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Interaction of Cardiovascular Risk Factors with Myocardial Ischemia/Reperfusion Injury, Preconditioning, and Postconditioning

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***Abstract*——Therapeutic strategies to protect the ischemic myocardium have been studied extensively. Reperfusion is the definitive treatment for acute coronary syndromes, especially acute myocardial infarction; however, reperfusion has the potential to exacerbate lethal tissue injury, a process termed "reperfusion injury." Ischemia/reperfusion injury may lead to myocardial infarction, cardiac arrhythmias, and contractile dysfunction. Ischemic preconditioning of myocardium is a well described adaptive response in which brief exposure to ischemia/reperfusion before sustained ischemia markedly enhances the ability of the heart to withstand a subsequent ischemic insult. Additionally, the application of brief repetitive episodes of ischemia/reperfusion at the immediate onset of reperfusion, which has been termed "postconditioning," reduces the extent of reperfusion injury. Ischemic pre- and postconditioning share some but not all parts of the proposed signal transduction cascade, including the activation of survival protein kinase pathways. Most experimental studies on cardioprotection have been undertaken in animal models, in which ischemia/reperfusion is imposed in the absence of other disease processes. However, ischemic heart disease in humans is a complex disorder caused by or associated with known cardiovascular risk factors including hypertension, hyperlipidemia, diabetes, insulin resistance, atherosclerosis, and heart failure; additionally, aging is an important modifying condition. In these diseases and aging, the pathological processes are associated with fundamental molecular alterations that can potentially affect the development of ischemia/reperfusion injury per se and responses to cardioprotective interventions. Among many other possible mechanisms, for example, in hyperlipidemia and diabetes, the pathological increase in reactive oxygen and nitrogen species and the use of the ATP-sensitive potassium channel inhibitor insulin secretagogue antidiabetic drugs and, in aging, the reduced expression of connexin-43 and signal transducer and activator of transcription 3 may disrupt major cytoprotective signaling pathways thereby significantly interfering with the cardioprotective effect of pre- and postconditioning. The aim of this review is to show the potential for developing cardioprotective drugs on the basis of endogenous cardioprotection by pre- and postconditioning (i.e., drug applied as trigger or to activate signaling pathways associated with endogenous cardioprotection) and to review the evidence that comorbidities and aging accompanying coronary disease modify responses to ischemia/reperfusion and the cardioprotection conferred by preconditioning and postconditioning. We emphasize the critical need for more detailed and mechanistic preclinical studies that examine car-**

dioprotection specifically in relation to complicating disease states. These are now essential to maximize the likelihood of successful development of rational approaches to therapeutic protection for the majority of patients with ischemic heart disease who are aged and/or have modifying comorbid conditions.

I. Introduction

A. Ischemic Heart Disease and Cardioprotection

Ischemic heart disease is the leading cause of death in the industrialized world. The treatment of acute ischemic heart disease has entered a new era in which mortality can be approximately halved by procedures that allow the rapid return of blood flow to the ischemic zone of the myocardium, i.e., reperfusion therapy. Reperfusion, however, may lead to further complications such as diminished cardiac contractile function (stunning) and arrhythmia. Moreover, there is experimental evidence that irreversible cell injury leading to necrosis and apoptosis may be precipitated by reperfusion. Therefore, development of cardioprotective agents to improve myocardial function, decrease the incidence of arrhythmias, delay the onset of necrosis, and limit the total extent of infarction during ischemia/reperfusion is of great clinical importance. Earlier pharmacological approaches to attenuate the consequences of ischemia/reperfusion injury have been of limited experimental efficacy or have failed to translate into useful clinical treatments. However, more recently 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 is a well described adaptive response in which brief exposure to ischemia/reperfusion markedly enhances the ability of the heart to withstand a subsequent ischemic injury. Moreover, brief cycles of ischemia/reperfusion applied after a longer period of ischemia also confer cardioprotection against the consequences of myocardial ischemia/reperfusion, a phenomenon called ischemic postconditioning (Fig. 1). The discovery of these two major forms of 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 injury and survival during ischemia/reperfusion.

Although ischemic heart disease in humans is a complex disorder caused by or associated with other systemic diseases and conditions, most experimental studies on cardioprotection have been undertaken in juvenile animal models, in which ischemia/reperfusion is imposed in the absence of other disease processes and risk factors for cardiovascular diseases. The aim of this review is not to summarize existing cardioprotective therapies, but to show the potential for development of cardioprotective drugs based on endogenous cardioprotection by pre- and postconditioning (i.e., drug applied as trigger or to activate signaling pathways associated with endogenous cardioprotection) and to emphasize that risk factors might largely affect endogenous cardioprotective pathways.

B. Risk Factors for Ischemic Heart Disease

Ischemic heart disease develops as a consequence of a number of etiological risk factors and always coexists with other disease states. These include systemic arterial hypertension and related left ventricular hypertrophy, hyperlipidemia, and atherosclerosis, diabetes and insulin resistance, heart failure, as well as aging. These systemic diseases with aging as a modifying condition, exert multiple biochemical effects on the heart that can potentially affect the development of ischemia/reperfusion injury per se and interfere with responses to cardioprotective interventions (Fig. 1). Therefore, the development of rational therapeutic approaches to protect the ischemic heart requires preclinical studies that examine cardioprotection specifically in relation to complicating disease states and risk factors.

II. Introduction to Endpoints of Ischemia/Reperfusion Injury and Experimental Approaches to Cardioprotection

Myocardial ischemia develops when coronary blood supply to myocardium is reduced, either in terms of absolute flow rate (low-flow or no-flow ischemia) or relative to increased tissue demand (demand ischemia). A pivotal feature of ischemia is that oxygen supply to the mitochondria is inadequate to support oxidative phosphorylation (Opie, 1990; Hearse, 1996; Ganz and Braunwald, 1997). In experimental models and in clinical situations, ischemia may be followed by reperfusion, that is, the re-admission of oxygen and metabolic substrates with washout of ischemic metabolites. The process of reperfusion is associated with further biochemical, structural, and functional changes in myocardium and may determine cell survival and cell death. It is common for commentators to consider ischemia/reperfusion injury as a composite entity with distinct components of injury associated specifically with ischemia and with reperfusion since it is a truism that reperfusion can never occur independently of ischemia.

A. Clinical and Experimental Endpoints of Injury

Irreversible cell injury leading to infarction, the development of arrhythmias, and the loss of myocardial contractility are all relevant as clinical consequences of occlusive coronary disease and they are also important as experimental correlates and endpoints. The following

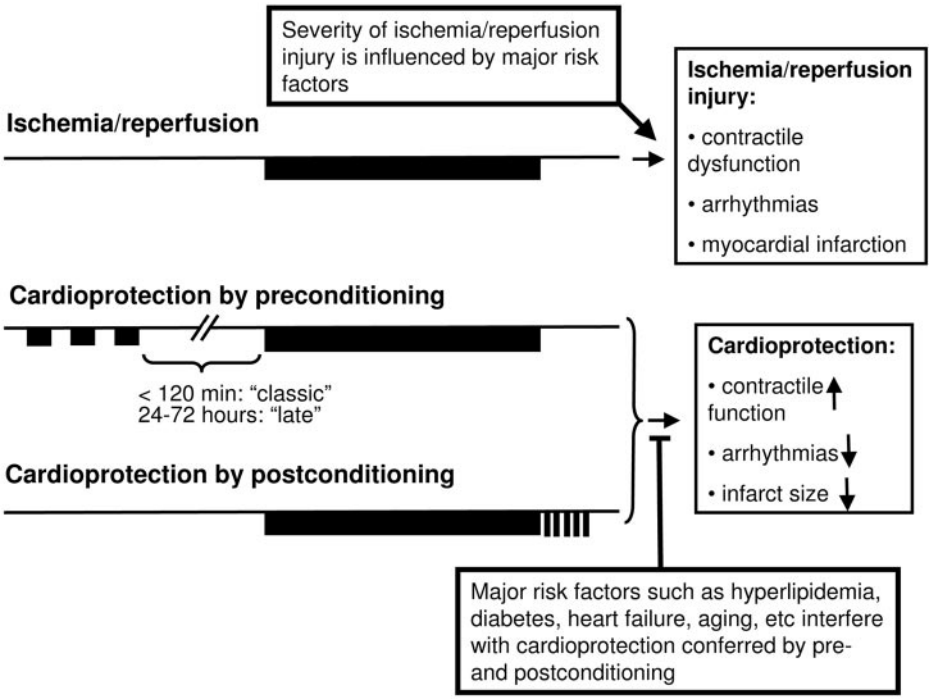

FIG. 1. The concept of ischemia/reperfusion injury and cardioprotection by pre- and postconditioning is expressed graphically in the figure, where shaded areas denote periods of ischemia. Myocardial ischemia and reperfusion leads to "ischemia/reperfusion injury" characterized by the development of contractile dysfunction, arrhythmias, and tissue necrosis (infarction) (for details see section III.). 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. The classic preconditioning protective response is seen within a few minutes after preconditioning ischemia. If the period between preconditioning ischemia and test ischemia is extended beyond 120 min, no protection is observed. If the heart is preconditioned with brief periods of ischemia and then left for 24 to 72 h before being subjected to test ischemia, protection is again observed, hence the term "late preconditioning." Brief cycles of ischemia/reperfusion applied after a longer period of ischemia also confer cardioprotection against the consequences of myocardial ischemia/reperfusion, a phenomenon called ischemic postconditioning (for details of experimental induction of preconditioning and assessment of cardioprotection, see section III.). The cardioprotective effect of pre- and postconditioning results in attenuation of ischemia/reperfusion injury characterized by improvement of postischemic contractile function, a decrease in the occurrence and severity of arrhythmias, and a reduction in infarct size. Major risk factors for ischemic heart disease such as systemic arterial hypertension, hyperlipidemia and atherosclerosis, diabetes and insulin resistance, heart failure, and aging influence the severity of ischemia/reperfusion injury and interfere with the cardioprotective effect of pre- and postconditioning.

is a synoptic description of the major endpoints, providing an introductory context for the experimental studies that we review subsequently. For the most part in this review, we refer to studies using markers of irreversible cell injury and tissue infarction.

1. Irreversible Cellular Injury and Infarction. Ultrastructural changes occur in myocardium rapidly after the onset of ischemia. These may be considered reversible alterations if reperfusion of the tissue can be effected promptly. However, ischemia lasting more than 20 to 30 min (without collateral flow or residual flow through the infarct-related artery) results in a transition from a state of reversible ultrastructural alterations to a state of irreversible tissue injury that is ultimately characterized as coagulative necrosis (Herdson et al., 1965; Reimer and Jennings, 1979; Jennings et al., 1981). A number of factors that influence the onset and extent of irreversible injury in experimental models have been identified. These include the size of the area at risk (Reimer and Jennings, 1979; Ytrehus et al., 1994), the extent of collateral blood flow or residual flow through the infarct-related artery (Reimer and Jennings, 1979), the duration of ischemia (Reimer and Jennings, 1979; Ytrehus et al., 1994), and myocardial temperature (Miki et al., 1998). There is also some evidence that systemic hemodynamic influences during ischemia, including heart rate, may contribute to the rate of development of irreversible injury (Schulz et al., 1995b).

In the absence of reperfusion, no intervention is able to limit infarct development, and it is clear that reperfusion is the sine qua non for tissue salvage. Reperfusion and revascularization therapies in acute myocardial infarction (fibrinolysis, percutaneous coronary intervention, and emergency coronary artery bypass grafting) have the primary aim of salvaging viable tissue, which may be reversibly injured, within the ischemic risk zone and thereby limiting the extent of necrosis. In clinical myocardial infarction, both early and late mortality are closely related to the duration of unrelieved coronary occlusion. This philosophy of prompt reperfusion/revascularization in acute myocardial infarction is succinctly defined in the axiom: "time is muscle and muscle is life" (Simoons et al., 1997).

Some cells subjected to ischemia/reperfusion display hallmarks of apoptosis. However, there is controversy as

to the extent of apoptosis in ischemia/reperfusion injury, when apoptosis occurs, and the relationships between apoptosis and necrosis (Kajstura et al., 1996; Misao et al., 1996; Olivetti et al., 1996; Saraste et al., 1997; Baliga, 2001; Bishopric et al., 2001). Early studies of apoptosis in experimental infarction using permanent coronary artery occlusion in the rat (i.e., ischemia without reperfusion) suggested that apoptosis represented the major form of myocyte death (Kajstura et al., 1996). Subsequently, the majority of evidence suggests that the number of cells undergoing apoptosis is likely to be relatively few compared with the number of necrotic cells. However, it is not clear at what stage apoptosis occurs in relation to necrosis or how these two processes are related. Although the mechanisms initiating apoptosis are still unknown, it seems likely that opening of the mitochondrial permeability transition pore (mPTP[1]) during reperfusion, after ischemia of sufficient duration, serves as a key mechanism of cell death, amplifying or accelerating cell death to produce a pattern of reperfusion-induced necrosis (section II.B.2.) (Crompton, 1999; Hajnóczky et al., 2000; Bishopric et al., 2001; Di Lisa et al., 2001; Pacher and Hajnóczky, 2001; Pacher et al., 2001; Halestrap et al., 2004).

2. Contractile Dysfunction and Ventricular Arrhythmias. Depression of myocardial contractility is an early consequence of myocardial ischemia (Tennant and Wiggers, 1935) and may result in acute cardiac failure. Impairment of contractility leading to left ventricular dysfunction and chronic cardiac failure may occur over extended periods after the development of infarction. In the absence of necrosis and with full reperfusion, myocardial contractility will recover completely. However, full recovery may take several hours or days in vivo. This condition of delayed, but ultimately complete, recovery of contractile function after reperfusion of viable tissue is called "myocardial stunning" (Braunwald and Kloner, 1982; Kloner and Jennings, 2001). The multifactorial mechanisms underlying myocardial stunning are complex and beyond the scope of the present survey. However, ROS generation and intracellular calcium overload as a direct result of reperfusion are pivotal aspects of this pathology (Kusuoka et al., 1987; Bolli et al., 1989a,b; Opie, 1989). It is relevant to note here that the rate and/or extent of recovery of postischemic function is widely used as an injury index in experimental ischemia/reperfusion studies; in many such cases, contractile recovery is a mixed endpoint, representing both loss of contractility due to irreversible injury and delayed recovery of viable myocardium due to stunning.

In myocardium subjected to reduced perfusion, a further pattern of depression of contractility is termed myocardial "hibernation" (Rahimtoola, 1999; Schulz and Heusch, 2000). The essential features of hibernating myocardium are that metabolism and contractility are reduced in response to a reduction in coronary blood flow but that reperfusion can restore contractile function to normal. However, hibernation is a complex pathology, exhibiting alterations of cellular metabolism, myocardial structure, myocardial perfusion, and subendocardial flow reserve. The interested reader is referred to Canty and Fallavollita (2005) and Heusch (1998) for detailed reviews of the condition.

During ischemia, arrhythmias may develop, ranging in severity from isolated ventricular premature beats, through runs of ventricular tachycardia, to ventricular fibrillation (Tennant and Wiggers, 1935; Curtis et al., 1987; Carmeliet, 1999). Early arrhythmias (phase I arrhythmias) after coronary artery occlusion may contribute to sudden cardiac death following coronary occlusion (Janse and Wit, 1989). In experimental models of coronary occlusion, the incidence and duration of arrhythmias has been used as an injury index although it is important to note that arrhythmias develop before the onset of irreversible tissue injury. Reperfusion of myocardium after relatively brief periods of ischemia may also precipitate a pattern of arrhythmia ranging in severity (Manning and Hearse, 1984; Carmeliet, 1999). Clinically reperfusion-induced arrhythmia may be observed during thrombolysis (Goldberg et al., 1983) and after percutaneous coronary intervention (Holdright et al., 1996).

B. Experimental Approaches to Infarct Size Limitation

1. Historical Background. Experimental research in the field of cardioprotection, which dates from the early 1970s when the concept of therapeutic infarct size limitation was first promoted by Eugene Braunwald and colleagues (Maroko et al., 1971), has resulted in a large and complex body of literature. Although a review of all the pharmacological approaches to infarct size limitation is impossible here, the quest during the 1970s and 1980s for drugs and other agents that could slow the

[1] Abbreviations: mPTP, mitochondrial permeability transition pore; ROS, reactive oxygen species; SOD, superoxide dismutase; PKC, protein kinase C; PI3K, phosphatidylinositol 3-kinase; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; JAK, Janus tyrosine kinase; STAT, signal transducer and activator of transcription; GSK-3β, glycogen synthase-3β; Kir, potassium inward rectifier; SUR, sulfonylurea receptor; K_{ATP}, ATP-sensitive potassium channel; HMR1098, 1-[[5-[2-(5-chloro-*o*-anisamido)ethyl]-2-methoxyphenyl]sulfonyl]-3-methylthiourea (sodium salt); HMR1883, 1-[[5-[2-(5-chloro-*o*-anisamido)ethyl]-2-methoxyphenyl]sulfonyl]-3-methylthiourea; 5-HD, 5-hydroxydecanoic acid; NS-1619, 1,3-dihydro-1-(2-hydroxy-5-(trifluoromethyl)-phenyl)-5-(trifluoromethyl)-2*H*-benzimidazol-2-one; NO, nitric oxide; eNOS, endothelial NO synthase; PKG, cGMP-dependent protein kinase G; RNS, reactive nitrogen species; HSP, heat shock protein; HSP72, 72-kDa heat shock protein; NF-κB, nuclear factor-κB; iNOS, inducible NO synthase; COX-2, cyclooxygenase-2; LVH, left ventricular hypertrophy; LV, left ventricular; SHR, spontaneously hypertensive rat(s); DOCA, deoxycorticosterone acetate; ACE, angiotensin-converting enzyme; T3, triiodothyronine; MI, myocardial infarction; AMP579, 1*S*-[1*a*,2*b*,3*b*,4*a*(*S**)]-4-[7-[[1-[(3-chloro-2-thienyl)methyl-propyl]propyl-amino]-3*H*-imidazo[4,5-*b*] pyridyl-3-yl]-*N*-ethyl-2,3-dihydroxycyclopentane carboxamide; LVEF, left ventricular ejection fraction; STZ, streptozotocin.

development of or prevent myocardial necrosis was largely unsuccessful. It is possible now to sketch the major historical developments and to discern the conceptual and technical obstacles to the successful development of infarct-limiting treatments.

First of all, although experimental models of coronary artery occlusion provided extensive descriptive accounts of the morphological changes associated with the development of necrosis (Herdson et al., 1965; Jennings et al., 1965, 1978; Jennings and Ganote, 1974; Whalen et al., 1974; Schaper, 1979, 1986; Schaper et al., 1992), they provided relatively few insights into the molecular mechanisms underlying cell death. As a consequence, the early conceptual approaches to infarct limitation in the 1970s centered on agents to reduce myocardial oxygen demand or vasodilators to increase oxygen and metabolic substrate delivery (Maroko et al., 1971; Braunwald and Maroko, 1975). Hence, agents such as β-adrenoceptor antagonists (Burmeister et al., 1981; Reynolds et al., 1981; Downey et al., 1982); calcium channel blockers (Reimer and Jennings, 1984; Reimer et al., 1985; Wende et al., 1975); and glyceryl trinitrate (Bleifeld et al., 1973; Malm et al., 1979; Fukuyama et al., 1980) were extensively investigated with no consistently reproducible evidence of infarct limitation.

Second, the fact that reperfusion was essential to halt the progressive wave front of necrosis and salvage ischemic myocardium—a concept so obvious to us now—was largely unrecognized until the late 1970s. In numerous studies, including some of those cited above, drugs were administered in animal models of infarction with permanent coronary occlusion, with the assumption that tissue could be saved from necrosis within a small but important "border zone" between normal and ischemic tissue. Although reperfusion with fibrinolytic agents became rapidly established in the early 1980s as a primary approach in the therapy of acute myocardial infarction, numerous experimental studies in the 1980s continued to use models with permanent coronary artery occlusion. The controversial concept of an infarct border zone was almost certainly incorrect (Hearse, 1983).

Third, the recognition that reperfusion was associated with specific patterns of injury, collectively termed reperfusion injury, identified reperfusion arrhythmias and stunning as clear therapeutic targets. However, the concept of irreversible injury occurring as a result of reperfusion proved very controversial. Several pathological mechanisms associated with reperfusion, including ROS generation, intracellular calcium overload, and the recruitment of inflammatory cells, became the foci of basic studies in which agents were administered as adjuncts to reperfusion. Examples include the application at reperfusion of SOD (Jolly et al., 1984; Uraizee et al., 1987; Przyklenk and Kloner, 1989; Downey et al., 1991), adenosine and adenosine receptor agonists (for a comprehensive account of the early experimental literature, see Baxter et al., 2000), nonsteroidal anti-inflammatory drugs (Romson et al., 1982; Mullane et al., 1984; Allan et al., 1985; Reimer et al., 1985; Crawford et al., 1988), and antineutrophil antisera (for a review, see Baxter, 2002a). The resulting literature for nearly two decades was characterized by no clear sense of experimental reproducibility or consistency of interpretation with regard to pharmacological infarct limitation.

In retrospect, the development during the 1970s and 1980s of experimental infarct size limitation as a plausible scientific concept was hampered by the relatively late recognition that reperfusion is an essential requirement, both clinically and experimentally; and by limited understanding of appropriate molecular targets. The recognition of ischemic preconditioning from the late 1980s onward proved to be the most significant development in the quest to identify rational approaches to infarct size limitation. An explosion of research effort has identified a number of molecular mechanisms pertinent to cell death and cytoprotection that form the basis of contemporary experimental approaches.

2. The Reperfusion Injury Paradigm of Irreversible Injury. There is little question that unrelieved (i.e., permanent) ischemia causes cell death by coagulative necrosis (section II.A.1.). However, controversy has surrounded the destructive role played by reperfusion (Fig. 2, a and b). Until recently the predominating view was that cell death occurred largely during ischemia, fundamentally as a result of ATP depletion and its multiple consequences. In this older paradigm, reperfusion is essential to restore ATP synthesis and thereby to salvage those cells uninjured or reversibly injured by ischemia. Some argued that any cells that died during reperfusion were already irreversibly injured and doomed to die and that the death of cells irreversibly injured by ischemia was merely accelerated by reperfusion.

Although changes in mitochondrial structure and respiratory function in ischemia/reperfusion have been documented for nearly five decades, the last 10 years have brought an understanding of the decisive role played by mitochondria in determining cell fate during and after cellular stresses (for a recent review, see Kroemer et al., 2007). A key component of mitochondrial response to stress is the formation of the mPTP, a nonspecific, multimeric pore structure spanning the mitochondrial inner and outer membranes (Halestrap and Brenner, 2003; Bernardi et al., 2006). The precise molecular composition of the pore is currently unknown and the subject of intense debate. However, a key component is cyclophilin-D, a peptidyl-prolyl *cis-trans* isomerase that binds and is inhibited by cyclosporin A (Crompton et al., 1988). mPTP serves as a high-conductance, nonselective, voltage-dependent channel and is closed under normal physiological conditions when the inner membrane is impermeable to most solutes. Under conditions of cell stress, however, the formation of the open pore in the inner membrane results in the loss of membrane impermeability and rapid collapse of the mitochondrial membrane

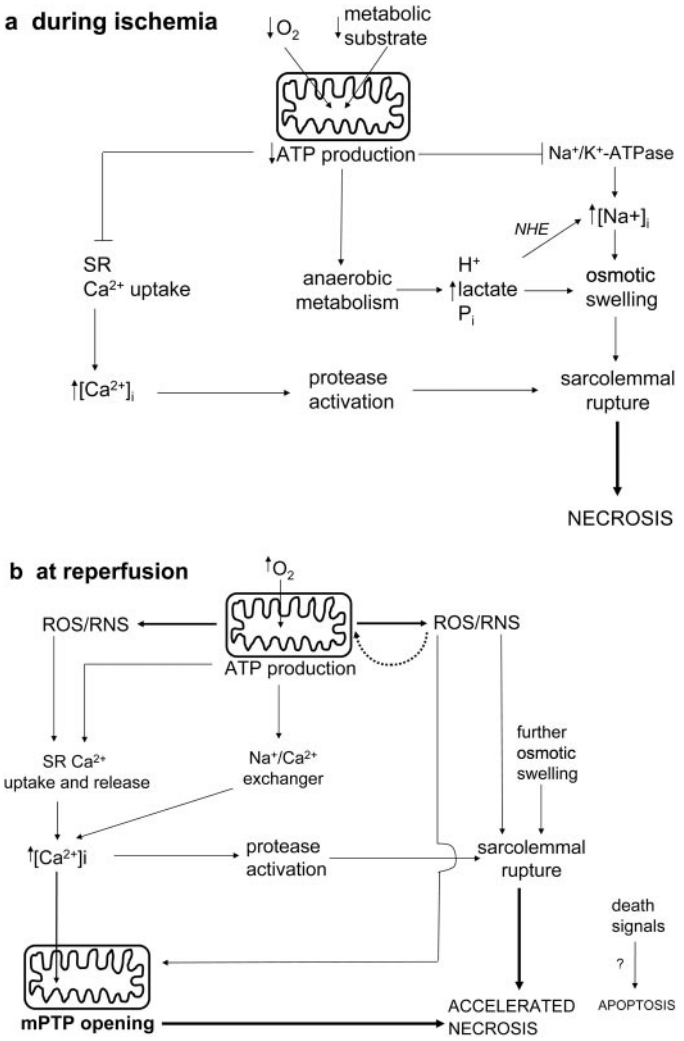

FIG. 2. Major cellular effects of ischemia and reperfusion leading to irreversible forms of injury. a, during ischemia, reduced availability of molecular oxygen and metabolic substrates results in a deficit of high-energy phosphates. Sarcoplasmic reticulum (SR) Ca^{2+} uptake mechanisms are impaired leading to intracellular Ca^{2+} accumulation. Anaerobic metabolism is associated with intracellular accumulation of inorganic phosphate, lactate, and H^+. Activation of the sodium-hydrogen exchanger (NHE) by intracellular acidosis leads to accumulation of intracellular Na^+. This Na^+ overload is exacerbated by inhibition of the sodium pump due to ATP depletion. Increasing intracellular concentrations of solutes results in osmotic swelling that may be sufficient to cause sarcolemmal fragility or disruption, further exacerbated by the activation of Ca^{2+}-dependent proteases and phospholipases. The process of irreversible injury is time-dependent and, in unrelieved ischemia, will result in the pathological features of necrosis. b, at reperfusion, cell death occurs predominantly by necrosis although some apoptosis may occur. The sudden reintroduction of molecular oxygen causes re-energization of mitochondria and reactivation of the electron transport chain with massive production of ROS, which may stimulate further ROS production (ROS-induced ROS release) and generation of RNS in the presence of NO. ROS/RNS cause oxidative and nitrosative damage to cellular structures, including the SR leading to Ca^{2+} release. Also, under conditions of restored ATP production, the activity of the Na^+/Ca^{2+} exchanger is restored, leading to the extrusion of Na^+ in exchange for Ca^{2+}, and SR Ca^{2+} release is further accentuated by restoration of ATP leading to cytosolic Ca^{2+} overload. The combined effects of Ca^{2+} accumulation in the mitochondrial matrix, ROS/RNS, and increasing intracellular pH due to H^+ washout favor the formation/opening of the mPTP. Opening of the mPTP is associated predominantly with necrotic cell death, most likely in those cells that have already sustained injury during ischemia. Some cells display hallmarks of apoptosis after reperfusion. The mechanisms leading to activation of the apoptotic program are unclear and could be related to either mitochondrial or extracellular death signals. The precise rate of injury or mode of cell death during reperfusion will be determined by the severity of changes during ischemia as well as by the extent of sarcolemmal fragility and disruption, which may be further exacerbated during reperfusion by further osmotic swelling and protease activity.

potential. The factors that determine whether and when mPTP opens during myocardial ischemia/reperfusion and evidence for mPTP opening contributing to ischemia/reperfusion injury have been studied extensively during the last two decades (Crompton et al., 1987; Duchen et al., 1993; Lemasters et al., 1997; Halestrap et al., 1998; Di Lisa et al., 2001; Halestrap et al., 2004; Di Lisa and Bernardi, 2006). The consensus of current opinions is that conditions during early reperfusion, but not during ischemia, favor the formation of the open pore and that inhibition of pore opening in reperfusion protects against cell death.

In the currently developing paradigm of ischemia/reperfusion injury and cardioprotection, formation or inhibition of mPTP at reperfusion is the primary determinant of cell death or survival. Evidence favors mPTP opening causing cell death by necrosis (Di Lisa et al., 2001), although it has been proposed that either apoptosis or necrosis might be precipitated, depending on the extent of mPTP opening (Halestrap et al., 2004). Although the molecular events occurring during ischemia determine whether mPTP opens during reperfusion, the corollary of this new view is that specific manipulation during reperfusion of conditions that inhibit mPTP opening offers the potential to attenuate cell death through reperfusion-specific cardioprotective strategies. As we shall discuss subsequently, ischemic preconditioning, pharmacological pretreatments that mimic preconditioning, ischemic postconditioning, and selected agents given at reperfusion may protect through a common mechanism of attenuating mPTP opening in early reperfusion.

In summary, the current view of reperfusion is that it is essential to salvage ischemic tissue. However, reperfusion has the potential to cause further irreversible cell injury, largely dependent on the duration of preceding ischemia, and this is closely linked to the extent of mPTP opening in early reperfusion. None of the recent information detracts from the proven therapeutic value of reperfusion, but it has prompted a reassessment of reperfusion injury and its mechanisms and the potential for therapeutic intervention to maximize the benefits of reperfusion in acute myocardial infarction.

3. Cardioprotection through Classic Preconditioning. The formal description by Murry et al. (1986) of ischemic preconditioning presented an experimental phenomenon that was the most markedly protective intervention able to limit infarct size in a consistent and reproducible manner. They showed in the anesthetized dog that four 5-min periods of left anterior descending coronary artery occlusion, interspersed with 5-min reperfusion periods, before a 40-min occlusion of the

same artery resulted in profound limitation of infarct size. This cardioprotective effect of ischemic preconditioning was independent of changes in transmural myocardial blood flow and Murry et al. proposed that the effect was a result of rapid metabolic adaptation of the ischemic myocardium. The wide reproducibility of this phenomenon using a variety of preconditioning protocols in a number of species and experimental preparations and with a number of endpoints of protection (section III.A.), rapidly led to ischemic preconditioning being established as a "gold standard" for cardioprotection. Various workers recognized that the cardioprotective potential of ischemic preconditioning is transient. For example, Van Winkle et al. (1991) showed that in rabbit myocardium, the protection against infarction afforded by a single 5-min preconditioning period was lost when the interval between the preconditioning stimulus and the infarct protocol was extended beyond 60 min. However, in 1993, two groups independently reported a recrudescence of protection 24 h after preconditioning in canine (Kuzuya et al., 1993) and rabbit (Marber et al., 1993) myocardium. Subsequently, the protection against infarction during this phase was shown to be present between 24 and 72 h after the preconditioning stimulus. The terms "second window of protection, " "delayed preconditioning," or "late-phase preconditioning" have been applied to distinguish this late-onset, long-lasting phenomenon from the "classic" or "early" preconditioning effect originally described by Murry et al. (1986).

Since 1990, more than 3000 original studies have been published addressing various aspects of the molecular mechanisms of ischemic preconditioning in myocardium and other tissues; the majority of these have related to classic preconditioning. A comprehensive review of all of the investigated mechanisms is beyond the scope of this review. It is possible here to provide a broad summary of the major mechanisms that have been investigated and to synthesize the developments within the major schools of thought that have influenced the extrapolation of preconditioning mechanisms to potential therapeutic strategies. The molecular adaptation that underlies the remarkably potent but short-lasting protective effect seen in classic preconditioning is not fully understood and is undoubtedly complex (Fig. 3). The interested reader is referred to comprehensive reviews published elsewhere (Yellon et al., 1998; Schulz et al., 2001; Yellon and Downey, 2003).

a. The role of protein kinases in classic preconditioning. It is highly likely that multiple signal transduction pathways converge on mitochondria, either preserving ATP synthesis or preventing the onset of mPTP formation after reperfusion or both. The identities and configurations of the signal transduction pathways that are activated in the preconditioned myocardium have been the subject of extensive study (for comprehensive reviews, see Schulz et al., 2001; Armstrong, 2004; Hausenloy and Yellon, 2006). The principal kinases that have been identified to exert obligatory roles in classic preconditioning are protein kinase C (PKC), the relevant isoforms of which may vary from one species to another (Liu et al., 1994; Meldrum et al., 1997; Budas et al., 2007), phosphatidylinositol 3-kinase (PI3K) and its substrate kinase Akt (also known as protein kinase B) (Tong et al., 2000; Mocanu et al., 2002; Mocanu and Yellon, 2007), p38 mitogen-activated protein kinase (MAPK) (Weinbrenner et al., 1997; Mocanu et al., 2000; Schulz et al., 2001; Steenbergen, 2002), p42/p44 MAPK/ERK (Ping et al., 1999; Strohm et al., 2000), receptor tyrosine kinases of the Src family (Maulik et al., 1996; Vahlhaus et al., 1998; Oldenburg et al., 2003), and the JAK/STAT pathway (Hattori et al., 2001; Barry et al., 2007). More recent interest has focused on glycogen synthase-3β (GSK-3β) as a distal kinase, phosphorylated (and hence inactivated) by other kinases including Akt and p42/p44 MAPK/ERK (Tong et al., 2002;

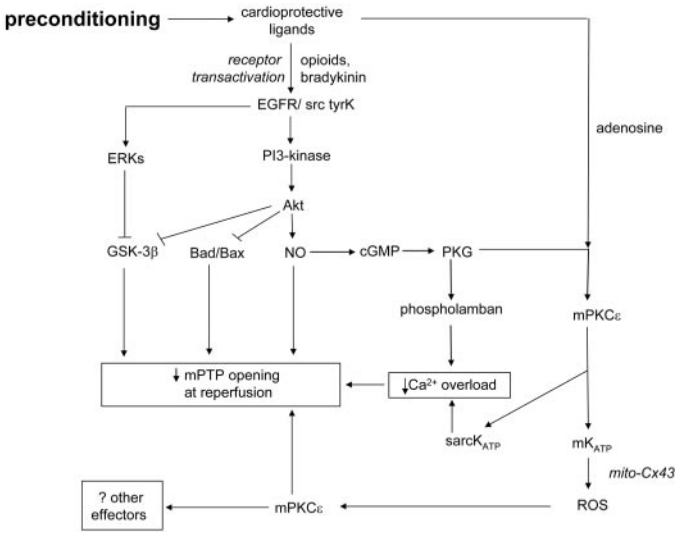

FIG. 3. Schematic representation of the major pathways of classic ischemic preconditioning. Classic preconditioning promotes the accumulation of various cardioprotective ligands for G-protein-coupled receptors, especially adenosine, bradykinin and opioid peptides. Evidence exists for the participation of receptor tyrosine kinase activity, possibly through transactivation, although adenosine may couple directly to PKC. The activation of numerous other protein kinases has been implicated, including the PI3K/Akt cassette, which is thought to be proximal in the signaling pathway. Akt phosphorylates a number of substrates including proapototic members of the Bcl-2 protein family and GSK-3β (causing inactivation) and eNOS (causing activation). NO generated from eNOS leads to activation of PKG via elevation of intracellular cGMP. Substrates for PKG may include the SR regulatory protein phospholamban, which promotes SR Ca^{2+} uptake, thereby reducing cytosolic Ca^{2+} overload. Recent evidence suggests that PKG is the final cytosolic signal transduction component and leads to activation of mitochondrial pools of PKCε. Downstream consequences of PKCε activation include activation of mitochondrial K_{ATP}, opening of which promotes ROS formation and further PKCε activation. Inhibition of mPTP opening can occur as a result of PKCε activation. Sarcolemmal K_{ATP} and mitochondrial connexin-43 have also been implicated in the mechanism of classical preconditioning. The latter is an essential component of the classical preconditioning mechanism. The generation of ROS and RNS appears to be a consequence of mitochondrial K_{ATP} opening and an obligatory part of the signaling cascade. It is likely that ROS/RNS signal the activation of distal kinases which may include p38 MAPK, PKC, and JAK/STAT. Although inhibition of mPTP opening in early reperfusion appears to be an important mechanistic feature of preconditioning, it is possible that other distal proteins may serve as effector mechanisms.

Juhaszova et al., 2004). Much of the evidence supporting involvement of these protein kinases has relied on the demonstration that they are phosphorylated, translocated, or display increased activity in preconditioned myocardium or that pharmacological inhibitors of their activation blunt or abrogate the protection afforded by preconditioning.

The upstream triggers for activation of these kinases and the sequence of their activation in a multistep cascade are not clear; interpretation of numerous experimental studies is complicated by variations in species and endpoints. However, with regard to infarct size limitation, it seems likely that adenosine, bradykinin, opioid peptides, and prostaglandins released or accumulating in ischemic myocardium during the preconditioning stimulus, bind to G-protein-coupled receptors, namely adenosine A_1 and A_3, kinin B_2, opioid δ_1 and EP_3, respectively (Gross and Gross, 2006). The relative importance of these autacoids may vary among species and according to the intensity of the preconditioning stimulus (Schulz et al., 1998). However, as a general summary, pharmacological blockade of individual receptors blunts or abolishes the protective effect of preconditioning whereas transient preischemic activation of any of the receptors with exogenous autacoid or synthetic receptor ligands induces protection ("pharmacological preconditioning") that is usually quantitatively similar to that seen with ischemic preconditioning.

b. ATP-sensitive potassium channels and classic preconditioning. An intriguing component of the classic preconditioning mechanism is involvement of the ATP-dependent potassium channels (K_{ATP}) (Gross, 1995; Oldenburg et al., 2002; Gross and Peart, 2003; Ardehali and O'Rourke, 2005). K_{ATP}, expressed at the sarcolemma of cardiomyocytes, opens during hypoxia, ischemia, or metabolic inhibition, thereby facilitating increased potassium influx and shortening of action potential duration. Increased cellular potassium influx increases osmotic load and shortening of action potential duration is arrhythmogenic. The sarcolemmal K_{ATP} consists of pore forming [potassium inward rectifier (Kir)] and receptor subunits [sulfonylurea receptors (SURs)], the latter being blocked by substances such as glibenclamide or more specifically by HMR1098 and HMR1883. Nicorandil, cromakalim, and pinacidil are examples of drugs that open sarcolemmal K_{ATP} channels (O'Rourke, 2000).

In most species examined, glibenclamide abolishes the protective effect of preconditioning (Gross and Auchampach, 1992; Ferdinandy et al., 1995), whereas pharmacological openers of K_{ATP} confer protection quantitatively similar to that obtained with ischemic preconditioning (Grover et al., 1989; for a review, see Auchampach and Gross, 1994). It has been proposed that a PKC-induced increased trafficking of K_{ATP} to the sarcolemma (Budas et al., 2004), which opens sarcolemmal K_{ATP} causing shortening of myocyte action potential duration and reduced Ca^{2+} influx (Rainbow et al., 2004), is responsible for preconditioning-induced protection (Gross, 1995). However, a number of observations are inconsistent with this conjecture, including the demonstration of protection independent of surface current changes in isolated nonbeating myocytes (Garlid et al., 1997; Liu et al., 1998b) and the ability of some K_{ATP} openers to protect against ischemia/reperfusion injury at doses or concentrations that do not influence the action potential duration (Yao and Gross, 1994; Grover et al., 1995, 1996). Nevertheless, the importance of sarcolemmal K_{ATP} activation for pharmacological preconditioning with diazoxide and pinacidil (Tanno et al., 2001) or desflurane (Toller et al., 2000) as well as a trigger for the delayed phase of ischemic (Patel et al., 2005) or opioid-induced (Chen et al., 2003) preconditioning remains a matter of debate.

At the level of mitochondria, potassium flux across the inner mitochondrial membrane influences mitochondrial membrane potential, volume regulation, energy production, and calcium homeostasis. To allow potassium cycling across the potassium-impermeable inner membrane, mitochondria express antiporter (hydrogen/potassium) and potassium channels, one of these being the calcium-activated potassium channel and the other one being a mitochondrial K_{ATP}. Indeed, in mitoplasts using single-channel recordings by means of the patch-clamp technique, a potassium current was measured (15–82 pS), which was blocked by ATP or 5-hydroxydecanoic acid (5-HD) (Dahlem et al., 2004). Under physiological conditions at a mitochondrial membrane potential of approximately −180 mV, however, the channel should express minimal ion conductance (Dahlem et al., 2004). In isolated rat heart mitochondria, openers of the mitochondrial K_{ATP} such as pinacidil, cromakalim, and levcromakalim reduced mitochondrial membrane potential and increased mitochondrial respiration and radical formation, matrix volume, and calcium release from calcium-preloaded mitochondria, with all effects being dependent on the extramitochondrial potassium concentration (Holmuhamedov et al., 1998). Furthermore, in the mitochondria-enriched fraction from PC12 cells, specific antibodies against SUR1 and Kir6.1 detected immunoreactive proteins of the apparent molecular masses of 155 and 50 kDa (Tai et al., 2003), although the latter finding was questioned using rabbit ventricular cardiomyocytes (Seharaseyon et al., 2000). Most recently, fluorescence imaging of isolated mitochondria from rat adult cardiomyocytes expressing recombinant Kir6.2/SUR2A showed that Kir6.2-containing K_{ATP} were localized to mitochondria and that mitochondrial localization was increased by PKC activation (Garg and Hu, 2007); indeed mice genetically deficient in Kir6.2 display no preconditioning response (Suzuki et al., 2002). In 1997, Keith Garlid and Gary Grover (Garlid et al., 1997) proposed that a mitochondrial K_{ATP} might be the molecular target of the cardioprotective K_{ATP}

opener drug diazoxide. Subsequently, several hundred studies have focused on opening of the mitochondrial K_{ATP} as a central mechanism of ischemic preconditioning (Ardehali and O'Rourke, 2005). Furthermore, opening of the calcium-activated potassium channel by NS-1619 before ischemia/reperfusion decreased infarct size in mice (Wang et al., 2004), highlighting the importance of mitochondrial potassium flux for cardioprotection.

Although there is little doubt that K_{ATP} opening is both protective and involved in the mechanism of preconditioning, some words of caution need to be made concerning the reliance on some of the pharmacological tools used to study mitochondrial K_{ATP}. These include openers of the mitochondrial K_{ATP} such as diazoxide, nicorandil, cromakalim, levcromakalim, bimakalim, and aprikalim; and inhibitors such as 5-HD, glibenclamide, and tetraphenylphosphonium (Table 1).

In summary, the existing literature overwhelmingly supports a central role of sarcolemmal and mitochondrial K_{ATP} for the cardioprotection obtained by classic ischemic preconditioning as well as certain pharmacological strategies based on the preconditioning paradigm. Not surprisingly, however, many drugs used to open or inhibit mitochondrial K_{ATP} elicit significant additional effects relevant to cardioprotection, such as augmented NO release or mitochondrial uncoupling (Brennan et al., 2006), making a definitive conclusion on the underlying mechanism difficult.

c. The evolving model of signal transduction in classic preconditioning. Jim Downey and Michael Cohen (Cohen et al., 2000a; Oldenburg et al., 2002; Critz et al., 2005) have proposed that adenosine, acting on A_1 or A_3 receptor subtypes, couples directly to PKC via phospholipase C and diacylglycerol formation. They proposed a hypothetical scheme wherein bradykinin and opioids, in contrast with adenosine, trigger a complex signal transduction pathway involving transactivation of receptor tyrosine kinase and subsequent activation of PI3K/Akt. Activated (phosphorylated) Akt phosphorylates endothelial NO synthase (eNOS) resulting in NO generation, activation of soluble guanylyl cyclase, cGMP accumulation, and activation of cGMP-dependent protein kinase (PKG). The roles of ROS, NO, reactive nitrogen species, and their downstream cellular targets are somewhat controversial in classic preconditioning (for extensive reviews, see Ferdinandy and Schulz, 2003; Jones and Bolli, 2006). However, it seems that ROS/RNS play a critical role in the signal transduction pathway, leading to activation of PKC (Otani, 2004).

Many potential substrates of PKC have been proposed as contributing to downstream mechanisms of preconditioning-induced protection and although PKC activation is clearly a necessary component of the mechanism of preconditioning in many studies, its functions and targets in classic preconditioning have until recently been unclear. Elegant studies by Garlid's group (Costa et al., 2005, 2006) have provided clearer insights and significant progress. PKG appears to be the terminal cytosolic step in the signal transduction cascade, phosphorylating an unknown target at the mitochondrial outer membrane. It is clear from the work of Costa et al. (2005) that mitoK_{ATP} opening is both PKG- and PKCε-dependent. However, PKG cannot phosphorylate mitoK_{ATP} channel proteins directly because it is too large to cross the mitochondrial outer membrane. They concluded that PKG phosphorylates an unknown target at the mitochondrial outer membrane that leads to subsequent activation of a PKCε pool within the intermembrane space. Subsequently, Costa et al. (2006) demonstrated that PKG inhibits mPTP opening through a mechanism involving activation of two discreet mitochondrial pools of PKCε. PKCε1 promotes mitoK_{ATP} opening, leading to a modest increase in matrix H_2O_2. H_2O_2 acts as a signaling intermediate, promoting further PKCε1 activation and activating PKCε2, which inhibits mPTP formation. This work constitutes a convincing scheme that connects PKG, mitochondrial PKCε, mitoK_{ATP} opening, ROS generation, and inhibition of mPTP.

TABLE 1
KATP openers and the mechanism of their cardioprotective effect

K_{ATP} Opener Drug	Mechanism of Effects on the Ischemic/Reperfused Heart	References
Nicorandil	Donates NO, which once applied exogenously can protect the heart against ischemia/reperfusion injury	Gomma et al. (2001)
Diazoxide	Suppresses mitochondrial respiration and increases formation of free oxygen radicals independent of the potassium concentration, an effect being blocked by 5-HD; inhibits succinate dehydrogenase; reduces infarct size which is blocked by L-NAME, implying a nitric oxide synthase-dependent effect	Minners et al. (2007), Dröse et al. (2006), Dröse et al. (2006), Ockaili et al. (1999)
Diazoxide/Pinacidil	Its uncoupling effect in isolated rat heart mitochondria respiring on pyruvate and malate was markedly reduced by inhibitors of the adenine nucleotide translocase, which also affected mitochondrial matrix volume regulation	Kopustinskiene et al. (2003), Das et al. (2003)
5-HD	As 5-HD is metabolized in the first 3 steps of the beta-oxidation, its metabolic intermediates, rather than blockade of K_{ATP}, could be involved in the inhibitory effect of 5-HD on preconditioning; 5-HD did not affect mitochondrial matrix volume in rat heart mitochondria	Hanley et al. (2003), Das et al. (2003)

Connexin-43 is another protein implicated in classic preconditioning. The protein forms the multimeric hemichannel structure of gap junctions in myocardium and appears to be obligatory for classic preconditioning, as hearts (Schwanke et al., 2002, 2003) or cardiomyocytes (Li et al., 2004) obtained from connexin-43 heterozygous knockout mice display no preconditioning response (Schulz and Heusch, 2004). Connexin-43, however, is, apart from its localization at the sarcolemma, also expressed in the inner membrane of cardiomyocyte mitochondria (Boengler et al., 2005), its transport being mediated by heat shock protein 90 and the translocator of the outer mitochondrial membrane (Rodriguez-Sinovas et al., 2006). Loss of connexin-43 decreases ROS formation secondary to diazoxide, leading to a loss of pharmacological preconditioning-induced protection (Heinzel et al., 2005).

4. Cardioprotection through Late Preconditioning. Mechanistic investigation of late preconditioning has been far less extensive than that for classic preconditioning, but a picture has emerged of a phenomenon no less complex than classic preconditioning and possibly having some mechanisms in common. At the time of its formal description, late preconditioning was considered to be an adaptive phenomenon, mechanistically distinct from classic preconditioning. The original study by Marber et al. (1993) examined the hypothesis that transient ischemia/reperfusion stress caused the de novo synthesis of the putative cytoprotective protein, inducible 72-kDa heat shock protein (HSP72), and they showed a correlation between HSP72 induction and infarct size limitation 24 h after ischemic preconditioning in the rabbit heart. The study by Kuzuya et al. (1993), detailing the time course of loss and recrudescence of protection after preconditioning in the dog, was complemented by a related study by the same group (Hoshida et al., 1993) describing the time course of induction of the intracellular antioxidant SOD. Induction of cytoprotective factors such as intracellular antioxidants and HSPs had long been recognized as a conserved stress response in eukaryotes subjected to transient oxidant cellular stresses. However, the mechanisms regulating the induction of these factors were not well characterized in mammalian systems although transcriptional regulation of stress response genes was recognized in lower organisms (Mestril and Dillman, 1995; Yellon and Baxter, 1995; Baxter and Yellon, 1996). Nevertheless, HSPs have become emerging molecular targets for cardioprotective drug development (for a review, see Sõti et al., 2005).

A major conceptual development in delayed preconditioning was the recognition that autacoid factors released during preconditioning play an important role in eliciting the late-appearing adaptive response. At the time that late preconditioning was formally described, the important role played by adenosine as a mediator of classic preconditioning was already widely acknowledged. In 1994, Baxter et al. demonstrated in the same rabbit model of delayed preconditioning used by Marber et al. (1993) that pharmacological blockade of adenosine receptors during ischemic preconditioning abolished the development of protection 24 h later. Conversely, administration of 2-chloro-N^6-cyclopentyladenosine, a selective A_1 receptor agonist to naive rabbits induced a state of cardioprotection against infarction 24 to 72 h later (Baxter et al., 1994; Baxter and Yellon, 1997), mimicking the time course of delayed protection induced by ischemic preconditioning (Baxter et al., 1997). This was the first indication that adenosine, a mediator with a brief biological half-life, could elicit a biological effect evident many hours later, although a delayed and long-lasting cardioprotection elicited by prostacyclin had been described several years previously in the work of Szekeres and colleagues (Szekeres, 2005). Bolli's group identified NO as a further obligatory trigger of late preconditioning in rabbit myocardium (Qiu et al., 1997), suggesting the participation of at least two independent autacoid triggers of delayed preconditioning in the rabbit. Subsequent work has identified the involvement of multiple endogenous triggers for delayed preconditioning, including adenosine, NO, bradykinin, cytokines and ROS, with some divergences related to species and experimental endpoint (e.g., infarct size versus myocardial stunning). Moreover, the exogenous administration of selective opioid δ receptor agonists is able to elicit a delayed cardioprotective response, although participation of endogenous opioid peptides in the late ischemic preconditioning response has not been specifically examined. For fuller descriptions, the reader is referred to focused reviews: Baxter and Yellon, 1998; Bolli, 2000; Baxter and Ferdinandy, 2001; Heusch, 2001; Baxter, 2002b; Dawn and Bolli, 2002; Ferdinandy and Schulz, 2003).

The nexus between upstream triggers of late preconditioning and the transcriptional and post-translational regulation of proteins associated with mediation of late protection involves a complex and poorly defined kinase cascade. Multiple studies using either ischemic preconditioning or pharmacological triggers of delayed protection have highlighted the involvement of PKC, especially PKCε (Baxter et al., 1995; Ping et al., 1997; Vondriska et al., 2001), Src and Lck tyrosine kinases, probably downsteam of PKC (Imagawa et al., 1997; Dawn et al., 1999; Ping et al., 1999; Vondriska et al., 2001), the JAK/STAT signaling pathway (Dawn et al., 2004), p38 MAPK (Dana et al., 2000; Lasley et al., 2004; Fryer et al., 2001), PI3K and p70s6 kinase/mammalian target of rapamycin (Kis et al., 2003); and p42/p44 MAPK/ERK (Fryer et al., 2001). Although early activation of these kinases may occur in response to ischemic or pharmacological preconditioning triggers, some studies provide evidence for altered kinase activity 24 h after the preconditioning stimulus (e.g., p38 MAPK activity 24 h after A_1 receptor agonist; Dana et al., 2000).

It is clear that delayed preconditioning recruits multiple signaling pathways that are highly dependent on the nature of the priming stimulus, e.g., transient ischemia or application of specific receptor ligands. The most complete account is given in the extensive studies of Bolli and colleagues (reviewed in Dawn and Bolli, 2002), a substantial body of work that has characterized late protection induced by ischemic preconditioning and NO donor compounds. The general scheme involves the interaction of eNOS-derived NO and superoxide to form peroxynitrite anion, which activates PKCε. PKCε activates Src and Lck tyrosine kinases. The activation of the transcription factor NF-κB occurs by dual serine and tyrosine phosphorylation of the inhibitor protein IKB-α by both PKC and tyrosine kinases. The cytoprotection-related proteins induced by NF-κB-regulated gene expression include inducible NOS (iNOS) and cyclooxygenase-2 (COX-2). Inducible NOS-derived NO appears to regulate the activation of COX-2 in the preconditioned myocardium, determining a pattern of prostanoid generation that is critical for the appearance of a cardioprotected phenotype (Bolli et al., 2002). This reliance of late preconditioning on the up-regulation of iNOS and COX-2 proteins is convincingly demonstrated by a series of pharmacological and functional genomic studies involving pharmacological inhibition and genetic deletion of iNOS and COX-2. How COX-2-derived prostanoids ultimately exert their cytoprotective action, and their relationships with other cytoprotective mechanisms such as antioxidant enzymes, heme oxygenase (Ockaili et al., 2005), HSPs, mitoK_{ATP} channel opening (Baxter and Yellon, 1999), and mPTP inhibition (Hausenloy and Yellon, 2004) remain unknown. DNA microarray studies have revealed that preconditioning changes the gene expression pattern of rat hearts extensively, which suggests that very complex cellular mechanisms are involved in the evolution of late cardioprotection conferred by preconditioning (Onody et al., 2003). It is likely that delayed preconditioning involves the recruitment of multiple mechanisms of genetic adaptation, many of which have not been studied yet.

5. Cardioprotection through Postconditioning. Despite the unquestioned need for reperfusion to prevent ischemic necrosis, in the past 5 years there has been accumulating experimental evidence that reperfusion per se is associated with the paradoxical activation of lethal signals that culminate in necrosis/apoptosis. The current development of concepts in this field of investigation is linked to the recent convergence of three major themes of investigation. The first is the promotion by Yellon and colleagues of the concept of antiapoptotic prosurvival kinases targeting against reperfusion injury (Yellon and Baxter, 1999; Hausenloy and Yellon, 2006). The second is a growing interest in and appreciation of the contribution of mPTP as a mediator of injury during reperfusion (Hausenloy et al., 2002). The third is the formal description in 2003 of a phenomenon termed ischemic postconditioning (Zhao et al., 2003). This area of investigation is now evolving rapidly and the current information suggests that reperfusion-induced cell injury contributes to a far greater extent than was previously accepted. Although many experimental studies during the last two decades have suggested that certain interventions administered immediately before or during the early moments of reperfusion could attenuate the extent of cell death and infarct size, these studies have been controversial in that their reproducibility was limited (section II.B.1.).

In a landmark study, Zhao et al. (2003) reported that three 30-s intermittent periods of left coronary artery occlusion in the dog, at the onset of reperfusion after a sustained 60-min occlusion, resulted in marked limitation of infarction. They coined the term "ischemic postconditioning" to describe this protective intervention. The protection induced by postconditioning was comparable with that observed after ischemic preconditioning with a single 5-min coronary occlusion before 60 min of ischemia. This study has ignited widespread interest in the possibility of reliably targeting reperfusion injury with a rational pharmacological intervention based on an understanding of the mechanism of protection.

At the time of writing, mechanistic investigation of ischemic postconditioning is at an early stage (Fig. 4). It is clear that the protection afforded by postconditioning (using suitable algorithms that may be model- and species-dependent) is reproducible and is observed in isolated buffer-perfused heart preparations, obviating any substantial primary role of blood-borne factors (Vinten-Johansen et al., 2005; Zhao and Vinten-Johansen, 2006) Attention thus far has focused on five major mechanistic themes: the involvement of endogenous adenosine and activation of adenosine receptor subtypes; the role of the NO/cGMP pathway; involvement of mitoK_{ATP} channels; the activation of reperfusion salvage kinase pathways (notably PI3K/Akt, p42/p44 MAPK/ERK, and PKG); and inhibition of mPTP opening at reperfusion.

a. Autacoid mediators of postconditioning. The observations that pharmacological adenosine receptor antagonism during reperfusion abolishes the protective effect of ischemic postconditioning (Kin et al., 2005; Yang et al., 2005; Philipp et al., 2006) confirm the obligatory role of endogenous adenosine, formed during index ischemia and whose washout is delayed by postconditioning ischemia. Furthermore, studies with selective adenosine receptor ligands suggest that the A_1 receptor activation is not involved in rabbit or mouse myocardium. Activation of A_2 receptor subtypes, either A_{2A} in mouse (Kin et al., 2005) or A_{2B} in rabbit (Philipp et al., 2006) as well as A_3 receptor in mouse (Kin et al., 2005), may play a role. However, these conclusions are based on the presumed selectivity of ligands in a notoriously controversial area of receptor pharmacology. These studies are likely to be associated with a resurgence of interest in the application of adenosine and adenosine receptor agonists as

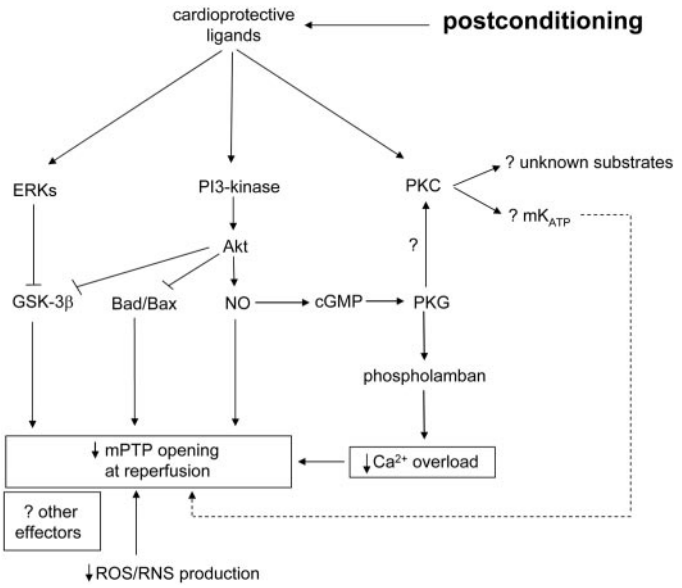

FIG. 4. Hypothetical scheme of the major pathways identified so far as contributing to postconditioning. Unlike classic preconditioning, the mechanisms of postconditioning have been relatively little investigated. Several autacoids and kinases appear to share common roles in classic preconditioning and postconditioning. Postconditioning may promote the accumulation or delay the washout of several autacoid mediators (extracellular cardioprotective ligands) whose participation in the mechanism is obligatory. It is proposed that ligands such as adenosine, bradykinin and opioids cause the activation of multiple kinases, including p42/p44 MAPK/ERKs, PI3K/Akt, and PKC. The substrates of PKC relevant to postconditioning protection are unknown. There is also evidence that PKG activation may occur as part of the postconditioning mechanism and this may lie downstream of Akt/NO/cGMP and play a role in promoting sarcoplasmic reticulum Ca^{2+} uptake. Activation of Akt also inhibits GSK-3β and members of the Bcl-2 protein family, thereby inhibiting mPTP formation/opening. Postconditioning is also associated with attenuation of ROS/RNS generation at reperfusion which may also serve to inhibit mPTP opening. Although in classic preconditioning a role of ROS signaling is recognized, it is not known if this is a significant feature of the postconditioning mechanism. A role of mK_{ATP} channel opening is implied by some pharmacological studies, but how this mediates protection at reperfusion is unknown although it is possible that mK_{ATP}-mediated inhibition of mPTP opening, as in the preconditioning model, plays a role. It is likely that other unidentified substrates and effector mechanisms play significant roles.

adjuncts to reperfusion despite a long and confused experimental history. The involvement in postconditioning of other endogenous autacoids, notably bradykinin and opioid peptides that have been implicated in ischemic preconditioning, has not yet been reported although there is evidence that pharmacological activation of bradykinin B_2 receptors or opioid δ receptors just before or during early reperfusion attenuates infarct size (Bell and Yellon, 2003a; Gross et al., 2004).

b. Role of the NO/cGMP pathway in postconditioning. The involvement of the NO/cGMP pathway in ischemic postconditioning is implied by studies in which NOS inhibition abolished the protection afforded by postconditioning (Yang et al., 2004, 2005; Penna et al., 2006) and by the observation that eNOS-ser1177 phosphorylation was increased in postconditioned myocardium (Tsang et al., 2004). Yang et al. (2005) also observed that [1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one], a selective soluble guanylyl cyclase inhibitor, abolished the infarct-limiting effect of ischemic postconditioning, whereas administration of atrial natriuretic peptide at reperfusion, which elevates cGMP through activation of particulate guanylyl cyclase mimicked postconditioning-induced protection. Other studies have observed that protective interventions given at reperfusion may rely on induction of NO synthesis. For example, adrenomedullin has been shown to limit infarct size when administered during early reperfusion in rat heart (Hamid and Baxter, 2005), an effect blocked by NOS inhibition. In mouse heart, adrenomedullin before reperfusion also limited infarct size (Hamid et al., 2007); an increase in myocardial NO generation was measurable after adrenomedullin administration and the protective effect was blocked by [1*H*-[1,2,4]oxadiazolo[4,3-*a*]-quinoxalin-1-one]. Similarly, the protective action of bradykinin at reperfusion has been reported to be through a NOS-dependent mechanism (Bell and Yellon, 2003a).

c. ATP-sensitive potassium channels and postconditioning. The requirement of $mitoK_{ATP}$ opening in the mechanism of postconditioning has been demonstrated through the observation that K_{ATP} blockers (glibenclamide or 5-HD) abolished the protection brought about by postconditioning in rabbit heart (Yang et al., 2004, 2005) and in pig heart (Iliodromitis et al., 2006a). There is some evidence that K_{ATP} openers such as bimakalim given before reperfusion are able to limit infarct size (Auchampach and Gross, 1994). Application of the halogenated volatile anesthetics such as isoflurane during ischemia/reperfusion is cardioprotective, an effect that has been ascribed to $mitoK_{ATP}$ opening. Interestingly, pharmacological postconditioning with isoflurane administered at reperfusion limited infarct size (Chiari et al., 2005; Feng et al., 2005; Krolikowski et al., 2005). This protective effect of anesthetic postconditioning was abolished by K_{ATP} blockade and was related to activation of PI3K/Akt signaling, inhibition of GSK-3β activation, and inhibition of mPTP opening.

d. The reperfusion injury salvage kinase pathway. The activation of reperfusion injury prosurvival kinase pathways by ischemic postconditioning and diverse pharmacological cardioprotective strategies has become an important contemporary motif in experimental cardioprotection. Yang et al. (2004) showed that the protective effect of postconditioning was abolished by inhibition of p42/p44 MAPK/ERK. However, other salvage kinases may be causally involved. For example, Tsang et al. (2004) and Yang et al. (2005) demonstrated the dependence of postconditioning on activation of the PI3K/Akt pathway through the ability of blockers of this pathway to abolish postconditioning-induced protection. PKG activation may be another signaling mechanism that limits reperfusion injury (Burley et al., 2007). Abdallah et al. (2006) have reported the activation of PKG downstream of PI3K/Akt/eNOS/NO as being critical to the protection afforded by insulin at reoxygenation in isolated cardiomyocytes, whereas pharmaco-

logical inhibition of PKG has been reported to abolish the protective effect of postconditioning against infarction (Burley and Baxter, 2005). The effects of many interventions at reperfusion rely on common activation of these survival pathways. They include classic activators such as insulin and other growth factors (Yellon and Baxter, 1999; Baxter et al., 2001; Jonassen et al., 2001). 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors (statins) (Bell and Yellon, 2003b; Wolfrum et al., 2004), adrenomedullin (Hamid and Baxter, 2005), natriuretic peptides (Inserte et al., 2000; Burley and Baxter, 2007), and ischemic preconditioning (Hausenloy et al., 2005a,b).

Thus, the signaling cascades activated by ischemic preconditioning (or pharmacological preconditioning mimetics) and by postconditioning (or pharmacological agents given at reperfusion) may be convergent. The prosurvival kinase pathways activated during ischemia and/or reperfusion have several known substrates that may be distal effector mechanisms of cytoprotection including activation of NOS (Zhang et al., 2005), inhibition of mPTP (Hausenloy et al., 2002, 2005b), up-regulation of antiapoptotic members, inhibition of proapoptotic members of the BclII family (Uchiyama et al., 2004), and inhibition of GSK-3β (Cross et al., 1995; Murphy and Steenbergen, 2005). However, although several steps in the signal transduction cascade of ischemic pre- and postconditioning are similar, there exists also a discrepancy in that connexin-43, which is essential to preconditioning-induced protection (see section II.B.3.), is not a prerequisite to ischemic postconditioning-induced protection. In mice in vivo, ischemic postconditioning reduces infarct size in wild-type as well as connexin-43 heterozygous deficient mice (Heusch et al., 2006). Finally, modified reperfusion to altered washout of metabolites and/or activation of the reperfusion injury salvage kinases might modify myocardial pH (Cohen et al., 2007)—as initially suggested by Heusch (2004)—thereby avoiding hypercontracture and irreversible cell injury (Garcia-Dorado, 2006).

III. Animal Models and Human Studies of Cardioprotective Strategies

Having described the major mechanisms of injury and survival in myocardial ischemia/reperfusion, we now survey some of the key experimental and clinical evidence that these mechanisms may be manipulated to yield cardioprotective strategies. In doing so, we limit the discussion to descriptions of how preconditioning and postconditioning strategies may influence the development of various forms of ischemia/reperfusion injury. For the most part, the experimental characterizations of the cardioprotective effects that we now describe have been determined using healthy juvenile animals or myocardial tissue from such animals. These descriptions of our contemporary knowledge of cardioprotective approaches serve as a basis for further consideration of cardioprotective strategies in comorbid conditions in section IV.

A. Classic Preconditioning

When first described by Murry et al. (1986), ischemic preconditioning was elicited by brief coronary occlusion interrupted by reperfusion, and the endpoint was reduced infarct size. Soon, however, a variety of preconditioning stimuli were uncovered including hypoxia (Cohen et al., 1995), rapid cardiac pacing (Szilvassy et al., 1994; Verdouw et al., 1996), thermal stress, and various pharmacological receptor-dependent and -independent agents (pharmacological preconditioning) (Yellon et al., 1998; Schulz et al., 2001; Yellon and Downey, 2003). For pharmacological preconditioning, the endogenous pathways of cardioprotection serve as the contemporary templates for therapeutic cardioprotection. Various endpoints of ischemic preconditioning have been used: ischemic preconditioning protects against infarction in all species tested so far, and there is evidence that it might be operative in humans (for reviews, see Yellon and Downey, 2003; Rezkalla and Kloner, 2005). Ischemic preconditioning also reduces the extent of apoptosis (for a review, see Zhao and Vinten-Johansen, 2002). On the other hand, it has been difficult to demonstrate an antistunning effect in the early phase of protection in rabbits, dogs, and pigs (for a review, see Schulz et al., 2001). In addition to infarct size reduction and apart from a potential attenuation of stunning, ischemic preconditioning diminishes adverse left ventricular remodeling after infarction and improves long-term functional recovery in chronically instrumented rabbits (Cohen et al., 2000b) and humans (Solomon et al., 2004). Ischemic preconditioning protects against arrhythmias in mice, rats, rabbits, and dogs, but not in pigs (for a review, see Schulz et al., 2001). In pigs, ischemic preconditioning not only fails to reduce the incidence of ventricular fibrillation during ischemia/reperfusion but even accelerates the onset of ventricular fibrillation during sustained ischemia and decreases the ventricular fibrillation threshold (Ovize et al., 1995).

1. Duration and Severity of Ischemia and Reperfusion in Preconditioning. Not all combinations and durations of ischemia and reperfusion will trigger the preconditioning phenomenon and protect ischemic myocardium. There appears to be a critical threshold of ischemia required to trigger the adaptive mechanism. A preconditioning regimen of only 1 or 2 min of ischemia with subsequent reperfusion before the index ischemia has no protective effect (Schulz et al., 1998; Matsubara et al., 2000). Above this threshold, the protection conferred by ischemic preconditioning is a graded phenomenon that depends on the intensity of the preconditioning stimulus: two cycles of 10 min of occlusion of a major epicardial branch of the left coronary artery each followed by 30 min of reperfusion before a 45-min coronary

occlusion and 2 h of reperfusion in anesthetized rabbits result in greater infarct size reduction than a single cycle of preconditioning ischemia and reperfusion before the index ischemia (Sandhu et al., 1997). Similarly, a single cycle of 10 min of low-flow ischemia followed by 15 min of reperfusion results in a greater limitation of infarct size than a single cycle of 3 min of low-flow ischemia and subsequent 15 min of reperfusion before 90 min of low-flow ischemia in anesthetized pigs (Schulz et al., 1998). However, the effect quickly saturates. In one study in dogs 1, 6, or 12 5-min preconditioning cycles offered similar protection (Li et al., 1990).

Apart from the number of preconditioning cycles and the duration of ischemia, the duration of the intermittent reperfusion also determines the protection achieved by ischemic preconditioning. In rats, protection is still evident when the reperfusion period is shortened to 1 min, although there is no protection if reperfusion is only 30 s in duration (Alkhulaifi et al., 1993). Typically, a 5-min period of ischemia followed by up to 60 min of reperfusion before the index ischemia results in salvage agents (for a review, see Schulz et al., 2001; Yellon and Downey, 2003). Although in rabbits protection can be reinstituted with a second cycle of preconditioning ischemia/reperfusion (Yang et al., 1993), this is not the case in pigs (Sack et al., 1993). To trigger preconditioning the extent of reperfusion appears to be less critical than its duration. Persistent blood flow reduction caused by a severe coronary artery stenosis does not prevent ischemic preconditioning (Kapadia et al., 1997).

Low-flow ischemia preceding the index ischemia can also successfully precondition the heart. A flow reduction to 30% of baseline for 30 min without intermittent reperfusion before 60 min of total coronary occlusion reduces infarct size in pigs (Koning et al., 1994); however, this effect is transmurally heterogeneous, insofar as the preconditioning ischemia reduces infarct size in the epicardium, but actually exacerbates infarction in the endocardium (Koning et al., 1995). Similarly, preconditioning without intermittent reperfusion before the index ischemia is seen when a 10-min coronary occlusion precedes 80 min of low-flow ischemia in pigs (Schulz et al., 1995a).

B. Remote Preconditioning

Preconditioning of the heart may even be evoked by brief episodes of ischemia and reperfusion in other organs, a phenomenon called remote preconditioning (for a review, see Heusch and Schulz, 2002). Przyklenk et al. (1993) first reported that four 5-min cycles of left circumflex coronary artery occlusion and 5 min of reperfusion reduced infarct size after 1 h of sustained left anterior descending coronary artery occlusion and 4.5 h of reperfusion in anesthetized dogs. In contrast, ischemic preconditioning by two 5-min cycles of regional ischemia and 5 min of reperfusion in rabbits in situ protected the preconditioned region but not the remainder of the heart when animals were subsequently subjected to 30 min of global ischemia and 2 h of reperfusion in the Langendorff mode ex vivo (Nakano et al., 2002). Again, in a rabbit isolated heart preparation, a humoral substance could be retrieved from the coronary effluent of a preconditioned donor heart, which induced protection when transferred to a virgin recipient. The detailed nature of this humoral substance, however, is unclear (for a review, see Heusch and Schulz, 2002). A reduction in myocardial infarct size is elicited by prior occlusion and reperfusion of a mesenteric or renal artery in rats, renal artery occlusion/reperfusion in rabbits, or stenosis of the femoral artery plus electrical stimulation of the gastrocnemius muscle in rabbits (for a review, see Heusch and Schulz, 2002). In the original study by Gho et al. (1996), remote preconditioning from the intestine or kidney was as protective as classic ischemic preconditioning; the protection was abolished by ganglionic blockade with hexamethonium, indicating the involvement of a neuronal pathway (Gho et al., 1996). Importantly, reperfusion of the mesenteric artery was mandatory to achieve protection, which could also reflect the involvement of a humoral mediator. In children undergoing repair of congenital heart defects, remote ischemic preconditioning was induced by four 5-min cycles of lower limb ischemia and reperfusion using a blood pressure cuff. Levels of troponin I postoperatively were greater in nonpreconditioned control patients compared with patients undergoing repetitive limb ischemia/reperfusion, indicating greater myocardial injury in control patients (Cheung et al., 2006).

Finally, remote ischemic preconditioning induced by limb ischemia/reperfusion protects against endothelial ischemia/reperfusion injury as a consequence of arm ischemia/reperfusion in patients. Flow-mediated dilatation was reduced by arm ischemia/reperfusion but not when immediately preceded by remote ischemic preconditioning. Remote ischemic preconditioning did not protect after 4 h, but once again induced protection at 24 and 48 h, suggesting that remote ischemic preconditioning in humans has two phases of protection against endothelial ischemia/reperfusion injury: an early (short) and late (prolonged) phase (Loukogeorgakis et al., 2005).

C. Late Preconditioning

Although not as powerful as classic preconditioning, the late phase of preconditioning-induced protection against infarction is more prolonged and lasts up to 72 h (Baxter et al., 1997). In addition to an enhanced tolerance to irreversible ischemic injury, late preconditioning confers protection against other endpoints of ischemia/reperfusion injury including ischemia- and reperfusion-induced ventricular arrhythmias (Vegh et al., 1992) and postischemic myocardial dysfunction, i.e., myocardial stunning (Sun et al., 1995; Takano et al., 2000). The delayed protective effects of preconditioning can be induced in human right atrial tissue (Loubani et al., 2004)

and isolated rat cardiomyocytes subjected to simulated ischemia or hypoxia (Yamashita et al., 1994).

Similar to classic preconditioning, various pharmacological agents can induce delayed cardiac protection against the effects of acute myocardial ischemia. Experimental protocols involve the administration of pharmacological agents (e.g., adenosine receptor agonists, NO donor compounds, and prostacyclin derivatives) 24 to 72 h before an ischemia/reperfusion protocol (Heusch, 2001). Under these conditions, pharmacological delayed preconditioning has been shown to protect against many consequences of ischemia/reperfusion, including attenuation of early morphological changes, limiting of infarct size, attenuation of stunning and suppression of arrhythmias (Baxter and Yellon, 1998; Bolli, 2001; Baxter, 2002). Prolongation of the effective refractory period and of the action potential duration may contribute to suppression of arrhythmias by delayed pharmacological preconditioning. The protection is time- and dose-dependent, with optimal effects 24 to 48 h after treatment (Szekeres, 2005).

Limited evidence is available for the existence of late preconditioning as a naturally occurring phenomenon in humans. The possibility that exercise-induced myocardial ischemia might trigger an adaptive response that attenuates the consequences of a subsequent episode of exercise-induced or percutaneous coronary intervention-induced ischemia has been examined in patients with stable angina (Bilinska et al., 2000; Lambiase et al., 2003).

D. Postconditioning

As for ischemic preconditioning, ischemic postconditioning reduces infarct size in rat isolated hearts (Tsang et al., 2004; Bopassa et al., 2006) and rabbit isolated hearts (Darling et al., 2005; Yang et al., 2005) and in mice (Heusch et al., 2006), rats (Kin et al., 2004, 2005; Tang et al., 2006; Zatta et al., 2006), rabbits (Yang et al., 2004; Argaud et al., 2005; Chiari et al., 2005; Couvreur et al., 2006; Philipp et al., 2006), dogs (Zhao et al., 2003; Halkos et al., 2004), and pigs (Iliodromitis et al., 2006a) in vivo. There is also recent evidence that postconditioning limits injury in the human heart (Staat et al., 2005; for reviews, see Valen and Vaage, 2005; Vinten-Johansen et al., 2005; Kloner and Rezkalla, 2006; Ramzy et al., 2006; Yellon and Opie, 2006). The extent of infarct size reduction achieved by ischemic postconditioning is similar to (Zhao et al., 2003; Halkos et al., 2004; Fantinelli and Mosca, 2006) or slightly smaller than (Kin et al., 2004) that obtained by ischemic preconditioning, and protection by ischemic postconditioning is not enhanced by ischemic preconditioning (Halkos et al., 2004).

Postconditioning by short episodes of hypoxia/reoxygenation attenuates rat cardiomyocyte apoptosis after prolonged periods of hypoxia (30 min–3 h)/reoxygenation (60 min–6 h) (Sun et al., 2005, 2006; Dosenko et al., 2006; Wang et al., 2006). Ischemic postconditioning possesses a strong antiarrhythmic effect against persistent reperfusion-induced tachyarrhythmias in isolated rat hearts (Galagudza et al., 2004) and in rats in vivo (Kloner et al., 2006). Although a controversy exists whether or not ischemic postconditioning protects against endothelial ischemia-reperfusion injury in humans (Loukogeorgakis et al., 2006; Dragoni et al., 2006), ischemic postconditioning, with a regimen of ischemia/reperfusion episodes reducing infarct size, does not protect against myocardial stunning in mouse isolated hearts (Kin et al., 2005) or rabbit and dog hearts in vivo (Couvreur et al., 2006).

For ischemic postconditioning the brief episodes of ischemia/reperfusion have to be applied just after the prolonged ischemic insult (for a review, see Heusch, 2004; Tsang et al., 2005; Vinten-Johansen, 2007; Crisostomo et al., 2006a; Garcia-Dorado et al., 2006). The protection from ischemic postconditioning depends on the number of short episodes of ischemia/reperfusion especially in pigs (Iliodromitis et al., 2006a; Schwartz and Lagranha, 2006), but once protection is achieved increasing the number of postconditioning ischemia/reperfusion episodes does not further decrease infarct size in isolated rat hearts (Tsang et al., 2004) and in rats (Kin et al., 2004) and rabbits (Yang et al., 2004) in vivo. The cardioprotection by ischemic postconditioning is limited to coronary occlusions of less than 45 min in conscious rats (Tang et al., 2006), and the extent of protection might differ between males and females (Crisostomo et al., 2006b). Renal artery occlusion and release 1 min before coronary artery reperfusion provided potent myocardial infarct size reduction in rats (Kerendi et al., 2005) showing that a "remote postconditioning" phenomenon also exists.

1. Pharmacological Postconditioning. The ischemic trigger during the first minute(s) of reperfusion to reduce infarct size can be substituted by pharmacological agents such as inhalational anesthetics, which are applied only during the initial minutes of reperfusion and rapidly washed out because of their short half-life (Chiari et al., 2005; Feng et al., 2005; for a review, see Weber et al., 2005). As with ischemia (Iliodromitis et al., 2006a; Schwartz and Lagranha, 2006), a threshold concentration of isoflurane is required to achieve cardioprotection, but when a subthreshold isoflurane concentration is combined with a subthreshold ischemic or pharmacological stimulus immediately at the onset of reperfusion, infarct size is again reduced in rabbit hearts (Chiari et al., 2005; Krolikowski et al., 2005; Weihrauch et al., 2005; Pagel et al., 2006). Although the effect on irreversible tissue injury appears to be similar between ischemic and pharmacological postconditioning, only pharmacological preconditioning with isoflurane prevents activation of the genomic postischemic remodeling program in rat hearts (Lucchinetti et al., 2005). Whereas ischemic pre- and postconditioning do not confer additive protection, infarct size reduction achieved by ischemic preconditioning in rabbit isolated

hearts (Tessier-Vetzel et al., 2006) or pharmacological preconditioning in rats in vivo (Obal et al., 2005) is further reduced by pharmacological postconditioning.

IV. Effects of Major Risk Factors on Ischemia/Reperfusion Injury and Cardioprotective Strategies

A. Left Ventricular Hypertrophy

The postgestational myocardium has an inherent capacity to undergo changes in size and shape in response to a variety of stimuli including hemodynamic stress, hormones, neural influences, and autacoid factors. The term "cardiac remodeling" is used to describe this plasticity of cardiac structure and is usually restricted to alterations of cardiac structure in disease states; the normal postgestational growth of the heart or the myocardial hypertrophy that results from extended exercise are not usually considered as examples of cardiac remodeling (for a comprehensive review of cardiac remodeling, see Swynghedauw, 1999). Left ventricular hypertrophy (LVH) as a consequence of sustained elevation of arterial blood pressure is an important form of cardiac remodeling (Massie et al., 1989; Diamond and Phillips, 2005; Prisant, 2005). Although LVH may be associated with conditions other than hypertension, including myocardial infarction, anemia, aortic valve disease, hyperthyroidism, obesity, and renal disease, hypertension is the most common cause of LVH. LVH associated with any pathological precursor may ultimately predispose to the development of congestive cardiac failure. In this section, we consider specifically the effects of compensated LVH (i.e., before the onset of cardiac failure) as a risk factor, its effects on responses to ischemia/reperfusion, and the effectiveness of cardioprotective strategies in compensated LVH. We also consider here experimental studies that have examined cardioprotective responses in hyperthyroid animals as thyroid hormone exerts a well recognized trophic effect on myocardium. However, cardioprotection in other remodeling states, namely postinfarct remodeling and congestive cardiac failure are considered separately in section IV.B.

1. Hypertensive Left Ventricular Hypertrophy as a Risk Factor. Estimates of the prevalence of LVH in the hypertensive population have varied widely and this variability is probably due to 1) the difficulty in defining the normal limits of LV size and hence in defining pathological deviations from these limits and 2) variability in the sensitivity of different methods of assessing LVH. Echocardiography is more sensitive than electrocardiography and estimates of LVH prevalence using echocardiography suggest that in unselected hypertensive populations, 20% have LVH (Kannel et al., 1969; Hammond et al., 1986; Savage et al. 1987; Diamond and Phillips, 2005). The prevalence is even higher in patients with severe hypertension or malignant hypertension (Shapiro et al., 1981) and in aged patients (Tuzcu et al., 1989).

The relationships between LVH and cardiovascular morbidity and mortality have been assessed extensively and most reliably in the Framingham cohort, a large general population, and in other smaller studies of selected hypertensive individuals. Among the traditional risk factors, with the exception of age, the presence of LVH on an electrocardiogram is the strongest independent predictor of cardiovascular events, including death from coronary heart disease, sudden cardiac death, congestive cardiac failure, and stroke (Kannel et al., 1975; Levy et al., 1988, 1990). For example, Framingham Study data show all-cause cardiovascular mortality increased 8-fold in men with electrocardiographically detected LVH relative to those without LVH, an 8-fold increase in the incidence of cardiac failure, and a 6-fold increase in the rate of sudden cardiac death (reviewed in Massie et al., 1989; Prisant, 2005).

2. Hypertensive Left Ventricular Hypertrophy and Myocardial Ischemia/Reperfusion. Explanations for the strong associations between hypertension, LVH, and ischemic heart disease are incomplete, and the relationship is undoubtedly complex. Hypertension and LVH exert specific effects that predispose the hypertensive heart to development of episodes of ischemia/reperfusion and a deleterious response to them. Experimentally, LVH is a universal feature of hypertension, irrespective of the widely differing pathophysiological mechanisms of pressure overload. The most commonly encountered models include the spontaneously hypertensive rat (SHR), originally developed by selective breeding of Wistar-Kyoto rats, renovascular stenosis in the rat, the Goldblatt two-kidney, one-clip model (originally developed in the dog), mineralocorticoid-induced hypertension [deoxycorticosterone acetate (DOCA)-salt]; the Dahl salt-sensitive rat; chronic inhibition of NO synthase with nitro-L-arginine methyl ester, abdominal aortic stenosis (see Doggrell and Brown, 1998, for a comprehensive survey and detailed bibliography of rat models of hypertensive LVH). In broad terms within any of these models, LV mass enlargement correlates well with the degree of pressure overload although there may be significant variations between models in the temporal onsets of LV enlargement and of decompensation and heart failure. For example, it has been reported that in the SHR, the onset of LV enlargement occurs before the detection of arterial hypertension (Sen et al., 1974).

a. Effects of left ventricular hypertrophy on the coronary circulation. Evidence from experimental and clinical studies suggests that hypertension accelerates the development of atherosclerosis in coronary arteries (Chobanian et al., 1986; Doyle, 1990). Additionally, LVH itself is associated with changes in the density, structure, and vasodilator capacity of the coronary vasculature so that although absolute coronary flow in hypertrophied hearts is increased, there is reduced cross-sectional density of endomyocardial capillaries and reduced coronary reserve even in the absence of

detectable coronary atherosclerosis (Vogt et al., 1990; Rakusan and Wicker, 1990).

b. Experimental ischemia/reperfusion injury in left ventricular hypertrophy. There is some experimental evidence that hypertrophied myocardium is at greater risk of sustaining injury and electrophysiological disturbances after ischemia/reperfusion. During global ischemia, the development of rigor contracture occurs earlier and is exaggerated in rat hearts with hypertensive LVH (Attarian et al., 1981; Peyton et al., 1982). Indeed, the clinical phenomenon of "stone heart" during cardiothoracic surgery was first described in the hypertrophied heart (Cooley et al., 1972). During reperfusion, recovery of contractile function is depressed and lactate dehydrogenase or creatine kinase release is increased in hypertrophied hearts subjected to global ischemia, suggestive of greater susceptibility to ischemia/reperfusion injury (Snoeckx et al., 1986; Anderson et al., 1987, 1990). Many biochemical and metabolic alterations have been advanced as explanations for the increased sensitivity of hypertrophied myocardium to ischemia/reperfusion injury. These include altered mitochondrial energetics and ATP production and changes in glycolytic metabolism and lactate accumulation during ischemia (Obata et al., 1990; Anderson et al., 1990; Osbakken et al., 1992) and increased oxidative stress characterized by increased ROS-generating capacity and reduced antioxidant potential (Batist et al., 1989), although these data have not been consistent (Singal et al., 1991).

It is important to note that there are very few studies of infarction in hypertensive animals with infarct size as the principal endpoint; the studies that have been undertaken have *not* clearly demonstrated increased infarct size in the hypertrophied heart. In a model of 48-h coronary ligation without reperfusion (a model now considered of questionable reliability and relevance in the reperfusion era), Koyanagi et al. (1982) showed that mortality and infarct size relative to the area at risk was increased in dogs with renal hypertension. However, the same group later showed that blood pressure reduction with sodium nitroprusside before coronary occlusion in animals with LVH reduced mortality and infarct size to levels measured in normotensive animals, suggesting that perfusion pressure rather than LVH per se was the most important factor potentiating the irreversible injury due to ischemia/reperfusion in hypertensive animals (Inou et al., 1987). In both the ex vivo rat heart infarct model (Ebrahim et al., 2007a) and an in vivo rat model of coronary occlusion with reperfusion (Speechly-Dick et al., 1994), infarct size was not different between DOCA-salt hypertensive and normotensive animals.

c. Arrhythmias in left ventricular hypertrophy models. As indicated in section IV.A.1., the incidence of sudden cardiac death, often presumed to be due to ventricular tachyarrhythmias (typically ventricular tachycardia progressing to ventricular fibrillation) is closely associated with hypertensive LVH in epidemiological studies (Schatzkin et al., 1984; Saadeh and Jones, 2001). Increased frequency of ventricular ectopic beats is also observed commonly in hypertensive patients. The electrophysiological changes underlying spontaneous ectopic activity in LVH are not well understood, but hypertrophied myocardium clearly represents a complex substrate for arrhythmic disturbance. Prolongation of the cardiac action potential has been reported in experimental animal models, and abnormalities in individual currents, including I_{CaL}, I_{Na}, and I_{to}, have been described in hypertrophied cardiac myocytes (Keung and Aronson, 1981; Keung, 1989; Ryder et al., 1993; Yokoshiki et al., 1997). Increased interstitial fibrosis, a universal feature of hypertensive LVH (Weber, 2000), may create zones of altered electrical conductance. Fibrosis may explain the increased dispersion of action potential duration seen in some studies and may predispose to arrhythmia development through reentrant mechanisms (Cameron et al., 1983).

There is evidence from experimental studies that the incidence and the severity of ischemia-induced tachyarrhythmias are augmented in LVH. For example, Bélichard et al. (1987) showed that the incidence and duration of ventricular fibrillation during 30 min of coronary artery occlusion was significantly greater in SHR than in normotensive controls. This greater sensitivity to ventricular fibrillation was apparently due to LVH rather than to hypertension because lowering blood pressure before coronary occlusion, by acute treatment with nicardipine, did not influence the development of ventricular fibrillation. In isolated hearts from aortic banded rats, the incidence of ventricular fibrillation during 30 min of coronary artery occlusion was increased from 27% in normotensive control hearts to 67% in moderately hypertrophied hearts and 100% in severely hypertrophied hearts (Kohya et al., 1988). Moreover, this increased incidence of ischemia-induced ventricular fibrillation was related to LVH specifically and not to LV mass as the incidence of ventricular fibrillation in hearts of comparable size from senescent normotensive rats was only 33% (compared with 67% in moderately hypertrophied hearts). Additionally, reperfusion-induced arrhythmias were increased in experimental LVH. In isolated hearts from DOCA-salt hypertensive rats, the incidence and duration of ventricular fibrillation during reperfusion after 10 min of coronary occlusion were higher than in normotensive control hearts. Moreover, after spontaneous regression of LVH in rats after withdrawal of DOCA-salt, the incidence of reperfusion-induced ventricular fibrillation returned to levels seen in normotensive controls (Baxter and Yellon, 1992).

In summary, many experimental studies of hypertrophied myocardium have demonstrated that pressure overload hypertrophy, even in the stage of compensation before the onset of dilatation and functional decline, is

accompanied by metabolic and other biochemical alterations. These could predispose the hypertrophied myocardium to more severe ischemia/reperfusion injury. Although experimental endpoints such as recovery of contractile function during reperfusion and arrhythmia development indicate a clearly deleterious interaction between hypertrophy and ischemia/reperfusion, the evidence that hypertrophied hearts sustain a greater degree of irreversible tissue injury during ischemia/reperfusion is less convincing.

3. Experimental Cardioprotection in Hypertensive Left Ventricular Hypertrophy. In turning to consider the effects of cardioprotective interventions in hypertensive LVH, it is important at the outset to distinguish between the effects of chronic treatment regimens that could influence the development or regression of hypertrophy and acute treatments that exert a primary action during ischemia/reperfusion. This distinction is critical because, as noted above, regression of LVH by blood pressure lowering leads to a reduction in susceptibility to ischemia/reperfusion endpoints, especially arrhythmias. In SHR rats pharmacological induction of LVH regression over 6 weeks with angiotensin-converting enzyme (ACE) inhibition or angiotensin II receptor antagonism was associated with normalization of action potential duration dispersion and other electrophysiological parameters and a reduction in susceptibility to ischemia-induced ventricular tachycardia and ventricular fibrillation (Kohya et al., 1995). There is evidence that this normalization of action potential duration is related to a restoration of I_{to} density after LVH regression (Yokoshiki et al.,1997). However, additional factors determining re-entrant arrhythmias, especially interstitial fibrosis, and stretch-activated ion channel activity, may be reversed or attenuated by LVH regression.

The influence of acutely applied cardioprotective maneuvers on susceptibility to ischemia/reperfusion injury in established LVH has been relatively little studied to date. Arguably the most informative studies have been undertaken in the last decade, examining the effects of preconditioning in hypertrophied myocardium, but it should be noted that there have been no published studies of postconditioning cardioprotection in hypertrophied myocardium so far.

a. Ischemic preconditioning in left ventricular hypertrophy. Several studies have reported the maintenance of classic ischemic preconditioning in different rodent models of pressure-overload hypertrophy. The first study of this type confirmed that ischemic preconditioning was able to elicit a full infarct-limiting effect in DOCA-salt hypertensive rats subjected to coronary occlusion and reperfusion in vivo (Speechly-Dick et al., 1994). In this study, 4 weeks of DOCA-salt treatment induced a 40% relative increase in heart weight/body weight ratio without any clinical signs of malignant hypertension or heart failure. A single 5-min coronary occlusion followed by a 10-min reperfusion, a classic preconditioning algorithm, before a 45-min coronary occlusion resulted in substantial limitation of infarction and a reduced incidence of ventricular tachyarrhythmias in both normotensive and hypertensive animals. The ability of ischemic preconditioning to limit infarct size in isolated hearts from DOCA-salt hypertensive rats has recently been confirmed (Ebrahim et al., 2007a). After the initial study of Speechly-Dick et al. (1994), subsequent studies in isolated heart preparations have confirmed the ability of classic ischemic preconditioning protocols to elicit a cardioprotective response, with postischemic recovery of contractile function as the principal endpoint, using other rodent models of hypertension including the SHR strain (Boutros and Wang, 1995), abdominal aortic banding (Pantos et al., 1996), the (mREN-2)27 transgenic rat (Randall et al., 1997), and the Dahl salt-sensitive rat strain (Butler et al., 1999). Rajesh et al. (2004) have reported that in pressure overload due to abdominal aortic banding, preconditioning with four 3-min coronary artery occlusion-reperfusion cycles before a 30-min coronary occlusion limited infarct size as effectively as it did in normotensive hearts in vivo and that the protection afforded by preconditioning in LVH was abolished by glibenclamide or 5-HD, implying that the involvement of K_{ATP} channels in the protection afforded by preconditioning extends to hypertrophied hearts.

Considered together, the above studies suggest that in moderate pressure-overload LVH, before the onset of decompensation, the ischemic preconditioning mechanism remains intact. However, this situation may not apply in heart failure (discussed in section IV.B.) or in hypertension of long duration but before the onset of decompensation. Moolman et al. (1997) reported that in 12-month-old New Zealand genetically hypertensive rats, preconditioning did not enhance postischemic functional recovery or attenuate creatine phosphate release during reperfusion in isolated hearts subjected to global ischemia and reperfusion, although preconditioning was protective in age-matched normotensive control hearts. This finding is in contrast to other studies and highlights an important distinction between preconditioning in early hypertension and chronic hypertension and, indeed, the potential interaction between aging and hypertension. In support of this contention, Ebrahim et al. (2007b) have characterized the preconditioning response in progressive experimental hypertension. In 3- to 4-month-old SHR with established LVH, ischemic preconditioning of isolated perfused hearts with two 5-min periods of global ischemia before a 30-min coronary artery occlusion limited infarct size as efficiently as in normotensive control animals. The efficacy of ischemic preconditioning was preserved in 7- to 8-month-old SHR and normotensive hearts but was lost in 11- to 13-month-old hearts.

b. Pharmacological preconditioning in left ventricular hypertrophy. Although experimental studies suggested that the classic ischemic preconditioning response is maintained in hypertensive LVH, few studies have examined

pharmacological preconditioning or cardioprotective strategies in hypertensive LVH. In 4-week DOCA-salt hypertensive rat hearts, Ebrahim et al. (2007a) showed that bradykinin responses are severely impaired in even moderate LVH. Coronary vasodilator responses to bradykinin were abrogated in hypertrophied hearts and the infarct-limiting action of preischemic bradykinin treatment was markedly attenuated, even though ischemic preconditioning was equally effective in normotensive and hypertrophied hearts. Boutros and Wang (1995) reported that preischemic adenosine or bethanechol treatment of SHR and normotensive hearts resulted in improved postischemic contractile recovery and coronary flow although the protective effects of the pharmacological agents were better in normotensive than in hypertensive hearts and adenosine was better than bethanechol.

Two cautionary notes should be sounded against extrapolation of the ischemic preconditioning studies in experimental LVH to clinical cardioprotection. First, the majority of these experimental studies have been undertaken in young animals with hypertension of relatively short duration and a modest degree of well compensated LVH. Second, experimental ischemic preconditioning, perhaps with multiple cycles of ischemia, recruits multiple autacoid mediators whereas a pharmacological preconditioning "mimetic" may rely on the manipulation of a single autacoid system. For example, the experimental infarct-limiting effects of ACE inhibitors may be in large part due to inhibition of kinin degradation (for a review, see Baxter and Ebrahim, 2002), and the acute infarct-limiting effect of statins, independent of serum cholesterol lowering, appears to be due to enhanced NO biosynthesis via promotion of PI3K/Akt signaling (Bell and Yellon, 2003b; Wolfrum et al., 2003; Elrod and Lefer, 2005). Yet, in hypertension, as in other cardiovascular diseases, a generalized endothelial dysfunction occurs (Drexler and Hornig, 1999); this dysfunction may extend to the myocardium itself where there is a reduction in the capacity of hypertrophied myocardium to synthesize NO (MacCarthy and Shah, 2000; Pacher et al., 2005). Moreover, alterations of the complex intracellular signaling cascades underpinning preconditioning and postconditioning have barely been addressed in the hypertensive heart. The clinical benefits of ACE inhibition in preventing cardiovascular events are not clearly related to the potentiation of kinin actions seen in experimental studies. However, it is theoretically conceivable that the anti-ischemic effects of ACE inhibitors and other kinin-potentiating therapeutic strategies could be limited in patients with coincident LVH. This is an issue of considerable complexity because ACE inhibitors promote regression of LVH, although the extent to which kinin-associated cardioprotection is restored in hypertrophied myocardium that has undergone regression has not been defined experimentally. Clearly, further experimental investigation of cardioprotective responses in LVH is warranted, and the limited experimental information available at present needs to be corroborated in translational studies of myocardial ischemia/reperfusion in hypertensive patients. However, at present, the clinical efficacy of preconditioning-like therapies in patients with hypertensive LVH cannot be assumed or taken for granted.

4. Hyperthyroid Left Ventricular Hypertrophy. The thyroid hormones, thyroxine and its biologically active metabolite triiodothyronine (T3), exert multiple and complex actions on the heart and circulation, and the heart is a principal target organ in both hypothyroid and hyperthyroid diseases (Woeber, 1992; Polikar et al., 1993; Klein and Ojamaa, 2001; Boelaert and Franklyn, 2005; Kahaly and Dillmann, 2005). Patients with overt or subclinical hyperthyroidism are at increased risk of cardiovascular morbidity and mortality, the most significant consequences of hyperthyroidism being atrial fibrillation leading to increased risk of arterial thromboembolism, and high-output congestive heart failure associated with chronic volume overloading. Although on its own, hyperthyroidism is not clearly identified as a risk factor for the development of coronary artery disease, the condition may coexist with other recognized vascular risk factors or may develop after the onset of coronary disease. Thus, some patients with hyperthyroidism may have coronary artery disease independent of the hyperthyroid state. The legitimate question arises as to whether the hyperthyroid heart displays altered responses to ischemia/reperfusion.

In relation to the present discussion, hyperthyroidism is of particular interest for the major reason that cardiomegaly with a hypertrophic component is a key feature of hyperthyroidism (Klein and Ojamaa, 2001). Moreover, the hypertrophied hyperthyroid heart displays remarkable metabolic alterations, including an increase in ATP consumption through increased glycolytic and glycogenolytic fluxes (Nishiki et al., 1978; Seymour et al., 1990), increased heart rate, increased oxygen consumption, and increased ROS production.

The structural and biochemical changes outlined in the preceding might be expected to render the hyperthyroid heart more sensitive to ischemia/reperfusion injury. Asahi et al. (2001) and Venditti et al. (2002) reported severely depressed postischemic functional recovery in hyperthyroid hearts. However, in the majority of experimental studies, the responses of hyperthyroid hearts to ischemia/reperfusion are not impaired, and there is some evidence that hyperthyroidism of intermittent duration (14–28 days) as well as acute pretreatment with thyroid hormones is protective against myocardial ischemia/reperfusion. Buser et al. (1990) showed that isolated hyperthyroid rat hearts displayed postischemic functional recovery comparable with euthyroid hearts, whereas hypertrophied hearts from rats with pressure overload due to aortic banding recovered poorly. Pantos et al. (1999, 2000, 2002) showed enhanced postischemic functional recovery in hyperthyroid rat hearts, associ-

ated with earlier and greater peak contracture than normal hearts, a consistent feature of global ischemia models of ischemic preconditioning. Interestingly, the effect on contracture seen in hyperthyroid hearts was additive to that of ischemic preconditioning (Pantos et al., 2001). Pantos and coworkers have proposed that hyperthyroid hypertrophy represents a cardioprotected phenotype rather than a cardiomyopathic state (for a review, see Pantos et al., 2004). It should be noted, however, that these experimental studies have been undertaken in animals subjected to relatively short-term thyroid hormone excess (typically 14 days).

The experience of Pantos's group with intermediate-term thyroid treatment summarized above is consistent with a large body of experimental evidence showing that thyroid hormones are acutely cardioprotective in ischemia/reperfusion through mechanisms that are not clearly defined (Novitzky et al., 1988; Dyke et al., 1991; Liu et al., 1998b). These studies, demonstrating very rapid protective actions of thyroid hormone, suggest an action mediated by binding to extranuclear receptors and distinct from actions mediated by transcriptional regulation through the classic nuclear receptors. However, subacute or intermediate-term treatment with T3 could also recruit other cardioprotective mechanisms via transcriptional up-regulation of various cytoprotective proteins, including some of those associated with the classic and late preconditioning pathways described in Section II. For example, there is evidence that in hyperthyroid rat myocardium, increased phosphorylation of PKC-ε and decreased phosphorylation of p38 MAP kinase are present (Pantos et al., 2001), whereas in isolated rat cardiomyocytes, subacute (24-h) application of T3 is associated with activation of the prosurvival kinase Akt (Kuzman et al., 2005). The 70- and 27-kDs heat shock proteins (HSP70 and HSP27) associated with late preconditioning have been shown to be up-regulated in the hyperthyroid heart (Pantos et al., 2006), although a causal relationship between their induction and the cardioprotected phenotype in hyperthyroid hypertrophy has not been conclusively demonstrated.

In summary, experimental studies suggest that hypertrophied myocardium in systemic arterial hypertension and hyperthyroidism display altered responses to ischemia/reperfusion compared with naive myocardium. Although myocyte hypertrophy is a common feature of these two pathological conditions, the biochemical changes underpinning the metabolic features in the two conditions show notable differences, with hypertensive myocardium displaying evidence of increased sensitivity to ischemia/reperfusion injury, whereas hyperthyroid myocardium has decreased sensitivity. With regard to the conditioning paradigms of cardioprotection, in hypertensive LVH before the onset of functional decompensation, classic ischemic preconditioning mechanisms remain intact. However, limited evidence suggests that perturbations of individual autacoid triggers, such as bradykinin, may be a feature of hypertensive LVH. In hyperthyroid hypertrophy, the majority of evidence suggests that myocardium displays features of an ischemia/reperfusion-tolerant phenotype, possibly as a result of up-regulation of cardioprotective kinases and/or cytoprotective proteins such as HSPs. It should be borne in mind that the ability of T3 or hyperthyroidism to protect against ischemia/reperfusion has been identified almost exclusively using postischemic functional recovery as the endpoint of protection. Arguably, the protective effect should be assessed using infarct size, as this is the standard endpoint. Moreover, the relationship between thyroid hormone and resistance to ischemia/reperfusion is likely to be even more complex than that described here because there is evidence that in experimental models of intermediate-term *hypo*thyroidism, the heart is better able to withstand an ischemia/reperfusion insult (Pantos et al., 2003). Arguably, further investigation of the actions of thyroid hormones and the interactions between hypertrophy mechanisms and myocardial ischemia/reperfusion mechanisms may provide important insights into cytoprotective mechanisms. However, it is important to acknowledge that most of the studies in this area to date have been descriptive in their approach and have had a very limited impact in linking the molecular pathology of hypertrophic diseases to the modification of cardioprotection.

B. Cardioprotection and Myocardial Infarction, Remodeling, and Heart Failure

Large multicenter studies on patients with cardiovascular disease, such as the Candesartan in Heart Failure Assessment of Reduction in Mortality and Morbidity (CHARM) trial, identified prognostic factors influencing the incidence of cardiovascular death and rate of hospitalization; factors with the highest odds ratios were patients' age, New York Heart Association class, and a history of previous myocardial infarction. Despite a better outcome with early reperfusion strategies for the treatment of acute myocardial infarction, mortality remains high and the incidence of congestive heart failure continues to increase (>140% within the last 30 years) (for a recent review, see Vinten-Johansen, 2007). An increased cardiovascular death rate in postinfarcted, failing hearts suggests that endogenous protective mechanisms against ischemia/reperfusion injury might be lost or attenuated by the ongoing disease, and, indeed, myocardial infarction with the subsequently occurring ventricular remodeling process as well as heart failure are characterized by a variety of morphological [hypertrophy (see above) and fibrosis] and biochemical alterations (receptor down-regulation, alteration in G-proteins and protein kinase activation) which per se might impact on the signal transduction cascade of pre- and postconditoning-induced cardioprotection. Furthermore, considerable attention has been given to the potential role of mitochondrial defects in the genesis and

progression of heart failure. Enzyme activities (complexes I, III, and IV) of the electron transport chain are decreased in failing hearts (Buchwald et al., 1990), and mitochondrial dysfunction has been proven to abolish endogenous cardioprotection in diabetic hearts (Hassouna et al., 2006) (see below).

Thus, there is clearly a need to improve cardioprotection, and a better understanding of the mechanisms involved in ischemic and especially reperfusion injury might help to define new therapeutic strategies. In this review, however, we will not address the issue of acute or continuous drug treatment but instead will address the issue of preconditioning (stimulus/drug removed before the prolonged ischemic period) or postconditioning (drug added immediately upon reperfusion).

1. Postinfarction Remodeling. In rabbits, coronary artery ligation was induced on a marginal branch of the left coronary artery and 2 weeks later hearts were isolated and subjected to a global ischemia (30 min)/reperfusion (2 h) protocol. Ischemic preconditioning failed to reduce infarct size in postinfarcted, remodeled hearts, whereas cardioprotection by pharmacological preconditioning with diazoxide remained unaffected (Miki et al., 2000), suggesting alterations in the upstream signaling cascade by the remodeling process. Indeed, activation of protein kinase C (measured as translocation) by ischemic preconditioning was decreased post-MI (Miki et al., 2003). Attenuating the remodeling process by administration of the angiotensin II type 1-receptor antagonist valsartan (Wong et al., 2002), an intervention known to reduce cardiovascular mortality in patients, (Maggioni et al., 2002) restored both PKC activation and infarct size reduction by ischemic preconditioning (Miki et al., 2000, 2003). Thus, continuous activation of the cardiac renin-angiotensin-system with a subsequent increase in the myocardial angiotensin II concentration post-MI might induce down-regulation of central parts of the upstream cardiomyocyte signal transduction cascade of ischemic preconditioning; however, direct activation of the downstream signaling cascade is once again capable of inducing cardioprotection Accordingly, ischemic postconditioning (six cycles of 10 s of ischemia/reperfusion each) (Zhu et al., 2006) as well as pharmacological postconditioning with isoflurane (2.1%, 15 min) (Feng et al., 2006) were effective in reducing infarct size and activating the salvage kinase pathway in rat hearts post-MI (6 weeks); blockade of PI3K, as in the intact hearts, abolished such cardioprotection.

In contrast with the effect on cardiomyocytes, hypoxic preconditioning before induction of ischemia decreased endothelial cell apoptosis, increased capillary and arteriolar density, and improved myocardial perfusion, thereby reducing the progression toward heart failure (better preserved left ventricular function) in rats 1, 2, and 3 weeks after permanent left anterior descending coronary artery occlusion (Sasaki et al., 2001, 2002).

2. Postinfarction Heart Failure. Patients with heart failure display a very high incidence of arrhythmias and sudden death that is often preceded by short episodes of ischemia. In a dog model of healed myocardial infarction and superimposed heart failure, acute ischemia (4 min of coronary artery occlusion) provoked ventricular tachycardia and fibrillation in 9 of 12 animals; intrathecal administration of clonidine reduced the incidence of arrhythmias to 25% (3 of 12 dogs) consistent with the idea that increased cardiac sympathetic nerve activity contributed to the development of ischemia-induced left ventricular arrhythmias in failing hearts (Issa et al., 2005).

Whereas ischemic preconditioning reduces ischemia/reperfusion-induced ventricular arrhythmias in intact hearts of most species except pigs (see section III.A.), ischemic preconditioning prolonged the duration of ischemia-induced electrical uncoupling in failing rabbit heart muscle (in contrast with shortening the duration of electrical uncoupling in intact hearts). The extent of pressure- and volume-induced heart failure correlated with the prolongation of electrical uncoupling (Dekker et al., 1998).

Right atrial appendages obtained from patients with left ventricular ejection fraction (LVEF) >50%, LVEF between 30 and 50%, and LVEF <30% were subjected to either aerobic conditions for 210 min, 90 min of ischemia followed by 120 min of reoxygenation or preconditioning by 5 min of ischemia/5 min of reoxygenation before 90 min of ischemia/120 min of reoxygenation. Ischemia caused creatine kinase leakage into the medium that was similar in normal and diseased tissue. Preconditioning attenuated creatine kinase release in LVEF >50% and LVEF 30 to 50% but not in LVEF <30%. In contrast, diazoxide induced similar protection in all groups (Ghosh et al., 2001). Thus, as in remodeled myocardium, the defect in the signal transduction cascade in failing hearts might be upstream of the level of mitochondrial K_{ATP} channels.

One potential difference in upstream signaling might relate to alterations in adenosine metabolism in diseased hearts. In patients with heart failure, ecto-5′-nucleotidase activity is increased leading to an increased serum adenosine level (Kitakaze and Hori, 1998; Kitakaze et al., 1999). Continuous adenosine receptor stimulation, however, was associated with the loss of cardioprotection afforded by ischemic preconditioning due to tachyphylaxis (Hashimi et al., 1998) potentially explaining the above finding as well as the failure of the adenosine A_1/A_{2A} receptor agonist AMP579 to reduce infarct size in patients with impaired left ventricular function undergoing percutaneous coronary intervention after acute ST-segment elevation [AMP579 Delivery for Myocardial Infarction Reduction (ADMIRE) study]. Only at very high concentrations of AMP579 was a trend toward greater

myocardial salvage detected in patients with an anterior acute MI (Kopecky et al., 2003).

Taken together, the existing data suggest that pre- and postconditioning protect the myocardium against irreversible injury in a setting of acute coronary artery occlusion/reperfusion in patients. However, in post-MI remodeled hearts with left ventricular dysfunction, there are defects in the upstream signaling cascade of ischemic preconditioning. Pharmacological approaches bypassing the initial steps within the signaling cascade or approaches such as postconditioning, however, may still be capable of reducing irreversible tissue injury in such diseased hearts.

C. Hyperlipidemia and Atherosclerosis

1. Hyperlipidemia as a Risk Factor. Hyperlipidemia, especially hypercholesterolemia, is regarded as an independent risk factor in the development of ischemic heart disease including myocardial infarction. Epidemiological studies showed that there is a strong relationship between the elevation of serum total cholesterol concentration and the morbidity and mortality of myocardial infarction (Kromhout et al., 1988; Roberts, 1995; Houterman et al., 1999; Fang and Alderman, 2006). Previously, this was attributed solely to the development of coronary atherosclerosis due to hypercholesterolemia; the possibility of hypercholesterolemia-induced deterioration of endogenous adaptive mechanisms against myocardial ischemia/reperfusion injury was not considered in hypercholesterolemic patients. In the last decade, the interest of the scientific community turned to the effect of hyperlipidemia on myocardial adaptation to ischemia and the underlying cardioprotective mechanisms. However, despite intensive research, the controversy still remains on whether experimental hyperlipidemia influences the severity of myocardial ischemia/reperfusion injury and whether it interferes with the cellular mechanisms of cardioprotection.

2. Ischemia/Reperfusion Injury in Hyperlipidemia. An early study by Golino et al. (1987) showed that acute hypercholesterolemia, induced by a 2% cholesterol-enriched diet for 3 days, independently from its atherogenic effect increased the extent of myocardial infarct size after acute coronary occlusion and reperfusion in rabbits in vivo. In other studies, infarct size was also increased in rabbits fed a high-cholesterol diet for 8 weeks (Ma et al., 1996), 4 weeks (Jung et al., 2000), or 4 days (Hoshida et al., 1996). Wang et al. (2003) showed that experimental hyperlipidemia induced by an 8-week hyperlipidemic diet increased infarct size and apoptotic cell death and demonstrated for the first time that these effects could be inhibited by pharmacological blockade of the caspase cascade in an open-chest acute coronary occlusion/reperfusion rabbit model. Ferdinandy's group have also shown that a cholesterol-enriched diet for 8 weeks led to increased ST-segment elevation in response to rapid pacing-induced ischemia (a model of demand ischemia) in conscious rabbits (Szilvassy et al., 1995). Hearts of apolipoprotein E and low-density lipoprotein receptor double knockout mice (ApoE/LDLr$^{-/-}$) fed an atherogenic diet for 6 to 8 months had worse postischemic function and increased infarct size and troponin T release compared with genetic controls (Li et al., 2001). In genetic noninsulin-dependent Zucker diabetic fatty rats, 4-week cholesterol feeding increased infarct size (Hoshida et al., 2000). Increased susceptibility of the heart to acute ischemia has been also confirmed in hyperlipidemic patients during coronary angioplasty (Ungi et al., 2005). The aforementioned studies showed that hyperlipidemia per se leads to a significant aggravation of myocardial ischemia/reperfusion injury.

Some studies, however, show that hyperlipidemia does not influence the outcome of ischemia/reperfusion injury or may even render the heart more resistant to ischemic stress. An interesting study by Girod et al. (1999) showed that 2 weeks of a high-cholesterol diet increased infarct size in low-density lipoprotein receptor knockout (LDLr$^{-/-}$) mice compared with wild-type mice after 30 min of ischemia and 120 min of reperfusion. However, a high-cholesterol diet for 12 weeks resulted in a significant decrease in infarct size in both wild-type and LDLr$^{-/-}$ mice. In another study, ischemia/reperfusion resulted in a deterioration of cardiac contractile function in isolated hearts of rabbits fed a 2% cholesterol-enriched diet for 2 to 3 weeks compared with rabbits fed the same diet for a longer duration (5–16 weeks) or rabbits fed a normal diet (Tilton et al., 1987). In hearts isolated from rats fed 2% cholesterol-enriched diet for 24 weeks, baseline left ventricular end-diastolic pressure was elevated; however, ischemic and postischemic cardiac functional parameters were not significantly impaired (Ferdinandy et al., 1997, 1998a). In hearts isolated from rabbits after a 6-week feeding with 2% cholesterol, although preischemic cardiac contractile function was significantly lower, no significant differences were observed upon reperfusion compared with controls (Le Grand et al., 1995). The reason that hyperlipidemia in some ex vivo rat and rabbit heart models and in LDLr$^{-/-}$ mice may not significantly impair or may even improve recovery of postischemic contractile function, especially after a long-term high-cholesterol diet, is not known. However, it should be noted that because of the development of severe atherosclerosis and liver failure, long-term hyperlipidemia may lead to a number of extracardiac pathological conditions that may further influence the susceptibility of the myocardium to ischemia in various experimental animal models of hyperlipidemia and atherosclerosis.

3. Cardioprotection by Pre- and Postconditioning in Hyperlipidemia. A number of studies have examined the ability of preconditioning and postconditioning to protect the heart in different experimental models of hyperlipidemia and in hyperlipidemic patients; however, discrepan-

cies exist in the literature as to whether hyperlipidemia interferes with cardioprotective strategies.

a. Classic preconditioning in hyperlipidemia. Szilvassy et al. (1995) reported for the first time that protection conferred by classic preconditioning against myocardial stunning and electrophysiological changes was lost when rabbits developed hypercholesterolemia and atherosclerosis after 8 weeks of exposure to 1.5% dietary cholesterol. When these animals were re-exposed to a normal diet, normalization of serum lipid levels recaptured the protective effect of preconditioning in the presence of a constant degree of intimal lesions (Szilvassy et al., 1995). This result indicated that hypercholesterolemia, irrespective of the development of atherosclerosis, interferes with the cardioprotective mechanisms of preconditioning (Szilvassy et al., 1995). The loss of classic preconditioning was subsequently confirmed in isolated hearts from rats with chronic exposure to dietary cholesterol without development of atherosclerosis because rats do not develop significant atherosclerosis from consumption of a high-cholesterol diet (Ferdinandy et al., 1997). Hyperlipidemia without atherosclerosis has also been shown to prevent the protective effect of classic preconditioning on the contractility and responsiveness to phenylephrine of rat papillary muscle (Kocić et al., 1999). The infarct size-limiting effect of classic preconditioning was also attenuated in rabbits fed 1% cholesterol in another study (Ueda et al., 1999). Furthermore, Juhasz et al. (2004) showed that increasing the number of preconditioning cycles even aggravated infarct size in rabbit isolated subjected to ischemia/reperfusion after 8 weeks of experimental hypercholesterolemia. The loss of the anti-ischemic effect of preconditioning (assessed by ST-segment elevation) in hyperlipidemia has been confirmed by two independent groups in patients undergoing repeated balloon inflations during coronary angioplasty (Kyriakides et al., 2002; Ungi et al., 2005). In one of these clinical studies, the loss of the anti-ischemic effect of early preconditioning was correlated with increased plasma cholesterol and LDL levels (Kyriakides et al., 2002). In contrast to the aforementioned studies, in rabbits fed a cholesterol-enriched diet for 8 weeks (Kremastinos et al., 2000), 6 weeks (Iliodromitis et al., 2006b), or 4 weeks (Jung et al., 2000) and in severely atherosclerotic ApoE/LDLr$^{-/-}$ double knockout mice fed an atherogenic diet for 6 to 8 months (Li et al., 2001) and 7 to 9 months (Tokuno et al., 2002) the infarct size-limiting effect of classic preconditioning was not attenuated. The reasons for these conflicting results are unknown, but it seems that in some hyperlipidemic models the presence of severe atherosclerosis and the resultant alterations in liver function may further affect the complex pathophysiology of hyperlipidemia and mechanisms of adaptive cardioprotection.

b. Late preconditioning in hyperlipidemia. The interaction of hyperlipidemia with late preconditioning has been examined in only a few studies. In a conscious rabbit model of rapid cardiac pacing-induced demand ischemia, the authors were able to induce late cardioprotection in hyperlipidemic animals only when the number of cycles of preconditioning ischemia was increased compared with that applied in normolipidemic animals to induce late cardioprotection (Szekeres et al., 1997). This finding shows that the threshold stress to trigger cardioprotection is increased in experimental hyperlipidemia. However, the bacterial endotoxin analog monophosphoryl lipid A-induced late protection in the same rabbit model remained unaffected by experimental hyperlipidemia and atherosclerosis (Szilvassy et al., 1998). The loss of the infarct size-limiting effect of late preconditioning in another conscious rabbit model of coronary occlusion and reperfusion was shown by Tang et al. (2005). The loss of NO donor-induced late preconditioning was shown in the same rabbit model by the same group (Tang et al., 2004). These results show that hyperlipidemia may interfere with the cardioprotective effect of late preconditioning and that the effectiveness of late preconditioning depends on the strengths of the ischemic stimulus and the type of the pharmacological stimulus.

c. Postconditioning in hyperlipidemia. Very little is known about the effect of postconditioning in hyperlipidemia. Iliodromitis et al. (2006b) have recently shown that the infarct size-limiting effect of postconditioning is lost in rabbits with experimental hyperlipidemia and atherosclerosis. The loss of the infarct size-limiting effect of postconditioning has been also confirmed in hearts isolated from cholesterol-fed rats (Kupai et al., 2006).

4. Interaction of Hyperlipidemia and Antihyperlipidemic Statins with Cardioprotective Cellular Mechanisms. The mechanism by which hyperlipidemia may influence the severity of myocardial ischemia/reperfusion injury and cardioprotection by pre- and postconditioning is not exactly known. Accumulation and redistribution of tissue/membrane cholesterol and the resulting changes in sarcolemmal and mitochondrial membrane microviscosity rather than the direct effect of high serum lipoprotein levels and coronary atherosclerosis may account for the altered severity of ischemia/reperfusion injury (Melax and Leeson, 1975; Venter et al., 1991; Hexeberg et al., 1993). Previous studies suggested that membranes can sense environmental changes and the resulting modulation of phase state and microdomain organization regulates the expression of several genes including heat shock proteins (Vigh et al., 1998). Ferdinandy's group have previously reported that the expression of the cardioprotective 70-kDa heat shock protein in response to ischemia and heat stress is markedly attenuated in hearts of hyperlipidemic rats (Csont et al., 2002).

A decrease in cardiac NO bioavailability due to increased nitrosative stress (Hoshida et al., 1996; Ferdinandy et al., 1997; Csonka et al., 2001; Tang et al., 2005;

for reviews, see Ferdinandy and Schulz, 2003; Pacher et al., 2007) and resulting activation of matrix metalloproteinases (Giricz et al., 2006) increased ecto-5′-nucleotidase activity (Ueda et al., 1999), as well as enhanced apoptotic cell death via the caspase-1 cascade (Wang et al., 2003), have been shown to contribute to increased ischemia/reperfusion injury and loss of classic preconditioning in hyperlipidemic animal models. Interestingly, inhibition of the mevalonate pathway by hyperlipidemia has been also described as a possible mechanism of the lost cardioprotection because mevalonate restored the effect of preconditioning on postischemic myocardial function and lactate dehydrogenase release in hyperlipidemic rats (Ferdinandy et al., 1998a). The loss of late preconditioning in hypercholesterolemia has been related to lack of up-regulation of tetrahydrobiopterin synthesis (an essential cofactor for iNOS and many other enzymes) (Tang et al., 2005) and disruption of biochemical pathways distal to the generation of NO that triggers the delayed adaptation (Tang et al., 2004) (section II.B.4.). The mechanism by which hyperlipidemia attenuates the efficacy of postconditioning has not been studied yet. It is possible that the reduced NO bioavailability may be at least partly responsible as the NO-soluble guanylyl cyclase pathway is implicated in the mechanism of postconditioning (section II.B.5.b.). However, in the light of DNA microarray studies on the changes in gene expression in rat hearts undergoing preconditioning (Onody et al., 2003) and hyperlipidemia (Puskás et al., 2004), many other cardioprotective mechanisms that have not yet been studied may be altered by hyperlipidemia in the ischemic heart.

The most frequently used antihyperlipidemic drugs, the 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) inhibit the synthesis of mevalonate, a rate-limiting step in cholesterol biosynthesis. A number of large clinical trials have shown that chronic administration of statins has potent cholesterol-lowering effects and reduces cardiovascular morbidity and mortality (Pedersen et al., 1994; Pfeffer et al., 1999; Heart Protection Study Collaborative Group, 2002; Law et al., 2003; Baigent et al., 2005). Because cardioprotection by statins was also observed in patients with normal cholesterol levels, it has been proposed that statins may exert a broad spectrum of cholesterol-independent protective effects including plaque stabilization, preservation of endothelial function, and scavenging of free radicals, as well as antiproliferative, anti-ischemic, antiinflammatory, and antiapoptotic effects (Kaneider et al., 2001; Laufs et al., 1998; Lefer et al., 1999; Leung et al., 1993; Weber et al., 1997). On the other hand, it is well known that chronic treatment with statins may exhibit a number of extrahepatic adverse effects, such as myopathy and rhabdomyolysis (Thompson et al., 2003; Jamal et al., 2004; Silva et al., 2006); however, the mechanisms of these side effects are not entirely clear. Di Napoli et al. (2001) showed that acute application of 25 μM simvastatin protects the ischemic/reperfused heart against contractile dysfunction, release of creatine kinase, and postischemic hyperpermeability. Interestingly, in that study protection by simvastatin became less evident at 50 μM and at 100 μM it exacerbated ischemia/reperfusion injury. Therefore, the possible interactions of statins with adaptive cardioprotective mechanisms are of great importance. However, few researchers have addressed this question so far. Ueda et al. (1999) have shown that pravastatin restored the infarct size-limiting effect of ischemic preconditioning blunted by cholesterol diet-induced hypercholesterolemia in a rabbit model of myocardial infarction. However, preliminary studies by Ferdinandy's group have recently shown that statins may also interfere with the cellular mechanisms of early preconditioning and postconditioning, i.e., statins may attenuate their infarct-size limiting effect in rat hearts (Fodor et al., 2006). The mechanism by which statins may interfere with cardioprotective mechanisms is unclear. Both mevalonate-pathway dependent and independent mechanisms can be involved, including alterations of oxidative and nitrosative stress (Singh et al., 1997; Sessa, 2001; Haendeler et al., 2004; Harris et al., 2004). Further clinical studies are necessary to investigate the optimal use of statins in the case of acute myocardial ischemia/reperfusion events. Development of novel lipid-lowering agents that do not interfere with innate mechanisms of cardiac stress adaptation such as preconditioning may further improve the efficacy of lipid-lowering therapy in the prevention of cardiovascular events in hypercholesterolemic patients.

In summary, the majority of preclinical and clinical studies show that hyperlipidemia, independently of the development of coronary atherosclerosis, worsens the outcome of ischemia/reperfusion injury and attenuates the cardioprotective effect of both early and late preconditioning as well as postconditioning. Statins, the most frequently used antihyperlipidemic drugs, although showing cardioprotective effects and decreasing cardiovascular mortality irrespective of the initial cholesterol level in large patient populations (Heart Protection Study Collaborative Group, 2002), may also interfere with cardioprotective mechanisms of pre- and postconditioning. These findings may serve to emphasize the necessity of lipid-lowering therapy. However, they also emphasize the need for development of new cardioprotective drugs that are able to reverse the increased susceptibility of hyperlipidemic hearts to ischemic stress and to enhance adaptive cardioprotective mechanisms in hyperlipidemic patients.

D. Diabetes

1. Diabetes as a Risk Factor. Epidemiological studies and clinical trials have clearly shown that both type 1 (insulin-dependent) and type 2 (noninsulin-dependent) diabetic individuals are more prone to developing isch-

emic heart disease, including acute myocardial infarction and postinfarct complications (Ramani et al., 1996; Aguilar et al., 2004; Stevens et al., 2004; Zairis et al., 2004). In fact, ischemic heart disease accounts for more than 50% of deaths in diabetic patients (Kannel and McGee, 1979) and mortality from acute myocardial infarction is almost doubled in diabetic patients compared with nondiabetic individuals (Abbott et al., 1988). Worldwide increases in obesity and diabetes have occasioned concern that increased morbidity and mortality will follow. Indeed, a recent epidemiological study documented a marked upsurge in diabetes-related mortality and morbidity in New York City, including a sharp increase in diabetic patients hospitalized for acute myocardial infarction (Fang and Alderman, 2006). Treatment of patients with diabetes who have underlying ischemic heart disease is a challenge of the new millennium because of the complex pathophysiology and the bad prognosis of these comorbidities (Klein and Gheorghiade, 2004; Murcia et al., 2004). Therefore, investigation of the cellular mechanisms of interaction of diabetes, as well as antidiabetic drugs, with ischemia/reperfusion injury and cardioprotective mechanisms is of particular interest.

2. 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 the heart to acute ischemia/reperfusion injury in various animal models of diabetes.

a. Preclinical studies. The most frequently used animal models of diabetes are generated by administration of toxins specific for insulin-producing pancreatic β cells, such as streptozotocin (STZ) or alloxan (Hayashi et al., 2006). Depending on the dosing regimen of these toxins, both type 1 and type 2 diabetic models can be generated (Szkudelski, 2001). Genetic animal models of diabetes are also available, such as the nonobese type 2 diabetic Goto-Kakizaki rats and the obese Zucker diabetic fatty rats. There is substantial controversy as to whether hearts from diabetic animals models are more sensitive or less sensitive to ischemia/reperfusion injury (for earlier reviews, see Ferdinandy et al., 1998b; Feuvray and Lopaschuk, 1997; Paulson, 1997).

Tosaki et al. (1996) documented the STZ-induced diabetic rat heart response to ischemia/reperfusion. They reported that in the early phase of experimental diabetes (2 weeks) the diabetic heart was more resistant to ischemia/reperfusion. However, this protection was not seen in the 4- and 6-week diabetic hearts and after 8 weeks, a worse outcome from ischemia/reperfusion assessed by the incidence of reperfusion-induced arrhythmias, cardiac function, and ion shifts was observed (Tosaki et al., 1996). In STZ-induced diabetes in rats, 1 week after the STZ injection, infarct size induced by 30 min of coronary occlusion and 4 h of reperfusion was less in diabetic rats than in controls, but this result was not observed 8 weeks later (Ravingerová et al., 2003). Similar results were obtained when reperfusion-induced arrhythmias were measured as an endpoint of injury in the same model (Ravingerová et al., 2000a). After 2 weeks of STZ-induced diabetes in rats, infarct size induced by 30 min of coronary occlusion and reperfusion was decreased but the same result was not seen after 6 weeks of diabetes (Ma et al., 2006). In a similar in vivo rat type 2 diabetes model, 2 weeks after STZ treatment infarct size was also decreased (Liu et al., 1993). In an alloxan-induced diabetes model in rabbits, 2 months after alloxan injection, diabetic rabbits showed smaller infarct sizes than controls (Hadour et al., 1998). The aforementioned studies show that experimental diabetes at least in the early stage may protect the heart against ischemia/reperfusion.

However, other studies in STZ-induced type 1 diabetes models in mice (Marfella et al., 2004; Liu et al., 2005) or rats (Xiao et al., 2004; Di Filippo et al., 2005) showed that infarct size, myocardial function, and mortality were significantly increased in the diabetic groups even 2 weeks after STZ treatment. Three weeks after streptozotocin-alloxan-induced diabetes, myocardial infarct size did not significantly change in dogs subjected to a 60-min coronary artery occlusion and 3 h of reperfusion (Kersten et al., 2001). Four weeks after i.v. STZ administration, the number of apoptotic cardiomyocytes was equally high in diabetic and nondiabetic rats 1 week after induction of permanent coronary occlusion. However, at 12 weeks after infarction the number of apoptotic cells was higher in the diabetic compared with the nondiabetic rats both in the border zone of infarction and in the noninfarcted area (Bäcklund et al., 2004).

b. Clinical studies. In contrast to the fairly inconclusive results in preclinical studies, the majority of clinical studies demonstrated worse outcome from acute myocardial infarction in diabetic patients. A study by Rytter et al. (1985) more than two decades ago involving 832 patients with acute myocardial infarctions showed that during the 1st month after infarction the mortality rate of diabetic patients was doubled compared with that of nondiabetic individuals. The acute in-hospital mortality rate of 9695 diabetic patients among the total 42,595 patients with myocardial infarction was moderately higher in both men and women in all age groups (Abbud et al., 1995). In another large study involving 11,667 diabetic patients, the increase in in-hospital mortality of diabetic patients was moderate and similar for men with insulin- and noninsulin-dependent diabetes. However, in women, mortality was markedly higher for insulin-dependent and only slightly higher for noninsulin-dependent diabetic patients (Zuanetti et al., 1993). Another study involving 883 patients also proved that in-hospital mortality of acute myocardial infarction was higher in diabetic patients (Barbash et al., 1993). How-

ever, in another clinical study, once the effects of age were accounted for, the risk of in-hospital mortality after acute myocardial infarction was not greater in patients with diabetes mellitus than in patients without diabetes (Chyun et al., 2000). In human atrial tissue ex vivo, ischemia caused similar injury in both normal and diabetic tissue (Ghosh et al., 2001). The latter two studies showed that it might be difficult to estimate the independent effect of diabetes on the outcome of patients with acute coronary syndromes. However, in a recent subgroup analysis, patients with acute coronary syndrome in 11 independent Thrombolysis in Myocardial Infarction (TIMI) Study Group clinical trials from 1997 to 2006 were pooled, including 62,036 patients [46,577 with ST-segment elevation myocardial infarction and 15,459 with unstable angina/non-ST-segment elevation myocardial infarction, of whom 10 613 (17.1%) had diabetes] revealed that diabetes is independently associated with 30-day or 1-year mortality after acute coronary syndrome (Donahoe et al., 2007). Similar conclusions were drawn from other recent retrospective analyses of large populations of diabetic patients with myocardial infarction (Cubbon et al., 2007; Norhammar et al., 2007).

In summary, the majority of large-scale human studies show that both type 1 and type 2 diabetes increase the susceptibility of the heart to ischemia/reperfusion injury as shown by worse outcome of diabetic patients after acute coronary syndromes and increased overall cardiovascular risk (for a review, see Jaffe et al., 2006). In contrast, preclinical studies of myocardial ischemia/reperfusion in diabetic animal models are rather controversial and inconclusive. The discrepancy of results in preclinical studies might be due to differences in species as well as in the dose and the duration of STZ or alloxan administration. It seems that in experimental models of diabetes, acute induction of diabetes with substances toxic for insulin-secreting cells may render the heart more resistant to ischemic injury. However, this protection is lost several weeks later and ischemic injury might even be increased possibly as diabetic cardiomyopathy develops, suggesting that the use of chronic diabetes models better reflects the clinical situation.

3. Cardioprotection by Pre- and Postconditioning in Diabetes. A number of studies have examined the ability of pre- and postconditioning to protect the heart in different experimental models of diabetes and in diabetic patients. The majority of the studies showed that diabetes and some antidiabetic drugs interfere with cardioprotective mechanisms, attenuating the effectiveness of cardioprotective strategies.

a. Preclinical studies. The first study to examine the classic preconditioning response in experimental STZ-induced diabetes was reported by Liu et al. (1993). Hearts of diabetic rats 2 weeks after STZ treatment were found to be more resistant to myocardial infarction in vivo than normal control hearts, and classic preconditioning conferred additional protection. A subsequent study examined the evolution of the STZ-induced diabetic response to classic preconditioning in isolated rat hearts. It was found that in contrast to nondiabetic hearts, four brief cycles of preconditioning ischemia did not afford protection against ischemia/reperfusion-induced arrhythmias, stunning, and intracellular Na^+ accumulation and K^+ efflux in 4- or 8-week diabetic rats (Tosaki et al., 1996). The lack of the infarct size-limiting effect of preconditioning was observed in dogs 3 weeks after induction of diabetes (Kersten et al., 2000). In hearts from obese Zucker diabetic fatty and lean Goto-Kakizaki type 2 diabetic rats, ischemic preconditioning did not afford protection against reperfusion injury (Kristiansen et al., 2004). The loss of the antiarrhythmic effect of ischemic preconditioning was observed in isolated hearts obtained from rats 1 week after STZ treatment (Ravingerová et al., 2000b). In a sheep model of alloxan-induced diabetes, classic preconditioning was shown to worsen the outcome of ischemia/reperfusion compared with nonpreconditioned diabetics, and the loss of late preconditioning as assessed by regional wall thickening of the myocardium has been also observed (del Valle et al., 2003). The loss of the infarct size-limiting effect of late ischemic preconditioning was observed in rabbits with alloxan-induced diabetes or experimental hyperglycemia induced by dextrose infusion, which was reversed by acute insulin treatment (Ebel et al., 2003). Preconditioning induced by the anesthetic isoflurane was also shown to be attenuated in diabetic animals (for a review, see Zaugg et al., 2003). The effect of postconditioning in diabetic animal models has not been thoroughly studied yet; however, according to some preliminary data, the effectiveness of postconditioning is diminished in diabetic rats (Hausenloy et al., 2006).

b. Clinical studies. Some clinical observations suggest that preconditioning or preconditioning-like phenomena are impaired in diabetic patients with ischemic heart disease. Prodromal (preinfarct) angina, thought to be a natural correlate of preconditioning in patients, did not limit infarct size, enhance recovery of myocardial function, or improve survival in diabetic patients with myocardial infarction compared with nondiabetic patients (Ishihara et al., 1997). Impairment of ischemic preconditioning during coronary angioplasty was observed in diabetic patients (Lee and Chou, 2003). Preconditioning did not reduce creatine kinase release in ex vivo right atrial appendices obtained from diabetic patients (Ovünç, 2000). In summary, both preclinical and clinical data show that the cardioprotective effect of ischemic preconditioning is impaired in preclinical animal models and in humans as well.

4. Interaction of Diabetes and Antidiabetic Drugs with Cardioprotective Cellular Mechanisms. Because insulin not only regulates the balance of energy substrates available to the heart but also regulates metabolism and myocardial perfusion via actions on various intracel-

lular regulatory proteins and messenger systems, it is conceivable that diabetes interferes with the biochemical pathways of cardioprotection (Abel, 2004). Beyond the possible detrimental effect of diabetes on preconditioning and postconditioning phenomena, the picture is further colored by the influence of antidiabetic drug treatment on ischemia/reperfusion injury and cardioprotection. Therefore, the mechanism by which diabetes interferes with ischemia/reperfusion injury and cardioprotective mechanisms has been extensively studied.

The role of K_{ATP} channels and the effect of the K_{ATP} inhibitor antidiabetic drugs have been much studied in the diabetic heart. Insulin secretagogues (sulfonylureas and glinides) increase insulin secretion by blocking the K_{ATP} channel in the pancreatic β-cell membrane. K_{ATP} channels subserve important functions in the heart also. First, K_{ATP} channels in coronary smooth muscle cells contribute to the control of coronary blood flow at rest and in hypoxia. Second, K_{ATP} channels in the sarcolemma of cardiomyocytes may contribute to the adaptation of the heart to stress. In addition, the opening of putative mitochondrial K_{ATP} channels plays a central role in cardioprotective mechanisms as discussed in section II.B.5.) (for reviews, see Grover, 1997; Liu et al., 1998a; Quast et al., 2004).

It has been well established that classic and late preconditioning in several species (Schulz et al., 1994; Ferdinandy et al., 1995; Miura et al., 1995; Hoag et al., 1997) including humans (Speechly-Dick et al., 1995; Cleveland et al., 1997; Ovünç, 2000; Ferreira et al., 2005) is abolished by K_{ATP} channel blockers such as glibenclamide and 5-hydroxydecanoate. Preconditioning with ischemia, phenylephrine, adenosine, or diazoxide failed to protect human diabetic atrial myocardium ex vivo. However, activation of PKC or p38 MAPK was still protective, showing that the cardioprotective deficit in diabetic myocardium arises upstream of PKC and p38 MAPK (Hassouna et al., 2006). Mitochondrial K_{ATP} dysfunction has been proposed to be involved in the loss of preconditioning in human atrial sections ex vivo by the same group (Ghosh et al., 2001). Therefore, inhibition of cardiovascular K_{ATP} channels by insulin secretagogues is considered to increase cardiovascular risk; e.g., these drugs increase mortality in diabetic patients after coronary angioplasty (Garratt et al., 1999) and the adverse cardiovascular outcomes with type 2 diabetics in a large cohort of patients (Evans et al., 2006). However, electrophysiological experiments have shown that the secretagogues differ in their selectivity for the pancreatic over the cardiovascular K_{ATP} channels (for a review, see Quast et al., 2004). Accordingly, in contrast with glibenclamide, glimepiride, which was shown to be more selective for pancreatic K_{ATP} channels, does not interfere with the cardioprotective effect of preconditioning either in preclinical (Mocanu et al., 2001; Nieszner et al., 2002) or clinical studies (Lee and Chou, 2003). Treatment of diabetes with insulin or pioglitazone, which activates prosurvival kinase pathways, namely PI3K/Akt, has been suggested as another option to avoid the negative effect of K_{ATP} blockade on cardioprotection (Scognamiglio et al., 2002; Forlani et al., 2004; Wynne et al., 2005). It should also be noted that in addition to the interaction of sulfonylurea derivatives with ischemia/reperfusion and preconditioning, these drugs may abolish the therapeutic effect of some frequently used antiischemic nitrates as well (for a review, see Csont and Ferdinandy, 2005).

In addition to K_{ATP}, other cellular mechanisms have been suggested as the background of diabetes-induced alterations in cardioprotection. It is of interest that the extent of hyperglycemia has been shown to be a major risk factor for mortality in a very extensive study involving elderly patients (n = 141,680) hospitalized with acute myocardial infarction (Kosiborod et al., 2005). These results are in line with preclinical studies showing that infarct size correlates with the extent of hyperglycemia in dogs irrespective of the presence or absence of diabetes (Kersten et al., 2000, 2001).

iNOS-mediated and heme oxygenase-1-mediated mechanisms have been shown to increase the susceptibility of $iNOS^{-/-}$ (Marfella et al., 2004) and heme oxigenase-1-deficient ($HO\text{-}1^{-/-}$)(Liu et al., 2005) mice with STZ-induced diabetes to the development of myocardial infarction. Impaired levels of HO-1 in cardiac tissue and increased myocardial infarct size after ischemia/reperfusion was shown in STZ-induced diabetic rats (Di Filippo et al., 2005). The contribution of poly(ADP)-ribose polymerase has been also shown in diabetes, as the poly(ADP)-ribose polymerase inhibitor INO-1001 inhibited diabetes-induced increases in infarct size and mortality (Xiao et al., 2004). After 2 weeks of STZ-induced diabetes rats were protected against ischemia/reperfusion injury and activation of vascular endothelial growth factor and increased eNOS expression, NO formation, activation of cell survival signals, and decreased oxidative stress was shown (Ma et al., 2006). These results may suggest that alterations in oxidative/nitrosative stress and their downstream cellular targets (Ferdinandy and Schulz, 2003; Ferdinandy, 2006) may account for the altered response of diabetic heart to ischemia/reperfusion and cardioprotective mechanisms (Haidara et al., 2006). Impaired Akt phosphorylation in response to ischemic preconditioning was also shown in STZ-diabetic rats (Tsang et al., 2005).

In summary, although the interaction of diabetes with ischemia/reperfusion injury is controversial in preclinical models of diabetes, the majority of studies show that the ability of the diabetic heart to adapt to ischemic stress is impaired. Human studies clearly show that the outcome of ischemia/reperfusion and the ability of the heart to adapt to ischemic stress are impaired. Intensive research on the mechanism of diabetic response to ischemia/reperfusion revealed that some sulfonylurea type

antidiabetic drugs may further impair cardioprotective mechanisms in diabetic patients. However, exploration of the exact mechanism of how diabetes interacts with other cardioprotective pathways needs further studies in the hope that new classes of antidiabetic agents may maintain or improve the ability of the heart to adapt to ischemic stress.

E. Aging and Cardioprotection

Mortality due to ischemic cardiovascular diseases is significantly higher in elderly than in young adults. Demographic changes brought about by increasing life expectancy in the global population will have a significant impact on the future of medical practice.

1. Aging and Ischemia/Reperfusion Injury. Aging is characterized by impaired diastolic and systolic function of the heart, and oxidative stress is one of the factors contributing to such alterations in cardiac function (Csiszar et al., 2005). Several mechanisms contribute to the increased oxidative stress in aged hearts: 1) upregulation of the angiotensin II type 1 receptors and subsequent activation of NADPH oxidases (Oudot et al., 2006); 2) increased cardiac monoamine oxidase A activity (Maurel et al., 2003); and finally 3) increased mitochondrial free oxyradical formation (Richter et al., 1988; Muscari et al., 1990) and decreased mitochondrial oxidative defense (Sivonova et al., 2007).

The major sites of oxyradical formation within mitochondria are complexes I and III of the electron transport chain. Oxyradicals released into the mitochondrial matrix are eliminated by manganese-containing mitochondrial SOD together with catalase and/or the glutathione redox system (for a detailed review, see Lesnefsky et al., 2001). Whereas an increase in oxidative stress in mitochondria from aged hearts is associated with increased manganese-containing SOD expression (Judge et al., 2005a,b), depending on the subpopulation of mitochondria either catalase expression (subsarcolemmal mitochondria) (Judge et al., 2005a) or glutathione expression (interfibrillar mitochondria) (Judge et al., 2005b) is decreased, resulting in reduced antioxidant capacity of senescent hearts (Sinonava et al., 2007). The overall increased oxidative stress causes protein, lipid, and DNA oxidation, potentially contributing to contractile failure (Kanski et al., 2005). Indeed, catalase overexpression in mice attenuates the age-dependent decline in contractile function (Ren et al., 2007; Wu et al., 2007) and increases life span (Schriner et al., 2005). Oxidative damage to mitochondria in concert with mitochondrial calcium overload favors the onset of a mitochondrial permeability transition and the subsequent release of cytochrome *c* (Lesnefsky et al., 2001). Cytochrome *c* release from mitochondria is a key step leading to programmed cell death, and, indeed, the rate of programmed cell death in the left ventricle increases with age and contributes to a reduction in cardiomyocyte number and an increase in the extent of fibrosis (for a review, see Higami and Shimokawa, 2000; Bernecker et al., 2003). Aging also aggravates the heterogeneities in cardiomyocyte size (Dyachenko et al., 2006). Finally, apart from the increased oxidative stress, mitochondria from aged hearts display reduced membrane potential (Savitha and Panneerselvam, 2006; Serviddio et al., 2007), which may contribute to reduced ATP synthesis (for reviews, see Pepe, 2000; Di Lisa and Bernardi, 2005).

On top of the age-related alterations, ischemia/reperfusion-induced mitochondrial changes occur. Free oxyradicals produced during ischemia/reperfusion reduce the activities of complexes I, III, and IV of the electron transfer chain as well as the mitochondrial cardiolipin content (Paradies et al., 2002, 2004; Petrosillo et al., 2003). Protein oxidation after ischemia/reperfusion is higher in senescent than in adult rat hearts (Besse et al., 2006) and is associated with a further reduced antioxidant capacity (Liu et al., 2004). When the response toward ischemia was studied, the aged myocardium showed a reduced tolerance to ischemic injury (Abete et al., 1999). In C57/BL6 mice the intrinsic myocardial tolerance to ischemia was decreased at 12 months of age (Willems et al., 2005). Retrospective analysis of the TIMI-4B trial revealed that patients who were aged 60 years and older had a higher rate of death and the combined endpoints of death, heart failure/shock, and/or reinfarction compared with younger patients, supporting the notion of reduced tolerance toward ischemic injury in aged hearts per se (Kloner et al., 1998a).

2. Aging and Ischemic Preconditioning. Several genes are differentially expressed in the aged myocardium (Volkova et al., 2005), among them genes encoding proteins involved in the signal transduction of preconditioning-induced cardioprotection (Taylor and Starnes, 2003). For example, the PKC content, which is central to the signal transduction cascade of ischemic preconditioning in juvenile animal hearts (section II.B.5.), was reduced in aged hearts (Korzick et al., 2001; Takayama et al., 2001); however, the importance of PKC for ischemic preconditioning-induced protection in aged hearts was questioned (Przyklenk et al., 2003). Another protein central for ischemic and pharmacological preconditioning-induced protection (Schwanke et al., 2002, 2003; Li et al., 2004; Heinzel et al., 2005; Rodriguez-Sinovas et al., 2006), namely connexin 43, was reduced with increasing age (Chen and Jones, 2000), especially at the level of the mitochondria (Boengler et al., 2006).

Whether cardioprotection by ischemic preconditioning is impaired in aged myocardium has been studied in animal models as well as in the human heart. However, the results on the effectiveness of ischemic preconditioning in the aged myocardium remain controversial (for a detailed review of in vitro studies, see Juhaszova et al., 2005). With a focusing on in vivo studies, ischemic preconditioning-induced cardioprotection was preserved in 4-year-old rabbits and 5- to 8-year-old sheep (Burns et

al., 1996; Przyklenk et al., 2001), whereas cardioprotection by ischemic preconditioning was impaired in senescent rat (24 months old) (Zheng et al., 2006) and mouse (13–16 months old) hearts (Boengler et al., 2006, 2007b).

In patients, ischemic preconditioning, as indicated by preinfarction angina within 24 h before infarction, reduces infarct size and left ventricular remodeling, thereby potentially improving the prognosis of patients with an acute myocardial infarction (Anzai et al., 1995; Kloner et al., 1995; Kloner et al., 1998b; Papadopoulos et al., 2003; Yellon and Downey, 2003; Solomon et al., 2004; Tokac et al., 2004). When preinfarction angina occurred within 48 h before acute myocardial infarction, no such difference in irreversible tissue injury and outcome was measured compared with patients without any ischemic symptoms before infarction (Psychari et al., 2004). However, in another study, the presence of preinfarction angina within 48 h before acute myocardial infarction conferred protection against in-hospital outcomes in adults younger than age 65, but the effect was less obvious in patients older than age 65 (Abete et al., 1997).

Loss of ischemic preconditioning-induced protection by a 120-s coronary occlusion before coronary angioplasty was also seen in senescent patients (mean age of 71 years), whereas it was present in adult hearts (mean age of 45 years). However, prolonging the preconditioning occlusion to 180 s restored protection also in hearts of senescent patients (Lee et al., 2002). Thus, loss of ischemic preconditioning-induced protection in aged hearts might be related to an attenuated signal transduction cascade, which could be overcome by strengthening the preconditioning stimulus.

Regular exercise, specifically endurance exercise, protects against ischemia/reperfusion injury in both young and old animals. Proposed mechanisms to explain the cardioprotective effect of exercise include the induction of myocardial heat shock proteins, improved cardiac antioxidant capacity, and/or elevation of other cardioprotective proteins (for a review, see Powers et al., 2004). Indeed more recently, 12 weeks of exercise training attenuated the age-induced increases in left ventricular cardiomyocyte apoptosis and subsequently remodeling (reactive hypertrophy of remaining cardiomyocytes with increased connective tissue content) in rats and improved the balance between apoptotic and antiapoptotic proteins (Bax/Bcl-2 ratio) in the left ventricle of the aging rat heart (Kwak et al., 2006). In accordance with this finding, in adult and trained but not in sedentary senescent rat hearts, preconditioning reduced postischemic left ventricular contractile dysfunction (although irreversible tissue injury was not assessed) (Abete et al., 2000). Similarly, the protective effect of preinfarct angina against the consequences of an acute myocardial infarction was preserved in elderly patients with a high level of physical activity (Abete et al., 2001).

Although a good body of literature exists on the effect of classic preconditioning on myocardial infarct size, the data on the other entities (late preconditioning or early and delayed pharmacological preconditioning) are sparse. In preliminary results, ischemic postconditioning by 3 cycles of 10 s of ischemia and 10 s of reperfusion immediately after 30 min of coronary artery occlusion failed to reduce infarct size in aged mice (>13 months), whereas the same protocol effectively reduced infarct size in young mice (<3 months). The failure to postcondition aged hearts was associated with reduced STAT3 expression, and this ischemic postconditioning protocol also failed to reduce infarct size in STAT3-deficient mice (Boengler et al., 2007a). Increasing the strength of the postconditioning stimulus (5 cycles of ischemia/reperfusion) partially restored infarct size reduction in aged and STAT3-deficient hearts. Interestingly, in patients with multiple risk factors (high blood pressure, smoking, dyslipidemia, and diabetes) and a mean age of approximately 60 years, ischemic postconditioning immediately after coronary angioplasty reduced infarct size (creatine kinase release) and improved the patient's prognosis (Staat et al., 2005; Darling et al., 2007).

3. Aging and Pharmacological Preconditioning. In male rats of three age groups (2–4, 10–12, and 20–24 months), pretreatment with 10 min of sevoflurane and a 5-min washout before 25 min of ischemia and 60 min of reperfusion decreased creatine kinase release and infarct size in the young adult and middle-aged groups but not in the aged group (Sniecinski and Liu, 2004). Similarly in isolated guinea pig hearts of different weights (1.1–2.2 g) and ages (2–7 weeks), sevoflurane for 15 min followed by a 30-min washout before 30 min of global ischemia and 120 min of reperfusion attenuated infarct size better in smaller/younger hearts than in larger/older hearts (Riess et al., 2005). Finally, aging abolished the cardioprotective effect on postischemic contractile function of heat shock and hypoxic preconditioning in rat hearts (Honma et al., 2002, 2003). Interestingly, whereas acute treatment with the ACE inhibitor captopril restored the efficacy of preconditioning in aged normotensive hearts to a modest degree (an effect presumed to be due to augmentation of tissue bradykinin levels), the combination of ischemic preconditioning and captopril was without effect in aged (11–13 months old) SHR hearts (Ebrahim et al., 2007b). This finding is consistent with the observation by the same group that cardioprotective responses to bradykinin, but not to ischemic preconditioning, are markedly attenuated in young hypertensive animals with modest pressure-overload hypertrophy (Ebrahim et al., 2007a). It also highlights the interaction of aging with hypertensive hypertrophy, a very common clinical condition.

Acute pharmacological treatment with the opioid δ-receptor agonist [D-Pen^2,D-Pen^5]-enkephalin was shown to enhance postischemic recovery of contractile function in young (10–14 weeks old) but not in aged (24–26 months old) mouse heart (Peart et al., 2007). The reason for this loss of sensitivity to opioid-induced car-

dioprotection might reside in a loss of coupling between the δ-opioid receptor and p38 MAPK because this kinase and a downstream substrate, HSP27, displayed less phosphorylation in response to [D-Pen^2,D-Pen^5]-enkephalin treatment in aged hearts. In contrast to the failure of acute opioid preconditioning to protect the aged heart, administration of opioids 24 h before a 20-min period of global ischemia followed by 20 min of reperfusion improved recovery of postischemic LV function and reduced the total release of creatine kinase and lactate dehydrogenase during reperfusion in young (12 weeks) and old (78 weeks) male rats (Shinmura et al., 2004), suggesting that the pathway for delayed cardioprotection remains intact. A fascinating observation is that chronic opioid receptor activation with morphine is profoundly cardioprotective, an effect that is completely dissociated from the acquisition of analgesic tolerance (Peart and Gross, 2004a) and mediated via protein kinase A activation whereas acute opioid-induced cardioprotection is PKC-dependent (Peart and Gross, 2006). Intriguingly, chronic opioid treatment confers marked cardioprotection in both young and senescent mouse heart, in contrast to the loss of protection with acute morphine treatment in aged hearts (Peart and Gross, 2004b). Thus, the chronic administration of appropriate opioid receptor agonists, lacking central effects, could provide a powerful means of cardioprotection for the aging heart.

In conclusion, aging or age-associated alterations (such as hypertrophy or remodeling) are associated with the loss or attenuation of cardioprotection by acute ischemic or pharmacological preconditioning as well as ischemic postconditioning. Increasing the strength of the preconditioning stimulus might re-install protection in some instances. Whether the cardioprotective properties of other approaches (e.g., late preconditioning and pharmacological postconditioning) are also lost in aged hearts remains to be established. Exercise might overcome some of the restrictions associated with aging, thereby restoring the cardioprotective phenomena.

V. Conclusions and Perspectives

The discovery of the remarkable cardioprotective effect of innate adaptive responses elicited by pre- and postconditioning fuelled intensive research in the last two decades to find key cardioprotective mechanisms against ischemia/reperfusion injury. Surprisingly, relatively little effort has been made to uncover the cellular mechanisms by which risk factors and systemic diseases such as hypertension, hyperlipidemia and atherosclerosis, diabetes, insulin resistance, heart failure, and aging interfere with cardioprotective mechanisms. However, ischemic heart disease in humans is a complex disorder caused by or associated with other systemic diseases and risk factors. Therefore, in this article we reviewed evidence that comorbidities and aging accompanying coronary disease modify responses to ischemia/reperfusion and the cardioprotection conferred by preconditioning and postconditioning. We emphasize the importance of preclinical studies that examine cardioprotection specifically in relation to complicating disease states to maximize the likelihood of identifying rational approaches to therapeutic protection of the aged or diseased ischemic heart.

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