Review Article

Ischaemic preconditioning: mechanisms and potential clinical applications

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Purpose: Brief ischaemic episodes, followed by periods of reperfusion, increase the resistance to further ischaemic damage. This response is called "ischaemic preconditioning." By reviewing the molecular basis and fundamental principals of ischaemic preconditioning, this paper will enable the anaesthetic and critical care practitioner to understand this developing therapeutic modality.

Source: Articles were obtained from a Medline review (1960-1997; search terms: ischaemia, reperfusion injury, preconditioning, ischaemic preconditioning, cardiac protection). Other sources include review articles, textbooks, hand-searches (Index Medicus), and personal files.

Principle finding: Ischaemic preconditioning is a powerful protective mechanism against ischaemic injury that has been shown to occur in a variety of organ systems, including the heart, brain, spinal cord, retina, liver, lung and skeletal muscle. Ischaemic preconditioning has both immediate and delayed protective effects, the importance of which varies between species and organ systems. While the exact mechanisms of both protective components are yet to be clearly defined, ischaemic preconditioning is a multifactorial process requiring the interaction of numerous signals, second messengers and effector mechanisms. Stimuli other than ischaemia, such as hypoxic perfusion, tachycardia and pharmacological agents, including isoflurane, have preconditioning-like effects. Currently ischaemic preconditioning is used during minimally invasive cardiac surgery without cardiopulmonary bypass to protect the myocardium against ischaemic injury during the anastomosis.

Conclusion: Ischaemic preconditioning is a powerful protective mechanism against ischaemic injury in many organ systems. Future clinical applications will depend on the clarification of the underlying biochemical mechanisms, the development of pharmacological methods to induce preconditioning, and controlled trials in humans showing improved outcomes.

Objectif : De brefs épisodes d'ischémie, suivis de périodes de reperfusion, accroissent la résistance à un dommage ischémique ultérieur. C'est ce qu'on appelle le «préconditionnement ischémique». En faisant un retour sur la base moléculaire et les principes fondamentaux du préconditionnement ischémique, le présent article fera mieux comprendre à l'anesthésiste et au praticien des soins intensifs les modalités de cette thérapeutique en évolution.

Sources documentaires : Des articles ont été obtenus à partir d'une recherche Medline (1960-1997; recherche de termes : ischémie, lésion de reperfusion, préconditionnement, préconditionnement ischémique, protection cardiaque). Les autres sources comprennent des articles de revues, des monographies, des recherches manuelles (Index Medicus) et une documentation personnelle.

Données principales : Le préconditionnement ischémique est un mécanisme protecteur puissant contre la lésion ischémique qui se produit, selon l'expérience, dans divers systèmes organiques, incluant le coeur, le cerveau, la moelle épinière, la rétine, le foie, les poumons et les muscles squelettiques. Le préconditionnement présente deux effets protecteurs, l'un immédiat et l'autre différé, dont l'importance varie entre les espèces et les systèmes organiques. Quoique les mécanismes exacts des deux composantes protectrices n'aient pas encore été clairement définis, on sait que le préconditionnement ischémique est un processus multifactoriel nécessitant l'interaction de nombreux signaux, de seconds messagers et de mécanismes effecteurs. Des stimuli différents de l'ischémie, comme la perfusion hypoxique, la tachycardie et des agents pharmacologiques, comprenant l'isoflurane, ont des effets similaires au préconditionnement. Le préconditionnement ischémique est actuellement utilisé pendant la chirurgie cardiaque mini-effractive, sans circulation extracorporelle pour protéger le myocarde contre une lésion ischémique lors de l'anastomose.

Conclusion : Le préconditionnement ischémique est un mécanisme protecteur puissant contre les lésions ischémiques dans de nombreux systèmes organiques. Les applications cliniques éventuelles vont dépendre de la clarification des mécanismes biochimiques sous-jacents, de l'évolution des méthodes pharmacologiques d'induction du préconditionnement et des essais contrôlés chez les humains démontrant de meilleurs résultats.

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Contents

OVER the past decade, the pathophysiological consequences of human myocardial ischaemia have received much attention. With the description of myocardial stunning[1] and of the hibernating[2] myocardium, it had been assumed, until recently, that intermittent episodes of ischaemia lead to cumulative myocardial damage.[3] Teleologically, this seems logical. As the life expectancy of early man was short, ischaemia would not have been a major problem and selection bias to prevent atherosclerotic diseases would have been unlikely. However, researchers have demonstrated that an endogenous myocardial protective mechanism does exist. Brief ischaemic episodes, followed by periods of reperfusion, paradoxically increase the resistance to further ischaemic damage.[4] This response is called ischaemic preconditioning (IP) (Figure 1). Subsequently, ischaemic preconditioning has been shown to have protective effects in several organ systems. It is likely that anaesthesia and critical care practitioners will be exposed to the potential therapeutic applications of this phenomenon in the future. As the biochemical basis for IP has been the subject of much investigation, this article will review the molecular mechanisms of IP and discuss possible future clinical applications.

History

In 1986, Reimer *et al.*, in an attempt to examine the contributions of high energy phosphate depletion and catabolite accumulation in the development of ischaemic injury, conducted a series of experiments in the dog heart where ATP and catabolite concentrations were measured after a series of four 10 min coronary artery occlusions. These were then compared with ATP concentrations measured after a single 40 min occlusion.[5] After 40 min, ATP levels in dogs subjected to serial ischaemia were higher than in those exposed to a single ischaemic event. Further, they noted that, of the seven dog hearts exposed to serial ischemic events, six did not develop myocardial necrosis.[5] This paradox, that intermittent ischaemia may not have an additive but rather a protective effect against subsequent ischaemia, was tested in 1986 by Murray *et al.*[4] They subjected dog hearts to four cycles of alternating five minute periods of ischaemia and reperfusion. This was followed by a prolonged ischaemic episode. With this protocol, infarct size was limited to 25% of that in the control group (which had no preceding cycles of ischaemia and reperfusion). This form of myocardial protection was termed ischaemic preconditioning. Myocardial IP has been shown to occur in several animal models (rabbits,[6] rats,[7] pigs,[8] humans[9]) and a variety of end-points have been used to reflect its efficacy as an intervention (incidence of arrhythmias, contractile parameters, infarct size). Other stimuli, including tachycardia, hypoxic perfusion and pharmacological agents, have been shown to precondition the myocardium.[10] The discovery of myocardial IP led investigators to study IP in other organs, and evidence now shows that this phenomenon occurs in the brain, spinal cord, retina, liver, lungs and skeletal muscle (see Clinical Applications).

The ischaemic times required to induce preconditioning are species-specific and organ system specific (Figure 2). Murray *et al.* established that four cycles of five minutes coronary occlusion and five minutes of reperfusion were sufficient to precondition the dog heart. Liu found that three cycles of three minutes of ischaemia and reperfusion preconditioned the rat heart.[11] A single five minute cycle of ischaemia and reperfusion was sufficient to precondition the rabbit heart.[6] A minimum of two minutes of ischaemia and reperfusion appears necessary to precondition the

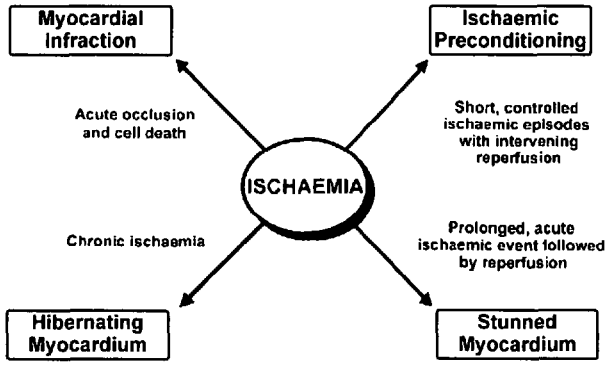

FIGURE 1 The spectrum of myocardial ischaemia.

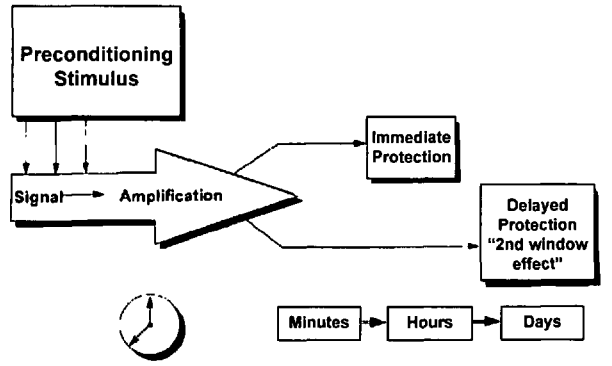

FIGURE 2 The time course of immediate and delayed ischaemic preconditioning.

human myocardium.[12] Yellon and colleagues found two cycles of three minutes ischaemia with two minutes reperfusion to be effective during CABG surgery.[9] The times to induce immediate protection in other organ systems shows a similar inter-species variation (see section on clinical applications).

There is considerable inter-species variation in the duration of protection provided by the immediate component of ischaemic preconditioning. While cardiac protection is lost after 30 min reperfusion in the rabbit heart,[13] one hour in rats,[7] and two hours in dogs,[14] the duration of immediate protection in humans is unknown.

The time to develop delayed protection is also variable (Figure 2). Marber *et al.* showed that rabbits preconditioned 24 hr before a 30 min coronary occlusion had a 40% decrease in infarct size.[15] Kuzuya *et al.* have demonstrated similar delayed cardiac protection in dogs.[16] It has not been determined whether there is a delayed preconditioning effect in human hearts. Similarly, the effectiveness of the delayed protection varies among organ systems (see clinical applications).

Proposed Mechanisms

A. Anatomical

Collateral Vessels

Initially, it was believed that IP protection was mediated by increased flow through the coronary collateral circulation. This theory has largely been abandoned. The temporal aspects of preconditioning (immediate onset, limited duration of cardiac protection and "second window" effect) cannot be explained by increased collateral flow alone. Murray *et al.* injected radiolabelled microspheres into the left atrial appendage of the dog heart and measured tissue radioactivity. They showed that there was no difference in collateral blood flow between preconditioned dogs and controls.[4] Finally, species with a limited collateral circulation (e.g. rabbit, rat) can be preconditioned.[6,7]

Myocardial Stunning

Sequential ischaemic events during IP produce reversible changes in contractility characteristic of myocardial stunning. It has been suggested that this period of reduced energy demand protects the myocardium against further ischaemic injury.[17] If this hypothesis were true, then the protective window of IP should coincide with the time that the myocardium is stunned. This is not the case. Myocardial protection is lost before return of normal contractile function.[14] In addition, the degree of stunning does not correlate with the degree of myocardial protection. Miura *et al.*, by measuring wall thickening relative to baseline in a rabbit model, found that the degree of stunning was greater with five minutes than with two minutes IP, but that the amount of protection afforded was identical.[18] Matsuda *et al.* demonstrated that overcoming the contractile dysfunction of the stunned myocardium with dobutamine did not decrease the protection afforded with ischaemic preconditioning.[19] These results suggest that stunning is not the direct mediator of preconditioning.

B. Molecular Mechanisms

As in any biochemical model, the molecular basis for IP must consist of an ordered series of events. In response to the IP stimulus, a *signal* must be rapidly generated that is *transduced* into an intracellular message and amplified to influence the *effector* mechanism (Figure 3). While support for each component of this activation cascade exists, IP is likely a complex multifactorial molecular process that involves a combination of the elements presented in this review. (Figure. 4)

SIGNALS

a) Adenosine

Previous studies have shown that adenosine, an endogenous nucleoside produced primarily through the degradation of ATP, can limit ischaemic injury (particularly reperfusion injury) through a variety of mechanisms, including: reduced neutrophil adherence and cytotoxicity to endothelial cells; decreased superoxide anion production; decreased lipolysis and increased membrane stability; reduced Ca^{2+} influx and hyperpolarization of myocytes through ATP-dependent K^+ channels.[20] Because adenosine is rapidly generated during ischaemia and reaches high concentrations, much of the IP research has focused on adenosine as the possible signal.

Adenosine exerts its physiological effect primarily through two purinergic extracellular membrane receptors: A_1 receptors located on myocardial cells, and A_2 receptors located on the coronary endothelial and leucocyte/platelet membranes. It is the A_1 receptor which plays a role in ischaemic preconditioning. Pretreatment of rabbit,[21] dog[22] and pig[23] hearts with selective A_1 receptor agonists prior to an ischaemic event mimics the protection derived from ischaemic preconditioning. Downey *et al.* found that pretreatment with adenosine receptor antagonists SPT and PD 115-199 inhibited the protective effect of IP in rabbits.[21] However, administration of these antagonists after the IP protocol but before the final ischaemic event, did not lead to loss of cardioprotection.[21] Therefore, adenosine is important in initiating IP but is not required to maintain cardiac protection once it is established.

There is some evidence that adenosine has a role in preconditioning the human heart. An adenosine infusion prior to CABG surgery improves haemodynamics after cardiopulmonary bypass and decreases CPK release.[24] Recently, Leesar *et al.* showed that an intracoronary adenosine infusion in patients undergoing angioplasty of the left anterior descending coronary artery reduced ST-segment changes and chest pain.[25] Acadesine, an inhibitor of adenosine breakdown, produces a 64% reduction in the incidence of perioperative myocardial infarction in patients undergoing elective CABG surgery.[26] Finally, Lawson has demonstrated that the enhanced resistance to hypoxia in isolated human atrial trabeculae is abolished in the presence of adenosine antagonists.[27]

While support for adenosine as a signal in IP is strong, its role as the sole mediator initiating IP has been questioned. First, the role for adenosine in the rat heart is unclear. While the addition of exogenous adenosine does confer protection, A_1 antagonists do not abolish the protective effect of IP in the rat heart. Yao *et al.* noted temporal differences between the duration of protection afforded by exogenous adenosine or by IP in the dog heart.[22] With exogenous

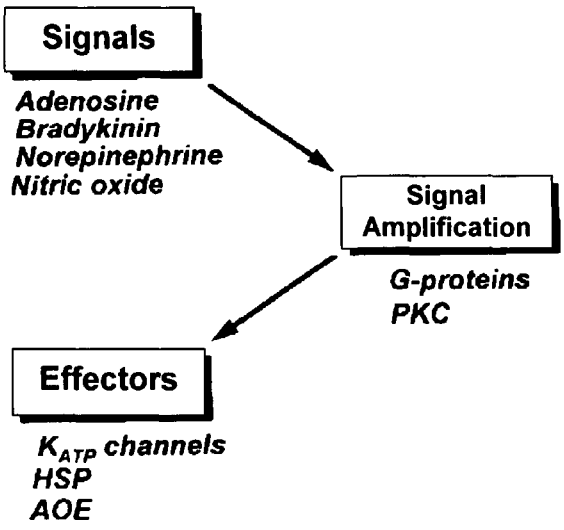

FIGURE 3 The molecular cascade of ischaemic preconditioning: signals amplification effectors. PKC = protein kinase C, HSP = heat stress proteins, AOE = antioxidant enzymes, K_{ATP} channels = ATP-dependent potassium channels.

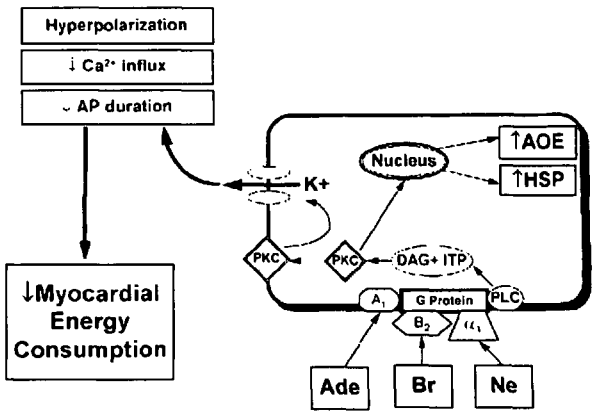

FIGURE 4 G-protein activation by adenosine (Ade), Bradykinin (Br), and possibly norepinephrine (Ne) stimulates phospholipase C (PLC), which then increases the production of diacetylglycerol (DAG) and inositol triphosphate (ITP). Diacylglycerol activates protein kinase C (PKC) which is then translocated to the cell membrane. Protein kinase C, through the opening of ATP-dependent potassium channels, hyperpolarizes the cell. This decreases calcium influx and therefore decreases myocardial contractility and energy consumption (immediate IP protection). Through a proposed translocation to the nucleus, PKC may increase the production of heat stress proteins (HSP) and antioxidant enzymes (AOE) (delayed IP protection). A_1 = Adenosine receptor type 1, $_1$ = alpha adrenergic receptor type 1, B_2 = bradykinin receptor type 2, K^+= potassium.

adenosine, protection faded in less than 60 min, while the protection provided by IP persisted beyond 60 min. It may be that multiple signals are required to induce maximal protection against further ischaemia.

b) Bradykinin

Goto *et al.* have demonstrated that, in the isolated rabbit heart, bradykinin infusions mimic ischaemic preconditioning.[28] A bradykinin B_2 receptor inhibitor, HOE 140, negated the protection induced by the bradykinin infusion. However, this inhibition could be overcome by amplifying the pre-conditioning stimulus with an ischaemic protocol using four cycles of five minutes ischaemia with 10 min intervening reperfusion. The protection afforded by IP was not abolished by HOE 140. While the potential role of bradykinin is intriguing, there is no evidence documenting its release during ischaemic preconditioning.

c) Catecholamines

There is conflicting evidence regarding the role of norepinephrine in ischaemic preconditioning. Toombs *et al.* showed that rabbit hearts depleted of norepinephrine stores with reserpine did not demonstrate any limitation in infarct size with ischaemic preconditioning.[29] Stimulation of rat α_1 -adrenoreceptors has been reported to confer ischaemic protection.[30] However, norepinephrine is not released into the coronary effluent of preconditioned rabbit hearts.[31] Seyfarth *et al.* reported that IP in fact suppressed norepinephrine release during subsequent prolonged ischaemic periods.[32] It is more likely that IP protects the heart not through the *release* of norepinephrine but by reducing norepinephrine-induced damage during prolonged myocardial ischaemia.

d) Nitric oxide

Vegh *et al.* reported that intracoronary administration of methylene blue (which interferes with the effect of nitric oxide on guanylate cyclase) and the intravenous administration of a nitric oxide synthase inhibitor, attenuated the antiarrhythmic effect of ischaemic preconditioning.[33] However, a recent study has failed to demonstrate involvement of nitric oxide in rabbit hearts.[34]

Signal Amplification

a) G-Proteins

The intracellular message in IP is generated through coupling of agonists to G-proteins that span the cell membrane. Adenosine (A_1), norepinephrine (α-1), and bradykinin receptors are coupled to G proteins which, when stimulated, increase activity of phospholipase C and D.[35] These enzymes catalyse hydrolysis of membrane phospholipids, increasing diacylglycerol and inositol triphosphate levels.[35]

b) Protein Kinase C

Diacylglycerol activates protein kinase C (PKC), an enzyme believed to play a key role in the biochemistry of IP through its phosphorylation of effector proteins. In the rabbit heart, administration of PKC inhibitors inhibits IP, while PKC activators mimic preconditioning.[36]

More importantly, the involvement of PKC has been demonstrated in the rat heart, a species in which all other proposed IP agonists to date have had variable reviews.[37] Goto *et al.* proposed that, for IP to occur, a threshold level of PKC activation must be attained.[28] Therefore, preconditioning may be a multifactorial process requiring the input of a variety of agonists in order to reach this threshold level of PKC activation.

Transfer of PKC to the membrane may be required to activate the enzyme.[38] Its persistence in the membrane may account for the duration of protection immediately after ischaemic preconditioning.[39] Immunocytochemical staining has identified PKC in the rat sarcolemma after ischaemic preconditioning.[40] Inhibition of microtubular activity with colchicine (a chemical that disrupts the cytoskeleton and prevents PKC transfer to the membrane) prevents cardiac protection with IP in the rabbit heart.[38] Some researchers have theorised that it is the transfer of PKC to the nucleus and its subsequent regulation of transcription that mediates the delayed protective effect of ischaemic preconditioning.

While it is possible that PKC is the unifying link in the effector pathway for IP, recent studies have called its role into question. Using staurosporine, a PKC inhibitor, Vahlhaus *et al.* showed no loss of IP protection in swine.[41] In rabbits, Simkhovich *et al.* could not demonstrate PKC translocation within a time frame that could explain ischaemic preconditioning.[42] Although these controversies may be based on species differences, PKC's role in IP remains uncertain.

Effector Mechanisms

The effector mechanisms of immediate and delayed ischaemic preconditioning are different.

Early Protection

K_{ATP} CHANNELS

In 1983, Noma identified the "outward injury current" observed in myocardial ischaemia as an efflux of potassium through ATP-dependent K^+ channels (K_{ATP}).[43] These channels open when ATP concentrations decrease. The resulting membrane hyperpolarization shortens the cardiac action potential, and through decreased Ca^{2+} influx, induces myocyte relaxation. These effects may be cardioprotective. However, K_{ATP} channels are inhibited by much lower concentrations of ATP than those measured during early ischaemia.[44] Therefore, the decrease in ATP levels is, by itself, insufficient to explain the K^+ efflux seen with ischaemia.

It is believed that IP leads to rapid phosphorylation of these K_{ATP} channels, increasing the cardioprotective K^+ efflux. Considerable evidence points to the involvement of K_{ATP} channels in IP in various animal models.[45] Speechly-Dick *et al.* have identified the role of K_{ATP} channels in humans by abolishing the protective effect of IP in isolated human atrial trabeculae with gilbenclamide.[46]

Delayed Protection

Although several mechanisms have been proposed to explain the delayed protection seen with IP, it is likely that changes in gene expression of protective proteins play a role.

HEAT STRESS PROTEINS

Exposure of cardiac myocytes to sublethal temperatures (generally 42°C for 15 min) has been shown to protect the myocytes against subsequent oxidative stresses.[47] This protection has been associated with the production of an inducible 70 kD stress protein (HSP70i).[48] Synthesis of HSP70i increases with a variety of other stimuli including haemodynamic overload, myocyte stretch, hypertension, ischaemia, exercise, and oxidant stress.[49] Elevated levels of HSP70i have been shown to occur 24 hr after ischaemic preconditioning.[47] More importantly, over-expression of the HSP70i gene in transgenic mice limits infarct size and improves post-ischaemic contractile recovery.[50] It is hypothesised that, in response to myocardial stresses, heat stress proteins help to identify damaged proteins, and facilitate the synthesis of new proteins.[49] However, before a direct link is established between the delayed protective effects of IP and HSP70i, the molecular mechanism by which heat stress proteins confer protection must be clarified.

ANTIOXIDANT ENZYMES

Preconditioning may play an important role in limiting reperfusion injury due to oxygen free radicals. A number of stresses, including hyperthermia and ischaemia, can increase the activity of endogenous antioxidant enzymes (AOE).[51] Hoshida *et al.* have shown that the levels of mitochondrial manganese-superoxide dismutase (MnSOD) are elevated 24 hr after IP in dog hearts.[51]

Clinical Applications

It is becoming apparent that IP is a protective mechanism common to a variety of tissues. A number of exciting clinical possibilities merit discussion.

A. Cardiac

There is increasing evidence that the human myocardium can be preconditioned. Patients with chronic angina pectoris have an improved short-term prognosis after thrombolytic therapy compared with patients with no preceding angina.[52] Recently, Tamura *et al.* have shown a reduction in life-threatening reperfusion arrhythmias in patients with angina preceding myocardial infarction compared with patients without angina.[53] While these improved outcomes may be due to IP, the retrospective analyses may not fully account for protective mechanisms other than IP, such as the higher frequency of anti-anginal medication and increased collateral flow in the patients with chronic angina.

Deutsch *et al.* have noted that each sequential 90 sec PCTA balloon inflation results in less chest pain, ST elevation and lactate production.[54] However, when repeating this study in a larger population group, DeJong *et al.* were unable to reproduce the results.[55] Other studies have demonstrated improved tolerance to ischaemia when sequential balloon inflations lasting two minutes were used.[12] These data suggest that PCTA balloon inflation of at least two minutes may precondition the human myocardium.

In 1993, Yellon subjected patients undergoing CABG surgery to an IP protocol involving three minutes aortic cross-clamping, followed by two minutes reperfusion.[9] This was repeated and the aortic cross-clamp was then applied for 10 min and myocardial tissue samples were taken from the left anterior descending coronary artery territory. The IP group had higher ATP levels at the end of the 10 min ischaemic period than the non-preconditioned group. Alkhulaifi, in a similar experiment, demonstrated lower levels of troponin T release in preconditioned human hearts, implying less myocardial damage.[56] However, these *in vivo* studies provide only indirect evidence of IP in the human myocardium.

In vitro studies have demonstrated more conclusively the protective effects of IP on the human myocardium. Walker *et al.*, using isolated human atri-

al trabeculae, demonstrated that IP improved functional recovery after 90 min ischaemia.[57] Ikonomidis showed that IP reduced infarct size in cultured human ventricular myocytes.[58] Although the data on IP in the human heart is increasing, due to the novelty of the concept, prospective trials are lacking. Direct measures demonstrating improvement in ischaemic cell damage and myocardial function, if ethically feasible, are needed to prove the existence of IP in the human myocardium.

Myocardial revascularization

It remains to be determined whether IP offers any *added* advantage to cardioplegia in protecting the myocardium. Kolocassides *et al.* demonstrated, in an isolated rat heart preparation that IP and warm cardioplegia provided similar protection of contractile function, but that there was no additional protection when they were combined.[59] In a human study, Kaukoranta *et al.*, using CK-MB and troponin T levels as their end-points, found that IP and retrograde normothermic cardioplegia provided no additional protection against myocardial damage when compared with retrograde normothermic cardioplegia alone.[60] Studying isolated human atrial trabeculae, Clevelend *et al.* demonstrated no difference in protection of myocardial function when IP and cold cardioplegia were combined.[61]

Is there a role for IP in situations where cardioplegia may be ineffective? Cardiac protection in CABG surgery is dependent on optimal delivery of the cardioplegia to all parts of the heart. Inadequate delivery through critical stenoses, non-homogeneous distribution with retrograde techniques and inadequate supply to hypertrophied ventricles, combined with the recent enthusiasm for warm cardioplegia, increases the potential for ischaemic injury. It is in these scenarios where IP may be beneficial. Galiñanes *et al.* studied the effects of IP by occluding one of the coronary arteries in an isolated rat heart, thereby impairing the delivery of cardioplegia.[62] Ischaemic preconditioning, by itself or in combination with cardioplegia, afforded better protection than cardioplegia alone. Similarly, when the volume of cardioplegia administered was inadequate (analogous to an inadequate supply to hypertrophied ventricles), IP improved cardiac protection.[63]

Recently, minimally invasive surgical techniques have been developed in the hope of avoiding the complications associated with cardiopulmonary bypass and minimising hospital costs. These procedures are particularly suited for isolated left anterior descending coronary artery lesions. In "Minimally Invasive Direct Coronary Bypass Grafting" (MIDCAB), isolated lesions of the LAD are by-passed using the left internal mammary artery, and surgery is performed on a normothermic, beating heart without cardiopulmonary bypass. There is an obligatory period of myocardial ischaemia while the vessel is occluded for the anastomosis to be performed (Figure 5). Ischaemic preconditioning has been used in some centres as a protective mechanism against ischaemia occurring during graft anastomosis. Jacobsohn *et al.* described the effect of IP on myocardial function during MIDCAB surgery.[64] During one such procedure, pressure-area loops were assessed using transoesophageal echocardiography at baseline, after each preconditioning stimulus and after revascularization (Figure 6). Contractility was expressed as the ratio of end-systolic blood pressure to left-ventricular area. The initial IP stimulus depressed contractility more than did the subsequent preconditioning stimulus. After revascularization, contractility dramatically improved. Lacking appropriate controls, preservation of myocardial function in this case cannot be conclusively determined but the results, combined with absence of electrocardiographic and cardiac

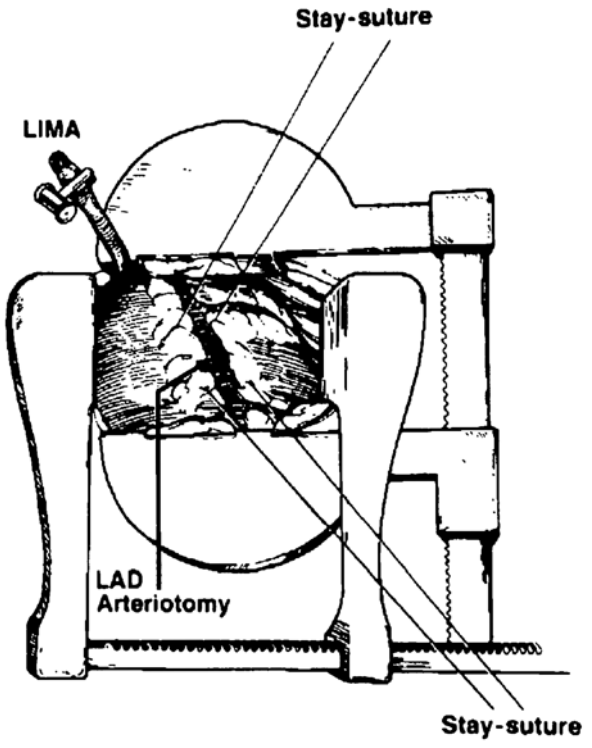

FIGURE 5 Minimally invasive direct coronary bypass surgery (MIDCAB). The mid-left anterior descending coronary artery (LAD) is exposed through a limited left anterior thoracotomy. Upward traction on stay-sutures placed proximal and distal to the arteriotomy site control blood flow in the LAD. Traction enables ischaemic preconditioning to be performed. The left internal mammary artery is shown with a vascular clamp attached.[64]

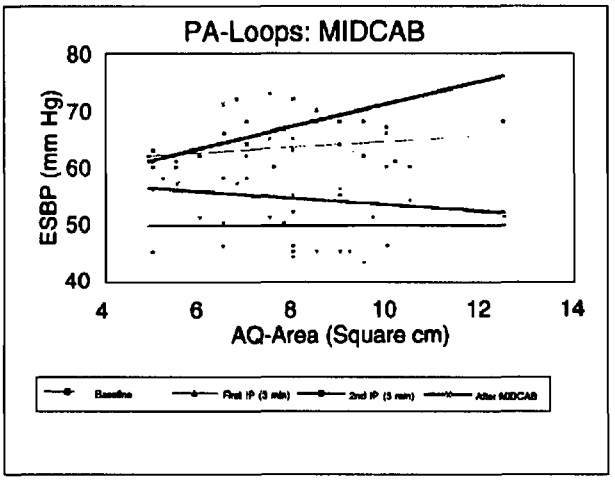

FIGURE 6 Contractility curves based on end-systolic pressure points from the pressure-area loops performed during MIDCAB surgery (baseline, during ischaemic preconditioning, and after myocardial revascularization). ESBP = end systolic blood pressure, AQ Area = measurement of end systolic left ventricular area by transesophageal echocardiography using acoustic quantification. ESBP/AQ Area plots are load insensitive measures of LV function.[64]

enzyme changes, indicate no deleterious consequences as a result of ischaemic preconditioning.

Established mechanisms of cardiac protection during cardiac surgery have proved to be effective and safe. While animal studies are encouraging, prospective, randomised clinical trials are needed to assess the role of IP in CABG surgery. The exact mechanism of IP and the conditions that confer optimal protection in the human heart need to be further defined. Pharmacological interventions that mimic IP may then be developed and used as an adjunct, rather than alternative to cardioplegia.

Heart Transplantation

Hypothermic ischaemia at 4°C is mandatory during donor heart procurement and efforts to minimise ischaemic contractile dysfunction in the transplanted human heart are important. Cleveland *et al.* examined whether normothermic IP conferred added benefit in the maintenance of contractile function in isolated human atrial trabeculae subjected to four hours hypothermic ischaemia (a time approaching the maximum allowed for storage and transport in transplantation).[61] After reperfusion, the developed force in the preconditioned trabeculae did not differ from controls. On the other hand, IP protected rat hearts against four hours hypothermic ischaemia.[65] While species differences and different study designs may account for these conflicting results, further research investigating the role of IP in human heart transplantation is needed.

B. Central Nervous System

Brain

The brain can be preconditioned, and there appears to be an immediate and a delayed component. Pérez-Pinzón and colleagues, studying the immediate effects of IP in the brain, subjected rats to 10 min global ischaemia.[66] Neuronal damage decreased in preconditioned animals relative to controls. Kitagawa *et al.*, studying neuronal necrosis and delayed IP in the gerbil hippocampus, demonstrated neuro-protection when IP was separated from the prolonged ischaemic insult by 24 hr.[67] An interval of 12 hr was not sufficient. When established, neuroprotection persisted for at least two days.

Recent evidence demonstrates that both global and focal ischaemia can induce ischaemic tolerance in the brain.[68] Chen *et al.* have shown that 30 min focal ischaemia, in single or divided doses, two days before an ischaemic insult, reduced cerebral infarct size by 30%.[69] They also demonstrated that changes in regional blood flow did not occur prior to or during the infarction-producing event.

Are the mechanisms underlying neuroprotection the same as those in the heart? There is evidence suggesting that adenosine has a role in IP of the brain. The A_1 agonist, 2-chloroadenosine, improves evoked potential recovery after prolonged cerebral ischaemia.[70] Pharmacological blockade of A_1 receptors or K_{ATP} channels negated any neuroprotection afforded by ischaemic preconditioning.[71] The onset of protection is delayed in many models, suggesting that the production of protective proteins is the likely effector mechanism. Although there is a general depression of protein synthesis as a consequence of cerebral ischaemia, increased levels of heat stress proteins have consistently been demonstrated in brains tolerant to ischaemia.[69] Furthermore, transient whole-body hyperthermia attenuated subsequent ischaemic brain injury in the rat.[72] Despite the raised levels of heat stress proteins in brain ischaemia, a cause and effect relationship has not been demonstrated.

With the identification of immediate and delayed IP in animal brains, many questions concerning neuro-IP remain: Do transient ischaemic attacks confer some protection against strokes in a way analogous to angina preceding myocardial infarction? Can IP protect the brain during carotid and other cerebrovascular surgery and, if so, how could IP be safely applied in the human brain?

SPINAL CORD

Spinal cord ischaemia is a devastating, usually irreversible, complication of thoracoabdominal aneurysm surgery. Despite numerous protective strategies, including shunts, increased perfusion pressure, CSF drainage, reimplantation of intercostal vessels and spinal cord cooling, the incidence of paraplegia remains between 6-10%.[73] Matsuyama *et al.* investigated whether ischaemic tolerance in the canine spinal cord could be increased by ischaemic preconditioning.[74] Twelve dogs were randomly assigned to receive either 20 min cross-clamping of the descending thoracic aorta distal to the subclavian artery or a sham operation with no aortic cross-clamping. After surgery, all animals had normal neurological function. Forty-eight hours after the initial operation, the aorta was cross-clamped for 60 min in both the test and control groups. Neurological recovery was assessed by a blinded observer and immuno-histochemical staining of the lumbar spinal cord was performed with anti-HSP70 monoclonal antibody. One day after the second operation, no preconditioned dogs were paraplegic, while three of six controls were paralysed. The immuno-histochemical staining demonstrated significant HSP70 levels in the spinal cord of the nine non-paraplegic dogs, while no reactivity was found in the cords of paralysed animals.

Although the sample size was small, this study suggests that delayed ischaemic tolerance can be induced in the canine spinal cord, without IP itself causing neuronal injury. It also suggests that HSP70 may be a mediator of this protection. While adding IP as a neuroprotective strategy in thoracoabdominal aortic surgery is enticing, these results may not be applicable to humans as the vascular anatomy between the species differs. To date, studies examining acute spinal cord IP have not been done. Preconditioning the human spinal cord would be impractical at this time as it would require percutaneous intraaortic balloon occlusion 24 hr before the procedure.

RETINA

Roth *et al.* have shown that adenosine enhances retinal blood flow during ischaemia. Larsen, using electroretinograms to identify retinal damage, showed that injection of an adenosine deaminase inhibitor, or an A_1 receptor agonist, prevented ischaemic damage in the rat retina.[76] Is this pharmacological preconditioning of the retina? There are no reports of IP on the retina.

C. Skeletal Muscle

Skeletal muscles are subjected to global ischaemia during many musculoskeletal procedures, including limb revascularization and free-flap muscle transplantation. To reduce intraoperative blood loss and improve the operative field, surgeons often expose skeletal muscle to periods of tourniquet-induced ischaemia. Although human skeletal muscle can tolerate up to 2.5 hr warm global ischaemia with minimal risk, subsequent reperfusion injury does occur, reducing post-ischaemic skeletal muscle function and impeding patient rehabilitation.[77] Several animal models have used IP as a mechanism to protect against ischaemic injury in skeletal muscle, and have shown favourable results. Carrol *et al.* studied the effect of IP on the preservation of skeletal muscle flap viability in rats.[78] Rat latissimus dorsi muscles were preconditioned immediately or 24 hr before muscle flap elevation. Necrosis, measured as a percentage of total muscle-flap area, was reduced from 24% (control) to 14% (immediate) and 11% (24 hr. previous). This showed that there was both an immediate and a delayed protective window in rat skeletal muscle. Muscle-flap blood flow, measured with a laser-doppler perfusion monitor, did not differ between the groups. Pang *et al.* similarly demonstrated a 44% decrease in skeletal muscle infarction by preconditioning pig latissimus dorsi immediately before muscle-flap elevation.[77] Gurke *et al.* have shown that IP not only limits infarction size but improves post-ischaemic skeletal muscle function as well.[79] After preconditioning with three cycles of IP (10 min ischemia each with 10 min reperfusion) prior to three hours of tourniquet inflation, they demonstrated improved endurance, contractility and generated force in the extensor muscles of rat hindlimbs.

Research into the mechanisms of skeletal muscle preconditioning is limited. While some suggest a role for adenosine[80] and K_{ATP} channels,[81] further studies delineating the biochemistry of IP in skeletal muscle are needed. Although it would be relatively easy to study the principles of IP in reconstructive surgery where considerable skeletal muscle ischaemia is expected, human trials examining this are lacking.

D. Liver

To achieve a bloodless field and to minimise intraoperative blood loss, clamping of the portal triad subjects the liver to regular ischaemic insults during resection and transplantation. It has been the practice of some surgeons to perfuse the liver at regular intervals to minimise total ischaemic time. As this is both inconvenient, bloody and of no proven benefit, most studies have focused on the ischaemic duration that the liver can tolerate. It is now estimated that the healthy liver, in a normotensive patient, can withstand warm ischaemia for an hour.[82] However, with the trauma of surgery and underlying hepatic disease, safe ischaemic times are not

known. In 1993, Lloris-Carsi *et al.* demonstrated that rats preconditioned with five minutes of portal triad clamping, showed improved survival and lower liver enzyme levels after 90 min ischaemia.[83] Hardy *et al.*, subjected rats to an IP protocol (five minutes ischaemia with 10 min reperfusion) prior to 45 min of ischaemia, during which 80% liver resection was performed.[84] Survival improved with IP (9/10 IP rats survived more than 24 hr *vs* 1/10 controls). Although hepatic function was better in the IP group (improved prothrombin times), no difference in serum alanine aminotransferase, a measure of immediate cell injury and hepatocyte membrane integrity, was shown. As the change in prothrombin time indicated some positive effect on hepatocyte function, it has been suggested that preconditioning affects hepatocytes indirectly through its effects on Kuppfer and endothelial cells.[84] As Kuppfer cells have been implicated in hepatic reperfusion injury, this may be a plausible hypothesis.

E. Lung

Du *et al.* studied the role of IP in lung transplantation. They occluded the rat pulmonary artery (and main bronchus) for five minutes and allowed for 10 min reperfusion (and ventilation) prior to donor harvest and storage.[85] Ischaemic preconditioning improved gas exchange after transplantation and decreased free radical formation. To date, human studies have not been done.

F. Kidney

After clamping the left renal artery for 40 min, Islam and colleagues compared the integrity of renal tissue in preconditioned (four minutes ischaemia and 11 min of reperfusion, repeated four times) and control (no preconditioning) rat kidneys.[86] Ischaemic preconditioning did not protect the kidney.

Anaesthesia and Preconditioning

Administration of volatile anaesthetics during transient myocardial ischaemia has been shown to enhance recovery of cardiac function and reduce the degree of subsequent myocardial infarction.[87] Because many isoflurane effects are mediated through K_{ATP} channel-dependent mechanisms, Cason *et al.* investigated whether isoflurane preconditioned the rabbit heart.[88] Rabbits were assigned to one of three groups: control (no pre-treatment), IP group (five minute coronary artery occlusion with 15 min reperfusion), and an isoflurane group (15 min isoflurane 1.1% end-tidal) prior to a 30 min coronary artery occlusion. Infarct size, as a percentage of the area at risk, was reduced from 33.1% (control) to 23.4% and 8.7% for the isoflurane and IP groups respectively (Figure 7). Although

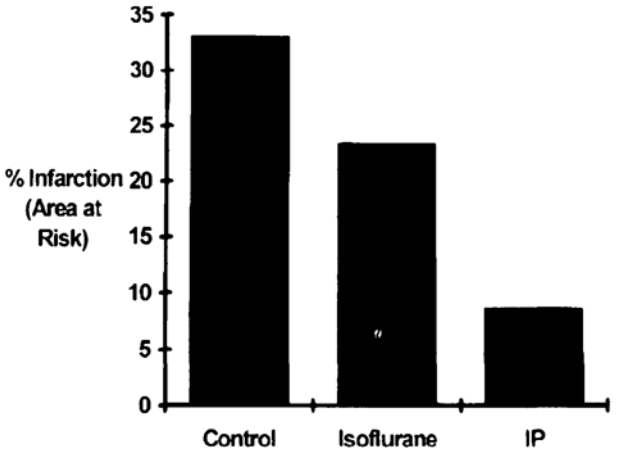

FIGURE 7 Comparison of infarct size (expressed as a % of the area at risk) in rabbit hearts that were either not pre-treated (controls), exposed to 15 min isoflurane 1.1% , or subjected to five minutes of ischaemic preconditioning with 15 min of reperfusion prior to 30 min of anterolateral coronary artery occlusion.[88]

protecting the heart to a lesser degree than IP, pretreatment of rabbit myocardium with isoflurane therefore induced a preconditioning-like effect.

Haessler *et al.* have also investigated the influence of anaesthetic agents on ischaemic preconditioning in the rabbit heart.[89] They determined that IP cardiac protection varies with the anaesthetic used. In comparison to non-preconditioned controls, IP reduced infarct size by 33% with ketamine/xylazine, 44% with isoflurane, and 81% with pentobarbitone anaesthesia. Therefore, future IP studies should consider the influence of the type of anaesthetic used.

Conclusions

Ischaemic preconditioning is a protective phenomenon that occurs in a variety of organ systems. While the understanding of ischaemic preconditioning has increased greatly over the past decade, only when the underlying molecular mechanisms are clearly defined can therapeutic interventions, including pharmacological methods aimed at increasing tissue ischaemic thresholds, be safely and effectively applied. Although IP is currently being used only in minimally invasive coronary artery bypass surgery, it is likely that in the near future anaesthesia practitioners will see the implementation of IP in a wide range of surgical patients.

References

1 *Braunwald E, Kloner RA.* The stunned myocardium: prolonged, postischemic ventricular dysfunction. Circulation 1982; 66: 1146–9.

2 *Rahimtoola SH.* The hibernating myocardium. Am Heart J 1989; 117: 211–21.

3 *Geft IL, Fishbein MC, Ninomiya K, et al.* Intermittent brief periods of ischemia have a cumulative effect and may cause myocardial necrosis. Circulation 1982; 66: 1150–3.

4 *Murray CE, Jennings RB, Reimer KA.* Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. Circulation 1986; 74: 1124–36.

5 *Reimer KA, Murray CE, Yamasawa I, Hill ML, Jennings RB.* Four brief periods of myocardial ischemia cause no cumulative ATP loss or necrosis. Am J Physiol 1986; 251: H1306–15.

6 *Cohen MV, Liu GS, Downey JM.* Preconditioning causes improved wall motion as well as smaller infarcts after transient coronary occlusion in rabbits. Circulation 1991; 84: 341–9.

7 *Li Y, Whittaker P, Kloner RA.* The transient nature of the effect of ischemic preconditioning on myocardial infarct size and ventricular arrhythmia. Am Heart J 1992; 123: 346–53.

8 *Schott RJ, Rohmann S, Braun ER, Schaper W.* Ischemic preconditioning reduces infarct size in swine myocardium. Circ Res 1990; 66: 1133–42.

9 *Yellon DM, Alkhulaifi AM, Pugsley WB.* Preconditioning the human myocardium. Lancet 1993; 342: 276–7.

10 *Lawson CS, Downey JM.* Preconditioning: state of the art myocardial protection. Cardiovasc Res 1993; 27: 542–50.

11 *Liu Y, Downey JM.* Ischaemic preconditioning protects against infarction in rat heart. Am J Physiol 1992; 263: H1107–12.

12 *Tomai F, Crea F, Gaspardone A, et al.* Effect of A_1 adenosine receptor blockade by bamiphylline on ischaemic preconditioning during coronary angioplasty. Eur Heart J 1996; 17: 846–53.

13 *Van Winkle DR, Thornton JD, Downey DM, Downey JM.* The natural history of preconditioning: cardioprotection depends on duration of transient ischemia and time to subsequent ischemia. Coronary Artery Dis 1991; 2: 613–9.

14 *Murray CE, Richard VJ, Jennings RB, Reimer KA.* Myocardial protection is lost before contractile function recovers from ischemic preconditioning. Am J Physiol 1991; 260: H796–H804.

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

16 *Kuzuya T, Hoshida S, Yamashita N, et al.* Delayed effects of sublethal ischemia on the acquisition of tolerance to ischemia. Circ Res 1993; 72: 1293–9.

17 *Kimura Y, Iyengar J, Subramanian R, Cordis GA, Das DK.* Preconditioning of the heart by repeated stunning: attenuation of post-ischemic dysfunction. Basic Res Cardiol 1992; 87: 128–38.

18 *Miura T, Goto M, Urabe K, Endoh A, Shimamoto K, Iimura O.* Does myocardial stunning contribute to infarct size limitation by ischemic preconditioning? Circulation 1991; 84: 2504–12.

19 *Matsuda M, Catena TG, Vander Heide RS, Jennings RB, Reimer KA.* Cardiac protection by ischaemic preconditioning is not mediated by myocardial stunning. Cardiovasc Res 1993; 27: 585–92.

20 *Forman MB, Velasco CE, Jackson EK.* Adenosine attenuates reperfusion injury following regional myocardial ischaemia. Cardiovasc Res 1993; 27: 9–17.

21 *Downey JM, Guang SL, Thornton JD.* Adenosine and the anti-infarct effects of preconditioning. Cardiovasc Res 1993; 27: 3–8.

22 *Yao Z, Gross GJ.* A comparison of adenosine-induced cardioprotection and ischemic preconditioning in dogs. Efficacy, time course and role of K_{ATP} channels. Circulation 1994; 89: 1229–36.

23 *Van Winkle D, Chien GL, Wolfe RA, Soifer BE, Kuzume K, Davis RF.* Cardioprotection provided by adenosine receptor activation is abolished by blockade of the K_{ATP} channel. Am J Physiol 1994; 264: H829–39.

24 *Lee HT, LaFaro RJ, Reed GE.* Pretreatment of human myocardium with adenosine during open heart surgery. J Card Surg 1995; 10: 665–76.

25 *Leesar MA, Stoddard M, Ahmed M, Broadbent J, Bolli R.* Preconditioning of human myocardium with adenosine during coronary angioplasty. Circulation 1997; 95: 2500–7.

26 *Leung J, Stanley T Mathew J, et al.* Effects of acadesine on perioperative cardiac morbidity in a placebo controlled double blind study. J Am Coll Cardiol 1992; 19: 112A.

27 *Lawson CS.* Does ischaemic preconditioning occur in the human heart? Cardiovasc Res 1994; 28: 1461–6.

28 *Goto M, Liu Y, Yang X-M, Ardell JL, Cohen MV, Downey JM.* Role of bradykinin in protection of ischemic preconditioning in rabbit hearts. Circ Res 1995; 77: 611–21.

29 *Toombs CF, Wilise AL, Shebuski RJ.* Ischemic preconditioning fails to limit infarct size in reserpinized rabbit myocardium. Implications of norepinephrine release in the preconditioning effect. Circulation 1993; 88: 2351–8.

30 *Hu K, Nattel S.* Mechanisms of ischemic preconditioning in rat hearts. Involvement of a_{1B}- adrenoreceptors, pertussis toxin-sensitive G proteins, and protein kinase C. Circulation 1995; 92: 2259–65.

31 *DeJong JW, Cargnoni A, Bradamante S, et al.* Intermittent *v* continuous ischemia decelerates adenylate breakdown and prevents norepinephrine release in reperfused rabbit heart. J Mol Cell Cardiol 1995; 27: 659–71.

32 *Seyfarth M, Richard G, Mizsnyak A, Kurz T, Schömig A.* Transient ischemia reduces norepinephrine release during sustained ischemia. Neural preconditioning in isolated rat heart. Circ Res 1996; 78: 573–80.

33 *Vegh A, Szekeres L, Parratt J.* Preconditioning of the ischaemic myocardium; involvement of the L- arginine nitric oxide pathway. Br J Pharmacol 1992; 107: 648–59.

34 *Patel VC, Woolfson RG, Singh KJ, Neild GH, Yellon DM.* Ischaemic preconditioning is not prevented by inhibition of endothelium-derived nitric oxide (Abstract). J Mol Cell Cardiol 1992; 24: S152.

35 *Sugden PH, Bogoyevitch M4.* Intracellular signalling through protein kinases in the heart. Cardiovasc Res 1995; 30: 478–92.

36 *Gho BCG, Eskildsen-Helmond YEG, de Zeeuw S, Lamers JMJ, Verdouw PD.* Does protein kinase C play a pivotal role in the mechanisms of ischemic preconditioning? Cardiovasc Drug Ther 1996; 10: 775–86.

37 *Speechly-Dick ME, Mocanu MM, Yellon DM.* Protein kinase C. Its role in ischemic preconditioning in the rat. Circ Res 1994; 75: 586–90.

38 *Liu Y, Ytrehus K, Downey JM.* Evidence that translocation of protein kinase C is a key event during ischemic preconditioning of rabbit myocardium. J Mol Cell Cardiol 1994; 26: 661–8.

39 *Bogoyevitch MA, Parker PJ, Sugden PH.* Characterization of protein kinase C isotype expression in adult rat heart. Circ Res 1993; 72: 757–67.

40 *Mitchell MB, Meng X, Ao L, Brown JM, Harken AH, Banerjee A.* Preconditioning of isolated rat heart is mediated by protein kinase C. Circ Res 1995; 76: 73–81.

41 *Vahlhaus C, Schulz R, Post H, Onallah R, Heusch G.* No prevention of ischemic preconditioning by the protein kinase C inhibitor staurosporine in swine. Circ Res 1996; 79: 407–14.

42 *Simkhovich BZ, Przyklenk K, Hale SL, Patterson M, Kloner RA.* Direct evidence that ischemic preconditioning does not cause protein kinase C translocation in rabbit heart. Cardiovasc Res 1996; 32: 1064–70.

43 *Noma A.* ATP-regulated K^+ channels in cardiac muscle. Nature 1983; 305: 147–8.

44 *Nichols CG, Lederer WJ.* Adenosine triphosphate-sensitive potassium channels in the cardiovascular system. Am J Physiol 1991; 261: H1675–86.

45 *Cason BA, Gordon HJ, Avery EG, Hickey RF.* The role of ATP sensitive potassium channels in myocardial protection. J Card Surg 1995; 10: 441–4.

46 *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: 1030–5.

47 *Currie RW* Effects of ischemia and perfusion temperature on the synthesis of stress-induced (heat-shock) proteins in isolated and perfused rat hearts. J Mol Cell Cardiol 1987; 19: 795–808.

48 *Yellon DM, Pasini E, Cargnoni A, Marber MS, Latchman DS, Ferrari R.* The protective role of heat stress in the ischaemic and reperfused rabbit myocardium. J Mol Cell Cardiol 1992; 24: 895–907.

49 *Richard V, Kaeffer N, Thuillez C.* Delayed protection of the ischemic heart-from pathophysiology to therapeutic applications. Fundam Clin Pharmacol 1996; 10: 409–15.

50 *Marber MS, Mestril R, Chi S-H, Sayen R, Yellon DM, Dillmann WH,* 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: 1446–56.

51 *Hoshida S, Kuzuya T, Fuji H, et al.* Sublethal ischemia alters myocardial antioxidant activity in the canine heart. Am J Physiol 1993; 264: H33–9.

52 *Muller DWM,* Topol *EJ, Califf RM, et al.* Relationship between antecedent angina pectoris and short-term prognosis after thrombolytic therapy for acute myocardial infarction. Am Heart J 1990; 119: 224–31.

53 *Tamura K, Tsuji H, Nishiue T, Tokunaga S, Iwasaka T.* Association of preceding angina with in-hospital life-threatening ventricular tachyarrhythmias and late potentials in patients with a first acute myocardial infarction. Am Heart J 1997; 133: 297–301.

54 *Deutsch E, Berger M, Kussmaul WG, Hirshfield JW Jr, Herrmann HC, Laskey WK.* Adaptation to ischemia during percutaneous transluminal coronary angioplasty. Clinical, hemodynamic and metabolic features. Circulation 1990; 82: 2044–51.

55 *De Jong JW, Cargnoni A, Huizer T, Ferrari R, Bonnier JJRM.* Lactate and hypoxanthine release after coronary angioplasty: no evidence of preconditioning. J Mol Cell Cardiol 1993; 25: S37.

56 *Alkhulaifi AM.* Preconditioning the human heart. Ann R Coll Surg Eng 1997; 79: 49–54.

57 *Walker DM, Walker JM, Pugsley WB, Pattison CW, Yellon DM.* Preconditioning in isolated superfused human muscle. J Mol Cell Cardiol 1995; 27: 1349–57.

58 *Ikonomidis JS, Tumiati LC, Weisel RD, Mickel DAG, Li R-K.* Preconditioning human ventricular cardiomyocytes with brief periods of simulated ischaemia. Cardiovasc Res 1994; 28: 1285–91.

59 *Kolocassides KG, Galiñanes M, Hearse DJ.* Ischemic preconditioning, cardioplegia or both? Differing approaches to myocardial and vascular protection. J Moll Cell Cardiol 1996; 28: 623–34.

60 *Kaukoranta PK, Lepojärvi MPK, Ylitalo KV, Kiviluoma KT, Peuhkurinen KJ.* Normothermic retro-

grade blood cardioplegia with or without preceding ischemic preconditioning. Am Thorac Surg 1997; 63: 1268–74.

61 *Cleveland JC Jr, Meldrum DR, Rowland RT Banerjee A, Harken AH.* Preconditioning and hypothermic cardioplegia protect human heart equally against ischemia. Ann Thorac Surg 1997; 63: 147–52.

62 *Galiñanes M, Argano V, Hearse DJ.* Can ischemic preconditioning ensure optimal myocardial protection when delivery of cardioplegia is impaired? Circulation 1995; 92(Suppl II): II389–94.

63 *Galiñanes M, Wilson ANA, Hearse DJ.* Impaired cardioplegic delivery and the loss of cardioprotection: a role for preconditioning. J Mol Cell Cardiol 1997; 29: 849–54.

64 *Jacobsohn E, Young CJ, Aronson S, Ferdinand FD, Albertucci M* The role of ischemic preconditioning during minimally invasive coronary artery bypass surgery. J Cardiothorac Vasc Anesth 1997; 11: 787–92.

65 *Engelman DT, Chen C, Watanabe M, et al.* Improved 4- and 6-hour myocardial preservation by hypoxic preconditioning. Circulation 1995; 92(Suppl II): II417–22.

66 *Péréz-Pinzón MA, Xu G-P, Dietrich WD, Rosenthal M, Sick TJ* Rapid preconditioning protects rats against ischemic neuronal damage after 3 but not 7 days of reperfusion following global cerebral ischemia. J Cereb Blood Flow Metab 1997; 17: 175–82.

67 *Kitagawa K, Matsumoto M, Tagaya M, et al.* 'Ischemic tolerance' phenomenon found in the brain. Brain Res 1990; 528: 21–24.

68 *Chen J, Simon R.* Ischemic tolerance in the brain. Neurology 1997; 48: 306–11.

69 *Chen J, Graham SH, Zhu RL, Simon RP.* Stress proteins and tolerance to focal cerebral ischemia. J Cereb Blood Flow Metab 1996; 16: 566–77.

70 *Péréz-Pinzón MA, Mumford PL, Rosenthal M, Sick TJ.* Anoxic preconditioning in hippocampal slices: role of adenosine. Neuroscience 1996; 75: 687–94.

71 *Heurteaux C, Lauritzen I, Widmann C, Lazdunski M.* Essential role of adenosine, adenosine A_1 receptors and ATP-sensitive K^+ channels in cerebral ischemic preconditioning. Proc Nad Acad Sci 1995; 92: 4666–70.

72 *Chopp M, Chen H, Ho K-L, et al.* Transient hyperthermia protects against subsequent forebrain ischemic cell damage in the rat. Neurology 1989; 39: 1396–8.

73 *Clark NJ, Stanley TH.* Anesthesia for vascular surgery. *In:* Miller RD (Ed.). Anesthesia, 4th ed. New York: Churchill Livingstone Inc., 1994: 1851–96.

74 *Matsuyama K, Chiba Y, Ihaya A, Kimura T, Tanigawa N, Muraoka R.* Effect of spinal cord preconditioning on paraplegia during cross-clamping of the thoracic aorta. Ann Thorac Surg 1997; 63: 1315–20.

75 *Roth S.* Post-ischemic hyperemia in the cat retina: the effects of adenosine receptor blockade. Curr Eye Res 1995; 14: 323–8.

76 *Larsen AK, Osborne NN.* Involvement of adenosine in retinal ischemia. Studies on the rat. Invest Ophthalmol Vis Sci 1996; 37: 2603–11.

77 *Pang CY, Yang RZ, Zhong A, Xu N, Boyd B, Forrest CR.* Acute ischaemic preconditioning protects against skeletal muscle infarction in the pig. Cardiovasc Res 1995; 29: 782–8.

78 *Carroll CMA, Carroll SM, Overgoor MLE, Tobin G, Barker JH.* Acute ischemic preconditioning of skeletal muscle prior to flap elevation augments muscle-flap survival. Plast Reconstr Surg 1997; 100: 58–65.

79 *Gürke L, Marx A, Sutter P-M, et al.* Ischemic preconditioning improves post-ischemic skeletal muscle function. Am Surg 1996; 62: 391–4.

80 *Lee HT, Schroeder CA, Shah PM, Babu SC, Thompson CI, Belloni FL.* Preconditioning with ischemia or adenosine protects skeletal muscle from ischemic tissue reperfusion injury. J Surg Res 1996; 63: 29–34.

81 *Weselcouch EO, Sargent C, Wilde MW, Smith AM.* ATP-sensitive potassium channels and skeletal muscle function *in vitro.* J Pharmacol Exp Ther 1993; 267: 410–6.

82 *Huguet C, Nordlinger B, Bloch P, Cornad J.* Tolerance of the human liver to prolonged norinothermic ischemia. Arch Surg 1978; 113: 1448–51.

83 *Lloris-Carsi JM, Cejalvo D, Toledo-Pereyra LH, Calvo MA, Suzuki S.* Preconditioning: effect upon lesion modulation in warm liver ischemia. Transplant Proc 1993; 25: 3303–4.

84 *Hardy KJ, McClure DN, Subwongcharoen S.* Ischaemic preconditioning of the liver: a preliminary study. Aust N Z J Surg 1996; 66: 707–10.

85 *Du ZY, Hicks M, Winlaw D, Spratt P, Macdonald P.* Ischemic preconditioning enhances donor lung preservation in the rat. J Heart Lung Transplant 1996; 15: 1258–67.

86 *Islam CF, Mathie RT, Dinneen MD, Kiely EA, Peters AM, Grace PA.* Ischaemia-reperfusion injury in the rat kidney: the effect of preconditioning. Br J Urol 1997; 79: 842–7.

87 *Kersten JR, Schmeling TJ, Hettrick DA, Pagel PS, Gross GJ, Warltier DC.* Mechanism of myocardial protection by isoflurane. Role of adenosine triphosphate-regulated potassium (K_{ATP}) Anesthesiology 1996; 85: 794–807.

88 *Cason BA, Gamperl AK, Slocum RE, Hickey RF.* Anesthetic-induced preconditioning. Anesthesiology 1997; 87: 1182–90.

89 *Haessler R, Kuzume K, Chien GL, Wolff RA, Davis RF, Van Winkle DM.* Anaesthetics alter the magnitude of infarct limitation by ischaemic preconditioning. Cardiovasc Res 1994; 28: 1574–80.