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Review

Ligand triggers of classical preconditioning and postconditioning

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Abstract

The cardioprotection afforded by ischemic preconditioning (IPC) and ischemic postconditioning (PC) are receptor mediated. In this review, we will focus on the major ligand classes and receptors that contribute to IPC and PC-induced cardioprotection. Ligand classes discussed include adenosine, bradykinin, opioids, erythropoietin, adrenergics and muscarinics. The cardioprotective therapeutic window of each ligand class will also be summarized, with particular focus as to whether ligands are protective when administered at or close to the time of reperfusion. Information will primarily be directed at studies in which infarct size reduction is the gold standard to assess the efficacy of IPC and PC. Myocardial stunning is a less robust endpoint for assessing cardioprotection and the use of this endpoint is only limited to studies with human tissue where infarct size assessment is not possible. Receptor cross-talk between ligands and the common signaling pathways involved for these ligands will also be briefly discussed.

Keywords: Preconditioning; Postconditioning; Opiods; Adenosine; Bradykinin

1. General introduction

Brief intermittent periods of ischemia and reperfusion are protective, both at a time prior to ischemia, known as ischemic preconditioning (IPC), or immediately after reperfusion, known as ischemic postconditioning (PC) [1,2]. Although these stimuli are powerful means of protecting the ischemic myocardium from irreversible injury, their clinical applicability may be limited since 1) the mechanical intervention may require precise, timed pulsations of ischemia and reperfusion, 2) a reservation of physicians to purposely create an ischemic myocardium and 3) training of emergency medical professionals in this technique to provide timely intervention. Therefore, an alternative means of harnessing this protection by the use of specific receptor agonists or antagonists may provide a feasible means of effectively producing cardioprotection clinically.

Within the last 15–20 years, a number of cardioprotective ligands were identified in animal models, including adenosine, bradykinin, opioids, erythropoietin, adrenergic and muscarinic agonists. Of particular interest is the ability for these ligands to initiate cardioprotective salvage pathways in a timely manner when administered after ischemia; i.e., reperfusion injury. Unlike a mechanical stimulus, it would appear that some ligands may produce greater cardioprotection when given after the initiation of ischemia or may lose their cardioprotective efficacy if administered too late after the initiation of reperfusion. In lieu of our present findings with opioids, ligand-mediated cardioprotection may vary based on the time of administration and the specific receptor subtype an agonist targets [3,4]. Therefore, this review will focus on the ability of different receptor-mediated ligands to achieve acute cardioprotection, with particular focus on studies conducted with agents administered after the initiation of ischemia or at the time of reperfusion. This review will also briefly discuss ligand receptor cross-talk and the signaling mechanisms responsible for ligand-mediated cardioprotection.

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2. Ligands that contribute to acute cardioprotection

In this section, we will discuss the different cardioprotective classes of ligands. For additional extensive and excellent reviews on ischemic and pharmacological preconditioning and postconditioning, please consult the following Refs. [5–7]. This section is by no means a complete review of studies concerning ligand-induced cardioprotection. This review focuses on studies where either a direct comparison of a ligand administered prior, during or after ischemia was conducted or in studies where ligands were administered during ischemia or during reperfusion. This section summarizes several factors for each ligand including 1) whether the endogenous ligand has been reported to be elevated during ischemia/reperfusion, 2) receptor subtypes identified, 3) transgenic or genetic knockout animal studies, 4) the cardioprotective efficacy of each ligand when administered during ischemia or reperfusion, 5) whether the ligand can mimic the effects of IPC or PC and 6) if an antagonist of the agent can block the effects of IPC or PC. A summary of 3) and 4) is presented in Table 1.

2.1. Adenosine

Adenosine is released during ischemia and reperfusion, with blood and interstitial concentrations elevated after ischemic insults [8–10]. Four adenosine receptor subtypes exist, which include A_1, A_{2A}, A_{2B} and A_3. In genetically modified mice, evidence suggests the A_1 and A_3 receptors may contribute to cardioprotection, since overexpression of either receptor improved functional recovery from ischemia in transgenic mice [11,12]. However, adenosine A_3 receptor knockout mice have also shown protection from ischemic insults [13]. If the degree of transgene A_3 overexpression is too excessive, the mice develop hypertrophy, bradycardia, hypotension and systolic dysfunction [11]. Hence, additional genetic studies are warranted with careful monitoring of receptor level expression and furthermore, whether the genetic manipulation alters additional receptor subtypes that may contribute to generating a cardioprotective phenotype.

Alternatively, the role for adenosine in cardioprotection has also been studied by pharmacological manipulation. Specific adenosine receptor agonists reduce infarct size just as effectively when administered either prior to ischemia or just prior to reperfusion, implying that the cardioprotective effects of adenosine receptor agonists occur at the time of reperfusion [14,15]. In the mouse, it even appears that adenosine administration enhances cardioprotection when administered at reperfusion, even more so than administration just prior to ischemia [16]. Adenosine given prior to or at the start of reperfusion in rabbit or canine models is cardioprotective in some studies [17–23] while others

Table 1
Summary of ligand-induced parameters of cardioprotection

Ligand	Genetic manipulation	Agents initiated during ischemia			Agents initiated during reperfusion			Window of protection
		Agent	Target	Species	Agent	Target	Species	During ischemia/reperfusion
Adenosine	Trans: A1, A3 [11,12] KO: A3 [13]	Adenosine	NS	Mouse [16] rabbit [19] canine [20–23]	Adenosine	NS	Mouse [16] canine [17] rabbit [18,19]	Isch to start of rep*
		BN-063	A1	Rat [14]	BN-063	A1	Rat [14]	Isch to start of rep*
		CPA	A1	Rabbit [18]				Isch*
					CHA	A1	Mouse [16]	Start of rep*
		NECA	A1/A2	Rabbit [31]				Isch*
		AMP-579	A1/A2	Porcine [15] rabbit [30,31,32]	AMP-579	A1/A2	Canine [17] rabbit [27,33]	Isch to <10 min post-rep
		CGS 21680	A2	Rabbit [18,35]	CGS 21689	A2	Porcine [34] canine [23]	Isch to start of rep*
		CLIB-MECA	A3	Canine [37]	CLIB-MECA	A3	Rats [36] porcine [34]	Isch to start of rep*
Bradykinin	Trans: NT KO: B1, B2 [54,53]	Bradykinin	B1, B2	Swine [55,56] rabbit [31,57]	Bradykinin	B1, B2	Mice [58]	Isch to start of rep*
Opioids	Trans: NT	Morphine	NS	Rat [74]				Isch
	KO: NT	BW373U86	δ	Rat [74]	BW373U86	δ	Rat [Fig. 1]	Isch to 10 sec post-rep
					FIT	δ	Rat [4]	Isch to 10 sec post-rep
		U50,488	κ	Rat [Fig. 1]				Isch
Erythropoietin	Trans: NT KO: NT	Erythropoietin	EPOR	Rabbit [85] rat [84,86] canine [87]	Erythropoietin	EPOR	Rabbit [82,83,85] rat [84]	Isch to 5 min post-rep
Adrenergics	Trans: β2 [92] KO: β2 [91]				Metoprolol	β1	Rabbit [94]	Start of rep*
					Bisprolol	β1	Rabbit [95]	Start of rep*
					Carvedilol	β1	Rabbit [94, 95]	Start of rep*

Trans: transgenic, KO: knockout, NT: not tested, NS: non-specific, EPOR: erythropoietin receptor, Isch: ischemia, rep: reperfusion, post-rep: after reperfusion, *: the complete window of protection for these agents has not been determined.

report adenosine having no effect [24–26]. The acute administrative window is less than 10 min after reperfusion for adenosine or adenosine receptor agonists to induce cardioprotection [27,28].

The selective A_1 receptor agonist, (*N*-[1S, (trans)-2-hydroxycyclopentyl] adenosine, GR79236, is cardioprotective in swine when administered either prior to ischemia or reperfusion [28]. Furthermore, the protective effect of GR79236 was abolished by prior administration of the selective A_1 antagonist, DPCPX [28]. Alternatively, activating the A_1 receptor just prior to reperfusion had no protective effect when GR79236 was given 10 min before reperfusion in rabbits [29], or when initiated 10 min prior to reperfusion and continued for 70 min in swine [15]. In rabbits, the A_1 receptor agonist CPA protected when administered for 65 min, when the dose was initiated 5 min prior to reperfusion [18]. Improved functional recovery was also reported with the A_1 agonist CHA [16].

Mixed A_1 and A_{2A} receptor agonists, AMP 579 and NECA, are cardioprotective when administered just prior to reperfusion [30,31]. However, AMP 579 administered 30 min prior to ischemia and continued through the first hour of reperfusion was markedly more effective in reducing infarct size as compared to administering AMP 579 10 min prior to reperfusion for 70 min in swine [15]. In rabbits, AMP 579 reduced infarct size equally when administered either prior to ischemia or prior to reperfusion, suggesting that the mechanism in rabbits may differ from swine [32]. A further study in rabbit hearts suggests that AMP 579, initiated at reperfusion, is protective only if AMP 579 is administered for longer than 40 min following reperfusion [27]. AMP 579 infusion started at reperfusion was also protective in canine hearts [17]. The protective effect of AMP 579 is suggested to be A_{2A} receptor mediated, since the putative A_{2A} receptor antagonist, ZM 241385 blocked the cardioprotective effect of AMP 579 [33].

The adenosine A_{2A} receptor agonist, CGS 21680, is also cardioprotective when administered at the time of reperfusion. A reduction in infarct size has been reported with CGS 21680 when administered 5 min before reperfusion and continued for 65 min in rabbits [18]. CGS 21680 administered at reperfusion for 60 min in swine was also reported to be cardioprotective [34]. Protection also occurred in canine hearts following administration of CGS 21680 both prior to and continued into reperfusion or at reperfusion [23,35].

Adenosine A_3 receptor activation at reperfusion is also cardioprotective. Administration of 2-chloro-IB-MECA (CLIB-MECA) in rats reduced infarct size when administered at reperfusion in a dose-dependent inverse bell-shaped curve. The protection was abolished by the adenosine A_3 receptor antagonist MRS 1191 [36]. However the effects of CLIB-MECA in rats and swine also appear to be mediated by the adenosine A_{2A} receptor, since inhibition of the A_{2A} receptor abrogates CLIB-MECA-induced cardioprotection [34,36]. In canine hearts, CLIB-MECA was as effective in reducing infarct size when administered 5 min before reperfusion as compared to administration prior to ischemia [37].

Adenosine administration mimics the effects of IPC in all animal models tested, including human atrial trabeculae [38–40]. Specific A_1 and A_3 receptor agonists also mimic the effects of IPC [39,40]. Inhibition of adenosine receptors with the nonspecific adenosine antagonist, 8-phenyltheophylline (SPT) blocked the ability of IPC to reduce infarct size when given either prior to IPC or following IPC [40,41]. The adenosine receptor antagonist PD 115,199 also abolished IPC-induced cardioprotection when given before IPC [40]. Receptor specific adenosine antagonists, targeting the A_1 receptor, including DPCPX, BG 9719, or BG 9928, did not abolish the protective effects of 4 cycles of IPC in the dog, while the adenosine A_1 receptor antagonist, DPCPX, abolished the cardioprotective effect of 2 cycles of IPC in pigs [28,42]. Alternatively, selective inhibition of the A_3 receptor by BW A1433 blocked the cardioprotection afforded by IPC in rabbits [39].

PC is abrogated with prior administration of the adenosine antagonist, SPT, in rabbit and rat hearts [43–45]. The specific adenosine receptors that mediate PC appear to involve both A_{2A} and A_3 receptors, since the putative A_{2A} receptor antagonist, ZM241385 and the putative A_3 receptor antagonist, MRS1523, blocked PC-induced infarct size reduction [45].

Although adenosine and adenosine receptor agonists are the most extensively studied cardioprotective ligands, there is no definitive consensus as to which adenosine receptor subtype contributes to cardioprotection during ischemia or reperfusion phases. Most likely the species of animal, dose, timing and receptor subtypes activated by agents all contribute to the variations between studies. It is also apparent that the IPC or PC cycle number may affect the outcome of cardioprotective blockade via adenosine receptor antagonists by affecting the release of adenosine, the adenosine receptor affinity or some other as yet to be defined mechanism.

2.2. Bradykinin

Bradykinin is elevated during and after an ischemic insult [46–48]. Two bradykinin receptors exist in cardiomyoctes, a constitutive B_2 receptor and a B_1 receptor that is induced after stress [49,50]. In rats, the induction of the B_1 receptor in the left ventricle occurs 6 h after reperfusion, with B_1 receptor expression increasing to four fold higher after 24 h, with similar trends reported for the B_2 receptor [51,52]. IPC-induced cardioprotection is abolished in B_2 receptor knockout mice [53]. Knockout of the B_1 receptor in female mice suggest B_1 receptors have no effect on remodeling after a myocardial infarction, however, the role of the B_1 receptor concerning cardioprotection is unknown [54].

Bradykinin administered 15 min after the start of 45 min of ischemia and continued into reperfusion reduced creatine kinase release and elevated catecholamine and renin levels in swine [55]. Additionally in swine, bradykinin administered 15 min before and continued into reperfusion was beneficial, based on an observed reduction in creatine kinase and an improved electrocardiogram. However, this study did not show any differences in mortality rate between the bradykinin and saline-treated groups after two weeks [56]. Bradykinin administered in rabbits or mice, starting 5 min before reperfusion also reduced infarct size [31,57,58]. Collectively, these data suggest that the protective effect of bradykinin occurs at reperfusion and mimics PC. However, it is yet to be determined whether bradykinin is protective when administered after reperfusion.

Bradykinin mimics IPC and the selective bradykinin B_2 receptor antagonist, HOE-140 (incatibant), abolished IPC-induced cardioprotection [59]. Bradykinin use in humans mimics the effects of IPC in patients undergoing percutaneous transluminal coronary angioplasty (PCTA) [60]. It is unknown whether bradykinin receptor inhibitors can abrogate the effects of PC.

One alternative strategy is to target the inhibition of enzymes responsible for the degradation of kinins. These are a family of kinin peptidases, which include angiotensin converting enzyme (ACE), neutral endopeptidase (NEP), kininase I, carboxypeptidase M, and aminopeptidase P [61]. Addition of the ACE inhibitor ramiprilat increased bradykinin concentrations in the perfusate of isolated rat hearts [46]. Inhibition of ACE or NEP prior to reperfusion was also effective in reducing infarct size [57,62], with combined inhibition of ACE or NEP enhancing infarct size reduction [62]. Prior administration of HOE-140 abolished the infarct size sparing effect of the ACE inhibitor ramiprilat [63,64] or the NEP inhibitors [57,62]. These effects were not attributed to angiotensin II [65], since angiotensin II or the angiotensin II antagonist, losartan, did not effect infarct size [64].

2.3. Opioids

Myocardial ischemia results in the synthesis and release of endogenous opioid peptides including both Met- and Leu-enkephalin and dynorphins [66]. The highest level of preproenkephalin mRNA is in rat ventricular tissue compared to the other rat organ systems, indicating that the heart may have a very significant endogenous opiate system [67]. Three opioid receptor subtypes, μ,κ and δ have been cloned. In adult ventricular cardiomyocytes, only the κ and δ receptor subtypes were reported [68–71]. Traditionally, μ receptors were reported to be absent in the heart [69], however, a more recent study suggests that this receptor is present within human atrial trabeculae [72]. Previously, administration of Met^5-enkephalin or Leu^5-enkephalin reduced the incidence of myocardial cell death, which did not occur with administration of β-endorphins that bind primarily to the μ opioid receptor [73]. Collectively, these studies support an important role for enkephalins, and perhaps dynorphins, as an endogenous opioid system responsible for cardioprotection.

Morphine is cardioprotective when administered just prior to reperfusion, with the efficacy of protection equivalent to that observed when morphine is administered prior to ischemia in rats [74]. The protective effect of morphine appears to only be beneficial when administered prior to reperfusion, since morphine administered only 10 s after reperfusion failed to reduce infarct size in rats [3]. In contrast, the selective irreversible δ agonist, fentanyl isothiocyanate (FIT), reduced infarct size equally when given prior to ischemia or reperfusion and this protection, assessed by infarct size reduction, was extended to 10 s after reperfusion [4]. BW373U86, a δ selective opioid agonist, also reduced infarct size when administered 5 min prior to reperfusion [74]. Taken together, these data suggest that opioids elicit or mimic PC in rat hearts.

To further investigate these findings, our laboratory subjected intact rats to 30 min of ischemia and 2 h of reperfusion, and rats were treated with either the selective κ opioid agonist, U50,488, or the selective reversible δ opioid agonist, BW373U86, administered as a single bolus during time points either prior to ischemia, prior to reperfusion or after reperfusion. As shown in Fig. 1, both opioids were able to reduce infarct size, as assessed by TTC staining 2 h after reperfusion, as effectively as when administered either just prior to ischemia or reperfusion. However, the infarct size sparing effects only occurred after reperfusion following administration of the selective δ opioid agonist, BW373U86. These data, in addition to our previous findings with morphine and FIT, would suggest that selective δ opioid agonists have a more extensive therapeutic window to produce PC than κ opioid agonists [3,4,74]. These findings will also need to be confirmed in additional animal species.

Administration of the non-selective opioid agonist morphine [77], the κ selective agonist U50,488 or the selective δ-receptor agonists DADLE, TAN-67 or BW373U86 mimicked the effects of IPC, while μ specific opioid agonists failed to produce cardioprotection [75,76,78]. The δ opioid agonist, D-Ala2-Leu-enkephalin, (DADLE), mimicked the effects of IPC in human atrial trabeculae [79]. Alternatively, the opioid receptor antagonist, naloxone, abrogated IPC-induced cardioprotection in rat and rabbit hearts [76,80]. Both κ and δ selective receptor antagonists also partially abrogated IPC-induced infarct size reduction [78]. Recent preliminary evidence also suggests that PC is mediated through endogenous opioid receptors, since both naloxone and the peripheral acting naloxone derivative, naloxone methiodide, both abrogated the effects of PC [81]. Further studies will be needed to determine the opioid receptor subtypes important in mediating PC.

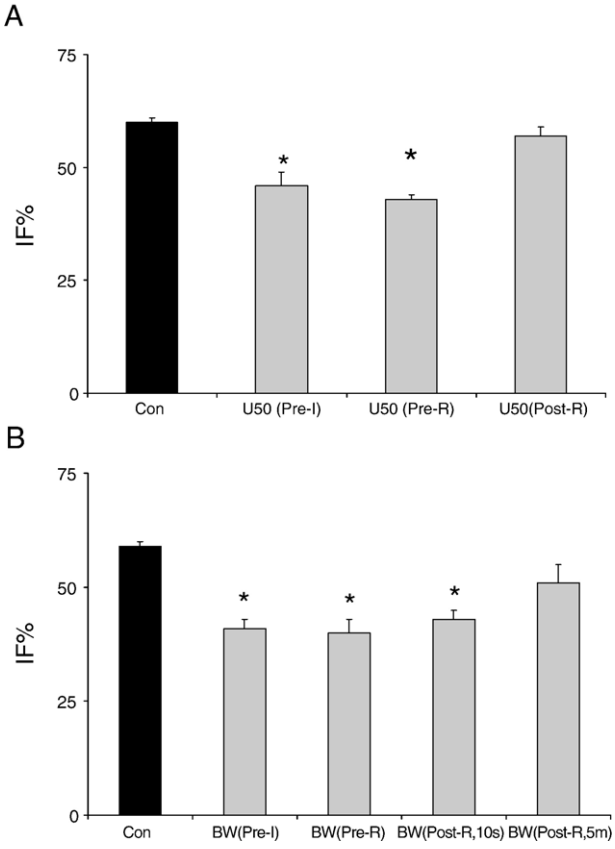

Fig. 1. Infarct size as a percent of area at risk (%IF) for rats ($n=6$/group) receiving either U50,488, BW373U86 or DMSO control (Panels A and B). Agents were administered either 10 min prior to ischemia (Pre-I), 5 min prior to reperfusion (Pre-R) or 10 s after reperfusion (Post-R, 10 s). BW373U86 was also administered 5 min after reperfusion (Post-R, 5 m). Significance is indicated by *($P<0.01$).

2.4. Erythropoietin

Erythropoietin administered in mice at the time of reperfusion produced a beneficial effect of normalizing LVEDP 1 week after infarction as well as normalizing ventricular wall stress [82], and reduced infarct size in rabbits [83]. Erythropoietin administered 5 min after reperfusion also reduced infarct size equally compared to erythropoietin administered either 2 h before ischemia or at the start of ischemia [84] indicating a PG-like effect. Comparative analysis of erythropoietin administered at either 1000 or 5000 U/kg also showed erythropoietin reduced infarct size when administered either at the time of ischemia or reperfusion [85]. Erythropoietin reduced infarct size when administered just prior to reperfusion in isolated rat hearts and dog hearts [86,87]. Most erythropoietin doses used for these studies ranged between 1000 and 5000 U/kg, however, a lower and perhaps more clinically relevant erythropoietin dose of 100 U/kg reduced infarct size when administered just prior to reperfusion in canine hearts [87].

The erythropoietin receptor is expressed in cardiac myocytes [88,89], however, no studies have examined whether receptor blockade can abrogate the cardioprotective effects of IPC or PC. The ability for erythropoietin to play a direct role in IPC or PC-induced cardioprotection is also unknown.

2.5. Adrenergic agents

The contribution of adrenergic receptors in cardioprotection was recently revisited. Adrenergic receptors include both α and β subtypes with α_1, β_1, β_2 and β_3 found to be present in cardiomyocytes [90]. Knockout mice deficient in the β_2 receptor lacked the ability to be preconditioned by IPC [91]. Over-expression of the β_2 receptor in transgenic mice worsened ischemic injury, suggesting chronic upregulation of the β_2 receptor may also deleteriously alter cardioprotective signaling pathways [92]. Administration of isoproterenol at the time of reperfusion improved both regional and global cardiac function in canine hearts, however, it failed to reduce infarct size, perhaps due to the fairly long 2 h ischemic period used [93].

Adrenergic receptor blocker administration at reperfusion yielded promising results. The selective β_1 receptor antagonists, bisoprolol and metoprolol, were found to produce a significant reduction of infarct size [94,95]. Carvedilol, a nonselective β receptor antagonist, α_1 receptor antagonist and free radical scavenger, produced more substantial infarct size reduction compared to other selective β_1 receptor antagonists, perhaps due to the free radical scavenging properties of carvedilol.

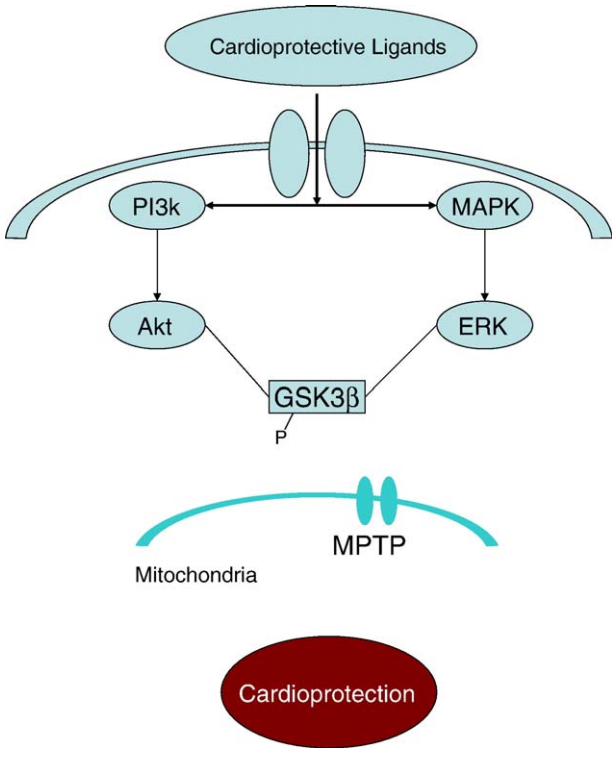

Fig. 2. Proposed signaling components of ligand-induced cardioprotection at the time of reperfusion.

Cardioprotection also occurs via α_1 adrenergic receptor activation, since norepinephrine reduced infarct size to an extent that mimicked IPC [96]. The cardioprotective effects of norepinephrine were also abolished by selective α_1 receptor antagonists in rabbits and rats [96,97]. Isoproterenol also mimicked the effects of IPC [91], however, contradictory findings suggest that inhibition of β receptors by atenolol or esmolol abrogate IPC-induced infarct size reduction [98]. Collectively, the importance for the availability of adrenergic receptors during ischemia and reperfusion is still in its infancy, and additional studies are required to investigate the role of adrenergic agents to effectively mimic or block IPC and PC.

2.6. Muscarinics

Myocardial interstitial levels of acetylcholine during IPC and during prolonged ischemia in felines were shown to be significantly elevated compared to baseline [99]. The addition of acetylcholine prior to ischemia also mimicked the effects of IPC in canine and rabbit hearts [100–102]. In rats, acetylcholine administered prior to ischemia and continued throughout ischemia and reperfusion reduced infarct size, but not as substantially as IPC. This study also showed that the muscarinic antagonist atropine did not abrogate IPC [103]. However, acetylcholine given 5 min prior to reperfusion for 60 min in isolated perfused rabbit hearts did not reduce infarct size [31]. Hence, the contributions of muscarinics in cardioprotection need further investigation.

3. Timing and dosing considerations of ligand administration

A direct comparison of the cardioprotective effects at each time of intervention in different animal species, similar to the study shown in Fig. 1, will be needed for each agent to discern whether the maximal efficacy is similar at different time points of administration. Additionally, the ceiling of cardioprotection produced by IPC and PC should warrant further investigation, since it would appear that the ability for ligands to salvage myocardium may be dependent upon the length of index ischemia experienced prior to ligand administration [104]. Characterization of these parameters in animal models would be fruitful in order to further design more effective clinical trials and maximize the efficacy of these ligands in humans. Once these parameters are established, whether a combination of ligands can act in synergy to more effectively reduce infarct size as compared to a single agent administered alone should also be determined.

Secondly, the dosing effects of ligands need more extensive investigation, since a number of ligands, including opioids and adenosine generate an inverse bell shaped dose response curves in relation to infarct size reduction. This would indicate that ligands have a finite dosing window that induces cardioprotection. The dose response curves also suggest that endogenous feedback systems are initiated when an agent is administered, and further studies should target ways to inactivate feedback systems in order to achieve greater ligand efficacy when ligands are given at higher doses.

4. Receptor cross-talk and importance in ligand-mediated protection

At the receptor level, receptors are somewhat promiscuous, since they can both homodimerize and heterodimerize with different receptor subtypes or receptor classes [105]. With this in mind, a number of studies suggest cross-talk occurs between receptors. For example, antagonism of δ opioid receptors blocks the protective effect of the adenosine A_1 receptor agonist, CCPA and in addition, morphine or fentanyl-induced infarct size reduction was abolished by an A_1 receptor antagonist [106,107]. The infarct size sparing effects of carvedilol were abrogated by an adenosine receptor antagonist [108]. It also appears that the κ opioid receptor subtypes and β adrenergic receptors share a cross-talk phenomenon [109]. The importance of receptor cross-talk in cardioprotection, particularly at reperfusion, will need further investigation, and is likely to be consistent with the "threshold hypothesis" of IPC previously hypothesized by Downey's laboratory [110].

5. Ligand-mediated signaling pathways initiated at the time of reperfusion

The molecular pathways involved in acute cardioprotection have been reviewed extensively [7,111–113]. Evidently, the means by which ligands induce protection are unclear and further consideration has to be made as to the cellular components initiated or inhibited during ischemia/reperfusion as well as their contribution to reducing necrosis, apoptosis, endothelial injury and/or microvascular and macrovascular injury.

Presently, evidence indicates that there are at least two molecular signaling pathways, the phosphatidylinositol-3 kinase (PI3k) and mitogen activated kinase pathways, responsible at reperfusion for relaying the ligand-induced cardioprotective effect from the receptor in the myocardium to a downstream end effector, as shown in Fig. 2. These pathways converge to cause inhibition of glycogen synthase kinase-3β (GSK3β) [111,112]. In the myocardium, PI3k has classically been shown to regulate GSK3β inhibition by activation of protein kinase B (Akt). Non-myocardial cell lines have also shown that extracellular regulated kinase (ERK) primes GSK3β to allow for phosphorylation and inhibition at the Ser^9 site [114].

Table 2
Future directions for ligand-induced cardioprotection research

- What receptor subtypes are important to post-conditioning?
  - Are these receptors important consistent with those required for IPC?
  - Are there different receptor subtypes important in ischemia compared to reperfusion?
- What are the most efficacious exogenous ligands?
  - Can an optimal cardioprotective cocktail be created and perhaps include IPC or PC?
  - Is the order of ligand administration important?
  - What experimental factors are important for a ligand to achieve maximal efficacy?
    - Dose?
    - Window of administration?
    - Ceiling (duration of ischemia)?
- Does receptor cross-talk occur between ligands at reperfusion?
- Is GSK3β/MPTP inhibition common signaling mediators or cardioprotective ligands?
  - Is inhibition connected to modulation of ROS burst at reperfusion?
  - Is this mechanism species dependent?
- Are these pathways and agents as effective in female species?
- How do diseases alter the efficacy of ligand-induced protection?
- What is the role for genetic background in ligand-induced myocardial protection?

GSK3β inhibition then leads to inhibition of the mitochondrial permeability transition pore (MPTP) [115]. Agonists including opioids, adenosine and bradykinin, in addition to IPC, are suggested to initiate cardioprotection via GSK3β and MPTP inhibition, suggesting this pathway may be a common mechanism for how ligands mediate their cardioprotective effect [74,115,116]. Both GSK3β inhibition and MPTP inhibition at the time of reperfusion reduce infarct size and may perhaps be the end effectors of cardioprotection [74,117].

One paradigm that presently seems to exist in cardioprotective signaling is the benefit of reactive oxygen species (ROS) generation. ROS generation was previously found to trigger cardioprotection when acetylcholine, bradykinin, opioids and phenylepherine are administered prior to ischemia, which in turn caused distal signaling events [118,119]. However, since agents such as bradykinin and opioids are cardioprotective when administered at the time of reperfusion, one would question whether these agents generate an initial burst of ROS that activate cardioprotective pathways and protect against the substantial ROS generation that occurs during reperfusion that leads to injury. For δ opioids, the infarct size sparing effect, at least in rats, is equally effective when administered at the time of reperfusion as compared to administration prior to or during ischemia. Therefore, agents that were previously shown to induce protection by an initial ROS burst prior to ischemia/reperfusion need to be re-examined in different animal species that have different free radical scavenging mechanisms, to determine whether ROS can trigger cardioprotective signaling events. Furthermore, whether the kinases altered at reperfusion, such as GSK3β inhibition, reduce the ROS generated at reperfusion that leads to injury needs further investigation.

6. Summary/conclusion

The resurgence of interest in effectively giving drugs at the time of reperfusion has suggested a promising avenue of research. The different cardioprotective agents discussed have potential as therapeutics to reduce the extent of myocardial infarction. Of importance is the characterization of the therapeutic window for each cardioprotective ligand, dosing efficacy, species dependent effects and whether synergism occurs with a cocktail of agents administered. With this in mind, many questions are unanswered in ligand-mediated cardioprotection and future directions, such as those in Table 2, should be pursued. By these means, we will be one step closer in harnessing the maximal cardioprotective efficacy of ligands that could one day be used as standard intervention for patients presenting with an acute myocardial infarction by using IPC and/or PC.

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