INTERACTION OF RISK FACTORS, COMORBIDITIES AND COMEDICATIONS WITH ISCHEMIA/REPERFUSION INJURY AND CARDIOPROTECTION BY PRECONDITIONING, POSTCONDITIONING, AND REMOTE CONDITIONING

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Abbreviations: 5-HD, 5-hydroxydecanoic acid; ACE, angiotensin-converting enzyme; AF, atrial fibrillation; AMPK, adenosine monophosphate activated kinase; CCB, L-type calcium channel blocker; Cx-43, connexin-43; COX-2, cyclo-oxygenase-2; Drp1, dynamin-related protein; DPP-4, dipeptidyl-peptidase-4; eNOS, endothelial NO synthase; ERK, extracellular regulated kinase; GLP-1, glucagon-like peptide-1; GSK-3β, glycogen synthase-3beta; HO-1, heme oxigenase-1; HSP72, 72 kDa heat shock protein; iNOS, inducible NO synthase; JAK, janus activated kinase; $K_{ATP,}$ ATP-sensitive potassium channel; LVEF, left ventricular ejection fraction; LVH, left ventricular hypertrophy; MAPK, mitogen activated protein kinase; MI, myocardial infarction; MKP, mitogen activated protein kinase phosphatase; MLKL, mixed lineage kinase domain-like protein; MMP, matrix metalloproteinase; MPTP, mitochondrial

permeability transition pore; NO, nitric oxide; PCI, percutaneous coronary intervention; PGAM5, mitochondrial phosphoglycerate mutase/protein phosphatase; PI3K, phosphatidylinositol 3-kinase; PKA, cAMP-dependent protein kinase; PKC, protein kinase C; PKG, cGMP-dependent protein kinase; PINK1, PTEN-induced kinase-1; PTEN, phosphatase and tensin homologue; RIPK, receptor-interacting protein kinase; RISK, reperfusion injury salvage kinase; RNS, reactive nitrogen species; ROS, reactive oxygen species; SAFE, survivor activating factor enhancement; SHR, spontaneously hypertensive rat strain; Sirt1, sirtuin deacetylase 1; SOD, superoxide dismutase; STEMI, ST-segment elevated MI; STZ, streptozotocin; STAT3, signal transducer and activator of transcription-3; TNFα, tumor necrosis factor-α; TRPV1, transient receptor potential vanilloid-1 channel

Abstract

Preconditioning, postconditioning and remote conditioning of the myocardium are well-described adaptive responses that markedly enhance the ability of the heart to withstand a prolonged ischemia/reperfusion insult and provide therapeutic paradigms for cardioprotection. Nevertheless, more than 25 years after the discovery of ischemic preconditioning, we still do not have established cardioprotective drugs on the market. Most experimental studies on cardioprotection are still undertaken in animal models, in which ischemia/reperfusion is imposed in the absence of cardiovascular risk factors. However, ischemic heart disease in humans is a complex disorder caused by or associated with cardiovascular risk factors and co-morbidities, including hypertension, hyperlipidemia, diabetes, insulin resistance, heart failure, altered coronary circulation, and aging. These risk factors induce fundamental alterations in cellular signaling cascades that affect the development of ischemia/reperfusion injury *per se* and responses to cardioprotective interventions. Moreover, some of the medications used to treat these risk factors, including statins, nitrates and anti-diabetic drugs, may impact on cardioprotection by modifying cellular signaling. The aim of this article is to review the recent evidence that cardiovascular risk factors and their medication may modify the response to cardioprotective interventions. We emphasize the critical need to take into account the presence of cardiovascular risk factors and concomitant medications when designing preclinical studies for the identification and validation of cardioprotective drug targets and clinical studies. This will hopefully maximize the success rate of developing rational approaches to effective cardioprotective therapies for the majority of patients with multiple risk factors.

Table of contents:

I. INTRODUCTION

Ischemic heart disease is one of the leading causes of death and disability in the industrialized societies. Effective treatment of acute myocardial infarction (MI) is based on procedures which promote the return of blood flow to the ischemic zone of the myocardium, i.e. reperfusion therapy. Reperfusion, however, may lead to further irreversible myocardial cell death, termed lethal myocardial reperfusion injury. Currently, there is no effective therapy for combined ischemia/reperfusion injury on the market, and routine pharmacological agents do not salvage the ischemic/reperfused myocardium. Therefore, the development of cardioprotective agents to limit the extent of infarcted tissue caused by ischemia/reperfusion injury is of great clinical importance.

Earlier pharmacological approaches to attenuate the consequences of ischemia/reperfusion injury were of limited experimental efficacy or failed to translate into useful clinical treatments. However, in the last three decades, the heart has been shown to possess a remarkable ability to adapt to ischemia/reperfusion stress and this molecular plasticity of the heart in ischemia/reperfusion has been the focus of intense research in the hope that the underlying mechanisms may be amenable to therapeutic exploitation. Ischemic preconditioning, postconditioning, and remote conditioning of myocardium are well-described adaptive responses in which there is brief exposure to ischemia/reperfusion prior to sustained ischemia (preconditioning), or at the immediate onset of reperfusion (postconditioning), or in a remote tissue prior to, during, or at reperfusion after sustained myocardial ischemia (remote conditioning). All forms of conditioning markedly enhance the ability of the heart to withstand a

prolonged ischemic insult (Fig 1). The discovery of these endogenous cardioprotective mechanisms has encouraged the exploration of new ways to protect the ischemic/reperfused myocardium and has amplified our knowledge of the molecular basis of cell injury and survival mechanisms during ischemia/reperfusion.

Ischemic heart disease develops as a consequence of a number of etiologic risk factors predisposing to atherosclerosis development; it always co-exists with other systemic disease states. These pre-disposing and/or co-existing conditions include systemic arterial hypertension with related left ventricular hypertrophy and disturbed coronary circulation (i.e. hypertensive heart disease); metabolic diseases such as hyperlipidemia, diabetes mellitus, obesity, insulin resistance, uremia; and heart failure. In addition, aging is a major risk factor predisposing to the development of ischemic heart disease. These risk factors and co-existing conditions exert multiple biochemical effects on the heart that affect the development of ischemia/reperfusion injury *per se* and interfere with responses to cardioprotective interventions. Moreover, routine drug therapies for these conditions, e.g. antihyperlipidemic, antidiabetic, antihypertensive, anti-anginal, and anti-platelet drugs, as well as drugs indicated for non-cardiovascular diseases may also interfere with cardioprotective interventions.

Since the original observations of the loss of preconditioning in hyperlipidemic rodents (Szilvassy et al., 1995, Ferdinandy et al., 2007), it has been well established that many of the cardiovascular risk factors may interfere with cardioprotection by conditioning strategies (see our earlier reviews: Ferdinandy et al., 1998; Ferdinandy, 2003; Ferdinandy et al., 2007, Ovize et al., 2010). Nevertheless, most experimental studies on cardioprotection are still undertaken in

juvenile healthy animal models, in which ischemia/reperfusion is imposed in the absence of the classical risk factors for cardiovascular disease. This has contributed, at least in part, to the slow progress of translation of preclinical results to clinical therapy. While some conditioning treatments in humans have shown promising results, other studies have shown no cardioprotective effect of conditioning in patients with acute MI (Heusch, 2013). Therefore, the development of rational therapeutic approaches to protect the ischemic heart requires preclinical studies that examine cardioprotection specifically in relation to cardiovascular risk factors and their medications. Moreover, to avoid unexpected ischemia-related safety problems, the interaction of drugs with endogenous cardioprotective mechanisms must be tested during preclinical and clinical phases of drug development as well as in post-marketing clinical studies.

The aim of this review is to update our previous review (Ferdinandy et al., 2007) on the effects of risk factors on ischemia/reperfusion injury and cardioprotection, and to emphasise the ongoing critical need for preclinical studies which model the presence of risk factors and their pharmacological treatments. Such studies are required for the proper validation of molecular targets for cardioprotection, thereby maximizing the chances of success for translation of cardioprotection into the clinical arena and for the benefit of the majority of ischemic heart disease patients who have multiple risk factors and associated medications. Furthermore, we highlight that routine medications for cardiovascular and other diseases may show undesirable effects on endogenous cardioprotective cellular signaling mechanisms, thereby possessing a "hidden cardiotoxicity" that may manifest latently in the ischemic heart as increased sensitivity to ischemic challenge or a decreased capability to adapt to an ischemic challenge, i.e. attenuated cardioprotection achieved by conditioning.

II. EXPERIMENTAL APPROACHES TO CARDIOPROTECTION

II.A. Cardioprotection through preconditioning

Cardioprotection elicited by ischemic preconditioning remains one of the most powerful therapeutic interventions for limiting infarct size following acute ischemia/reperfusion injury. Despite ongoing intensive investigation, the actual mechanisms underlying its cardioprotective effect and their interaction remain largely unclear. A large number of signaling pathways are recruited at the cardiomyocyte sarcolemma through the activation of cell surface receptors by their endogenous ligands. Many of these signal transduction pathways appear to terminate at the mitochondria, and it is in this area where most of the recent research has been focused (Fig 2). A comprehensive review of all of the investigated mechanisms is beyond the scope of this review. The interested reader is referred to comprehensive reviews published elsewhere on the topics of ischemic preconditioning and its signal transduction (Heusch et al.,2008; Hausenloy, 2013).

II.A.1. Mitochondria and preconditioning

Mitochondria appear to play two critical roles in the setting of ischemic preconditioning. Prior to the index ischemic event and in response to the preconditioning stimulus, mitochondria are known to release signaling reactive oxygen species (ROS) which then activate key mediators of cardioprotection, which subsequently prevent the opening of the mitochondrial permeability transition pore (MPTP) in the first few minutes of myocardial reperfusion, thereby attenuating myocardial reperfusion injury and limiting infarct size.

II.A.1.a. The MPTP and preconditioning

The mechanism through which the signaling ROS are generated in response to the ischemic preconditioning stimulus is not clear but one suggestion has implicated the activation of the mitochondrial ATP-sensitive potassium channel (K_{ATP}), which is related to mitochondrial connexin 43 (Cx43) (Heinzel et al., 2005) and appears to be mediated via protein kinase G (PKG) and mitochondrial protein kinase C (PKC)-ε (Costa & Garlid, 2008). The K^+ influx into mitochondria is believed to induce matrix alkalinization which then results in the production of superoxide from complex I of the electron transport chain.

A number of experimental studies have linked ischemic preconditioning-induced cardioprotection to the inhibition of MPTP opening at the onset of reperfusion. The precise mechanism through which this is achieved remains undetermined but may involve the following: (1) activation of pro-survival pathways such as the reperfusion injury salvage kinase (RISK) or survivor activating factor enhancement (SAFE) signaling pathways which then act to prevent MPTP opening either in a direct or indirect manner (Hausenloy et al., 2009; 2011b). There is evidence to suggest that one particular downstream mediator, glycogen synthase kinase-3β (GSK-3β), appears to mediate cardioprotection through the inhibition of the MPTP, although the mechanism through which this is achieved is unclear (Juhaszova et al., 2004); (2) activation of the mitochondrial K_{ATP} channel, which via mitochondrial PKC-ε, results in ROS-mediated inhibition of MPTP opening (Costa & Garlid, 2008); (3) attenuation of oxidative stress generated during myocardial ischemia thereby preventing MPTP opening at reperfusion (Clarke et al., 2008).

Since 2007, several key developments have arisen with respect to the MPTP and its role in acute ischemia/reperfusion injury. Although mitochondrial cyclophilin D has been established as a regulator of the MPTP, the precise identities of the components of the MPTP remain unknown. Recent experimental studies have suggested that dimers of mitochondrial ATP synthase may constitute the MPTP (Giorgio et al., 2013; Bonora et al., 2013). Interestingly, an insight into the potential physiological role of the MPTP was provided by Elrod et al. (2010) who reported that mice deficient in mitochondrial cyclophilin D were more susceptible to calcium-overload, suggesting that the MPTP may mediate mitochondrial calcium efflux, a mechanism which had been first proposed in 1992 (Altschuld et al., 1992). Another important discovery was the identity of the mitochondrial calcium uniporter (De Stefani et al., 2011; Baughman et al., 2011), and the surprising observation that mice deficient in the uniporter were not protected from myocardial infarction despite being resistant to MPTP opening (Pan et al., 2013).

II.A.1.b. Mitochondrial Connexin-43 and preconditioning

Recent experimental data have suggested that the gap junction sarcolemmal protein, Cx43, is also present in cardiac sub-sarcolemmal inner mitochondrial membranes, where it acts as a signaling mediator of ischemic preconditioning but not postconditioning (reviewed in Schulz et al., 2007). Cx43 is believed to form hemi-channels in the inner mitochondrial membrane thereby facilitating complex I function and the influx of K^+ into mitochondria in response to the ischemic preconditioning stimulus (Boengler et al., 2012; Boengler et al., 2013a). The activation of the RISK or SAFE pathways is not involved in the protective function of Cx43 in ischemic preconditioning (Sanchez et al., 2013).

II.A.1.c. Mitochondria and new forms of cell death

The majority of experimental studies investigating the beneficial effects of conditioning the heart have focused on preventing cardiomyocyte death due to necrosis and/or apoptosis, and in this regard the mitochondria play a pivotal role. More recently, two further forms of cell death have been described. Both autophagy (including mitophagy) and regulated cell necrosis appear to be relevant to cardiomyocyte death induced by acute ischemia/reperfusion injury. Only an overview can be provided here, and the interested reader is referred to more comprehensive review articles (Giricz et al., 2012; Kaczmarek et al., 2013).

Autophagy is an evolutionarily conserved process that mediates the degradation of cytoplasmic components via the lysosomal pathway under conditions of cellular stress. It has been suggested that autophagy may be an adaptive response to protect the cell against myocardial ischemia. Autophagy can be activated in response to ischemic preconditioning, whereas its activation at the time of reperfusion is thought to be deleterious (reviewed in Giricz et al., 2012). Mitophagy allows the removal of defective mitochondria and may also provide a cardioprotective response (Kubli & Gustafsson, 2012). This process is initiated by mitochondrial fragmentation and mitochondrial membrane depolarization which induces the translocation of the cytosolic ubiquitin ligase, parkin, to the mitochondrial outer membrane where it binds to mitofusin 2, which in itself has to be phosphorylated by phosphatase and tensin homologue (PTEN)–induced kinase 1 (PINK1) (Chen & Dorn, 2013), resulting in the removal of the damaged mitochondria. Abolishing mitophagy by knock-out of parkin abolishes cardioprotection by ischemic preconditioning (Huang et al., 2011).

Necrosis was previously considered to be an accidental, unregulated form of cell death. However, there also appears to be a regulated form of necrotic cell death, termed 'necroptosis' or 'programmed necrosis' (reviewed by (Kaczmarek et al., 2013)). It is initiated by tumor necrosis factor-α (TNF-α) death domain receptor activation, the receptor-interacting protein 1 and 3 kinases (RIPK1 and 3), the mixed lineage kinase domain-like protein (MLKL), and mitochondrial phosphoglycerate mutase/protein phosphatase (PGAM5), which then activates dynamin-related protein 1 (Drp1)-mediated mitochondrial fission resulting in cell death (Wang et al., 2012). Importantly, pharmacological inhibition of this novel death pathway has been reported to limit MI size and prevent adverse post-MI left ventricular remodeling (Lim et al., 2007; Oerlemans et al., 2012).

II.A.1.d. Mitochondrial dynamics and cardioprotection

Mitochondria are no longer considered to be static organelles but are dynamic structures which are able to change their morphology by undergoing either fusion to generate elongated mitochondria which allows replenishment of damaged mitochondrial DNA, or fission to produce fragmented mitochondria to replace damaged mitochondria by mitophagy (reviewed in Ong & Hausenloy, 2010; Ong et al., 2013). Interestingly, cardiac mitochondria have been demonstrated to undergo fragmentation during myocardial ischemia under the control of the mitochondrial fission protein, Drp1 (Ong et al., 2010). Pharmacological or genetic inhibition of Drp1-mediated mitochondrial fission induced by ischemia has been reported to prevent MPTP opening and reduce MI size (Ong et al., 2010; Disatnik et al., 2013; Wang et al., 2011a). Somewhat surprisingly, the ablation of cardiac mitofusin 1 and 2 (known mitochondrial fusion proteins) also prevented MPTP opening and rendered hearts resistant to acute ischemia/reperfusion injury

(Papanicolaou et al., 2011; Papanicolaou et al., 2012). This unexpected result may be due to the pleiotropic non-fusion effects of these mitochondrial fusion proteins which include apoptosis induction, mediation of mitophagy, and tethering the sarcoplasmic reticulum to the mitochondria (de Brito & Scorrano, 2008a; de Brito & Scorrano, 2008b; Wang et al., 2012). A recent study has shown that pharmacological preconditioning using nitrite protected a cardiac-cell line by inhibiting ischemia-induced mitochondrial fission through the activation of protein kinase A (Pride et al., 2014). Whether ischemic preconditioning and postconditioning exert their cardioprotective effect by modulating mitochondrial morphology is not known.

II.B Cardioprotection through postconditioning

One major limitation of ischemic preconditioning has been the necessity to apply the therapeutic intervention prior to the sustained index myocardial ischemia, the onset of which is unpredictable in patients presenting with MI. The discovery in 2003 of ischemic postconditioning by interrupting myocardial reperfusion with several cycles of short-lived ischemia has overcome this limitation (Zhao et al., 2003). The clinical applicability of ischemic postconditioning was realized only two years later in ST-segment elevated MI (STEMI) patients treated by percutaneous coronary intervention (PCI) using re-inflation of the coronary angioplasty balloon to interrupt myocardial reperfusion (Staat et al., 2005) (see section III for further clinical application of ischemic postconditioning). The protection afforded by ischemic postconditioning has been reproduced in most species tested although suitable algorithms may be model and species dependent (Skyschally et al., 2009). The modification of the reperfusion phase had been reported previously to confer cardioprotection by more gentle reperfusion (Musiolik et al., 2010).

In terms of the mechanistic pathway underlying ischemic postconditioning, many of the signaling pathways, but not all (Heusch et al., 2006), are shared with ischemic preconditioning. In brief, autacoids activate pro-survival signal transduction pathways, the majority of which converge on mitochondria and prevent MPTP opening at the time of reperfusion. A comprehensive review of all of the investigated mechanisms is beyond the scope of this review and we will only focus on the major developments since 2007. The interested reader is referred to comprehensive reviews published elsewhere on the topics of ischemic postconditioning (Hausenloy, 2013; Shi & Vinten-Johansen, 2012; Ovize et al., 2010; Burley & Baxter, 2009).

II.B.1. Autacoid mediators of postconditioning

Initial experimental studies using pharmacological antagonists had implicated adenosine to be a key mediator of postconditioning through activation of the adenosine A_{2A} receptor (Kin et al., 2005), A_{2B} receptor (Philipp et al., 2006), or A_3 receptor (Kin et al., 2005; Philipp et al., 2006), but not the A_1 receptor (Kin et al., 2005; Donato et al., 2007; Xi et al., 2008). A subsequent study found that mice deficient for the myocardial adenosine A_{2A} receptor were resistant to ischemic postconditioning (Morrison et al., 2007). Since 2007, an increasing number of autacoid mediators of postconditioning have been described, including bradykinin (via the B_2 receptor) (Penna et al., 2007; Xi et al., 2008), opioids (Pateliya et al., 2008; Jang et al., 2008; Zatta et al., 2008), TNFα (Lacerda et al., 2009), and sphingosine (Vessey et al., 2008a, b; Jin et al., 2008).

II.B.2. Delaying the correction of pH at reperfusion

The acidic intracellular conditions produced during myocardial ischemia exert a strong inhibitory effect on the MPTP, keeping it closed during ischemia, despite calcium overload, increased inorganic phosphate, oxidative stress and ATP depletion. In the first few minutes of reperfusion

the wash-out of myocardial lactate and activation of the Na^+-H^+ exchanger and Na^+-HCO_3^- co-transporter rapidly correct the intracellular acidosis, thereby releasing the inhibition on the MPTP and allowing the latter to open at the time of reperfusion (Halestrap et al., 2004; Yellon & Hausenloy, 2007). A number of experimental studies have reported that ischemic postconditioning may prevent MPTP opening by delaying the restoration of physiological pH at the onset of reperfusion (Fujita et al., 2007; Cohen et al., 2007), although the actual mechanism through which this might be achieved is not clear. Whether the stuttering reperfusion of the postconditioning protocol inhibits MPTP opening by delaying the washout of the myocardial lactate, attenuating oxidative stress production or activating the RISK or SAFE pathway is unclear.

II.B.3. Mitochondria and postconditioning

Experimental studies suggest that ischemic postconditioning prevents myocardial reperfusion injury and limits infarct size by inhibiting MPTP opening (Argaud et al., 2005). As with ischemic preconditioning, the mechanism through which this is achieved is not clear but a number of potential signaling pathways have been proposed: (1) the activation of the pro-survival cardioprotective pathways such as the RISK, SAFE and NO-cGMP-PKG pathways at the onset of reperfusion inhibit MPTP opening (Andreadou et al., 2014; Hausenloy et al., 2005; Boengler et al., 2011a; Bopassa et al., 2006; Hausenloy et al., 2011b; Heusch et al., 2008; Heusch et al., 2011); (2) the delayed restoration in intracellular pH may inhibit MPTP opening (Cohen et al., 2007); (3) the reduction in ROS generated at reperfusion may prevent MPTP opening (Clarke et al., 2008).

II.C. Cardioprotection through pharmacological conditioning

Elucidation of the signaling pathways underlying ischemic conditioning in the heart has helped to identify a number of novel therapeutic targets for cardioprotection. These include targets in the signal transduction pathways linking the cell membrane to the mitochondria, and direct targets in the mitochondria. A number of pharmacological agents capable of mimicking the cardioprotective effects of ischemic conditioning continue to be investigated in the experimental setting, but there appear to be species differences, e.g. cyclosporine-A does not protect the rat heart (De Paulis et al., 2013). Some of these agents have been investigated in the clinical setting already. The most promising pharmacological cardioprotective agents and their potential targets include: cyclosporine-A (MPTP inhibition); metoprolol, matrix metalloproteinase (MMP) inhibition, glucagon-like peptide 1 (GLP-1) analogues (RISK pathway); nitrite/nitrates and soluble guanylate cyclase activators (NO-cGMP-PKG pathway) (reviewed in Andreadou et al, 2014; Rassaf et al., 2014; Bice et al., 2014; Sharma et al., 2012; Stasch et al., 2011; Evgenov et al., 2006).

II.D. Cardioprotection through remote conditioning

The major disadvantage of ischemic preconditioning and postconditioning as therapeutic interventions for limiting acute myocardial ischemia/reperfusion injury is that they both require the intervention to be applied directly to the heart, thereby limiting their clinical applicability. In this regard, the discovery in 1993 (Przyklenk et al., 1993), that the cardioprotective stimulus could be applied to remote myocardium, and later to a remote organ away from the heart, was a major advance. This phenomenon has been termed ‘remote ischemic conditioning’ (reviewed in

(Hausenloy & Yellon, 2008; Vinten-Johansen & Shi, 2013)). However, the major breakthrough which facilitated the translation of remote ischemic conditioning into the clinical setting was the discovery in the experimental setting that the cardioprotective stimulus could be applied to the musculoskeletal tissue of the hind-limb (Oxman et al., 1997; Birnbaum et al., 1997). This was followed by the discovery in human volunteers that the cardioprotective stimulus could be applied to the arm or leg in a non-invasive manner by simply inflating and deflating a blood pressure cuff or similar device (Gunaydin et al., 2000; Kharbanda et al., 2002).

An additional advantage with remote ischemic conditioning is its ability to confer cardioprotection when initiated at different time-points in relation to acute ischemia/reperfusion injury. It can be applied prior to myocardial ischemia (remote ischemic **pre**conditioning) (Przyklenk et al., 1993); after the onset of myocardial ischemia but prior to reperfusion (remote ischemic **per**conditioning)(Schmidt et al., 2007); at the onset of myocardial reperfusion (remote ischemic **post**conditioning)(Andreka et al., 2007); and even after 15 min of reperfusion has elapsed (remote ischemic delayed **post**conditioning) (Basalay et al., 2012). Remote conditioning interventions thereby lend themselves to application in a number of different clinical settings of acute ischemia/reperfusion injury (see section III). Moreover, repeated daily episodes of remote ischemic **post**conditioning over a period of 28 days following MI in a rat model of acute ischemia/reperfusion injury have been reported to have beneficial effects on post-MI remodeling (Wei et al., 2011).

Despite its discovery in 1993, the actual mechanism underlying the cardioprotective effect of remote conditioning remains unclear. The signal transduction pathway can be divided into three

stages: (1) the application of the 'conditioning' stimulus to the remote organ or tissue results in the generation of a cardioprotective signal, the nature of which is unclear; (2) the mechanism through which the cardioprotective signal is conveyed to the heart is currently unclear, but is believed to involve both neural and circulating humoral components; and (3) the recruitment of established cardioprotective signaling pathways within the cardiomyocyte (reviewed in (Hausenloy & Yellon, 2008)). Dissection of the individual contributions of these three sequential signaling steps has been an experimental challenge which remains unsolved. The current paradigm suggests that the conditioning stimulus within the remote organ or tissue generates autacoids such as adenosine, bradykinin and opioids, which result in the stimulation of the neural pathway to that remote organ or tissue (Liem et al., 2002; Redington et al., 2012; Jensen et al., 2012). The neural pathway then relays the cardioprotective signal to the brainstem nuclei (Lonborg et al., 2012), where a humoral factor(s), as yet unidentified, is released into the circulation and carried to the heart to mediate the cardioprotective effect. Recently, involvement of the SDF-1α/CXCR4 axis has been shown (Davidson et al., 2013). Also, cardioprotection by remote ischemic preconditioning of the rat heart has recently been shown to be mediated by extracellular microvesicles released by brief periods of ischemia and acting as potential carriers of cardioprotective substances (Giricz et al., 2014). A comprehensive discussion of the potential mechanisms underlying remote ischemic conditioning is beyond the scope of this review and the reader is referred to comprehensive reviews on the subject (Hausenloy & Yellon, 2008; Vinten-Johansen & Shi, 2013). The clinical application of this phenomenon is dealt with in the next section.

III. CLINICAL APPROACHES TO CARDIOPROTECTION

There are now a number of studies which have examined cardioprotection by ischemic preconditioning, ischemic postconditioning and remote conditioning in various clinical scenarios (Heusch, 2013).

III.A. Ischemic preconditioning

Conceptually, ischemic preconditioning has been associated with pre-infarction angina i.e. unstable angina preceding acute MI. It is known that pre-infarction angina is associated with better clinical outcome than an abrupt acute MI without preceding episodes of angina (Heusch, 2001; Rezkalla and Kloner, 2004). The causal attribution of protection with pre-infarction angina to ischemic preconditioning rather than collateral recruitment or more rapid reperfusion, as well as to the early versus the delayed form of ischemic preconditioning remains unclear. Although conceptually inferred for pre-infarction angina, ischemic preconditioning has been more empirically studied in interventional and surgical revascularization protocols (Fig. 3).

During repeated balloon angioplasty, ECG alterations, pain sensation, lactate production, and creatine kinase release were found to be attenuated during the second as compared to the first coronary occlusion period, and this was taken as evidence of ischemic preconditioning (Heusch, 2001). With the use of pharmacological antagonists, the causal involvement of adenosine, opioids, α-adrenoceptor activation and K_{ATP} was demonstrated. However, a caveat must be noted since reduced ST-segment elevation can be dissociated from reduced infarct size (Birincioglu et al., 1999) such that the selected endpoint of ischemic preconditioning's protection may be

critical for successful clinical translation. There are also a number of studies where an ischemic preconditioning algorithm was used in coronary artery bypass graft or valvular surgery and protection was seen in terms of reduced release of serum biomarkers (creatine kinase-MB, troponin I or T) (Lu et al., 1997; Jenkins et al., 1997; Szmagala et al., 1998; Li et al., 1999; Teoh et al., 2002a, b; Ghosh and Galinanes, 2003; Buyukates et al., 2005; Codispoti et al., 2006; Ji et al., 2007; Amr and Yassin, 2010). However, not all studies were positive (Alkhulaifi et al., 1994; Perrault et al., 1996; Cremer et al., 1997; Kaukoranta et al., 1997; Illes and Swoyer, 1998; Pego-Fernandes et al., 2001; Wu et al., 2001; Ghosh and Galinanes, 2003; Jebeli et al., 2010). Both positive and negative studies suffer from small cohort sizes and lack of clinical outcome as endpoint. Nevertheless, the impression is that ischemic preconditioning can be utilized to induce protection in elective cardiac surgery, and a meta-analysis of published studies suggests clinical benefit in terms of reduced arrhythmias, less inotrope support requirement and reduced intensive care unit stay (Walsh et al., 2008).

III.B. Ischemic postconditioning

Ischemic postconditioning has been used in patients undergoing primary PCI for an acute MI (Fig. 4). The landmark study by Staat et al. (Staat et al., 2005) appeared only two years after the original experimental report of ischemic postconditioning in dogs (Zhao et al., 2003). Several studies demonstrated reduced infarct size by reduced biomarker release (creatine kinase, creatine kinase-MB, troponin I) or by gadolinium-contrast MRI (Ma et al., 2006; Yang et al., 2007; Luo et al., 2007; Thibault et al., 2008; Laskey et al., 2008; Zhao et al., 2009b; Lonborg et al., 2010; Sorensson et al., 2010; Xue et al., 2010; Garcia et al., 2011; Luo et al., 2011; Ji et al., 2011; Liu et al., 2011a; Thuny et al., 2012; Durdu et al., 2012; Ugata et al., 2012; Mewton et al., 2013; Liu

et al., 2013). However, not all studies have reported positive findings (Freixa et al., 2011; Tarantini et al., 2012; Hahn et al., 2013; Dwyer et al., 2013; Elzbieciak et al., 2013). The sample size of the study cohorts was small, making them sensitive to false-negative type II errors. A systematic underestimation of the protective potential of ischemic postconditioning may result from lack of direct stenting. Direct stenting removes any residual stenosis and prevents coronary microembolization from the culprit lesion (Loubeyre et al., 2002) when further manipulated by the postconditioning manoeuvre (Heusch, 2012). With use of direct stenting, the consequences of immediate full reperfusion are compared to those of a postconditioning algorithm, without any interference by a residual stenosis or by coronary microembolization. Also, Ovize and colleagues who consistently reported protection with ischemic postconditioning always inflated the balloon upstream of the stent (Staat et al.,2005; Thibault et al.,2008; Thuny et al., 2012). A larger clinical trial recently failed to observe reduced peak creatine kinase-MB or a significant benefit in clinical outcome from ischemic postconditioning, but unfortunately this trial did not use direct stenting in most patients (Hahn et al., 2013).

III.C. Remote ischemic conditioning

In recent years, remote ischemic conditioning has become the most popular form of mechanical cardioprotection, since the procedure is non-invasive, predictable, precise, safe and notably avoids manipulation of the coronary culprit lesion (Fig. 5). Remote ischemic preconditioning has been used in elective interventional revascularization (Iliodromitis et al., 2006; Hoole et al., 2009; Prasad et al., 2013; Ahmed et al., 2013; Luo et al., 2013) and in surgical coronary revascularization (Gunaydin et al., 2000; Hausenloy et al., 2007; Venugopal et al., 2009; Thielmann et al., 2010; Rahman et al., 2010; Wagner et al., 2010; Ali et al., 2010; Hong et al.,

2010; Karuppasamy et al., 2011; Kottenberg et al., 2012; Heusch et al., 2012b; Lucchinetti et al., 2012; Young et al., 2012; Hong et al., 2012; Kottenberg et al., 2012, 2014; Thielmann et al., 2013; Saxena et al., 2013). The procedure has also been applied in other forms of cardiac surgery (Cheung et al., 2006; Li et al., 2010; Zhou et al., 2010; Wu et al., 2011a; Xie et al., 2012; Young et al., 2012; Pavione et al., 2012; Lee et al., 2012; Albrecht et al., 2013; Jones et al., 2013; Pepe et al., 2013; Meybohm et al., 2013). Not all studies reported infarct size reduction, using biomarker release or imaging as endpoints. A common feature of all the negative studies appears to be the use of propofol anesthesia in some form; propofol has been demonstrated to abrogate the protection by remote ischemic preconditioning (Kottenberg et al., 2012, 2014; Bautin et al., 2013).

A few studies have also used a remote conditioning procedure during an ongoing acute MI before primary PCI; increased myocardial salvage was seen in one study (Botker et al., 2010), but no significant reduction in infarct size by biomarker release or imaging (Botker et al., 2010; Rentoukas et al., 2010; Munk et al., 2010). One recent study has demonstrated reduced infarct size, as assessed by biomarker release and MRI, when the remote lower limb conditioning protocol was started in a postconditioning mode at the onset of reperfusion in patients with acute myocardial infarction (Crimi et al., 2013). Three further studies have even reported reduced all-cause mortality (secondary endpoint) in patients undergoing a remote conditioning protocol before elective PCI (Davies et al., 2013), emergency PCI (Sloth et al., 2014) or surgical coronary revascularization (Thielmann et al., 2013). Another recent study reported no clinical benefit in patients undergoing elective cardiac surgery with a combined remote ischemic pre- and postconditioning protocol; however, this study also used propofol and did not report

protection in terms of biomarker release or imaging endpoints (Hong et al., 2014). Also, no additive protection of local ischemic postconditioning with remote ischemic preconditioning was seen in the small-scale RIPOST-MI study in patients undergoing primary PCI for acute MI (Prunier at al., 2014). We therefore await the results of several ongoing multi-center trials on remote conditioning where mortality is a primary endpoint; these include ERICCA (Hausenloy et al., 2011a) (NCT 1247545), RIPHeart (NCT 01067703), or CONDI II (NCT 01857414).

III.D. Pharmacological postconditioning

Our increasing understanding of the mechanisms underlying ischemic postconditioning has identified a vast array of signalling mediators, which can be targeted by pharmacological agents to recapitulate the cardioprotective effects of ischemic postconditioning. In this regard, a number of pharmacological approaches to limiting infarct size in STEMI patients undergoing primary PCI have been investigated (reviewed in Sharma et al., 2012; Hausenloy et al., 2013a). Unfortunately, many of these studies have failed to demonstrate any cardioprotective effect in the clinical setting, despite promising experimental animal data. This apparent failure can be attributed to a number of different factors. These include the use of animal models which do not adequately represent clinical reality e.g. due to lack of co-morbidities; and poor study design (Ludman et al., 2010; Ovize et al., 2010; Schwartz-Longacre et al., 2011; Hausenloy et al., 2010; Bell et al., 2012; Hausenloy et al., 2013a).

More recently, several novel pharmacological approaches have been reported to limit infarct size when administered prior to reperfusion in primary PCI-treated STEMI patients (Table 1). Most promising among these therapies are cyclosporine-A, exenatide and metoprolol. Whether these

pharmacological postconditioning agents can actually improve clinical outcomes remains to be investigated, and in this regard, a large multi-center clinical outcome study is currently underway investigating cyclosporine-A (NCT 01502774).

In summary, mechanical and pharmacological conditioning strategies are promising therapeutic options for cardioprotection in patients undergoing elective or emergency coronary revascularization, although there are several negative studies. Most of the clinical trials, both positive and negative, have been small. The positive trials have been conducted in selected patients under well controlled conditions, whereas the negative trials (e.g. on remote preconditioning) have been less selective in terms of patient recruitment and procedures (anesthesia, surgery). The observed lack of protection in the negative studies can in part be attributed to the presence of different risk factors, comorbidities and their medications in different patient cohorts, as well as to poorly validated drug targets in juvenile and healthy animal models, and poorly designed clinical studies (Ferdinandy et al, 2007; Ovize et al., 2010; Hausenloy et al., 2013a). None of the existing studies has really raised a safety concern for the conditioning strategies. Larger studies with clinical outcome endpoints are necessary to gain more insight into the clinical applicability of conditioning strategies in different patient populations with different medications and confounding factors.

IV. EFFECTS OF MAJOR RISK FACTORS ON ISCHEMIA-REPERFUSION INJURY AND CARDIOPROTECTIVE STRATEGIES

In the mid 1990s, hyperlipidemia was the first cardiovascular risk factor to be associated with the loss of preconditioning cardioprotection in rabbits and rats (Szilvassy et al., 1995; Ferdinandy et al., 1998). Since then, it has been well established that in addition to hyperlipidemia, most of the other major risk factors and/or medications that target them may modify cardioprotective signaling leading to the loss or attenuation of cardioprotection by ischemic or pharmacological conditioning (see for extensive earlier reviews: Ferdinandy et al, 1998; Ferdinandy 2003; Ferdinandy et al, 2007). In this chapter, we review more recent evidence of the impact of the most important risk factors on ischemia/reperfusion injury and cardioprotection (Table 2).

IV.A. Aging and Cardioprotection

IV.A.1. Ischemic/pharmacological preconditioning in aging

While ischemic and pharmacological preconditioning attenuate ischemia/reperfusion injury in juvenile hearts, most studies suggest a loss of protection in aged hearts (for review, see Ferdinandy et al., 2007; Boengler et al., 2009; Przyklenk, 2011). Using endothelial function rather than myocardial infarct size as endpoint of protection in humans in vivo, increased age was associated with loss of protection by ischemic preconditioning against endothelial dysfunction after ischemia/reperfusion in the brachial artery (van den Munckhof et al., 2013).

A number of studies have focused on different components of the signaling cascades (for review, see Heusch et al., 2008), assessing differences between young and aged hearts that might explain the observed loss of cardioprotection with aging.

IV.A.1.a Effect of aging on cardioprotective signaling

Cytosolic signaling. Blockade of the Na^+/H^+ exchanger protected myocardium from ischemia/reperfusion injury in aged rats, whereas anesthetic preconditioning did not (Liu and Moore, 2010). Cyclic AMP-dependent protein kinase (PKA) activation and Akt activation are critical for ischemic preconditioning-induced cardioprotection (Yang et al., 2013). The adenylyl cyclase activator forskolin, which promotes subsequent PKA activation, reduced infarct size in young but not in aged rat hearts (Huhn et al., 2012). The loss of cardioprotection in aged, diabetic Goto-Kakizaki rats was associated with a chronic up-regulation of Akt phosphorylation and a lack of further activation of Akt by ischemic preconditioning (Whittington et al., 2013b). The myocardial Akt isoforms Akt1 and Akt2 must be distinguished in their function for ischemic preconditioning's protection. The lack of a protective response to ischemic preconditioning in Akt1 knockout mice was accompanied by impaired phosphorylation (and thus inactivation) of GSK-3β (Kunuthur et al., 2012). Similarly, lack of pharmacological preconditioning by isoflurane in aged rat hearts was associated with differences in the Akt/GSK-3β signaling pathway (Zhu et al., 2010), and pharmacological GSK-3β inhibition decreased infarct size in young but not in old rat hearts (Zhu et al., 2011). In aged rat hearts, sirtuin deacetylase-1 (Sirt1) activity was increased following ischemia/reperfusion as compared to young hearts (Adam et al., 2013). While young Sirt1 knockout mice hearts could not be preconditioned (Nadtochiy et al., 2011b), a drug-induced increase in Sirt1 activity did not elicit cardioprotection following

ischemia/reperfusion, suggesting that Sirt1 activity is necessary but not sufficient for the cardioprotective effects of ischemic preconditioning (Nadtochiy et al., 2011a) and is most likely not responsible for any observed age-related differences.

Mitochondria. The different cytosolic signaling pathways activated by ischemic or pharmacological preconditioning converge at the level of mitochondria (for review, see Heusch et al, 2008; Boengler et al., 2011b; Wojtovich et al., 2012), and the opening of certain mitochondrial ion channels alone is sufficient to elicit protection (for review, see Wojtovich et al., 2012). Pharmacological preconditioning by helium, which protected young but not old rat hearts, could be abolished by blockade of the mitochondrial calcium-sensitive potassium channel (Heinen et al., 2008). As expected, pharmacological activation of the mitochondrial calcium-sensitive potassium channel reduced irreversible injury induced by ischemia/reperfusion in young rat hearts but surprisingly was also effective in reducing infarct size in aged rat hearts (Huhn et al., 2012). GSK-3β inhibition significantly prolonged the time to MPTP opening induced by ROS in cardiomyocytes isolated from young but not from aged rat hearts (Zhu et al., 2011; Zhu et al., 2013b). Attenuation of ischemic or pharmacological preconditioning's protection in the aged heart was associated with failure to reduce adenine-nucleotide-translocase -cyclophilin-D interactions, a critical modulator of MPTP opening (Zhu et al, 2013b). Cyclosporine A, which binds cyclophilin D thereby delaying MPTP opening , reduced myocardial infarct size and time to MPTP opening in young rats, whereas it failed to significantly affect either infarct size or time to MPTP opening in old rats (Liu et al., 2011b). Four weeks of treatment with the superoxide scavenger tempol restored pharmacological preconditioning and cardioprotection by cyclosporine-A in old rats, and the reinstatement of the

cardioprotected condition was associated with delayed onset of MPTP opening (Zhu et al., 2013a).

IV.A.1.b. Gender paradox

Most of the experimental studies (for review, see Ostadal et al., 2009) confirm the clinical observations (Canali et al., 2012) that female hearts have an increased resistance to ischemia/reperfusion injury, associated with an altered distribution of PKC and ERK isoforms as compared to male hearts (Hunter et al., 2005). The already high tolerance of the adult female heart can be increased further by ischemic preconditioning. However, it seems that this protective effect of preconditioning in female animals depends on age: it was absent in the young female rat heart but it appeared with the decrease of resistance towards ischemia/reperfusion injury during aging (Ostadal et al., 2009). An increased resistance towards ischemia/reperfusion injury in aged female hearts could also be restored by a PKCε-activator administered prior to ischemia and restoration of protection was associated with an enhanced mitochondrial PKCε-translocation (Lancaster et al., 2011).

IV.A.1.c Delayed preconditioning (second window of protection)

Twenty-four hour delayed anesthetic preconditioning with sevoflurane reduced infarct size in young but not in old rat hearts. Anesthetic preconditioning affected gene expression profiles (functional categories of cell defense/death, cell structure, gene expression/protein synthesis, inflammatory response/growth/ remodeling, and signaling/communication) of the cardiomyocyte in an age-associated pattern (Zhong et al., 2012).

IV.A.2. Aging and ischemic and pharmacological postconditioning

Ischemic or pharmacological postconditioning attenuates ischemia/reperfusion injury in young animal hearts (Skyschally et al., 2009). However, in most, although not all (Yin et al., 2009) studies, the protection is lost in aged hearts (for review, see Boengler et al., 2009 and Przyklenk et al., 2011). Comparing ischemic preconditioning and postconditioning, one study suggested that ischemic postconditioning was less affected by aging than ischemic preconditioning (Vessey et al., 2009). However, genetic characteristics, a minor difference in age, or the number of postconditioning cycles are all critical factors for the successful effect of ischemic postconditioning and must be taken into consideration (Boengler et al., 2008a; Boengler et al., 2009; Skyschally et al., 2009; Somers et al., 2011). While there is no doubt that postconditioning protects human hearts (Heusch et al., 2013) there is some evidence that the extent of protection might depend on age. In a retrospective analysis, postconditioning the human heart by multiple balloon inflations failed to reduce irreversible injury in patients above the age of 65 years (Darling et al., 2007). Using the improvement of left ventricular function by postconditioning as endpoint, rather than reduction of infarct size, a recent meta-analysis also suggested a beneficial effect of postconditioning only in patients younger than 62 years (Zhou et al., 2012).

As with ischemic preconditioning, the more recent experimental studies have attempted to define specific alterations in the signaling mechanims leading to the failure of protection by postconditioning in aged as compared to young hearts.

IV.A.2.a Cytosolic signaling

Ischemic postconditioning reduced infarct size in young mice hearts and the protection was associated with an up-regulation of extracellular regulated kinase (ERK), but not Akt signaling. In contrast, postconditioning failed to limit infarct size in aged hearts, possibly as a consequence of the defect in ERK phosphorylation and increased MAPK phosphatase (MKP)-1 expression. Indeed, MKP inhibition restored the ischemic postconditioned phenotype in aged mice hearts (Przyklenk et al., 2008). Similarly, pharmacological postconditioning with isoflurane protected the heart in young but not in senescent rats; again the failure to activate the RISK pathway might have contributed to the attenuation of isoflurane-induced postconditioning effect in senescent rats (Chang et al., 2012). In one study with maintained reduction of infarct size by ischemic postconditioning in aged rat hearts (16-18 months), protection was accompanied by an increase in phosphorylation of Akt and GSK-3β similar to that measured in young rat hearts (Yin et al., 2009). In addition to the RISK pathway, the SAFE pathway also appears to be affected by age. The signal transducer and activator of transcription-3 (STAT3), which is involved in ischemia/reperfusion injury and cardioprotection by conditioning protocols (Boengler et al., 2008b), was less highly expressed and activated in aged mice hearts (Boengler et al., 2008a). Possibly, STAT3 plays a role in modifying mitochondrial function during ischemia/reperfusion such as ROS formation (Boengler et al., 2013b) and opening of the MPTP (Boengler et al., 2010; Heusch et al., 2011). While many postconditioning interventions are affected by age, pharmacological postconditioning with sphingosine reduced infarct size to the same extent in young and aged rat hearts (Vessey et al., 2009). Blockade of PKG or PKA attenuated the cardioprotection by sphingosine, suggesting that the cyclic nucleotide-dependent signaling

pathway utilized by sphingosine remains unaffected by age (Vessey et al., 2008a, b; Vessey et al., 2009).

IV.A.2.b. Mitochondria

Similarly to what has been described for ischemic preconditioning, direct pharmacological inhibition of electron transport at reperfusion using amobarbital protected mitochondria and decreased myocardial injury in isolated aged rat hearts, even when signaling-induced pathways of postconditioning that are upstream of mitochondria were ineffective (Chen et al., 2012).

IV.A.3. Aging and remote ischemic preconditioning

While remote ischemic preconditioning protects young and aged human hearts from ischemia/reperfusion-induced irreversible injury and improves patient outcomes after coronary artery bypass grafting, little is known about the age-dependency of the process. In a recent experimental study, remote ischemic preconditioning by lower limb ischemia did not protect against ischemia/reperfusion injury in isolated newborn rabbit hearts and even caused deleterious effects in these hearts, while it effectively reduced infarct size in adult rabbit hearts (Schmidt et al., 2014).

Using endothelial function rather than irreversible myocardial injury as endpoint, healthy elderly people had a greater relative increase of flow-mediated vasodilatation after remote ischemic preconditioning than young individuals (Moro et al., 2011). Thus, whether or not an age-dependency of remote ischemic preconditioning exists remains unknown at present.

Taken together, many studies demonstrate that protection by ischemic and pharmacological preconditioning (early and delayed phase) and postconditioning is lost in aged hearts. Loss of cardioprotection is related to alterations in cytosolic signaling cascades leading to modification in the opening of MPTP. However, direct stimulation of mitochondrial targets might be capable of inducing protection even in aged hearts.

IV.B. Hypertension, cardiac hypertrophy and remodeling

Ischemic or pharmacological (e.g. adenosine-receptor agonist, propofol) preconditioning reduced infarct size in normotensive and hypertensive, hypertrophied rat hearts in vitro (Ebrahim et al., 2007; Hochhauser et al., 2007; King et al., 2012) and in vivo (Dai et al., 2009). Similarly, pharmacological preconditioning with isoflurane six weeks after permanent coronary artery ligation reduced infarct size following ischemia/reperfusion in the remaining myocardium although hearts exhibited a substantial compensatory hypertrophy. The cardioprotection by isoflurane was abolished by inhibition of PI3K or K_{ATP} blockade, indicating that the established signaling cascade of protection was intact in the remodeled myocardium (Lucchinetti et al., 2008). In contrast, ischemic postconditioning reduced infarct size in normotensive but not hypertensive rat hearts (Penna et al., 2010; Wagner et al., 2013). The phosphorylation of GSK-3β was increased by ischemic postconditioning in normotensive rats. However, this increase was completely absent in hypertensive, hypertrophied rat hearts (Wagner et al., 2013). In anabolic steroid-induced cardiac hypertrophy, ischemic postconditioning failed to reduce infarct size following ischemia/reperfusion; postconditioning increased Akt phosphorylation regardless of its protective effects, but reduced expression of protein phosphatase expression was measured in protected hearts (Penna et al., 2011). Chronic captopril treatment significantly reduced left

ventricular hypertrophy in hypertensive rats and reduced infarct size following ischemia/reperfusion in isolated hearts from both normotensive and hypertensive rats. Ischemic postconditioning was unable to add its protective effect to that of chronic captopril even though treatment induced hypertrophy regression and almost completely normalized left ventricular pressure (Penna et al., 2010).

Taken together, while preconditioning's protection is still present in animals with hypertension and/or left ventricular hypertrophy, infarct size reduction by ischemic postconditioning appears to be lost. Once again the lack of protection by ischemic postconditioning relates to changes in the cytosolic signaling pathway. Treatment of the primary disease (hypertension, hypertrophy) does not restore the cardioprotection by ischemic postconditioning.

IV.C. Hyperlipidemia and atherosclerosis

IV.C.1. Ischemia/reperfusion injury, ischemic pre-, post, and remote conditioning in hyperlipidemia

In epidemiological studies, there is a well-recognized relationship between serum total cholesterol concentration and the morbidity and mortality due to MI. Previously, this was attributed solely to the development of coronary atherosclerosis as a result of hypercholesterolemia. However, in the last two decades, a significant volume of evidence has accumulated showing that hyperlipidemia exerts direct effects on the myocardium that may interfere with cardioprotective mechanisms. Although there are some conflicting results, most of the preclinical studies, together with some small scale clinical studies, have shown that

hyperlipidemia *per se*, but not atherosclerosis, leads to a significant aggravation of myocardial ischemia/reperfusion injury and to attenuation of the cardioprotective effect of both early and late preconditioning. These studies were reviewed by us previously (Ferdinandy et al., 2007).

More recent studies confirm the deleterious effects of hyperlipidemia on susceptibility to ischemia/reperfusion injury and on ischemic preconditioning. Expansion of infarct size in a hyperlipidemic pig model was shown by Osipov et al. (2009). The loss of the infarct limiting effect of ischemic preconditioning (Gorbe et al., 2011; Landim et al., 2013; Babbar et al., 2013; Yadav et al., 2010b; Xu et al., 2013) and late ischemic preconditioning (Yadav et al., 2012) have been shown in different models of diet-induced hyperlipidemia in rats.

The loss of the infarct size limiting effect of ischemic postconditioning has been also confirmed in the last five years by several studies in hypercholesterolemic rats (Kupai et al., 2009; Landim et al., 2013; Wu et al., 2014) and in rabbit models (Andreadou et al., 2012; Iliodromitis et al., 2010). In the fructose-fed hypertriglyceridemic rat heart, cholesterol lowering by fenofibrate restored ischemic preconditioning-mediated cardioprotection (Babbar et al, 2013). However, in one study ischemic postconditioning was still effective in hyperlipidemic rats (Zhao et al., 2009a). The effect of hyperlipidemia on remote conditioning has not yet been reported.

IV.C.2. Pharmacological cardioprotection in hyperlipidemia: implications for target validation

One of the most extensively investigated cardioprotective cellular signaling pathways is the NO-cGMP-PKG pathway and elements of this signaling pathway are thought to be promising drug targets (Andreadou et al., 2014; Bice et al., 2014; Burley et al., 2007; Gorbe et al., 2010; Garcia-

Dorado et al., 2009). However, Giricz et al. (2009) showed that although the NO donor SNAP which activates soluble guanylate cyclase, or BNP which activates particulate guanylate cyclase, or the stable cGMP analogue 8-bromo cGMP significantly reduced infarct size in normal rat hearts, none of these treatments was effective in hearts of rats fed a cholesterol-enriched diet. The loss of cardioprotection by activators of the cGMP-PKG pathway was possibly due to inactivation of PKG by oxidative dimerization of the kinase under hyperlipidemic conditions. These results indicate that drug targets upstream of PKG are less likely targets for cardioprotection, since activation of such targets does not provide cardioprotection in the presence of hyperlipidemia. Another recognized cardioprotective pathway includes K_{ATP} channel activation. However, it has recently been shown that the infarct limiting effect of either the non-selective K_{ATP} activator cromakalim or the selective mitochondrial K_{ATP} activator diazoxide was abrogated in hyperlipidemic, cholesterol-fed rats (Csonka et al., 2014). Sevoflurane-induced delayed cardioprotection against ischemia/reperfusion injury was also lost in hypercholesterolemia, potentially via interference of hyperlipidemia with the iNOS/mitochondrial K_{ATP} channel pathway in rats (Zhang et al., 2012). Moreover, in hyperlipidemic Zucker obese rats, neither postconditioning nor the MPTP inhibitor cyclosporine-A could exert cardioprotection (Huhn et al., 2010). These results indicate that activation of the NO-cGMP-PKG pathway or K_{ATP} channels, and inhibition of the MPTP may not be ideal cardioprotective drug targets, since they are ineffective in the presence of hyperlipidemia, a frequent attendant condition in patients with ischemic heart disease.

In contrast, the use of hyperlipidemic animal models of cardioprotection reveals some pharmacological targets that remain effective in the presence of hyperlipidemia.

Cardioprotection by preconditioning involves an approximately 20% inhibition of myocardial matrix metalloproteinase-2 (MMP2) (Lalu et al., 2002; Giricz et al., 2006). However, MMP2 inhibition by preconditioning is absent in hyperlipidemic rats. Giricz et al. (2006) showed that by mimicking the moderate MMP2 inhibitory effect of preconditioning with the inhibitor ilomastat, cardioprotection occurred both in normal and hyperlipidemic hearts. Effective cardioprotection by a moderate inhibition of intracellular MMP2 was confirmed recently by Bencsik et al., (2014). MMP2 inhibition as a pathway parallel to the known cardioprotective pathways has been confirmed by Bell et al. (2013). Dietary supplementation of red palm oil in rats with established hyperlipidemia was able to reduce infarct size associated with MMP2 inhibition (Szucs et al, 2011). These results show that MMP2 inhibition might be a valid cardioprotective target, as moderate inhibition of MMP2 in the heart confers cardioprotection in both normal and hyperlipidemic animals subjected to MI (Dorman et al., 2010). Independent of MMP2 inhibition, Yadav et al. (2012) have shown that pharmacological inhibition of GSK-3β produced a late cardioprotected state in both normal and hyperlipidemic rats, possibly associated with HSP72 induction. This may also suggest an MPTP-independent effect of GSK3β inhibition.

IV.C.3. Effect of hyperlipidemia on cardioprotective cellular mechanisms

The mechanism by which hyperlipidemia may influence the severity of myocardial ischemia/reperfusion injury and cardioprotection is not fully understood. However, decreased cardiac NO content, increased oxidative/nitrosative stress, inactivation of PKG, impairment of the mevalonate pathway, decreased heat-shock response, increased ecto-5'-nucleotidase activity, enhanced apoptotic cell death, as well as dramatic changes in cardiac gene expression profile have all been demonstrated as consequences of hyperlipidemia (see for earlier reviews:

Ferdinandy et al., 1998; Ferdinandy, 2003; Ferdinandy et al., 2007). Moreover, there is evidence that the microRNA expression pattern of hyperlipidemic rat hearts (Varga et al., 2013) and the gene expression patterns in the Zucker Diabetic Fatty rat strain are changed (Sarkozy et al., 2013), indicating that the heart responds to the systemic hyperlipidemic state by concerted alterations in gene expression including genes controlling metabolic functions. We have also shown that hyperlipidemia changes Cx43 distribution in the sarcolemma and in the mitochondria (Gorbe et al., 2011). Hyperlipidemia also interferes with the nitrosative triggering signal of postconditioning in rats (Kupai et al., 2009)

In summary, the majority of preclinical studies show that hyperlipidemia worsens the outcome of ischemia/reperfusion injury and attenuates the cardioprotective effect of both early and late preconditioning, postconditioning, and pharmacological conditioning via hyperlipidemia-induced changes in cardioprotective signaling pathways. Nevertheless, there are some promising targets that may be still effective in the hyperlipidemic heart e.g. MMP-2. These findings emphasize the necessity for the development of new cardioprotective drugs that are able to reverse the increased susceptibility of hyperlipidemic hearts to ischemia/reperfusion stress and to enhance adaptive cardioprotective mechanisms in hyperlipidemic patients.

IV.D. Diabetes

Epidemiological studies and clinical trials have clearly shown that both type I (insulin-dependent) and type II (non-insulin-dependent) diabetics are more prone to developing ischemic heart disease, including acute MI and post-MI complications (Baars et al., 2013; Otto et al., 2012). The diabetic heart is more susceptible to acute myocardial ischemia/reperfusion injury (Marso et al., 2007; Alegria et al., 2007).

IV.D.1. Ischemia/reperfusion injury in diabetes

Although it has long been known that diabetes is an independent risk factor for the development of ischemic heart disease and that the long term outcome of ischemic heart disease is worsened by diabetes in humans, inconsistency exists in the literature regarding the susceptibility of myocardium to acute ischemia/reperfusion injury in various animal models of diabetes (reviewed in Ferdinandy et al., 2007; Whittington et al., 2012; Miki et al., 2012). With respect to the preclinical animal studies, it appears that the susceptibility of the diabetic heart to acute ischemia/reperfusion injury is critically dependent on the duration and severity of the diabetes model and the experimental conditions of the acute ischemia/reperfusion injury model.

In experimental studies, the diabetic heart has been shown to be *less* sensitive to acute ischemia/reperfusion injury in those experimental studies which used: (i) a short duration of diabetes (<6 weeks); (ii) glucose as the only substrate; and (iii) a no-flow acute ischemia/reperfusion injury protocol. In contrast, in studies where the diabetes model was more prolonged and severe, fatty acids were present in the substrate, and a low-flow acute ischemia/reperfusion injury protocol was used, the diabetic heart was found to be *more* sensitive

to acute ischemia/reperfusion injury (Whittington et al., 2012; Miki et al., 2012). In contrast to the inconsistent preclinical studies, the majority of clinical studies demonstrate worse outcome from acute myocardial infarction in diabetic patients. This suggests that the use of chronic diabetes animal models better reflects the clinical situation.

A number of studies have examined cardioprotection in different experimental animal models of diabetes and in diabetic patients. The majority of studies show that the presence of diabetes may interfere with the cardioprotective mechanisms, attenuating the effectiveness of these therapeutic strategies. As expected in some of the animal diabetic models used in the experimental studies, there is some overlap with the related co-morbidities of obesity and metabolic syndrome, both of which may also impact on the efficacy of cardioprotection. Many of the therapies used to treat diabetes may also impact on the cardioprotective intervention (discussed in a later section V.C). Finally, some experimental studies are beginning to investigate the effect of one or more co-morbidities on the efficacy of cardioprotective strategies, which better reflects the clinical setting in which multiple co-morbidities often co-exist with ischemic heart disease.

IV.D.2. Cardioprotection by preconditioning in diabetes

The majority of preclinical experimental studies investigating the effect of diabetes on the myocardial response to either ischemic or pharmacological preconditioning have found that the presence of diabetes renders the heart more resistant to the infarct-limiting effects of preconditioning (reviewed in Ferdinandy et al., 2007; Miki et al., 2012). Recent experimental studies have also shown that the cardioprotective efficacy of pharmacological preconditioning using a variety of different agents including erythropoietin (Hotta et al., 2010; Miki et al., 2009),

δ-opioid receptor agonist (Hotta et al., 2010), isoflurane (Matsumoto et al., 2009), L-glutamate (Povlsen et al., 2009), remifentanil (Kim et al., 2010), helium (Huhn et al., 2009), is also impaired in the diabetic heart. Whittington et al. (2013b) investigated the combined effect of diabetes and age on the response of the diabetic heart to acute ischemia/reperfusion injury. As expected, the combination of aging (up to 18 months in the rat) and diabetes (Goto-Kaziaki rat) increased infarct size in response to acute ischemia/reperfusion and raised the threshold for ischemic preconditioning in a predictably additive manner.

IV.D.2.a. Mechanisms contributing to resistance of the diabetic heart to preconditioning

A variety of different mechanisms have been suggested to contribute to the impaired response of the diabetic heart to preconditioning. These include impaired activation of known intracellular pro-survival signaling pathways such as the Akt and ERK1/2 components of the RISK pathway (Hausenloy & Yellon, 2007) and its downstream targets such as GSK3β, and the janus-activated kinase (JAK)-STAT3 components of the SAFE pathway (Lecour, 2009). More recent experimental studies have investigated the effect of chronic Akt activation on the myocardial response to preconditioning. Whittington et al. (2013b) found that in the aged rat heart (12-18 months old) there was chronic activation of Akt, which was not further augmented in response to ischemic preconditioning, suggesting that in this setting Akt activation may not mediate cardioprotection. In this regard, Fullmer et al. (2013) showed that pharmacological (insulin) or genetic activation of Akt in the heart blocked the infarct-limiting effects of ischemic preconditioning.

Since 2007, experimental studies have investigated several novel mechanisms to explain the impaired response of the diabetic heart to preconditioning. Miki et al. (2009) reported that the presence of augmented endoplasmic reticulum stress in the diabetic heart blocked ERK1/2-mediated phosphorylation of GSK3β, leading to increased susceptibility to MPTP opening and mitochondrial calcium overload. Hotta et al. (2010) showed that the angiotensin-II subtype 1 receptor-mediated upregulation of calcineurin in the diabetic rat heart interfered with the phosphorylation of JAK2 and PI3K-Akt signaling, thereby affecting the efficacy of pharmacological preconditioning. Ajmani et al. (2011) provided data implicating caveolin, which was increased in the diabetic heart and interfered with eNOS activity, contributing to the inability of the diabetic heart to respond to ischemic preconditioning. Impaired mitochondrial biogenesis, secondary to a dysfunctional adiponectin-adenosine monophosphate activated kinase (AMPK) axis (Yan et al., 2013), and increased TNFα- induced oxidative stress (Su et al., 2013) were also proposed as mechanisms underlying the greater sensitivity of the diabetic heart to ischemia/reperfusion injury and its resistance to ischemic preconditioning. A recent study by Gurel et al. (2013) has provided preliminary data suggesting that in the diabetic heart the failure of ischemic preconditioning to localize hexokinase to mitochondria may contribute to the lack of cardioprotection. Finally, an intriguing study by Vinokur et al. (2013) suggests that altered iron metabolism may contribute to the response of the diabetic heart to acute ischemia/reperfusion injury and ischemic preconditioning. Under basal conditions diabetic hearts sustained less acute ischemia/reperfusion injury, a finding which was associated with basally increased levels of myocardial ferritin, a cardioprotective factor. Conversely, the failure of the diabetic heart to respond to ischemic preconditioning was found to be associated with an accelerated loss of myocardial ferritin during ischemia.

In order to overcome the resistance of the diabetic heart to preconditioning, several studies have used pharmacological agents to target the effectors of cardioprotection downstream of the impaired signaling pathways or have used pharmacological modulation of alternative cardioprotective pathways. In this regard, it has been shown that pharmacological inhibition of GSK-3β, a downstream target of Akt and ERK1/2, was able to reduce MI size in the diabetic heart (Miki et al., 2009; Yadav et al., 2010a). Other experimental studies have reported being able to pharmacologically precondition the diabetic heart using a variety of diverse agents including olprinone (a phosphodiesterase type 3 inhibitor) (Matsumoto et al., 2009), and even ginsenoside Rb1 (a pharmacologically active component of ginseng) (Wu et al., 2011b).

IV.D.2.b. Restoring myocardial sensitivity to preconditioning in the presence of diabetes

Whether anti-diabetic therapy or another pharmacological agent can restore the sensitivity of the diabetic heart to preconditioning has been investigated. Gu et al. (2008) found that simvastatin treatment was able to restore cardioprotection elicited by ischemic preconditioning in the presence of hyperglycemia, and this effect was associated with the generation of NO. Pre-treatment of diabetic Goto-Kakizaki rats with the anti-diabetic sulfonylurea glimepiride (which did not reduce infarct size itself) was demonstrated to restore the sensitivity of the myocardium to ischemic preconditioning, such that one cycle instead of three cycles of ischemic preconditioning was sufficient to limit infarct size (Hausenloy et al., 2013c). However, this effect of glimepiride in lowering the threshold for ischemic preconditioning appeared to be independent of serum glucose levels as the latter remained unchanged with glimepiride treatment (Hausenloy

et al., 2013c). Finally, chronic insulin treatment increases cardiac adiponectin and restores cardioprotective AMPK signaling (Pei et al., 2013).

Experimental studies using human right atrial tissue harvested from patients undergoing coronary artery bypass grafting surgery have reported that myocardial tissue from diabetic patients is resistant to ischemic preconditioning when subjected to simulated acute ischemia/reperfusion injury (Ovunc, 2000; Barua et al., 2011). Barua et al. (2011) showed that the resistance to ischemic preconditioning could be overcome by modulating NO availability in atrial tissue from diabetic patients. Interestingly, Sivaraman et al. (2010) demonstrated that intensifying the ischemic preconditioning stimulus protected isolated human right atrial trabeculae harvested from diabetic patients against simulated acute ischemia/reperfusion injury. In this study, the inability to precondition the diabetic atrial tissue using a standard preconditioning protocol was associated with lower basal levels of Akt activation (Sivaraman et al., 2010). Furthermore, Wang et al. (2011b) reported enhanced expression of PTEN (a suppressor of Akt activity) through microRNA interference (Ling et al., 2013)) associated with reduced Akt and downstream eNOS expression in diabetic human atrial tissue.

In vivo human data have confirmed the resistance of diabetic patients to the protective effect of ischemic preconditioning. Engbersen et al. (2012) have demonstrated using a forearm model of endothelial injury that the efficacy of ischemic preconditioning to reduce acute ischemia/reperfusion injury was lower in type 1 diabetic patients and was completely abolished in these patients during hyperglycemia.

IV.D.3. Cardioprotection by postconditioning in diabetes and metabolic syndrome

Experimental animal data suggest that the presence of diabetes and related conditions such as obesity and the metabolic syndrome may impact on the cardioprotective efficacy of both ischemic and pharmacological postconditioning. For ischemic postconditioning, using either a genetic or a chemically-induced murine model of diabetes, Przyklenk et al. (2011) found that the isolated perfused diabetic heart was resistant to the infarct-limiting effects of ischemic postconditioning when compared to the normoglycemic heart. In this study, the lack of response to ischemic postconditioning in the diabetic heart was associated with a failure to activate the ERK1/2 component of the RISK pathway. Using an isolated rat heart model of acute ischemia/reperfusion injury, Ren et al. (2011) demonstrated that the failure to postcondition the diabetic heart appeared to be linked to reduced myocardial expression of transient receptor potential vanilloid 1 channel (TRPV1), calcitonin gene related peptide and substance P (Babiker et al., 2012) have shown that the diabetic heart was resistant to a novel form of postconditoning using pacing (ten cycles of 30 seconds left ventricular pacing alternated with 30 seconds right atrial pacing) when compared to the non-diabetic heart.

Further studies have demonstrated that pharmacological postconditioning using a variety of agents can also be affected by the presence of diabetes. Raphael et al. (2010) found that hyperglycemia abrogated the cardioprotection elicited by the inhaled anesthetic isoflurane, administered at the onset of myocardial reperfusion, using a rabbit *in vivo* model of acute ischemia/reperfusion injury, a finding which was associated with impaired activation of Akt-eNOS. Similarily, the infarct-limiting effect of the inhaled anesthetics desflurane (Tai et al., 2012) and sevoflurane (Drenger et al., 2011), administered at the onset of myocardial reperfusion

to rats in vivo, was abolished in the presence of diabetes. This was associated with a failure of these anesthetic agents to activate Akt, ERK1/2, GSK-3β (Tai et al., 2012) and STAT3 (Drenger et al., 2011). An interesting study by Potier et al. (2013) has suggested that there may be differential effects in the response to cardioprotection using different bradykinin receptor agonists in the presence of diabetes. It was shown that ischemic postconditioning, the ACE inhibitor ramiprilat or a bradykinin B_2 receptor agonist, all reduced infarct size and activated Akt, ERK1/2 and GSK-3β in the non-diabetic heart but failed to do so in the diabetic heart (Potier et al., 2013). In contrast, the diabetic heart, but not the non-diabetic heart, was found to be amenable to pharmacological postconditioning using a bradykinin B_1 receptor agonist (Potier et al., 2013).

A number of experimental studies have investigated the cardioprotective effects of combining ischemic and pharmacological postconditioning in the diabetic heart. Badalzadeh et al. (2012) demonstrated that the isolated diabetic rat heart was resistant to cardioprotection by either ischemic postconditioning or cyclosporine A alone, but with the combination of interventions, a significant reduction in infarct size was observed. In contrast, Fan et al. (2012) found that atorvastatin was able to postcondition the diabetic heart, despite it being resistant to ischemic postconditioning; when given in combination, an additive cardioprotective effect was seen. These data suggest that the diabetic heart may be amenable to cardioprotection if the intensity of the postconditioning stimulus is enhanced.

Attempts have been made to recapture the cardioprotective effects of postconditioning by correcting the hyperglycemic state. In this regard, although insulin pre-treatment failed to restore

the sensitivity of the diabetic heart to postconditioning (Drenger et al., 2011), the transplantation of pancreatic islet tissue was able to reverse the effect of diabetes on the myocardial response to postconditioning (Przyklenk et al., 2011).

In contrast to the above findings, Lacerda et al. (2012) found that despite the presence of diabetes, the murine heart was equally sensitive to ischemic postconditioning (6 x 10 second cycles of coronary arterial occlusion/reperfusion) as the non-diabetic heart. However, this discordant finding may in part be explained by the fact that the streptozotocin-induced diabetes was only initiated 5-10 days prior to MI. A recent study by Oosterlinck et al. (2013), using a genetic murine model of diabetes (ob/ob and DKO strains), demonstrated that the cardioprotective effect of ischemic postconditioning was attenuated, although this may in part relate to the use of a non-standard ischemic postconditioning protocol (3x10 second cycles of coronary arterial occlusion/reperfusion).

Bouhidel et al. (2008) investigated the effect of obesity alone on the efficacy of ischemic postconditionin. They found that the leptin-deficient obese (ob/ob) mice was resistant to cardioprotection by ischemic postconditioning, and that this was associated with an inability to activate Akt, ERK1/2, p70S6 kinase, and AMPK. Using a rat model of the metabolic syndrome, Wagner et al. (2008) found that the Wistar-Ottawa-Karlsburg W (WOKW) rats were resistant to the cardioprotection elicited by ischemic postconditioning, and this was associated with an inability to phosphorylate ERK1/2 and its downstream effector GSK-3β. Huhn et al. (2009b; 2010) reported that the Zucker obese rat was resistant to pharmacological postconditioning using either sevoflurane or cyclosporine A . The finding that the presence of a co-morbidity could

impact on the cardioprotective efficacy of cyclosporine-A is somewhat surprising as its target, the MPTP, is believed to be downstream of the signaling pathways affected by the co-morbidity. However, one can speculate that the co-morbidity may interfere with cardioprotection by modulating mitochondrial function. An interesting study by Pons et al. (2013) showed that regular treadmill exercise was able to protect the heart in obese (ob/ob) mice through the activation of pro-survival kinases (Akt, ERK1/2, GSK-3β, p70S6 kinase, AMPK) and in the absence of any improvement in metabolic profile. These findings suggest that regular exercise may have beneficial effects on the heart independently of any effect it may have on the metabolic profile.

Despite the preclinical data demonstrating the resistance of the diabetic heart to both ischemic and pharmacological postconditioning, there have been no clinical trials investigating the effect of diabetes on the cardioprotective efficacy of postconditioning. This is despite a number of proof-of-concept clinical studies, comprising a significant proportion of diabetic patients, reporting beneficial effects of postconditioning in STEMI and coronary artery bypass graft surgery patients (see section III). Interestingly, Lemoine et al. (2008; 2010; 2011) found that human atrial trabeculae subjected to simulated acute ischemia/reperfusion injury were equally sensitive to the cardioprotective effects of pharmacological postconditioning using desflurane, whether harvested from diabetic or non-diabetic patients undergoing coronary artery bypass graft surgery.

IV.D.4. Cardioprotection by remote ischemic conditioning in diabetes

Despite the clinical potential of remote ischemic conditioning, there is a paucity of animal data investigating whether the diabetic heart is amenable to this cardioprotective intervention. Investigating the effect of diabetes in this setting can be quite challenging, given that the presence of diabetes may modulate signaling at any one or more of the following stages of the mechanistic pathway: the remote organ or tissue in which the conditioning stimulus is applied, the neuro-hormonal pathway which conveys the cardioprotective stimulus to the heart, and finally, the activation of intracellular pro-survival signaling pathways within the heart (reviewed in Hausenloy & Yellon, 2008).

There has only been one experimental animal study published specifically investigating the cardioprotective efficacy of remote ischemic preconditioning in the presence of diabetes. Zhu et al. (2011b) found that the diabetic rat heart *in vivo* was amenable to infarct size reduction elicited by limb preconditioning (3x5 min cycles of hind-limb cuff inflation/deflation). However, it is important to note that in this study streptozotocin-induced diabetes was only initiated one week prior to MI. Whether remote ischemic preconditioning would have been effective in a chronic diabetes animal model remains to be determined.

Remote ischemic conditioning, using a cuff placed on either the upper arm or leg to induce three or more cycles of brief ischemia and reperfusion, has been reported to have beneficial effects in a number of clinical settings including STEMI patients treated by primary PCI, coronary artery bypass graft patients and patients undergoing elective PCI (reviewed in (Hausenloy & Yellon, 2008; Candilio et al., 2011; Heusch, 2013)). Although many of these clinical studies included

diabetic patients they did not specifically address the question of whether the diabetic heart was amenable to remote ischemic conditioning.

Xu et al. (2014) found that a standard remote ischemic conditioning stimulus (3x5 min upper arm cuff inflations/deflation) did not reduce the magnitude or incidence of peri-procedural myocardial injury during elective PCI in older patients (mean age 69 years) with diabetes. However, it is difficult to distinguish the effect of diabetes from that of age on the remote ischemic preconditioning protocol. Furthermore, the authors of this study did not demonstrate that their remote ischemic preconditioning protocol was effective in a younger non-diabetic population (Xu et al., 2014). In an intriguing clinical study by Jensen et al. (2012) it was reported that diabetic patients with a peripheral neuropathy failed to produce a cardioprotective humoral factor in response to a standard remote ischemic preconditioning protocol (4x5 min upper arm cuff inflation/deflation), when compared to non-diabetic and diabetic patients who did not have a peripheral neuropathy. This finding supports the notion that an intact neural pathway from/to the remotely conditioned organ or tissue is required to elicit the cardioprotective humoral factor (Jensen et al., 2012). In this particular study, the cardioprotective efficacy of the humoral factor was tested in an isolated perfused rabbit heart ischemia/reperfusion model, and therefore whether the diabetic heart was amenable to remote ischemic preconditioning cardioprotection was not actually investigated (Jensen et al., 2012). A later study by the same research group investigated the impact of myocardial O-linked β-N-acetylglucosamine (O-GlcNAc) in remote ischemic preconditioning-induced protection (Jensen et al., 2013). They found that plasma dialysate harvested from either normal volunteers or diabetic patients treated with a standard protocol of remote ischemic preconditioning (3x5 cycles of upper am cuff inflation/deflation) was able to

protect naïve human right atrial trabeculae subjected to simulated ischemia/reperfusion injury, findings which were associated with increased levels of myocardial O-GlcNAc (Jensen et al., 2013). These findings appear to suggest that increased myocardial levels of O-GlcNAc may indicate a cardioprotective phenotype.

In summary, the majority of both preclinical and clinical data suggest that the diabetic heart is more susceptible to ischemia/reperfusion injury and that the cardioprotective effect of ischemic and pharmacological preconditioning and of postconditioning is impaired in the presence of diabetes. This impairment appears to be associated with deficient activation of pro-survival signaling pathways such as Akt, ERK1/2, AMPK and STAT3. Due to the limited pre-clinical and clinical studies available, whether or not the diabetic heart is amenable to remote ischemic conditioning is currently not known.

IV.E. Kidney failure and uremia

Uremia, resulting from kidney failure, is a metabolic disease associated with a high prevalence of ischemic heart disease (Levey and Coresh, 2012). Therefore, one may speculate that a uremic state may also lead to the attenuation of endogenous cardioprotective mechanisms. However, Byrne et al. (2012) reported that ischemic preconditioning, remote conditioning, and postconditioning were still cardioprotective after 4 weeks of subtotal nephrectomy or adenine-enriched diet-induced acute uremia in rats. They also showed in this model that the RISK and SAFE signaling pathways were unaffected. However, experimental models of acute uremia may not properly reflect the clinical situation, since uremia frequently remains unrecognised until its late stages (Levey and Coresh, 2012). Kocsis et al. (2012) studied if prolonged experimental

uremia of 30 weeks duration affects ischemic preconditioning. They found that although prolonged experimental uremia led to severe metabolic changes and mild myocardial dysfunction, the cardioprotective effect of ischemic preconditioning was still preserved 30 weeks after partial nephrectomy in rat hearts. However, it is not known if longer durations of uremia may interfere with postconditioning or remote conditioning. Human conditioning studies in uremia have not been reported.

Taken together, the limited preclinical studies suggest that in spite of the complex systemic metabolic changes in uremia, cardioprotection by pre- and postconditioning is preserved. Nevertheless, further preclinical studies in long-term experimental uremia models, as well as clinical studies will be necessary to show if mechanical or pharmacological conditioning can still protect the heart in uremic patients.

IV.F. The diseased coronary circulation

Experimental studies on myocardial ischemia/reperfusion are usually performed in healthy young animals; moreover, irrespective of age, these animals have a virgin coronary circulation. In clinical reality, atherosclerosis develops progressively over time. Plaque fissure and rupture are then acutely superimposed on the underlying atherosclerosis and further complicated by intraluminal platelet aggregation and coagulation. In contrast, experimental studies usually rely on abrupt closure and reopening of the epicardial coronary artery with external devices. Therefore, the status of both the epicardial coronary arteries and the coronary microcirculation is vastly different between clinical reality and most experimental models. We need to consider

these differences as confounders in translation of cardioprotective strategies (Heusch et al., 2012a). In fact, in most but not all of the more clinically relevant conditions, a diseased coronary circulation tends to attenuate the efficacy of cardioprotection.

IV.F.1. Epicardial coronary arteries

Atherosclerotic plaque rupture in an epicardial coronary artery with superimposed intraluminal thrombotic occlusion of the coronary arterial lumen is the culprit event which causes acute MI in the clinical setting. Primary PCI removes the occlusive thrombus, but – unless there is direct stenting – leaves the underlying atherosclerotic vascular lesion unaffected. Therefore, reperfusion does not occur through a fully patent epicardial coronary artery, but one with a residual stenosis. As a consequence, there is not abrupt, but attenuated, more gentle reperfusion. This gentle reperfusion will even be more prominent when thrombolysis is applied, giving slow thrombus dissolution. Gentle reperfusion *per se* attenuates reperfusion injury and reduces infarct size (Heusch, 2004), as has been demonstrated by gradual restoration of coronary perfusion pressure in isolated rodent heart preparations (Nemlin et al., 2009) and slow restoration of coronary blood flow in larger mammals *in vivo* (Musiolik et al., 2010). Therefore, when comparing a group of patients undergoing a conditioning intervention to a purported control group, which inadvertently undergoes gentle reperfusion through a residual stenosis and thereby experiences some protection, the difference between the two groups which reflects the magnitude of cardioprotection by the conditioning intervention is diminished. Use of direct stenting removes any residual stenosis and coronary microembolization from the culprit lesion such that the consequences of immediate full reperfusion in the control group are compared to those of the postconditioning algorithm in the intervention group (Loubeyre et al., 2002; Heusch, 2012).

IV.F.2. The coronary microcirculation

Plaque rupture is not only the causal index event which leads to acute MI; minor plaque fissure or rupture may also precede or follow the index event, initiated either spontaneously and/or following mechanical intervention. In the absence of coronary occlusion, plaque fissure/rupture releases particulate debris from the underlying atherosclerotic lesion which is superimposed by intraluminal platelet aggregates and coagulation material. These atherothrombotic particles are then washed into the coronary microcirculation where they cause microembolization. Coronary microembolization with inert particles in dogs and pigs induces microinfarcts and an inflammatory reaction (Heusch et al., 2009). However, in patients the ruptured atherosclerotic plaque also releases soluble vasoconstrictor, pro-inflammatory and pro-thrombotic mediators which contribute to the impairment of coronary microvascular perfusion (Kleinbongard et al., 2011). Both, the particulate debris and the soluble mediators not only impair coronary microvascular perfusion *per se* but may also interfere with cardioprotective conditioning strategies.

As discussed previously (section III A), pre-infarction angina is associated with cardioprotection and probably represents a clinical correlate of ischemic preconditioning. In a pig model, coronary microembolization, which may occur clinically during pre-infarction angina, induces microinfarcts that add to the aggregate infarct size caused by the index MI. Coronary microembolization with inert particles in pigs neither induces acute ischemic preconditioning nor does it interfere with acute ischemic preconditioning (Heusch et al., 2009). However, on a somewhat more prolonged time frame, coronary microembolization causes the upregulation of

TNF-α over several hours which then induces cardioprotection (Skyschally et al., 2007); this form of protection following several hours after coronary microembolization was termed a "third window of ischemic preconditioning" (Heusch et al., 2007).

The no-reflow phenomenon is the most severe form of coronary microvascular obstruction that occurs during reperfusion following acute MI and is associated with adverse prognosis. Embolization of atherothrombotic debris, platelet and platelet/leukocyte aggregates, vasoconstrictor substances, edema and physical destruction of the capillary bed all contribute to the no-reflow phenomenon. Minor forms of microvascular obstruction are frequently observed following elective and emergency PCI (Heusch et al., 2013). Coronary microembolization which occurs during early reperfusion, may even be initiated by interventional manipulation of the culprit lesion, increasing infarct size. In a pig model of coronary microembolization during early reperfusion, a redistribution of embolizing particles into the infarct border zone is causal for lateral extension of the infarct (Skyschally et al., 2013). Many interventionalists abstain from postconditioning maneuvres by further manipulation of the culprit lesion after primarily successful reperfusion for fear of causing coronary microembolization and additional damage (Heusch, 2012). However, in pigs ischemic postconditioning is still effective in reducing infarct size even when associated with coronary microembolization, albeit to a lesser extent (Skyschally et al., 2013). Therefore, again it appears wise to perform postconditioning after direct stenting which prevents coronary microembolization from the culprit lesion (Loubeyre et al., 2002; Heusch, 2012).

Finally, we have to consider the impact of soluble mediators released into the coronary circulation, which not only contribute to microvascular obstruction *per se*, but may also induce cardioprotection. These include notably TNFα (Skyschally et al., 2007; Kleinbongard et al., 2010; Kleinbongard et al., 2011) and endothelin (Kleinbongard et al., 2013a) but others might also be implicated.

In summary, the coronary circulation is not only the primary origin of myocardial infarction and reperfusion, but also a major confounder of local cardioprotective strategies i.e. ischemic preconditioning and postconditioning.

V. Effects of concomitant medications used to treat risk factors and co-morbidities on cardioprotection: hidden cardiotoxicity?

Here we review recent data on the effect of the most frequently-encountered drug therapies for cardiovascular risk factors that may modify cardioprotective mechanisms and thereby modifying the efficacy of cardioprotection (Table 3). Very little is known on the possible unwanted effect of medications on the ischemic heart and endogenous cardioprotection, which may result in a latently developing cardiotoxicity (Golomb et al, 2009), which is termed now as “hidden cardiotoxicity”. Hidden cardiotoxicty of a medication may not be seen in the healthy heart, but may manifest only in the ischemic heart as an increased tissue injury due to an increased sensitivity to an ischemic challenge or a decreased capability to adapt to an ischemic challenge.

V.A. Nitrates and nitrate tolerance

Organic nitrates have been used for more than 100 years to prevent and alleviate symptoms of angina pectoris and to reduce blood pressure. However, the main limitation of long-term prophylactic nitrate therapy is the development of vascular nitrate tolerance, which leads to the loss of clinical efficacy (see for review: Csont and Ferdinandy, 2005; Csont, 2010). It has been shown in preclinical studies that the presence of nitrate tolerance aggravates ischemia/reperfusion injury and leads to loss of the cardioprotective effect of preconditioning (see for review Ferdinandy et al., 2007). Since then, little progress has been made in this field. Nevertheless, Fekete et al. (2013) have recently shown that the presence of nitrate tolerance inhibits the infarct-size limiting effect of postconditioning in rats. Moreover, in a recent human study, Gori et al. (2010) have reported that the endothelial preconditioning effect of a single dose of nitroglycerin is lost upon a prolonged exposure to nitroglycerin. Nevertheless, the acute administration of nitrates appears not to interfere with remote conditioning in patients undergoing coronary artery bypass graft surgery (Kleinbongard et al., 2013b).

V.B. Statins and antihyperlipidemic medication

A number of large clinical trials and meta-analyses have shown that chronic administration of the most frequently used antihyperlipidemic drugs, statins (hydroxymethylglutarate co-A reductase inhibitors i.e. inhibitors of the mevalonate pathway), have potent cholesterol-lowering effects and reduce cardiovascular morbidity and mortality (Mills et al., 2011) even in low-risk patients

(Mihaylova et al., 2012). However, it seems that statin therapy is less effective in women (Gutierrez et al., 2012). Since the anti-ischemic effect of rosuvastatin, as shown by improved flow-mediated dilation after upper arm ischemia, may disappear after chronic treatment in patients (Liuni et al., 2012), acute statin loading therapy has been recommended for PCI recently (Benjo et al., 2014). However, acute statin therapy was found ineffective in a recent clinical trial in low risk PCI patients (Ludman et al., 2011). The possible mechanisms of the statin-induced cardioprotective effect are reviewed elsewhere (Ludman et al., 2009; Mahalwar and Khanna, 2013).

We have previously reviewed that statins protect the heart against ischemia/reperfusion injury in preclinical studies but may interfere with the infarct size limiting effect of preconditioning (see Ferdinandy et al., 2007). However, very little is known on the possible interactions of statins with cardioprotection by conditioning strategies. Kocsis et al. (2008) showed that preconditioning failed to decrease infarct size in rat hearts treated acutely with lovastatin, and that postconditioning failed to decrease infarct size after chronic lovastatin treatment. In this study, myocardial levels of coenzyme Q9 and phosphorylation of Akt were decreased due to lovastatin-treatment. Moreover, acute, but not chronic, lovastatin treatment increased the phosphorylation of p42 ERK. Szucs et al. (2013) have recently shown that activation of the mevalonate pathway with farnesol is cardioprotective; thus, one may speculate that inhibition of this pathway by statins may interfere with cardioprotective mechanisms. Accordingly, chronic atorvastatin treatment in diabetic rats with postconditining failed to show any cardioprotection (Fan et al., 2012). Upregulation by statins of PTEN, which then dephosphorylates PI3K, might contribute to the lack of endogenous cardioprotection following long-term statin-treatment

(Schulz, 2005). In rabbits, short-term simvastatin treatment and postconditioning were unable to reduce infarct size, but pravastatin decreased infarct size, possibly via eNOS activation (Andreadou et al., 2012). Chronic simvastatin treatment, in contrast to postconditioning, was able to reduce infarct size in both normocholesterolemic and hypercholesterolemic rabbits (Iliodromitis et al., 2010). The effect of statins on remote conditioning has not yet been reported.

In summary, although statins show a potential cardioprotective effect, some statins, depending on the duration of treatment, may interfere with the mechanisms of cardiac adaptation to ischemia/reperfusion stress. This may represent an uncharacterized deleterious effect of statins. Further preclinical and clinical studies are needed to explore the effect of statins on cardioprotection by conditioning strategies. Nevertheless, the development of novel lipid lowering agents that do not interfere with innate mechanisms of cardiac stress adaptation may further improve the efficacy of lipid lowering therapy in the prevention of cardiovascular events in hypercholesterolemic patients.

V.C. Anti-diabetic therapy

The treatments used to lower blood glucose levels in diabetic patients may impact on the cardioprotective efficacy of 'conditioning' strategies by modulating the underlying intracellular signaling pathways within the heart in two major ways. The anti-diabetic therapy may either interfere with the cardioprotective signalling pathway, thereby blocking the 'conditioning' strategy; or it may mimic the 'conditioning' strategy, thereby inducing cardioprotection

(reviewed in (Ferdinandy et al., 2007; Ye et al., 2011)). Through these effects, there is the potential for anti-diabetic therapies to impact on long-term cardiac outcomes in diabetic patients.

In 2008, the US Food and Drug Administration issued requirements that novel anti-diabetic drugs must be shown not to increase the risk of cardiovascular events. It is well-established that anti-diabetic therapy can prevent microvascular complications of diabetes, but whether it can also reduce macrovascular complications and improve major cardiovascular outcomes such as myocardial infarction, stroke, heart failure, and fatal arrhythmias is unresolved.

V.C.1. Anti-diabetic therapy and K_{ATP} channels

The activation of either the sarcolemmal or mitochondrial K_{ATP} channel within the cardiomyocyte is a critical step in the signaling pathway underlying both preconditioning and postconditioning (Ferdinandy et al., 2007; Hausenloy, 2013). It is well established in the preclinical literature that some long-acting anti-diabetic sulfonylureas (such as tolbutamide and glibenclamide), which act by blocking K_{ATP} channel activity in the pancreas to promote insulin release, also interfere with the signaling pathway underlying preconditioning and postconditioning by antagonizing K_{ATP} channel activity within the cardiomyocyte (reviewed in (Ye et al., 2011)). However, some of the newer shorter acting anti-diabetic sulfonylureas such as gliclazide and glimepiride do not appear to interfere with cardioprotection (Wu et al., 2007). A recent meta-analysis has confirmed that sulphonylurea use may elevate the risk of cardiovascular disease among patients with diabetes (Phung et al., 2013), although one long-term clinical outcome study failed to report any increase in cardiovascular events in diabetic patients treated with glibenclamide when compared to gliclazide (Juurlink et al., 2012).

V.C.2. Anti-diabetic therapy with mixed actions on cardioprotection

A number of anti-diabetic agents which are used to treat hyperglycemia in diabetic patients are known to confer cardioprotection; many of these appear to act through the activation of the signaling pathways underlying 'conditioning' interventions.

Experimental studies have previously established that the anti-diabetic agents insulin and metformin can confer cardioprotection in animal MI models (Whittington et al., 2013a; Bhamra et al., 2008). Some of the newer anti-diabetic agents have also been reported to limit infarct size in the diabetic and non-diabetic heart, including: (1) thiazolidinediones such as rosiglitazone (Morrison et al., 2011; Palee et al., 2013), pioglitazone (Ye et al., 2010a; Ye et al., 2008); (2) GLP-1 analogues such as liraglutide (Noyan-Ashraf et al., 2009), exenatide (Timmers et al., 2009); and (3) dipeptidyl peptidase-4 (DPP-4) inhibitors such as sitagliptin (Ye et al., 2010a; Hausenloy et al., 2013b; Sauve et al., 2010), and vildagliptin (Hausenloy et al., 2013b). The novel anti-diabetic agent, mitiglinide (Ogawa et al., 2007), has been found to affect the efficacy of preconditioning or postconditioning in the diabetic heart. However, not all studies have reported cardioprotective effects with these anti-diabetic agents (Kristensen et al., 2009; Yin et al., 2011).

A number of clinical studies have either investigated whether anti-diabetic agents abolish cardioprotection or have investigated the anti-diabetic agent as a therapeutic intervention for limiting MI size. Large long-term clinical outcome studies have examined the effect of the anti-diabetic therapy on major cardiovascular events. Hueb et al. (2007) have demonstrated in

diabetic patients that treatment with repaglinide (a K_{ATP} channel antagonist) blocked the ischemic preconditioning effect against exercise-induced myocardial ischemia. Using a similar clinical model, Rahmi et al. (2013) confirmed the negative effect of repaglinide on preconditioning but also found that vildagliptin did not block preconditioning´s protection.

Administration of the GLP-1 analogue exenatide at the time of myocardial reperfusion has been reported to reduce infarct size in STEMI patients treated by primary PCI in two proof-of-concept clinical studies (Lonborg et al., 2012; Woo et al., 2013). The recent IMMEDIATE trial suggests that the beneficial effect of glucose-insulin-potassium therapy in STEMI patients may indeed be operative if administered in the ambulance en route to the primary PCI center while ischemia is ongoing (Selker et al., 2012).

A number of large multicenter long-term clinical outcome studies have reported mixed results in terms of their impact on major ischemia-related cardiovascular outcomes with either better (metformin (Roussel et al., 2010)), worse (rosiglitazone (Nissen & Wolski, 2007)) or no effect (alogliptin (White et al., 2013) and saxagliptin (Scirica et al., 2013)).

In summary, it is important to appreciate that many anti-diabetic therapies can either interfere with or mimic the cardioprotective intervention under investigation. It is important to take this into account when designing clinical studies of cardioprotection in diabetic patients.

V.D. Beta-adrenoceptor antagonists

The effect of ischemic preconditioning was abolished in isolated rat hearts following long-term oral treatment with propranolol or nipradilol (Suematsu et al., 2004). In contrast, landilol treatment during ischemic preconditioning in isolated rat hearts (Yu et al., 2010) or intravenous atenolol or esmolol, given before ischemia or at reperfusion, did not interfere with protection afforded by ischemic preconditioning in rabbit hearts *in vivo* (Iliodromitis et al., 2004). However, esmolol abolished the infarct size reduction achieved by flurane-induced anesthetic preconditioning (Lange et al., 2006). In isolated rat cardiomyocytes, hypoxia or anesthesia-induced 24 hour delayed preconditioning was abolished by metoprolol (Goetzenich et al., 2011). Similarily, anesthesia-induced postconditioning was mediated in part by β-adrenergic signaling as infarct size reduction was again blocked by esmolol (Lange et al., 2009). While β-blockers might thus interfere with the protection afforded by preconditioning and postconditioning in healthy hearts in vivo, carvedilol restored the protection by ischemic preconditioning (Watanabe et al., 2006) or postconditioning (Oikawa et al., 2008) that was otherwise lost in hearts with a chronic coronary artery stenosis.

Most recently, a meta-analysis of fifteen trials with a total of 1155 patients confirmed that remote ischemic preconditioning significantly reduced postoperative biomarkers of myocardial injury, and this effect was most significant in valve surgery and to a lesser extent in coronary artery bypass graft surgery. One major source of heterogeneity in the protective effect was the use of β-blockers, suggesting that these drugs might attenuate the cardioprotective effect of remote ischemic preconditioning in patients (Zhou et al., 2013).

Currently, there is no clear answer yet as to whether β-blockers impact on endogenous cardioprotective signaling. However, the data provided so far highlight the need for further studies and more detailed analyses, given the wide-spread use of β-blocker therapy.

V.E. ACE-inhibitors/AT1-receptor antagonists

ACE inhibitors and AT1-receptor antagonists when administered prior to ischemia and/or reperfusion reduce irreversible myocardial injury (Jalowy et al., 1998; Jancso et al., 2004; Messadi et al., 2010). However, the beneficial effects of ACE inhibitors and/or AT1-receptor antagonists become attenuated when COX2 inhibitors are simultaneously applied (Jalowy et al., 1998). Both ACE inhibitors and AT1-receptor antagonists lower the threshold to achieve endogenous cardioprotection, especially in hearts with co-morbidities (Jaberansari et al., 2001; Ebrahim et al., 2007; Penna et al., 2010).

V.F. Calcium channel blockers

L-type calcium channel blockers (CCBs) are widely used as anti-anginal and antihypertensive drugs. Experimentally, they reduce infarct size in almost all animal models of MI, with no apparent chemical class differences between different CCBs (i.e. 1,4-dihydropyridines, verapamil or diltiazem). However, protection is consistently higher when treatment is started before or during early ischemia with little benefit seen when the agents are given at reperfusion (Kleinbongard et al., 2012). Contrary to the promising experimental data, clinical trials in acute MI have been disappointing (Opie et al., 2000), probably because the agents need to be given prior to the onset of ischemia, or during the early ischemic phase. While studies on post- or

remote conditioning and CCBs are lacking, a single study in pigs demonstrated no interference of CCBs with cardioprotection by ischemic preconditioning (Wallbridge et al., 1996).

V.G. Cyclooxygenase inhibitors

Cyclooxygenase (COX)-2 is involved in the protective signaling of preconditioning and postconditioning (Alcindor et al. 2004; Penna et al., 2008; Sato et al., 2007; Tosaka et al., 2011). Blockade of COX-2 can abolish the cardioprotective phenomena, most importantly delayed or second window preconditioning (Przyklenk and Heusch, 2003; Huhn et al., 2009a; Guo et al., 2012). High dose aspirin – which blocks both COX-1 and COX-2 – interferes with endogenous cardioprotection (Ye et al., 2010b). Nevertheless, many patients with an acute MI receive high dose aspirin (>300 mg) orally or intravenously. Whether or not patient outcome would be improved with lower doses of aspirin remains to be elucidated, although recently COX-2 inhibition was found to increase mortality in patients post-MI (Olsen et al., 2012). Again, whether such increase in mortality relates to the attenuation of endogenous cardioprotective phenomena, or is a consequence of reduced endothelial prostacyclin production on susceptibility to thrombosis, remains unanswered.

VI. Conclusions and future perspectives

The discovery of the remarkable cardioprotective effect of innate adaptive responses elicited by different conditioning strategies has fuelled intensive research in the last three decades to find key cellular mechanisms and drug targets for pharmacological cardioprotection as well as clinically applicable protocols for mechanical cardioprotection. Given the global scale of ischemic heart disease, surprisingly little effort has been made to uncover the cellular mechanisms by which major cardiovascular risk factors (see chapter IV.) and their medications (see chapter V.) may interfere with cardioprotective mechanisms.

Most of the clinical trials with potential cardioprotective drugs have been unsuccessful so far. One of the reasons might be that target validation and preclinical efficacy and safety studies have been performed in juvenile, healthy animals subjected to ischemia/reperfusion injury. Here we have indicated data suggesting that validation of drug targets in co-morbid animal models would be essential for successful clinical translation (see chapter IV.C.2). Furthermore, we have highlighted that routine medications for cardiovascular and other diseases may exert undesirable effects on the ischemic heart and cardioprotective signaling mechanisms, thereby possessing an as yet largely unexplored “hidden cardiotoxicity” (see chapter V.).

Proof-of-principle studies using mechanical conditioning strategies in patients undergoing elective, emergency or surgical coronary revascularization have successfully translated cardioprotection from animal experiments to human studies, although there are several negative

studies (see chapter III). The reasons for the negative studies can be attributed to the presence of risk factors, comorbidities and their medications in different patient cohorts. So far, such clinical studies have been completed in small cohorts of selected patients under tightly controlled conditions, usually with surrogate rather than clinical outcome endpoints. However, ongoing clinical outcome studies (e.g. CIRCUS, ERICCA, RIPHeart) will further determine whether ischemic conditioning can improve clinical outcomes in different patient cohorts.

The body of evidence we have reviewed here underscores the critical importance of preclinical models and study designs that address cardioprotection specifically in relation to complicating disease states and risk factors. This more sophisticated approach is now an urgent necessity in experimental cardioprotection research in order to maximize the likelihood of identifying translatable effective approaches to therapeutic protection of the aged or diseased ischemic heart.

VII. Acknowledgement

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VIII. Authorship contributions

Participated in design of the paper: Péter Ferdinandy, Rainer Schulz

Wrote or contributed to the writing of the manuscript: Péter Ferdinandy, Derek Hausenloy, Gerd Heusch, Gary Baxter, Rainer Schulz

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Legends for figures

Figure 1.

The concept of ischemia/reperfusion injury and cardioprotection by pre-, post and remote conditioning is expressed graphically in the figure, where shaded areas denote periods of ischemia. Myocardial ischemia and reperfusion leads to 'ischemia/reperfusion injury' characterized by the development of contractile dysfunction, arrhythmias, and tissue necrosis (infarction). Ischemic preconditioning is a well-described acute and subacute adaptive response in which brief exposure to ischemia/reperfusion markedly enhances the ability of the heart to withstand a subsequent ischemia/reperfusion injury. In this diagram, three brief periods of ischemia are used to precondition the myocardium against a subsequent period of 'test' ischemia that is longer than the preconditioning periods. Preconditioning induces protection in a biphasic pattern. Brief cycles of ischemia/reperfusion applied following a longer period of ischemia also confer cardioprotection against the consequences of myocardial ischemia/reperfusion, a phenomenon called ischemic postconditioning. Brief cycles of ischemia/reperfusion applied in a remote cardiac tissue or remote organ, e.g. kidney, limbs, before, during or right after a longer period of cardiac ischemia also provides cardioprotection, a phenomenon called remote conditioning. The cardioprotective effect of conditioning strategies results in attenuation of ischemia/reperfusion injury characterized by improvement of postischemic contractile function, decrease in the occurrence and severity of arrhythmias, and reduction of infarct size. Major cardiovascular risk factors and their medications influence the severity of ischemia/reperfusion injury and interferes with the cardioprotective effect of conditioning.

Figure 2.

Mitochondrial reactive oxygen species (ROS, including O_2^-, H_2O_2) are at the center of cardioprotection and/or irreversible injury depending on the timing and quantity of their generation. Several mitochondrial proteins contribute to the generation of ROS through modulation of proteins of the respiratory chain (connexin 43 [Cx43], STAT3, p66shc) or directly (monoamine oxidases, MAO). High amounts of ROS at the time of reperfusion contribute to irreversible tissue injury, probably by facilitating opening of the mitochondrial permeability transition pore (MPTP). The proteins contributing to the formation of MPTP are still under investigation, but dimerization of complex V or protein complexes involving adenine-nucleotide transporter (ANT), hexokinase (HK) and the phosphate carrier (PiC) have been proposed. Many factors apart from ROS are important for MPTP opening including binding of cyclophilin D (CypD), calcium (Ca^{2+}), and ADP. Mitochondrial Ca^{2+} concentration and homeostasis is influenced by the close interaction with the SR/ER and specialized proteins for such interaction like mitofusins (Mfn). Under pathophysiological conditions, ADP can be generated by the reversed mode of complex V using ATP as substrate to maintain the inner mitochondrial membrane proton gradient. ATP will pass the outer mitochondrial membrane through the voltage-gated anion channel (VDAC) and proteins such as Bcl2 or Bax, affecting channel open probability. Some other proteins modifying MPTP opening have been described such as glycogen synthase kinase 3β (GSK-3β), aldehyde dehydrogenase 2 (ALDH2) and protein kinase C-ε (PKCε). While high concentrations of ROS are detrimental, low amounts of ROS can trigger a cardioprotective state and are central to the endogenous protection by pre- and postconditioning. In this context, increases in mitochondrial potassium (K^+) leads to increased

ROS formation and is central to endogenous cardioprotection. Here, mitochondrial ATP-sensitive potassium channels (K_{ATP}) but also Cx43 play important roles. Nitrosylation of thiol groups (SNO) are also important for protein activity, and nitric oxide can be derived either by a mitochondrial nitric oxide synthase (mtNOS) or by a NOS isoform in close proximity to mitochondria, transported by caveolae-like structures into the mitochondria.

Figure 3. Forest plot on the available clinical studies (state December 2013) on ischemic preconditioning. Grey bars indicate the standard error of the mean in the placebo group, black bars the % infarct size reduction with its standard error in the conditioned group (updated from Heusch, 2013).

Figure 4. Forest plot on the available clinical studies (state December 2013) on ischemic postconditioning. Grey bars indicate the standard error of the mean in the placebo group, black bars the % infarct size reduction with its standard error in the conditioned group (updated from Heusch, 2013).

Figure 5. Forest plot on the available clinical studies (state December 2013) on remote ischemic conditioning. Grey bars indicate the standard error of the mean in the placebo group, black bars the % infarct size reduction with its standard error in the conditioned group (updated from Heusch, 2013).

Clinical study	Pharmacological postconditioning agent	number of patients	Effect	Mechanism of cardioprotection
	Atrial natriuretic peptide			
J-WIND-ANP (Kitakaze *et al.*, 2007)	IV carperitide 72 hr infusion started **after** reperfusion	569	15% reduction in MI size (72 hr AUC total CK) 2.0% absolute increase in LVEF	Atrial natriuretic peptide is a pharmacological activator of a number of pro-survival signalling pathways including the RISK and cGMP-PKG pathways.
	Cyclosporin A			
Piot *et al.* (2008)	IV CsA (2.5mg/kg) 10 min **prior** to primary PCI	58	44% reduction in MI size (72hr AUC total CK) 20% reduction in MI size (CMR in subset of 27 patients) 28% reduction in MI size and smaller LVESV on CMR at 6 months (Mewton *et al.*, 2010)	Cyclosporin-A is a known inhibitor of the mitochondrial permeability transition pore, a critical determinant of cardiomyocyte death.
	Exenatide			
Lonborg *et al.* (2012)	IV infusion of exenatide started 15 min **prior** to primary PCI for 6 hr	107	Increase in myocardial salvage index at 90d by CMR Reduced MI size as % of AAR at 90 days by CMR Patients presenting with short ischemic times (≤132 min) had greater myocardial salvage	Exenatide is a long-acting analogue of glucagon-like peptide-1 (GLP-1) which lowers blood glucose as well as limiting MI size through the activation of the RISK pathway.
	Glucose insulin potassium (GIK) therapy			
IMMEDIATE (Selker *et al.*, 2012)	Intravenous **GIK** infusion for 12 hrs started by paramedics in ambulance- **prior** to reperfusion	357	Reduction in MI size and less in-hospital mortality and cardiac arrest	GIK promotes glucose metabolism during myocardial ischemia which has beneficial effects of cellular function.
	Metoprolol			

METOCARD-CNIC 2013 (Ibanez *et al.*, 2013)	Intravenous metoprolol three-5mg boluses administered in ambulance **prior** to PPCI.	220	20% reduction in MI size (5-7 days by CMR)	Metoprolol reduces myocardial oxygen consumption and may have direct cardioprotective effects on the cardiomyocyte.
	Nitrite (sodium)			
Frennaux *et al*., NIAMI NCT01388504	Intravenous bolus of sodium nitrite given 5 min **prior** to primary PCI.	200	MI size as a % of AAR on CMR Results awaited	Sodium nitrite releases nitric oxide which activates pro-survival kinases and protects against mitochondrial dysfunction.
Mathur *et al.,* NITRITE-AMI NCT01584453	Intracoronary bolus of sodium nitrite over 30-60s at the time of primaryPCI.	80	MI size (48hr Total CK) Results awaited	As above.
	Adenosine			
Garcia-Dorado *et al.,* PROMISE NCT0781404	Intracoronary bolus of 4mg adenosine	201	MI size on CMR; NS	Adenosine has multiple cardioprotective effects

Table 1: Clinical studies of PPCI-treated STEMI patients which have reported beneficial effects with a pharmacological agent administered at early reperfusion. AAR: Area at risk measured; AUC: area under the curve; CK: creatinine kinase; CMR: cardiac MRI; LVESV: left ventricular end-systolic volume; MI: myocardial infarct; NS non significant

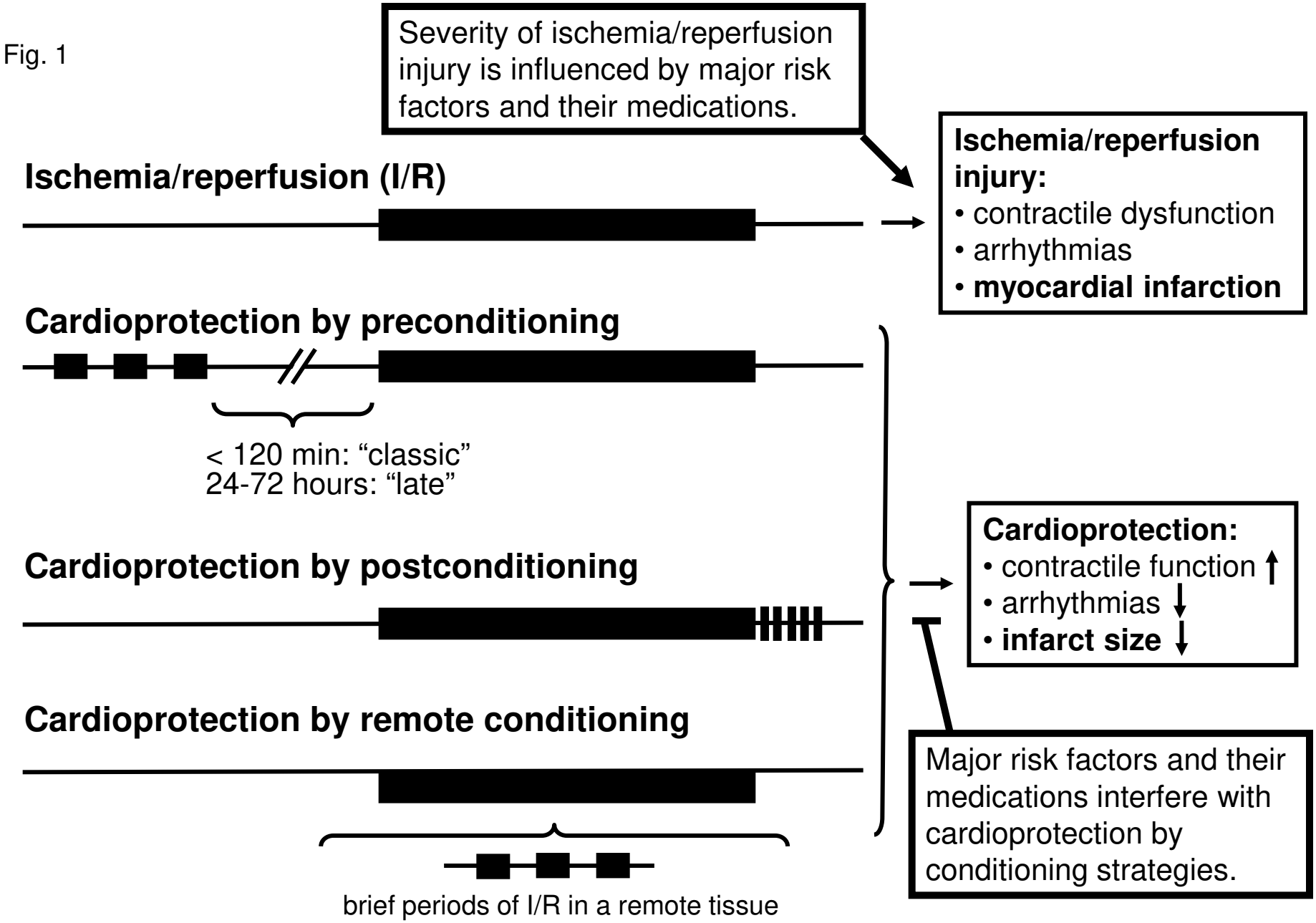
Risk factor	I/R injury	preconditioning	postconditioning	remote conditioning
Ageing	↑	↓	↓	− CL
Hypertension, hypertrophy & remodelling	−	−	↓	ND
Hyperlipidemia	↑	↓ CL	↓	ND
Diabetes	↑	↓ CL	↓	↓CL
Uremia, kidney failure	↑	−	−	ND
Impaired coronary microcirculation	↑	− ↑	↓	ND

Table 2. Effect of major risk factors on ischemia/reperfusion (I/R) injury as well as pre-, post-, and remote conditioning in the majority of the studies. CL: some clinical data are also available. ND: no data available. ↑ enhance, ↓ attenuate, − no effect

Medication	I/R injury	preconditioning	postconditioning	remote conditioning
Nitrate tolerance	↑	↓	↓	ND
Statins	↓	↓	↓	ND
Antidiabetics:				
KATP inhibitors	↑	↓	↓	ND
others	↓	–	–	ND
Beta blockers	↓	↓–	–	ND
ACE inhibitors	↓	↑	ND	ND
AT_1 antagonists	↓	↑	ND	ND
Calcium channel blockers	↓–	–	ND	ND
COX inhibitors	↑	ND	ND	ND

Table 3. Effect of most common medications of cardiovascular risk factors on ischemia/reperfusion (I/R) injury as well as pre-, post-, and remote conditioning in the majority of the studies. It should be noted that very few data are availabe so far on the effect of medications on conditioning strategies.
ND: no data available. ↑ enhance, ↓ attenuate, – no effect,

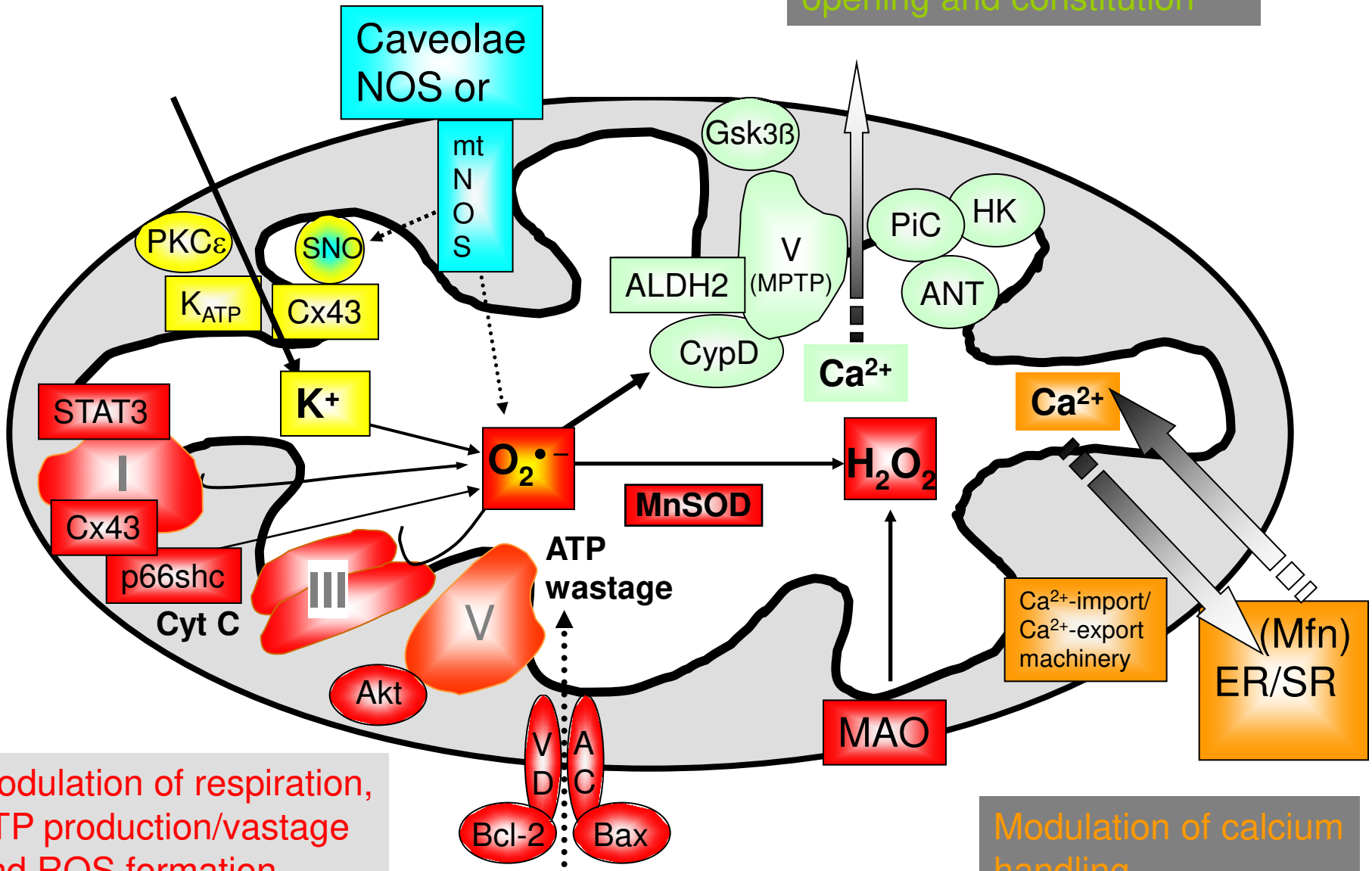
Fig. 1


Severity of ischemia/reperfusion injury is influenced by major risk factors and their medications.
Ischemia/reperfusion injury:
• contractile dysfunction
• arrhythmias
• myocardial infarction
Ischemia/reperfusion (I/R)
Cardioprotection by preconditioning
< 120 min: "classic"
24-72 hours: "late"
Cardioprotection by postconditioning
Cardioprotection by remote conditioning
brief periods of I/R in a remote tissue
Cardioprotection:
• contractile function ↑
• arrhythmias ↓
• infarct size ↓
Major risk factors and their medications interfere with cardioprotection by conditioning strategies.

Modulation of matrix potassium flux

Modulation of S-nitrosation of numerous proteins

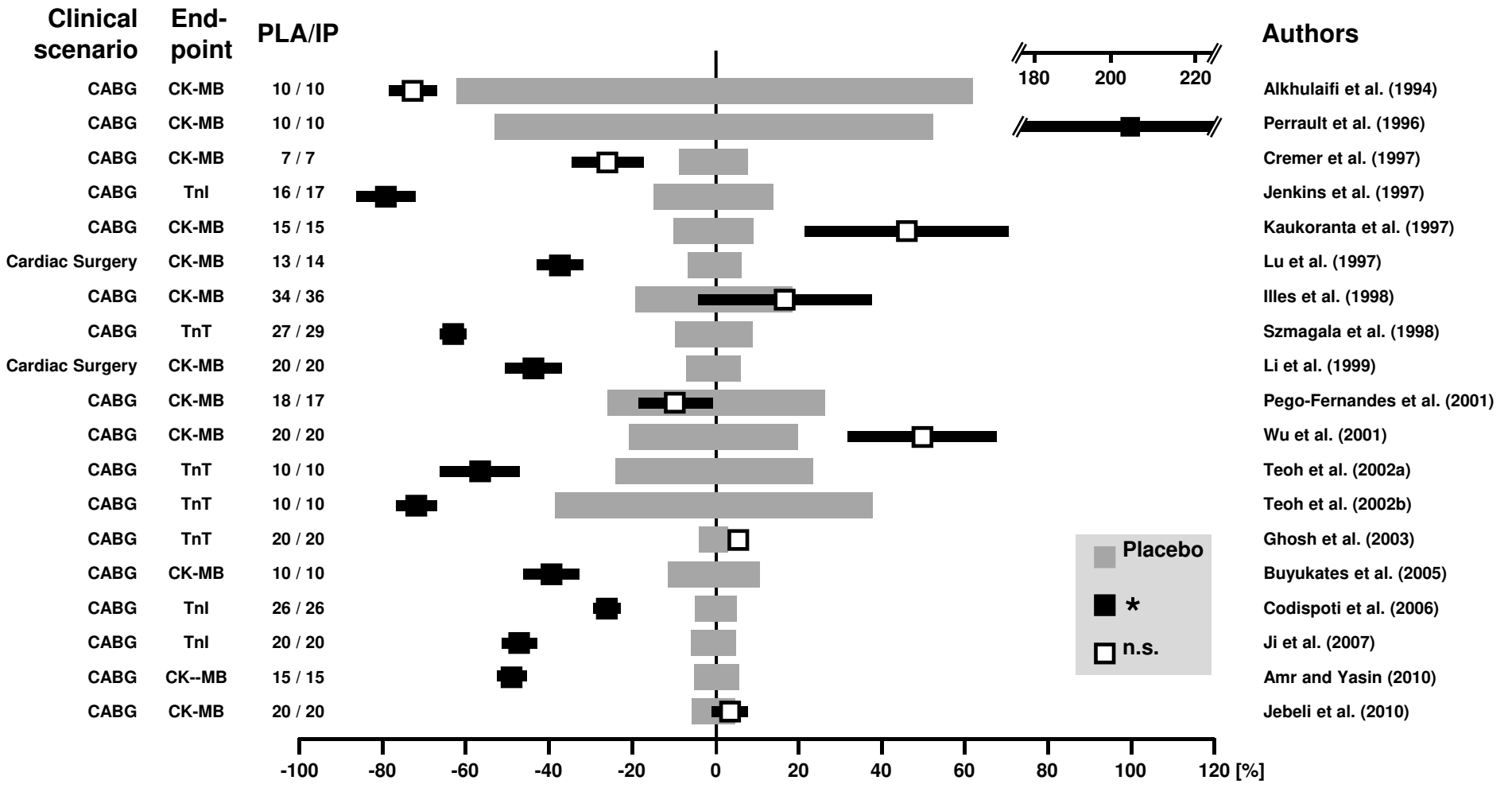
Modulation of mitochondrial permeability transition pore opening and constitution



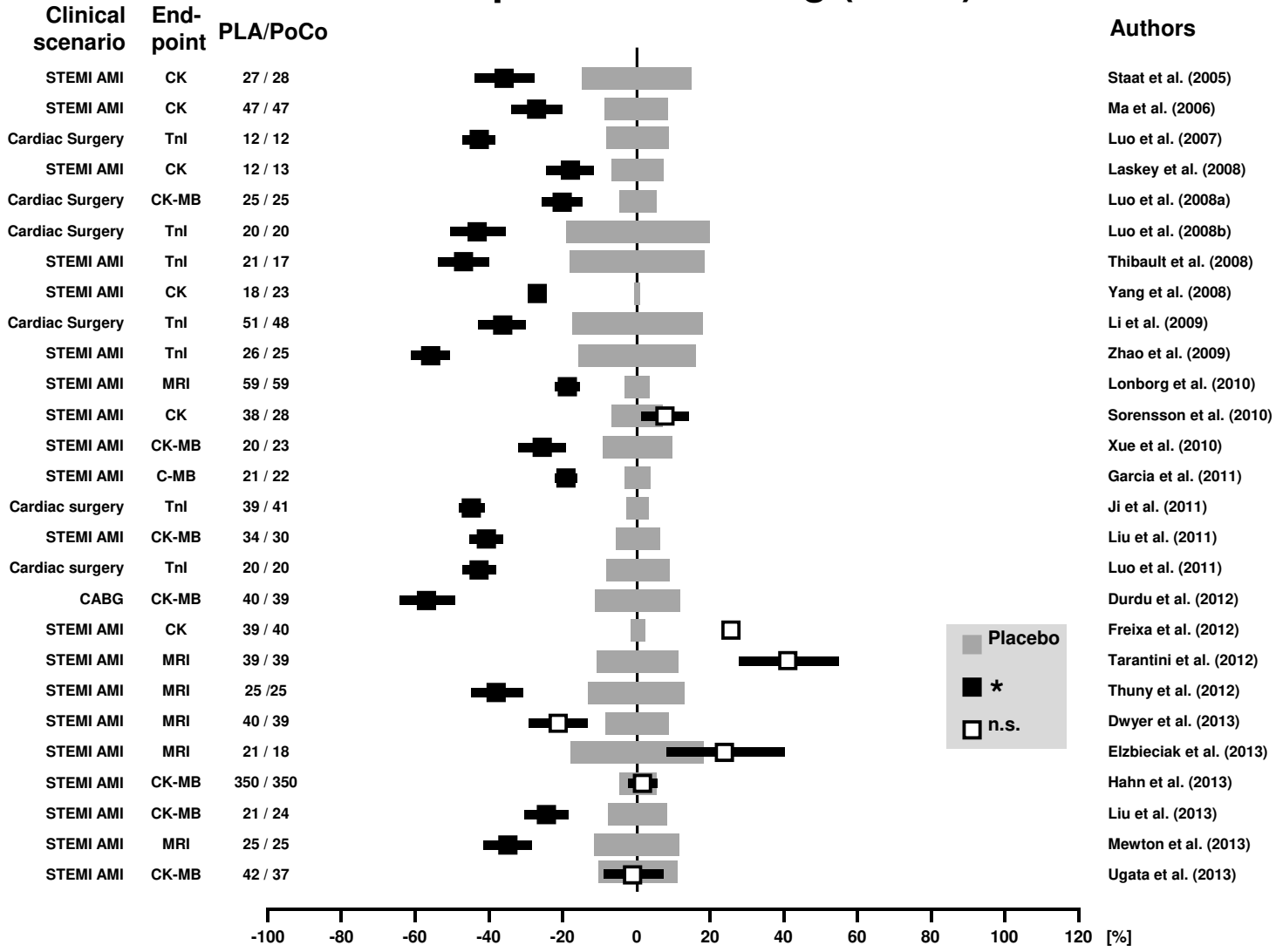
Modulation of respiration, ATP production/vastage and ROS formation

Modulation of calcium handling

Ischemic preconditioning (IP)



Ischemic postconditioning (PoCo)



Remote ischemic conditioning (RIC)