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REVIEW ARTICLES

Anaesthetics and cardiac preconditioning. Part I. Signalling and cytoprotective mechanisms

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Cardiac preconditioning represents the most potent and consistently reproducible method of rescuing heart tissue from undergoing irreversible ischaemic damage. Major milestones regarding the elucidation of this phenomenon have been passed in the last two decades. The signalling and amplification cascades from the preconditioning stimulus, be it ischaemic or pharmacological, to the putative end-effectors, including the mechanisms involved in cellular protection, are discussed in this review. Volatile anaesthetics and opioids effectively elicit pharmacological preconditioning. Anaesthetic-induced preconditioning and ischaemic preconditioning share many fundamental steps, including activation of G-protein-coupled receptors, multiple protein kinases and ATP-sensitive potassium channels (K_{ATP} channels). Volatile anaesthetics prime the activation of the sarcolemmal and mitochondrial K_{ATP} channels, the putative end-effectors of preconditioning, by stimulation of adenosine receptors and subsequent activation of protein kinase C (PKC) and by increased formation of nitric oxide and free oxygen radicals. In the case of desflurane, stimulation of α- and β-adrenergic receptors may also be of importance. Similarly, opioids activate δ- and κ-opioid receptors, and this also leads to PKC activation. Activated PKC acts as an amplifier of the preconditioning stimulus and stabilizes, by phosphorylation, the open state of the mitochondrial K_{ATP} channel (the main end-effector in anaesthetic preconditioning) and the sarcolemmal K_{ATP} channel. The opening of K_{ATP} channels ultimately elicits cytoprotection by decreasing cytosolic and mitochondrial Ca^{2+} overload.

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The heart possesses a remarkable ability to adapt to stress by changing its phenotype in a manner that makes it more resistant to further damage. Verdouw and colleagues[111] and Reimer and colleagues[89] reported favourable ATP handling in response to brief episodes of ischaemia. In 1986, Murry and colleagues[69] described, for the first time, the phenomenon of ischaemic preconditioning in canine myocardium. Subjecting hearts to four brief ischaemic episodes (ligation of the circumflex coronary artery) interspersed with 5-min periods of reperfusion before a prolonged 40-min ischaemic insult reduced myocardial infarct size from 30% to only 7% of the area at risk. Since then, this potent endogenous protective mechanism has been confirmed in almost all species, including the mouse, rat, guinea-pig, rabbit, dog, pig and, at least indirectly, the human (reviewed by Przyklenk and Kloner).[87] Moreover, ischaemic preconditioning was consistently observed in single cells,[60] in superfused myocardial samples,[112] in the whole heart and in all types of *in vivo* experiments. A few years later, in 1993, Marber and colleagues[61] and Kuzuya and colleagues[49] described another remarkable phenomenon, called late preconditioning, which reflects a second delayed window of protection 12–72 h after initiation of preconditioning.

Under most experimental conditions, preconditioning not only reduces infarct size but also alleviates post-ischaemic cardiac dysfunction and arrhythmias. The possibility of an effective clinical use for this innate cardioprotective mechanism has generated enormous interest (more than 3000 articles published). It has elucidated the underlying signalling pathways, with the final aim of mimicking the preconditioned state and its benefits by means of pharmacological agents. Pharmacological preconditioning, in place

of ischaemia, may represent a safer way of eliciting protection, particularly in the diseased myocardium. Two anaesthesia research groups (Cason and colleagues[9] and Kersten and colleagues[42]) independently described the preconditioning-mimicking effects of isoflurane for the first time in 1997. However, infarct-limiting properties of halothane and isoflurane were reported as early as 1983 and 1989 respectively by Davis and colleagues.[18 19] Subsequent extensive experimental work aimed at elucidating the complex signalling cascade involved in anaesthetic-induced preconditioning. Today, we know that most anaesthetics elicit, enhance or inhibit preconditioning.[119] Unlike most other preconditioning-inducing agents, which must be administered directly into the coronary arteries to be effective, anaesthetics can be administered non-selectively with relatively low toxicity. Anaesthetic-mediated or -facilitated cardiac preconditioning around the stressful time of surgery would be particularly beneficial in high-risk cardiac patients.

Mechanisms of cardiac preconditioning

Definition and time course of preconditioned states

The classic or early preconditioned state of cardiac tissue is an immediate consequence of multiple brief episodes of sublethal cardiac ischaemia, and is defined as a state of marked protection against subsequent prolonged ischaemia. While multiple, brief antecedent ischaemic episodes may have additive effects,[91] too many repetitive stimuli abolish the protection.[37] Of note, preconditioning *per se* does not prevent myocardial cell death, but significantly delays its occurrence during the first 2–3 h of sustained ischaemia (temporal limitation of protection). Although the gold standard end-point for assessing the preconditioned state and its protection is the reduction in infarct size, preconditioning also improves post-ischaemic functional recovery and decreases arrhythmogenicity in many experimental approaches. Improved preservation of ATP reserves and attenuated ST-segment alterations in the ECG, though considered to be surrogate markers of effective cardiac protection, are highly species-dependent and therefore may not serve as valid markers for preconditioning.[21 46] The protection elicited by preconditioning is typically present immediately after the stimulus, but vanishes after 2–3 h (classic or early preconditioning).[87] Additional, though less pronounced, protection occurs 12–24 h after the initial preconditioning stimulus and lasts for up to 72 h (second window of protection or late/delayed preconditioning).[6] Consistent with this delayed type of protection, late preconditioning is dependent on *de novo* synthesis of cardioprotective proteins. In contrast to most classic or early preconditioning models, late preconditioning consistently protects against stunning.[100] Lastly, and most importantly, a multitude of additional stressful stimuli (apart from ischaemia), including oxidative (hyperoxia), mechanical (stretch),[75] electrical (rapid pacing), thermal and chemical (hormonal, ionic (calcium)[66] and pharmacological) stressors, can induce the same archetypal early and late protective response in cardiac tissue.

Signalling cascades and amplification of the preconditioning stimulus

Receptors

The concept that brief renal, mesenteric or skeletal muscle ischaemia of remote origin can effectively precondition the heart is consistent with humoral induction of the preconditioned state ('remote preconditioning') (reference 116 and references therein). Also consistent with this notion is the fact that regional cardiac ischaemia can initiate global protection and render remote myocardium resistant to infarction ('preconditioning at a distance').[86] Ischaemic preconditioning is mediated via several sarcolemmal receptors, which are mostly linked to inhibitory G-protein (Gi),[72] namely adenosine (A-1, A-3), purinoceptors (P2Y), endothelin (ET1), acetylcholine (M2), α_1- and β-adrenergic, angiotensin II (AT1), bradykinin (B2) and opioid (δ_1, κ) receptors, which couple to a highly complex network of kinases (for review see reference 3). The involvement of many receptors or triggers in mediating preconditioning reflects the biological redundancy in this life-saving signal transduction pathway. The importance of the individual receptors depends heavily on the species and the preconditioning stimulus itself.[103] The main signalling steps and components of early and delayed preconditioning are summarized schematically in Figure 1.

Amplification of the preconditioning signal

G-proteins link the initial stimulus from the individual receptors to phospholipases C and D but have several additional functions, such as inhibition of Ca^{2+} influx during ischaemia, regulation of cellular metabolism and activation of K_{ATP} channels, the putative main end-effectors of preconditioning. Activation of phospholipases C and D leads to formation of inositol triphosphate (IP_3) for the release of Ca^{2+} from the sarcoplasmic reticulum via the IP_3 receptor, and production of diacylglycerol, which, in turn, activates different isoforms of protein kinase C (PKC). PKC is activated by a large number of phosphorylating enzymes, including G-proteins, phospholipids, diacylglycerol, increased intracellular Ca^{2+}, and nitric oxide (NO), which is derived from intracellular constitutively active NO synthase (NOS) or from extracellular sources. PKC can be activated by reactive oxygen species (ROS) arising from mitochondria either during the short ischaemic or the subsequent repetitive reperfusion episodes. Activation of this key enzyme leads to isoform-specific and cytoskeleton-mediated translocation of cytosolic PKC, ultimately inducing phosphorylation and thus activation of the sarcolemmal and mitochondrial K_{ATP} channels, the putative end-effectors of early but also delayed preconditioning[56] (Fig. 1). Isoform-

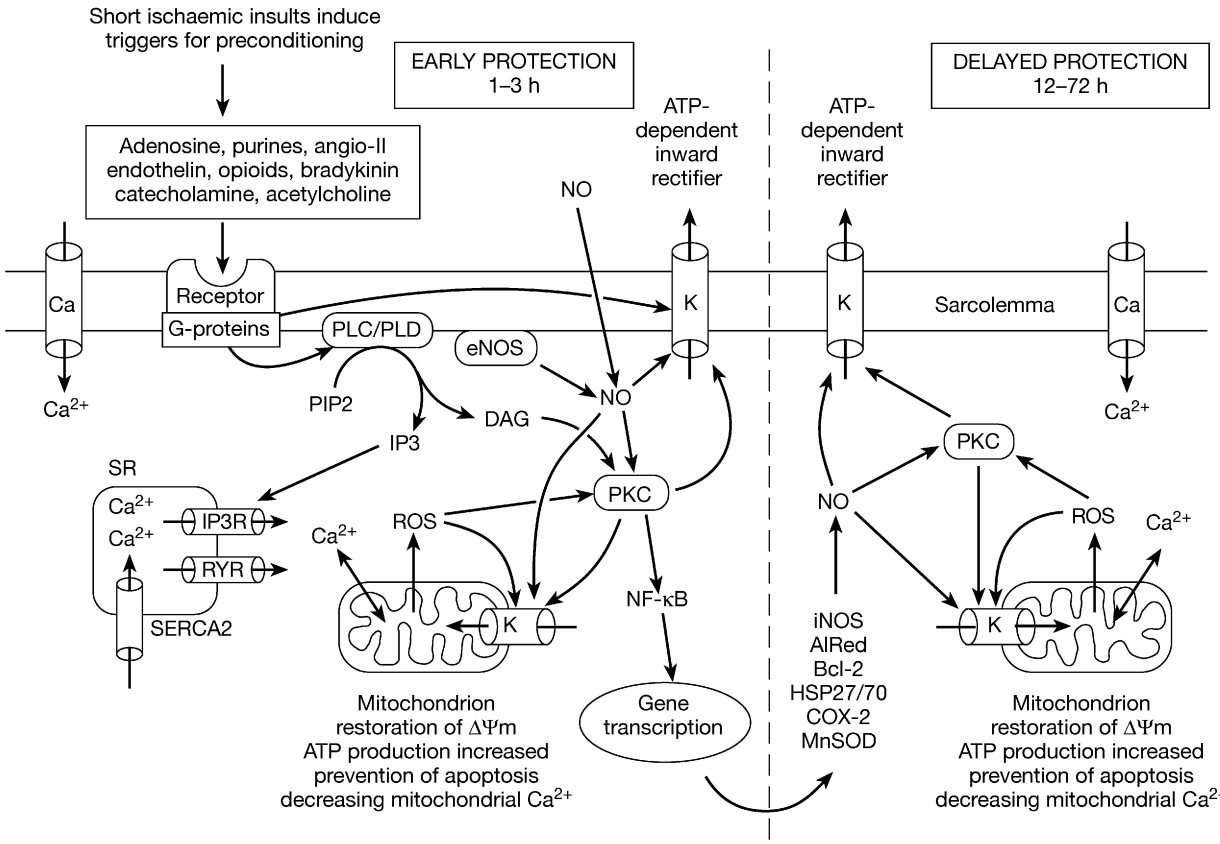

Fig 1 Signalling for cardiac preconditioning. The signalling components depicted illustrate current knowledge regarding the mechanisms of early (left of dashed line) and delayed (right of dashed line) cardiac preconditioning. ΔΨm=inner mitochondrial membrane potential; AlRed=aldose reductase; Bcl-2=anti-apoptotic protein; Ca=sarcolemmal voltage-dependent Ca^{2+} channels; DAG=diacylglycerol; COX-2=cyclooxygenase type 2; eNOS=endothelial NO synthase; G-proteins=heterotrimeric G-proteins; HSP27 and HSP70=heat shock proteins; iNOS=inducible NO synthase; IP3=inositol triphosphate; IP3R=inositol triphosphate receptor; K=sarcolemmal and mitochondrial K_{ATP} channels; MnSOD=manganese superoxide dismutase; NF-κB=nuclear factor-κB; NO=nitric oxide; PIP2=phosphatidylinositol bisphosphate; PKC=protein kinase C; PLC/PLD=phospholipases C and D; ROS=reactive oxygen species; RYR=ryanodine Ca^{2+}-release channel; SERCA2=Ca^{2+} pump of the SR; SR=sarcoplasmic reticulum.

specific translocation of PKC allows the enzyme to take up the correct position to phosphorylate the targets. After only 10 min of ischaemic preconditioning, PKC activity in the cytosol decreases, whereas PKC in the particulate fraction (i.e. nuclei, mitochondria and membranes) increases.[98] PKC-δ translocation seems to be responsible for activating mitochondrial K_{ATP} channels and PKC-ε translocation for the establishment of late preconditioning by phosphorylating nuclear targets.[39] However, activation of specific PKC isoforms is species-dependent and further depends on the pathological stimuli.[103] The observation that PKC inhibition may not necessarily completely block the preconditioning stimulus[110] supports the concept that additional intracellular kinases downstream, upstream or in parallel to PKC signalling contribute to the amplification and establishment of the preconditioned state (the 'molecular chain reaction of kinases'). As PKC inhibitors failed to affect preconditioning in dogs and pigs,[88 110] alternative kinases may be involved in preconditioning. Inhibition of protein tyrosine kinases clearly abrogates the reduction in infarct size in rabbits, and may thus be as important as or more important than PKC under certain circumstances.[83] Additionally, some studies propose activation of ERK (extracellular signal-regulated kinase), JNK (c-jun N-terminal kinase) and p38MAPK (mitogen-activated protein kinase) as mediators of preconditioning, while others suggest the inhibition of these kinases as favourable (for review see reference 3). These contradictory results may be due to the existence of various isoenzymes of specific kinases that possess differential biological functions and to the restricted selectivity of the various pharmacological blockers used in the reported experiments. Finally, the involvement of multiple phosphatases, which are the physiological counterplayers of kinases and are responsible for their deactivation, may play an additional role in cardiac protection by facilitating higher activity levels and/or prolonged phosphorylation states of individual kinases.

Specific aspects

Adenosine is considered to be one of the most relevant triggers of early and late preconditioning.[87] Adenosine is released from cells in the ischaemic zone by upregulation of

ectosolic or membrane-associated 5′-nucleotidase (responsible for adenosine phosphate ester dephosphorylation)[44] and activates the respective receptors on cardiomyocytes in an autacoid manner. A_3 receptor-initiated protection seems to last longer than A_1 receptor-initiated protection, which may be explained by the differential coupling of the A_1 receptor to phospholipase C and the A_3 receptor to phospholipase D.[12] Consistent with the pivotal role of adenosine, Haedrick and colleagues[28] reported marked cardioprotection against ischaemia in murine hearts overexpressing A_1 receptors. Adenosine may be less important as an effective preconditioning trigger in the rat heart model,[54] where α-adrenergic receptors (mainly α1B) play a major role.[36] In contrast, in rabbit and canine hearts α-adrenergic receptor agonists abolish preconditioning. These observations support the concept that multiple receptor systems of varying importance can contribute to cardioprotection in different animal models (Fig. 1).

ROS are important intracellular signalling molecules and are increased during sublethal oxidative stress (preconditioning stimulus). They play a pivotal role in triggering early and delayed cardioprotection and are probably derived from mitochondria.[13] ROS activate phospholipase C and PKC, which, in turn, amplify the preconditioning stimulus. Murry and colleagues[70] first demonstrated that administration of radical scavengers blocks the beneficial effects of early ischaemic preconditioning. Evidence for an essential role of ROS in the establishment of late preconditioning was reported by Sun and colleagues.[100] Thus, generation of ROS during the initiation of preconditioning represents an essential trigger for early and delayed cardioprotection.

NO is able to induce a cardioprotective effect against myocardial stunning and infarction. Recent studies provided direct evidence of enhanced biosynthesis of NO in the myocardium subjected to brief episodes of ischaemia and reperfusion, probably via increased (endogenous constitutive) NOS activity.[7] Although most studies indicate that endogenous NO is not necessary for ischaemia-induced early preconditioning, exogenous or pharmacologically increased endogenous NO production elicits an early preconditioning effect (i.e. NO is sufficient but not necessary for early preconditioning).[7] Conversely, NO has an obligatory role in late preconditioning.[27]

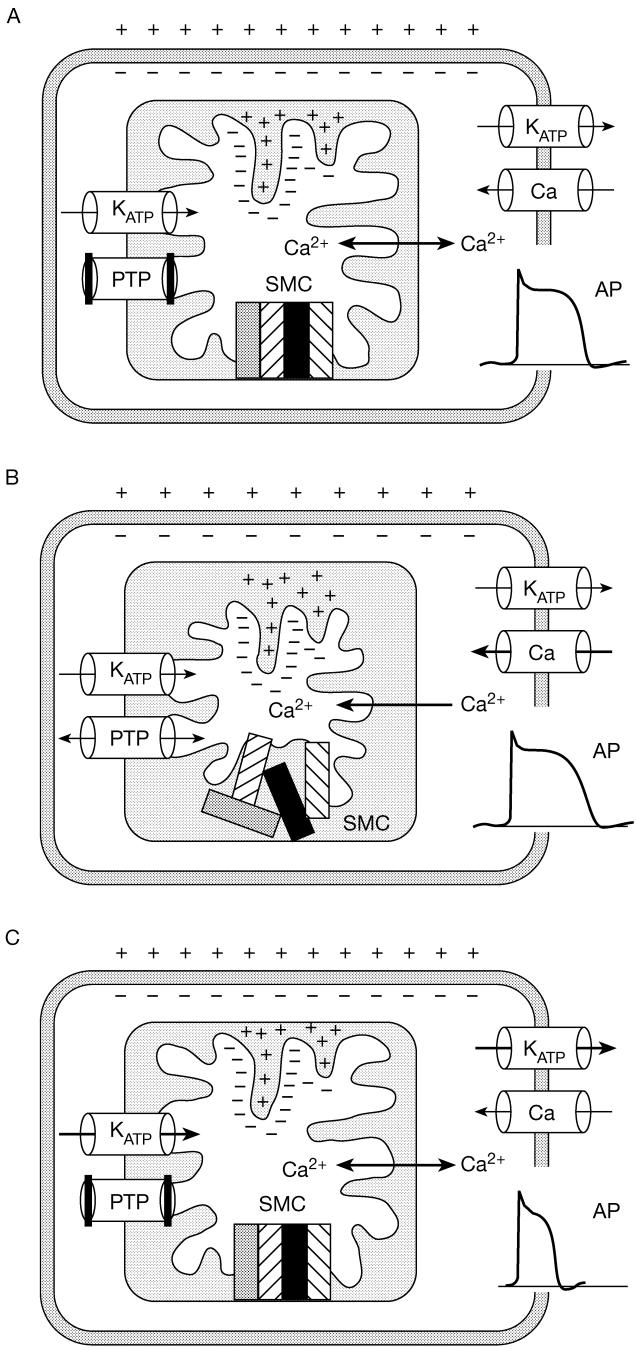

Fig 2 Cytoprotective mechanisms of cardiac preconditioning. The sarcolemmal and mitochondrial K_{ATP} channels, the sarcolemmal voltage-dependent Ca^{2+} channel and the mitochondrial permeability transition pore (PTP) are shown. The differently marked bars in the mitochondrial intermembrane space represent the supramolecular complex (SMC) containing the proton pump, ATP synthase, the adenine nucleotide transporter and mitochondrial creatine kinase. Boldness and direction of arrows represent relative intensity and direction of ion flux. (A) Under normal conditions, the cytosolic Ca^{2+} concentration is governed mainly by duration of the action potential (AP) and the resting membrane potential. The Ca^{2+} uniporter is dependent on the mitochondrial inner membrane potential. (B) During ischaemia–reperfusion injury, the increased cytosolic Ca^{2+} concentration induces high metabolic activity with accumulation of inorganic phosphate and reactive oxygen species. This is accompanied by swelling of the intermembrane space, with subsequent shrinkage of the mitochondrial matrix and disruption of the SMC. This impairs energy production and nucleotide transport, resulting in cellular ATP depletion. The PTP opens and induces dissipation of the inner membrane potential, leading to cell death. (C) Ischaemic and pharmacological preconditioning are thought to exert cardioprotection, first by activation of the sarcolemmal K_{ATP} channel, which reduces the intracellular Ca^{2+} by stabilization of the resting membrane potential below –80 mV and shortening of the AP. Secondly, activation of the mitochondrial K_{ATP} channel leads to an increase in the mitochondrial matrix volume and concomitantly reduces the intermembrane space, which leads to reassembly of the SMC, closure or maintenance of the closed state of the PTP, and restoration of mitochondrial energy production.[99 109]

Late or delayed preconditioning (delayed acquisition of tolerance to ischaemia)

In contrast to early preconditioning, late preconditioning requires NO formation and increased synthesis of protective proteins (for detailed review see reference 7). Again, PKC and multiple kinases are involved in the signalling cascade, leading to activation of several transcription factors, such as nuclear factor-κB (NF-κB), which leads to the sustained expression of a number of proteins thought to be responsible for the delayed protection phase (right part of Fig. 1). Disruption of the inducible NOS (iNOS) gene completely abrogates the delayed infarct-sparing effect, which points to the obligatory role of inducible NOS in the cardioprotection afforded by delayed preconditioning.[27] Because of the dominant role of NO, late preconditioning is viewed as a state of enhanced NO synthesis, NO acting as a trigger (produced by constitutive NOS) initially and subsequently as an effector (produced by inducible NOS). The most likely cardioprotective effects of NO in late preconditioning are: (i) inhibition of Ca^{2+} influx; (ii) antagonism of β-adrenergic stimulation; (iii) reduced contractility and myocardial oxygen consumption; (iv) opening of K_{ATP} channels; (v) antioxidant actions; and (vi) activation of COX-2 with the synthesis of prostanoids. Other enzymes associated with delayed protection are aldose reductase, which catalyses sorbitol to glucose and detoxifies ROS-derived lipid aldehydes, manganese superoxide dismutase (MnSOD), and the anti-apoptotic protein Bcl-2. Biosynthesis of heat shock proteins (Hsp27, Hsp70) is not increased, but this family of protective proteins is subject to post-translational modification.[17] Recent findings also indicate that activation of mitochondrial K_{ATP} channels plays a role in delayed protection, as 5-hydroxydecanoate (a specific mitochondrial K_{ATP} channel blocker), administered immediately before sustained ischaemia, can inhibit protection elicited 24 h after the initial preconditioning stimulus.[5]

Pharmacology of sarcolemmal and mitochondrial K_{ATP} channels, the putative end-effectors of preconditioning

Cardiomyocytes have two distinct types of K_{ATP} channels, one located in the surface membrane and another in the inner mitochondrial membrane (Figs 1 and 2) (for review see reference 26). Preconditioning can be pharmacologically mimicked by K_{ATP} channel openers and abolished by K_{ATP} channel inhibitors (Table 1). Sarcolemmal K_{ATP} channels are colocalized (i.e. physically bound)[15] with the creatine phosphate–creatine kinase system and provide a direct link between the metabolic state and cellular excitability. Mitochondrial K_{ATP} channels regulate mitochondrial volume state, mitochondrial membrane potential, formation of ROS and energy production. The molecular structure of mitochondrial K_{ATP} channels is largely unknown. In contrast, sarcolemmal K_{ATP} channels are composed of hetero-octamers of four pore-forming subunits (four Kir6.1 or Kir6.2 subunits form an inward-rectifying K^+ channel with a conductance of 80 pS) and four sulfonylurea receptors (SUR1, SUR2A or SUR2B). The most common expression pattern of sarcolemmal K_{ATP} channels is Kir6.2/SUR1 in the pancreas, Kir6.1/SUR2B and Kir6.2/SUR2B in vascular smooth muscle, and Kir6.2/SUR2A in the heart. Kir6.2/SUR2A is responsible for the early depolarization in ischaemic heart tissue, which represents the basis for the clinically observed 'injury current' of ischaemia (molecular basis of ischaemic ECG ST-segment changes).[53]

Table 1 Pharmacology of mitochondrial and sarcolemmal K_{ATP} channels. ATP=adenosine triphosphate; CoA=coenzyme A; GDP=guanosine diphosphate; GTP=guanosine triphosphate; UDP=uridine diphosphate

Selectivity	Agonists	Antagonists
Sarcolemmal	Long-chain CoA esters	HMR-1098
	P-1075	
	ADP	
Mitochondrial	GTP	ADP
	GDP	Long-chain-CoA esters
	UDP	5-Hydroxydecanoate
	Superoxide anions	
	Diazoxide	
	Nicorandil	
	BMS-191095	
Non-selective	Cromakalim	ATP
	Bimakalim	Glibenclamide
	Aprikalim	Glyburide
	Diethylaminoethylbenzoate	
	Pinacidil	

Recent studies demonstrate that mitochondrial K_{ATP} channels closely resemble Kir6.1/SUR1 sarcolemmal K_{ATP} channels, at least in their pharmacological profile. Diazoxide, a highly specific mitochondrial channel activator (2000-fold more specific for the mitochondrial channel), opens mitochondrial channel activity, 5-hydroxydecanoate inhibits it and HMR-1098 does not affect it.[57] This does not mean that mitochondrial channels are identical with Kir6.1/SUR1. Kir6.1 is not colocalized with mitochondria, and dominant-negative Kir6.1 or Kir6.2 constructs did not affect mitochondrial flavoprotein oxidation,[94] a direct and highly specific indicator of mitochondrial K_{ATP} channel activity. Patch-clamp studies in isolated mitoplasts (planar lipid bilayers of mitochondrial membranes) also demonstrated a much smaller conductance for mitochondrial K_{ATP} channels (~10–56 pS).[38] In the heart, the sarcolemmal K_{ATP} channels (and most probably also the mitochondrial K_{ATP} channels) are not active under physiological conditions, where ATP at a concentration of ~1 mM allosterically inhibits the Kir6.*x* subunits, thus binding to and stabilizing the closed state of the channel (Table 1). A low cellular ATP content under ischaemic conditions effectively activates the K_{ATP} channels. Conversely, SUR*x* subunits confer high-affinity blockade by sulfonylurea agents and stimulation by K_{ATP} channel openers and MgADP. Mitochondrial K_{ATP} channels are regulated differently and are inhibited by ATP and ADP, but activated by GTP and GDP.[25] Also, while palmitoyl- and

Table 2 Volatile anaesthetics, opioids, ethanol-based anaesthetics, and urethane with mostly enhancing effects on mitochondrial and sarcolemmal K_{ATP} channels. ←→=no effect; ↑=increased effect; ↓=decreased effect; *increased open probability of sarcolemmal K_{ATP} channels for a given ATP concentration in response to isoflurane, but isoflurane itself inhibits the sarcolemmal K_{ATP} channel or has no effect on the channel; **induces stunning, which could result from inhibitory effects on sarcolemmal and/or mitochondrial K_{ATP} channels[96]

Anaesthetic drug	Mitochondrial K_{ATP} channel activity	References	Sarcolemmal K_{ATP} channel activity	References
Isoflurane	↑	45, 120	↑ / ↓ / ←→*	24, 30, 50, 90
Sevoflurane	↑	45, 120	?	
Desflurane	↑	31, 104	↑	104
Halothane	?		↓	50, 90
Enflurane	?		?	
Nitrous oxide**	?		?	
Morphine	↑	55	?	
Fentanyl	↑	119	?	
Sufentanil	?		?	
Remifentanil	?		?	
Trichloroethanol (chloral hydrate, α-chloralose)	↑	119	?	
Ethanol	↑	77	↑	77
Urethane	↑	119	?	

oleyl-CoA facilitate opening of the sarcolemmal K_{ATP} channel, they have inhibitory effects on the mitochondrial K_{ATP} channel[82] (Table 1). Recent research has uncovered the dominant role of the mitochondrial K_{ATP} channels in mediating the preconditioned state (for review see reference 74). The sarcolemmal K_{ATP} channel, which was thought initially to be the main end-effector of preconditioning, did not explain why reduction in infarct size was observed even in the case of unchanged action potential duration. Also, defetilide, a K^+ channel blocker (class III antiarrhythmic) with no effects on K_{ATP} channels, abolished shortening of the action potential but failed to remove the protection provided by ischaemic preconditioning. However, recent research in transgenic mice lacking the sarcolemmal K_{ATP} channels ($Kir6.2^{-/-}$) indicates that sarcolemmal K_{ATP} channels may be important, at least in the murine heart, which has a high baseline heart rate (5–10 Hz).[101] Interestingly, Toyoda and colleagues[108] suggested a differential role of sarcolemmal and mitochondrial K_{ATP} channels in preconditioning, whereby infarct size reduction is mediated largely by mitochondrial K_{ATP} channels, but functional recovery (in which heart rate plays a pivotal role) is mediated by sarcolemmal K_{ATP} channels. Mitochondrial K_{ATP} channels also play an important role in the prevention of cardiomyocyte apoptosis[1] and in delayed preconditioning protection.[7] Considerable cross-talk was recently reported between sarcolemmal and mitochondrial K_{ATP} channels.[92] Accordingly, increased ATP consumption through uncoupled mitochondria leads to activation of sarcolemmal K_{ATP} channels. Thus, although a preponderance of experimental work favours the mitochondrial K_{ATP} channels as the main end-effector of preconditioning, the role of sarcolemmal K_{ATP} channels cannot be dismissed totally.

How might the K_{ATP} channels modulate infarct size?

The cellular consequences of the opening of the K_{ATP} channels are depicted in Figure 2A–C.

Sarcolemmal K_{ATP} channels

Although the intracellular Ca^{2+} transients for contraction and relaxation are governed mainly by the Ca^{2+} pump (SERCA2) and the ryanodine Ca^{2+}-release channel (RYR) in the sarcoplasmic reticulum, a significant amount of the cytosolic Ca^{2+} is recruited from the extracellular space, mainly via the Na^+/Ca^{2+} exchanger and the voltage-gated L-type Ca^{2+} channels (Fig. 1). Membrane hyperpolarization, by the opening of sarcolemmal K_{ATP} channels, as in cardioplegia, may shorten action potential duration (Fig. 2C). As a consequence, less Ca^{2+} enters the myocytes from outside and attenuates Ca^{2+} overload.

Mitochondrial K_{ATP} channels

Three main concepts, which do not exclude, but rather complement, each other, are currently under investigation (Fig. 2). According to Marbán and his group, opening of the mitochondrial K_{ATP} channel leads to depolarization of the inner mitochondrial membrane.[68] Uncoupling of mitochondria and increased formation of ROS have been reported previously.[22] Although this change is modest, it has a significant effect on mitochondrial Ca^{2+} load because of the non-linear dependence of Ca^{2+} flux on the membrane potential. It is hypothesized that depolarization of the inner mitochondrial membrane (by 12% at a diazoxide concentration of 10 μmol litre^{-1}, corresponding to a change from –200 to –176 mV) attenuates mitochondrial Ca^{2+} accumulation by lowering the driving force for Ca^{2+} uptake. The decreased mitochondrial Ca^{2+} overload during ischaemia[114] may prevent opening of the mitochondrial permeability transition pores and guarantee optimal conditions for ATP production.[35] Conversely, mitochondrial matrix contraction (~30%) and intermembrane space expansion is a direct consequence of anoxic blockade of electron transport during ischaemia (Fig. 2B). While mitochondrial K^+ influx ceases (a compensatory consequence of the H^+ pumping from the matrix into the intermembrane space), mitochondrial K^+ efflux (K^+/H^+ antiporter) continues until a new equilibrium

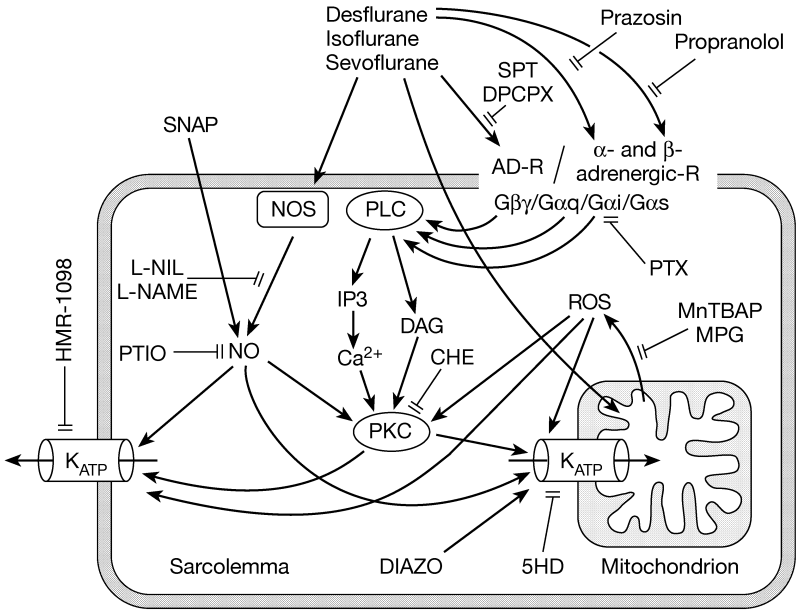

Fig 3 Signalling pathways involved in volatile anaesthetic-induced preconditioning. The signalling cascades that are listed derive from the results of recently published studies using the indicated blockers and activators.[24 31 45 50 67 90 104 119 120] Multiple signalling cascades prime the sarcolemmal and mitochondrial K_{ATP} channels, allowing prompt opening at the initiation of ischaemia. Arrows indicate positive activity and lines with blunted ends (=) indicate inhibition. Ad=adenosine; CHE=chelerythrine (protein kinase C inhibitor); DAG=diacylglycerol; DIAZO=diazoxide (specific opener of the mitochondrial K_{ATP} channel); DPCPX=8-cyclopentyl-1,3-dipropylxanthine (specific adenosine 1 receptor blocker); Gαs/Gαi/Gαq/Gβγ=different G-protein species; 5HD=5-hydroxydecanoate (selective mitochondrial K_{ATP} channel blocker); HMR-1098=specific blocker of the sarcolemmal K_{ATP} channel; L-NIL=L-N6-(1-iminoethyl)lysine; IP3=inositol triphosphate; L-NAME=N^G-nitro-L-arginine methyl ester (L-NIL and L-NAME are nitric oxide synthase inhibitors); MnTBAP=Mn(III)tetrakis(4-benzoic acid)porphyrine chloride; MPG=*N*-(2-mercaptopropionyl)glycine (MnTBAP and MPG are free radical scavengers); NO=nitric oxide; NOS=nitric oxide synthase; PKC=protein kinase C; PLC=phospholipase C; PTIO=2-(4-carboxyphenyl)-4,4′,5,5′-tetramethylimidazole-1-oxyl-3-oxide (nitric oxide scavenger); PTX=pertussis toxin (Gαi-protein inhibitor); R=receptor; ROS=reactive oxygen species; SNAP=*S*-nitroso-*N*-acetyl-DL-penicillamine (nitric oxide donor); SPT=8-sulfophenyl theophylline (non-specific adenosine receptor blocker).

at a lower matrix volume is reached. This leads to dissociation of the mitochondrial creatine kinase octameric complex from the outer and inner membranes.[51] Garlid and colleagues now propose that opening of the mitochondrial K_{ATP} channel decreases the ischaemia-induced swelling of the mitochondrial interspace, which would preserve functional coupling between adenosine nucleotide translocase and mitochondrial creatine kinase (preservation of structure/function).[47 51] This, in turn, secures the transport of newly synthesized ATP from the site of production by ATP synthase on the inner mitochondrial membrane to the cytosol. Thus, both mechanisms contribute to an uninterrupted supply of high-energy phosphate substrates from the mitochondria to the sites of energy consumption. A third possibility for the means by which mitochondrial K_{ATP} channels may elicit protection is based on the observation of the increased formation of ROS (generation of a pro-oxidant environment probably derived from the cytochrome b-c1 segment of complex III of the respiratory chain).[22] Pain and colleagues[78] demonstrated that diazoxide is protective even if it is only present before (rather than during) the sustained ischaemia, which implies that mitochondrial K_{ATP} channel opening would serve as a trigger of preconditioning, but would not mediate cardioprotection *per se* (effector). According to this concept, ROS (or the intracellular redox state) would stimulate the activation of multiple transcriptional factors (NF-κB, activator protein-1, hypoxia-inducible factor, protein kinases, protein phosphatases and various ion channels ($Ca^{2+}/K^+/Na^+$ channels, Na^+/Ca^{2+} or Na^+/H^+ exchanger)), ultimately leading to cardioprotection.

Other mechanisms involved in cardiac preconditioning

In whole-organ preparations and *in vivo* experiments, preconditioning protection is also attributable to the protective effects on the endothelium of the coronary vasculature,[52] the inhibition of platelet aggregation[34] and leucocyte adhesion.[43] Thus, the benefits of preconditioning clearly extend beyond the cardiomyocyte.

Anaesthetic-induced preconditioning

Preconditioning can be pharmacologically elicited by anaesthetics. Volatile anaesthetics, opioids and a small group of anaesthetics primarily used in animal experiments were found to induce or enhance preconditioning in cardiac tissue. Table 2 summarizes the effects of these anaesthetics on mitochondrial and sarcolemmal K_{ATP} channels. Current knowledge about anaesthetic-induced preconditioning from *in vitro* systems and animal experiments will now be discussed. Evidence for anaesthetic-induced preconditioning in humans will be presented in Part II of this review.[118]

Volatile anaesthetics

Most studies have evaluated the cardiac preconditioning effects of isoflurane, enflurane and halothane,[64 115] and more recently those of sevoflurane[2 106] and desflurane.[104] The favourable oxygen supply/demand ratio elicited by volatile anaesthetics is not required for preconditioning because volatile anaesthetic-induced protection also occurs under cardioplegic arrest.[58] Many characteristics of preconditioning by volatile anaesthetics are similar to those of ischaemic preconditioning. These involve activation of A_1 adenosine receptors, PKC and K_{ATP} channels. Of note, ischaemic preconditioning and anaesthetic preconditioning (2×2 min with sevoflurane at 3.5 vol/vol % interspersed with 5 min of reperfusion) similarly reduce Ca^{2+} loading, augment post-ischaemic contractile responsiveness to Ca^{2+} and decrease infarct size.[2] Whether volatile anaesthetics also elicit late preconditioning is not yet known, but preliminary data indicate that volatile anaesthetics may exert additive effects in combination with ischaemic preconditioning.[106] Isoflurane and sevoflurane induce an acute 'memory phase', which is more pronounced with isoflurane (>30 min) than sevoflurane (<30 min).[106] For both anaesthetics, dose-dependent protection was demonstrated in a cellular model of isolated adult rat ventricular myocytes.[120] Dose-dependent protection by isoflurane was also reported in an *in vivo* dog model of regional ischaemia, in which as little as 0.25 MAC isoflurane (0.3 vol/vol %, which is close to 1 MAC_{awake} in humans) significantly reduced infarct size.[40] Interestingly, isoflurane protection depended on collateral blood flow at low MAC values but was independent of collateral flow at higher MAC values. The maximum preconditioning protection was reached at an isoflurane concentration of approximately 1.5–2 vol/vol %.[40 120]

Key signalling components involved in preconditioning elicited by volatile anaesthetics were unravelled recently by means of specific blockers for signalling steps (Fig. 3). Using an *in vivo* rabbit model with regional ischaemia combined with a Langendorff model, Cope and colleagues[14] showed that 8-sulfophenyl theophylline, a non-specific adenosine receptor blocker, inhibited the preconditioning effect of halothane. The effect was also inhibited by chelerythrine, a highly specific PKC blocker. Similarly, high doses of bisindolylmaleimide, a PKC inhibitor, blocked isoflurane-enhanced recovery of canine stunned myocardium.[107] Isoflurane and halothane are known to affect PKC activity. PKC-induced coronary vasoconstriction is inhibited by halothane, but enhanced by isoflurane.[79] Another study indicated that neither isoflurane nor halothane inhibited PKC-induced alterations in coronary vascular tone.[76] As in Cope's study,[14] Cason and colleagues[9] demonstrated in an *in vivo* rabbit model with regional ischaemia that 8-sulfophenyl theophylline could inhibit isoflurane-mediated preconditioning. In a dog model of regional ischaemia, Kersten and colleagues[41] reported that the effects of isoflurane on post-ischaemic recovery were partially inhibited by 8-cyclophenyl-1,3-dipropylxanthine, an adenosine 1-specific receptor blocker. In this study, isoflurane-induced protection was associated with decreases in endogenous adenosine release, which is the opposite of what is observed in ischaemic preconditioning. A_1 receptors were also found to mediate protection in isoflurane- but not halothane-treated human atrial trabecular strips[31] and myocytes.[90] Toller and colleagues[105] reported the important role of Gi-proteins in establishing the isoflurane-induced preconditioned state by showing complete blockade of preconditioning in response to pertussis toxin before treatment in an *in vivo* dog model of regional ischaemia. The significance of all these signalling components could be confirmed at the cellular level using adult rat ventricular myocytes.[120]

In many studies, non-specific blockers of the sarcolemmal and mitochondrial K_{ATP} channel identified these channels as important in volatile anaesthetic-induced preconditioning. The question of whether the sarcolemmal K_{ATP} channel or the mitochondrial K_{ATP} channel is more important in mediating volatile anaesthetic-induced preconditioning has been addressed in several experimental studies.[33 104 120] It is important to note that considerable cross-talk is now documented between sarcolemmal and mitochondrial K_{ATP} channels,[92] and that the importance of the individual K_{ATP} channels may vary among experimental approaches and animal models. Zaugg and colleagues[120] showed that protection by isoflurane and sevoflurane is selectively mediated by activation of mitochondrial K_{ATP} channels in a cellular model of non-beating cardiomyocytes. Also, Hanouz and colleagues[31] demonstrated that desflurane-induced preconditioning is inhibited by 5-hydroxydecanoate (a selective mitochondrial K_{ATP} channel inhibitor) but not HMR-1098 (a selective sarcolemmal K_{ATP} channel inhibitor) in human right atria. Conversely, Toller and colleagues[104] reported that both K_{ATP} channels may be involved in desflurane-mediated preconditioning in an *in vivo* dog model of regional ischaemia. In accordance with the results of Zaugg and colleagues,[120] mitochondrial K_{ATP} channels were found to mediate isoflurane protection selectively in an *in vivo* rabbit model.[85] Similarly, Shimizu and colleagues[95] reported the inhibition of isoflurane preconditioning by 5-hydroxydecanoate (a specific blocker of the mitochondrial K_{ATP} channel) in isolated perfused rat hearts, and Nakae and colleagues[71] reported that nicorandil (a specific activator of mitochondrial K_{ATP} channels) did not have infarct-size limiting effects in halothane anaesthetized rabbits. Conversely, Piriou and colleagues[85] demonstrated additive beneficial effects when coadministering nicorandil (a specific activator of mitochondrial K_{ATP} channels) with isoflurane. This discrepancy among reports showing that either mitochondrial or sarcolemmal K_{ATP} channels are more important may be due to the different experimental approaches, species differences (rat *vs* dog), or fundamental differences between anaesthetics.

Results from patch-clamp experiments demonstrated either an increased open probability of the sarcolemmal K_{ATP} channel for a given ATP concentration in response to isoflurane but inhibitory effects on overall channel activity (excised patch configuration),[30] or no effect on sarcolemmal K_{ATP} channel activity at all (whole-cell and excised patch configuration).[24 90] In contrast to isoflurane, halothane inhibits the sarcolemmal K_{ATP} channel in human[90] and guinea-pig myocytes.[50] Isoflurane facilitates cardiac sarcolemmal K_{ATP} channels preactivated by the non-specific K_{ATP} channel opener pinacidil,[50] the PKC activator phorbol 12,13-dibutyrate and the metabolic inhibitor 2,4-dinitrophenol,[24] indicating a priming effect of these agents on this channel. Two recent studies addressed the effect of volatile anaesthetics on mitochondrial K_{ATP} channels. In both studies, myocyte-inherent flavoprotein-induced fluorescence (autofluorescence) was used to measure mitochondrial K_{ATP} channel activity. Marbán and his group[93] showed that the redox state of these endogenous fluorophores directly reflected mitochondrial K_{ATP} channel activity, and that the opening of this channel was closely associated with significant protection against ischaemia. Kohro and colleagues[45] demonstrated in guinea-pig myocytes that mitochondrial K_{ATP} channel activity was increased by 10% when exposed to isoflurane 0.7 mM or sevoflurane 1 mM. Administration of propofol or pentobarbital abrogated this effect. However, the concentrations used in these experiments were high (2–3 vol/vol % for isoflurane and 4–5 vol/vol % for sevoflurane, corresponding to more than 2 MAC isoflurane or sevoflurane at 37°C), and the effect on the mitochondrial K_{ATP} channel was small compared with other preconditioning drugs. These observations may reflect toxic effects of volatile anaesthetics on oxidative phosphorylation rather than pharmacologically relevant effects on mitochondrial K_{ATP} channels. In the study by Zaugg and colleagues,[120] isoflurane and sevoflurane did not elicit increased channel activity *per se* at lower concentrations, but enhanced diazoxide-induced flavoprotein oxidation. These results support the concept of channel priming by volatile anaesthetics, which was described recently by Sato and colleagues.[93] They proposed a resting, primed and open state of the mitochondrial K_{ATP} channel on the basis of the observation that adenosine did not affect basal mitochondrial K_{ATP} channels but significantly enhanced opening by diazoxide, a highly selective opener of mitochondrial K_{ATP} channels. This concept of channel priming (including the sarcolemmal and the mitochondrial K_{ATP} channel) also appears to extend to isoflurane- and sevoflurane-induced preconditioning. The primed channel state allows easy and rapid opening at the initiation of ischaemia. In their studies, Zaugg and colleagues[120] also presented evidence that volatile anaesthetics mediate their protection by selectively enhancing mitochondrial K_{ATP} channels through the triggering of multiple PKC-coupled signalling pathways, namely NO and adenosine/Gi signalling pathways. An overwhelming body of evidence now demonstrates that biosynthesis of NO plays a pivotal role in decreasing ischaemic damage in heart tissue.[7] It is not surprising, therefore, that NO and cGMP may be major players in volatile anaesthetic-induced protection. NO/cGMP signalling and basal NOS activity were reported to play a fundamental role in pacing associated-preconditioning in the isolated heart.[102] It may well be that volatile anaesthetics differentially modulate the activity of the various isoenzymes of NOS (nNOS, eNOS, iNOS), which are ubiquitous but heterogeneously distributed in myocytes. The observation that isoflurane-induced preconditioning (2% vol/vol) in α-chloralose-anaesthetized rabbits is inhibited by free radical scavengers supports the concept that generation of radicals, either by means of altered NO synthesis or by enhanced formation of ROS/NO (possibly by opening mitochondrial K_{ATP} channels), is important.[67] Recently, desflurane-induced preconditioning was shown to be inhibited by phentolamine (α-adrenergic blockade) and propranolol (β-adrenergic blockade), which is in accordance with the notion that desflurane releases a significant amount of catecholamines in cardiac tissue.[32] It may be that low and high concentrations of volatile anaesthetics activate distinct signalling pathways, resulting in sequential activation of the two K_{ATP} channel subtypes. Taken together, these results show that the preconditioning effects of volatile anaesthetics are triggered by multiple signalling cascades and mediated mainly by mitochondrial K_{ATP} channels, but sarcolemmal K_{ATP} channels may also contribute to the protection elicited by volatile anaesthetics.

The cardioprotective effects of volatile anaesthetics can be enhanced by cariporide, a Na^+–H^+ exchange inhibitor.[62] One additional interesting mechanism by which volatile anaesthetics could elicit their protection was described by Piriou and colleagues.[84] In this study, the use of gadolinium to block mechano-gated channels, which are activated by isoflurane,[80] abolished isoflurane-induced preconditioning. Pre-ischaemic administration of volatile anaesthetics is also known to decrease the incidence of post-ischaemic ventricular arrhythmias, particularly in small animals,[8] whereas in larger animals (dogs, pigs) this is more controversial.[29] Volatile anaesthetics also vasodilate coronary arteries by activation of endothelium K_{ATP} channels,[16] increase endothelial-dependent and endothelial-independent post-ischaemic basal coronary flow, and promote endothelial NO release.[73] Preserved NO generation also prevents leucocyte adhesion and migration (inflammatory response), and blocks expression of adhesion molecules. One study demonstrated improved vascular endothelial protection if volatile anaesthetics were administered during low-flow perfusion for 1 day at 3°C.[97] This indicates significant microvascular protection by volatile anaesthetics. Most recently, pretreatment with isoflurane before cytokine exposure increased the survival of human endothelial and smooth muscle cells, an affect that was abrogated by PKC or K_{ATP} channel inhibition.[20] Importantly, protective effects by endothelial precondition-

ing appear to be beneficial for up to 1 month and may apply to a wide variety of tissues, including all vital organs.[59]

Opioids

The existence of κ- and δ-opioid receptors, but not μ-receptors, has been reported in rat atrial and ventricular tissue.[48] In addition, cardiomyocytes constantly release opioids into the circulation, particularly during stressful stimuli, and thereby serve as an endocrine organ.[65] Activation of opioid receptors results in a potent cardioprotective effect similar to classical and delayed preconditioning. Currently, it is thought that selective activation of δ_1 opioid agonists exert this protection via interaction with Gi-proteins and activation of PKC, tyrosine kinases (and possibly other kinases, such as MAPK), and ultimately K_{ATP} channels.[23] Some studies report opioid-induced preconditioning effects, which are independent of direct receptor stimulation and are mediated solely by free radical formation.[81] As with ischaemic preconditioning, activation of δ-opioid receptors also protects the heart from arrhythmias. Although the δ-opioid receptor is the most prominent receptor subtype in opioid-induced cardioprotection,[4] some role for κ-receptors cannot be dismissed, particularly in protection against ventricular fibrillation.[113] Opioid receptor-mediated protection is stereoselective, as protection can be abolished only by the (–)-active stereoisomer of naloxone.[11] Using a cellular model of simulated ischaemia in chicken cardiomyocytes, Liang and colleagues[55] showed that morphine 1 μM elicited the same protection as preconditioning with 5 min of ischaemia. In this study, morphine effects were abolished by 5-hydroxydecanoate (a specific mitochondrial K_{ATP} channel blocker), which again emphasizes the dominant role of mitochondrial K_{ATP} channels in preconditioning. Interaction of δ-opioid agonists with K_{ATP} channels was first shown in neuronal tissue, in which analgesia produced by δ-opioid receptor activation could be antagonized by glibenclamide.[117] Recently, Zaugg and colleagues[119] demonstrated that fentanyl enhanced diazoxide-induced mitochondrial K_{ATP} channel activity, which was inhibited by the PKC inhibitor chelerythrine. Again, this observation is consistent with a priming effect of fentanyl on mitochondrial K_{ATP} channels. Conversely, in an isolated perfused rat heart model, sufentanil did not improve post-ischaemic recovery, but produced an increase in left ventricular end-diastolic pressure during reperfusion.[63] The clinical relevance of opioid-induced cardioprotection is not yet clear. This topic will be discussed in Part II of this review.[118]

Ethanol-based anaesthetics (chloral hydrate, α-chloralose) and urethane

Zaugg and colleagues[119] recently showed that 2,2,2-trichloroethanol, a halogenated analogue of ethanol and the active metabolite of chloral hydrate and α-chloralose, enhanced mitochondrial K_{ATP} channel activity via activation of PKC. Though not commonly used clinically, these two anaesthetics are still used extensively in experimental investigations. The cardioprotective role of ethanol in ischaemia–reperfusion has been reported previously. In a cellular model of simulated ischaemia, ethanol mimicked preconditioning by activation of PKC.[10] Its preconditioning effects could also be demonstrated in dogs fed chronically with ethanol.[77] Zaugg and colleagues[119] further reported cardiac preconditioning effects by urethane, mediated by mitochondrial K_{ATP} channels in a PKC-dependent manner. As many anaesthetics clearly affect K_{ATP} channels (Table 2) and thereby modify preconditioning, investigations into the precise mechanisms of preconditioning need to consider the effects of background anaesthesia. Experimental results need to be interpreted in the light of these findings.

Conclusions

This review summarizes current knowledge about the key cellular events involved in ischaemic and anaesthetic preconditioning. Although many characteristics of anaesthetic preconditioning are similar to ischaemic preconditioning, there may be fundamental differences with respect to signal intensity and the potential to concomitantly harm cardiac tissue. Understanding of the multiple signalling steps and the ultimate cytoprotective mechanisms is an important prerequisite for both the design of future basic research studies and the evaluation of the clinical effects of ischaemic and anaesthetic-induced preconditioning.

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Addendum

During the review process for this article, important studies on basic principles underlying cardiac preconditioning and, in particular, anaesthetic preconditioning were published. The most salient findings of these are briefly summarized here.

Controversy surrounding the main end-effector in early preconditioning: mitochondrial vs *sarcolemmal K_{ATP} channels—a never-ending story*

A recent study by Suzuki and colleagues[121] demonstrated loss of diazoxide-mediated post-ischaemic functional

improvement in mice lacking the sarcolemmal K_{ATP} channels. This study has the following important implications. First, although it cannot be totally excluded that knockout of sarcolemmal K_{ATP} channels may artificially increase ischaemic damage and thereby cancel the effect of ischaemic preconditioning, the results of this study reinforce the importance of sarcolemmal K_{ATP} channels in the development of cardioprotection in the murine model. Secondly, the findings of this study provide evidence that diazoxide may have significant effects on sarcolemmal K_{ATP} channels, questioning its mitochondrial selectivity in some species (mouse *vs* rat). Finally, results obtained from mouse hearts should not be extrapolated directly to larger animal models. In another recent study,[122] a mixed agonist/antagonist (MCC-134) with opposing effects on sarcolemmal and mitochondrial K_{ATP} channels was identified. MCC-134 inhibited diazoxide-induced flavoprotein oxidation and at the same time opened sarcolemmal K_{ATP} channels in mouse and rabbit cardiomyocytes. In the *in vivo* mouse model, MCC-134 abolished the effect of ischaemic preconditioning against infarction, suggesting that, even in mice, mitochondrial K_{ATP} channels are the key players in cardioprotection. In accordance with this concept, several recent publications suggest that mitochondrial K_{ATP} channels may be more important than sarcolemmal K_{ATP} channels in anaesthetic-induced preconditioning.[123–125]

The metabolic concept of cardiac preconditioning

This concept provides an alternative explanation for the preconditioning process without assuming the existence of mitochondrial K_{ATP} channels, which have not yet been cloned. According to this model, agents such as pinacidil, nicorandil and volatile anaesthetics would target enzymes of the respiratory chain directly. A recent report by Hanley and colleagues[126] showed that diazoxide and 5-hydroxydecanoate have K_{ATP} channel-independent targets in the heart, diazoxide inhibiting succinate dehydrogenase and 5-hydroxydecanoate serving as a substrate for acyl-CoA synthetase (fatty acid oxidation). These observations raise the possibility that inhibition of key enzymes in the tricarboxylic acid cycle and the respiratory chain may initially lead to a reduction in ROS formation and mitochondrial ATPase activity and subsequently (at the time of washout of the agent) induce a burst of free radicals. The sequence of these events would mimic the preconditioning process and ultimately induce protection.[127 128] In this scenario, 5-hydroxydecanoate would abrogate preconditioning by increasing the electron flow to the respiratory chain, thereby circumventing the inhibitory effects of diazoxide on succinate dehydrogenase. In fact, 5-hydroxydecanoate is metabolized by acyl-CoA-dehydrogenase and may thus increase the supply of electrons to the respiratory chain.

Lack of delayed protection in volatile anaesthetic-induced preconditioning?

Kehl and colleagues[129] showed that isoflurane (1 MAC, corresponding to 1.28 vol%) administered for 6 h in dogs does not produce a second window of protection (SWOP) against coronary occlusion 24 h later. However, other temporal relationships for the occurrence of a SWOP were not tested. Also, this study failed to show effective delayed preconditioning by ischaemia as an important positive control in the experimental setting used.

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