ELSEVIER

Cardiovascular Research 55 (2002) 520–525

Cardiovascular Research

www.elsevier.com/locate/cardiores

Review

Therapeutic receptor targets of ischemic preconditioning

Ryan M. Fryer, John A. Auchampach, Garrett J. Gross*

Department of Pharmacology and Toxicology, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53226 USA

Received 8 November 2001; accepted 8 February 2002

Abstract

This review focuses on target receptors that have been shown to have the potential to mimic the cardioprotective effect of ischemic preconditioning (IPC). There is an abundance of information concerning the intracellular mechanisms and membrane-bound receptors responsible for IPC. Important intracellular mediators of this cardioprotection likely reside in the activation of multiple kinase cascades. The major players in IPC are thought to include protein kinase C, tyrosine kinases, and members of the mitogen-activated protein kinase signaling family and these topics will be covered in more detail in other papers of this focused issue. However, many of these kinase-mediated mechanisms are triggered by the activation of transmembrane spanning receptors, some of which may be manipulated therapeutically to induce cardioprotection in humans with unstable angina or who are at risk for myocardial infarction. In this review, we will discuss the evidence supporting the possibility of manipulating several of these G protein-coupled receptors as potential therapeutic targets. Stimulation of numerous receptors has been targeted as possible triggers for IPC. Some of those that have been identified include A_1 adenosine, α_1 adrenergic, M_2 muscarinic, B_2 bradykinin, δ_1 opioid, AT_1 angiotensin, and endothelin-1 receptors. In general, these receptors are thought to couple to inhibitory G proteins. In this review, we will focus on the most likely therapeutic candidates for cardioprotection, namely adenosine, opioid, and bradykinin receptors since selective agonists and antagonists, either alone or in combination, have most often been shown to mimic or block IPC in numerous animal models and man, respectively. This is not meant to completely rule out other receptors since it is clear that IPC is a phenomenon with multiple pathways that appear to be responsible for the cardioprotection observed.

Keywords: Adenosine; Infarction; Preconditioning; Receptors

1. Adenosine receptors as therapeutic targets of cardioprotection

Adenosine is generated in the heart at high levels during myocardial ischemia from the metabolism of ATP. It has numerous physiological actions in the cardiovascular system, which were first described by Drury and Szent-Gyorgi in 1929 [1]. Adenosine produces vasodilation by interacting with A_{2A} adenosine receptors in smooth muscle cells, decreases heart rate by interacting with A_1 adenosine receptors in the sino–atrial node, and causes negative inotropy by interacting with A_1 adenosine receptors in ventricular cardiomyocytes. All of these actions of adenosine are beneficial in the ischemic myocardium by improving the oxygen supply–demand balance.

In 1991 [2], it was first demonstrated that another action of adenosine is to induce adaptive mechanisms that increase the intrinsic resistance of the heart to subsequent ischemic insults, i.e. to trigger IPC. Liu et al. [2] demonstrated that the cardioprotection induced by IPC can be abrogated by the administration of nonselective adenosine receptor antagonists, implicating that adenosine produced during IPC acts on cell-surface receptors to induce the adaptive response. Subsequent studies have implicated the A_1 adenosine receptor as being most important in preconditioning, based on the observations that exogenous administration of agonists selective for the A_1 receptor can induce cardioprotection similar to IPC [2]. While these initial findings pointed towards an exclusive role of

*Corresponding author. Tel.: +1-414-456-8627; fax: +1-414-456-6545.

E-mail address: ggross@mcw.edu (G.J. Gross).

Time for primary review 27 days.

0008-6363/02/$ – see front matter
PII: S0008-6363(02)00316-4

adenosine in mediating IPC, subsequent work has shown that multiple factors are capable of inducing IPC including bradykinin and opioids, and that the relative importance of these mediators depends on the duration and the number of preconditioning cycles [51].

IPC is well known to consist of two phases, an early phase that appears immediately ('classical' preconditioning) and a delayed phase known as the 'second window' of protection. Baxter et al. [3] first characterized the time-course of the second phase of IPC and demonstrated that it appears 24, 48 and 72 h following brief periods of preconditioning ischemia. This time-course and duration of the second window of IPC implicates the involvement of de novo synthesis of cardioprotective proteins as a primary mechanism of the phenomena. Proteins known to be important in mediating the second window of protection include nitric oxide synthase, cyclooxygenase-2, heat shock proteins, and Mn-superoxide dismutase [4]. Similar to the early phase of IPC, adenosine also triggers the second phase of IPC, which appears to involve A_1 adenosine receptors [5]. Interestingly, however, although the second phase of IPC can protect against myocardial infarction as well as against myocardial stunning, adenosine receptor antagonists only block the effect of IPC against infarction [6,7]. Thus, adenosine plays a selective signaling role in the development of the second phase of IPC against irreversible ischemic injury, but not against reversible injury.

The discovery of adenosine as a trigger of IPC renewed interest in the therapeutic potential of adenosine as a cardioprotective agent. Previously, adenosine has been considered for use in cardioplegic solutions [8], during coronary angioplasty [9,10], and as an adjunct to thrombolytic therapy [11] where it acts acutely to reduce ischemic injury or to reduce injury caused by reperfusion. Indeed, the results of the AMISTAD trial [11] suggest that administration of adenosine or adenosine receptor mimetics may be useful in the treatment of acute myocardial infarction by attenuating reperfusion injury. The observation that adenosine also induces IPC opened the possibility that adenosinergic agents could potentially be administered continually to induce the heart into a preconditioned state, and thus provide protection if an ischemic event occurs. Since treating with agents prior to ischemia typically provides a more robust cardioprotective effect compared with therapies applied during reperfusion, it would be expected that adenosine agonists acting as preconditioning mimetics could significantly improve therapies currently available for patients with ischemic heart disease. One of the problems associated with this treatment approach, however, is that continued use of adenosine receptor agonists may result in the loss of efficacy due to receptor desensitization/down-regulation. Indeed, this problem has been encountered in experimental animal studies [12,13], where chronic administration of an adenosine receptor agonist not only negated the beneficial effects of the agonist but it also resulted in the loss of the protective effects of IPC. To avoid this problem of receptor inactivation, Dana et al. [14] devised a treatment regimen in rabbits in which the A_1 adenosine receptor agonist CCPA (2-chloro-*N*-[6]-cyclopentyladenosine) was administered every other day. It was hypothesized that this protocol would provide sustained cardioprotection by repeated activation of the second window of protection, but that the low frequency of drug administration would not result in receptor inactivation. After treating rabbits every 48 h for 10 days with CCPA, it was found that the hearts continued to be protected (infarct size was reduced ~42%) and the hemodynamic responses to the agonist remained intact [14]. Thus, it may be possible to take advantage of the long period of protection provided by adenosine receptor agonists (or other agonists capable of inducing preconditioning) to overcome problems often associated with the use of agonists of G-protein-coupled receptors as therapeutic agents.

Systemic side effects pose another obstacle towards the use of adenosine receptor agonists as preconditioning mimetic agents. A_1 adenosine receptor agonists produce substantial hemodynamic effects including bradycardia and hypotension, which limit the widespread clinical use of these agents. However, it should be noted that full dose–response curves with A_1 adenosine receptor agonists focused on cardioprotection have yet to be performed. Since the potency of agonists to mediate a response depends on the number of receptors that are expressed and their coupling efficiency within specific tissues [15–17], it is possible that a cardioprotective response could be achieved with A_1 adenosine receptor agonists at low doses that do not affect systemic hemodynamics.

More recently, agonists with high potency for the A_3 adenosine receptor (principally N^6-(3-iodobenzyl)adenosine-5′-*N*-methylcarboxamide, IB-MECA] have been tested in animal models of ischemia–reperfusion injury [7,18,19]. Although the A_1 adenosine receptor has been implicated as the primary receptor involved in IPC, it has recently been proposed that the A_3 adenosine receptor is expressed in the myocardium and that activation of this receptor may also induce a preconditioning response, based on studies with embryonic chick cardiomyocytes as well as mammalian myocardium [20–23]. In a conscious rabbit model, pretreatment with IB-MECA was found to provide marked protection against myocardial stunning and myocardial infarction [19]. In addition, IB-MECA was shown to induce a second window of cardioprotection [7], which provided protection against myocardial infarction that was similar in magnitude to that produced by CCPA. Remarkably, in all of these studies IB-MECA was effective at doses (100–300 μg/kg) that produced no detectable changes in heart rate or arterial blood pressure [7,18,19]. Taken together, these studies support the concept that adenosine receptor agonists can be effective as cardioprotective agents at hemodynamically

inert doses. These studies also suggest that therapies targeting the A_3 adenosine receptor may be useful in the clinical setting. It should be noted, however, that IB-MECA has recently been shown to be only moderately selective for the A_3 adenosine receptor [7], suggesting that it may act in vivo via other adenosine receptor subtypes. Thus, additional studies with more selective A_3 receptor agonists and antagonists are needed to define more clearly the importance of this receptor in the ischemic myocardium. Furthermore, it has been shown that infarct size is reduced in A_3 receptor knockout mice, implicating that the A_3 adenosine receptor may also produce deleterious actions in the ischemic myocardium via mechanisms that remain unclear [24,25].

2. Opioid receptors as therapeutic targets of cardioprotection

Traditionally, the importance of opioid receptor agonists and antagonists has focused on the treatment of pain. However, it has been recently found that the heart may be modulated by opioids both in physiological and pathophysiological states [26]. Additionally, it is now known that the heart is an abundant source of opioid precursors and it has been suggested that the heart, due to its limited capacity to store opioid peptides, may actually be an endocrine organ that supplies the rest of the body with enkephalins.

2.1. Opioids in myocardial protection

The first evidence of the importance of opioid receptors as an integral component of preconditioning-induced cardioprotection was published in 1995. Schultz et al. [27] demonstrated that naloxone, a nonspecific opioid receptor antagonist, could blunt the cardioprotective effects of IPC in a rat model. The following year, the same group demonstrated that infusion of morphine in the absence of IPC could induce cardioprotection in the in vivo rat heart [28]. While probing the identity of the receptor subtype responsible for IPC, Schultz et al. [29] demonstrated that the effect of IPC to reduce infarct size was mediated by the δ_1-, but not the δ_2-, μ- or κ-opioid receptors since the effects of IPC were blunted by the selective δ_1-receptor antagonist, BNTX, but not the δ_2-antagonist, naltriben. Additionally, they demonstrated that cardioprotection was not induced by the administration of multiple doses of the selective μ-agonist, DAMGO, and that IPC was not attenuated by the μ-antagonist, β-FNA [29]. Finally, they excluded the involvement of the κ receptor since two doses of the κ-selective antagonist, nor-BNI, could not reverse the effects of IPC to reduce infarct size [29]. Later, Miki et al. [30] demonstrated in a rabbit model that opioids contribute to cardioprotection via a PKC-sensitive mechanism. The effect of opiates to induce cardioprotection was also found to be mediated by a peripheral, rather than a centrally acting mechanism [31,32] and may be related to the ability of opiates to attenuate neutrophil activation [33] which likely contributes to cell death following myocardial reperfusion. Additionally, in a porcine model of myocardial ischemia, Schulz et al. [34] demonstrated the importance of endogenous opioids in myocardial salvage since naloxone could abolish cardioprotection induced by IPC as assessed by infarct size reduction.

More recently, the contribution of the δ_1-opioid receptor to cardioprotection has been shown to be induced by the δ_1-selective agonist, TAN-67, both in an in vivo rat model [35] and in a model of isolated chick cardiomyocytes [36]. In chick myocytes, TAN-67 application protected cells from hypoxia and reoxygenation as assessed by a pronounced reduction in the percentage of cells killed and the amount of creatine kinase release versus control cells. This effect was also shown to be mediated by PKC and K_{ATP} channel activation [36].

Additionally, Fryer et al. [37] have demonstrated that TAN-67 is anti-arrhythmic during myocardial ischemia as assessed by arrhythmia score and completely abolished the incidence of ventricular fibrillation during coronary artery occlusion and reperfusion in rats. Similarly, Pepe et al. [38] have demonstrated that the δ-opioid receptor agonist, leucine enkephalin, inhibited β_1-adrenoceptor stimulation of adenylate cyclase, and that β_1-adrenoceptor/δ-opioid receptor 'cross-talk' occurs via a pertussis toxin sensitive G-protein. Since cAMP production is thought to be a major factor contributing to arrhythmias [39], this may also suggest that δ-opioid receptor stimulation is anti-arrhythmic. Therefore, considerable evidence exists in animal models that suggest that opioids induce potent cardioprotection, and there is evidence that this may occur in humans [40].

Recently, evidence has been presented to suggest the importance of the κ-receptor in opioid-induced cardioprotection. Although this is in opposition to many reports of δ-mediated cardioprotection and to that originally found by Schultz et al. [29], it is not certain which receptor subtype is responsible for cardioprotection in humans. Wang et al. [41] demonstrated that U50,488H, a selective κ-receptor agonist, induced cardioprotection and was anti-arrhythmic during myocardial ischemia. Furthermore, they suggested that stimulation of the δ-receptor was not a component of IPC [41]. This is difficult to reconcile with the findings of Fryer et al. [37] who demonstrated that the δ_1 agonist, TAN-67 was anti-arrhythmic and two other reports which both suggested that stimulation of the κ receptor is proarrhythmic [42,43]. Additionally, a recent report by Aitchison et al. [44] suggested that κ- and δ-receptors had opposing effects during myocardial infarction in the isolated rat heart whereby κ-receptor activation exacerbated infarct size and δ-receptor activation mediated a marked reduction in infarct size.

2.2. Opioids and the 'second window' of cardioprotection

IPC also induces a 'second window' of cardioprotection. Following the demonstration that adenosine could pharmacologically mimic this cardioprotection, Fryer et al. [45] demonstrated that TAN-67, a δ_1-opioid agonist, could also induce cardioprotection during this delayed phase. They demonstrated that stimulation of this receptor induced cardioprotection 24 h later that was maximal at 48 h. However, unlike that of IPC [3], cardioprotection following opioid administration faded by 72 h. Interestingly, they also demonstrated that this was dependent upon δ_1 receptor stimulation immediately following administration of the opioid agonist and upon receptor reoccupation immediately prior to index ischemia [45]. It was later shown that this cardioprotective effect was mediated by the activation of the MAP kinases, ERK and p38 [46].

2.3. Evidence in humans

Opioids are thought to mediate cardioprotection in rats [47], pigs [34], and rabbits [30]. Tomai et al. [48] have demonstrated in humans that opioids also are likely to mediate preconditioning. They demonstrated that naloxone could abrogate the reduction in ST-segment elevation normally observed during a second balloon inflation during coronary angioplasty. Additionally, they demonstrated that in naloxone-treated patients, the severity of cardiac pain and time to pain onset at the end of the second balloon inflation were similar to that of the first inflation, while in placebo-treated patients the severity of cardiac pain during the second inflation was reduced and the time to onset of pain was lengthened versus the first inflation [48]. This suggests a preconditioning-like effect in humans undergoing coronary angioplasty that could be attenuated by the opioid antagonist naloxone. Bell et al. [40] have also demonstrated that δ-opioid receptor stimulation mimics IPC in human atrial trabeculae and could induce protection to a similar extent as that obtained via IPC. They showed that the δ_1/δ_2 agonist, DADLE, induced cardioprotection against simulated ischemia and reoxygenation as determined by an increase in developed force, an effect which could be abolished by a mitochondrial K_{ATP} channel antagonist, 5-HD [40]. Interestingly, it has also been demonstrated that morphine administration to patients with acute myocardial infarction attenuated neutrophil activation via an increase in neutrophil endopeptidase and a decrease in the shedding of L-selectin and ICAM-1 [49].

2.4. Current clinical use of opioid receptor agonists

Opioid analgesics are widely used for the treatment of pain. Commonly utilized opioid agonists in clinical medicine include morphine, levorphanol, meperidine, fentanyl, and methadone. Although these agents are predominately μ-opioid receptor agonists, cross-talk with δ-opioid receptors has been demonstrated to occur. However, the FDA has not approved these drugs for use in patients with unstable angina or who are predisposed to myocardial infarction. This is likely to due to the limited research in humans concerning the importance of opioid receptors in the myocardium and the high potential for dependence, abuse, and respiratory depression. Future avenues of research should focus on the identification of orally-active compounds with high δ-opioid receptor affinity to be used as cardioprotective agents since such drugs are currently lacking.

3. Bradykinin receptors as therapeutic targets of cardioprotection

Wall et al. [50] first demonstrated in 1994 the importance of bradykinin in myocardial preconditioning. They demonstrated in an open-chest rabbit model that the bradykinin B_2 receptor antagonist, HOE 140, could abolish the protective effects of IPC suggesting that bradykinin may be a physiological mediator of IPC [50]. Additionally, they demonstrated that intra-atrial infusion of bradykinin (250 μg/kg/min) prior to ischemia and reperfusion reduced infarct size in the absence of IPC, an effect that could be blocked by pretreatment with HOE 140 [50]. Later, Goto et al. [51] demonstrated in an in situ rabbit heart preparation that HOE 140 abolished IPC-induced cardioprotection when administered before a single cycle IPC stimulus, but not when administered after preconditioning before the index ischemia. They also demonstrated that the effect of HOE 140 could be overcome following multiple cycles of preconditioning which suggested that bradykinin is only one of several substances that are important for triggering IPC [51]. Brew et al. [52] demonstrated that administration of exogenous bradykinin improved functional recovery as assessed by an increase in developed pressure and dP/dt and a reduction in LVEDP after ischemia and reperfusion.

There is likely a release of bradykinin from the ischemic myocardium during the IPC stimulus, acting as an endogenous trigger of cardioprotection. That bradykinin is released from ischemic tissue was first demonstrated by Matsuki et al. [53] in 1987. More recently, Schulz et al. [54] demonstrated that IPC induced by 2, 3 and 10 min of ischemia induced an increased resistance against prolonged ischemia that was positively associated with an increase in interstitial bradykinin concentration as determined by microdialysis and radioimmunoassay. Recently, Pan et al. [55] corroborated this finding by demonstrating that bradykinin released during prolonged ischemia was actually enhanced in animals previously subjected to IPC versus nonpreconditioned controls.

Utilizing the commonly prescribed antihypertensive agent, losartan, Sato et al. [56] demonstrated that blockade

of the angiotensin II type 1 receptor is cardioprotective and can reduce the extent of infarct size and myocyte apoptosis while improving myocardial function. Additionally, it has been demonstrated that ACE inhibitors, like captopril, are also cardioprotective during ischemia [57]. In fact, it has been reported that ACE inhibitors may preserve bradykinin and therefore potentiate the cardioprotective effects of IPC via bradykinin B_2 receptor activation. More recently, Schriefer et al. [58] found that ramiprilat and an endopeptidase inhibitor, cFP-AAF-pAB, produced a long-lasting protection against myocardial infarction in rabbit hearts following 3–7 days of reperfusion. These results suggest that administration of inhibitors of bradykinin-inactivating enzymes can produce a sustained cardioprotective effect. However, in spite of these encouraging results, the above studies have all been performed in animal models of myocardial infarction. However, a recent clinical study published by Leesar et al. [59] has shown that intracoronary infusion of bradykinin preconditions human myocardium against ischemia as effectively as ischemia per se in patients undergoing coronary angioplasty. These beneficial effects occurred in the absence of hemodynamic changes and suggest that bradykinin may be used prophylactically to mimic ischemic PC in humans undergoing PTCA.

In conclusion, this review has only focused on three receptors which have been shown to be triggers of IPC, adenosine, opioid, and bradykinin receptors. These receptors appear to be the ones closer to clinical use as preconditioning mimetics since drugs that mimic or enhance the activation of these receptors are already clinically available and include adenosine, morphine, and ACE inhibitors. There may be other possible receptors or drug targets which have not been included in this review which may also prove to be potential therapeutic targets for future investigation. Since multiple receptors are likely to work in concert to produce IPC, an approach which may be particularly attractive includes the use of combinations of two or more of these preconditioning mimetics for maximal clinical efficacy. In this way, smaller nontoxic doses of each drug may have synergistic effects to produce cardioprotection and in this way reduce the risk of drug interactions or toxicity.

References

[1] Drury AN, Szent-Gyorgi A. The physiological activity of adenine compounds with especial reference to their action upon the mammalian heart. J Pysiol (Lond) 1929;68:213–237.

[2] Liu GS, Thornton J, Van Winkle DM et al. Protection against infarction afforded by preconditioning is mediated by A_1 adenosine receptors in rabbit heart. Circulation 1991;84:350–356.

[3] Baxter GF, Goma FM, Yellon DM. Characterisation of the infarct-limiting effect of delayed preconditioning: timecourse and dose-dependency studies in rabbit myocardium. Basic Res Cardiol 1997;92:159–167.

[4] Bolli R. The late phase of preconditioning. Circ Res 2000;87:972–983.

[5] Baxter GF, Marber MS, Patel VC, Yellon DM. Adenosine receptor involvement in a delayed phase of myocardial protection 24 h after ischemic preconditioning. Circulation 1994;90:2993–3000.

[6] Maldonado C, Qiu Y, Tang XL et al. Role of adenosine receptors in late preconditioning against myocardial stunning in conscious rabbits. Am J Physiol 1997;273:H1324–1332.

[7] Takano H, Bolli R, Black Jr. RG et al. A_1 or A_3 adenosine receptors induce late preconditioning against infarction in conscious rabbits by different mechanisms. Circ Res 2001;88:520–528.

[8] Mentzer Jr. RM, Rahko PS, Molina-Viamonte V et al. Safety, tolerance, and efficacy of adenosine as an additive to blood cardioplegia in humans during coronary artery bypass surgery. Am J Cardiol 1997;79:38–43.

[9] Marzilli M, Orsini E, Marraccini P, Testa R. Beneficial effects of intracoronary adenosine as an adjunct to primary angioplasty in acute myocardial infarction. Circulation 2000;101:2154–2159.

[10] Leesar MA, Stoddard M, Ahmed M, Broadbent J, Bolli R. Preconditioning of human myocardium with adenosine during coronary angioplasty. Circulation 1997;95:2500–2507.

[11] Mahaffey KW, Puma JA, Barbagelata NA et al. Adenosine as an adjunct to thrombolytic therapy for acute myocardial infarction: results of a multicenter, randomized, placebo-controlled trial: the Acute Myocardial Infarction STudy of ADenosine (AMISTAD) trial. J Am Coll Cardiol 1999;34:1711–1720.

[12] Cohen MV, Yang XM, Downey JM. Conscious rabbits become tolerant to multiple episodes of ischemic preconditioning. Circ Res 1994;74:998–1004.

[13] Hashimi MW, Thornton JD, Downey JM, Cohen MV. Loss of myocardial protection from ischemic preconditioning following chronic exposure to $R(-)$-N^6-(2-phenylisopropyl)adenosine is related to defect at the adenosine A_1 receptor. Mol Cell Biochem 1998;186:19–25.

[14] Dana A, Baxter GF, Walker JM, Yellon DM. Prolonging the delayed phase of myocardial protection: repetitive adenosine A_1 receptor activation maintains rabbit myocardium in a preconditioned state. J Am Coll Cardiol 1998;31:1142–1149.

[15] Baker SP, Scammells PJ, Belardinelli L. Differential A_1-adenosine receptor reserve for inhibition of cyclic AMP accumulation and G-protein activation in DDT_1 MF-2 cells. Br J Pharmacol 2000;130:1156–1164.

[16] Shryock JC, Snowdy S, Baraldi PG et al. A_{2A}-adenosine receptor reserve for coronary vasodilation. Circulation 1998;98:711–718.

[17] Srinivas M, Shryock JC, Dennis DM, Baker SP, Belardinelli L. Differential A_1 adenosine receptor reserve for two actions of adenosine on guinea pig atrial myocytes. Mol Pharmacol 1997;52:683–691.

[18] Kodani E, Bolli R, Tang XL, Auchampach JA. Protection of IB-MECA against myocardial stunning in conscious rabbits is not mediated by the A_1 adenosine receptor. Basic Res Cardiol 2001;96:487–496.

[19] Auchampach JA, Rizvi A, Qiu Y et al. Selective activation of A3 adenosine receptors with N^6-(3-iodobenzyl)adenosine-5′-*N*-methyluronamide protects against myocardial stunning and infarction without hemodynamic changes in conscious rabbits. Circ Res 1997;80:800–809.

[20] Strickler J, Jacobson KA, Liang BT. Direct preconditioning of cultured chick ventricular myocytes. Novel functions of cardiac adenosine A_{2a} and A_3 receptors. J Clin Invest 1996;98:1773–1779.

[21] Liu GS, Richards SC, Olsson RA et al. Evidence that the adenosine A_3 receptor may mediate the protection afforded by preconditioning in the isolated rabbit heart. Cardiovasc Res 1994;28:1057–1061.

[22] Hill RJ, Oleynek JJ, Magee W, Knight DR, Tracey WR. Relative importance of adenosine A_1 and A_3 receptors in mediating physiological or pharmacological protection from ischemic myocardial injury in the rabbit heart. J Mol Cell Cardiol 1998;30:579–585.

[23] Safran N, Shneyvays V, Balas N et al. Cardioprotective effects of adenosine A_1 and A_3 receptor activation during hypoxia in isolated rat cardiac myocytes. Mol Cell Biochem 2001;217:143–152.

[24] Guo Y, Bolli R, Bao W et al. Targeted deletion of the A_3 adenosine receptor confers resistance to myocardial ischemic injury and does not prevent early preconditioning. J Mol Cell Cardiol 2001;33:825–830.
[25] Cerniway RJ, Yang Z, Jacobson MA, Linden J, Matherne GP. Targeted deletion of A_3 adenosine receptors improves tolerance to ischemia–reperfusion injury in mouse myocardium. Am J Physiol Heart Circ Physiol 2001;281:H1751–1758.
[26] Barron B. Cardiac opioids. Proc Soc Exp Biol Med 2000;224:1–7.
[27] Schultz JE, Rose E, Yao Z, Gross GJ. Evidence for involvement of opioid receptors in ischemic preconditioning in rat hearts. Am J Physiol 1995;268:H2157–2161.
[28] Schultz JE, Hsu AK, Gross GJ. Morphine mimics the cardioprotective effect of ischemic preconditioning via a glibenclamide-sensitive mechanism in the rat heart. Circ Res 1996;78:1100–1104.
[29] Schultz JE, Hsu AK, Gross GJ. Ischemic preconditioning in the intact rat heart is mediated by δ_1- but not μ- or κ-opioid receptors. Circulation 1998;97:1282–1289.
[30] Miki T, Cohen MV, Downey JM. Opioid receptor contributes to ischemic preconditioning through protein kinase C activation in rabbits. Mol Cell Biochem 1998;186:3–12.
[31] Chien GL, Mohtadi K, Wolff RA, Van Winkle DM. Naloxone blockade of myocardial ischemic preconditioning does not require central nervous system participation. Basic Res Cardiol 1999;94:136–143.
[32] Schultz JJ, Hsu AK, Gross GJ. Ischemic preconditioning is mediated by a peripheral opioid receptor mechanism in the intact rat heart. J Mol Cell Cardiol 1997;29:1355–1362.
[33] Wang TL, Chang H, Hung CR, Tseng YZ. Morphine preconditioning attenuates neutrophil activation in rat models of myocardial infarction. Cardiovasc Res 1998;40:557–563.
[34] Schulz R, Gres P, Heusch G. Role of endogenous opioids in ischemic preconditioning but not in short-term hibernation in pigs. Am J Physiol Heart Circ Physiol 2001;280:H2175–2181.
[35] Schultz JE, Hsu AK, Nagase H, Gross GJ. TAN-67, a d1-opioid receptor agonist, reduces infarct size via activation of $G_{i/o}$ proteins and K_{ATP} channels. Am J Physiol 1998;274:H909–H914.
[36] Huh J, Gross GJ, Nagase H, Liang BT. Protection of cardiac myocytes via δ_1-opioid receptors, protein kinase C, and mitochondrial K_{ATP} channels. Am J Physiol Heart Circ Physiol 2001;280:H377–383.
[37] Fryer RM, Hsu AK, Nagase H, Gross GJ. Opioid-induced cardioprotection against myocardial infarction and arrhythmias: mitochondrial versus sarcolemmal ATP-sensitive potassium channels. J Pharmacol Exp Ther 2000;294:451–457.
[38] Pepe S, Xiao RP, Hohl C, Altschuld R, Lakatta EG. 'Cross talk' between opioid peptide and adrenergic receptor signaling in isolated rat heart. Circulation 1997;95:2122–2129.
[39] Podzuweit T, Els DJ, McCarthy J. Cyclic AMP mediated arrhythmias induced in the ischaemic pig heart. Basic Res Cardiol 1981;76:443–448.
[40] Bell SP, Sack MN, Patel A, Opie LH, Yellon DM. δ-Opioid receptor stimulation mimics ischemic preconditioning in human heart muscle. J Am Coll Cardiol 2000;36:2296–2302.
[41] Wang GY, Wu S, Pei JM, Yu XC, Wong TM. κ- but not δ-opioid receptors mediate effects of ischemic preconditioning on both infarct and arrhythmia in rats. Am J Physiol Heart Circ Physiol 2001;280:H384–391.
[42] Bian JS, Zhang WM, Xia Q, Wong TM. Phospholipase C inhibitors attenuate arrhythmias induced by κ-receptor stimulation in the isolated rat heart. J Mol Cell Cardiol 1998;30:2103–2110.
[43] Bian JS, Pei JM, Cheung CS, Zhang WM, Wong TM. κ-Opioid receptor stimulation induces arrhythmia in the isolated rat heart via the protein kinase $C/Na^+–H^+$ exchange pathway. J Mol Cell Cardiol 2000;32:1415–1427.
[44] Aitchison KA, Baxter GF, Awan MM et al. Opposing effects on infarction of δ- and κ-opioid receptor activation in the isolated rat heart: implications for ischemic preconditioning. Basic Res Cardiol 2000;95:1–10, discussion 11.
[45] Fryer RM, Hsu AK, Eells JT, Nagase H, Gross GJ. Opioid-induced second window of cardioprotection: potential role of mitochondrial K_{ATP} channels. Circ Res 1999;84:846–851.
[46] Fryer RM, Hsu AK, Gross GJ. ERK and p38 MAP kinase activation are components of opioid-induced delayed cardioprotection. Basic Res Cardiol 2001;96:136–142.
[47] Fryer RM, Wang Y, Hsu AK, Gross GJ. Essential activation of PKC-δ in opioid-initiated cardioprotection. Am J Physiol Heart Circ Physiol 2001;280:H1346–1353.
[48] Tomai F, Crea F, Gaspardone A et al. Effects of naloxone on myocardial ischemic preconditioning in humans. J Am Coll Cardiol 1999;33:1863–1869.
[49] Wang TL, Chang H, Hung CR, Tseng YZ. Attenuation of neutrophil and endothelial activation by intravenous morphine in patients with acute myocardial infarction. Am J Cardiol 1997;80:1532–1535.
[50] Wall TM, Sheehy R, Hartman JC. Role of bradykinin in myocardial preconditioning. J Pharmacol Exp Ther 1994;270:681–689.
[51] Goto M, Liu Y, Yang XM et al. Role of bradykinin in protection of ischemic preconditioning in rabbit hearts. Circ Res 1995;77:611–621.
[52] Brew EC, Mitchell MB, Rehring TF et al. Role of bradykinin in cardiac functional protection after global ischemia–reperfusion in rat heart. Am J Physiol 1995;269:H1370–1378.
[53] Matsuki T, Shoji T, Yoshida S et al. Sympathetically induced myocardial ischaemia causes the heart to release plasma kinin. Cardiovasc Res 1987;21:428–432.
[54] Schulz R, Post H, Vahlhaus C, Heusch G. Ischemic preconditioning in pigs: a graded phenomenon: its relation to adenosine and bradykinin. Circulation 1998;98:1022–1029.
[55] Pan HL, Chen SR, Scicli GM, Carretero OA. Cardiac interstitial bradykinin release during ischemia is enhanced by ischemic preconditioning. Am J Physiol Heart Circ Physiol 2000;279:H116–121.
[56] Sato M, Engelman RM, Otani H et al. Myocardial protection by preconditioning of heart with losartan, an angiotensin II type 1-receptor blocker: implication of bradykinin-dependent and bradykinin-independent mechanisms. Circulation 2000;102:III346–III351.
[57] Miki T, Miura T, Ura N et al. Captopril potentiates the myocardial infarct size-limiting effect of ischemic preconditioning through bradykinin B_2 receptor activation. J Am Coll Cardiol 1996;28:1616–1622.
[58] Schriefer JA, Broudy EP, Hassen AH. Inhibitors of bradykinin-inactivating enzymes decrease myocardial ischemia–reperfusion injury following 3 and 7 days of reperfusion. J Pharmacol Exp Ther 2001;298:970–975.
[59] Leesar MA, Stoddard MF, Manchikalapudi S, Bolli R. Bradykinin-induced preconditioning in patients undergoing coronary angioplasty. J Am Coll Cardiol 1999;34:639–650.