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Themed Section: Conditioning the Heart – Pathways to Translation

REVIEW

Extracellular signalling molecules in the ischaemic/reperfused heart – druggable and translatable for cardioprotection?

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In patients with acute myocardial infarction, timely reperfusion is essential to limit infarct size. However, reperfusion also adds to myocardial injury. Brief episodes of ischaemia/reperfusion in the myocardium or on organ remote from the heart, before or shortly after sustained myocardial ischaemia effectively reduce infarct size, provided there is eventual reperfusion. Such conditioning phenomena have been established in many experimental studies and also translated to humans. The underlying signal transduction, that is the molecular identity of triggers, mediators and effectors, is not clear yet in detail, but several extracellular signalling molecules, such as adenosine, bradykinin and opioids, have been identified to contribute to cardioprotection by conditioning manoeuvres. Several trials have attempted the translation of cardioprotection by such autacoids into a clinical scenario of myocardial ischaemia and reperfusion. Adenosine and its selective agonists reduced infarct size in a few studies, but this benefit was not translated into improved clinical outcome. All studies with bradykinin or drugs which increase bradykinin's bioavailability reported reduced infarct size and some of them also improved clinical outcome. Synthetic opioid agonists did not result in a robust infarct size reduction, but this failure of translation may relate to the cardioprotective properties of the underlying anaesthesia *per se* or of the comparator drugs. The translation of findings in healthy, young animals with acute coronary occlusion/reperfusion to patients of older age, with a variety of co-morbidities and co-medications, suffering from different scenarios of myocardial ischaemia/reperfusion remains a challenge.

LINKED ARTICLES

This article is part of a themed section on Conditioning the Heart – Pathways to Translation. To view the other articles in this section visit http://dx.doi.org/10.1111/bph.2015.172.issue-8

Abbreviations

AMI, acute myocardial infarction; AT_1 receptor, angiotensin II type 1 receptor; CABG, coronary artery bypass surgery; eNOS/PKG, endothelial NOS/PKG; GLP, glucagon-like peptide; K_{ATP}, mitochondrial ATP-sensitive K^+ channel; MPTP, mitochondrial permeability transition pore; PCI, percutaneous coronary intervention; RIPC, remote ischaemic preconditioning; RISK, reperfusion injury salvage kinase; ROS, reactive oxygen species

Tables of Links

TARGETS	
CGRPs[a]	**Enzymes**[d]
β-adrenoceptor	ACE
δ opioid receptor	Adenosine deaminase
κ opioid receptor	Adenosine kinase
μ opioid receptor	Adenylate cyclase
A_1 receptor	Akt (PKB)
A_{2A} receptor	Aminopeptidase M
A_{2B} receptor	COX
A_3 receptor	eNOS
AT_1 receptor	ERK
AT_2 receptor	GSK3β
B_1 receptor	JAK
B_2 receptor	PI3K
GLP-1 receptor	PKA
Ion channels[b]	PKC
K_{ATP} channel	PKG
Catalytic receptors[c]	sGC
gp 130 (IL-6β) receptor	
Natriuretic peptide receptor	
TNF receptor	

LIGANDS	
Acadesine	Halothane
Adenosine	Isoflurane
ATP	Ketamine
Bradykinin	Losartan
Buprenorphine	Methadone
cAMP	Metoprolol
Candesartan	Midazolam
Captopril	Morphine
cGMP	Naloxone
Cyclosporine A	Olmesartan
Diazepam	Pancuronium
Dipyridamole	Propofol
Enalapril	Prostacyclin
Enalaprilat	Ramipril
Enflurane	Remifentanil
Fentanyl	Scopolamine
Flunitrazepam	Sufentanil
	Telmisartan

These Tables list key protein targets and ligands in this article which are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Pawson *et al.*, 2014) and are permanently archived in the Concise Guide to PHARMACOLOGY 2013/14 ([a,b,c,d]Alexander *et al.*,2013a,b,c,d).

Cardioprotection

Infarct size determines the prognosis of patients suffering an acute myocardial infarction (AMI) (Burns *et al.*, 2002) and it is therefore important to reduce infarct size as much as possible (Braunwald, 1974). Reperfusion of the ischaemic region is mandatory to halt the progression of infarction during sustained myocardial ischaemia (Ginks *et al.*, 1972; Maroko *et al.*, 1972). Reperfusion, however, is a double-edged sword and not only terminates myocardial ischaemic injury, but also induces additional myocardial injury (Heusch, 2004; Yellon and Hausenloy, 2007). Therefore, there is a need for further cardioprotection beyond that by reperfusion (Heusch, 2013a).

Brief episodes of myocardial ischaemia/reperfusion before or shortly after a sustained myocardial ischaemia effectively reduce infarct size, but only when there is ultimately reperfusion. These protective phenomena are termed ischaemic pre- or postconditioning respectively. Likewise, cardioprotection can be achieved by inducing brief episodes of ischaemia/reperfusion in a myocardial territory remote from the infarcting myocardium or in an organ remote from the heart – that is remote ischaemic conditioning. The conditioning phenomena were originally reported in dogs (Murry *et al.*, 1986; Przyklenk *et al.*, 1993; Zhao *et al.*, 2003), but have meanwhile been confirmed in many species, including humans (Heusch, 2013a). Apart from and beyond the reduction in infarct size, conditioning strategies also reduce arrhythmias, preserve ventricular function, prevent the development of heart failure and even improve clinical outcome (Davies *et al.*, 2013; Heusch, 2013a; Thielmann *et al.*, 2013; Heusch *et al.*, 2014; Sloth *et al.*, 2014).

Common to all conditioning phenomena is the recruitment and activation of cardioprotective signalling by the mechanical intervention which induces brief cycles of ischaemia and reperfusion in the heart or in an organ remote from the heart (Heusch *et al.*, 2008). The underlying signal transduction of mechanically induced endogenous cardioprotection has been studied extensively with the aim to develop the identified signals into pharmacological conditioning strategies which could then be translated to patients suffering from myocardial ischaemia/reperfusion events. The different conditioning manoeuvres appear to act to a certain extent through common cardioprotective signalling steps (Downey and Cohen, 2005). However, the notion of common signalling steps for all conditioning strategies was largely derived by inference from experiments using different setups, animal models and conditioning manoeuvre. Currently, no data are available which directly compare the cardioprotective signalling under different conditioning strategies in the same animal model. With such caveats in mind, the signal transduction of the conditioning manoeuvres can be conceptually

classified either by the timing of their operation (trigger, mediator and effector) or their (sub-) cellular localization (extracellular molecules, cytosolic signal transduction and target organelle/structure).

With respect to the temporal sequence of cardioprotective signalling, a *trigger* is released during the repeated cycles of ischaemia/reperfusion before or after the sustained myocardial ischaemia which act as the stimulus for cardioprotection. The trigger then activates a receptor-dependent or receptor-independent signalling cascade. A *mediator* is activated by the trigger and actively transmits the cardioprotective signal during the sustained ischaemia/reperfusion. An *effector* is the target of the protective signalling which when activated during the sustained ischaemia or during early reperfusion ultimately attenuates myocardial injury (Yellon and Downey, 2003). Such temporal classification of signalling steps was originally developed for ischaemic preconditioning (Downey *et al.*, 2008); it never really became clear where the 'memory' resided which remembered the trigger during the preconditioning cycles and then activated the mediator during the sustained ischaemia. Also, such temporal classification of cardioprotective signal transduction is conceptually more difficult to use during ischaemic postconditioning when trigger, mediator and effector must all be sequentially activated over a very short period during early reperfusion.

Therefore, the local classification of cardioprotective signalling is more popular now and we will use it here. *Extracellular molecules* are released during the brief conditioning cycles of ischaemia/reperfusion from various cellular compartments (cardiomyocytes, endothelium, nerve endings, etc.) and activate the protective signal cascade through sarcolemmal receptors or independently of receptors. Autacoids, such as adenosine, bradykinin and opioids, activate GPCRs; natriuretic peptides activate their specific receptors and cytokines activate gp130 (IL-6, β subunit) receptors. Reactive oxygen species (ROS) and NO can initiate receptor-independent protective signalling (Heusch *et al.*, 2008; Tullio *et al.*, 2013; Rassaf *et al.*, 2014). Within the cardiomyocyte, a variety of proteins are activated as *cytosolic signal transducers*. They interact at different levels and at different time points during the conditioning and the sustained index ischaemia/reperfusion. Three major intracellular signal transduction pathways are apparent: the endothelial NOS/PKG (eNOS/PKG) pathway (Cohen and Downey, 2007), the reperfusion injury salvage kinase (RISK) pathway (Hausenloy and Yellon, 2004) and the survivor activating factor enhancement pathway (Heusch *et al.*, 2008; Lecour, 2009) (Figure 1). These complex signalling cascades interact at different levels (Vahlhaus *et al.*, 1996; 1998; Cohen and Downey, 2007; Cohen *et al.*, 2007) (Figure 1). The mitochondria have been identified as a major common intracellular *target structure* (Heusch *et al.*, 2008). Mitochondria are the major energy source (aerobic ATP production) of the cell and hence relevant for all cellular functions and cell survival in general. Beyond and apart from energy metabolism, mitochondria have a decisive role in apoptosis, autophagy and necrosis (Heusch *et al.*, 2008; 2010). Various PKs activate the mitochondrial ATP-sensitive K^+ (K_{ATP}) channel, thereby trigger a modest amount of ROS formation (Pain *et al.*, 2000; Heinzel *et al.*, 2005; Downey *et al.*, 2007; Costa and Garlid, 2008) and inhibit the opening of the mitochondrial permeability transition pore (MPTP) (Hausenloy *et al.*, 2002; 2004b; Argaud *et al.*, 2004; 2005; Juhaszova *et al.*, 2004; Heusch *et al.*, 2010). Nitrosation (Methner *et al.*, 2013; Rassaf *et al.*, 2014) and nitrosylation (Penna *et al.*, 2013) of mitochondrial membrane proteins are causally involved in cardioprotection.

In the following, we will focus on the extracellular signalling molecules which are potentially druggable and translatable for cardioprotection, and more specifically those which act through receptors and for which drugs are available that interact with them. Knowledge about such receptor ligands is derived from a multitude of studies, ranging from *in vitro* studies of isolated subcellular elements, cells or heart preparations to different *in vivo* models from different species and using a variety of techniques, ranging from immunoblotting, biochemical analyses to pharmacological agonist and antagonist approaches and molecular genetic approaches (e.g. knockout, knockdown and transgenic overexpression).

Extracellular receptor ligands in conditioning

Ischaemic preconditioning was originally reported as an-all-or-none phenomenon. Protection by ischaemic preconditioning in rats (Barbosa *et al.*, 1996), rabbits (Goto *et al.*, 1995) and pigs (Schulz *et al.*, 1998), however, depends on the strength (number and duration of ischaemia/reperfusion cycles) of the preconditioning stimulus, reflecting a dose–response relationship between the trigger/released ligand during the preconditioning cycles of ischaemia/reperfusion and the resulting infarct size reduction (Yellon and Downey, 2003). A protective response is already induced by one short (between 1.5 and 2.5 min) or more cycles of ischaemia followed by a minimum reperfusion period of about 30 s to 1 min (Alkhulaifi *et al.*, 1993). Ligands involved in such ischaemic preconditioning protection are adenosine (Liu *et al.*, 1991; Schulz *et al.*, 1995), bradykinin (Goto *et al.*, 1995; Schulz *et al.*, 1998) and opioids (Schultz *et al.*, 1995; Cohen *et al.*, 2001; Schulz *et al.*, 2001). For these three ligands, an increased release/bioavailability has been detected during/after the preconditioning procedure (Goto *et al.*, 1995; Schulz *et al.*, 1995; 1998; Younes *et al.*, 2005). A second window of protection is apparent between 12 and 72 h after the preconditioning stimulus; the ligands which are involved as trigger and/or mediator are mostly identical to those in acute ischaemic preconditioning (Bolli, 2000; Yellon and Downey, 2003; Stein *et al.*, 2004).

Adenosine was the first signalling molecule reported to trigger and mediate cardioprotection by ischaemic preconditioning in the rabbit heart (Liu *et al.*, 1991). Myocardial interstitial adenosine levels increase during ischaemic preconditioning in rats (Kuzmin *et al.*, 2000), rabbits (Lasley *et al.*, 1995), dogs (Mei *et al.*, 1998) and pigs (Schulz *et al.*, 1995). Adenosine is formed intracellularly through ecto-5′-nucleotidase and S-adenosylhomocysteine hydrolase in vascular cells and in cardiomyocytes. During hypoxia, the cellular formation and release of adenosine are increased. The half-life of extracellular adenosine is short, the cellular uptake of interstitial and plasmatic adenosine is fast and the enzymatic metabolism of adenosine through adenosine kinase

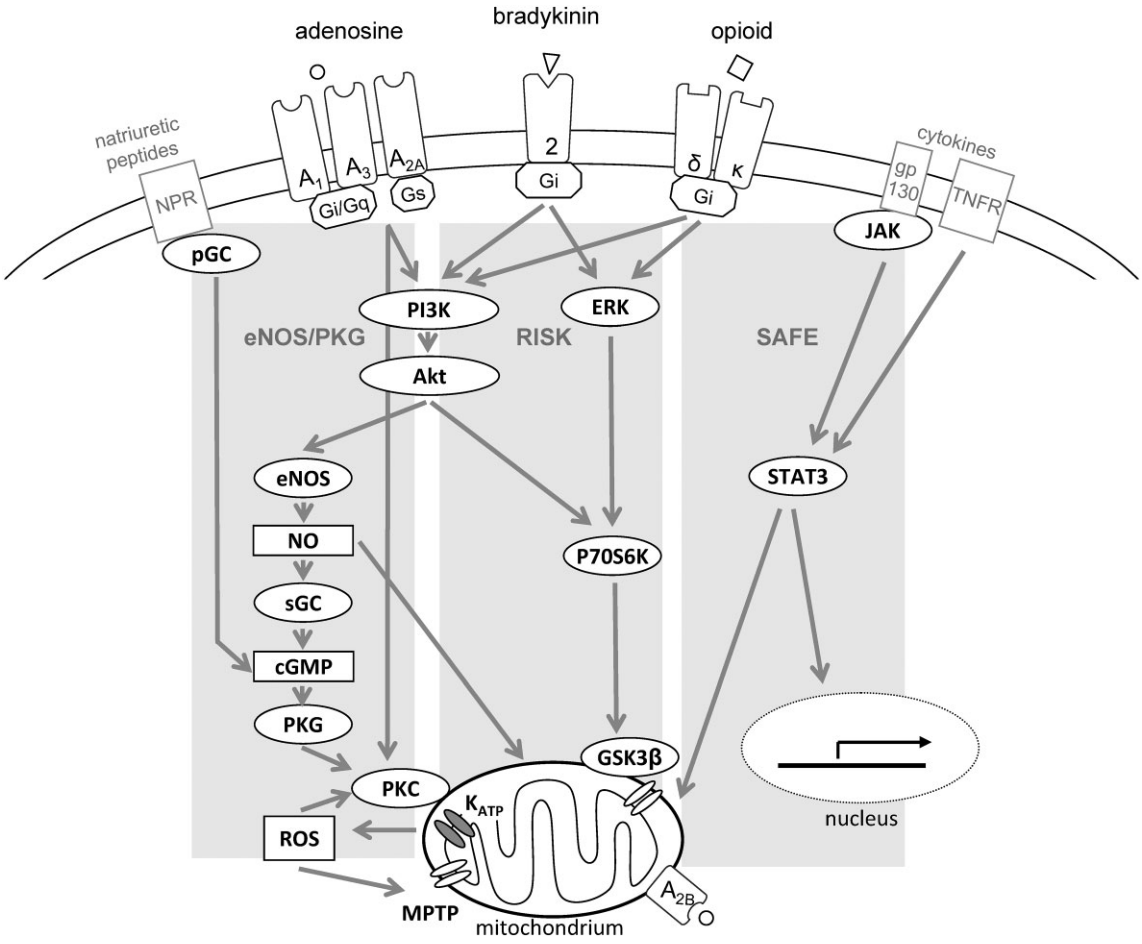


Figure 1

Signal transduction in cardioprotection: the extracellular signalling molecules adenosine, bradykinin and opioids act as triggers, the respective receptors and downstream activated protein kinases as mediators and the mitochondria as effectors (modified from Heusch *et al.*, 2008). eNOS/PKG, endothelial NOS/PKG pathway; gp130, glycoprotein 130; GSK3β, glycogen synthase kinase 3β; K_{ATP}, mitochondrial ATP-sensitive K^+ channel: MPTP, mitochondrial permeability transition pore; NPR, natriuretic peptide receptor; P70S6K, p70 ribosomal S6 protein kinase; pGC, particulate guanylate cyclase; RISK, reperfusion injury salvage kinase pathway; ROS, reactive oxygen species; SAFE, survivor activating factor enhancement pathway; sGC, soluble guanylate cyclase; TNFR, tumour necrosis factor receptor.

and adenosine deaminase is high (Deussen, 2001). A decrease in interstitial adenosine with intracoronary adenosine deaminase in pigs prevents (Schulz *et al.*, 1995), whereas an increase in interstitial adenosine with uptake inhibition by dipyridamole enhances the myocardial protection by ischaemic preconditioning in rabbits and dogs (Auchampach and Gross, 1993; Suzuki *et al.*, 1998). Four adenosine receptor subtypes (A_1, A_{2A}, A_{2B}, A_3) have been identified which are expressed on most cells of the body. In the cardiovascular system, adenosine receptors exist on cardiomyocytes, endothelial cells, fibroblasts, smooth muscle cells and also on blood cells (Forman *et al.*, 2006; McIntosh and Lasley, 2012). In the vasculature, all four receptor subtypes are expressed on vascular smooth muscle cells; A_{2A} and A_{2B} receptors are also expressed on endothelial cells. The A_{2A} receptor is the predominant receptor for coronary vasomotor effects and its activation induces coronary vasodilation (Mustafa *et al.*, 2009). On cardiomyocytes, A_1, A_{2A} and A_3 receptors are expressed (Xin *et al.*, 2012); for the A_{2B} receptor, an intracellular, mitochondrial localization has been demonstrated in rat cardiomyocytes which serves a cardioprotective function (Grube *et al.*, 2011). The A_1 receptor is abundantly localized on cardiomyocytes and mediates the negative chronotropic and dromotropic effects of adenosine (Mustafa *et al.*, 2009). All adenosine receptors are coupled to G proteins (Mustafa *et al.*, 2009; McIntosh and Lasley, 2012). Whereas A_{2A} and A_{2B} receptors couple to Gs proteins (Xin *et al.*, 2012), which activate PKA via adenylate cyclase activation and accumulation of cAMP, A_1 and A_3 receptors couple to Gi and Gq proteins and thereby inhibit the activity of adenylate cyclase (Mustafa *et al.*, 2009; Xin *et al.*, 2012). Downstream of the different G proteins, PKC is directly activated through adenosine, which appears to be a unique pathway for adenosine (Downey *et al.*, 2007). Also, the eNOS/PKG and RISK signal transduction pathways are activated by adenosine (Heusch *et al.*, 2008) (Figure 1). Cardioprotection during ischaemia and reperfusion is mediated through activation of different adenosine receptors (Heusch, 2010). Blockade of A_1 and A_3 receptors prevents the

protection by ischaemic preconditioning. In contrast, A_2 receptor blockade does not affect infarct size in rabbits (Liu *et al.*, 1991; 1994; McCully *et al.*, 2001). Different from ischaemic preconditioning, A_{2A} and A_{2B} receptors are involved in the cardioprotection by postconditioning, and A_1 and A_3 receptors are irrelevant here (McIntosh and Lasley, 2012). A selective A_{2A} and A_{2B} receptor agonist, when given at early reperfusion, induces protection in different species (mouse, rat, rabbit, dog and pig) (McIntosh and Lasley, 2012). The timing and cooperation of A_{2A} and A_{2B} receptor activation are critical (Methner *et al.*, 2010), that is they must be activated during the last minutes of ischaemia and the first minutes of reperfusion in mouse (Methner *et al.*, 2010) and rat hearts (Xi *et al.*, 2009) to induce cardioprotection during ischaemic postconditioning. Blockade of the A_1 receptor during ischaemic postconditioning does not affect infarct size in rats (Kin *et al.*, 2005) and rabbits (Philipp *et al.*, 2006). Non-selective blockade of adenosine receptors does not influence the protection by remote ischaemic preconditioning (RIPC) in pigs (Hausenloy *et al.*, 2012). The causal involvement of adenosine in the conditioning phenomena is established in most species (Forman *et al.*, 2006; Cohen and Downey, 2008), except the rat (Li and Kloner, 1993). However, the reduction of infarct size by exogenous adenosine or its selective agonists remains controversial. Exogenous adenosine or its selective agonists given before ischaemia in rats induced protection (Dai *et al.*, 2009) or did not (Li and Kloner, 1993), and when given just before reperfusion in rabbits induced protection (Xu *et al.*, 2000) or did not (Baxter *et al.*, 2000). Notably in larger animals, adenosine reduced infarct size in pigs (van Winkle *et al.*, 1994), but when given on top of lidocaine, adenosine either reduced infarct size in dogs (Homeister *et al.*, 1990) or did not (Vander Heide and Reimer, 1996).

Ischaemic preconditioning increases the interstitial bradykinin concentration in rabbits (Goto *et al.*, 1995) and pigs (Schulz *et al.*, 1998). Bradykinin is cleaved from kininogens. Lys-bradykinin is the major kinin peptide in the interstitium which is then converted to bradykinin by the aminopeptidase M (Kokkonen *et al.*, 1999). The kinin metabolism is linked to the angiotensin metabolism. Bradykinin is rapidly degraded by ACE. In parallel to ACE, neutral endopeptidase degrades bradykinin to a receptor-inactive kinin metabolite. The kinin metabolism is predominantly localized in the vascular bed; however, there is also a bradykinin synthesis in cardiomyocytes. There are two bradykinin receptors, B_1 and B_2 receptors. The B_2 receptor is localized on cardiomyocytes, endothelial cells and fibroblasts (Kokkonen *et al.*, 2000), whereas the B_1 receptor is usually absent on all cell types and its expression is only up-regulated with inflammation and/or severe tissue damage (Burley *et al.*, 2007). Thus, the B_2 receptor (Kokkonen *et al.*, 2000) is the relevant one for acute protection in rats and pigs (Linz *et al.*, 1997; Schulz *et al.*, 1998; Kokkonen *et al.*, 2000). The B_2 receptor is linked to Gi proteins and signals downstream to the intracellular eNOS/PKG and RISK signal transduction pathways (Heusch *et al.*, 2008) (Figure 1). Exogenous bradykinin induces myocardial protection in rats (Linz *et al.*, 1997), and ACE inhibitors mimic the cardioprotective effects of bradykinin (Wall *et al.*, 1994). Increasing the plasma angiotensin II concentration by antagonism of angiotensin II type 1 (AT_1) receptors activates the AT_2 receptor, subsequently initiates a kininogen activity, which finally results in increased bradykinin formation (Jalowy *et al.*, 1999). In this way, AT_1 receptor antagonists also mimic the cardioprotective effects of bradykinin (Jalowy *et al.*, 1998). Bradykinin increases prostacyclin synthesis; activation of the B_2 receptor results in activation of the COX and *de novo* synthesis of prostacyclin, which then attenuates ischaemia/reperfusion injury (Jalowy *et al.*, 1998). In pigs, the combination of an ACE inhibitor and an AT_1 receptor antagonist enhances the reduction of infarct size over that by monotherapy with each single drug (Weidenbach *et al.*, 2000). Ischaemic postconditioning is also mediated through bradykinin and the respective downstream pathways, including prostacyclin synthesis (Penna *et al.*, 2008). B_2 receptor blockade prevents remote preconditioning cardioprotection by mesenteric ischaemia/reperfusion in rats (Schoemaker and van Heijningen, 2000). In humans undergoing coronary artery bypass surgery (CABG), B_1 and B_2 receptor expression on neutrophils is down-regulated by remote ischaemic conditioning (Saxena *et al.*, 2013), indicating altered bradykinin metabolism.

Opioid peptides are secreted from cardiac nerves or produced in the cardiomyocyte itself. Apart from and beyond their synthesis, the myocardium is capable of storage and release of opioid peptides; however, enzymatic proteolysis of the stored peptides is required before active peptides are released (Pugsley, 2002). The endogenous opioid peptides act through activation of μ, δ and κ receptors respectively. In the adult myocardium, the δ and κ receptors are expressed, whereas the μ receptor is absent (Peart *et al.*, 2005). The opioid receptors are coupled to Gi proteins and share parts of the intracellular signalling with that of adenosine and bradykinin. Downstream of opioid receptor activation, also the activation of ERK, mediates cardioprotection (Ikeda *et al.*, 2006; Shimizu *et al.*, 2009) and ERK projects further downstream onto p70 ribosomal protein S6 kinase and glycogen synthase kinase 3ß (Figure 1). δ And κ receptor agonists, such as remifentanil which activates δ and κ receptors, methadone which activates the δ receptor (Gross *et al.*, 2009) and U50488H which activates the κ receptor (Wang *et al.*, 2014), induce myocardial protection when given before ischaemia (Wang *et al.*, 2014) or during reperfusion (Gross *et al.*, 2009; Wong *et al.*, 2010b) in rats. The non-selective opioid receptor antagonist naloxone prevents the protection by ischaemic preconditioning in rats (Schultz *et al.*, 1995), rabbits (Miki *et al.*, 1998) and pigs (Schulz *et al.*, 2001) and by RIPC in rabbits (Shimizu *et al.*, 2009). The protection by ischaemic preconditioning is prevented by selective antagonism of δ and κ receptors, but not by antagonism of μ receptors (Gross, 2003; Peart *et al.*, 2005). The most important receptor for protection is the δ receptor. A specific δ receptor antagonist reduces cardioprotection markedly, whereas a κ receptor antagonist has little effects on ischaemic preconditioning (Schultz *et al.*, 1998; Aitchison *et al.*, 2000) and ischaemic postconditioning in rats (Jang *et al.*, 2008; Zatta *et al.*, 2008; Guo *et al.*, 2011), and during RIPC in rats (Wong *et al.*, 2010b).

The available evidence suggests that the above-mentioned autacoids are involved in the different ischaemic conditioning manoeuvres and possibly share parts of their signalling. However, that notion does not imply that the underlying mechanism(s) are indeed identical. Autacoids appear to initiate protection in an interactive manner (Figure 1). At

weaker stimuli (shorter duration or less cycles of preconditioning ischaemia), bradykinin is more important; at stronger stimuli (longer duration or more cycles of preconditioning ischaemia), adenosine becomes more important (Goto *et al.*, 1995; Barbosa *et al.*, 1996; Schulz *et al.*, 1998). Fentanyl-induced myocardial protection is abolished by pretreatment with an adenosine receptor antagonist in rats (Kato *et al.*, 2000), emphasizing the interactive signalling of cardioprotection.

Drugs based on receptor ligands

Numerous experimental studies suggest that adenosine, bradykinin and endogenous opioids participate in myocardial protection during ischaemia/reperfusion. These studies encouraged the use of the respective drugs in clinical settings. Here, we focus on studies reporting settings of acute myocardial ischaemia/reperfusion, either spontaneous or elective, during percutaneous coronary intervention (PCI) or cardiac surgery involving ischaemic cardioplegic arrest (CABG and/or valve replacement). We selected studies using the autacoids as such, specific agonists of the autacoids or drugs increasing their bioavailability respectively. We considered only placebo-controlled (or for opioids: alternative drug) studies using myocardial infarct size as the primary and most robust endpoint for cardioprotection. Infarct size has been characterized in these studies by biomarkers reflecting myocardial damage (such as creatine kinase or troponins) or by imaging techniques (such as thallium or sestamibi single-photon emission CT or gadolinium contrast MRI). We used the following terms for systematic search in Medline, Current Contents and PubMed: 'adenosine/bradykinin, ACE inhibitor (specifically: captopril, enalapril, ramipril), AT_1 receptor antagonist (specifically: candesartan, losartan, olmesartan, telmisartan)/opioid, opioids (specifically: buprenorphine, butorphanol, fentanyl, levorphanol, morphine, remifentanil, sufentanil)' and 'myocardial infarction/coronary artery bypass surgery/percutaneous coronary intervention/reperfusion/revascularisation' and 'infarct size/creatine kinase/troponin' and 'human/patient'. We included those reports published up to May 2014 in English, or with an abstract in English, which provided data for infarct size in humans. Studies analysing only subgroups of larger trials were not systematically considered.

Adenosine has been tested in several clinical trials in the scenario of AMI, elective PCI, CABG or other cardiac surgery. Mode (bolus vs. infusion), dose and timing (before or during ischaemia and reperfusion) of administration differed (Table 1). Taken together and considering such differences, there are several positive studies, but there is no consistent evidence that adenosine reduces infarct size in clinical settings of myocardial ischaemia/reperfusion (Table 1). However, even in studies with reduced infarct size (e.g. AMISTAD II), there was no benefit in clinical outcome with adenosine or its selective agonists (Ross *et al.*, 2005). Exogenous bradykinin or acutely increasing bradykinin's bioavailability by ACE inhibitors or angiotensin receptor blockers has also been tested in several clinical scenarios (Table 2). Independent of mode, dose or timing, infarct size was consistently reduced in these studies (Table 2). Data on clinical outcome from these studies are lacking. Different synthetic opioid agonists have been tested in several clinical scenarios of myocardial ischaemia/reperfusion (Table 3). Drugs, dose, mode (bolus vs. infusion) and timing (before or during ischaemia) of administration differed (Table 3). From these studies, there is no evidence that synthetic opioid agonists reduce infarct size in clinical settings of myocardial ischaemia/reperfusion (Table 3).

Cardioprotection by other receptor-dependent and non-receptor-dependent signalling molecules and its clinical translation

Activation of brain natriuretic peptide receptors recruits a cardioprotective signal transduction cascade which involves increased myocardial cGMP and activation of mitochondrial K_{ATP} channels to reduce infarct size in isolated rat hearts (D'Souza *et al.*, 2003). In the J-WIND trial in more than 500 patients with reperfused AMI, i.v. infusion of atrial natriuretic peptide (ANP) for 3 days after reperfusion reduced infarct size, as assessed from the AUC of creatine kinase (Kitakaze *et al.*, 2007).

Recently, i.v. infusion of the ß-blocker metoprolol just before reperfusion in patients with AMI undergoing primary PCI reduced infarct size, as assessed by MRI (Ibanez *et al.*, 2013), and the benefits for ventricular function and survival persisted more long-term (Pizarro *et al.*, 2014). It is currently unclear, whether the observed benefit from metoprolol is shared by other ß-blockers and to which particular property of metoprolol or ß-adrenoceptor blockade the protection might relate.

Exenatide is a mimetic of human glucagon-like peptide (GLP)-1, activates the G-protein coupled GLP-1 receptor, recruits a protective signal transduction and reduces infarct size in an anaesthetized pig model of myocardial ischaemia/reperfusion (Timmers *et al.*, 2009). Such cardioprotection was also recently reported in patients with AMI undergoing primary PCI who had reduced infarct size with i.v. exenatide just before reperfusion, as assessed by MRI (Lønborg *et al.*, 2012).

There is currently a notion that cardioprotective signal transduction converges onto the mitochondria, more specifically the MPTP (Heusch *et al.*, 2008; 2010). c inhibits MPTP opening and reduces infarct size in mice (Boengler *et al.*, 2010) and pigs (Skyschally *et al.*, 2010; Gedik *et al.*, 2013), but not in rats (De Paulis *et al.*, 2013). Also, in patients with AMI undergoing primary PCI, an i.v. bolus of cyclosporine A just before reperfusion reduced infarct size, as assessed from the AUC of creatine kinase release (Piot *et al.*, 2008); similarly, protection by cyclosporine A was also reported for patients undergoing CABG (Hausenloy *et al.*, 2014).

Conclusion and perspectives for the future of pharmacological cardioprotection

The translation of extracellular signalling molecules with established cardioprotective potential in experimental

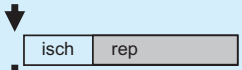
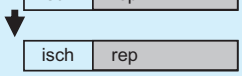
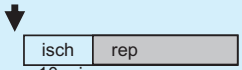
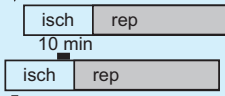
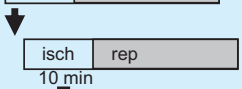
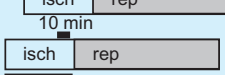
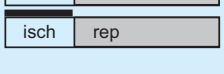
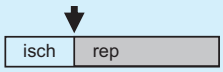
Table 1

Effects of adenosine or its agonists on infarct size in patients

Study	Clinical scenario	Method to assess endpoint	Dosage	Time of application	Intervention [*n*]	Placebo [*n*]	Infarct size [Δ% of placebo]
Mahaffey *et al.*, 1999 AMISTAD I	STEMI AMI	SPECT (at day 5–9)	Adenosine, i.v. (70 μg·kg^{-1}·min^{-1})	3 h; isch rep	101	96	↓33*
Marzilli *et al.*, 2000	AMI	CK (max within 24 h)	Adenosine, i.v. (4 mg)	↓; isch rep	27	27	↓22
		CK-MB (max within 24 h)					↓55
Kopecky *et al.*, 2003 ADMIRE	STEMI AMI	SPECT (at day 5–9)	AMP579, i.v. (15 μg·kg^{-1})	6 h; isch rep	78	74	↑17
			AMP579, i.v. (30 μg·kg^{-1})		83		↓22
			AMP579, i.v. (60 μg·kg^{-1})		76		↓28
Ross *et al.*, 2005 AMISTAD II	STEMI AMI	SPECT (at day 5–9)	Adenosine, i.v. (50 μg·kg^{-1}·min^{-1})	15 min; isch rep	~60	~60 (243)	↓15
			Adenosine, i.v. (70 μg·kg^{-1}·min^{-1})		~60		↓61*
Fokkema *et al.*, 2009	STEMI AMI	CK (max within 48 h)	Adenosine, i.c. (2 × 120 μg)	↓↓; isch rep	226	222	↑2
		CK-MB (max within 48 h)					↑15
Desmet *et al.*, 2011	STEMI AMI	MRI (at day 5–9)	Adenosine, i.c. (4 mg)	↓; isch rep	51	49	↑15
		CK (max within 24 h)					↑15
		CK (AUC 24 h)					↑31
		CK-MB (max within 24 h)					↑7
		CK-MB (AUC 24 h)					↑2
		Troponin I (max within 24 h)					↑16
		Troponin I (AUC 24 h)					↑16
Grygier *et al.*, 2011	STEMI AMI	CK (max within 24 h)	Adenosine, i.c. (1–2 mg)	↓; isch rep	35	35	↓21
		CK-MB (max within 24 h)					↑13
		Troponin I (max within 24 h)					↑8
Zhang *et al.*, 2012	STEMI AMI	SPECT (at day 1)	Adenosine, i.v. (50 μg·kg^{-1}·min^{-1})	3 h; isch rep	19	17	↓15
			Adenosine, i.v. (70 μg·kg^{-1}·min^{-1})		18		↓21
		SPECT (after 6 months)	Adenosine, i.v. (50 μg·kg^{-1}·min^{-1})		19	17	↓13
			Adenosine, i.v. (70 μg·kg^{-1}·min^{-1})		18		↓25*
		CK-MB (max within 24 h)	Adenosine, i.v. (50 μg·kg^{-1}·min^{-1})		32	31	↓18
			Adenosine., i.v. (70 μg·kg^{-1}·min^{-1})		27		↓35*

Table 1

Continued

Study	Clinical scenario	Method to assess endpoint	Dosage	Time of application	Intervention [*n*]	Placebo [*n*]	Infarct size [Δ% of placebo]
Lee *et al.*, 2007	Elective PCI	CK-MB (max within 24 h)	Adenosine, i.c. (50 μg)		31	31	↓50*
De Luca *et al.*, 2012	Elective PCI	Troponin I (max within 24 h)	Adenosine, i.c. (120 μg and 180 μg)		130	130	↑11
Kim *et al.*, 2012	Elective PCI	CK-MB (max within 12 h)	Adenosine, i.c. (50 μg)		54	55	↑89
Belhomme *et al.*, 2000	CABG	Troponin I (AUC 48 h)	Adenosine, i.v. (140 μg·kg^{-1}·min^{-1})		22	22	↑22
Rinne *et al.*, 2000	CABG	CK-MB (max within 48 h)	Adenosine, i.v. (12 mg)		20	20	↑36
Teoh *et al.*, 2002	CABG	Troponin T (mean of 72 h)	GR79236X, i.v. (100 μg·mL^{-1})		10	10	↓7
Jakobsen *et al.*, 2013	CABG	Troponin T (max within 48 h) CK-MB (max within 48 h)	Adenosine, with cardioplegic solution (1.2 mmol·L^{-1})		30	30	–
Jin *et al.*, 2007	Valve surgery	Troponin I (AUC 24 h)	Adenosine, i.c. (1.5 mg·kg^{-1})		30	30	↓31*

*$P < 0.05$ versus placebo.

AMI, acute myocardial infarction; CABG, coronary artery bypass surgery; CK-MB, creatine kinase – muscle, brain; i.c., intracoronary; isch, ischaemia; max, maximum; PCI, percutaneous coronary intervention; rep, reperfusion; SPECT, thallium or sestamibi single-photon emission CT; STEMI AMI, ST elevation myocardial infarction, acute myocardial infarction.

animal models to cardioprotection in clinical scenarios of myocardial ischaemia and reperfusion has been largely disappointing, so far.

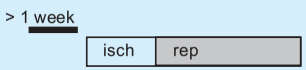
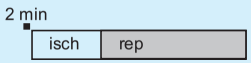
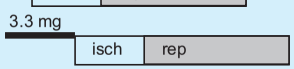
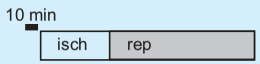
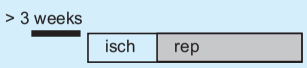
The obstacles to successful translation have often been emphasized (Ovize *et al.*, 2010; Schwartz Longacre *et al.*, 2011; Bell *et al.*, 2012; Heusch *et al.*, 2012; Hausenloy *et al.*, 2013; Heusch, 2013a; Kloner, 2013). Most of the experimental studies were performed in young and healthy animals, often only in rodents and not in larger mammals. Apart from the obvious species differences in cardiac size and geometry, as well as in haemodynamics, notably heart rate (Heusch, 2008), there are substantial species differences in cardioprotective signal transduction: for example, adenosine appears to be not important for ischaemic preconditioning in rats (Li and Kloner, 1993) and the RISK pathway is important for ischaemic postconditioning in rodents (Hausenloy and Yellon, 2004; Hausenloy *et al.*, 2004a), but not in pigs (Skyschally *et al.*, 2009). Age (Boengler *et al.*, 2008), co-morbidities and co-medications (Heusch, 2012; Ferdinandy *et al.*, 2014) are additional confounders of translation of cardioprotection to the clinical scenario. Also, the clinical scenario *per se* differs between CABG where there is controlled global myocardial ischaemia and reperfusion under cardioplegic protection (Thielmann *et al.*, 2013) and primary PCI where there is plaque rupture with release of atherosclerotic debris, thrombotic material and soluble vasoconstrictor, thrombogenic and inflammatory factors (Kleinbongard *et al.*, 2011).

Adenosine was the first signalling molecule of ischaemic preconditioning to be identified (Liu *et al.*, 1991), and ever since there has been great enthusiasm to recruit its cardioprotective potential in patients with ischaemic heart disease. As detailed earlier, a consistent reduction in infarct size was not observed with adenosine or its agonists in various clinical trials, and even those with reduced infarct size did not report improved clinical outcome. In retrospect, not all studies using adenosine, notably not those in larger animals, were positive. It is therefore surprising that apparently people have not given up on adenosine. Preliminary data of the PROMISE trial again just report reduced infarct size in a subgroup of patients with ischaemia of less than 200 min duration (Garcia-Dorado *et al.*, 2013). Acadesine, a substance that increases adenosine's bioavailability, again did not improve all-cause mortality, non-fatal stroke and severe left ventricular dysfunction in patients undergoing CABG (Newman *et al.*, 2012).

As compared with adenosine, only few clinical studies investigated the acute use of bradykinin or of drugs increasing bradykinin's bioavailability in scenarios of myocardial ischaemia/reperfusion. ACE inhibitors and AT1 antagonists, substances which increase bradykinin's bioavailability, are typically used chronically for the treatment of hypertension or heart failure. With chronic use of ACE inhibitors and AT_1 receptor antagonists, the incidence of myocardial infarction is reduced (McAlister, 2012; Savarese *et al.*, 2013), and apart

Table 2

Effects of acute ACE inhibitor, ARB or bradykinin on infarct size in patients

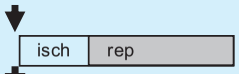
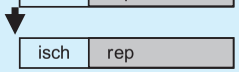
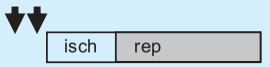
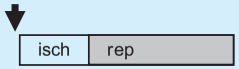
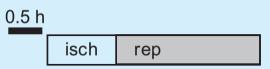
Study	Clinical scenario	Method to assess endpoint	Dosage	Time of application	Intervention [*n*]	Placebo [*n*]	Infarct size [Δ% of placebo]
Bussmann *et al.*, 1995	AMI	CK (max within 48 h)	Captopril, i.v. (2.5–5 mg and 1.5–2 mg·h^{-1})		22	24	↓13
		CK-MB (max within 48 h)			22	24	↓1
Kurz *et al.*, 2001	AMI	CK (max within 36 h)	Enalaprilat, i.c. (50 μg)		11	11	↓18
		CK-MB (max within 36 h)			11	11	↓17
Shariff *et al.*, 2010	STEMI AMI	Troponin I (max within 24 h)	ACE inhibitor or ARB, oral (drugs and dosage not specified)		66	445	↓33*
Mangiacapra *et al.*, 2013	Elective PCI	hs troponin T (mean of 24 h)	Enalaprilat, i.c. (50 μg)		20	20	↓63*
Boldt *et al.*, 1996	CABG	CK-MB (max within 72 h)	Enalaprilat, i.v. (5 μg·kg^{-1}·min^{-1})		22	22	↓62*
		Troponin T (max within 72 h)					↓87*
Walter *et al.*, 2002	CABG	CK (within 120 h)	Enalaprilat, oral (20 mg·day^{-1})		22	21	–
		CK-MB (within 120 h)			22	21	–
		troponin T (within 120 h)			22	21	–
Wei *et al.*, 2004	CABG	CK-MB (within 48 h)	Bradykinin, i.v. (4 μg·min^{-1})		21	20	↓37*
		Troponin I (within 48 h)			21	20	↓32
Benedetto *et al.*, 2008	CABG	Troponin I (max within 48 h)	ACE inhibitor, oral (drugs and dosage not specified)		245	236	↓33*
Wang *et al.*, 2009	CABG	CK-MB (max within 48 h)	Bradykinin, i.v. (4 μg·min^{-1})		19	19	↓40*
		Troponin T (max within 48 h)			19	19	↓16

*$P < 0.05$ versus placebo.

AMI, acute myocardial infarction; ARB, angiotensin receptor blocker; CABG, coronary artery bypass surgery; CK-MB, creatine kinase – muscle, brain; i.c., intracoronary; isch, ischaemia; max, maximum; PCI, percutaneous coronary intervention; rep, reperfusion; STEMI AMI, ST elevation myocardial infarction, acute myocardial infarction.

Table 3

Effects of opioids on infarct size in patients

Study	Clinical scenario	Method to assess endpoint	Drug and dosage	Time of application	Opioid [*n*]	Alternative anaesthesia or placebo [*n*]	Infarct size [Δ% of placebo]
Rentoukas *et al.*, 2010	STEMI AMI	Troponin I (max during hospitalization)	Morphine, i.v. (5 mg) + RIPC vs. RIPC, no further medication		33	33	↓38
Abdel-Wahab *et al.*, 2008 PROFIT	Elective PCI	Troponin T (max within 12 h)	Fentanyl (0.05 mg) vs. diazepam, both i.v.		94	90	↓31
		CK-MB (max within 12 h)					↓5
		Troponin T (max within 12 h)	Fentanyl (0.1 mg) vs. diazepam, both i.v.		92		↓8
		CK-MB (max within 12 h)					↑5
Slogoff *et al.*, 1989	CABG	CK-MB (mean within 16 h)	Sufentanil (15–25 μg·kg^{-1} and 5 mg·kg^{-1}) vs. enflurane or halothane or isoflurane on top of diazepam, pancuronium, fentanyl, all i.v.		254	257/253/248	–
Tuman *et al.*, 1989	CABG	CK-MB (max within 72 h)	Fentanyl (>50 μg·kg^{-1}) vs. diazepam (0.4–1 mg·kg^{-1}) or halothane (0.5–2.5%) on top of benzodiazepine, scopolamine, isoflurane, enflurane, halothane all i.v. – except halothane volatile		240	250	↑67 vs. diazepam ↑3 vs. halothane
			Fentanyl (<50 μg·kg^{-1}) vs. diazepam (0.4–1 mg·kg^{-1}) or halothane (0.5–2.5%) on top of benzodiazepine, scopolamine, isoflurane, enflurane, halothane all i.v. – except halothane volatile		345		↑57 vs. diazepam ↑9 vs. halothane
			Sufentanil (3–8 μg·kg^{-1}) vs. diazepam (0.4–1 mg·kg^{-1}) or halothane (0.5–2.5%) on top of benzodiazepine, scopolamine, isoflurane, enflurane, halothane all i.v. – except halothane volatile		212		↑53 vs. diazepam – vs. halothane
Neuhäuser *et al.*, 2008	CABG	Troponin T (max within 24 h)	Sufentanil (0.25–1 μg·kg^{-1}) on top of flunitrazepam, morphine, midazolam, pancuronium vs. ketamine (1–3 mg·kg^{-1}) on top of flunitrazepam, morphine, midazolam, propofol, pancuronium all i.v.		108	101	↑50*
Wong *et al.*, 2010a	CABG	Troponin I (max within 24 h)	Remifentanil (1 and 0.5 μg·kg^{-1}) vs. placebo on top of morphine, scopolamine, entomidate, fentanyl, pancuronium, propofol all i.v.		20	20	↓38*
		CK-MB (max within 24 h)					↓39*

*$P < 0.05$ versus alternative anaesthesia/placebo.

AMI, acute myocardial infarction; CABG, coronary artery bypass surgery; CK-MB, creatine kinase – muscle, brain; isch, ischaemia; max, maximum; PCI, percutaneous coronary intervention; rep, reperfusion; RIPC, remote ischaemic preconditioning; STEMI AMI, ST elevation myocardial infarction, acute myocardial infarction.

from more favourable haemodynamics, bradykinin may contribute to such benefit.

There is no consistent evidence for cardioprotection by opioids in clinical settings. This lack of evidence for cardioprotection may relate to the underlying background anaesthesia, as most anaesthetics are *per se* cardioprotective (e.g. halothane, isoflurane, ketamine, propofol, sevoflurane, sufentanil) (Kato and Foex, 2002; Zaugg *et al*., 2014). Also, in studies where the opioid was not compared with strict placebo but to another drug [e.g. diazepam (Obame *et al*., 2007)], the potential cardioprotection by the comparator drug may obscure the cardioprotection by the opioid.

Given the recent evidence that remote ischaemic preconditioning and preconditioning by repeated inflation/deflation of a blood pressure cuff around a limb reduces infarct size during elective interventional and surgical coronary revascularization (Hausenloy *et al*., 2007; Thielmann *et al*., 2010) as well as in patients with reperfused AMI (Bøtker *et al*., 2010) and may even improve clinical outcome (Davies *et al*., 2013; Thielmann *et al*., 2013; Sloth *et al*., 2014), the question arises whether we should abandon the search for cardioprotective drugs and embark on remote ischaemic conditioning as a simple, safe, cheap and effective cardioprotective strategy (Heusch, 2013b).

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Conflict of interest

None.

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