Review

Ischaemic preconditioning: present position and future directions

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Abstract

Preconditioning the myocardium using short episodes of sublethal ischaemia will delay the onset of necrosis during a subsequent lethal ischaemic insult. This powerful protective adaptation of the myocyte has also been observed in other cell types. The potential for clinical application to benefit patients with a variety of pathological conditions has led to an expansion in our knowledge concerning the pathophysiology of ischemia-reperfusion injury and the regulatory mechanisms underlying cellular metabolism. We feel it is timely to assess the current position in this field and provide a critical appraisal to facilitate future research. © 1998 Elsevier Science B.V.

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1. Introduction

It is now 11 years since the phenomenon termed “ischaemic preconditioning” was formally recognised [1]. There can be little doubt that our understanding of the mechanisms underlying the pathogenesis of ischaemia-reperfusion injury has been enhanced significantly by the extensive research stimulated by interest in endogenous myocardial protection. In the basic experimental setting, the triggers, mediators and effectors of the preconditioning phenomenon are being extensively investigated. The results of recent clinical experiments suggest that preconditioning can protect against ischaemic injury, although at this stage they must be interpreted with caution. In view of the enormous interest, we believe it is timely to undertake a reasoned evaluation of the key information in order to prevent unrealistic expectations and more specifically to identify areas of future focused research.

2. What is ischaemic preconditioning?

In 1986 Reimer et al. [2] reported a series of experiments in the dog heart designed to dissect the contributions of ATP depletion from catabolite accumulation in the genesis of lethal ischaemic injury. Their experimental model involved repetitive brief ischaemic episodes, working on the premise that each ischaemic episode would cause cumulative ATP depletion while the intermittent reperfusion would wash out ischaemic catabolites. To their surprise they found that, following the initial ischaemic period, ATP levels were not depleted further by subsequent similar ischaemic challenges. They also noted that no infarction occurred in six of the seven dogs studied. This result was contrary to the previously accepted view that repetitive ischaemia would cumulatively lead to infarction. The observation led the same group [1] to test the hypothesis that the preservation of high energy phosphates was due to a slowing of consumption during ischaemia associated with a rapid and protective adaptation of the
myocyte. They tested this hypothesis by subjecting the myocardium to four 5 minute coronary occlusions, separated by 5 minutes’ reperfusion, before a sustained 40 minute ischaemic insult. They found that the preceding brief periods of ischaemia and reperfusion were protective, reducing infarct size to 25% of that seen in the control group. This phenomenon was termed “preconditioning with ischaemia”. Following these initial studies, the protection obtained has been further characterised both in terms of time course and various end-points of cellular injury.

3. Induction and end-points of classic preconditioning

In the strictest sense, ischaemic preconditioning refers to the delay of infarct development by one or more preceding cycles of ischaemia and reperfusion. It is important to realise that the evolution of necrosis is delayed but not prevented. Preconditioning will limit infarct size during a temporary coronary occlusion but not during a prolonged or permanent occlusion. Classically, the stimulus for preconditioning is a critical reduction of myocardial blood flow, and the end-point is infarct size. However, in a broader sense, preconditioning is also induced by a reduction of coronary flow in buffer perfused heart preparations [3], as well as by hypoxic perfusion of buffer perfused [3] and blood perfused hearts [4]. Finally, preconditioning is also induced in isolated cardiac muscle preparations when ischaemia is simulated by a combination of hypoxia, substrate free perfusion and pacing stress [5] and even in isolated cardiomyocytes subjected to hypoxia and lack of glucose substrate [6]. Infarct size (the extent of irreversible damage) is usually determined by quantitative morphological techniques such as triphenyltetrazolium chloride (TTC) staining. In some studies, morphological alterations or indicator uptake are determined on the single cell level [6]. Other studies, however, have used recovery of contractile function as an end-point of ischaemic preconditioning [7]. This is a critical distinction because there is good evidence that classic preconditioning does not protect against stunning [8]. Thus, as long as the signal cascade of ischaemic preconditioning is not entirely clear, great caution should be used when extrapolating findings from simulated ischaemia and improved recovery of contractile function to true ischaemia and reduction of infarct size. Yet another end-point of ischaemic preconditioning is the incidence and severity of ischaemia- and reperfusion-induced arrhythmias, and both a reduction [9] and an aggravation of arrhythmias [10] have been reported.

It appears that there is a bimodal distribution of protection; the initial phase described by Murry, Reimer and Jennings lasts around one to three hours, depending on species and model, whilst a delayed preconditioning or “second window of protection” (SWOP) originally identified and described in 1993 [11], exists between 12 and 72 hours following the initial ischaemic insult. Since the underlying pathophysiology and mechanisms of these two phases of endogenous cardioprotection may be different, it is important to make critical distinctions between the two. Accordingly we refer to the early phase of protection as “classic preconditioning”.

4. Triggers and mediators of classic ischaemic preconditioning

Classic ischaemic preconditioning is not dependent on the existence of collateral vessels [12] and occurs in the presence of a protein synthesis inhibitor [13]. A number of neuroendocrine and paracrine triggers have been identified which are released and/or operative during the preconditioning ischaemia, including adenosine [14], acetylcholine [15,16], catecholamines [17], angiotensin II [18], bradykinin [19], endothelin [20], and opioids [21]. Which triggers and to what extent they contribute to preconditioning may vary between species. For example, adenosine was not found to be involved in preconditioning in the rat heart [22], but it is important in rabbit [14], dog [23], pig [24] and human myocardium [25]. The nature of the trigger varies also with respect to the end-point of protection, as prostanoids appear not to be involved in infarct size reduction [26] but are involved in the attenuation of arrhythmias [27].

Downey and co-workers [19] have proposed an additive interaction of preconditioning triggers such that a threshold must be reached to achieve protection. At the cellular level the metabolic adaptation induced is characteristic of an ‘all or none’ phenomenon, and the situation would seem to be akin to that seen in synaptic transmission of neural impulses, with summation of different impulses until firing threshold is attained. This concept is well illustrated by a recent study using human atrial muscle [28]. Two individual stimuli (a sublethal hypoxic insult, and elevated bradykinin levels by ACE inhibition) were insufficient to induce a preconditioning response when administered separately, but, in combination, a full protective effect was observed.

It should be emphasized that triggers must be distinguished from mediators of preconditioning. For example, bradykinin is a trigger as it is important during the preconditioning episode but not during the sustained ischaemia [19]. In contrast, adenosine is important, both as a potential trigger and also as a mediator during the sustained ischaemia, at least in the rabbit [29].

5. Post-receptor signal cascade and end-effector(s) of classic ischaemic preconditioning

A number of the triggers mentioned above including noradrenaline, bradykinin and adenosine, are coupled to G-proteins, and pretreatment with pertussis toxin blocks
the protective effect of ischaemic preconditioning [30]. Downey has proposed a scheme whereby the activated receptors couple through G-proteins to phospholipase C, and the formed diacylglycerol activates protein kinase C (PKC) [31] (see Fig. 1). Indeed, ischaemic preconditioning was blocked by the PKC inhibitor staurosporine [32], as well as by more specific PKC inhibitors such as polymyxin B [32,33] and chelerythrine [34,35]. Also, ischaemic preconditioning may be substituted by activation of PKC using phorbol myristate acetate or a diacylglycerol analogue [32,35]. In contrast, two recent studies failed to demonstrate prevention of ischaemic preconditioning in anaesthetised dogs [36] and pigs [37], using either polymyxin B [36] or staurosporine [37]. It should be appreciated that phorbol ester-induced translocation of PKC also leads to increases in intracellular cAMP and to cellular calcium overload, both of which would be detrimental to cardiac function. In addition, PKC aggravates hypoxic injury [38] and is proarrhythmic [39]. These inconsistent results have lead some commentators to suggest that the activation of PKC following ischaemic preconditioning may simply be an epiphenomenon [40]. However, the use of poorly selective activators and antagonists of PKC may generate misleading data. A wide variety of isoenzymes of PKC may be activated by phorbol esters, whereas translocation and activation of one specific isoenzyme may follow an ischaemic preconditioning stimulus. Therefore assays of total PKC activity may fail to detect changes in the activity of one or two isoforms which may form only a fraction of the PKC pool.

Recent work suggests that kinases other than PKC play a role in classic preconditioning. Experiments with the tyrosine kinase inhibitor genistein [41] suggest that tyrosine kinase activity is a crucial component of the signalling cascade. This inhibitor is reported to be selective for tyrosine kinase relative to other kinases but ultimately some measure of tyrosine kinase activity is required to support the pharmacological data. The positions of receptor and cytosolic (protein) tyrosine kinases, PKC and any other kinases in the signalling cascade remain undetermined and add a further tier of complexity to the mechanisms of classic preconditioning. The mitogen-activated protein kinases (MAPKs) are discussed below under ‘‘signalling aspects of delayed preconditioning’’, but may well be involved in the signalling mechanism of classic preconditioning. Further work in this area may be important for directing us toward more specific ways of manipulating the preconditioning phenomenon.

Several studies have reported that blockade of ATP-sensitive K+ channels (KATP) abolishes ischaemic preconditioning in rats [21,42], rabbits [43,44], dogs [45], pigs [46,47] and man [5], using KATP blockers such as glibenclamide or the more ischaemia-specific inhibitor sodium 5-hydroxydecanoate. Conversely, KATP activation using bimakalim mimics ischaemic preconditioning [48] or lowers the threshold for induction [49]. Activation of KATP is associated with a reduction in action potential duration [47] which is thought to reduce cellular calcium overload and preserve viability. Of further significance is the finding that adenosine receptors couple to the phospholipase C-diacylglycerol second messenger system through G-proteins [50]. Furthermore, PKC activates KATP at near physio-

![Fig. 1. Diagrammatic representation of the cellular events thought to occur following a preconditioning stimulus. Solid arrows represent pathways established by a significant body of experimental evidence. Dashed arrows represent pathways for which there is suggestive evidence only.](https://academic.oup.com/cardiovascres/article-abstract/37/1/21/540753)
logical levels of ATP [51], and it has been shown that the protection induced by the PKC activator dioctanoylglyceral is abolished by the $K_{\text{ATP}}$ blocker glibenclamide in human muscle [5]. A synergistic action of adenosine and PKC on $K_{\text{ATP}}$ and action potential duration shortening has been reported [52]. The hypothesis that $K_{\text{ATP}}$ channel openers exert their cardioprotective effect by accelerating sarcolemmal channel opening during ischaemia has been challenged by the observation that low dose bimakalim may induce cardioprotection without any effect on action potential duration [53]. Thus the involvement of $K_{\text{ATP}}$ channels in other cell membranes, such as the mitochondrial plasma membrane, may be worthy of investigation. End-effectors other than the $K_{\text{ATP}}$ have been suggested, including a critical step in energy expenditure [54] or alterations of the cytoskeleton [55].

6. Is reperfusion mandatory for classic preconditioning?

A 15 minute partial coronary artery occlusion which reduced blood flow by 50% in anaesthetised dogs was not sufficient to reduce infarct size after a subsequent 60 minute coronary occlusion when there was no intermittent reperfusion [56]. However, a 30 minute partial coronary artery occlusion which reduced blood flow by 70% in anaesthetised pigs reduced infarct size after a subsequent 60 minute coronary occlusion without intermittent reperfusion [57]. Also, a 10 minute no-flow ischaemic episode preceding 80 minute of sustained low-flow ischaemia with a reduction of blood flow by 70% reduced infarct size as compared to 90 minute low-flow ischaemia alone [58].

Whereas these studies appear to be controversial with respect to the need for reperfusion, the 15 minute reduction of coronary blood flow by 50% may have been insufficient to reach the critical threshold for preconditioning. In that case, classic ischaemic preconditioning does not require reperfusion as long as some residual flow is maintained.

7. Metabolic considerations in classic preconditioning

The relatively small influence of classic ischaemic preconditioning on the rate of energy depletion during prolonged ischaemia is probably not sufficient to explain its anti-infarct effect. Several observations support an important role of reduced catabolite accumulation in the genesis of the anti-infarct effect of classic ischaemic preconditioning. During brief preconditioning episodes of ischaemia, glycogen stores are depleted and lactate and protons accumulate. During the reperfusion periods following these episodes, accumulated catabolites are rapidly washed out [59,60]. The synthesis of glycogen proceeds at a much slower rate, and glycogen stores remain substantially depleted during a period of time which coincides quite closely with the duration of the first window of ischaemic preconditioning [61]. When glycogen-depleted myocardium is exposed to prolonged ischaemia, lactate accumulation is slowed [59]. The consequent attenuation of proton production is the main reason for the slower rate of progression of ischaemic acidosis in preconditioned myocardium. This effect of classic preconditioning on ischaemic acidosis is much more prominent than that on energy depletion, and the delay in pH fall is typically much more prolonged than the delay in ATP decay [62]. Complete catabolite washout by effective reperfusion seems to be an essential condition for preconditioning, and the restoration of myocardial glycogen content after preconditioning is temporally paralleled by a loss of the anti-infarct effect [61]. However, reduced lactate accumulation per se does not explain the anti-infarct effect of classic ischaemic preconditioning. Glycogen-depleted myocardium can still be preconditioned, and washout of catabolites during ischaemia by means of anoxic intracoronary perfusion has no effect on infarct size [63]. The limitation of proton production in preconditioned cardiomyocytes during ischaemia could have important consequences on cell viability. The excess of protons produced during ischaemia or anoxia stimulates $Na^+-K^+$ exchange even in the absence of a transsarcolemmal pH gradient. The resulting $Na^+$ gain can accelerate ATP consumption by increasing $Na^++K^+$-ATPase activity during the initial phases of ischaemia and contribute to deleterious $Ca^{2+}$ overload via $Na^+/Ca^{2+}$ exchange during ischaemia and/or during the initial phase of reperfusion [64]. Although reduced $Na^+/H^+$ exchange could play a role in the genesis of the anti-infarct effect of classic ischaemic preconditioning, it is probably not the only or major cause, since the beneficial effects of classic preconditioning and $Na^+/H^+$ exchange inhibition seem to be partially additive [65]. Thus, reduced proton and catabolite accumulation during prolonged myocardial ischaemia are prominent effects of classic preconditioning. These effects could contribute to the anti-infarct effect of preconditioning, but only in cooperation with other mechanisms.

8. Remote preconditioning and stretch-induced preconditioning

Stimuli other than ischaemia of the myocardial risk territory may confer cardioprotection against damage by subsequent coronary artery occlusion. Brief circumflex coronary occlusions in canine myocardium induce protection of remote myocardium subtended by the left anterior descending coronary artery, suggesting that unidentified diffusible factors or neuronal mechanisms may influence remote tissue [66]. A further, and more intriguing, example of remote preconditioning is that brief renal ischaemia or mesenteric artery occlusion leads to protection against coronary artery occlusion [67]. The mechanisms of ‘inter-organ’ preconditioning are unknown but it is possible that...
there is a neuronal component. Transient stretch of myocardium by acute volume overload has been shown to confer protection against myocardial ischaemia by Ovize and co-workers [68]. This phenomenon is abolished by gadolinium, a stretch activated ion channel blocker, and by a PKC blocker [69]. These investigations expand the strictly defined concept of ischaemic preconditioning.

9. Ischaemic preconditioning of non-cardiac tissues

The induction of endogenous protective mechanisms by exposure to a stressful stimulus is not a phenomenon unique to myocardium. Using a porcine model Mounsey et al. [70] were able to demonstrate less necrosis of skeletal muscle (latissimus dorsi flap) when subjected to prolonged ischaemia following three brief cycles of antecedent ischaemia [71]. This adaptive response in skeletal muscle is blunted by adenosine receptor blockade and elicited by A1 receptor activation [72]. Moreover, preconditioning of skeletal muscle attenuates capillary no refill following ischemia in this tissue, an effect which is KATP channel-dependent [73]. A delayed phase of protection has been observed in rat hippocampus two to three days following a short period of sublethal cerebral ischaemia which was associated with increased expression of HSP72 [74]. More recent evidence has emerged that both adenosine and KATP channels are involved in the mechanism of delayed preconditioning in neuronal tissue [75]. Delayed protection against ischaemic injury in rat small intestine has been observed 24 hours after an ischaemic episode. This effect is not related to epithelium and would appear to be dependent on adaptive changes in the lamina propria. This observation may have relevance for the disappointing results of attempts to precondition the kidney using models which rely on a functional endpoint of tubular damage. In a rat study using two cycles of 5 minutes renal artery occlusion followed by 10 minutes reperfusion the functional and histological consequences of a 30 minute ischaemic insult were exacerbated [76]. Induction of the heat stress response was not associated with functional protection against ischaemia reperfusion injury in either in situ or isolated rat kidney [77]. Thus, the cell types in which either early or delayed preconditioning phenomena, or both, occur would appear to share a common feature of being terminally differentiated, or at least slowly regenerated following injury, unlike renal tubular and intestinal epithelia which can undergo rapid restitution.

10. General characteristics of delayed preconditioning

Investigation of delayed preconditioning is still at an early stage but the available evidence suggests that this phenomenon is a form of sub-acute myocardial adaptation. The second window of protection was originally described as a period of enhanced tolerance to lethal ischaemia in open-chest rabbit [11] and canine [78] models of coronary artery occlusion 24 hours after a preconditioning stimulus of brief repetitive cycles of coronary occlusion. This delayed anti-infarct effect has subsequently been confirmed in further open-chest rabbit [79–81] and dog studies [82], in chronically-instrumented conscious rabbits [83] and in the rat [84,85]. The duration of the delayed anti-infarct effect of preconditioning has been characterised in the rabbit and protection extends between 1 and 3 days [80]. The prolonged duration of protection makes delayed preconditioning particularly interesting for its potential clinical relevance.

There is evidence also that the delayed protection conferred by preconditioning extends to other indices of ischaemic and post-ischaemic myocardial dysfunction. Vech’s group reported a delayed anti-arrhythmic effect following preconditioning [86] with ventricular rapid pacing in the dog. A late phase of protection against coronary occlusion- and reperfusion-induced tachyarrhythmias was observed around 20–24 hours after the pacing stimulus. Further evidence for an anti-arrhythmic effect 24 hours after preconditioning has been obtained in conscious rabbits [83]. In the dog the anti-arrhythmic protection is almost completely lost by 48 hours [87], in contrast to the anti-infarct effect in the rabbit which, as mentioned above, extends to 72 hours.

A further endpoint of delayed preconditioning protection has been described. Bolli’s group [88] used conscious pigs to investigate post-ischaemic myocardial dysfunction following repeated 2 minute coronary artery occlusions (inducing myocardial stunning lasting 3 to 4 hours). Repetition of the protocol in the same animals 24 hours later revealed that post-ischaemic recovery of thickening fraction was significantly accelerated compared with recovery following the initial protocol. It appeared that the initial stunning protocol preconditioned against a subsequent stunning protocol 24 hours later. This anti-stunning effect of delayed preconditioning persists for several days [89] and has also been observed in conscious rabbits [90]. Evidence for a delayed effect of preconditioning in vitro has been obtained in isolated cardiomyocyte studies using hypoxic and simulated ischaemic preconditioning media [91,92]. These studies have demonstrated attenuated ischaemic injury by enzyme leakage and dye exclusion techniques.

Some studies designed to examine the second window of protection in vivo have reported negative findings. Tanaka et al. [93] showed that a preconditioning protocol that induced early protection in the rabbit induced no protection 24 or 48 hours later. Reasons for this absence of protection are not clear. A further two studies have examined preconditioning protocols in pig myocardium and have not seen limitation of infarction 24 hours later [94,95]. It is possible that 24 hours after preconditioning may not be the ideal time for examination of the delayed phase of...
protection against infarction and it is conceivable that in the pig maximum infarct limitation may be observed beyond 24 hours. Alternatively, these failures to observe delayed protection may be due to selection of relatively severe index ischaemic periods (60 minutes and 40 minutes respectively) during which endogenous mechanisms of delayed protection may be overwhelmed, even though classic preconditioning still works with these ischaemic periods. A further study has reported no delayed preconditioning limitation of necrosis in the rat [96].

11. Triggers of delayed preconditioning

As with investigations of classic preconditioning, the mechanisms of delayed preconditioning may be conceptually divided into ‘upstream’ and ‘downstream’ components. In the rabbit, adenosine A1 receptor activation during preconditioning is an important trigger of delayed protection against infarction. Adenosine receptor blockade during preconditioning abolishes the protective response 24 hours later [79] and conversely, stimulation of A1 receptors with a selective agonist, CCPA, results in marked protection against infarction 24–72 hours later [79,97,98].

In the pig, delayed preconditioning against stunning does not appear to involve adenosine, but free radicals and nitric oxide are important triggers [99,100]. At present it is not possible to say if this divergence is due to differences in experimental endpoint (infarction versus stunning) or species (rabbit versus pig).

Since it is clear that delayed myocardial protection can be induced by means other than transient ischaemia, investigation of these stimuli may ultimately be relevant, not only for our understanding of the mechanisms of delayed preconditioning but to the development of practical therapeutic approaches. For example, bacterial endotoxin treatment is known to induce delayed myocardial protection, probably by upregulating various cytoprotective proteins, including antioxidants and inducible nitric oxide synthase. The endotoxin derivative monophosphoryl lipid-A induces myocardial protection 24 hours after administration and the opening of the $K_{ATP}$ channel may be integral to this late protective response [101].

12. Signalling aspects of delayed preconditioning

Activation of PKC appears to be a crucial intermediate step since pharmacological inhibition of PKC during the preconditioning stimulus abolishes protection 24 hours later in the rabbit infarct model [81] (see Fig. 1). Direct measurements of PKC activity and translocation have not been widely studied so far but Parratt’s group have recently provided evidence that sustained PKC-ε translocation to the membrane fraction occurs in the hearts of dogs subjected to rapid cardiac pacing [102], a stimulus that induces delayed protection against ischaemia-reperfusion arrhythmias. It has also been reported that brief repeated periods of coronary artery occlusion in the conscious rabbit cause the translocation of PKC-ε [103], and that this can be blocked with chelerythrine [104].

The involvement of other parallel and downstream kinases is under investigation. Considerable interaction exists between PKC and other kinase systems including tyrosine kinase and MAP kinase cascades (see below). Tyrosine kinase activation may be an obligatory component of the signalling cascade since administration of genistein during preconditioning in rabbits abrogates the delayed anti-infarct effect [105]. Interestingly, delayed protection induced by adenosine A1 agonist in rabbits is dependent on both PKC and tyrosine kinase activation since protection can be abolished by pretreatment with either chelerythrine (a PKC inhibitor) or lavendustin-A (a tyrosine kinase inhibitor) [106].

In addition to PKC and tyrosine kinases, other kinase cascades are likely to be important, especially those in MAPK families. Three major MAPK families exist in eukaryotic cells; the ‘classic’ p42/p44 MAPKs (also known as p42/p44 ERKs); p38 kinase; and the stress activated c-jun N-terminal kinase (JNK, SAPK). PKC is known to phosphorylate and activate raf-1 kinase which provides a direct link to the p42/p44 MAPK family. There is evidence that transient ischaemia rapidly increases total MAPK activity in rat heart [107]. Other studies suggest the activation, by ischaemia or reactive oxygen species, of p38 kinase and JNK SAPK [108,109] which are known to phosphorylate factors that co-ordinate gene transcription. The involvement of these kinases in delayed preconditioning is set to become the focus of increasing attention. The interactions of these complex signalling systems and their involvement or interaction with membrane channels or new protein synthesis still needs to be evaluated.

13. Protein effectors of delayed protection

The prevailing hypotheses imply that delayed protection is related to the acquisition of cytoprotective proteins in preconditioned myocardium or to alterations in the activity of such proteins by post-translational mechanisms. The time course of delayed preconditioning is suggestive of a mechanism involving new protein synthesis. However, the identities of these putative effectors of cytoprotection are presently unknown.

Two early reports raised the possibility that the delayed phase of protection involves either increased activity of manganese-superoxide dismutase (SOD) [78] or elevation of the myocardial content of the major inducible heat shock protein, HSP72 [11]. Both proteins are stress-induced proteins that have cytoprotective properties. Manganese-SOD is a mitochondrial antioxidant which detoxifies superoxide anions. HSP72 is a chaperone protein...
involved in regulation of protein folding, transport and denaturation during the cellular response to injury. Hoshida et al. [110] described the temporal dynamics of manganese-SOD activity following preconditioning in canine myocardium and reported a biphasic pattern of enzyme activity over a 24 hour period similar to the biphasic timecourse of the anti-infarct effect [78]. Similarly, myocardial content of HSP72 was elevated in rabbits 24 hours after preconditioning, a time when increased tolerance to infarction was observed [11]. Relationships between enhanced ischaemic tolerance and stress-inducible cytoprotective protein activity have been pursued most convincingly in gene transfection studies [111,112], antisense oligonucleotide studies [91] and studies with transgenic mice constitutively over-expressing human HSP72 [113]. These studies tend to suggest, but do not confirm, that the appearance of the delayed protection could be related to changes in activities of stress-inducible cytoprotective proteins in preconditioned myocardium. However, it is important to note that since the regulation of a large number of proteins is altered by sublethal ischaemia, it is likely that delayed preconditioning involves other proteins in addition to anti-oxidants and heat shock proteins. A number of new gene products not yet identified may be involved and techniques such as differential gene display and other advanced molecular techniques will be very relevant to future research directions.

14. Preconditioning the human heart

The evidence in support of the occurrence of the preconditioning phenomenon in human myocardium arises from laboratory and clinical experiments. Studies in isolated human ventricular myocytes [114], and isolated atrial trabeculae [25] both suggest that protection can be induced in vitro using metabolic and functional end-points respectively. In the clinical setting there is some evidence to suggest that preconditioning may occur naturally. Patients suffering angina prior to a myocardial infarction have a better in-hospital prognosis, a reduced incidence of cardiogenic shock and congestive cardiac failure, and smaller infarcts as assessed by release of cardiac enzymes [115]. The phenomenon of warm-up angina, in which patients complain that their anginal symptoms are worse in the morning but improve during the course of the day has been studied [116]. There is evidence of increased efficiency of myocardial metabolism during a second episode of exercise in terms of reduced oxygen consumption at a given work load as well as less anginal symptoms and ST segment changes. PTCA studies, in which the effect of serial balloon inflations can be examined, have provided further support [117] but, as with all of the above examples, results may be confounded by the effects of collateral recruitment despite efforts to control for this effect [118].

More direct evidence for preconditioning in man has emerged from a study in patients undergoing cardiac surgery in which resistance to global ischaemia was assessed [119]. In this situation changes in collateral flow do not play a role. Intermittent application of the aortic cross clamp was used to deliver repeated episodes of global ischaemia to provide the preconditioning stimulus. Patients subjected to this protocol had better preservation of ATP levels in myocardial biopsies during a subsequent 10 minute global ischaemic period. These metabolic changes were almost identical to those seen in dogs by Jennings group [2]. However, as discussed later, total myocardial ATP content may not reflect local turnover within subcellular compartments, and certainly does not provide information about the efficiency of cellular metabolism in terms of ATP requirements. In a more recent study [120], involving a larger group of patients, serum levels of troponin-T were used as an indicator of myocardial cell necrosis. Using this end-point, patients subjected to the same preconditioning protocol suffered less necrosis as determined by release of troponin-T. Of considerable interest, however, was the finding that the ATP levels did not differ between preconditioned and control groups. This emphasises the need for multiple end-points to be used, especially in studies where small differences in myocardial viability without overt clinical effects are expected.

15. Who should we treat with therapeutic approaches based on preconditioning?

It would appear from the evidence outlined above that human myocardium is amenable to preconditioning and that preconditioning may occur as a natural feature of some ischaemic syndromes. However, even with the development of pharmacological agents that can mimic or evoke the protection of ischaemic preconditioning, the timing of administration will be critical. Prompt reperfusion will always remain the most effective method of limiting ischaemic injury and is, therefore, the most important determinant of prognosis. However, there are certain situations in which the timing of treatment before the onset of ischaemia can be controlled to some extent.

Patients presenting with unstable angina are at high risk of myocardial infarction and would form a reasonably well-defined group for pre-emptive treatment. A therapy that stimulated or augmented the cellular preconditioning mechanisms over a period of several days or weeks could keep the myocardium protected. In the event of the patient suffering an acute myocardial infarction the treatment would enhance tissue tolerance and slow the rate of necrosis. Such a treatment would ‘buy time’ for the administration of revascularisation therapies. A major theoretical hurdle is maintaining myocardium in a protected state by preconditioning. Experiments in Downey’s laboratory suggest that continuous adenosine A1 receptor activation with high dose chronic infusion of CCPA leads to down-
regulation of the signalling mechanism and loss of protection [121]. However, more encouraging data have been obtained recently using a different dosing schedule. CCPA was administered to rabbits by intermittent dosing over a 10 day period, and the persistence of myocardial protection assessed 48 hours after the final dose. The expected down-regulation of adenosine A_1 receptors was not observed (since the haemodynamic responses to administration of the agonist were preserved) and infarct size remained significantly reduced in the drug treated group [122].

Preconditioning strategies might also be applied prior to a planned procedure involving a potentially injurious ischaemic insult. An example is coronary artery bypass graft (CABG) surgery. Highly effective strategies for myocardial preservation have already been developed including the use of various cardioplegic solutions, topical and systemic hypothermia, and intermittent aortic cross-clamping with ventricular fibrillation. In general, the rationale behind the use of cardioplegic techniques includes rapid diastolic arrest, membrane stabilisation, hyperosmolarity (to prevent intracellular oedema), acid buffering, and hypothermia. Additional strategies such as continuous coronary perfusion, warm instead of cold cardioplegia (to avoid cold injury), and the use of blood instead of crystalloid solutions (to improve oxygen delivery) have all added to the choices available to the cardiac surgeon. Having said that present cardioprotective measures are highly effective at minimising irreversible injury that might occur during these periods of imposed ischaemia, they are not without their limitations. Even with carefully controlled intra-operative ischaemic periods and hypothermia, sensitive markers of tissue injury such as troponin-T indicate that discrete necrosis occurs [120,123–125]. Moreover, as surgeons undertake more complex and higher risk operations, so the need for better preservation methods increases. In a situation like CABG, the administration of an agent prior to surgery that could enhance myocardial defences would reduce susceptibility to focal necrosis during surgery and permit the extension of the intra-operative ischaemic period. High risk patients with poor pre-operative left ventricular function might certainly benefit if the degree of protection could be improved by invoking endogenous cellular adaptive mechanisms. The possibility that organ preservation prior to transplantation might be amenable to the same improved protection is also of significant interest.

16. Clinical endpoints for assessment of efficacy

Any clinical trial involving the use of a potential pharmacological agent designed to mimic the protection of ischaemic preconditioning will have to demonstrate its value in terms of relevant clinical end-points such as preservation of left ventricular function, attenuation of stunning, need for inotropic or balloon support, incidence of clinically detectable infarction, left ventricular failure, and post-operative death. However, studies so far have concentrated on low risk patients with good pre-operative status that would be expected to do well in any event. The benefit derived from ischaemic preconditioning in this group of patients is likely to be marginal. The end-points used presently are relatively insensitive; they provide us with indirect information on myocardial viability and are no substitute for direct measurement of infarct size. Measurement of total myocardial ATP content is not universally accepted as a sensitive marker of cell viability and the concept of a critical tissue concentration of ATP, below which cell death occurs, is now known to be incorrect [126]. If it were possible to measure sub-cellular levels of ATP within different compartments (such as the mitochondrial fraction), and thereby assess local turnover, then more useful information might be available. End-points of clinical outcome are more likely to demonstrate a difference in studies conducted in a group of patients at higher risk, but these can only be performed once safety and tolerability have been established.

17. The future

Direct activation of the cellular pathways involved in ischaemic preconditioning by pharmacological manipulation would allow improved myocardial protection without the need for an ischaemic preconditioning insult. A clear understanding of the mechanisms involved in either form of protection (early or late) is essential to allow a reasoned approach to drug design.

There are several classes of pharmacological agents that may be able to mimic the protection conferred by ischaemic preconditioning and provide some basis for optimism that a beneficial and clinically detectable improvement in myocardial protection may be possible. Some feasible pharmacological approaches are discussed below.

17.1. Mimic the trigger

Adenosine released during ischaemia is one of the most well-researched candidates for the role of preconditioning trigger, and adenosine A_1 receptor agonists represent a promising therapy for improved myocardial resistance to ischaemia. However, there are several potential problems with this approach. Down-regulation of the receptor population and uncoupling from the intracellular message may occur with continued agonist occupancy, although intermittent administration with more modest doses to maintain delayed protection may circumvent this problem (as discussed above).

The potential role of mediators derived from the endothelium has also been investigated. In anaesthetised mongrel dogs, the antiarrhythmic effect of preconditioning can be attenuated or abolished by an inhibitor of the
L-arginine nitric oxide (NO) pathway and by intracoronary administration of methylene blue (preventing the effects of released NO on soluble guanylate cyclase) [127]. Bradykinin is released early in ischaemia [128] and has been postulated as a trigger for the release of NO and prostacyclin. Various attempts have been made to synthesise stable analogues of prostacyclin (e.g. iloprost), and drugs known as “nitric oxide donors” have been studied for many years in an attempt to ameliorate ischaemic injury. It seems more likely, however, that progress in cardioprotection will result from manipulation of cellular mediators down stream of bradykinin and NO/prostacyclin release.

17.2. Modulate the trigger

If the release, transport, uptake, or metabolism of the putative trigger could be deliberately modified then a tissue targeted effect would be more feasible, and systemic side-effects avoided. This approach has been used in order to increase the amount of locally synthesised adenosine present during myocardial ischaemia. Nucleoside transport inhibitors have already been shown to potentiate the antiarrhythmic effects of adenosine [129]. The time threshold for the limitation in myocardial infarct size following classic preconditioning is lowered by drafazine (R75231) and acadesine (another agent that regulates local adenosine levels). Drafazine has also been shown to potentiate the effects of preconditioning [130] and reduce the severity of ischaemia-induced arrhythmias in the pig [129].

It is conceivable that this approach to the pharmacological induction of preconditioning has been utilised unwittingly. There is considerable evidence that bradykinin (synthesised by endothelial cells) is involved as a trigger in preconditioning, and may contribute to the cardioprotective effects of ACE inhibitors [28,131]. ACE inhibition results reduced breakdown of bradykinin. However, an effect on augmented bradykinin levels during myocardial ischaemia has not yet been demonstrated clinically.

17.3. Activate the intracellular signal

The mechanism by which the triggers mentioned above interact with the cell to cause generation of an intracellular message is thought to involve coupling of the receptor to G-proteins that span the cell membrane (see previous discussion). Surprisingly, there has been little published work examining the possibility of deliberate upregulation of G protein function or quantity in cardiac muscle in order to enhance myocardial resistance to ischaemia. As described above, inhibitory G proteins couple to PKC and the possibility of manipulating PKC activity directly is a further theoretical approach. It seems likely that certain isoforms have more importance than others in the mechanisms of classic and delayed preconditioning, necessitating specific targeting of these isoforms to avoid unwanted more generalised cellular effects associated with global PKC activation. At present, however, no selective pharmacological agents are available. Similar considerations may be extended to other downstream kinases which are receiving attention at present in mechanistic studies of preconditioning.

Although the PKC hypothesis has many protagonists another intracellular pathway has been proposed to explain the marked antiarrhythmic effects of preconditioning. There is some convincing evidence to suggest that bradykinin synthesis and release is markedly increased during an ischaemic insult from studies in which coronary sinus blood is sampled during coronary angioplasty [132]. Bradykinin is thought to act via B1 receptors to activate nitric oxide synthase in endothelial cells, leading to stimulation of soluble guanylate cyclase in myocytes with a resultant increase in intracellular cGMP. Elevated cGMP may reduce calcium influx, stimulate cGMP phosphodiesterase (PDE) and therefore reduce cAMP levels, and decrease myocardial oxygen demand via nitric oxide mediated reduction in myocardial contractility [134]. Clearly this pathway may be amenable to pharmacological manipulation. Selective inhibitors of PDE (e.g. zaprinast) might prevent breakdown of cGMP. Activation of the PDE enzymes responsible for breakdown of cAMP might also be possible. It is interesting to note that prostacyclin derivatives have early and late myocardial protective effects [135] and are also selective activators of specific isotypes of PDE.

17.4. Modulate the end-effector

The impressive experimental evidence implicating KATP channel in the mechanism of classic preconditioning, possibly as the end effector of protection, would suggest that there are possibilities for therapeutic exploitation. Several K_{ATP} channel openers are under development as antiischaemic agents at present and the hybrid nitrate-K_{ATP} channel opener, nicorandil, is licensed in some countries. The potential of this approach to cardioprotection may be greatly enhanced by ongoing work examining the role of mitochondrial rather than sarcolemmal K_{ATP} channels in the mechanism of classic preconditioning [136]. Targetting the organelle that is specifically involved in cellular respiration has obvious theoretical advantages as well as avoiding unwanted effects on sarcolemmal transmembrane potential.

In conclusion, we feel that exploitation of endogenous cardioprotective mechanisms may be possible in the context of carefully conducted clinical studies. There have been significant advances in our understanding of the mechanisms underlying ischaemia-reperfusion injury as a result of preconditioning research and potential pharmacological approaches to protection seem feasible. However further development of pharmacological therapies should be based on sound experimental investigation and assessed in the context of other effective therapeutic strategies.
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