Ischaemic preconditioning is defined as an increased tolerance to ischaemia and reperfusion induced by a previous sublethal period of ischaemia. Since this is the most powerful mechanism for limiting infarct size, other than timely reperfusion, an overwhelming number of studies have addressed the way in which this form of protection occurs. During the short preconditioning period of ischaemia, several trigger substances are released (adenosine, bradykinin, norepinephrine, opioids). By activation of membrane-bound receptors, these substances activate a complex intracellular signalling cascade, which converges on mitochondrial end-effectors, including the ATP-sensitive potassium channel and the mitochondrial permeability transition pore. Activation of this pathway protects cardiomyocytes against both necrosis and apoptosis during a subsequent more prolonged ischaemic episode. The protection afforded by preconditioning lasts only two to three hours, but reappears 24 hours after the preconditioning stimulus. This ‘delayed preconditioning’ requires synthesis of new proteins, including inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2) and heat shock proteins. Additionally, preconditioning is not confined to one organ, but can also limit infarct size in remote, non-preconditioned organs (‘remote preconditioning’). Knowledge of these mechanisms mediating ischaemic preconditioning is essential to understand which drugs are able to mimic preconditioning or interfere with preconditioning in patients at risk for myocardial ischaemia. This review aims to summarise current knowledge regarding the different forms and mechanisms of ischaemic preconditioning.

INTRODUCTION

Despite major advances in prevention and treatment, ischaemic heart disease, and in particular acute myocardial infarction with its late sequelae, remains the leading cause of morbidity and mortality in the Western world and is rapidly gaining its leading position in the developing world. Moreover, due to improved survival from acute myocardial infarction, more and more patients suffer from chronic heart failure, which is an important late complication of infarction. In this regard, continued improvement of strategies aimed at primary and secondary prevention of myocardial infarction is essential. To define suitable targets for intervention, three factors can be identified that ultimately determine the development and outcome of coronary occlusion. The occurrence of coronary artery occlusion is determined by ‘vulnerable plaques’ (prone to thrombotic complications) and ‘vulnerable blood’ (prone to thrombosis). Once coronary occlusion has occurred, the clinical outcome is dependent on the ‘vulnerability’ of the myocardium. Complementary to primary prevention, limitation of infarct size, once occlusion has occurred, is an interesting target which could ultimately attenuate the development of subsequent heart failure.

Until 1986, it was not known whether therapeutic limitation of infarct size was possible at all. In that year, the landmark study by Murry et al. was published, in which they described that brief periods of ischaemia (preconditioning ischaemia) in a dog model render the myocardium resistant to a subsequent more prolonged ischaemic period (index ischaemia), since then known as ‘ischaemic preconditioning’. Four cycles of five minutes of coronary occlusion prior to 40 minutes occlusion reduced infarct size induced...
by these 40 minutes of occlusion by 75% (figure 1). However, the infarct sparing effect was lost when three hours of occlusion was applied, emphasising that timely reperfusion remains indispensable for preconditioning to limit myocardial damage. Since then, an overwhelming number of studies have investigated the underlying mechanism, with the ultimate aim of exploiting this powerful protective mechanism in clinical practice. It was found that ischaemic preconditioning offers two windows of protection in time, called ‘early’ or ‘classical’ preconditioning, providing protection immediately after the preconditioning stimulus, and ‘late’ or ‘delayed’ preconditioning. It was also found that preconditioning ischaemia is able to protect remote cells and organs, which have not been preconditioned by themselves (‘remote preconditioning’). It is essential to realise that most of these studies were conducted in animal models and that important inter-species differences might exist concerning the mechanism of protection, although the effect of preconditioning could be reproduced in all species studied so far. In addition, various in vitro and in vivo human models have been developed, often using surrogate endpoints to study the effect of preconditioning. This review is the first of two parts that deal with ischaemic preconditioning. In this first part, we focus on the mechanisms responsible for ischaemic preconditioning. Knowledge of these signalling cascades is essential to understand how various drugs could mimic ischaemic preconditioning or interfere with ischaemic preconditioning. Indeed, many drugs that are currently used in clinical practice have the potential to interfere with ischaemic preconditioning, which is especially relevant in patients who are at risk for ischaemia. In the second part we will focus on this pharmacological modulation of ischaemic preconditioning and we will describe the potential therapeutic applications of preconditioning in the near future.

**EARLY ISCHAEMIC PRECONDITIONING**

In the original paper by Murry et al. it was stated that ischaemic preconditioning reduces infarct size, expressed as percentage of the area at risk, by approximately 75%. Ever since, this has remained the primary endpoint to describe the effect of ischaemic preconditioning. Moreover, using this endpoint, classical preconditioning has limited infarct size in every species tested so far. That this infarct size limitation would, indeed, be able to attenuate the progression to heart failure after myocardial infarction is suggested by the study by Cohen et al. who showed that in rabbits early ischaemic preconditioning not only reduces infarct size, but also improves systolic myocardial function, measured three weeks after the index ischaemic insult. For studying ischaemic preconditioning in humans, especially in vivo, several surrogate endpoints have been developed, such as ECG changes and coronary lactate, which will be discussed in more detail in the second part of the review. Besides infarct size limitation, ischaemic preconditioning has also been shown to attenuate other forms of ischaemic injury, such as stunning and ventricular arrhythmias, although the evidence is less convincing than for infarct size limitation. In the present review, we will focus primarily on necrosis and apoptosis of cardiomyocytes as primary endpoint of ischaemia and reperfusion injury.

The duration of the preconditioning ischaemia as well as the period of reperfusion before the index ischaemia is applied show fairly rigid time frames in order to give full protection. Concerning the preconditioning ischaemic period, protection has been described for periods ranging from one cycle of 1.25 minutes to five five-minute ischaemia/five-minute reperfusion cycles. It is important to realise that the nature of the preconditioning ischaemic stimulus (amount and duration of ischaemic episodes) influences not only the amount of protection but also the signalling pathways involved. Too many repetitive stimuli might actually abolish preconditioning. Concerning the reperfusion period before the index ischaemia is applied,
the minimum duration lies between 30 seconds and one minute\textsuperscript{17} and when the reperfusion period is extended beyond one to two hours, the infarct-limiting effect is no longer evident.\textsuperscript{18,19} At this point, it is interesting to mention that in animal models also triggers other than complete ischaemia are able to bring myocardium into the preconditioned state. The observation that myocardium can also be preconditioned by a partial coronary occlusion without reperfusion preceding a sustained period of total occlusion has potential clinical significance considering the nature of thrombus formation in acute myocardial infarction.\textsuperscript{20} Also, a brief period of acute volume loading resulting in myocardial stretch,\textsuperscript{21,22} a brief period of rapid pacing\textsuperscript{23} or transient hyperthermia\textsuperscript{24} preceding a sustained period of myocardial ischaemia are all shown to limit infarct size, sharing largely similar signalling pathways as classic ischaemic preconditioning.

In recent years, much research has been devoted to elucidating the mechanisms which are responsible for the preconditioning-induced protection to ischaemia/reperfusion injury. When considering the signalling cascade, triggers and mediators that ultimately converge on end-effectors can be differentiated. Triggers are released during the short preconditioning ischaemia and exert their activity only during this period, whereas end-effectors are solely active during the prolonged index ischaemia and actually cause the protection when needed (figure 2). The first identified and probably most important trigger of classic preconditioning is the endogenous nucleoside adenosine. Myocardial interstitial adenosine concentration increases rapidly during ischaemia.\textsuperscript{25} In 1991 it was discovered that adenosine A1 receptor stimulation during preconditioning ischaemia is essential for protection to occur\textsuperscript{26} and that intravenous administration of selective adenosine A1 receptor agonists instead of preconditioning ischaemia offers similar protection (pharmacological preconditioning).\textsuperscript{27} Similarly, local intracoronary adenosine administration offers protection similar to ischaemic preconditioning in dog hearts.\textsuperscript{28} Later it was found both in vitro and in vivo that A1 receptor stimulation also contributes to ischaemic preconditioning.\textsuperscript{21,22,26} Additional evidence for an important role for adenosine as a trigger of early preconditioning is derived from the observation that pharmacological potentiation of the ischaemia-induced increase in adenosine concentration during preconditioning, by pretreatment with the adenosine-uptake inhibitor dipyridamole, significantly increases the infarct size limiting effect of preconditioning.\textsuperscript{29} Considering the protective role of adenosine in ischaemia/reperfusion injury, it is important to realise that, in addition to its role as a trigger of ischaemic preconditioning, endogenous adenosine also provides direct protection against both ischaemia and reperfusion injury, independent of preconditioning, which involves stimulation of adenosine A2A receptors (figure 3).\textsuperscript{30}

**Figure 2**
Simplified representation of the mechanism of classical preconditioning

During the preconditioning stimulus, several triggers are released which activate a complex signalling cascade, including phosphatidyl-inositol-3-kinase (PI3-kinase), protein kinase C (PKC), protein tyrosine kinases (PTK) and mitogen-activated-protein kinases (MAPKs). This signalling cascade inhibits opening of the MPTP via mitoKATP channel opening and ROS formation.

**Figure 3**
Simplified illustration of the cardioprotection by endogenous adenosine

In addition to the protection afforded by ischaemic preconditioning, adenosine also provides direct cardioprotection during ischaemia and reperfusion.
Later it was found that, in addition to adenosine, several other trigger substances such as bradykinin, opioids, norepinephrine and reactive oxygen species (ROS) are released during preconditioning ischaemia and contribute to the infarct-sparing effect. Regarding ROS, this seems paradoxical, as ROS are generally assumed to contribute to ischaemia/reperfusion injury. Indeed, ROS act as a trigger to protection during the preconditioning stimulus, whereas during the index ischaemia and reperfusion they contribute to injury. Also, a transient elevation in calcium during the preconditioning stimulus might contribute to the protection observed. Whereas an important role for nitric oxide (NO) has unequivocally been shown in delayed preconditioning, its role in classic preconditioning is more controversial. Although exogenous administration of NO donors prior to ischaemia can limit infarct size, endogenous NO-synthase derived NO is probably not involved in classic preconditioning.

It is suggested that because of this redundancy concerning the preconditioning triggers, blockade of one single receptor type only raises the ischaemic threshold required to provide protection, rather than completely blocking protection. Moreover, several studies suggest that the contribution of each of these trigger substances to the induction of preconditioning depends on the nature of the stimulus, which should be realised when comparing results from different study protocols.

As previously mentioned, it is also possible to pharmacologically precondition myocardium. Besides the above-mentioned triggers this can also be achieved with norepinephrine, endothelin-1, acetylcholine and angiotensin II, but these substances are not released in sufficient quantities during ischaemia to contribute to endogenous protection. After this triggering phase, an intracellular cascade of events finally brings the cell into its protected phenotype (figure 2). Several essential components of this cascade have been identified, although the exact sequence has not yet been fully elucidated. The activation of the intracellular enzyme protein kinase C (PKC) is essential for ischaemic preconditioning. Several studies have shown that PKC activation is mediated via activation of phosphatidilylinositol-3-kinase (PI3K), which is an important upstream signalling molecule. PI3K activates the serine/threonine kinase Akt, which subsequently inactivates the proapoptotic kinase glycogen synthase kinase-3 (GSK-3).

Following activation, PKC actually translocates from the cytosol to the particulate fraction where phosphorylation of specific substrates can occur. Specific activation and translocation of the isoform PKC-ε seems to be responsible for ischaemic preconditioning. Interestingly, in some animal models only inhibition of PKC during the index ischaemia aborts preconditioning, suggesting that PKC is a mediator and not a trigger. Additionally, activation of a tyrosine kinase mediates early preconditioning, either downstream or in parallel with PKC. Also, each subfamily of the mitogen-activated protein kinases (MAPKs), namely the 42/44-kDa extracellular receptor kinase (ERK), 46/54-kDa c-jun kinase (JNK) and 38-kDa p38 MAPK, has been proposed to be involved in the signalling cascade of ischaemic preconditioning (reviewed by Michel et al. and Armstrong).

Another essential component of the mechanism leading to early protection after preconditioning is the ATP-sensitive potassium channel (KATP channel). This channel, which opens when intracellular levels of ATP decline, is the known target of sulphonylureas in the pancreas, but is also present in cardiomyocytes and vascular smooth muscle cells. Cardiomyocytes contain KATP channels located on both the sarcolemma (sarKATP channels) and the mitochondrial inner membrane (mitoKATP channel). These channels have different pharmacological profiles. Both channels are blocked by glibenclamide whereas the mitoKATP channel is selectively blocked by 5-hydroxydecanoate (5-HD). Diazoxide opens the mitoKATP channel with far greater affinity than the sarKATP channels. Gross and Auchampach first described the critical role of KATP channel opening in ischaemic preconditioning, because early preconditioning was completely inhibited by the administration of glibenclamide either before or immediately after the preconditioning ischaemia. Initially, sarKATP channels were held responsible for preconditioning, but recent evidence increasingly favours a role for mitoKATP channels (already extensively reviewed). Several studies have shown that the administration of diazoxide is able to mimic ischaemic preconditioning and that 5-HD inhibits preconditioning. However, some recent studies still suggest that sarKATP channels are also involved. It appears likely that opening mitoKATP channels is not only an end-effector of preconditioning, but also a trigger, as opening is also essential during the preconditioning stimulus.

Which end-effectors are involved and how these end-effectors ultimately provide protection is the most elusive part of ischaemic preconditioning. Inhibition of the sodium/hydrogen exchanger, prevention of osmotic swelling and prevention of cytoskeleton disruption by heat shock protein HSP27 have all been proposed to act as end-effectors. Lately, however, accumulating evidence strongly suggests that the various upstream signalling pathways all converge on mitochondrial proteins aimed at limiting in particular reperfusion injury. In order to adequately understand this complex part of the preconditioning cascade, we will briefly focus on mitochondrial function, with particular emphasis on the role of mitochondria in reperfusion injury. Although reperfusion is essential for cardiomyocytes to survive a period of ischaemia, it is well appreciated that
reperfusion itself can expedite cell death, which is known as reperfusion injury. The mechanism of reperfusion injury differs from ischaemic injury, best illustrated by the role of apoptosis in both forms of injury. The vast majority of studies on this topic conclude that apoptosis, in contrast to necrotic cell death, only occurs or is accelerated during reperfusion and not during ischaemia. Reperfusion is characterised by a boost of ROS, which are important mediators of reperfusion injury, as antioxidants, applied during reperfusion, limit cellular death. Moreover, as apoptosis is an energy-requiring form of cell death, it has been postulated that reperfusion is essential to generate the necessary amount of ATP molecules. Mitochondria play a prominent role in reperfusion. The most important function of mitochondria is the generation of ATP, by the transfer of electrons on oxygen. This transfer is associated with a transfer of H⁺ ions from the inside to the outside of the mitochondrial inner membrane, thus establishing the mitochondrial transmembrane potential. Subsequently, the passive inward flux of H⁺ ions forms the driving force for ATP production. Moreover, during electron transfer, 1 to 5% of ions lose their way and participate in the formation of ROS. The mitochondrial permeability transition pore (MPTP) is formed by multiprotein complexes capable of forming large nonselective pores in the otherwise highly impermeable inner mitochondrial membrane. There is a large body of evidence that this pore, which remains closed during ischaemia, opens during reperfusion. This pore is characteristically opened by high mitochondrial [Ca²⁺], oxidative stress, ATP depletion and mitochondrial depolarisation, all pre-eminently present during reperfusion. Mitochondrial permeability transition during reperfusion results in uncoupling of the respiratory chain, ultimately resulting in ATP depletion and necrosis on the one hand and in matrix swelling and subsequent rupture of the outer membrane leading to release of proapoptotic proteins and apoptosis on the other hand. That opening of the MPTP indeed contributes to reperfusion injury is convincingly demonstrated by showing that inhibition of MPTP opening at reperfusion, typically with cyclosporine A (CsA), significantly reduces ischaemia/reperfusion injury.

A series of recent studies has shown that ischaemic and pharmacological preconditioning ultimately provide protection by inhibiting ROS-induced opening of the MPTP during reperfusion. Very recently, an extensive and elegant study by Juhaszova et al. showed that ischaemic preconditioning as well as pharmacological preconditioning by a wide variety of drugs act by inhibiting ROS-induced MPTP opening at reperfusion and this study elucidated a great part of the signalling cascade responsible for MPT inhibition. They showed that cardioprotection with a memory (e.g. by ischaemia, diazoxide, pinacidil, bradykinin) opens mitoK<sub>ATP</sub> channels, resulting in a subtle mitochondrial swelling, which increases electron transport and gives rise to a small burst of ROS production, which acts as a messenger to activate PKC, which ultimately converge on phosphorylation of GSK-3β. Phosphorylation of GSK-3β inhibits its function and inhibits MPTP opening during reperfusion. Interestingly, GSK-3β can be inhibited by lithium, which has previously been shown to reduce infarct size.

In conclusion, the infarct size limiting effect of ischaemic preconditioning seems to be largely mediated by inhibition of reperfusion injury and subsequent apoptosis. There is convincing evidence that in myocardial infarction, both necrosis and apoptosis are involved. Various animal studies have shown significant reduction in myocardial infarct size using inhibitors of apoptosis, such as caspase inhibitors, during reperfusion. Moreover, caspase or endonuclease inhibition after myocardial infarction attenuates ventricular remodelling and improves contractile function. Gottlieb et al. were the first to show that in an in vitro model of rabbit cardiomyocytes, ischaemic preconditioning inhibits ischaemia/reperfusion-induced apoptosis. Later, this was confirmed in vivo in a rat model of myocardial ischaemic preconditioning.

With increasing emphasis on the pivotal role of limitation of reperfusion injury in the infarct size limitation by ischaemic preconditioning, several studies explored whether interventions during reperfusion, rather than before ischaemia, could also limit infarct size. This is of great potential importance, as ischaemic insults are seldom predictable and therefore interventions at the time of reperfusion are more suited to most clinical scenarios. Indeed, intermittent short repetitive interruptions to reperfusion at the very onset of reperfusion were shown to provide similar protection to ischaemic preconditioning in dogs and rats, via activation of the PI3K-Akt pathway (reviewed by Hausenloy et al).

**Delayed Ischaemic Preconditioning**

In 1993, it was first described that the protective effect of ischaemic preconditioning, which was previously thought to be a transient phenomenon, reappears 24 hours after the preconditioning ischaemic period and results in a delayed protected phenotype. Although not as powerful as the early protection provided by preconditioning (infarct size reduction on average 50%), this delayed phase of protection lasts up to 72 hours and, in that respect, might be more therapeutically applicable in clinical practice. Moreover, this late phase of preconditioning also provides robust protection against myocardial stunning. This delayed phase of protection is also called ‘late’ preconditioning.
tioning or the ‘second window of protection’ (SWOP).

Although classical and delayed protection largely share common signalling pathways, several essential differences are present (figure 4). In this review, we only briefly highlight the differences between classical and delayed preconditioning, the latter being more extensively reviewed elsewhere. The distinctive time course of delayed preconditioning, the latter being more extensively reviewed elsewhere.

The ultimate infarct limitation, but how these channels are opened is still a matter of debate.

In 1993, Przyklenk et al. extended the initial view on ischaemic preconditioning tremendously by demonstrating that brief preconditioning occlusions of the circumflex
The Journal of Medicine

N. P. Riksen is an MD clinical research trainee financially supported by the Netherlands Organisation of Scientific Research (ZonMw). The contribution of G. A. Rongen was made possible by a fellowship of the Royal Netherlands Academy of Arts and Sciences.

NOTE

This review is the first of two parts. The second part will appear in the December issue of this Journal.

REFERENCES


The mechanism underlying remote ischaemic preconditioning is not yet as well defined as the mechanisms mediating classic preconditioning. Interestingly, in the first study on inter-organ remote preconditioning, Gho et al. already identified two important clues for understanding the mechanism of protection. First, ganglionic blockade with hexamethonium prior to the preconditioning stimulus abolished cardioprotection, suggesting neuronal involvement. Secondly, reperfusion after the preconditioning ischaemia was essential, suggesting that at reperfusion substances are released in the mesenteric bed that stimulate afferent neurofibres or directly protect the heart. Although several other studies confirmed involvement of a neurogenic pathway in mesenteric preconditioning of the myocardium, the mechanism underlying remote ischaemic preconditioning was not abolished by hexamethonium. Additional evidence that a humoral factor is also involved in remote preconditioning comes from the observation that in rabbits cardioprotection by a preceding short period of coronary occlusion can be transferred to a nonpreconditioned heart via coronary effluent transfusion and even transfusion of whole blood. This transferred protection is not mediated via adenosine or norepinephrine in the effluent and can be abolished by the opioid-antagonist naloxone. Additional studies on mesenteric preconditioning of the myocardium showed that capsaicin-sensitive sensory nerves might be involved and that the protection is abolished by pretreatment with naloxone and a bradykinin receptor antagonist before the transient mesenteric occlusion. Moreover, signal transduction via PKC is proposed, based on the findings that inhibition of PKC before as well as after the preconditioning stimulus inhibits protection and that brief mesenteric artery occlusion induces a rapid translocation of PKC-e from the cytosol to membrane fractions in cardiomyocytes. In a rabbit model, it was shown that cardioprotection by a brief renal artery occlusion is totally abolished by adenosine antagonism either before the renal occlusion or before the subsequent coronary occlusion, proposing a dual role for adenosine as trigger and mediator of remote preconditioning. In line with these observations, Liem et al. recently described evidence that in remote preconditioning with small intestine ischaemia, locally released adenosine triggers afferent nerves which in turn leads to stimulation of cardiac adenosine receptors. Finally, very limited evidence suggests that remote preconditioning also occurs in humans in vivo, using a surrogate marker of ischaemic damage. Kharbanda et al. showed that three five-minute cycles of forearm ischaemia prevents reduction in acetylcholine-induced vasodilation after 20 minutes of ischaemia of the contralateral arm.

ACKNOWLEDGEMENTS

This review is the first of two parts. The second part will appear in the December issue of this Journal.


47. Murphy E. Primary and secondary signaling pathways in early preconditioning that converge on the mitochondria to produce cardioprotection. Circ Res 2004;94:7-16.


89. Marber MS, Latchman DS, Walker JM, Yellon DM. Cardiac stress protein elevation 24 hours after brief ischemia or heat stress is associated with resistance to myocardial infarction. Circulation 1993;88:1264-72.


