect.

In contrast, a more recent study by Schultz and co-workers (1996) showed that intravenous administration of morphine mimicked the effect of PC to reduce infarct size in anesthetized open-chest rats subjected to 30 min of ischemia and 2 hr of reperfusion. In this study, morphine was given by three 5-min infusions of 100 μg/kg (total dose of 300 μg/kg), separated by 5 min of no drug between each infusion. Administration of naloxone or glibenclamide blocked the cardioprotective effect of morphine and PC, which suggested that the infarct size reduction produced by morphine and PC is produced by an opioid receptor–K_{ATP} channel linked mechanism in the rat heart. These results are in agreement with those of Raffa and Martinez (1995), who showed that glibenclamide produced a rightward shift in the antinociceptive effect of morphine and several other opioid analgesics in rats, which suggested that a K_{ATP} channel may also be involved in mediating the supraspinal antinociceptive effect of certain opioid analgesics. The reasons for the differences in the study by Schultz and colleagues (1996) and that of Markiewicz and co-workers (1982) are unclear, but may be related to the route of administration, dose of morphine used, or the infarct model. In the study by Markiewicz and group (1982), no reperfusion was allowed, whereas in the study by Schultz and co-workers (1996), hearts were reperfused after 30 min of ischemia. These results suggest that a reperfusion period may be necessary to observe the cardioprotective effects of morphine in the intact animal.

Morphine has high affinity for the μ-opioid receptor, and is generally classified as a μ-receptor agonist, but evidence exists to suggest that morphine can interact with κ- and δ-opioid receptors as well (Martin, 1983; Paterson et al., 1983; Ela et al., 1997). It has been documented that crosstalk occurs between δ- and μ-opioid receptors (Sheldon et al., 1989; Jiang et al., 1990; Schoffelmeer et al., 1990; Traynor & Elliott, 1993). In this regard, Schultz and colleagues (1997b) tested the hypothesis that the cardioprotective effects of PC and morphine were mediated in the rat heart by the δ-opioid receptor. To test this hypothesis, we (Schultz et al., 1997b) administered the selective δ receptor antagonist naltrindole (5 mg/kg, i.v.) to rats 10 min prior to PC or morphine infusion. In both cases, naltrindole completely abolished the cardioprotective effect of PC or morphine, which suggested that the δ-opioid receptor was the major one responsible for the cardioprotection observed. Naltrindole had no effect on infarct size when given alone. With evidence of opioid receptor crosstalk and the absence of μ-opioid receptors on cardiac myocytes, our work (Schultz et al., 1996, 1997b) clearly indicates that morphine's activity to induce cardioprotection is via the δ-opioid receptor.

Although these previous studies provided suggestive evidence that opioids produce cardioprotective effects in the heart, it still was not certain that these effects occurred directly on cardiac myocytes or perhaps at some other tissue in the immediate vicinity. In this regard, Liang and Gross (1999) recently addressed this issue by investigating whether opioid receptor stimulation could mimic the cardioprotective effect of PC in an isolated myocyte model. Cardiac ventricular myocytes were cultured from chick embryos 14 days in ovo, and were used as a model of simulated PC. They found that a 5-min exposure of the myocytes to 1 μM of morphine produced a cardioprotective effect equivalent to that of PC during a subsequent 90-min period of hypoxia. This effect of morphine was concentration-dependent, and was blocked by the opioid receptor antagonist naloxone (0.1–10 μM). In addition, glibenclamide, a nonselective K_{ATP}-channel antagonist, and 5-hydroxydecanoic acid (5-HD), a putative mitochondrial selective K_{ATP}-channel antagonist, both abolished the cardioprotective effect of morphine. Furthermore, Liang and Gross (1999) also showed that a δ_1 selective antagonist, 7-benzylidenenaltrexone (BNTX), blocked the effect of morphine, and that the selective δ_1 selective agonist 2-methyl-4a-α-(3-hydroxyphenyl)-1,2,3,4,4a,5,12,12a-α-octahydroquinolino[2,3,3-g]isoquinoline (TAN-67) mimicked the effect of morphine in the chick myocyte model (unpublished results). These results are in agreement with those of Schultz and colleagues (1996, 1997b, 1998b, 1998c), and extend their work by providing direct evidence that the PC-like effect of morphine in the intact heart is primarily exerted at the level of the cardiomyocyte. Wang and co-workers (1997, 1998) have presented evidence that morphine attenuates neutrophil and endothelial activation in patients with an acute myocardial infarction and reduces the amount of adhesion molecules in rat vena caval blood, which suggests that morphine may be exerting cardioprotective effects to reduce reperfusion injury. Similarly, morphine has been shown to prevent peroxynitrite-induced death of a human neuroblastoma cell line by a direct scavenging action (Kanesaki et al., 1999). Morphine has also been demonstrated to stimulate the release of adenosine, a well-documented cardioprotective agent, in hypotension (Calignano et al., 1992) and antinociception (Sweeney et al., 1987; Cahill et al., 1995). The results obtained by Liang and Gross (1999) with 5-HD also suggest that morphine is exerting its cardioprotective effect via activating the mitochondrial K_{ATP} channel in the myocyte.

8. Opioid receptor subtype responsible for cardioprotection

Although evidence presented thus far suggests that the δ-opioid receptor is responsible for the cardioprotective effect of PC and morphine, both κ- and δ-opioid receptors are

abundant in the ventricle of several species, with the κ receptor being present in the greatest amount (Ventura et al., 1989; Xia et al., 1996). With this and other results in mind that support a primary role of the δ receptor in PC and morphine-induced cardioprotection, Schultz and colleagues (1998b) undertook a detailed look at the specific subtype of opioid receptor involved in mediating the cardioprotective effect of PC in the intact Wistar rat subjected to 30 min of ischemia and 2 hr of reperfusion. Infarct size reduction was used as an index of cardioprotection in these studies. PC was produced by three 5-min occlusion periods, interspersed with 5 min of reperfusion prior to the 30-min occlusion period. Two doses of BNTX (1 and 3 mg/kg), a selective δ_1 antagonist, or naltriben (1 and 3 mg/kg), a δ_2 selective antagonist, were given prior to PC. BNTX produced a dose-related reduction in the protective effects of PC, whereas naltriben had no effect. We (Schultz et al., 1998b) also tested the effects of the selective μ and κ receptor antagonists β-funaltrexamine and nor-binaltorphimine, respectively, and observed no effect on PC. These results indicate that δ_1-opioid receptors play the major role in the cardioprotective effects of PC in the intact rat heart. These results agree with those previously published by our laboratory (Schultz et al., 1997b), and those published more recently by Tsuchida and co-workers (1998) and Aitchison et al. (2000).

Conversely, several papers suggest that κ receptors may be involved in PC in rat hearts (Xia et al., 1996; Wu et al., 1999). Xia and co-workers (1996) suggested that the cardioprotective effects of PC against ventricular fibrillation may be related to a decrease in affinity of κ receptor binding. More recently, Wu et al. (1999) showed that the κ receptor mediated the cardioprotective effect of PC produced by sublethal metabolic inhibition in rat ventricular myocytes against severe metabolic inhibition. Therefore, these results do not completely rule out a role for the κ receptor in cardioprotection, and the divergent results obtained in different laboratories may be the result of differences in experimental model, or perhaps the strain of rat used (Baker et al., 2000).

9. Signaling pathways involved in opioid-induced cardioprotection

Opioid-induced cardioprotection and PC seem to share a common pathway, in that the δ-opioid receptor and the K_{ATP} channel, possibly the putative mitochondrial K_{ATP} channel, appear to be involved in the beneficial effects observed. However, the intracellular signaling pathways that transduce the effects of δ_1 receptor stimulation to the possible end effector, perhaps the mitochondrial K_{ATP} channel, are less well understood. There is some evidence for the involvement of $G_{i/o}$ proteins in PC (Schultz et al., 1998a) and PKC is well established as a messenger in PC, at least in small animals such as rat and rabbit (Fryer et al., 1999b; Ytrehus et al., 1994). Recent work of Schultz and colleagues (1998c) indicated that several of the known mediators of IPC, such as the K_{ATP} channel and $G_{i/o}$ proteins, are involved in the cardioprotective effect produced by stimulation of the δ_1-opioid receptor. TAN-67, the selective δ_1 agonist, produced a marked cardioprotective effect that was blocked by the selective δ_1 antagonist BNTX. Pretreatment with pertussis toxin (PTX) (25 μg/kg) for 48 hr also completely blocked the effect of TAN-67, as did 30 min of pretreatment with glibenclamide, a K_{ATP}-channel antagonist. These results (Fig. 2) clearly demonstrate that the δ_1-opioid receptor elicits a cardioprotective effect that is mediated by activation of a $G_{i/o}$ protein and the K_{ATP} channel in the intact rat heart. PTX has also been shown to block the effects of κ receptor stimulation in cardiac myocytes (Sheng et al., 1997; Wenzlaff et al., 1998). However, it is not clear how this relates to κ receptors and cardioprotection. Subsequently, Fryer and co-workers (2000) have found that 5-HD blocks the cardioprotective effect of acute administration of TAN-67 in the intact rat myocardium, whereas HMR 1098, a new selective sarcolemmal K_{ATP}-channel antagonist (Wirth et al., 2000), did not. These data further support a role for the mitochondrial K_{ATP} channel in opioid-induced cardioprotection.

PKC and TKs have been demonstrated to be important intracellular messengers in PC-mediated cardioprotection in rats and rabbits (Ytrehus et al., 1994; Fryer et al., 1998, 1999b). Little work, however, has been performed to

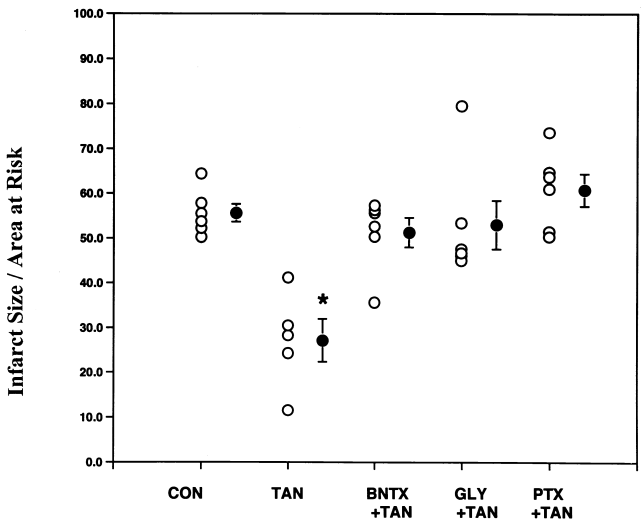

Fig. 2. Infarct size in rat hearts subjected to vehicle (saline, CON); TAN-67 (TAN), a δ_1 receptor agonist (10 mg/kg), infused for 15 min immediately prior to a 30-min coronary artery occlusion; BNTX, a δ_1 selective antagonist (3 mg/kg) given 10 min prior to TAN-67 infusion (BNTX + TAN); glibenclamide, a K_{ATP}-channel antagonist (0.3 mg/kg) given 45 min prior to TAN-67 infusion (GLY + TAN); and PTX administered (10 μg/kg) 48 hr prior to TAN-67 infusion (PTX + TAN). Open circles indicate infarct sizes from individual hearts and solid circles indicate group mean infarct size expressed as a percent of the area at risk. Values are the mean ± SE. * $P < 0.05$ vs. control. Reproduced from Schultz et al. (1998c), with permission of the copyright holder, The American Physiological Society, Bethesda.

determine the role of these kinases in the heart following opioid administration. Kita et al. (1995) demonstrated that opioid receptors are involved in the antiarrhythmic effect of PC in the rat heart independent of PKC activation. Recently, Miki and co-workers (1998) found that morphine (0.3 μM) significantly reduced infarct size in isolated rabbit hearts, and that this effect was blocked by the PKC inhibitor chelerythrine at a concentration that had no effect on infarct size in nontreated hearts. Subsequently, Wu and group (1999) provided evidence that the κ-opioid receptor mediates cardioprotection from severe metabolic inhibition via a pathway involving PKC and intracellular Ca^{2+}. Preliminary results from our laboratory (unpublished data) recently have shown that chelerythrine and bisindolemaleimide, two selective PKC inhibitors, and genistein, a TK inhibitor, all blocked the infarct-reducing effect of TAN-67, a selective δ_1-opioid agonist, and DADLE, a nonselective δ-opioid receptor agonist, in intact rat hearts. These results suggest that opioid receptors signal via pathways similar to those previously shown to mediate the effects of PC. Currently, we are investigating the role of specific isoforms of PKC that may be responsible for the cardioprotective effects of opioid receptor stimulation.

10. Role of opioids in delayed preconditioning

Baxter and co-workers (1994) originally demonstrated a second window of cardioprotection that occurred 24–48 hr following an IPC stimulus or following administration of an adenosine Type 1 receptor agonist in rabbits. This same group (Baxter et al., 1995; Imagawa et al., 1997) also demonstrated that delayed cardioprotection produced by PC could be blocked by either a PKC or TK inhibitor, suggesting that some important similarities exist between the early phase and late phases of PC. It has been hypothesized that this delayed protection may result from translocation of PKC from the cytosol to the nucleus (Yellon & Baxter, 1995) and the subsequent transcription and synthesis of cardioprotective molecules, such as heat shock proteins (Qian et al., 1998) and inducible nitric oxide synthases (Bolli et al., 1997). Although no previous work had been performed showing a delayed cardioprotective effect of opioids, work of Ventura and colleagues (1995, 1998) demonstrated that κ-opioid receptor stimulation resulted in a translocation of PKC to the nucleus and produced increased opioid peptide gene transcription. Furthermore, Gutstein and colleagues (1997) have studied the involvement of opioids on the mitogen-activated protein kinase (MAPK) pathway, and have shown that opioids may induce the activation of extracellular signal-regulated kinase and p38 MAPK. Based upon these intriguing findings, our laboratory examined the possibility that opioids might produce delayed cardioprotection in our rat model of ischemia/reperfusion injury. We (Fryer et al., 1999a) found that TAN-67, a selective δ_1-opioid agonist, had no protective effect to reduce infarct size 12 hr after administration. However, it produced a marked cardioprotective effect at 24–48 hr following drug administration, which disappeared at 72 hr (Fig. 3). These cardioprotective effects were blocked by pretreatment with BNTX, a selective δ_1 antagonist. In addition, we were also able to block these protective effects of TAN-67 by glibenclamide, a nonselective K_{ATP}-channel antagonist, and by 5-HD, a mitochondrial selective K_{ATP}-channel blocker, when the antagonists were administered 30 or 5 min prior to the long ischemic period following 48 hr of TAN-67 pretreatment (Fryer et al., 1999a), respectively (Fig. 4). These results suggest that δ_1-opioid receptor activation 24–48 hr prior to an ischemic insult results in a delayed cardioprotective effect that

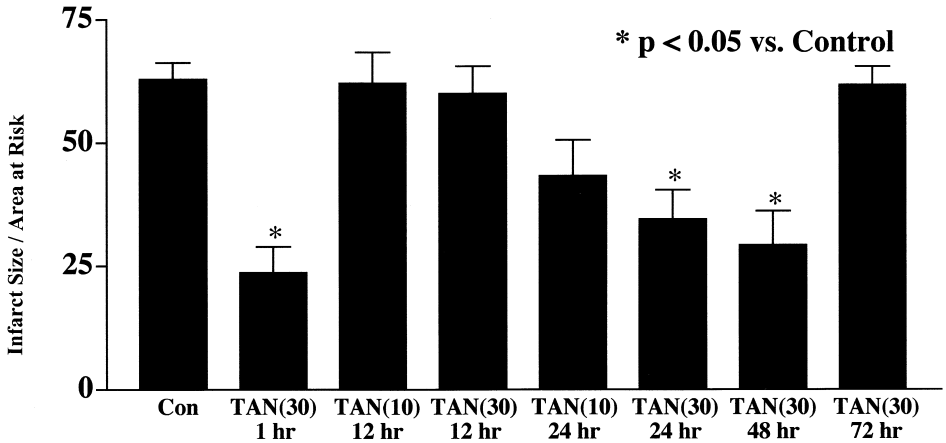

Fig. 3. Infarct size expressed as a percent of the area at risk in rats administered 10 or 30 mg/kg of TAN-67, either 1, 12, 24, 48, or 72 hr before 30 min of ischemia and 2 hr of reperfusion. A 1-hr pretreatment with TAN-67 produced a significant reduction in infarct size/area at risk. Pretreatment with both doses of TAN-67 12 hr prior to ischemia/reperfusion or low dose TAN-67 24 hr prior to ischemia/reperfusion had no significant effect on infarct size/area at risk. However, pretreatment with the large dose of TAN-67 24–48 hr prior to ischemia/reperfusion significantly reduced infarct size/area at risk. This cardioprotective effect was lost following 72 hr of pretreatment. All values are the mean ± SE. $*P < 0.05$. Reproduced from Fryer et al. (1999a), with permission of the copyright holder, Lippincott, Williams and Wilkins, Baltimore.

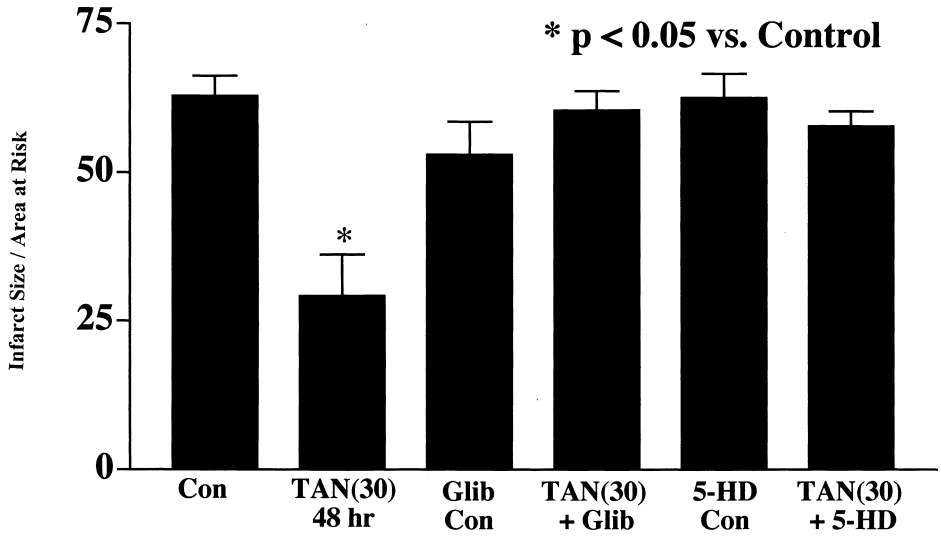

Fig. 4. Infarct size expressed as a percent of the area at risk in rats administered TAN-67 (30 mg/kg) in the presence or absence of glibenclamide (Glib) or 5-HD. Both Glib and 5-HD blocked the cardioprotective effect of TAN-67 48 hr after its administration at doses that had no effect on infarct size/area at risk in nontreated animals. All values are the mean ± SE. $*P < 0.05$ vs. control. Reproduced from Fryer et al. (1999a), with permission of the copyright holder, Lippincott, Williams and Wilkins, Baltimore.

appears to be mediated by the mitochondrial K_{ATP} channel. Studies are ongoing in our laboratory to more precisely determine the exact intracellular signaling pathways involved in producing the acute and delayed cardioprotective effects of endogenous and exogenous opioids. Recent work of Wu and colleagues (1999) revealed that κ-opioid receptor-induced cardioprotection occurred via two phases: the first window occurred ~ 1 hr after receptor activation and the second window developed 16–20 hr following drug administration in isolated rat ventricular myocytes. This delayed κ receptor-mediated cardioprotection was attenuated by inhibiting PKC (Wu et al., 1999).

11. Opioid receptors and cardioprotection in humans

Although the animal and cell work presented, which implicates a cardioprotective effect of opioid receptor acti-

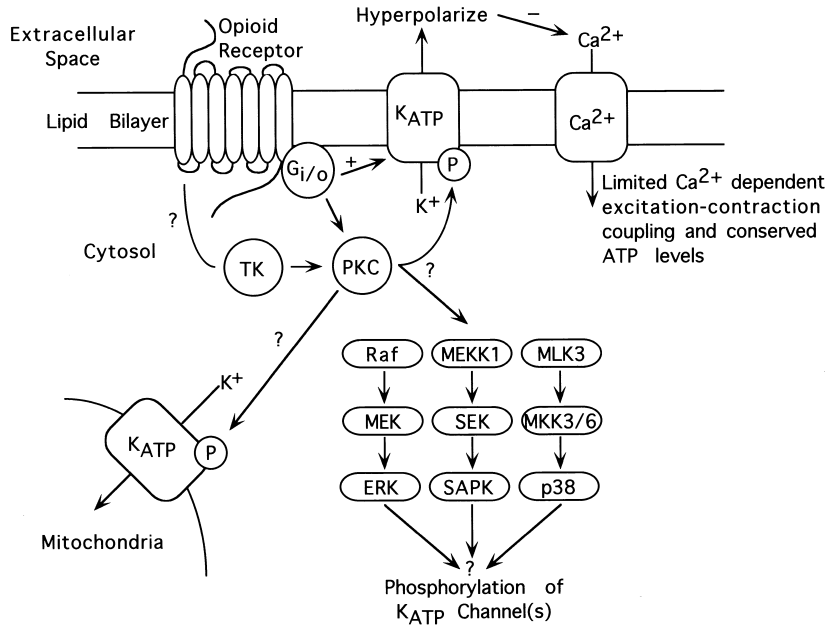

Fig. 5. A schematic diagram of some of the important signaling pathways involved in mediating opioid-induced cardioprotection. Findings obtained thus far suggest that opioids exert their cardioprotective effects by activating δ_1-opioid receptors, $G_{i/o}$ proteins, PKC, and the mitochondrial K_{ATP}-channel. Other pathways of the MAPK family are currently under investigation.

vation, is intriguing, it is important to demonstrate that a similar system exists in humans, if these basic studies can be extrapolated to the clinical arena. In this regard, Tomai and colleagues (1999) recently have shown that the nonselective opioid receptor antagonist naloxone was able to abolish the adaptation to ischemia in humans undergoing repeat balloon angioplasty. These data are encouraging, and suggest that IPC in humans may be at least partly the result of opioid receptor stimulation. Similarly, Xenopoulos and co-workers (1998) have shown that intracoronary morphine (15 μg/kg) mimics PC, as assessed by changes in ST segment shifts in humans undergoing percutaneous transluminal coronary angioplasty. Finally, preliminary results of Yellon's group (Bell et al., 1998) have shown that opioids mimic and appear to mediate PC-induced cardioprotection via δ receptor stimulation and K_{ATP}-channel activation in isolated right atrial trabeculae obtained from humans. In addition, these investigators have demonstrated the presence of δ receptors in human atrial and ventricular tissue by reverse transcription-polymerase chain reaction. These results are encouraging, and may suggest a possible clinical use for opioids in the therapy of acute or chronic myocardial ischemia.

Another area of clinical medicine where opioids may be used might be in preserving organs targeted for cardiac transplantation. In animal studies, Bolling and co-workers (1997a, 1997b) recently have shown that the δ-opioid receptor agonist DADLE protects hearts that were subjected to 18 hr of cold storage at 4°C or 2 hr of global ischemia, respectively, in the presence of a standard cardioplegia. This group also showed that HIT produced a protective effect similar to that produced by DADLE, which led to the hypothesis that this unknown hibernation factor may be some type of endogenous opioid. These authors concluded that the use of HIT or a δ-opioid agonist with similar properties is a promising new approach for enhancing organ preservation. Recently, Bolling's group (Schwartz et al., 1999) provided evidence that pentazocine, a δ-opioid agonist, enhanced the myocardial protection of standard cardioplegia at temperatures ranging from 0°C to 34°C. Subsequently, Kevelaitis and colleagues (1999) have demonstrated that stimulation of δ-opioid receptors improves recovery of cold-stored rat hearts to a similar level as PC. These investigators showed that this opioid-induced cardioprotection is mediated through K_{ATP}-channel activation.

12. Conclusions and clinical relevance

The results obtained from a number of animal and clinical studies clearly show that stimulation of δ-opioid receptors is cardioprotective, and that this effect appears to be mediated via signaling pathways similar to that of PC. Activation of these receptors produces both an acute, as well as a chronic, cardioprotective effect. The pathways involved in producing these protective effects include $G_{i/o}$ proteins, PKC, TK, MAPK, and the K_{ATP} channel, most likely the mitochondrial K_{ATP} channel (Fig. 5). Based upon these intriguing findings, further animal, cellular, molecular, and clinical studies are needed to determine whether this concept has important clinical ramifications.

Opioids have been used clinically to manage pain peri- and postoperatively. The demonstration that opioid receptors, most notably δ_1, which not only have analgesic properties, but may now have the potential to protect the myocardium during cardiac surgical interventions, suggests a possible new pharmacological approach for the treatment of patients suffering from acute or chronic myocardial ischemia. Therefore, since many of these drugs are already approved for the clinical treatment of pain, a long period of time of new drug development may not be necessary before bringing this concept to the bedside.

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