# Therapeutic potential of ischaemic preconditioning

**Richard J. Edwards, Adrian T. Saurin, Roby D. Rakhit & Michael S. Marber**

*Department of Cardiology, KCL, St Thomas' Hospital, London SE1*

## Brief history of infarct size limitation

During the 1970s and 1980s it was a major goal of academic cardiologists to discover a pharmacological method to protect the infarcting myocardium [1]. A considerable volume of work was published on this subject and a plethora of agents from calcium antagonists to cobra venom and β-adrenoceptor blockers to ibuprofen were repeatedly tried and tested [2–4]. A variety of animal models was employed and drugs were infused pre, peri and post ischaemia. Some studies showed dramatically positive results, whereas others showed no benefit [5, 6]. Results were often conflicting, confusing and inconsistent. Even the traditional 'anti-infarct drugs', such as β-adrenoceptor blockers, which at first gave hopeful results [7], in later, more robust, models, were unable to show a significant reduction in infarct size [6, 8]. Several reasons were mooted for the conflicting data [1]. Firstly, there was a failure of investigators to distinguish between the ability of a drug to delay the onset of cell death and the ability of a drug to bring about an absolute reduction in the extent of cell death. Secondly, in some studies the crucial role of coronary collateral flow was not taken into account when calculating infarct size. Thirdly, surrogate end points of infarction such as enzyme derived infarct size or ST changes on the ECG, were imprecise. Even tetrazolium staining, that remains the 'gold standard' as a measure of infarct size, is prone to serious error and all results have to be interpreted with caution [5]. Consistency and reproducibility are vital when interpreting the significance of an unexpected isolated finding. Studies have to be examined collectively and only if results are repeatedly positive can they be interpreted with confidence. By 1986, despite a wealth of research data, it seemed that no pharmacological agent could claim to consistently limit infarction. The question being asked, was 'whether infarct size reduction was really possible'?

## History of preconditioning

It was in this climate that Murry *et al.* published a paper stating that antecedent short ischaemia could protect against the deleterious consequences of subsequent more prolonged ischaemia, and could dramatically reduce infarct size [9]. These investigators had been attempting to understand the metabolic consequences of brief episodes of ischaemia [10]. They knew that prolonged (40 min) cardiac ischaemia led to depletion of ATP and myocyte necrosis; what they did not know however, was whether it was the run down of ATP stores, or the build up of toxic catabolites that was deleterious to the heart. Hence, they devised an experiment whereby the heart was subjected to four repeated relatively short periods of 10 min ischaemia (but equal in *total* time to a single sustained period of ischaemia) interspersed with short periods of reperfusion to wash out, and prevent accumulation of toxic catabolites. An open chest canine model was employed and myocardial perfusion was controlled with a circumflex artery ligature. They showed that ATP levels fell during the first ischaemic episode, but they were surprised to find that during subsequent ischaemic episodes ATP levels were preserved. It was as though the first ischaemic insult had instructed the myocardium to conserve energy and become tolerant to subsequent ischaemia. Crucially, they next showed that ischaemic preconditioning protected myocytes against ischaemic cell death [9]. In this experiment the ligature was tied for 5 min and then released for 5 min to allow reperfusion. This was repeated 4 times prior to an ischaemic insult lasting 40 min. As a result the preconditioned group had infarct sizes, as a percentage of anatomic area at risk, four times smaller than controls (7% *vs* 29%). Different models were employed in different species in different laboratories and all confirmed similar levels of protection. For the first time a convincing method of reducing myocardial cell death had been discovered.

The protective effects of preconditioning have been repeated in all species thus far tested including rabbit [11], pig [12], and rat [13] with a typical fourfold reduction in infarct volume. In most circumstances preconditioning exhibits an all or none effect, a short single ischaemic episode being just as effective as repeated episodes and a trigger of at least 3 min is required to induce protection [14]. The protective effects last for about an hour [15] but protection disappears altogether 2 h [16] after the preconditioning ischaemia. In addition, although initially

*Received 11 January 2000, accepted 26 April 2000.*

© 2000 Blackwell Science Ltd 

ischaemic preconditioning can be renewed by further ischaemic stimuli, eventually tolerance occurs [17].

Since these early findings the pace of preconditioning research has increased dramatically and over a thousand papers have been published on the subject since 1986. Some believe it may be the panacea of infarct size limitation. However, others think that, despite its academic interest, preconditioning will never have a clinical role. This review, discusses the mechanisms, weighs up its clinical role and examines the therapeutic potential of ischaemic preconditioning.

## Preconditioning is receptor mediated

Downey *et al.* [18], were the first to demonstrate that protection was receptor mediated. Adenosine is a breakdown product of ATP and occurs in high concentrations in ischaemic tissue [19]. They have shown that in the rabbit isolated heart the protective effects of preconditioning are inhibited if adenosine receptor antagonists are infused before the preconditioning stimulus is given [17]. Also, a 5 min infusion of adenosine or the A1 receptor agonist N6–1-(phenyl-2R-isopropyl) adenosine is as effective as 5 min of ischaemia in protecting against infarct size [17].

Results from experiments which use a variety of receptor agonists and antagonists suggest several other substances are also involved including: bradykinin [20], catecholamines [21], free radicals [22], angiotensin II [23], nitric oxide [24], and opiates [25]. All these agonists are products of ischaemia in animals and are therefore well placed to play a role in initiating the protective effects. It seems likely that they are released from ischaemic myocardium and act in a paracrine fashion to activate the protective mechanism.

In the rat, adrenergic and opioid signalling seem dominant, whilst adenosine and bradykinin signalling are more important in rabbit myocardium. Notably, if the action of one agonist is blocked protection can still be induced by boosting the preconditioning stimulus or infusing a different agonist [26].

As well as being an important trigger of preconditioning, adenosine is also required to mediate the protective effects during the prolonged ischaemia. Thornton *et al.* [27] showed that blocking adenosine during this stage also inhibits protection.

## Intracellular signalling and protein kinase C

Most of the agonists, which generate the signal of preconditioning, bind to heptahelical transmembrane receptors [28]. The intracellular second messenger systems linked to these receptors are relatively well characterized and have now been investigated with regard to myocardial preconditioning [29].

When agonist binds, a receptor-coupled G protein is activated [28]. This dissociates and in turn activates a membrane bound phospholipase, which cleaves phosphotidylinositol bisphosphate into inositol trisphosphate and diacylglycerol (DAG). DAG then activates protein kinase C that is believed to have a central role in ischaemic preconditioning [28].

Protein kinase C (PKC) is well placed to have a key role in cellular protection. It is known to regulate numerous biological processes such as metabolism, myocyte contraction, ion transport, gene expression [28] and is coupled to the receptors of many reported agonists of preconditioning [29]. PKC is a complex protein both structurally and pharmacologically. Typically it contains four constant regions responsible for activation and enzymatic action as well as five variable regions responsible for translocation, substrate binding and perhaps specificity [30]. There are at least 11 isomers in existence, each having several different functions [30]. It is this complexity which makes its role in preconditioning difficult to assess experimentally and its involvement controversial [31].

Some groups are able to show that activation of PKC induces protection [32, 33] and inhibition blocks it [34]. However, others report that PKC inhibitors do not block preconditioning [35–37]. Problems interpreting these data stem from the nonspecificity to individual PKC isotypes of pharmacological agents which may also activate or inhibit other protein kinases [31]. Indeed, activation may have a bimodal effect first stimulating then down regulating PKC activity. Also PKC activity and preconditioning induction are seldom assessed in the same experiment, so that PKC's involvement can only be inferred.

Its role can be further interrogated using gene transfer techniques. Recently PKC isotypes, which have their catalytic site rendered constitutively active either by small deletions or point mutations in the pseudosubstrate domain have become available [38]. Their effect can be assessed by using *in vitro* models of preconditioning [39].

Work in this laboratory by Zhao *et al.* [40] has shown that transfection with the active isotype of PKC δ in rat-isolated neonatal cells consistently increases resistance to simulated ischaemia. Transfection is apparent in only 5–10% of cells and yet the remaining cells are also protected against ischaemia. This suggests cellular cross talk as a novel mechanism of preconditioning.

Experiments suggest that PKC activity, *per se*, may not be increased by preconditioning but translocation of the relevant isomers from cytosol to membranes is important. The protection conferred be preconditioning can be inhibited by colchicine [41] supporting the involvement of microtubule mediated translocation. Western blotting [42] and immunofluoresence [43] studies suggest that PKC-δ,


© 2000 Blackwell Science Ltd 

PKC-α and PKC-ε are translocated to the sarcolemma for approximately 60 min after the initial preconditioning stimulus, providing evidence of these isomers' involvement and explaining the memory of the second reperfusion phase of preconditioning.

### *Downstream signalling and mitogen-activated protein kinases*

The neonatal rat cardiac myocyte model, where cardiac myocyte protection is mediated through overexpression of PKC-δ, has provided insights into distal signalling pathways. By examining cross talk between protein kinase C and limbs of the mitogen-activated protein kinase (MAPK) cascade, we have shown that p38-MAPK is activated during ischaemia and that this activation is reduced in cells that have been preconditioned with ischaemia or express active PKC-δ [44]. These data are in broad agreement with those of other groups using similar techniques. For example, Mochley-Rosen's group also suggest that p38-MAPK increases cell death during ischaemia [45] although the relationship to preconditioning was not examined.

The tachyphylaxis that occurs with repeated episodes of preconditioning [46] does not appear to relate to loss of PKC responsiveness. In hearts made tolerant to an adenosine analogue, treatment with phenylepinephrine in lieu of global ischaemia, restores protection [47], implying that PKC activation is intact and tolerance occurs at the receptor level.

## End effectors

Protein kinases exert control on cellular function by phosphorylation of relevant proteins [30]. Proteins that slow cellular metabolism during ischaemia would conserve energy and protect against further ischaemic stress. ATP-sensitive $K^+$ channels are found in high concentrations in the myocyte membrane and are a favoured end effector [48]. Opening of these channels causes efflux of $K^+$ ions which leads to a reduced inward calcium current, which may conserve energy by decreasing the force of contraction [49]. The $K^+_{ATP}$ channel is blocked by glibenclamide and this drug is able to block the protective effects of preconditioning in guinea pig [50], dog [51] and rabbit [52]. However, $K^+_{ATP}$ blockers do not prevent preconditioning in all species, in particular the rat [53]. Also, the cardioprotective effects of the $K^+_{ATP}$ channel openers cromakalin and aprikalim occur at doses lower than those required to elicit other effects consistent with sarcolemmal $K^+_{ATP}$ channel activation, such as vasodilatation and action potential duration shortening [54]. In the rabbit, PKC activation increases the likelihood of the channel opening which significantly shortens the action potential duration. Light *et al.* [52] were able to show, using excised membrane patches, that PKC activity mediates a change in the stoichiometry of ATP binding to this channel causing it to open. Other groups have also demonstrated a link between these two components in the signalling pathway of preconditioning [55]. Of course, the ATP sensitive $K^+_{ATP}$ channel may not be the only target of the PKC isomers. It has also been suggested that strengthening the cellular cytoskeleton may make cells more resistant to ischaemic damage [15].

This initial work has placed the emphasis on the sarcolemmal $K^+_{ATP}$ channel; however, Garlid & Marban have demonstrated that the mitochondrial $K^+_{ATP}$ channel may be the more important player. Diazoxide is 1000 times more potent at opening mitochondrial than sarcolemmal channels and is cardioprotective in isolated cell models [56, 57]. It has also been shown that 5-hydroxydecanoate, a specific mitochondrial $K^+_{ATP}$ channel blocker, inhibits preconditioning [58] and that this protection is enhanced by PMA, implicating PKC in the pathway [59]. A popularly hypothesized mechanism of protection is that, by partially depolarizing the mitochondria, calcium entry is diminished at reperfusion allowing more rapid re-energization in favour of mitochondrial and myocyte survival.

## Second window of protection

After 2 h the protective effects of ischaemic preconditioning wears off. However, there is a biphasic response and protection re-emerges 24 h later [60, 61] and lasts for a further 3 days [62–64]. This subsequent delayed phase of protection has been termed the 'second window'. The protection it affords against infarct size is not as robust as that seen during classical preconditioning. Nevertheless, some groups are able to demonstrate a 50% reduction in infarct size compared with controls [62, 65]. Although others have been unable to repeat these findings [66–68]; there does appear to be consistent protection against dysrhythmia [69] and stunning [67].

The same second messenger cascade that is involved in classical preconditioning may also mediate the second window of protection. It, too, can be abolished if adenosine antagonists [70], free radical scavengers [71] or nitric oxide synthase inhibitors [72] are applied during the initiating ischaemia. Baxter *et al.* have also shown that PKC inhibition blocks the second window of protection [73]. PKC-ε is known to migrate to the nucleus after preconditioning and remain there for 24 h [42, 74]. Transfection of PKC plasmids leads to expression of reporter genes, which suggest all subgroups of PKC have the propensity to contribute to protection by inducing protein transcription [38]. Notably, the second window of protection can be blocked by cyclohexamide [75] and, as

such, protein transcription appears to be a requirement. Heat shock proteins (HSP) and antioxidant proteins increase in concentration 12–24 h after heat stress or ischaemia [60, 76]. The roles of such proteins as intracellular protein chaperones and free radical scavengers belie their potential role as mediators of the mechanism. Notably, the loss in protection at 72 h corresponds with decay in their concentration [77], and transgenic mice can be protected against myocardial infarction when inducible HSP70 is over expressed [78]. Inducible nitric oxide synthase also increases in concentration during the second window and increased nitric oxide production during the subsequent ischaemia may also contribute to the cardioprotection [79].

Theoretically, manipulation of delayed preconditioning offers a wider therapeutic window, unlike classical preconditioning, however, its existence in humans awaits thorough investigation.

## Evidence of preconditioning in humans

Due to ethical considerations, demonstrating preconditioning in humans can pose problems; nevertheless six models have been described in the literature. The discomfort of angina pectoris is mediated by cardiac sympathetic afferent neurones [80]. The component or consequence of ischaemia that stimulates these neurones is not known. However, interstitial adenosine and bradykinin accumulation during myocardial ischaemia are thought to be important mediators. Thus, in patients with ischaemic heart disease, the discomfort of angina can be precisely mimicked by infusion of adenosine into the diseased coronary artery [81] and attenuated by the use of relatively specific adenosine type 1 (A1) receptor antagonists [82]. The sensation of angina might be a surrogate indicator of the accumulation within the myocardium of the agonists that trigger preconditioning. There are a number of circumstances in which angina precedes further ischaemia. These circumstances could provide an opportunity to investigate the mediators of preconditioning in patients.

### *Preinfarction angina*

Retrospective and prospective data suggest that angina prior to myocardial infarction is beneficial [83–89]. There is evidence of reduced enzyme-derived infarct size [83, 86, 89] with improved short-term and long-term prognosis [90]. Two studies suggest this might be due to a greater chance of patency being achieved in the infarct-related artery if there is antecedent angina [89, 90]. The mechanism therefore may be due to thrombosed atherosclerotic plaques of patients with unstable angina being more amenable to thrombolysis, rather than due to preconditioning of distal myocardium. Hata *et al.* using a canine model of spontaneous, platelet-mediated coronary thrombosis, have shown that ischaemic preconditioning improves vessel patency and is inhibited by administration of an adenosine antagonist [91]. However, multivariate analysis of human studies suggest that, despite the improved vessel patency, angina, itself, is independently associated with improved survival at 5 years [90].

In an ancillary study to TIMI-9B, in a prospective analysis, 3002 patients were asked to report symptoms of angina before their infarct. This study showed that patients with symptoms within 24 h of infarction had a lower 30-day event rate than those with angina greater than 24 h before the event. These findings could support a role for classical preconditioning or the second window [92]. However, it is difficult to conclude that the benefit of preinfarction angina is entirely related to preconditioning as patients with more frequent angina may differ substantially from patients without angina prior to infarction. In particular, differences in concomitant medications, collateral circulation and time to presentation after the onset of chest pain all confound data interpretation.

### *Percutaneous transluminal coronary angioplasty*

Since 1990 there have been several studies using PTCA as a model of preconditioning [93–97]. During this procedure the first balloon inflation mimics the preconditioning stimulus and ischaemic parameters such as chest pain, ST segment deviation and lactate production are lessened during subsequent inflations. However, not all groups have shown a benefit after first inflation [97, 98]. There are two problems with this model; firstly, the preconditioning stimulus only lasts around 2 min whereas in animal models a preconditioning stimulus of at least 3 min is required to achieve protection [14]. Secondly, it is not clear whether recruitment of collaterals following the first inflation is the main factor responsible for the improved ischaemic parameters. Cribier *et al.* [93] showed that collateral score as measured by an increase in occlusion pressure and radiographic visualization correlates well with those patients who adapt favourably to myocardial ischaemia. In contrast, Sakata [99] using myocardial contrast echo, showed that ischaemic preconditioning occurs in the absence of visible collaterals. By positioning a pressure sensor distal to the lesion (RADI Medical Systems) to allow accurate measurement of balloon occlusion pressure, we have demonstrated that myocardial protection during PTCA can occur in the absence of any significant collateral recruitment [100], thus providing strong evidence of ischaemic preconditioning in man.

Further evidence in favour of classical preconditioning, rather than opening of collaterals, comes from pharma-


© 2000 Blackwell Science Ltd 

cological manipulation of the preconditioning pathway. Infusion of an adenosine antagonist [95] or pretreatment with the $K^+_{ATP}$ channel blocker, glibenclamide [94], are able to prevent the conditioning effect of the first balloon inflation. Moreover, Lesser *et al.* [49] have shown that a 5-minute intracoronary infusion of adenosine preconditions human myocardium even more effectively than ischaemia. There may also be a clinical benefit, as it seems that following a strict preconditioning protocol during PTCA results in reduced CK release [101].

### *Warm-up in angina*

First effort, warm-up or first hole angina describes the ability of some patients to exercise to angina, rest and then continue exertion with few or no symptoms. The relationship between warm-up angina and ischaemic preconditioning has been a subject of interest [102].

Traditionally, warm-up angina was ascribed to coronary vasodilatation [103], with perhaps the concomitant opening of collateral vessels to support the ischaemic myocardium [103]. This explanation was accepted despite the observations that arterial vasodilators such as aminophylline had little effect on exercise tolerance [104], and that the presence of collaterals on angiography were not a predictor of walk through or warm-up in angina [105]. In addition, there were other features to warm-up that were difficult to explain, such as the finding that second effort would reproducibly exceed first effort providing the two were separated by a rest period of at least 2–5 min but not more than 30–60 min [104]. These time constraints are consistent with warm-up being a form of ischaemic preconditioning [14, 41]. However, exercise-induced ischaemia does not appear to trigger the second window of protection 24 h later [105, 106].

The suggestion that warm-up, like ischaemic preconditioning, represents an adaptive phenomenon is further supported by invasive investigations which indicate that the first episode of angina conditions the myocardium so that it becomes resistant to further ischaemia. This enhanced resistance is reflected by reduced symptoms, ST segment change, myocardial lactate production and oxygen consumption at corresponding rate pressure products on second compared with first effort [105, 107]. We have also found (unpublished data) that the incidence of ectopic beats is significantly reduced during the second exertion implying an antiarrhythmic effect of warm-up angina. In addition, the measurements of regional oxygen consumption and flow made it unlikely that collateral myocardial blood flow increased on second effort. Thus warm-up is likely to be a form of metabolic myocardial adaptation akin to classic ischaemic preconditioning [102].

We have used a pressure wire to measure balloon occlusion pressure during PTCA, which has previously been shown to provide a sensitive measure of coronary collateral perfusion [108]. Using this method we have identified patients with coronary artery disease whom, despite a total of 6 min balloon occlusion, have no demonstrable collateral circulation. These patients were able to warm up just as effectively as those with collaterals (unpublished data) implicating preconditioning as the dominant mechanism in the phenomenon.

A previous pharmacological study of warm up in angina that has attempted to address the underlying mechanism suggests that adenosine receptor blockade interferes with the benefit of first effort [109]. However, the interpretation of this study is complicated by the direct antianginal/analgesic action of the antagonist used (bamiphylline) [81].

### *Cardiac surgery*

Cross clamping of the aorta during coronary artery bypass isolates the coronary circulation to allow fashioning of the grafts. This model has been exploited by Yellon *et al.* [110] who were able to show that two 3 min cross clamping periods slows the rate of ATP depletion compared with controls, resembling the pattern of change originally described by Murry *et al.* in the dog [9]. However, Yellon *et al.* were unable to show a difference in CKMB release between the two groups but it has since been found that preconditioned hearts release less troponin T [6, 111], a more sensitive marker of myocardial damage. The difference in enzyme derived myocardial damage between groups is not large, which might indicate that the prolonged ischaemic stimulus is only 10 min, not long enough to cause significant necrosis. It is known that use of cold cardioplegic solutions during surgery conserves ATP by reducing cellular metabolic demand. As ischaemic preconditioning is acting in the same way it is not surprising that some groups do not find an additive protective effect of preconditioning in this model [112]. Lee *et al.* [113] did successfully precondition using a presurgical infusion of adenosine, which improved post operative haemodynamic function and reduced ischaemic cell damage.

### *Isolated muscle and cultured myocyte experiments*

Small atrial trabeculae harvested during cardiac surgery exhibit ischaemic preconditioning *in vitro* [114] as measured by improved contractile function. This protective effect was also induced by PKC activation and $K^+_{ATP}$ openers and inhibited by their closure [114]. Also angiotensin converting enzyme inhibitors, which are known to inhibit bradykinin breakdown, enhance the preconditioning effect in human atrial trabeculae [115]. Human adult cardiac myocytes have been successfully cultured from patients with congenital heart disease and

successfully preconditioned [116]. Isolated human ventricular fetal myocytes also appear to be protected against ischaemic cell death during the second window of protection [117].

*Remote preconditioning*

In animal models when organs remote from the heart such as kidney [118], intestine [119] and skeletal muscle [120] are rendered ischaemic through arterial ligation, the myocardium, itself, becomes preconditioned. The heart subsequently preserves high-energy phosphates [118] and is protected against infarction [119, 120]. These experiments confirm that preconditioning is probably mediated through substances released into the blood such as adenosine [118] and or catecholamines [119]. This cross talk between organs may be of clinical relevance in patients who have an episode of remote organ ischaemia, such as claudication, prior to acute myocardial infarction.

## Therapeutic potential of preconditioning

*Surgically induced ischaemia*

The global ischaemia deliberately induced during cardiac surgery offers the ideal opportunity to precondition the myocardium. The heart is readily accessible, the ischaemia is anticipated, of relatively short duration and always associated with reperfusion. However, the very successful use of buffered hypothermic solutions whilst keeping the heart in diastolic arrest means that irreversible myocardial injury fortunately rarely occurs [1]. During prolonged complex procedures troponin-*t* is released from the myocardium, indicating discrete necrosis and in this instance preconditioning may have an additional role to play, delaying cell death.

A recent advance in cardiothoracic surgery is the use of minimally invasive techniques for coronary bypass grafting. During this procedure a much smaller operating window is used and a single internal mammary artery is rapidly sewn directly on to the coronary artery. The heart is not put on bypass and there is no cardioplegia so surgical speed is of the essence to prevent myocardial necrosis. Preconditioning could have a simple prophylactic role by preinfusing with adenosine prior to the procedure to reduce the risk of irreversible injury.

Transplant surgery could be another therapeutic target of preconditioning. For a heart to remain in top condition for successful transplantation it should be cold stored for a maximum of 4–6 h; longer than this and irreversible damage occurs. Animal studies suggest that preconditioning may increase the therapeutic window which, may provide a therapeutic target as yet untested in man [138, 139].

*Acute myocardial infarction*

This poses a difficult therapeutic challenge. For preconditioning to be successful, the duration of the index ischaemia must be less than 90 min and have a predictable onset. However, acute myocardial infarction can not be predicted, ischaemic duration is at best variable and often permanent. Due to its unpredictable nature, some groups have tested, in animal models, whether an infusion of adenosine given at the time of acute myocardial infarction or early reperfusion can be protective [121, 122]. There are two problems with this; firstly, unless there is sufficient collateral flow the drug cannot get to the site of intended action and secondly, in most studies [123], a short period of reperfusion is required for the protective mechanisms to be generated. Todd *et al.* [124] showed in a rabbit model of myocardial infarction that an adenosine receptor agonist given *during* the index ischaemia exerted a cardioprotective effect and reduced infarct size. Other groups have also shown a protective benefit [121, 122], but in similar experimental models other groups [125, 126] could show no benefit of adenosine infusion. Nevertheless, the technique has been tested in man [127]. The AMISTAD trial was a prospective, open label trial of thrombolysis with randomization to adenosine or placebo in 236 patients within 6 h of infarction onset. Infarct size was determined by Tc-99 m sestamibi single-photon emission computed tomography (SPECT) imaging 6 days after enrolment. In this study adenosine resulted in a 33% relative reduction in infarct size and supports the need for a large clinical outcome trial.

*Prophylactic myocardial protection*

If the heart were in a permanently protected state the problems of pre-emting myocardial infarction would be avoided. A preconditioning drug could be given to an at risk population, so their hearts were continuously in a protected state. Unfortunately adenosine, which is the obvious candidate, has to be given as an infusion and if given repeatedly down regulates its receptor [128]. One way around this problem is to include an adenosine free period into the regime and maintain the heart in a permanently protected state [129].

One option is to bolster endogenous adenosine during ischaemia and reperfusion. Dralfalazine inhibits nucleoside transport, bolstering adenosine concentration during low flow ischaemia and in isolated porcine hearts elicits cardioprotection during ischaemia [121]. This has the advantage of providing a time and site specific therapy which avoids tolerance and systemic side-effects.

ACE inhibitors may exert some of their beneficial actions in a similar way. The main side-effect of ACE inhibitors experienced by patients is a dry cough, which is


© 2000 Blackwell Science Ltd 

mediated through the inhibition of bradykinin breakdown. However, accumulation of this important agonist of preconditioning may also mediate important anti-ischaemic effects. Large clinical trials such as SOLVD [130], SAVE [131], GISSI-3 [132] and ISIS-4 [133] which were designed to assess the ability of these agents as effective heart failure drugs revealed unexpected findings. Patients postmyocardial infarct who had received these drugs had a decreased incidence of myocardial ischaemic events and a significant reduction in early mortality, at a time when the remodelling effects had probably not yet become effective.

Animal hearts exposed to ACE inhibitors prior to an ischaemic episode have a reduced infarct size, decreased reperfusion dysrhythmias and improved function compared withtrols [20, 23, 134, 135]. Matoba *et al.* [136] have shown in a rat cultured cardiac myocyte model the protective effect of ACE inhibitors is indeed mediated through bradykinin accumulation and the attendant production of nitric oxide. These beneficial effects have also been demonstrated in human isolated atrial trabeculae [115]. The time may be approaching when all patients with coronary artery disease will be prescribed ACE inhibitors and not just those post myocardial infarction [137].

Further excitement in clinical cardiology is provided by nicorandil. This drug which is licensed for the treatment of angina has two moieties, a nitrate group and a $K^+_{ATP}$ opener. It is the latter effect that has generated most interested. A nicorandil infusion prior to an ischaemic episode successfully protects against infarction and stunning in a variety of animal models [138, 139]. These beneficial effects do not appear to be shared by pure nitrate donors and it is thought they are mediated through the $K^+_{ATP}$ opening [140]. Notably $K^+_{ATP}$ channel antagonists entirely block the protective effects of nicorandil [138, 141, 142]. Several papers have documented its efficacy as an antianginal [143] and in a recent placebo controlled study was administered to patients admitted with unstable angina [144]. It was found that when nicorandil was added to the treatment regime of unstable angina there was a reduction in myocardial ischaemia and dysrhythmias, compared with placebo. The authors suggested that pharmacological preconditioning was responsible for these cardioprotective effects.

Since its discovery in 1986 we have come a long way in understanding the mechanisms involved in preconditioning as well as its existence in man. Several potential agonists have been characterized but we still await full delineation of intracellular mechanisms and end effectors. The phenomenon of ischaemic preconditioning is beginning to make inroads in various clinical settings and its eventual clinical impact will become clearer when the results of further clinical trials are published.

## References


1 Hearse DJ, Yellon DM. Why are we still in doubt about infarct size limitation? The experimentalist's viewpoint. *Therapeutic Approaches to Myocardial Infarct Size Limitation,* 17–41, 1984.

2 Opie LH. Myocardial infarct size. Part I. Basic considerations. *Am Heart J* 1980; **100**: 355–372.

3 Opie LH. Myocardial infarct size. Part 2. Comparison of anti-infarct effects of beta-blockade, glucose-insulin-potassium, nitrates, and hyaluronidase. *Am Heart J* 1980; **100**: 531–552.

4 Reimer KA, Jennings RB, Cobb FR, *et al.* Animal models for protecting ischemic myocardium: results of the NHLBI Cooperative Study. Comparison of unconscious and conscious dog models. *Circ Res* 1985; **56**: 651–665.

5 Darsee JR, Kloner RA, Braunwald E. Early recovery of regional performance in salvaged ischemic myocardium following coronary artery occlusion in the dog [retracted by Braunwald E, Kloner RA. In J Clin Invest 1982 October; 70: following 915] *J Clin Invest* 1981; **68**: 225–239.

6 Jennings RB, Reimer KA. Effects of beta adrenergic blockade on acute myocardial injury. In *Modulation of sympathetic tones in the treatment of cardiovascular diseases* ed Gross F. Berne: Hans Huber, 1979; 103–114.

7 Reimer KA, Rasmussen MM, Jennings RB. Reduction by propranolol of myocardial necrosis following temporary coronary artery occlusion in dogs. *Circ Res* 1973; **33**: 353–363.

8 Hearse DJ, Yellon DM, Downey JM. Can beta-blockers limit myocardial infarct size? *Eur Heart J* 1986; **7**: 925–930.

9 Murry CE, Jennings RB, Reimer KA. Preconditioning with ischaemia: a delay of lethal cell injury in ischaemic myocardium. *Circulation* 1986; **74**: 1124–1136.

10 Reimer KA, Murry CE, Yamasawa I, *et al.* Four brief periods of myocardial ischemia cause no cumulative ATP loss or necrosis. *Am J Physiol* 1986; **251**: H1306–H1315.

11 Liu SG, Richards SC, Olsson RA, *et al.* Evidence that the adenosine $A_3$ receptor may mediate the protection afforded by preconditioning in the isolated rabbit heart. *Cardiovasc Res* 1994; **28**: 1057–1061.

12 Schott RJ, Rohmann S, Braun ER, *et al.* Ischemic preconditioning reduces infarct size in swine myocardium. *Circ Res* 1990; **66**: 1133–1142.

13 Li Y, Kloner RA. The cardioprotective effects of ischemic 'preconditioning' are not mediated by adenosine receptors in rat hearts. *Circulation* 1993; **87**: 1642–1648.

14 Marber MS, Walker DM, Yellon DM. Ischaemic preconditioning. *Br Med J* 1994; **308**: 1–2.

15 Downey JM, Cohen MV. Signal transduction in ischemic preconditioning. *Adv Exp Med Bioy* 1997; **430**: 39–55.

16 Van Winkle DM, Downey JM, Cohen MV. Cardioprotection from ischaemic preconditioning is lost following prolonged reperfusion in the rabbit. *Cor Art Dis* 1991; **2**: 613–619.

17 Cohen MV, Yang XM, Downey JM. Conscious rabbits become tolerant to multiple episodes of ischaemic preconditioning. *Circ Res* 1994; **74**: 998–1004.

18 Liu GS, Van Thornton J, WD, *et al.* Protection against infarction afforded by preconditioning is mediated by A1 adenosine receptors in rabbit heart. *Circulation* 1991; **84**: 350–356.

19 Van Wylen D, Willis J, Shodi J. Cardiac microdialysis to estimate interstitial adenosine and coronary blood flow. *Am J Physiol* 1990; **258**: H1642–H1649.
20 Wall TM, Sheehy R, Hartman JC. Role of bradykinin in myocardial preconditioning. *J Pharmacol Exp Ther* 1996; **78**: 443–454.
21 Bankwala Z, Hale SL, Kloner RA. Alpha-adrenoceptor stimulation with exogenous norepinepherine or release of endogenous catecholamines mimics ischaemic preconditioning. *Circulation* 1998; **90**: 1023–1028.
22 Baines CP, Goto M, Downey JM. Oxygen radicals released during ischemic preconditioning contribute to cardioprotection in the rabbit myocardium. *J Mol Cell Car* 1997; **29**: 207–216.
23 Noda K, Sasaguri M, Ideishi M, *et al*. Role of locally formed angiotensin II and bradykinin in the reduction of myocardial infarct size in dogs. *Cardiovas Res* 1993; **27**: 334–340.
24 Vegh A, Papp JG, Szekeres L, *et al*. R. Prevention by an inhibitor of the L-arginin-nitric oxide pathway of the antiarrythmic effects of bradykinin in anaesthetized dogs. *Br J Pharmacol* 1993; **110**: 18–19.
25 Schultz JJ, Hsu AK, Gross GJ. Ischemic preconditioning is mediated by a peripheral opioid receptor mechanism in the intact rat heart. *J Mol Cell Cardiol* 1997; **29**: 1355–1362.
26 Goto M, Yongge L, Xi-ming Y. Role of bradykinin in protection of ischaemic preconditioning in rabbit hearts. *Circ Res* 1995; **77**: 611–621.
27 Thornton JD, Thornton CS, Downey JM. Effect of adenosine receptor blockade: preventing protective preconditioning depends on time of initiation. *Am J Physiol* 1993; **265**: H504–H508.
28 Sugden PH, Bogoyevitch MA. Intracellular signalling through protein kinases in the heart. *Cardiovasc Res* 1995; **30**: 478–492.
29 Cohen MV, Downey JM. Myocardial preconditioning promises to be a novel approach to the treament of ischaemic heart disease. *Ann Rev Med* 1996; **47**: 21–29.
30 Newton AC, Protein Kinase C. structure, function and regulation. *J Biol Chem* 1996; **270**: 28495–28498.
31 Brooks G, Hearse DJ. Role of protein kinase C in ischaemic preconditioning: Player or spectator? *Circ Res* 1996; **79**: 627–630.
32 Speechly-Dick ME, Mocanu MM, Yellon DM, Protein Kinase C. Its role in ischaemic preconditioning in the rat. *Circ Res* 1994; **75**: 586–590.
33 Mitchell MB, Meng X, Brown JM, *et al*. A. Preconditioning of isolated rat heart is mediated by protein kinase C. *Circ Res* 1995; **76**: 73–81.
34 Ytrehus K, Liu Y, Downey JM. Preconditioning protects ischemic rabbit heart by protein kinase C activation. *Am J Physiol* 1994; **266**: H1145–H1152.
35 Vahlhaus C, Schulz R, Post H, *et al*. No prevention of ischemic preconditioning by the protein kinase C inhibitor staurosporine in swine. *Circ Res* 1996; **79**: 407–414.
36 Przyklenk K, Sussman MA, Simkhovich BZ, *et al*. Does ischemic preconditioning trigger translocation of protein kinase C in the canine model? *Circulation* 1995; **92**: 1546–1557.
37 Simkhovich BZ, Przyklenk K, Kloner RA. Role of protein kinase C as a cellular mediator of ischemic preconditioning: a critical review. *Cardiovasc Res* 1998; **40**: 9–22.
38 Genot EM, Parker PJ, Cantrell DA. Analysis of the role of protein kinase C-alpha-epsilon, and -zeta in T cell activation. *J Biol Chem* 1995; **270**: 9833–9839.
39 Decock JBJ, Gillespie-Brown J, Parker PJ, *et al*. Classical, novel and atypical isoforms of PKC stimulate ANF- and TRE/AP-1-regulated-promoter activity in ventricular cardiomyocytes. *Febs Letts* 1994; **356**: 275–278.
40 Zhao J, Renner O, Wightman L, *et al*. The expression of constitutively active isotypes of protein kinase C to investigate preconditioning. *J Biol Chem* 1998; **273**: 23072–23079.
41 Cohen MV, Downey JM. Ischaemic preconditioining: can the protection be bottled? *Lancet* 1998; **342**: 8862–8866.
42 Ping P, Zhang J, Qiu Y, *et al*. Ischemic preconditioning induces selective translocation of protein kinase C isoforms epsilon and eta in the heart of conscious rabbits without subcellular redistribution of total protein kinase C activity. *Circ Res* 1997; **81**: 404–414.
43 Mitchell MB, Meng X, Ao L, *et al*. Preconditioning of isolated rat heart is mediated by protein kinase C. *Circ Res* 1995; **76**: 73–81.
44 Saurin A, Zhao J, Mockridge JW, *et al*. Negative regulation of p38 and p42/44 MAPK by PKC in a surrogate model of ischaemic preconditioning. *Circulation* 1998; **98**: I–72.
45 Mackay K, Mochly-Rosen D. An inhibitor of p38 mitogen-activated protein kinase protects neonatal cardiac myocytes from ischemia. *J Biol Chem* 1999; **274**: 6272–6279.
46 Tsuchida A, Thompson R. The anti-infarct effect of an adenosine A1-selective agonist is diminished after prolonged infusion as is the cardioprotective effect of ischaemic preconditioning in the rabbit heart. *J Mol Cell Cardiol* 1994; **26**: 303–311.
47 Hashimi W, Thornton JD, Downey JM, *et al*. Chronic adenosine A1 agonist exposure results in adenosine receptor rather than G protein down regulation (Abstract). *Circulation* 1994; **90**: 107.
48 Sumeray MS, Yellon DM. Myocardial preconditioning. What have we learned? *Eur Heart J* 1997; **18**(Suppl A): A8–A14.
49 Leesar MA, Stoddard M, Ahmed M, *et al*. Preconditioning of human myocardium with adenosine during coronary angioplasty [see comments]. *Circulation* 1997; **95**: 2500–2507.
50 Cole WC, McPherson CD, Sontag D. ATP-regulated K+ channels protect the myocardium against ischemia/reperfusion damage. *Circ Res* 1991; **69**: 571–581.
51 Gross GJ, Auchampach JA. Blockade of ATP-sensitive potassium channels prevents myocardial preconditioning in dogs. *Circ Res* 1992; **70**: 223–233.
52 Light PE, Sabir AA, Allen BG, *et al*. Protein kinase C-induced changes in the stoichiometry of ATP binding activate cardiac ATP-sensitive K+ channels. A possible mechanistic link to ischemic preconditioning. *Circ Res* 1996; **79**: 399–406.
53 Liu Y, Downey JM. Ischemic preconditioning protects against infarction in rat heart. *Am J Physiol* 1992; **263**: H1107–H1112.
54 Grover GJ. Pharmacology of ATP-sensitive potassium channel (KATP) openers in models of myocardial ischemia and reperfusion. [Review] [49 refs]. *Can J Physiol Pharmacol* 1997; **75**: 309–315.
55 Liu Y, Gao WD, O'Rourke B, *et al*. Synergistic modulation of ATP-sensitive $K^+$ currents by protein kinase C and adenosine: implication for ischaemic preconditioining. *Circ Res* 1996; **78**: 443–454.


© 2000 Blackwell Science Ltd 

56 Garlid KD, Paucek P, Yarov-Yarovoy V, *et al.* Cardioprotective effect of diazoxide and its interaction with mitochondrial ATP-sensitive K+ channels. Possible mechanism of cardioprotection. *Circ Res* 1997; **81**: 1072–1082.

57 Liu Y, Sato T, O'Rourke B, *et al.* Mitochondrial ATP-Dependant Potassium Channels Novel Effectors of Cardioprotection. *Circulation* 1998; **97**: 2463–2469.

58 Jaburek M, Yarov-Yarvoyoy V, Paucek P, *et al.* State-dependant Inhibition of the Mitochondrial $K_{ATP}$ Channel by Glyburide and 5-Hydroxydecanoate. *J Biol Chem* 1998; **273**: 13578–13582.

59 Sato T, O'Rourke B, Marban E. Modulation of Mitochondrial ATP-dependant $K^+$ Channels by Protein Kinase C. *Circ Res* 1998; **83**: 110–114.

60 Marber MS, Latchman DS, Walker JM, *et al.* Cardiac stress protein elevation 24 hours after brief ischemia or heat stress is associated with resistance to myocardial infarction. *Circulation* 1993; **88**: 1264–1272.

61 Kuzuya T, Hoshida S, Yamashita N, *et al.* Delayed effects of sublethal ischemia on the acquisition of tolerance to ischemia. *Circ Res* 1993; **72**: 1293–1299.

62 Baxter GF, Goma FM, Yellon DM. Characterisation of the infarct-limiting effect of delayed preconditioning: timecourse and dose-dependency studies in rabbit myocardium. *Bas Res Cardiol* 1997; **92**: 159–167.

63 Kaszala K, Vegh A, Papp JG, *et al.* Time course of the protection against ischaemia and reperfusion-induced ventricular arrhythmias resulting from brief periods of cardiac pacing. *J Mol Cell Cardiol* 1996; **28**: 2085–2095.

64 Tang XL, Qiu Y, Park SW, *et al.* Time course of late preconditioning against myocardial stunning in conscious pigs. *Circ Res* 1996; **79**: 424–434.

65 Bernardo NL, D'Angelo M, Okubo S, *et al.* Delayed ischemic preconditioning is mediated by opening of ATP-sensitive potassium channels in the rabbit heart. *Am J Physiol* 1999; **276**: H1323–H1330.

66 Qian YZ, Bernardo NL, Nayeem MA, *et al.* Induction of 72-kDa heat shock protein does not produce second window of ischemic preconditioning in rat heart. *Am J Physiol* 1999; **276**: H224–H234.

67 Qiu Y, Tang XL, Park SW, *et al.* The early and late phases of ischemic preconditioning: a comparative analysis of their effects on infarct size, myocardial stunning, and arrhythmias in conscious pigs undergoing a 40-minute coronary occlusion. *Circ Res* 1997; **80**: 730–742.

68 Miki T, Swafford AN, Cohen MV, *et al.* Second window of protection against infarction in conscious rabbits: real or artifactual. *J Mol Cell Cardiol* 1999; **31**: 809–816.

69 Yamashita N, Hoshida S, Taniguchi N, *et al.* A 'second window of protection' occurs 24 h after ischemic preconditioning in the rat heart. *J Mol Cell Cardiol* 1998; **30**: 1181–1189.

70 Baxter GF, Marber MS, Patel VC, *et al.* Adenosine receptor involvement in a delayed phase of myocardial protection 24 hours after ischemic preconditioning. *Circulation* 1994; **90**: 2993–3000.

71 Sun JZ, Tang XL, Park SW, *et al.* Evidence for an essential role of reactive oxygen species in the genesis of late preconditioning against myocardial stunning in conscious pigs. *J Clin Invest* 1996; **97**: 562–576.

72 Qiu Y, Rizvi A, Tang XL, *et al.* Nitric oxide triggers late preconditioning against myocardial infarction in conscious rabbits. *Am J Physiol* 1997; **273**: H2931–H2936.

73 Baxter GF, Goma FM, Yellon DM. Involvement of protein kinase C in the delayed cytoprotection following sublethal ischaemia in rabbit myocardium. *Br J Pharmacol* 1995; **115**: 222–224.

74 Wilson S, Song W, Karoly K, *et al.* Delayed cardioprotection is associated with the sub-cellular relocalisation of ventricular protein kinase C epsilon, but not p42/44MAPK. *Mol Cell Biochem* 1996; **160–161**: 225–230.

75 Meldrum DR, Cleveland JCJ, Rowland RT, *et al.* Early and delayed preconditioning: differential mechanisms and additive protection. *Am J Physiol* 1997; **273**: H725–H733.

76 Karmazyn M, Mailer K, Currie RW. Acquisition and decay of heat-shock-enhanced postischemic ventricular recovery. *Am J Physiol* 1990; **259**: H424–H431.

77 Marber MS, Walker JM, Latchman DS, *et al.* Myocardial protection after whole body heat stress in the rabbit is dependent on metabolic substrate and is related to the amount of the inducible 70-kD heat stress protein. *J Clin Invest* 1994; **93**: 1087–1094.

78 Marber MS, Mestril R, Chi SH, *et al.* Overexpression of the rat inducible 70-kD heat stress protein in a transgenic mouse increases the resistance of the heart to ischemic injury. *J Clin Invest* 1995; **95**: 1446–1456.

79 Bolli R, Dawn B, Tang XL, *et al.* The nitric oxide hypothesis of late preconditioning [editorial; see comments]. *Bas Res Cardiol* 1998; **93**: 325–338.

80 Meller ST, Gebhart GF. A critical review of the afferant pathways and the potential chemical mediators involved in cardiac pain. *Neuroscience* 1992; **48**: 501–524.

81 Crea F, Gasparadone A, Kaski JC, *et al.* A. Relation between stimulation site of cardiac afferent nerves by adenosine and distribution of cardiac pain: results of a study with stable angina. *J Am Coll Cardiol* 1992; **20**: 1498–1502.

82 Gasparadone A, Crea F, Tomai F, *et al.* Muscular and cardiac adenosine-induced pain is mediated by A1 receptors. *J Am Coll Cardiol* 1995; **25**: 251–257.

83 Iwasaka T, Nakamura S, Karakawa M, *et al.* Cardioprotective effect uf unstable angina to acute anterior myocardial infaction. *Chest* 1994; **105**: 57–61.

84 Anzai T, Yoshikawa T, Asakuru Y, *et al.* Efects on short-term prrognosis and left ventricular function of angina pectoris prior to first Q-wave anterior wall acute myocardial infarction. *Am J Cardiol* 1994; **74**: 755–759.

85 Ottani F, Galvini M, Ferrini D, *et al.* Prodromal angina limits infarct size. A role for ischaemic preconditioining. *Circulation* 1995; **91**: 291–297.

86 Kloner RA, Shook T, Pryzklenk K, *et al.* Previous angina alters in-hospital outcome in TIMI-4. A clinical correlate to preconditioning. *Circulation* 1995; **91**: 37–47.

87 Nakagawa Y, Ito H, Kitakaze M, *et al.* Effect of angina pectoris an myocardial protection in patients with reperfused anterior wall myocardial infarction: Retrospective clinical evidence of preconditioning. *J Am Coll Cardiol* 1995; **25**: 1076–1083.

88 Maybaum S, Ilan M, Mogilevsky J, *et al.* Improvement in ischaemic parameters during repeated exercise testing: a possible model for myocardial preconditioning. *Am J Cardiol* 1996; **78**: 1087–1091.

89 Andreotti F, Pasceri V, Hackett DR, *et al.* Preinfarction angina as a predictor of more rapid coronary thrombolysis in patients

with acute myocardial infarction. *N Eng J Med* 1996; **334**: 7–12.
90 Ishihara M, Sato H, Tateishi H, *et al.* Implications of prodromal angina pectoris in anterior wall acute myocardial infarction: acute angiographic findings and long-term prognosis. *Am Col Cardiol* 1997; **30**: 970–975.
91 Hata K, Whittaker P, Kloner RA, *et al.* Brief antecedent ischemia attenuates platelet-mediated thrombosis in damaged and stenotic canine coronary arteries: role of adenosine. *Circulation* 1998; **97**: 692–702.
92 Kloner RA, Shook T, Antman EM, *et al.* Prospective temporal analysis of the onset of preinfarction angina versus outcome: an ancillary study in TIMI-9B. *Circulation* 1998; **97**: 1042–1045.
93 Cribier A, Korsatz L, Koning R, *et al.* Improved myocardial response and enhanced collateral circulation with long repetitive coronary occlusion during angioplasty. A prospective study. *J Am Coll Cardiol* 1992; **20**: 578–586.
94 Tomai F, Crea F, Gaspardone A. Ischaemic preconditioning during coronary angioplastyis prevented by glibenclamide, a selective ATP-sensitive $K^+$ channel blocker. *Circulation* 1994; **90**: 700–705.
95 Claeys MJ, Vrints CJ, Bosmans JM, *et al.* Aminophylline inhibits adaptation to ischaemia during angioplasty. Role of adenosine in ischaemic. *Eur Heart J* 1996; **17**: 539–544.
96 Kerensky RA, Kutcher MA, Braden GA, *et al.* The effects of intracoronary adenosine on preconditioning during coronary angioplasty. *Clin Cardiol* 1995; **18**: 91–96.
97 Dupouy P, Geschwind H, Pelle G. *et al.* L. Repeated coronary artery occlusions during routine angioplasty do not induce myocardial preconditioning in humans. *J Am Coll Cardiol* 1996; **27**: 1374–1380.
98 de Jong JW, Cargnoni A, Huizer T. *et al.* Lactate and hypoxyxanthine release after coronary angioplasty: No evidence of preconditioning. *J Mol Cell Cardiol.*: 25(suppleI): S37, 1993.
99 Sakata Y, Kodama K, Kitakaze M, *et al.* Different mechanisms of ischemic adaptation to repeated coronary occlusion in patients with and without recruitable collateral circulation. *J Am Coll Cardiol* 1997; **30**: 1679–1686.
100 Edwards RJ, Redwood S, Rakhit RD, *et al.* Myocardial Protection during Angioplasty (PTCA): enhancement of collaterals or preconditioning? *Heart* 1999; suppl. 1: 54–183.
101 Laskey WK. Beneficial impact of preconditioning during PTCA on creatine kinase release. *Circulation* 1999; **99**: 2085–2089.
102 Marber MS, Joy MD, Yellon DM. Warm-up in angina: is it ischaemic preconditioning? *Br Heart J* 1994; **72**: 213–215.
103 Wayne EJ, Graybiel A. Observation on the effect of food, gastric dilitation, external temperature, and repeated exercise on angina of effort, with a note on angina sine dolore. *Clin Sci* 1934; **1**: 287–304.
104 Wayne EJ, Laplace LB. Observations of angina of effort. *Clin Sci* 1933; **1**: 103–129.
105 Macalpin RN, Kattus AA. Adaptation to exercise in angina pectoris. *Circulation* 1966; **33**: 183–201.
106 Tomai F, Perino M, Ghini AS, *et al.* Exercise-induced myocardial ischemia triggers the early phase of preconditioning but not the late phase. *Am J Cardiol* 1999; **83**: 586–588.
107 Williams DO, Bass TA, Gewirtz T, *et al.* Adaptation to the stress of tachycardia in patients with coronary artery disease: insight into the mechanism of the warm up phenomenon. *Circulation* 1985; **71**: 687–692.
108 Pijls NHSJ, Kirkeeide RL, *et al.* Experimental basis of determining maximum coronary, myocardial, and collateral blood flow by pressure measurements for assessing functional stenosis severity before and after percutaneous transluminal coronary angioplasty. *Circulation* 1993; **87**: 1354–1367.
109 Tomai F, Crea F, Danesi A, *et al.* Effects of A1 adenosine receptor blockade on the warm-up phenomenon. *Cardiologica* 1997; **42**: 385–392.
110 Yellon DM, Alkhulaifi AM, Pugsley WB. Preconditioning the human myocardium. *Lancet* 1993; **342**: 276–277.
111 Alkhulaifi AM. Preconditioning the human heart. *Ann Roy Coll Surg Eng* 1997; **79**: 49–54.
112 Cleveland JCJ, Meldrum DR, Rowland RT, *et al.* Preconditioning and hypothermic cardioplegia protect human heart equally against ischemia. *Ann Thorac Surg* 1997; **63**: 147–152.
113 Lee HT, LaFaro RJ, Reed GE. Pretreatment of human myocardium with adenosine during open heart surgery. *J Cardiac Surgery* 1995; **10**: 665–676.
114 Speechly-Dick ME, Grover GJ, Yellon DM. Does ischemic preconditioning in the human involve protein kinase C and the ATP-dependent K+ channel? Studies of contractile function after simulated ischemia in an atrial *in vitro* model. *Circ Res* 1995; **77**: 1030–1035.
115 Morris SD, Yellon DM. Angiotensin-converting enzyme inhibitors potentiate preconditioing through bradykinin $B_2$ receptor activation in human heart. *J Am Coll Cardiol* 1997; **29**: 1599–1606.
116 Ikonomidis JS, Tumiati LC, Weisel RD, *et al.* Preconditioning human ventricular cardiomyocytes with brief periods of simulated ischaemia. *Cardiovasc Res* 1994; **28**: 1285–1291.
117 Arstall MA, Zhao YZ, Hornberger L, *et al.* Human ventricular myocytes *in vitro* exhibit both early and delayed preconditioning responses to simulated ischemia. *J Mol Cell Cardiol* 1998; **30**: 1019–1025.
118 Takaoka A, Nakae I, Mitsunami K, *et al.* Renal ischemia/reperfusion remotely improves myocardial energy metabolism during myocardial ischemia via adenosine receptors in rabbits: effects of 'remote preconditioning'. *J Am Coll Cardiol* 1999; **33**: 556–564.
119 Gho BC, Schoemaker RG, van den Doel MA, *et al.* Myocardial protection by brief ischemia in noncardiac tissue. *Circulation* 1996; **94**: 2193–2200.
120 Birnbaum Y, Hale SL, Kloner RA. Ischemic preconditioning at a distance: reduction of myocardial infarct size by partial reduction of blood supply combined with rapid stimulation of the gastrocnemius muscle in the rabbit. *Circulation* 1997; **96**: 1641–1646.
121 Sommerschild HT, Grund F, Offstad J, *et al.* Importance of endogenous adenosine during ischemia and reperfusion in neonatal porcine hearts. *Circulation* 1997; **96**: 3094–3103.
122 Pitarys CJ, Virmani R, Vildibill HDJ, *et al.* Reduction of myocardial reperfusion injury by intravenous adenosine administered during the early reperfusion period. *Circulation* 1991; **83**: 237–247.
123 Schulz R, Post H, Sakka S, *et al.* Intraischemic preconditioning. Increased tolerance to sustained low-flow ischemia by a brief episode of no-flow ischemia without intermittent reperfusion. *Circ Res* 1995; **76**: 942–950.


© 2000 Blackwell Science Ltd 

124 Todd J, Zhao ZQ, Williams MW, *et al*. Intravascular adenosine at reperfusion reduces infarct size and neutrophil adherence. *Ann Thorac Surg* 1996; **62**: 1364–1372.
125 Vander HR, Reimer KA. Effect of adenosine therapy at reperfusion on myocardial infarct size in dogs. *Cardiovasc Res* 1996; **31**: 711–718.
126 Homeister JW, Hoff PT, Fletcher D, *et al*. Combined adenosine and lidocaine administration limits myocardial reperfusion injury. *Circulation* 1990; **82**: 595–608.
127 Mahaffey KW, Puma JA, Barbagelata A, *et al*. Adenosine as an adjunct to thrombolytic therapy for acute myocardial infarction. *J Am Coll Cardiol* 2000; **34**: 1711–1720.
128 Tsuchida A, Thompson R, Olsson RA, *et al*. The anti-infarct effect of an adenosine A1-selective agonist is diminished after prolonged infusion as is the cardioprotective effect of ischaemic preconditioning in rabbit heart. *J Mol Cel Cardiol* 1994; **26**: 303–311.
129 Dana A, Baxter GF, Walker JM, *et al*. Prolonging the delayed phase of myocardial protection: repetitive adenosine A1 receptor activation maintains rabbit myocardium in a preconditioned state. *J Am Coll Cardiol* 1998; **31**: 1142–1149.
130 Anonymous. Effect of enalapril on mortality and the development of heart failure in asymptomatic patients with reduced left ventricular ejection fractions. The SOLVD Investigators published erratum appears in *N Engl J Med* 1992; 327: 1768.
131 Rutherford JD, Pfeffer MA, Moye LA, *et al*. Effects of captopril on ischemic events after myocardial infarction. Results of the Survival and Ventricular Enlargement trial. SAVE Investigators. *Circulation* 1994; **90**: 1731–1738.
132 Anonymous. GISSI-3: effects of lisinopril and transdermal glyceryl trinitrate singly and together on 6-week mortality and ventricular function after acute myocardial infarction. *Lancet* 1994; **343**: 1115–1122.
133 Anonymous. ISIS-4: a randomised factorial trial assessing early oral captopril, oral mononitrate, and intravenous magnesium sulphate in 58,050 patients with suspected acute myocardial infarction. *Lancet* 1995; **345**: 669–685.
134 Parratt JR. Cardioprotection by angiotensin converting enzyme inhibitors – the experimental evidence. *Cardiovasc Res* 1994; **28**: 183–189.
135 Miki T, Miura T, Ura N, *et al*. Captopril potentiates the myocardial infarct size-limiting effect of ischemic preconditioning through bradykinin B2 receptor activation. *J Am Col Cardiol* 1996; **28**: 1616–1622.
136 Matoba S, Tatsumi T, Keira N, *et al*. Cardioprotective effect of angiotensin-converting enzyme inhibition against hypoxia/reoxygenation injury in cultured rat cardiac myocytes. *Circulation* 1999; **99**: 817–822.
137 Kleinert S. HOPE for cardiovascular disease prevention with ACE-inhibitor ramipril. Heart Outcomes Prevention Evaluation [news]. *Lancet* 1999; **354**: 841.
138 Menasche P, Kevelaitis E, Mouas C, *et al*. Preconditioning with potassium channel openers. A new concept for enhancing cardioplegic protection?. *J Thoracic Cardiovasc Surg* 1995; **110**: 1606–1613.
139 Menasche P, Mouas C, Grousset C. Is potassium channel opening an effective form of preconditioning before cardioplegia? *Ann Thoracic Surg* 1996; **61**: 1764–1768.
140 Gross GJ, Auchampach JA, Maruyama M, *et al*. Cardioprotective effects of nicorandil. *J Cardiovasc Pharmacol* 1992; **20**(Suppl 3): S22–S28.
141 Sugimoto S, Iwashiro K, Monti F, *et al*. The risk of myocardial stunning is decreased concentration-dependently by KATP channel activation with nicorandil before high K+ cardioplegia. *Int J Cardiol* 1995; **48**: 11–25.
142 Imagawa J, Baxter GF, Yellon DM. Myocardial protection afforded by nicorandil and ischaemic preconditioning in a rabbit infarct model *in vivo*. *J Cardiovasc Pharmacol* 1998; **31**: 74–79.
143 Rajaratnam R, Brieger DB, Hawkins R, *et al*. Attenuation of anti-ischemic efficacy during chronic therapy with nicorandil in patients with stable angina pectoris. *Am J Cardiol* 1999; **83**: 1120–1124.
144 Patel DJ, Purcell HJ, Fox KM. Cardioprotection by opening of the K (ATP) channel in unstable angina. Is this a clinical manifestation of myocardial preconditioning? Results of a randomized study with nicorandil. CESAR 2 investigation. Clinical European studies in angina and revascularization. *Eur Heart J* 1999; **20**: 51–57.