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Mechanisms of Cardioprotection by Volatile Anesthetics

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A RAPIDLY growing body of evidence indicates that volatile anesthetics protect myocardium against reversible and irreversible ischemic injury. Identifying the mechanisms by which volatile agents mediate these antiischemic actions is the subject of intense research. This objective has been difficult to accomplish because volatile anesthetics also profoundly affect cardiovascular function. Volatile agents reduce arterial and coronary perfusion pressure, cause dose-related depression of myocardial contractility, produce coronary vasodilation, affect electrophysiologic function, and modify autonomic nervous system activity to varying degrees. Therefore, the antiischemic effects of volatile anesthetics may be mediated, at least in part, by favorable alterations in myocardial oxygen supply–demand relations, preservation of energy-dependent cellular functions, and increased coronary blood flow. However, it seems unlikely that changes in myocardial metabolism and coronary perfusion caused by volatile anesthetics are solely responsible for protection against ischemic damage. Instead, several endogenous signal transduction pathways, acting through the adenosine triphosphate (ATP)–sensitive potassium (K_{ATP}) channel and involving the generation of reactive oxygen species (ROS), have been implicated in mediating the antiischemic actions of volatile anesthetics. The experimental and clinical findings documenting the phenomenon of volatile anesthetic preconditioning against ischemic injury of myocardium are evaluated. Recent findings *in vitro* and *in vivo* that seek to define the intracellular mechanisms responsible for these beneficial actions are also summarized.

Historical Perspective

The antiischemic effects of volatile anesthetics were initially proposed more than 20 yr ago. Lowenstein *et al.*[1] demonstrated that halothane reduced ST segment elevation in a canine model of brief coronary artery occlusion. These data were consistent with the hypothesis that exposure to halothane reduced acute ischemic injury. A subsequent study by this research group also demonstrated that halothane reduced myocardial infarct size when administered before prolonged coronary artery occlusion in dogs.[2] Lactate production was decreased in the presence as compared with the absence of enflurane during demand-induced ischemia produced by a critical coronary artery stenosis and ventricular pacing when coronary perfusion pressure was maintained.[3] These results suggested that myocardial metabolism may be improved by administration of a volatile agent during an ischemic episode independent of alterations in hemodynamics. The relative importance of these early findings was initially overshadowed[4] by a series of reports published in the mid-1980s[5–8] suggesting that isoflurane may be capable of producing an abnormal redistribution of coronary blood flow away from ischemic toward normal myocardium.[9–11] This "coronary steal" phenomenon was attributed to the coronary vasodilating properties of isoflurane that are known to occur primarily in arterioles of less than 100 μm in diameter.[12] Isoflurane was thought to be capable of directly producing myocardial ischemia in susceptible patients with "steal-prone" coronary artery anatomy under certain hemodynamic conditions[13] in a fashion similar to that of potent coronary vasodilators (*e.g.*, adenosine, chromonar, dipyridamole).

The implication that isoflurane might produce myocardial ischemia through such a steal mechanism was subsequently dispelled by several investigations conducted in animal models[14–17] and humans with coronary artery disease.[18–20] For example, isoflurane did not selectively redistribute blood flow away from the collateral-dependent region in a chronically instrumented canine model

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of multivessel coronary artery disease.[15] In contrast, adenosine produced marked coronary steal by preferentially shunting blood flow away from collateral-dependent myocardium in this model.[15] Other studies[14,16–20] also suggested that isoflurane-induced hypotension may reduce myocardial perfusion, but true coronary steal did not occur when coronary perfusion pressure was maintained. Subsequent investigations with the newer volatile anesthetics sevoflurane[21,22] and desflurane[23] showed that these drugs also did not reduce or abnormally redistribute coronary collateral blood flow. Therefore, despite initial concerns, volatile anesthetics were subsequently shown to be relatively weak coronary vasodilators that are incapable of causing coronary steal under the vast majority of clinical conditions.[24]

Contrary to the hypothesis that the use of volatile anesthetics may be potentially deleterious in some patients with coronary artery disease, many laboratory and clinical investigations conducted since the resolution of the coronary steal controversy have convincingly shown that volatile anesthetics *protect* the heart against ischemia and reperfusion injury. In addition to previously cited studies suggesting that halothane[1,2] and enflurane[3] exerted antiischemic effects, halothane was also shown to preserve contractile function and ultrastructural integrity during cardioplegic arrest.[25] This latter study was of considerable interest because these data indicated that halothane was capable of exerting a cardioprotective effect completely independent of improvements in myocardial oxygen supply–demand balance. In addition, halothane,[26] enflurane,[26] desflurane,[26–28] and sevoflurane[26–31] have been shown to reduce myocardial damage when administered during reperfusion after prolonged coronary occlusion or cardioplegic arrest. Another study showed that preservation of high-energy phosphate concentrations was coupled to the protective effects of enflurane.[32] Isoflurane and desflurane did not depress but modestly enhanced left ventricular diastolic function during acute coronary occlusion in dogs.[33] Halothane,[34–36] enflurane,[34,37,38] isoflurane,[34,35,37] and sevoflurane[39] were shown to improve the functional recovery of isolated hearts subjected to global ischemia and reperfusion. Halothane and isoflurane markedly augmented the recovery of regional contractile function of stunned myocardium *in vivo*.[40,41] Halothane[2] and isoflurane[42] reduced myocardial infarct size in dogs, and this beneficial action was found to persist despite discontinuation of the volatile anesthetic before coronary artery occlusion.[42] The myocardium acted as if it had "remembered" the previous exposure to the volatile agent. This phenomenon was termed *anesthetic-induced preconditioning* (APC)[43] and was characterized by a short-term memory phase similar to that observed during ischemic preconditioning (IPC).

Anesthetic-induced preconditioning has also been described in other animal species, including rats[44] and rabbits.[43] The efficacy of APC conferred by isoflurane to reduce infarct size has been shown to be dose dependent in rats,[44] an animal model with minimal coronary collateral flow.[45] High concentrations of isoflurane may also have greater efficacy to protect myocardium during conditions of low coronary collateral blood flow in dog myocardium.[46] Similarly, isoflurane and sevoflurane dose-dependently preserved the viability of isolated cardiac myocytes during ischemia.[47] Isoflurane has been shown to elicit cardioprotective effects after discontinuation for 15 min or 30 min before coronary artery occlusion in rats and rabbits[43,44] or dogs,[42] respectively. In contrast, sevoflurane did not exert antiischemic actions after a 30-min washout period.[48] Taken together, these data suggest that the memory period associated with APC may differ between volatile anesthetics and among species. Interestingly, recent findings showed that isoflurane reduced myocardial damage when administered 24 h before coronary artery occlusion and reperfusion in rabbit hearts *in vivo*.[49] Pretreatment with isoflurane also preserved endothelial and vascular smooth muscle cell viability 12–48 h after cytokine-induced injury.[50] Therefore, volatile anesthetics also produce a late phase (*i.e.*, a second window) of myocardial protection similar to IPC. In addition, sevoflurane reduced the duration of a brief ischemic episode required to protect against infarction during IPC.[48] Sevoflurane also enhanced cardioprotection when administered 24 h after an initial IPC stimulus.[51] These important findings showed that administration of a volatile anesthetic combined with a brief ischemic event synergistically protects myocardium against subsequent damage as well.

Additional data about the effects of volatile agents on the coronary circulation also stand in contrast with the conclusions implicated by the coronary steal hypothesis. These results indicated that volatile agents are certainly not deleterious to but may instead exert beneficial actions on coronary collateral perfusion to ischemic myocardium. Volatile anesthetics have been shown to produce coronary vasodilation by activating K_{ATP} channels[39,52–55] or by favorably affecting intracellular Ca^{2+} homeostasis in vascular smooth muscle.[56] Halothane attenuated reductions in coronary collateral perfusion associated with acute coronary occlusion and also improved the myocardial oxygen supply–demand relation in collateral-dependent myocardium.[57] In addition, halothane reduced cyclical changes in coronary blood flow and prevented the development of platelet thrombi in the presence of a critical coronary artery stenosis.[58] Sevoflurane increased collateral blood flow to ischemic myocardium when perfusion pressure was maintained.[21,59] Sevoflurane also improved the functional recovery of coronary vascular reactivity and nitric oxide release in isolated hearts after global ischemia.[39] Lastly, volatile anesthetics attenuated neutrophil and platelet aggregation[60] and also inhibited cytokine-induced cell

death[50,61] after ischemia–reperfusion injury *in vitro*. The results of these studies collectively show the protection against ischemia and reperfusion injury may be at least partially based on favorable effects of volatile agents on coronary perfusion.

The precise mechanisms responsible for volatile anesthetic–induced protection against ischemic injury remain unclear despite extensive study. Although it is clear that volatile anesthetics may indirectly improve myocardial oxygen supply–demand relations or enhance coronary collateral perfusion, it is equally clear that these actions are not entirely responsible for the antiischemic effects of these agents. This contention is emphasized by findings showing that volatile anesthetics conferred protection during cardioplegic arrest[25] and during reperfusion,[26–30] conditions in which myocardial oxygen supply–demand relations play little if any role. Similarly, isoflurane and sevoflurane increased the viability of isolated cardiac myocytes,[47] and sevoflurane[62] and desflurane[63] improved contractility of isolated cardiac muscle exposed to simulated ischemia. These results were initially attributed to reductions in excessive intracellular Ca^{2+} concentrations during ischemia and reperfusion[64] produced by partial inhibition of Ca^{2+} channel activity.[65–68] However, this relatively generic Ca^{2+} hypothesis did not address the precise mechanisms or provide deeper insight into the intracellular processes by which volatile anesthetics exert protective effects in the intact heart.

K_{ATP} Channels

The signal transduction pathways involved in APC bear striking similarity to those responsible for IPC. It is hypothesized that volatile anesthetics stimulate a trigger that initiates a cascade of events leading to activation of an end-effector that is responsible for resistance to injury. To date, adenosine type 1 (A_1) receptors,[34,69,70] protein kinase C (PKC),[34,71,72] inhibitory guanine nucleotide binding (G_i) proteins,[73] ROS,[74–76] and mitochondrial and sarcolemmal K_{ATP} (mito K_{ATP} and sarc K_{ATP}, respectively) channels[42,77–79] have been shown to mediate APC (fig. 1). K_{ATP} channels are heteromultimeric complexes containing an inward-rectifying potassium (K_{ir}) channel and a sulfonylurea receptor (SUR).[80] Pharmacologic and recombinant techniques indicate that sarc K_{ATP} and mito K_{ATP} channels[81,82] are composed of the $K_{ir}6.2$/SUR2A and $K_{ir}6.1$/SUR1 isoforms,[83] respectively. K_{ATP} channel opening was initially implicated as the central end-effector during APC,[84] similar to the findings during studies of the mechanisms responsible for IPC.[85,86] Isoflurane and sevoflurane preserved myocardial viability in a cellular model of ischemia, and this protective effect was abolished by the selective mito K_{ATP} channel antagonist 5-hydroxydecanoate (5-HD) but not the selective sarc K_{ATP} channel antagonist HMR-1098.[47] Isoflurane,[69] sevoflurane,[62] and desflurane[63] but not halothane[69] enhanced the recovery of contractile force of isolated human right atrial trabeculae after hypoxia and reoxygenation. The nonselective K_{ATP} channel blocker glyburide (glibenclamide) or 5-HD inhibited this protective effect. HMR-1098 also attenuated the beneficial actions produced by sevoflurane in isolated human atria.[62] Glyburide blocked the enhanced recovery of contractile function produced by isoflurane in stunned myocardium *in vivo*.[41,87] Reductions in canine myocardial infarct size produced by isoflurane[42] and the ATP-sparing effects of this agent[88] have been shown to be blocked by glyburide as well. 5-HD also inhibited preconditioning by isoflurane in rats[44] and rabbits.[77] Both 5-HD and HMR-1098 abolished the protective effects of desflurane against ischemia and reperfusion injury in dogs,[78] supporting a role for both mito K_{ATP} and sarc K_{ATP} channels in APC. In contrast, another study showed that HMR-1098 did not modify desflurane-induced preconditioning in isolated human right atria *in vitro*.[63] Therefore, some controversy continues to exist about the relative contribution of sarc K_{ATP} and mito K_{ATP} channels in APC.

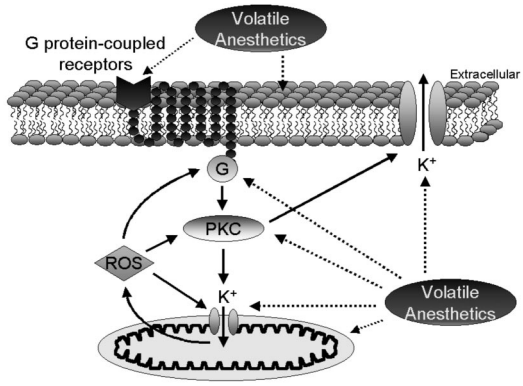

Fig. 1. Multiple endogenous signaling pathways mediate volatile anesthetic–induced myocardial protection. A trigger initiates a cascade of signal transduction events, resulting in the activation of an end-effector that promotes resistance against ischemic injury. Mitochondrial adenosine triphosphate–sensitive potassium (K_{ATP}) channels have been implicated as the end-effector in this protective scheme, but sarcolemmal K_{ATP} channels may also play a role. Volatile anesthetics signal through adenosine and opioid receptors, modulate G proteins, stimulate protein kinase C (PKC) and other intracellular kinases, or have direct effects on mitochondria to generate reactive oxygen species (ROS) that ultimately enhance K_{ATP} channel activity. Volatile anesthetics may also directly facilitate K_{ATP} channel opening. *Dashed arrows* delineate the intracellular targets that may be regulated by volatile anesthetics; *solid arrows* represent potential signaling cascades.

Carefully conducted *in vitro* experiments suggest that volatile anesthetics are capable of modifying K_{ATP} channel activity. Isoflurane stimulated outward K^+ current through sarc K_{ATP} channels in isolated ventricular myocytes during patch clamping.[89,90] Volatile anesthetics also reduced sarc K_{ATP} channel sensitivity to inhibition by ATP, thereby increasing open state probability.[91] In contrast, other patch clamp results suggested that vola-

tile agents alone did not open K_{ATP} channels. Isoflurane did not affect sarc K_{ATP} channel current in human atrial cells[69] and also inhibited sarc K_{ATP} channel activity in rabbit ventricular myocytes.[91] However, some volatile anesthetics were able to enhance sarc K_{ATP} channel current by facilitating channel opening after initial activation.[89,90] Isoflurane enhanced sarc K_{ATP} channel opening in the presence of the mitochondrial uncoupler 2,4-dinitrophenol, the K_{ATP} channel opener pinacidil, and the protein tyrosine kinase (PTK) inhibitor genistein in a whole cell patch clamp model.[89,92] Activation of PKC,[90] adenosine receptors,[93] and phosphatidylinositol kinase[93] seemed to be necessary for this process to occur. Isoflurane also directly opened sarc K_{ATP} channels during intracellular acidosis, a condition that is known to occur during ischemia.[94] These data suggest that volatile anesthetics may not directly interact with sarc K_{ATP} channels but instead may affect other signaling elements that modulate sarc K_{ATP} channel activity. In contrast with the findings with isoflurane, halothane had no effect on pinacidil-induced increases in sarc K_{ATP} channel current and even inhibited K_{ATP} channel current that had been maximally activated by 2,4-dinitrophenol.[89] The anesthetic specificity for APC remains to be well characterized, although studies such as these do suggest important differences in efficacy may exist among individual agents.

The ability of volatile anesthetics to directly open mito K_{ATP} channels has also been examined. Isoflurane and sevoflurane increased mitochondrial flavoprotein oxidation, an index of mito K_{ATP} channel activity, in guinea pig cardiac myocytes.[95] This process was inhibited by 5-HD.[95] Flavoprotein fluorescence may not be entirely specific for mito K_{ATP} channel opening,[96] but isoflurane has also been shown to directly activate mito K_{ATP} channels reconstituted in lipid bilayers.[97] In contrast with these intriguing findings,[97] Zaugg *et al.*[47] recently demonstrated that although isoflurane or sevoflurane did not directly enhance flavoprotein oxidation in rat ventricular myocytes, these volatile agents did potentiate increases in fluorescence produced by the selective mito K_{ATP} channel agonist diazoxide. These results suggested that volatile anesthetics may not directly open but instead act to prime mito K_{ATP} channels, thus enhancing their ability to open in response to an agonist. Sarc K_{ATP} channels may also be linked to the function of the mitochondrial inner membrane. For example, ROS generated by mitochondria may act to open sarc K_{ATP} channels.[98] 2,4-Dinitrophenol–induced activation of sarc K_{ATP} channel current was reversible and accompanied by nicotinamide adenine dinucleotide oxidation, suggesting the existence of cross-talk between mito K_{ATP} and sarc K_{ATP} channels.[99] Taken as a whole, the preponderance of evidence collected to date implies that volatile anesthetics do not necessarily directly open K_{ATP} channels but instead prime the activation of these channels in both sarcolemmal and mitochondrial membranes.

Adenosine triphosphate–sensitive potassium channels in vascular smooth muscle cells have been shown to be essential regulators of coronary vascular tone when ATP production is reduced.[100] Volatile anesthetic–induced coronary vasodilation[39,52–55] was attenuated by glyburide, indicating an important role for K_{ATP} channels in this process. These data suggest that the beneficial actions of volatile agents during myocardial ischemia may be partially attributed to increased oxygen supply mediated *via* K_{ATP} channel–dependent coronary vasodilation. However, sevoflurane increased coronary collateral blood flow in the presence of glyburide *in vivo*, indicating that volatile anesthetics enhance collateral perfusion independent of K_{ATP} channel activation.[59] In fact, sevoflurane-induced increases in collateral perfusion were recently shown to occur as a result of Ca^{2+}-regulated potassium and not K_{ATP} channel activation.[101] Based on these findings and results obtained in isolated cardiac myocytes where blood flow is not a factor,[47] it seems highly unlikely that myocardial protection produced by volatile anesthetics is solely related to favorable alterations in coronary vascular tone mediated by K_{ATP} channels.

G Protein–coupled Receptors

Volatile anesthetics may activate parallel or redundant signaling pathways that involve K_{ATP} channel opening to generate a physiologically meaningful cellular response. The sequential activation of several intracellular elements within a given transduction pathway may facilitate signal amplification and interaction between other redundant signaling systems. For example, administration of isoflurane in the presence of the K_{ATP} channel openers nicorandil[102] or diazoxide[103] markedly enhances protection against ischemic injury beyond that observed with either drug alone. Several receptor-mediated events and intracellular signaling elements that converge on the K_{ATP} channel have been implicated in APC. Pretreatment with pertussis toxin abolished any reduction in infarct size produced by isoflurane, indicating that G_i proteins are linked to the signal transduction pathways that mediate APC.[73] In contrast, pertussis toxin did not alter the beneficial effects of direct K_{ATP} channel opening produced by nicorandil. These data strongly support the contention that volatile anesthetics modulate K_{ATP} channel activity through second messenger signaling.

Halothane-induced protection against infarction was completely abolished by blockade of the adenosine A_1 receptor.[34] The nonselective adenosine receptor antagonist 8-(p-sulfophenyl)-theophylline abolished isoflurane-induced preconditioning in rabbits.[79] The selective A_1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine partially attenuated the beneficial effects of isoflu-

rane in canine stunned myocardium.[70] A role for adenosine receptors in APC was also identified in isolated human right atrial myocardium *in vitro*.[69] Isoflurane eliminated increases in interstitial adenosine during repetitive periods of coronary artery occlusion and reperfusion using a microdialysis technique.[70] These findings suggest that ATP preservation and a subsequent reduction of adenosine released into the interstitium occur during isoflurane anesthesia.[70] In addition, the data imply that volatile agents may either directly activate A_1 receptors or indirectly enhance A_1 receptor sensitivity to diminished endogenous adenosine concentrations.[70] These results were also similar to those observed during IPC[104] or bimakalim-induced pharmacologic preconditioning.[105] The preservation of cardiac myocyte viability during ischemia produced by volatile anesthetics was also sensitive to adenosine receptor and G_i protein inhibition in rats.[47]

Stimulation of the δ_1-opioid receptor has been shown to produce a cardioprotective effect that is abolished by selective opioid antagonists[106–110] or K_{ATP} channel blockers.[107,108,110–113] The acute and delayed phases of IPC are also mediated by activation of the δ_1-opioid receptor.[114] Recent results indicated that the combined administration of isoflurane and selective δ_1-opioid receptor agonists TAN-67 or BW373U86 potentiated K_{ATP} channel opening and enhanced protection against myocardial ischemia and reperfusion injury.[103] Combined administration of isoflurane and morphine, a μ receptor agonist with δ_1 receptor agonist properties, also reduced the extent of myocardial infarction to a greater degree than either drug alone.[44] This beneficial effect was shown to be mediated by mito K_{ATP} channels and opioid receptors. Interestingly, the nonselective opioid antagonist naloxone abolished isoflurane-induced preconditioning.[44] These intriguing data suggest an important link between volatile anesthetics and the opioid family of G protein–coupled receptors. Another recent study also indicated that halothane competitively inhibited the ligand-binding site of G protein–coupled receptors.[115] Adrenergic receptor blockade was shown to abolish desflurane-induced preconditioning in isolated human right atria[63] but had no effect on the antiischemic actions of sevoflurane in isolated rat cardiac myocytes.[47] Overall, APC seems to be associated with the activation of separate receptor-mediated pathways that are linked to G_i proteins.

Protein Kinases

Translocation and phosphorylation of multiple protein kinases are known to be involved in signal transduction pathways involved in protecting myocardium against cell death after ischemia and reperfusion.[116–118] In particular, PKC is an essential component of the signaling pathways associated with preserving cellular viability.[119] The diverse PKC isoform family is a large group of serine/threonine protein kinases that are distinguished by variable regulatory domains and cofactors and also display diverse tissue and species distributions.[120] Activation of G protein–coupled receptors (*e.g.*, A_1,[121,122] bradykinin,[123,124] δ_1 opioid[125,126]) stimulate PKC during IPC. Volatile anesthetics have also been shown to stimulate PKC translocation and activity,[127] possibly by interacting with the regulatory domain of the enzyme.[128] Inhibition of PKC attenuated isoflurane-enhanced recovery of contractile function in canine stunned myocardium.[71] The antiischemic actions of halothane were abolished by selective PKC antagonism in rabbits.[34] The δ and ϵ isoforms of PKC translocated to mitochondria and sarcolemma, respectively, 10 min after discontinuation of isoflurane in isolated rat hearts.[129] In contrast, isoflurane stimulated translocation of PKC-δ and -ϵ to sarcolemmal and mitochondrial membranes, respectively, in the *in vivo* rat heart.[72] These discrepancies may be attributed to differences in experimental model or time of tissue sampling. The microtubule depolymerizing drug, colchicine, prevented isoflurane-induced reductions in myocardial infarct size in rabbits,[130] suggesting that an intact cytoskeleton is essential for translocation of these protein kinases.[72,129]

Recent findings strongly suggest that volatile anesthetic–induced PKC activation is required to open K_{ATP} channels and produce myocardial protection. For example, the nonselective PKC antagonist chelerythrine abolished sevoflurane-induced increases in mito K_{ATP} channel activity in rat ventricular myocytes and prevented protection against simulated ischemia.[47] Patch clamp experiments showed that isoflurane did not facilitate K_{ATP} channel opening in excised membrane patches but enhanced K_{ATP} channel current in a whole cell configuration concomitant with PKC stimulation.[90] These observations were supported by other studies showing that adenosine and PKC increased K_{ATP} channel activity.[103,131–134] Specific PKC consensus sites have been identified on K_{ATP} channels, indicating a molecular basis for phosphorylation and activation of the channel by the enzyme.[135] Mito K_{ATP} channel opening also occurred after PKC activation during IPC in isolated rabbit hearts.[136] In contrast, recent evidence indicates that 5-HD inhibited PKC translocation,[72] suggesting that mito K_{ATP} channel opening may be upstream of PKC activation. Therefore, a possible feed-forward system between PKC and K_{ATP} channel activation may occur during APC.

Protein kinase C has been shown to stimulate PTK[137,138] and mitogen-activated protein kinases (MAPKs),[139] and volatile anesthetics may modulate several of these critical intracellular signaling proteins independent of direct receptor activation as well. Ischemic and pharmacologic preconditioning have been shown to be mediated by activation of PKC,[34,71,106,124,140] PTK,[116,137,138,141,142] and MAPK.[117,118,143–145] A recent investigation showed that the PTK inhibitor lavendustin

A and the Src-selective inhibitor PP1 abolished isoflurane-induced preconditioning in rats.[72] The MAPK family plays an important role in signal transduction from the cell surface and the nucleus and has been strongly implicated in the initiation and progression of cell death (*i.e.*, apoptosis).[146–148] The p38 MAPK subfamily mediated IPC of myocardium in dogs, and activation of p38 MAPK was also associated with phosphorylation and translocation of heat shock protein 27 *in vivo*.[144] In contrast with the findings during IPC, recent data suggest that p38 MAPK may not play a role in APC in isolated rat hearts.[149] Nonetheless, volatile anesthetics modulate activity of one or more intracellular kinases to produce APC, and it seems that activation of PKC is critical to cardioprotection.

Reactive Oxygen Species

Large quantities of ROS are released during reperfusion of ischemic myocardium that damage proteins responsible for intracellular homeostasis, depress contractile function, and produce membrane damage.[150–152] Halothane, isoflurane, and enflurane have been shown to attenuate the toxic effects of ROS on left ventricular pressure development in isolated hearts.[153] Isoflurane decreased hydroxyl radical generation in the ischemic rat heart,[154] and halothane had a similar effect in dogs.[155] The protective effects of sevoflurane were associated with reduced dityrosine formation, an indirect marker of ROS and reactive nitrogen species.[76] These results support the hypothesis that volatile anesthetics reduce the release of deleterious quantities of ROS associated with coronary artery occlusion and reperfusion. Isoflurane also inhibited superoxide anion production by activated neutrophils, an action that occurred independent of K_{ATP} channel opening.[156] In addition, isoflurane and sevoflurane have been shown to abolish activated neutrophil-induced myocardial dysfunction.[157] These effects were associated with reductions in superoxide anion production and neutrophil adherence to coronary vascular endothelium. Therefore, volatile anesthetics also seem to exert beneficial actions by inhibiting neutrophil-induced injury during reperfusion.

In contrast with data implicating a pathologic role of large amounts of ROS, other findings strongly suggest that a variety of preconditioning stimuli, including brief ischemia, direct mito K_{ATP} channel openers, opioids, and volatile anesthetics, stimulate a small burst of ROS that initiate downstream signaling events and produce protection from subsequent ischemic injury.[158] For example, pretreatment with low concentrations of ROS have been shown to mimic the beneficial actions of IPC.[159,160] Free radical scavengers administered before or during brief ischemia markedly attenuated the protective effect of the preconditioning stimulus on infarct size.[161,162] These findings indicate that IPC is mediated by small quantities of ROS released during the preconditioning stimulus. The beneficial actions of sevoflurane against ischemic damage were abolished by scavengers of superoxide anion and inhibition of nitric oxide synthase.[76] These results suggest that superoxide anion may act to trigger APC and further indicated that nitric oxide may scavenge superoxide anion on reperfusion to reduce injury. ROS scavengers attenuated isoflurane-induced reductions in myocardial infarct size in rabbits[74,75] and also inhibited the beneficial effects of direct mito K_{ATP} channel activation.[163] Isoflurane has been shown to directly increase superoxide anion formation *in vivo* independent of an ischemic episode by use of the fluorescent probe dihydroethidium and laser confocal microscopy.[75] These data[75] indicated for the first time that volatile anesthetics were capable of producing small amounts of ROS that were correlated with a reduction in myocardial infarct size after prolonged ischemia. Taken as a whole, these reports provide compelling evidence that small quantities of ROS also play a critical role in APC.

Reactive oxygen species have been shown to act as regulatory mediators in many signaling processes that protect the cells against oxidative stress.[164] ROS-induced activation of PKC[159] and MAPK[165–167] have been implicated in both ischemic and pharmacologic preconditioning. Hydrogen peroxide activated all three MAPK subtypes in neonatal rat ventricular myocytes, but stimulation of the p38 MAPK family and the consequent phosphorylation of heat shock protein 25/27 seemed to be of critical importance during cardioprotection.[165] ROS have also been shown to activate $G\alpha_i$ and $G\alpha_o$ proteins.[168,169] Recent findings showed that sevoflurane-induced ROS generation was unaffected by PKC inhibition,[170] but ROS scavengers inhibited isoflurane-induced PKC translocation.[72] These findings provide indirect evidence linking ROS production by volatile anesthetics to subsequent activation of protein kinases implicated in the signal transduction responsible for APC.

A controversy continues to exist regarding the temporal relation between mito K_{ATP} channel opening and ROS production during ischemic or pharmacologic preconditioning.[171] Although sarc K_{ATP} channel opening was initially assumed to be the end-effector of IPC,[81,85] mito K_{ATP} channel activation may instead trigger preconditioning by generating ROS.[163,172] Mito K_{ATP} channel opening produced by selective agonists generates ROS[163,172] that seem to be essential for activation of MAPK[166] and are also required for beneficial effects on myocardium.[173] The mito K_{ATP} channel agonist diazoxide caused oxidation of the ROS probe MitoTracker® orange (Molecular Probes, Eugene, OR) and enhanced cell viability after hypoxia and reoxygenation *in vitro*.[172] These actions were attenuated by pretreatment with 5-HD or ROS scavengers. Therefore, the protective effects of mito K_{ATP} channel agonists may occur as a consequence of triggering by ROS that subsequently

reduces myocyte injury, including the release of large quantities of these reactive intermediates during reperfusion injury. Morphine increased cardiomyocyte viability and the fluorescence intensity of the hydrogen peroxide–sensitive probe, 2'7'-dichlorofluorescin.[111] These actions were abolished by 5-HD pretreatment, suggesting that activation of mito K_{ATP} channels by opioids results in ROS production. Conversely, other studies[174,175] have indicated that ROS modulate mito K_{ATP} channel activity[174] to provide a beneficial effect, indirectly suggesting that mito K_{ATP} channels may also function as an end-effector of preconditioning. For example, superoxide anion generated by xanthine oxidase activated mito K_{ATP} channels from bovine ventricular myocardium reconstituted from lipid bilayers.[174] Lebuffe *et al.*[175] demonstrated that ROS may serve as a trigger by opening mito K_{ATP} channels, which subsequently generates additional ROS and nitric oxide that are both required for preconditioning in isolated chick neonatal myocytes. Therefore, whether mito K_{ATP} channel opening serves as a trigger or end-effector of ischemic or pharmacologic preconditioning remains unclear.[176] Nevertheless, the apparently complimentary interaction between ROS and mito K_{ATP} channels suggest the intriguing possibility that positive feedback loops may exist between these elements that contribute to myocardial protection.

It also remains unclear whether volatile anesthetic–induced mito K_{ATP} channel opening precedes or follows ROS generation. Pretreatment with 5-HD or the ROS scavengers N-acetylcysteine or N-2-mercaptopropionyl glycine before administration of isoflurane abolished ROS generation *in vivo*.[177] In contrast, administration of 5-HD after discontinuation of isoflurane but before prolonged ischemia only partially attenuated this effect. These data suggest that mito K_{ATP} channel opening acted as a trigger of APC by generating ROS. Conversely, another recent investigation conducted in isolated guinea pig hearts showed that sevoflurane-induced generation of ROS was not inhibited by 5-HD before ischemia.[178] Therefore, experimental findings remain equivocal in support of the hypothesis that mito K_{ATP} channel opening is the major trigger of APC.

Reactive oxygen species derived from the mitochondrial respiratory chain have been shown to play important roles during IPC or pharmacologic preconditioning.[111,160,179,180] The complex III inhibitor myxothiazol blocked hypoxia-[160] or acetylcholine-induced[179] ROS generation and abolished preconditioning in isolated chick cardiac myocytes. The precise source of volatile anesthetic–induced production of ROS has yet to be finally established, but volatile agents have been previously shown to inhibit electron transport chain complexes I and II of cardiac mitochondria.[181,182] Interestingly, sevoflurane-induced complex I inhibition was attenuated by the superoxide dismutase mimetic Mn(III)tetrakis(4-benzoic acid)porphyrin chloride.[182] These results suggested that ROS may inhibit mitochondrial respiration through a positive feedback mechanism to amplify the ROS signal for triggering APC. In contrast, a recent investigation showed that the complex III inhibitor myxothiazol, but not the complex I inhibitor diphenyleneiodonium, abolished isoflurane-induced reductions in myocardial infarct size and generation of ROS.[183] These preliminary data indicated that mitochondrial electron transport chain complex III may be the source of ROS production induced by isoflurane during APC. Taken together, it is possible that volatile anesthetics may modulate multiple sites of the electron transport chain either directly or indirectly *via* a ROS-mediated feedback mechanism. Despite these compelling results, other potential enzymatic sources of ROS (*i.e.*, nicotinamide adenine dinucleotide oxidase, cyclooxygenase, lipoxygenase, xanthine oxidase, nitric oxide synthase, cytochrome P450[184–188]) may play a role in APC and have yet to be excluded from this process.

The precise identities of the specific ROS involved in APC have yet to be defined, and the signaling pathways that may be modulated by these ROS are also largely unknown. ROS activated PKC, restored contractility, and reduced infarct size in rabbit hearts.[159] Superoxide anion also opened mito K_{ATP} channels.[174] Hydrogen peroxide stimulated PTK-dependent activation of phospholipase C in mouse embryonic fibroblasts, rendering these cells resistant to stress.[189] Hydrogen peroxide has also been shown to directly activate G_i and G_o proteins[168,169] and other protein kinases involved in reducing cellular injury.[166,190–192] Hydrogen peroxide may also be converted to other more reactive species that modify cysteine residues of specific G proteins, resulting in their selective activation.[169] Recent results showed that isoflurane administration produced ethidium fluorescence in rabbit myocardium.[75,177] Dihydroethidium is oxidized by intracellular superoxide anion to produce ethidium that subsequently binds to DNA, further amplifying its fluorescence.[193] These results strongly suggest that superoxide anion is the particular ROS involved in isoflurane-induced preconditioning. Sevoflurane also generated superoxide anion before ischemia and reperfusion in isolated hearts.[178] Alternatively, different ROS may exert opposing actions on mito K_{ATP} channel activity. Dismutation of superoxide anion leads to production of secondary ROS, including hydrogen peroxide, hydroxyl radical, and peroxynitrite,[194] and these radicals may differentially alter channel activity. For example, superoxide anion and hydrogen peroxide enhanced but peroxynitrite decreased Ca^{2+}-regulated potassium channel activity.[195] Therefore, it remains possible that volatile anesthetics may also generate ROS other than superoxide that activate mito K_{ATP} channels, or these agents may inhibit the formation of intermediates such as peroxynitrite that adversely affect mito K_{ATP} channel function. Further research is needed to clarify this issue.

Mechanisms of Protection

Opening of sarc K_{ATP} channels was originally implicated in IPC and pharmacologic preconditioning by shortening the action potential duration,[81,196] thereby reducing intracellular Ca^{2+} overload during ischemia.[81] However, subsequent studies conducted after the discovery of mito K_{ATP} channels[82] indicated that the antiischemic actions of K_{ATP} channel activation occurred independent of action potential duration.[197–199] Nevertheless, IPC did not occur in $K_{ir}6.2$-deficient mice, suggesting that the presence of the sarc K_{ATP} channel was still required for myocardial protection to occur.[200] Despite these latter data, the majority of findings accumulated indicate that preservation of mitochondrial bioenergetic function that occurs as a consequence of mito K_{ATP} channel opening seems to be of critical importance for protection against ischemia.[201–204] Selective pharmacologic openers of mito K_{ATP} channels (*e.g.*, diazoxide) maintain mitochondrial Ca^{2+} homeostasis and inhibit Ca^{2+} overload within the organelle.[203,204] Alteration of the mitochondrial oxidation–reduction balance by mito K_{ATP} channel opening may also act to promote cellular protection.[204,205] Membrane depolarization, matrix swelling, and uncoupling of ATP synthesis occur as a result of mito K_{ATP} channel opening that may mediate cellular viability during IPC.[205] Mito K_{ATP} channel opening depolarizes the inner mitochondrial membrane and causes a transient swelling of the mitochondrial matrix,[206] resulting from a shift in the ionic balance.[207] These actions initially reduce ATP production[204] but subsequently stimulate a compensatory increase in respiration that optimizes the efficacy of oxidative phosphorylation in part through energy-dependent matrix volume regulation.[208] Therefore, the moderate disturbance of mitochondrial homeostasis caused by mito K_{ATP} channel opening (fig. 2) may promote tolerance to subsequent ischemic damage by reducing Ca^{2+} overload,[203,204] preventing activation of necrotic or apoptotic pathways,[209,210] or attenuating oxidative stress.[211]

Mitochondrial ATP synthesis has also been shown to be preserved after prolonged as compared with brief ischemia and reperfusion.[212] This beneficial effect was abolished by 5-HD, suggesting that activation of mito K_{ATP} channels improves mitochondrial energy production.[213] Opening of mito K_{ATP} channels has been hypothesized to preserve outer mitochondrial membrane permeability to ATP precursors (*e.g.*, adenosine, adenosine diphosphate) and cytochrome c. The structure of the intermembrane space may also be maintained as a consequence of mito K_{ATP} channel activation despite generalized swelling of the mitochondrial matrix.[201] Preservation of ATP substrates and mitochondrial structure may facilitate more efficient energy transfer between the mitochondria and the cytosol immediately after ischemia. Sevoflurane was recently shown to preserve ATP synthesis in isolated cardiac mitochondria obtained during early reperfusion after ischemia *in vivo*, and this beneficial effect was abolished by pretreatment with a ROS scavenger.[214] Sevoflurane-induced preconditioning improved mitochondrial bioenergetics through mito K_{ATP} channel activation in isolated guinea pig hearts as assessed using flavoprotein fluorescence.[215] Therefore, it seems likely that mito K_{ATP} channel opening by volatile anesthetics may be associated with preservation of mitochondrial function during reperfusion and, further, that this maintenance of mitochondrial performance contributes to cardioprotection.

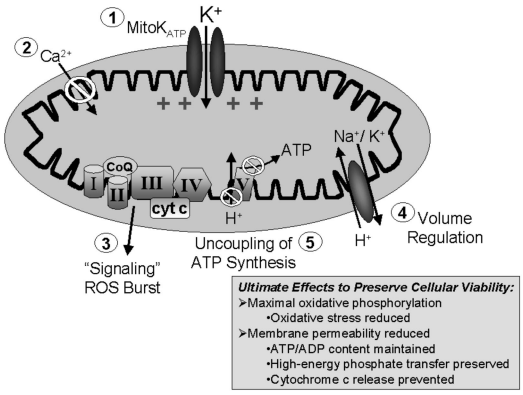

Fig. 2. Cellular preservation during ischemia–reperfusion injury may be mediated by the opening of mitochondrial adenosine triphosphate–sensitive potassium (mito K_{ATP}) channels concomitant with modest modulation of mitochondrial function. Depolarization of the inner mitochondrial membrane and matrix swelling occur as a consequence of mito K_{ATP} channel opening (*1*). Membrane depolarization due to a shift in the ionic balance prevents Ca^{2+} overload (*2*). Initial disruption of the electron transport chain may generate signaling amounts of reactive oxygen species (ROS; *3*). Volume regulatory mechanisms (*4*) dissipate the proton gradient and uncouple adenosine triphosphate (ATP) synthesis (*5*) that ultimately maximizes oxidative phosphorylation and reduces oxidative stress. Mito K_{ATP} channel opening can also reduce membrane permeability and prevent apoptosis and/or necrosis by maintaining nucleotide content, preserving high-energy phosphate transfer, and reducing cytochrome c release. ADP = adenosine diphosphate.

Experiments conducted in isolated mitochondria have shown that a triggering quantity of ROS exceeding a critical threshold results in a transition in mitochondrial inner membrane permeability and the subsequent release of a burst of ROS in a process that has been termed *ROS-induced ROS release*.[216] This mitochondrial permeability transition (MPT) has been shown to precede necrotic or apoptotic cell death,[217] and glutathione is a primary defense against this event.[216,218] These data suggest that volatile anesthetics or other mito K_{ATP} channel agonists may prevent MPT in an oxidant-sensitive fashion, but this intriguing hypothesis has yet to be tested. Opening of an MPT pore using the agonist atractyloside during reperfusion was recently shown to abolish IPC and diazoxide-induced preconditioning in isolated rat hearts.[219] These findings suggested that inhibition of MPT pore opening may represent a distal effector responsible for preconditioning, whereas mito K_{ATP} acti-

vation functions as a trigger or a mediator. Most recently, rabbit hearts pretreated with desflurane before ischemia and reperfusion exhibited resistance to MPT pore opening.[220] Future investigations are necessary to delineate the role of MPT during APC.

Cytosolic and mitochondrial Ca^{2+} overload during prolonged ischemia and reperfusion have been shown to be associated with mitochondrial damage and myocardial cell death.[221–223] Ischemic and sevoflurane-induced preconditioning were shown to reduce cytosolic Ca^{2+} overload and improve the recovery of contractile function during reperfusion.[64] Administration of sevoflurane after ischemia also reduced cytosolic Ca^{2+} and myocardial damage.[31] IPC and APC attenuated mitochondrial Ca^{2+} overload during ischemia in rat and guinea pig hearts,[224,225] actions that were abolished by 5-HD. These data suggested that volatile anesthetics protect against ischemia–reperfusion injury, at least in part, by attenuating cytosolic and mitochondrial Ca^{2+} overload through a mito K_{ATP} channel–dependent mechanism. Volatile anesthetics have also been shown to suppress sarcoplasmic reticulum Ca^{2+} release[226,227] and depress myofilament Ca^{2+} sensitivity.[227] Therefore, modulation of the sarcoplasmic reticulum to reduce cellular Ca^{2+} overload and alterations of myofilament Ca^{2+} sensitivity under conditions of excess Ca^{2+} have been implicated in cardioprotection.[228,229] The inhibitory actions of the volatile anesthetics on the voltage-dependent Ca^{2+} channel are also well known,[65–68] and reductions in cytosolic and mitochondrial Ca^{2+} overload during ischemia and reperfusion injury may also occur through this mechanism.

Myocardial Protection in Clinical Conditions

Compelling experimental data in multiple animal models regarding the protective effects of volatile anesthetics remain to be translated into therapeutic approaches to reduce morbidity and mortality in patients with ischemic heart disease. However, evidence accumulated to date strongly suggests that APC occurs in human myocardium. Repetitive, brief (60- to 90-s) balloon inflations and deflations performed during percutaneous transluminal coronary angioplasty were associated with progressive reductions in the severity of chest pain and in the extent of ST segment elevation, decreases in myocardial lactate production, and declines in cardiac enzyme and troponin release.[230–233] Adenosine[234] or the mito K_{ATP} channel opener nicorandil[235] administered before the first balloon inflation during percutaneous transluminal coronary angioplasty was also shown to reduce the severity of ST changes during subsequent occlusions. Pretreatment with nicorandil before percutaneous transluminal coronary angioplasty also attenuated the release of troponin T, an indicator of myocyte necrosis.[236] These findings suggest that both ischemic and pharmacologic preconditioning can be elicited during percutaneous transluminal coronary angioplasty. Consecutive exercise stress tests (separated by 15 min) performed in patients with critical left anterior descending coronary artery stenoses showed that anginal symptoms, ST segment depression, and myocardial oxygen consumption were reduced during the second as compared with the first exercise period for an equivalent amount of work.[237] This "warm-up" phenomenon occurred independent of coronary vasodilation[237] and also provides evidence of IPC in humans.[238,239]

Ischemic preconditioning has also been shown during cardiac surgery in patients with coronary artery disease. Yellon *et al.*[239] used intermittent aortic cross clamping to produce IPC during coronary artery bypass graft surgery (CABG) and found enhanced preservation of ATP content in preconditioned hearts as compared with those that did not receive preconditioning stimuli. These investigators also showed that less troponin T was released in the presence as compared with the absence of IPC in this model.[240] The incidence of ventricular tachyarrhythmias was reduced after cardiopulmonary bypass in patients undergoing CABG when cold blood cardioplegia was used for myocardial preservation.[241–243] Regional IPC produced by brief coronary occlusion has also been shown to result in improved hemodynamic recovery and reduced release of cardiac troponin I during off-pump CABG.[244] In contrast with the findings of these investigations, other studies have failed to show that IPC exerts beneficial effects during CABG in the presence of cardioplegia and cardiopulmonary bypass.[245–247] Therefore, although the myocardial protective effects of IPC have been clearly identified in the experimental laboratory, further large scale clinical trials are needed to definitively demonstrate the beneficial actions of IPC in humans.

Documentation of APC in patients has been complicated by alterations in systemic and coronary hemodynamics; the use of other anesthetics, analgesics, or vasoactive drugs; preexisting disease states; and the acute influence of surgery on cardiovascular homeostasis. Nevertheless, isoflurane,[69] desflurane,[63] and sevoflurane[62] enhanced the recovery of contractile function of human atrial trabeculae *in vitro* by stimulation of adenosine receptors and opening of K_{ATP} channels. Other studies have previously shown a role for adenosine receptors, MAPK,[248] and ROS[172] in other forms of preconditioning concomitant with opening of mito K_{ATP} channels in human atrial myocytes. Isoflurane increased the tolerance to pacing-induced ischemia in patients with coronary artery disease.[249] Isoflurane also decreased postoperative release of troponin I and creatine kinase-MB in patients undergoing CABG.[250] Although the aforementioned results[250] were not statistically significant, these data suggest that reduction in the extent of myocardial necrosis had occurred. Administration of isoflurane immediately before aortic cross clamping in patients un-

dergoing CABG was shown to decrease the severity of subsequent ST segment changes and preserve cardiac index to a greater extent than that observed in patients who did not receive pretreatment with the volatile anesthetic.[251] Administration of enflurane before cardioplegic arrest enhanced recovery of postischemic contractile function assessed using pressure–area relations in CABG patients.[252] Sevoflurane[253–255] and desflurane[253,255] but not the intravenous anesthetic propofol was shown to preserve myocardial function in patients undergoing CABG as well as a reduction in troponin I release. Most recently, preconditioning with sevoflurane reduced a biochemical marker of myocardial dysfunction (*i.e.*, N-terminal pro–brain natriuretic peptide) in patients undergoing CABG concomitant with translocation of PKC-δ and -ϵ.[256] This compelling evidence strongly suggests that volatile anesthetics exert beneficial effects against ischemic injury in humans.

In contrast with the aforementioned results, no differences in PKC and p38 MAPK activity or peak troponin I release were observed between patients undergoing cardioplegic arrest in the presence or absence of sevoflurane pretreatment.[257] The activities of PKC, PTK, and p38 MAPK were increased equally in both groups, suggesting that cardiopulmonary bypass and cardioplegic arrest may produce a preconditioning-like effect that obscured the antiischemic actions of sevoflurane in this setting. However, sevoflurane was recently shown to reduce myocardial injury to a greater degree than propofol in patients undergoing off-pump CABG,[254] a clinical setting that does not require cardiopulmonary bypass. Most investigations conducted in humans seem to indicate that volatile anesthetics represent an important therapeutic modality to reduce the sequelae of perioperative myocardial ischemia and infarction.[258,259] A large-scale, randomized clinical trial is clearly needed to firmly establish this conclusion. Given the recent data suggesting that cardiopulmonary bypass and cardioplegia may exert a protective effect,[257] such a clinical trial may best be conducted in patients undergoing off-pump CABG[254] or those with documented coronary artery disease undergoing noncardiac surgery.[4] Furthermore, no clinical study has shown that the use of volatile anesthetics in patients with coronary artery disease contributes to reduced cardiac morbidity or perioperative mortality. Additional multicenter trials also need to be conducted to identify the relative impact of APC on clinical outcome.

Future Perspectives

Experimental evidence collected indicates that volatile anesthetics exert important cardioprotective effects that reduce the consequences of reversible and irreversible ischemia and reperfusion injury. Differences in the efficacy of APC and timing of administration among volatile anesthetics alone and in combination with other cardioprotective drugs remain to be fully distinguished. Another important aspect is to determine the effect of aging on APC.[255,260] Characterization of a late phase or second window of APC may be of special clinical significance to protect against ischemic events that frequently occur in the postoperative period. Several endogenous signaling elements seem to mediate APC, and mito K_{ATP} channels, PKC, and ROS have emerged as central features in this process. Future investigations are needed to further delineate and identify essential components in these complex signal transduction cascades that mediate the early and late phases of APC. In this regard, microarray technology may prove useful in ascertaining candidate genes that are responsible for APC.[261] Use of other fundamental molecular and biochemical tools is needed to determine whether preservation of mitochondrial integrity and metabolic homeostasis ultimately enhances tolerance to myocardial ischemia. Volatile anesthetics also act to elicit signaling that is probably present in many types of cells. Therefore, it is not surprising that these agents may reduce injury to other tissues.[256,262] This may also be based on preventing cytokine-induced injury in endothelial and vascular smooth muscle cells.[50,61] Finally, recent investigations have also strongly implied that APC occurs in humans and may represent an important therapeutic approach to reduce morbidity and mortality in patients with coronary artery disease. Nevertheless, further investigation is needed to firmly link this emerging body of clinical evidence to the already strongly established experimental data about the protective effects of volatile anesthetics in myocardium.

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