

# Cardiac Preconditioning by Anesthetic Agents: Roles of Volatile Anesthetics and Opioids in Cardioprotection

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**Cardiac preconditioning is the most potent and consistently reproducible method of protecting heart tissue against myocardial ischemia-reperfusion injury. This review discussed about the signaling and amplification cascades from either ischemic preconditioning stimulus or pharmacological preconditioning stimulus, the putative end-effectors and the mechanisms involved in cellular protection. The pharmacological preconditioning induced by volatile anesthetics and opioids is very similar to the ischemic preconditioning. It includes activation of G-protein-coupled receptors, multiple protein kinases and ATP-sensitive potassium channels ( $K_{ATP}$  channels). Volatile anesthetics prime the activation of the sarcolemmal and mitochondrial  $K_{ATP}$  channels, which are 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. Similarly, opioids activate  $\delta$ - and  $\kappa$ -opioid receptors leading to activation of PKC. The open state of the mitochondrial  $K_{ATP}$  channel and sarcolemmal  $K_{ATP}$  channel ultimately induces cytoprotection by decreasing  $Ca^{2+}$  overload in the cytosol and mitochondria.**

**Key words:** ATP-sensitive potassium channel; ischemic preconditioning; pharmacological preconditioning; volatile anesthetic; opioid

Anesthesiologists frequently meet perioperative cardiac ischemic events in the clinical anesthesia and also treat patients with ischemic heart disease. Myocardial ischemic events lead to severe complications and delay the postoperative recovery, thereby worsening the prognosis of the patients who underwent surgery. To minimize the damage or injury of myocardium in the perioperative period is a very important factor to improve outcome of surgery. It has been known well that anesthetics have abilities to prevent ischemic myocardial injury. Therefore, understanding the role of anesthetics including volatile anesthetics

and opioids in myocardial protection is likely to show the strategies of anesthetic management to reduce the incidence of cardiac ischemic events in the perioperative period. This short review reveals the role of volatile anesthetics and opioids in prevention myocardial ischemic injury due to cardiac preconditioning.

## Ischemic preconditioning

Ischemic preconditioning is the concept introduced by Murry et al. (1986) that four cycles of 5-min

Abbreviations: CABG, coronary artery bypass grafting; DAG, diacylglycerol; Gi, inhibitory G; ICAM-1, intercellular adhesion molecule-1; iNOS, inducible NOS; IP, inositol triphosphate;  $K_{ATP}$ , ATP-sensitive potassium; LCx, left circumflex coronary artery; NF- $\kappa$ B, nuclear factor- $\kappa$ B; NO, nitric oxide; NOS, NO synthase; PKC, protein kinase C; ROS, reactive oxygen species

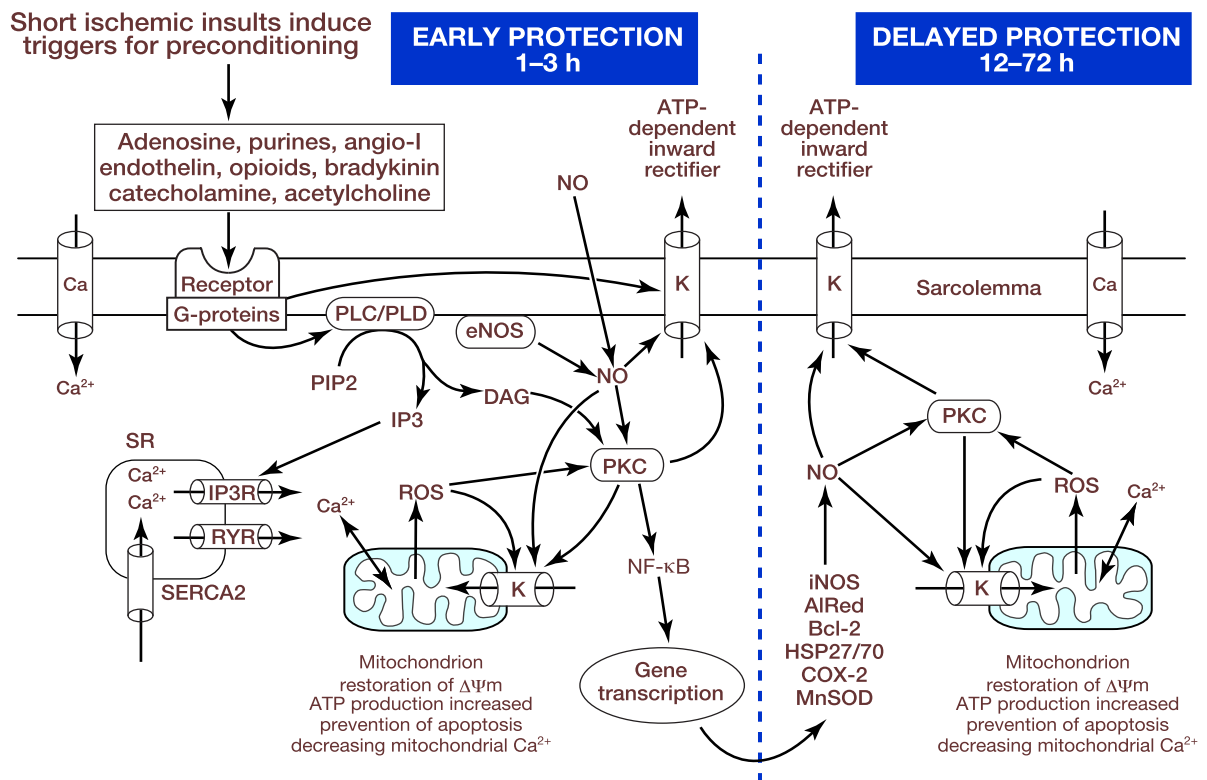
left circumflex coronary artery (Lcx) occlusion, in advance of 40-min Lcx occlusion, reduced infarct size by 75% in a canine model. Thereafter, there have been many reviews on ischemic preconditioning (Okubo et al., 1999; Nakano et al., 2000; Rubino and Yellon, 2000). Ischemic preconditioning can be observed from isolated cardiomyocytes and vascular endothelial cells to hearts in situ in various species (Okubo et al., 1999; Tomai et al., 1999a; Nakano et al., 2000; Rubino and Yellon, 2000). In humans, ischemic preconditioning enhanced postischemic contraction in ventricular trabeculae muscle and improved survival rate of isolated cardiomyocytes (Tomai et al., 1999a). Moreover, in clinical application, ischemic preconditioning elicited by two periods of 3-min aortic cross clamping before cardiopulmonary bypass for valve replacement reduced myocardial enzyme leakage, free radical production and histological degeneration and increased contractility after cardiopulmonary bypass (Lu et al., 1998; Li et al., 1999). Szmagala et al. (1998) applied 4-min aortic cross clamping and 6-min reperfusion prior to coronary artery bypass grafting (CABG), thereby reducing troponin from blood samples. The present author addresses the mechanisms of ischemia-reperfusion injury before showing the possible mechanisms of ischemic preconditioning.

Ischemia precludes adequate oxygen supply, which rapidly results in depletion of ATP. This inhibits ATP-driven  $\text{Na}^+\text{-K}^+$  pumps, increasing  $[\text{Na}^+]_i$ .  $[\text{H}^+]_i$  is increased due to poor washout of metabolites and inhibition of mitochondrial oxidation of  $\text{NADH}_2$ . Increased  $[\text{H}^+]_i$  enhances  $\text{Na}^+\text{-H}^+$  exchange to retain normal  $\text{pH}_i$ , leading to increased  $[\text{Na}^+]_i$ . Accordingly,  $[\text{Ca}^{2+}]_i$  is augmented via  $\text{Na}^+\text{-Ca}^{2+}$  exchange (Opie, 1998a, 1998b). High  $[\text{Ca}^{2+}]_i$  degrades proteins and phospholipids (Opie 1998c; Maxwell and Lip, 1997). Onset of ischemia increased the production of free radicals derived mainly from neutrophils and mitochondria (Opie 1998a; Maxwell and Lip, 1997). When coronary arteries are damaged, ischemia-related injury prevents swift gas exchange by swollen endothelial cells. Vessels with

malfunctioning endothelium and smooth muscle cannot dilate when necessary. Moreover, neutrophils/platelets aggregating in the lumen decrease adequate coronary flow (Opie 1998c; Maxwell and Lip, 1997). Neutrophils release oxygen free radicals, cytokines and other proinflammatory substances, which injure the endothelium, vascular smooth muscle and myocardium (Jordan et al., 1999). A pathway for neutrophil sequestration is the specific interaction of adhesion molecules whose expression is promoted by ischemia-reperfusion. Adhesion molecules, for example, intercellular adhesion molecule-1 (ICAM-1), L-selectin and CD11b/CD18 are expressed on neutrophils and endothelium. On reperfusion,  $[\text{H}^+]_i$  outside the cell is rapidly decreased to normal levels because of wash-out. This results in an increase in  $[\text{Ca}^{2+}]_i$  due enhanced  $\text{Na}^+\text{-H}^+$  and  $\text{Na}^+\text{-Ca}^{2+}$  exchange (Opie 1998b; Opie 1998c). Reperfusion also results in a burst of free radical generation because oxygen abundantly supplied (Opie 1998c; Maxwell and Lip, 1997). Both increased  $[\text{Ca}^{2+}]_i$  and free radicals harm the myocardium during reperfusion (Opie 1998c; Maxwell and Lip, 1997). Damage of the vascular system is more prominent during reperfusion than ischemia (Maxwell and Lip, 1997; Jordan et al., 1999). Infarction is one of the major events of ischemia-reperfusion injury during anesthesia. Another major event is myocardial stunning, which is defined as reversible myocardial dysfunction that persists after reperfusion (Opie 1998c; Bolli and Marban, 1999; Braunwald and Kloner, 1982).

### Mechanisms of early preconditioning

Preconditioning is a treatment before an ischemic event while ischemia-reperfusion injury is developed during and after an ischemic period. The signals were generated by short period of ischemia in ischemic preconditioning. Ischemic preconditioning is mediated via several sarcolemmal receptors, which are mostly linked to inhibitory G (Gi)-protein (Ninomiya et al., 2002), namely



**Fig. 1.** Signaling for cardiac preconditioning. The left of dashed line represents mechanisms of early preconditioning and the right represents those of late (delayed) preconditioning. This figure is quoted from the reference of Zaugg et al., 2003.  $\Delta\Psi_m$ , inner mitochondrial membrane potential; AlRed, aldose reductase; Bcl-2, anti-apoptotic protein; Ca, sarcolemmal voltage-dependent  $Ca^{2+}$  channels; COX-2, cyclooxygenase type 2; DAG, diacylglycerol; eNOS, endothelial NO synthase; HSP, 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- $\kappa$ B, nuclear factor- $\kappa$ B; NO, nitric oxide; PIP2, phosphatidylinositol biphosphate; 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.

adenosine (A-1, A-3), purinoceptors (P2Y), endothelin (ET1), acetylcholine (M2),  $\alpha_1$ - and  $\beta$ -adrenergic, angiotensin II (AT1), bradykinin (B2) and opioid ( $\delta_1$ ,  $\kappa$ ) receptors, which couple to a highly complex network of kinases. The involvement of many receptors or triggers in mediating preconditioning reflects the biological redundancy in this life-saving signal transduction pathway. Figure 1 shows the main signaling steps and components of early and delayed preconditioning (Zaugg et al., 2003).

G-proteins link the initial stimulus from the individual receptors to phospholipase C and D. They have several additional functions such as inhibition of  $Ca^{2+}$  influx during ischemia, regula-

tion of cellular metabolism and activation of ATP-sensitive potassium channels ( $K_{ATP}$  channels), the putative main end-effectors of preconditioning. Activation of phospholipase C and D introduces formation of inositol triphosphate (IP3) for the release of  $Ca^{2+}$  from the sarcoplasmic reticulum via the IP3 receptor, and production of diacylglycerol (DAG). DAG activates different isoforms of protein kinase C (PKC). PKC is activated by a large number of phosphorylating enzymes, including G-proteins, phospholipids, DAG, 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) derived from mitochondria either during the short ischemic or the subsequent repetitive reperfusion episodes. Activation of this key enzyme leads to isoform-specific and cytoskeleton-mediated translocation of cytosolic PKC, inducing phosphorylation and thus activation of the sarcolemmal and mitochondrial  $K_{ATP}$  channels (Light et al., 2000). After only 10 min of ischemic preconditioning, PKC activity in the cytosol reduces, whereas PKC in the particulate fraction (i.e., nuclei, mitochondria and membranes) increases (Strasser et al., 1992). PKC- $\delta$  translocation seems to be responsible for activating mitochondrial  $K_{ATP}$  channels and PKC- $\epsilon$  translocation for the establishment of late preconditioning by phosphorylating nuclear targets (Kawamura et al., 1998). However, the observation that PKC inhibition may not completely block the preconditioning stimulus (Vahlhaus et al., 1996) supports the concept that additional intracellular kinases downstream, upstream or in parallel to PKC signaling contribute to the amplification and establishment of the preconditioned state. Recent studies suggested that mitochondrial  $K_{ATP}$  channels play a greater role than sarcolemmal  $K_{ATP}$  channels (Nakano et al., 2000; Rubino and Yellon, 2003).

ROS, important intracellular signaling molecules derived from mitochondria, are increased during sublethal oxidative stress (preconditioning stimulus) and play a pivotal role in triggering early and delayed cardioprotection (Cohen et al., 2001). ROS activate phospholipase C and PKC, which, in turn, amplify the preconditioning stimulus. Generation of ROS during the initiation of preconditioning represents an essential trigger for early and delayed cardioprotection. NO can induce a cardioprotective effect against myocardial stunning and infarction. Recent studies revealed direct evidence of enhanced biosynthesis of NO in the myocardium subjected to brief episodes of ischemia and reperfusion, probably via increased NOS activity (Bolli, 2001). Although NO is not necessary for ischemia-induced early preconditioning, exogenous or pharmacologically increased endogenous NO production elicits an

early preconditioning effect, that is, NO is sufficient but not necessary for early preconditioning (Bolli, 2001). Conversely, NO has an obligatory role in late preconditioning (Guo et al., 1999).

### **Mechanisms of late preconditioning**

Late preconditioning requires NO formation and increased synthesis of protective proteins (Bolli, 2001). PKC and multiple kinases are involved in the signaling cascade, leading to activation of several transcription factors, such as nuclear factor- $\kappa$ B (NF- $\kappa$ B), which leads to the sustained expression of a number of proteins considered to be responsible for the delayed protection phase. Disruption of the inducible NOS (iNOS) gene completely abolished the delayed infarct-sparing effect, which indicates the obligatory role of iNOS in the cardioprotection afforded by delayed preconditioning (Guo et al., 1999). The most likely cardioprotective effects of NO in late preconditioning are: i) inhibition of  $Ca^{2+}$  influx; ii) antagonism of  $\beta$ -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. Activation of  $K_{ATP}$  channels also plays a role in delayed protection (Bernardo et al., 1999).

### **Sarcolemmal and mitochondrial $K_{ATP}$ channels**

Cardiomyocytes have two distinct types of  $K_{ATP}$  channels, one located in the surface membrane (sarcolemmal  $K_{ATP}$  channels) and another in the inner mitochondrial membrane (mitochondrial  $K_{ATP}$  channels). Sarcolemmal  $K_{ATP}$  channels are physically bound with the creatine phosphate-creatine kinase system and provided a direct link between metabolic state and cellular excitability. Mitochondrial  $K_{ATP}$  channels regulate mitochondrial volume state, mitochondrial membrane po-

tential, formation of ROS and energy production. Toyoda et al. (2000) suggested differential role of sarcolemmal and mitochondrial  $K_{ATP}$  channels in preconditioning. Reduction of myocardial infarct size is mediated largely by mitochondrial  $K_{ATP}$  channels, but functional recovery is mediated by sarcolemmal  $K_{ATP}$  channels. Mitochondrial  $K_{ATP}$  channels also play an important role in the prevention of cardiomyocyte apoptosis (Akao et al., 2001) and in late preconditioning protection (Bolli, 2001). Considerable cross-talk was reported between sarcolemmal and mitochondrial  $K_{ATP}$  channels (Sasaki et al., 2001). A lot of experimental studies indicate the mitochondrial  $K_{ATP}$  channels as the main end-effector of preconditioning, but role of sarcolemmal  $K_{ATP}$  channels cannot be dismissed totally.

Sarcolemmal  $K_{ATP}$  channels may modulate myocardial infarct size by reducing  $Ca^{2+}$  entrance into the myocytes from outside and by attenuating  $Ca^{2+}$  overload. There are three possible explanations about reduction of infarct size by mitochondrial  $K_{ATP}$  channels. First, the decreased mitochondrial  $Ca^{2+}$  overload during ischemia (Wang et al., 2001) may prevent opening of the mitochondrial permeability transition pores and guarantee optimal conditions for ATP production (Holmuhamedov et al., 1998). Second, Garlid and Pancek (2003) proposed that opening of the mitochondrial  $K_{ATP}$  channel decreases the ischemia-induced swelling of the mitochondrial interspace, which would preserve functional coupling between adenosine nucleotide translocase and mitochondrial creatine kinase (prevention of structure/function) (Kowaltowski et al., 2001; Laclau et al., 2001). This secures the transport of newly synthesized ATP from the site of production by ATP synthase on the inner mitochondrial membrane to the cytosol. Thus, high-energy phosphate substrates are supplied continuously from the mitochondria to the sites of energy consumption. Third, mitochondrial  $K_{ATP}$  channels may elicit protection in basis of the observation of increased formation of ROS (Fobes et al., 2001). ROS would stimulate the activation of multiple

**Table 1. Volatile anesthetics and opioids with mostly enhancing effects on mitochondrial and sarcolemmal  $K_{ATP}$  channels**

Anesthetic agent	$K_{ATP}$ channel	
	Mitochondrial	Sarcolemmal
Isoflurane	↑	↓/→
Sevoflurane	↑	?
Desflurane	↑	↑
Morphine	↑	?
Fentanyl	↑	→
Remifentanyl	↑	→

→, no effect; ↑, increased effect; ↓, decreased effect.  $K_{ATP}$ , ATP-sensitive potassium.

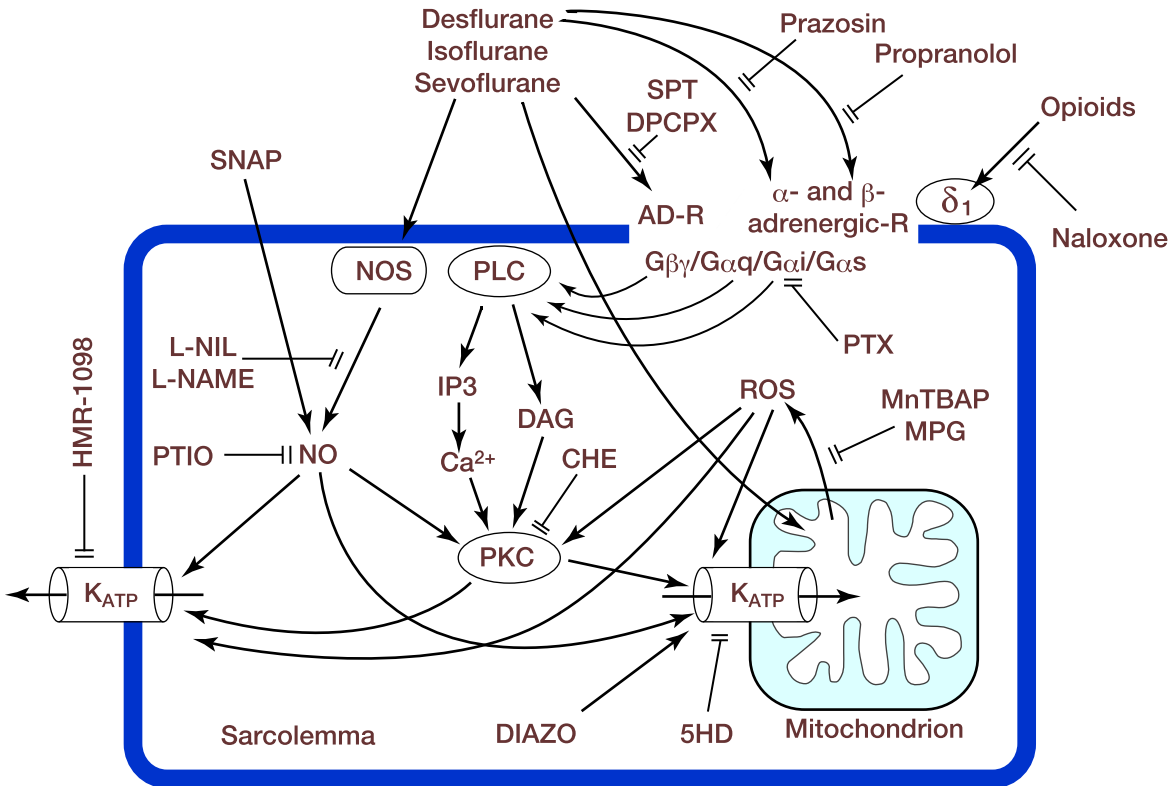
transcriptional factors (NF- $\kappa$ B, activator protein-1, protein kinases, protein phosphatase, etc.), ultimately leading to cardioprotection.

## Pharmacological preconditioning

Preconditioning can be pharmacologically induced by anesthetics. Volatile anesthetics, opioids and other anesthetics were found to induce or enhance preconditioning in cardiac tissue.

### Volatile anesthetics

Lots of studies have evaluated the cardiac preconditioning effects of isoflurane, enflurane and halothane (Mattheussen et al., 1993; Warltier et al., 1988). Sevoflurane, the most frequently used volatile anesthetic in Japan, has also improves postischemic mechanical and coronary function, and reduces infarct size (Novalija and Stowe, 1998; Toller et al., 1999b). Desflurane, a volatile anesthetic used outside of Japan, is suggested the beneficial cardioprotection (Toller et al., 2000b). The beneficial effects of volatile anesthetics on myocardial protection by their pharmacological preconditioning have been evaluated by reduction in infarct size, postischemic contractility and coronary vasculature. Halothane, isoflurane and sevoflurane reduced the number of neutrophils sequestered in the coronary vasculature



**Fig. 2.** Signaling pathways involved in volatile anesthetic- and opioid-induced preconditioning. Multiple signaling cascades prime the sarcolemmal and mitochondrial K<sub>ATP</sub> channels, allowing rapid opening at the initiation of ischemia. This figure is changed slightly from the original figure quoted from the reference of Zaugg et al., 2003. Abbreviations of the blockers and signaling components are referred to Table 2 and the legend of Fig. 1.

after ischemia (Kowalski et al., 1997; Heindl et al., 1999a). A similar effect was also shown for platelets (Heindl et al., 1998; Heindl et al., 1999b). Reduced neutrophils/platelet entrapment by anesthetics was accompanied by enhancement of postischemic mechanical function (Heindl et al., 1999a; Heindl et al., 1999b). Novalija et al. (1999) measured coronary flow changes in response to endothelial-dependent and independent vasodilators. Sevoflurane preserved the reaction elicited by both types of vasodilators during the reperfusion period better than no treatment.

The favorable oxygen supply/demand ratio provided by volatile anesthetics is not required for preconditioning because volatile anesthetic-induced protection occurs under cardioplegic arrest (Lochner et al., 1994). Many characteristics of preconditioning by volatile anesthetics are similar to those of ischemic preconditioning. These

involve activation of A<sub>1</sub> adenosine receptors, PKC and K<sub>ATP</sub> channels. Ischemic preconditioning and anesthetic preconditioning similarly reduce Ca<sup>2+</sup> loading, augment post-ischemic contractile responsiveness to Ca<sup>2+</sup> and decrease infarct size (An et al., 2001). Whether volatile anesthetics induce late preconditioning is still unknown.

Key signaling components involved in preconditioning elicited by volatile anesthetics were unraveled recently by means of specific blockers for signaling steps (Fig. 2) and the specific openers and blockers for signaling steps are shown in Table 2. The main routes of activation by volatile anesthetics involve the G<sub>ai</sub> protein-coupled adenosine receptor and the production of NO, probably by modulation of NOS activity (Zaugg et al., 2002). These two signaling pathways converged at the level of PKC, although alternative routes for NO could be operative as well. Finally

**Table 2. Specific openers and blockers for signaling steps of pharmacological preconditioning**

Selectivity	Opener	References	Blocker	References
Adenosine receptors			SPT DPCPX	Cope et al., 1997 Kersten et al., 1997
PKC			CHE Bicindolylmaleimide	Toller et al., 1999a Toller et al., 1999a
Gi-proteins			PTX	Toller et al., 1999a
Mitochondrial K <sub>ATP</sub> channel	Nicorandil DIAZO	Piriou et al., 1997 Sato et al., 2000	5HD	Toller et al., 1999a; Piriou et al., 1997; Hanouz et al., 2002; Zaugg et al., 2002; Shimizu et al., 2001
Sarcolemmal K <sub>ATP</sub> channel			HMR-1098	Hanouz et al., 2002
NOS			L-NIL, L-NAME	Müllenheim et al., 2002
NO	<i>S</i> -nitroso- <i>N</i> -acetyl- DL-penicillamine		PTIO	
ROS			MnTBAP, MPG	Müllenheim et al., 2002
$\alpha$ -adrenergic receptor			Phentramine, Prazosin	Hanouz et al., 2000
$\beta$ -adrenergic receptor			Propranolol	Hanouz et al., 2000
$\delta$ -opioid	DADLE	McPherson and Yao, 2001	Naloxone	Tomai et al., 1999b
$\delta_1$ -selective	TAN-67	Fryer et al., 1999		

CHE, chelerythrine; DADLE, D-Ala<sup>2</sup>-D-Leu<sup>5</sup>-enkephalin; DIAZO, diazoxide; DPCPX, 8-cyclophenyl-1,3-dipropyl-xanthine; 5HD, 5-hydroxydecanoate; Gi, inhibitory G; L-NIL, L-N<sup>6</sup>-(1-iminoethyl)lysine; L-NAME, N<sup>G</sup>-nitro-L-arginine methyl ester; MnTBAP, Mn(III)tetrakis(4-benzoic acid)porphyrine chloride; MPG, N-(2-mercaptopropionyl)glycine; NO, nitric oxide; NOS, NO synthase; PKC, protein kinase C; PTIO, 2-(4-carboxyphenyl)-4,4',5,5'-tetramethylimidazole-1-oxyl-3-oxide; PTX, pertussis toxin; ROS, reactive oxygen species; SPT, 8-sulfophenyl theophylline.

volatile anesthetics activate mitochondrial and sarcolemmal K<sub>ATP</sub> channels, thereby providing cardioprotection. There is a question of whether the sarcolemmal K<sub>ATP</sub> channel or mitochondrial K<sub>ATP</sub> channel is more important in mediating volatile anesthetic-induced preconditioning. Although several experimental studies have addressed this question (Toller et al., 2000; Zaugg et al., 2002; Hara et al., 2001), it is important to note that considerable cross-talk is documented between sarcolemmal and mitochondrial K<sub>ATP</sub> channels (Sasaki et al., 2001) and the importance of the individual K<sub>ATP</sub> channels may vary among experimental approaches and species differences. Sato et al. (2000) proposed the concept of channel priming (including the sarcolemmal and mitochondrial K<sub>ATP</sub> channels) by volatile anesthetics. The primed channel state allows easy and rapid opening at the initiation of ischemia. On

the other hand, volatile anesthetics mediate their protection by selectively enhancing mitochondrial K<sub>ATP</sub> channels through the triggering of multiple PKC-coupled signaling pathways, namely NO and adenosine/Gi signaling pathways (Zaugg et al., 2002). Biosynthesis of NO plays a pivotal role in reducing ischemic damage in heart tissue. Moreover, NO and cGMP may be major players in volatile anesthetic-induced cardioprotection. Both NO/cGMP signaling and basal NOS activity play a fundamental role in pacing associated-preconditioning. Volatile anesthetics may 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 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 (Müllenheim et al., 2002). These results show that the preconditioning effects of volatile anesthetics are triggered by multiple signaling cascades and mediated mainly by mitochondrial  $K_{ATP}$  channels, but sarcolemmal  $K_{ATP}$  channels may also contribute to the protection induced by volatile anesthetics.

Volatile anesthetics can elicit coronary protection through an ischemic (pharmacological) preconditioning-like effect. Ischemic preconditioning is known to reduce ICAM-1 production and neutrophil entrapment, and to preserve the response to vasodilators (Rubino and Yellon., 2000). Treatment with volatile anesthetics decreased neutrophil adhesion on the endothelium and expression of CD11b, which forms an integrin with CD18, while the anesthetic did not affect endothelial cell activation vis-à-vis neutrophils (Mobert et al., 1999). These findings supports that administration of volatile anesthetics prior to reperfusion maintains coronary vasculature.

### **Opioids**

The involvement of opioid receptors in ischemic preconditioning has been demonstrated in various animal species (Schultz and Gross 2001) and humans (Bell et al., 2000). Among opioid receptor subtypes,  $\delta$ -opioid receptors are responsible for ischemic preconditioning in rats and humans. Although opioid receptors are located more abundant in the central nervous system, they are also located in the heart (Bell et al., 2000). Opioid receptor subtype distribution in heart is considered to differ between species;  $\delta$ - and  $\kappa$ -, but not  $\mu$ -opioid receptors are expressed in the rat heart (Schultz and Gross, 2001),  $\delta$ - and  $\mu$ -opioid receptors are dominant compared with  $\kappa$ -opioid receptors in human atrium (Schultz and Gross, 2001). Naloxone blocked the effect of ischemic preconditioning in isolated hearts, and quaternary naloxone, which does not cross the blood-brain barrier, eliminated the protection by ischemic pre-

conditioning in in vivo models (Chien et al., 1999). These findings suggest that it is in the heart itself that opioid receptors play a role in protection by ischemic preconditioning.

Morphine and fentanyl are capable of binding to  $\delta$ - and  $\kappa$ -receptors although they bind dominantly with  $\mu$ -receptors (Jaffe and Martin, 1990). Selective  $\delta$ - (McPherson and Yao, 2001) and  $\delta_1$ - (Huh et al., 2001) agonists induce cardioprotection. Conversely protection by morphine and fentanyl is abolished by  $\delta$ -antagonists (McPherson and Yao, 2001). The role of  $\kappa$ -receptors remains controversial. Activation of opioid receptors results in a potent cardioprotection effect similar to classical and late preconditioning. Currently, it is considered that selective activation of  $\delta_1$  opioid agonists exert this protection through an interaction with Gi-proteins and activation PKC, tyrosine kinases (and possibly other kinases, such as MAPK), and ultimately  $K_{ATP}$  channels, especially mitochondrial  $K_{ATP}$  channels (Fryer et al., 1999). Morphine 1 mM induced the same protection as preconditioning with 5 min of ischemia and that protection were abolished by 5-hydroxydecanoate (a specific mitochondrial  $K_{ATP}$  channel blocker), which emphasizes the dominant role of mitochondrial  $K_{ATP}$  channels in preconditioning (Liang and Gross, 1999).

Remifentanyl, a new comer of fentanyl family, induces also the pharmacological preconditioning effect as well as morphine and fentanyl through the same mechanism (Zang et al., 2004, 2005).

**Conclusions:** This review summarizes recent knowledge about the key cellular events involved in ischemic and pharmacological preconditioning. Many characteristics of anesthetic-induced preconditioning are similar to ischemic preconditioning. However, there may be fundamental differences in terms of signal intensity and the potential to concomitantly injured cardiac tissue. Of many anesthetics, volatile anesthetics are arguably the most promising agents as cardiopro-



tectors. They demonstrated the beneficial effect against ischemic-reperfusion injury better than any other anesthetic. Volatile anesthetics provide cardioprotection at clinically relevant concentrations and morphine has also been to be protective at clinical concentrations. Therefore, volatile anesthetic and morphine might be good choice for the patients at risk of myocardial ischemia.

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