Marquette University
e-Publications@Marquette

Biomedical Engineering Faculty Research and Publications | Biomedical Engineering, Department of

1-1-2003

Mechanisms of Volatile Anesthetic-Induced Myocardial Protection

Lynda M. Ludwig
Medical College of Wisconsin

Judy R. Kersten
Medical College of Wisconsin

Katsuya Tanaka
Medical College of Wisconsin

Weidong Gu
Medical College of Wisconsin

John F. LaDisa
Marquette University, john.ladisa@marquette.edu

See next page for additional authors

Published version. *Current Topics in Pharmacology*, Vol. 7, No. 2 (2003): 195-207. Publisher Link. © 2003 Research Trends. Used with permission.
John F. LaDisa was affiliated with Medical College of Wisconsin at the time of publication.

Authors
Lynda M. Ludwig, Judy R. Kersten, Katsuya Tanaka, Weidong Gu, John F. LaDisa, David C. Warltier, and Paul S. Pagel

This article is available at e-Publications@Marquette: https://epublications.marquette.edu/bioengin_fac/233

Current Topics in
Pharmacology
Vol. 7, 2, 2003

Mechanisms of volatile anesthetic-induced myocardial protection

Lynda M. Ludwig[1], Judy R. Kersten[2], Katsuya Tanaka[3], Weidong Gu[3], John F. LaDisa Jr[1], David C. Warltier[4] and Paul S. Pagel[5,*]

[1]Graduate Student, [2]Professor of Anesthesiology, Pharmacology and Toxicology, [3]Research Fellow in Anesthesiology, [4]Professor of Anesthesiology, Pharmacology and Toxicology, and Medicine (Division of Cardiovascular Diseases) and Vice Chairman for Research of Anesthesiology, [5]Professor and Director of Cardiac Anesthesia

From the Departments of Anesthesiology, Pharmacology and Toxicology, and Medicine (Division of Cardiovascular Diseases), the Medical College of Wisconsin, and the Clement J. Zablocki Veterans Affairs Medical Center, Milwaukee, Wisconsin, USA

ABSTRACT

Volatile anesthetics protect myocardium against reversible and irreversible ischemic injury. Experimental evidence from several *in vitro* and *in vivo* animal models demonstrates that volatile agents enhance the recovery of stunned myocardium and reduce the size of myocardial infarction after brief or prolonged coronary artery occlusion and reperfusion, respectively. This protective effect persists after the anesthetic has been discontinued, a phenomenon known as anesthetic-induced preconditioning (APC). Recent clinical data also demonstrates evidence of APC in patients during cardiac surgery. Thus, administration of volatile anesthetics may represent a novel therapeutic approach that reduces morbidity and mortality associated with perioperative myocardial ischemia and infarction. The mechanisms responsible for APC appear to be similar to those implicated in ischemic preconditioning, but nonetheless have subtle differences. Accumulating evidence indicates that APC is characterized by complex signal transduction pathways that may include adenosine receptors, G proteins, protein kinase C, reactive oxygen species, and sarcolemmal or mitochondrial K_{ATP} channels. Opioid analgesics may further enhance APC as well. This article will review recent advances in the understanding of mechanisms responsible for volatile anesthetic-induced myocardial protection.

*Address all correspondence to: Paul S. Pagel, Medical College of Wisconsin, MEB-M4280, 8701 Watertown Plank Road, Milwaukee, WI 53226, USA
e-mail: pspagel@mcw.edu

INTRODUCTION

A growing body of experimental and clinical evidence indicates that volatile anesthetics protect myocardium against ischemic injury. Delineating the mechanisms that mediate this beneficial anti-ischemic effect has been a difficult task because volatile agents profoundly affect cardiovascular function. Volatile anesthetics cause dose-related depression of myocardial contractility, reduce arterial and coronary perfusion pressure, produce coronary vasodilation, affect electrophysiological function, and modify autonomic nervous system activity to varying degrees. The anti-ischemic effects of volatile anesthetics may be partially attributed to a favorable reduction in myocardial oxygen consumption, preservation of energy-dependent cellular functions, and increases in coronary blood flow. Nevertheless, it is highly unlikely that alterations in myocardial metabolism and coronary perfusion are solely responsible for myocardial protection during administration of these drugs. Multiple endogenous signal transduction pathways, all of which appear to act through the adenosine triphosphate-sensitive

potassium (K_{ATP}) channel as an integral component, have been implicated in mediating the salutary effects of volatile anesthetics. In this review, we will discuss recent developments in the understanding of mechanisms responsible for volatile anesthetic-induced myocardial protection.

BACKGROUND

Laboratory investigations performed over the past 15 years repeatedly demonstrated that volatile anesthetics attenuate reversible and irreversible damage associated with myocardial ischemia. Halothane was shown to reduce ST segment elevation during brief coronary artery occlusion in dogs [1], consistent with a decrease in acute ischemic injury. Halothane also preserved contractile function and ultrastructural integrity during cardioplegic arrest [2]. Enflurane decreased lactate production during coronary artery stenosis when perfusion pressure was maintained [3], and preservation of high-energy phosphate levels was coupled to these protective effects [4]. Isoflurane and desflurane enhanced left ventricular diastolic function during acute coronary artery occlusion [5]. Halothane [6, 7, 8], enflurane [6, 9, 10], isoflurane [6, 7, 9], and sevoflurane [11] were shown to improve the functional recovery of isolated hearts subjected to global ischemia and reperfusion. Halothane and isoflurane also enhanced the functional recovery of stunned myocardium *in vivo* [12, 13]. Halothane [14] and isoflurane [15] reduced myocardial infarct size in dogs, and this protective effect persisted despite discontinuation of the volatile agent before prolonged coronary artery occlusion [15]. This phenomenon (figure 1) was termed "anesthetic-induced preconditioning" (APC), and was characterized by a short-term memory phase similar to that of ischemic preconditioning (IPC). APC has also been described in rats [16] and rabbits [17]. Sevoflurane decreased the time threshold level of the ischemic stimulus required to protect against infarction during IPC [18]. These results suggested that volatile agents and brief ischemic episodes may produce synergistic protection against subsequent irreversible ischemic damage.

Volatile anesthetics may also exert

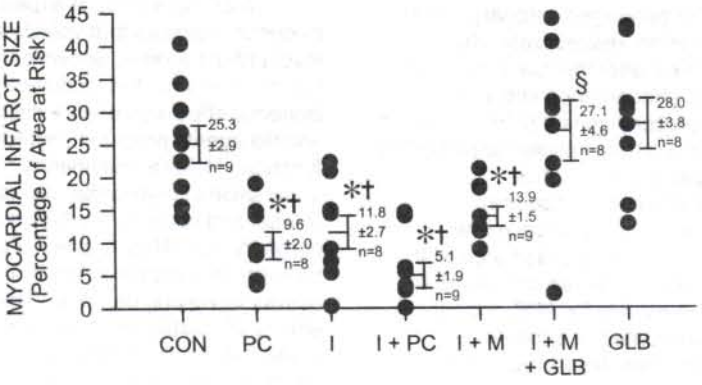

Figure 1. Isoflurane preconditions myocardium by activation of K_{ATP} channels in dogs subjected to coronary artery occlusion and reperfusion. Isoflurane significantly ($P < 0.05$) reduced infarct size in the absence (I) and presence (I+PC) of ischemic preconditioning (PC). Isoflurane produced profound reductions in myocardial infarct size, and this protective effect was associated with an acute memory period (I+M). Isoflurane-induced preconditioning was attenuated by the K_{ATP} channel antagonist glyburide (I+M+GLB). Other groups: control (CON), glyburide alone (GLB). (Adapted with permission of authors and publisher [15].)

beneficial actions on coronary collateral perfusion to ischemic myocardium. Volatile anesthetics produce coronary vasodilation by activating K_{ATP} channels [11, 19, 20, 21, 22, 23] or by favorably affecting intracellular Ca^{2+} handling in vascular smooth muscle cells [24]. Halothane has been shown to attenuate reductions in coronary collateral perfusion associated with acute coronary occlusion [25]. Halothane also enhances the ratio of myocardial oxygen delivery to consumption in collateral-dependent myocardium [25]. Sevoflurane increases coronary collateral blood flow to ischemic myocardium when perfusion pressure is maintained at a constant level [23, 26]. Sevoflurane also improves functional recovery of coronary vascular reactivity and nitric oxide release in isolated hearts after global ischemia [11]. Halothane has been shown to reduce cyclical changes in coronary blood flow and prevent the development of platelet thrombi in the presence of a critical coronary artery stenosis [27]. Volatile anesthetics attenuate neutrophil and platelet aggregation [28] and also inhibit cytokine-induced cell death [29] after ischemia-reperfusion injury in coronary vessels.

The precise mechanisms responsible for anesthetic-induced myocardial protection against ischemia remain unclear despite extensive study. While it is clear that volatile anesthetics produce complex cardiovascular actions that may improve the myocardial oxygen supply and demand relationship, it is equally clear that such salutary alterations in the determinants of myocardial metabolism and coronary perfusion are not solely responsible for the anti-ischemic actions of these agents. For example, halothane confers protection during cardioplegic arrest [2] and upon reperfusion [30], circumstances in which myocardial metabolism plays little if any role. Isoflurane and sevoflurane also increase viability of isolated cardiac myocytes exposed to a cellular model of ischemia [31]. These results were initially attributed to reductions in excessive intracellular Ca^{2+} during ischemia and reperfusion [32] by partial inhibition of Ca^{2+} channel activity during administration of these drugs [33, 34, 35, 36]. However, these data did not address specific mechanisms by which anesthetics produced protection nor did they provide insight into the intracellular processes responsible for these beneficial effects.

MITOCHONDRIAL K_{ATP} CHANNELS: MEDIATORS OF IPC

K_{ATP} channels are heteromultimeric complexes consisting of an inward-rectifying K^+ channel (Kir) and a sulfonylurea receptor (SUR) [37]. Pharmacological and recombinant techniques indicate that the cardiac sarcolemmal K_{ATP} channel [38] is composed of the Kir6.2/SUR2A isoforms. In contrast, the mitochondrial K_{ATP} channel [39] consists of the Kir6.1/SUR1 isoforms [40]. It was originally hypothesized that opening of sarcolemmal K_{ATP} channels protects ischemic myocardium by shortening the action potential duration and preventing intracellular Ca^{2+} overload [38]. However, other studies indicated that the beneficial actions of K_{ATP} channel activation occurred independent of the action potential duration [41, 42]. In fact, the vast majority of recent evidence suggests that mitochondrial K_{ATP} channels are the dominant mediators of IPC [43] and further, that preservation of mitochondrial bioenergetic function is vital for cytoprotection against myocardial ischemia [44, 45, 46, 47]. Mitochondrial K_{ATP} channel openers maintain intracellular Ca^{2+} homeostasis and inhibit mitochondrial Ca^{2+} overload [46, 47]. These actions may enhance myocyte survival by preventing tissue necrosis or apoptosis [48]. Opening of mitochondrial K_{ATP} channels has been shown to inhibit apoptosis in rat ventricular myocytes [49] by attenuating oxidant stress upon reperfusion [50]. Alteration of the mitochondrial redox state by K_{ATP} channel opening may also promote cellular protection [47, 51]. Experiments conducted in isolated cardiac mitochondria [47] indicate that membrane depolarization, matrix swelling, and the uncoupling of ATP synthesis that occurs as a result of increased oxygen consumption are effects associated with the opening of mitochondrial K_{ATP} channels that may mediate cellular viability during IPC [51]. Opening of mitochondrial K_{ATP} channels depolarizes the inner mitochondrial membrane and causes a

transient swelling of the mitochondrial matrix [52], resulting from a shift in the ionic balance [53]. Mitochondrial K_{ATP} channel opening initially reduces ATP production as a result of membrane depolarization [47], but also subsequently stimulates a compensatory increase in respiration that optimizes oxidative phosphorylation efficiency in part through energy-dependent matrix volume regulation [54]. Thus, a moderate disturbance of mitochondrial homeostasis may promote myocardial tolerance to ischemic stress by altering energetic systems to reduce Ca^{2+} overload, prevent the activation of necrotic or apoptotic pathways, or attenuate oxidant stress.

SIGNAL TRANSDUCTION IN APC

K_{ATP} CHANNELS

Experimental evidence accumulated in the past seven years indicates that the endogenous mechanisms involved in APC bear striking similarity to those identified during IPC. It is hypothesized that a "trigger" initiates a cascade of signaling events leading to activation of an "end-effector" that is responsible for resistance to injury. K_{ATP} channel activation has been implicated as the end-effector (figure 2) in this protective scheme during APC, as these channels have been shown to play a fundamental role in mediating myocardial protection during IPC [55, 56, 57]. A recent investigation using a cellular model of ischemia reported that administration of isoflurane and sevoflurane preserved myocyte viability compared to cells that were not exposed to a volatile agent. This protective effect was abolished by the selective mitochondrial K_{ATP} channel antagonist 5-hydroxydecanoic acid (5-HD)

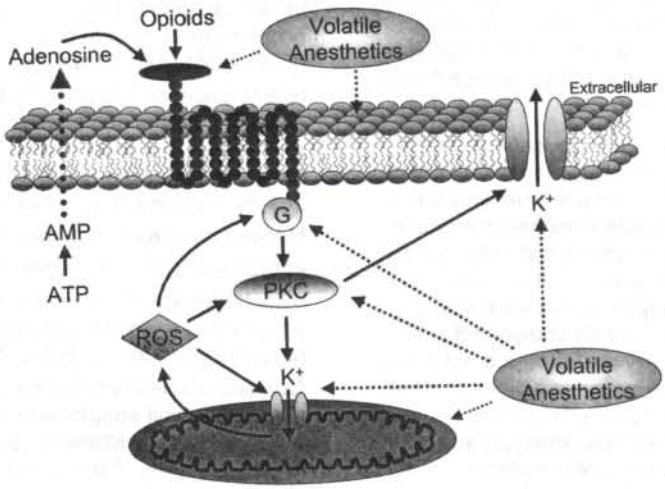

Figure 2. Schematic diagram illustrating volatile anesthetic-induced stimulation of endogenous signaling mechanisms responsible for myocardial protection. A "trigger" initiates a cascade of signal transduction events resulting in the activation of an "end-effector" that promotes resistance against cellular injury. Mitochondrial 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, or activate protein kinase C (PKC) and other intracellular kinases that subsequently open K_{ATP} channels. Volatile anesthetics also directly generate reactive oxygen species (ROS) in mitochondria that modulate K_{ATP} channel activity.

but not the selective sarcolemmal K_{ATP} channel antagonist HMR-1098 [31]. The nonselective K_{ATP} channel blocker glyburide completely attenuated the recovery of contractile function produced by isoflurane in stunned myocardium [13, 58]. Isoflurane-induced reductions in canine myocardial infarct size [15] and the ATP-sparing effects of this agent [59] are also abolished by glyburide. 5-HD inhibits preconditioning by isoflurane *in vivo* in rats [16], rabbits [60], and isolated human atria [61, 62]. Both HMR-1098 and 5-HD abolished the protective effects of desflurane in dogs [63], supporting a role for both sarcolemmal and mitochondrial K_{ATP} channels in APC. These latter data contrast to some degree with findings that implicate mitochondrial K_{ATP} channels alone as the predominant mediators of IPC [43].

Results from carefully conducted *in vitro* experiments using pharmacological techniques strongly suggested that volatile anesthetics directly activate K_{ATP} channels. Isoflurane stimulated outward K^+ current through sarcolemmal K_{ATP} channels in isolated ventricular myocytes during patch-clamping [64, 65]. Volatile anesthetics also reduced sarcolemmal K_{ATP} channel sensitivity to inhibition by ATP, thereby increasing the open state probability [66]. In contrast, other studies indicate volatile anesthetics alone were unable to elicit channel opening, but these agents did enhance sarcolemmal K_{ATP} channel current by facilitating channel opening after initial activation [64, 65]. Isoflurane and sevoflurane increased mitochondrial flavoprotein oxidation, an index of mitochondrial K_{ATP} channel activity, in guinea pig cardiac myocytes, and this endogenous autofluorescence was inhibited by 5-HD [67]. Isoflurane and sevoflurane also enhanced diazoxide-induced flavoprotein fluorescence in isolated rat ventricular myocytes [31]. Interestingly, isoflurane failed to augment K_{ATP} channel current in excised membrane patches [64]. These data suggest that volatile anesthetics may not directly interact with sarcolemmal K_{ATP} channels, but instead act upon other intracellular signaling elements that modulate K_{ATP} channel activity. In contrast to the findings with isoflurane, halothane had no effect on pinacidil-induced increases in sarcolemmal K_{ATP} channel current. Halothane also inhibited K_{ATP} channel current that had been maximally activated by 2,4-dinitrophenol [65]. Thus, the preponderance of evidence collected to date suggest that volatile anesthetics may not directly open K_{ATP} channels, but instead prime the activation of these channels in both sarcolemmal and mitochondrial membranes. This hypothesis is partially supported by the observation during IPC that adenosine facilitates K_{ATP} channel activation during myocardial ischemia through a PKC-dependent mechanism [68]. Taken as a whole, the experimental results with volatile anesthetics are consistent with previous findings demonstrating that pharmacological stimulation of K_{ATP} channels with other drugs mimics the conditions present during IPC [69].

Volatile anesthetics may activate parallel or redundant signal transduction 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 opener nicorandil [70] or diazoxide [71] markedly enhances protection against ischemic injury beyond that observed with either drug alone. Interestingly, the combination of isoflurane and a selective δ_1-opioid receptor agonist amplifies the preconditioning response in the rat [71]. This effect is synergistic, and is sensitive to inhibition by glyburide [71]. Recent experiments also demonstrate that combined administration of isoflurane and morphine also markedly reduces infarct size *in vivo*. This enhanced protective effect is abolished by 5-HD [16]. These data suggest that combined administration of a volatile anesthetic and an opioid may stimulate similar or cooperative signaling cascades that amplify K_{ATP} channel activation to profoundly augment myocardial protection beyond that produced by either drug alone.

K_{ATP} channels in vascular smooth muscle cells have been shown to be essential regulators

of coronary vascular tone when ATP production is reduced [72]. Volatile anesthetic-induced coronary vasodilation [11, 19, 20, 21, 22, 23] is attenuated by glyburide, indicating an important role for K_{ATP} channels in this process. Thus, it remains possible that the beneficial actions of APC may be partially attributed to increased oxygen supply mediated by K_{ATP} channel-dependent coronary vasodilation. However, sevoflurane increases coronary collateral blood flow in the presence of glyburide *in vivo*, indicating that volatile anesthetics enhance coronary collateral blood flow independent of K_{ATP} channel activation [23]. In fact, we have recently shown that sevoflurane-induced increases in collateral perfusion occurs as a result of Ca^{2+}-regulated potassium (BK_{Ca}) and not K_{ATP} channel activation [73]. In addition, isoflurane and sevoflurane directly activate mitochondrial K_{ATP} channels in isolated cardiac myocytes, and this action is linked to enhanced cell viability [31]. Based on these data, it appears highly unlikely that myocardial protection produced by volatile anesthetics is solely related to favorable alterations in coronary vascular tone mediated by K_{ATP} channels.

ADENOSINE AND OTHER G-PROTEIN COUPLED RECEPTORS

Several receptor-mediated events and intracellular signaling elements that converge on the K_{ATP} channel have been implicated in APC. Pertussis toxin abolished reductions in infarct size produced by isoflurane, indicating that inhibitory guanine (G_i) nucleotide-binding proteins are linked to the signal transduction pathways that mediate APC [74]. In contrast, pertussis toxin did not prevent the beneficial effects of direct K_{ATP} channel opening produced by nicorandil. These data strongly supported the contention that volatile anesthetics modulate K_{ATP} channel activity through a second messenger. Halothane-induced protection against infarction was completely abolished by blockade of the adenosine type 1 (A_1) receptor [6], and a selective A_1 receptor antagonist also partially attenuated the beneficial effects of isoflurane in stunned myocardium [75]. Isoflurane eliminated increases in interstitial adenosine during repetitive periods of coronary artery occlusion and reperfusion as demonstrated using a myocardial microdialysis technique [75]. These novel findings suggest that ATP preservation and a subsequent reduction of adenosine released into the interstitium occur during isoflurane anesthesia. The results are also very similar to findings during IPC [76] and pharmacological preconditioning produced by bimakalim [77]. The results further indicate that volatile anesthetics may either directly activate A_1 receptors or indirectly enhance A_1 receptor sensitivity to diminished endogenous adenosine concentrations [75]. The preservation of cardiac myocyte viability during ischemia produced by volatile anesthetics is also sensitive to adenosine receptor and G_i protein-mediated signaling blockade [31]. Additional findings indicate that the nonselective opioid antagonist naloxone abolishes isoflurane-induced preconditioning in the rat [16]. These intriguing data suggest an important link between volatile anesthetics and another family of G protein-coupled receptors. In fact, new evidence reveals that volatile agents competitively inhibit the ligand-binding site of G protein-coupled receptors [78]. Thus, APC appears to be associated with the activation of at least two separate receptor-mediated pathways (A_1 and δ-opioid) that are linked to G_i proteins.

PROTEIN KINASE C

APC may also produce translocation and phosphorylation of several protein kinases involved in signal transduction [79, 80, 81]. PKC is an essential component of the signaling pathway involved in protecting the myocardium against cell death after ischemia and reperfusion [82]. 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 [83]. Volatile anesthetics stimulate PKC translocation and activity [84], possibly by interacting with the regulatory domain of the enzyme[85]. Inhibition of PKC attenuates isoflurane-induced augmentation of the functional recovery of stunned myocardium in dogs [86]. The beneficial actions of halothane are entirely blocked by selective PKC antagonism in rabbits [6]. The microtubule depolymerizing drug, colchicine, prevents isoflurane-induced reductions

in myocardial infarct size in rabbits [87]. This interesting result suggests that an intact cytoskeleton may be essential for translocation of these protein kinases to occur.

Recent findings strongly suggest that volatile anesthetic-induced PKC translocation and activation is required to open K_{ATP} channels and produce myocardial protection. For example, the nonselective PKC antagonist chelerythrine abolished sevoflurane-induced increases in mitochondrial K_{ATP} channel activity in rat ventricular myocytes [31] and prevents protection from ischemic damage. Patch-clamp experiments demonstrate that isoflurane does not facilitate K_{ATP} channel opening in excised membrane patches, but enhances K_{ATP} channel current in a whole-cell configuration concomitant with PKC stimulation [64]. These observations are supported by additional evidence illustrating that both adenosine and PKC enhance K_{ATP} channel activity [71, 88, 89, 90, 91]. In fact, specific PKC consensus sites are located on K_{ATP} channels, indicating a molecular basis for phosphorylation and activation of the channel by the enzyme [92]. Mitochondrial K_{ATP} channel opening occurs after PKC activation during IPC in isolated rabbit hearts [93]. Other intracellular kinases may also be involved in the signaling pathways mediating the cellular response to ischemia because PKC stimulates tyrosine [94] and mitogen-activated protein (MAP) kinases [95] as well. Thus, volatile anesthetics also appear to modulate several critical intracellular signaling proteins independent of direct receptor activation (figure 2).

REACTIVE OXYGEN SPECIES

Reperfusion of ischemic myocardium is associated with the release of large quantities of reactive oxygen species (ROS) [96, 97, 98] that disrupt intracellular homeostasis, depress contractility, and produce tissue injury. Halothane, isoflurane, and enflurane has been shown to attenuate the toxic effects of oxygen-derived free radicals on left ventricular pressure development in isolated hearts [99]. Isoflurane decreases hydroxyl radical generation in the ischemic rat heart [100] and halothane has a similar effect in dogs [101]. The protective effects of sevoflurane are associated with reduced dityrosine formation, an indirect marker of reactive oxygen and nitrogen species [102]. These results support the contention that volatile anesthetics may reduce the release of deleterious quantities of reactive oxygen species immediately after coronary artery occlusion.

In contrast to these data implicating a pathological role of large amounts of ROS, new evidence strongly suggests that a variety of preconditioning stimuli, including brief ischemia, mitochondrial K_{ATP} channel openers, opioids, and volatile anesthetics, stimulate a small burst of ROS that paradoxically appears to initiate downstream signaling events and produce protection from subsequent ischemic injury. The beneficial actions of sevoflurane against ischemic damage are abolished by scavengers of superoxide and inhibition of nitric oxide synthase [102]. These results suggest that superoxide may act to trigger APC and also indicate that nitric oxide may scavenge superoxide upon reperfusion to reduce injury. ROS scavengers attenuate isoflurane-induced reductions in myocardial infarct size in rabbits [103, 104] as shown in figure 3, and also inhibit the salutary effects of IPC [105, 106] or mitochondrial K_{ATP} channel activation [107]. We have recently demonstrated that isoflurane directly increases superoxide formation *in vivo* (figure 4) independent of ischemia and reperfusion [104]. These data [104] indicate that volatile anesthetics are capable of producing small amounts of ROS that may exert protective effects during subsequent ischemia. Notably, pretreatment with low concentrations of ROS mimic the beneficial actions of IPC and reduce infarct size through a PKC-mediated mechanism [108]. These reports provide compelling evidence that small quantities of ROS play a critical role in APC.

Activation of mitochondrial K_{ATP} channels is associated with the ROS generation [109]. Mitochondrial K_{ATP} channel opening produced by selective agonists generates ROS that appear to be essential for activation of MAP kinase [110] and beneficial effects on myocardium [111]. Morphine increases cardiac myocyte viability and the fluorescence intensity of the hydrogen peroxide-sensitive probe, 2'7'-dichlorofluorescin. These actions are blocked by 5-HD [112], suggesting that activation of mitochondrial K_{ATP} channels by

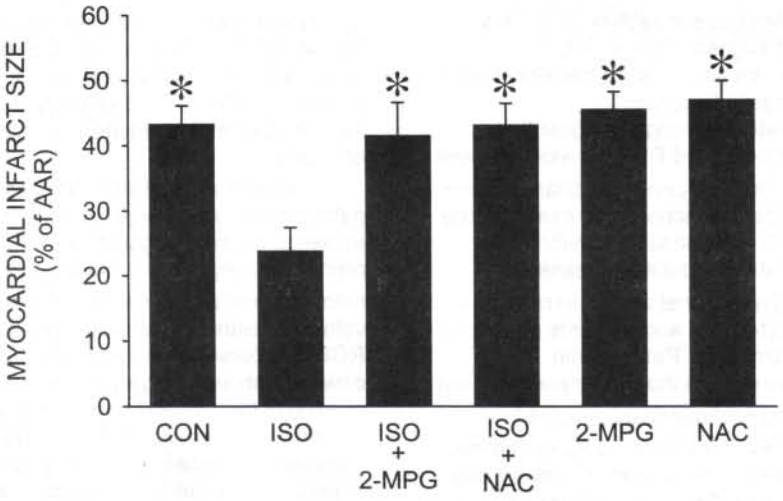

Figure 3. Histogram depicting myocardial infarct size expressed as the percentage of the left ventricular area at risk (AAR) in rabbits exposed to isoflurane in the presence and absence of the reactive oxygen species (ROS) scavengers N-acetylcysteine (NAC) and 2-mercaptopropionylglycine (2-MPG). NAC and 2-MPG attenuated isoflurane-induced myocardial protection (ISO + NAC and ISO + 2-MPG, respectively). Other groups: control (CON), isoflurane alone (ISO), NAC alone (NAC), 2-MPG alone (2-MPG). (Adapted with permission of authors and publisher [104].)

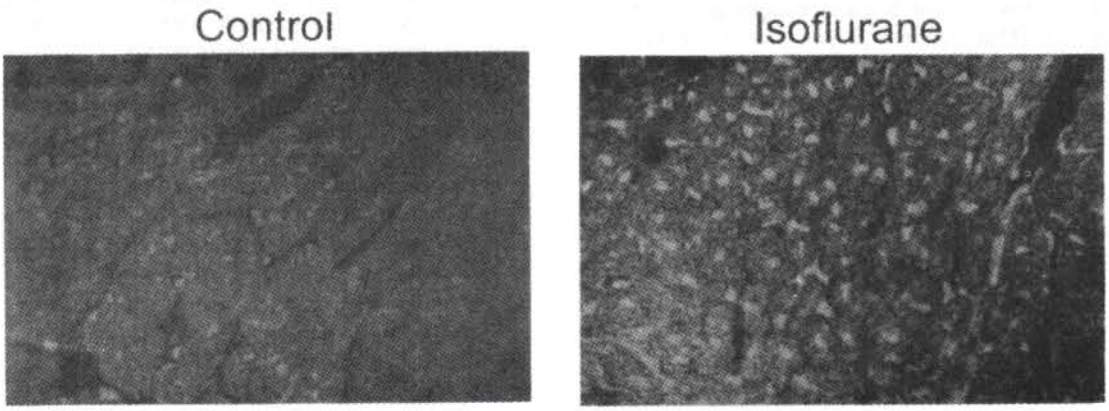

Figure 4. Photomicrographs demonstrating superoxide anion production using dihydroethidium fluorescence in rabbits. Isoflurane significantly ($P < 0.05$) increased fluorescence intensity of stained myocardial nuclei compared to control. (Adapted with permission of authors and publisher [104].)

opioids results in the ROS production. Mitochondria have been hypothesized to be a source of ROS production [106, 113, 114, 115, 116], but whether opening of mitochondrial K_{ATP} channels directly contribute to free radical generation remains unknown. Conversely, ROS may also modulate K_{ATP} channel activity [117] to provide a beneficial effect.

The identities of the ROS involved in APC and IPC and the signaling cascades that are modulated by these free radicals also remain unclear. ROS have been shown to activate PKC, restore contractility, and limit the extent of myocardial infarction [108]. Hydrogen peroxide stimulates tyrosine kinase-dependent activation of phospholipase C (PLC) in mouse embryonic fibroblasts, rendering these cells resistant to stress [118]. ROS may also directly stimulate PKC activity [119] or indirectly enhance the activity of the enzyme by activation of PLC [118]. Interestingly, hydrogen peroxide activates G_i and G_o proteins [120, 121], as well as other protein kinases involved in reducing cellular injury [110, 122, 123, 124]. Recent evidence suggests that hydrogen peroxide may be converted to more reactive species that modify cysteine residues specific to $G\alpha_i$ and $G\alpha_o$, resulting in selective activation of these proteins [120]. Therefore, volatile anesthetic-induced production of ROS may directly activate intracellular mediators of endogenous protection against ischemic injury (figure 2). This tantalizing hypothesis will require further research to confirm.

MYOCARDIAL PROTECTION BY VOLATILE ANESTHETICS IN HUMANS

Evidence accumulated to date strongly suggests that APC occurs in human myocardium, but evaluation of this process in patients is complicated by alterations in systemic and coronary hemodynamics, the use of other anesthetics, analgesics, or vasoactive drugs, preexisting disease states, and the influence of surgery on cardiovascular homeostasis. Isoflurane [62] and desflurane [125] enhance the recovery of contractile function of human atrial trabeculae by stimulation of adenosine receptors and opening of K_{ATP} channels [62]. Other studies have previously demonstrated a role for adenosine receptors, MAP kinases [61], and ROS [126] in other forms of preconditioning in human atrial myocytes concomitant with opening of mitochondrial K_{ATP} channels. Isoflurane increases the tolerance to pacing-induced ischemia in patients with coronary artery disease [127]. Isoflurane also decreases postoperative release of troponin I and creatine kinase-MB in patients undergoing coronary artery bypass graft (CABG) surgery, suggesting a reduction in the severity of myocardial necrosis [128]. Administration of enflurane before cardioplegic arrest also enhances postischemic contractile functional recovery in CABG patients [129]. Most recently, sevoflurane but not the intravenous anesthetic propofol was shown to preserve myocardial function in patients undergoing CABG concomitant with a reduction in troponin I release [130]. This compelling evidence emphasizes that volatile anesthetics exert beneficial effects against ischemic injury in humans and dispels the previously held contention that these agents may produced adverse redistribution of coronary collateral blood flow away from ischemic myocardium ("coronary steal") in certain patients with coronary artery disease. It has become increasingly clear that preexisting ischemia on arrival to the operating room and not anesthetic technique is the strongest predictor of intraoperative ischemia [131, 132]. However, volatile anesthetics may represent an important therapeutic modality to reduce the risk of perioperative myocardial ischemia and infarction [133], although this conclusion has yet to be confirmed in a large-scale, randomized, clinical trial.

SUMMARY

Experimental and clinical evidence indicates that volatile anesthetics exhibit important anti-ischemic actions that reduce the sequelae of reversible and irreversible ischemic injury. Several endogenous signaling elements mediate APC, but the mitochondrial K_{ATP} channel appears to be a central feature involved in maintaining function and promoting myocyte survival. Preservation of mitochondrial integrity and metabolic homeostasis by volatile anesthetics may ultimately enhance tolerance to myocardial ischemia. Translation of strong experimental data

about the protective effects of volatile anesthetics and other protective drugs, including opioids, into therapeutic approaches to reduce morbidity and mortality in patients with coronary artery disease remains to be established.

REFERENCES

1. Bland, J. H., and Lowenstein, E. 1976, Anesthesiology, 45, 287.
2. Lochner, A., Harper, I. S., Salie, R., Genade, S., and Coetzee, A. R. 1994, Anesth. Analg., 79, 226.
3. van Ackern, K., Vetter, H. O., Bruckner, U. B., Madler, C., Mittman, U., and Peter, K. 1985, Br. J. Anaesth., 57, 497.
4. Kanaya, N., and Fujita, S. 1994, Anesth. Analg., 79, 447.
5. Pagel, P. S., Hettrick, D. A., Lowe, D., Tessmer, J. P., and Warltier, D. C. 1995, Anesthesiology, 83, 1021.
6. Cope, D. K., Impastato, W. K., Cohen, M. V., and Downey, J. M. 1997, Anesthesiology, 86, 699.
7. Marijic, J., Stowe, D. F., Turner, L. A., Kampine, J. P., and Bosnjak, Z. J. 1990, Anesthesiology, 73, 976.
8. Coetzee, A., Brits, W., Genade, S., and Lochner, A. 1991, Anesth. Analg., 73, 711.
9. Coetzee, A., Skein, W., Genade, S., and Lochner, A. 1993, Anesth. Analg., 76, 602.
10. Freedman, B. M., Hamm, D. P., Everson, C. T., Wechsler, A. S., and Christian, C. M., 2nd. 1985, Anesthesiology, 62, 29.
11. Novalija, E., Fujita, S., Kampine, J. P., and Stowe, D. F. 1999, Anesthesiology, 91, 701.
12. Warltier, D. C., al-Wathiqui, M. H., Kampine, J. P., and Schmeling, W. T. 1988, Anesthesiology, 69, 552.
13. Kersten, J. R., Lowe, D., Hettrick, D. A., Pagel, P. S., Gross, G. J., and Warltier, D. C. 1996, Anesth. Analg., 83, 27.
14. Davis, R. F., DeBoer, L. W., Rude, R. E., Lowenstein, E., and Maroko, P. R. 1983, Anesthesiology, 59, 402.
15. Kersten, J. R., Schmeling, T. J., Pagel, P. S., Gross, G. J., and Warltier, D. C. 1997, Anesthesiology, 87, 361.
16. Ludwig, L. M., Gross, G. J., Kersten, J. R., Pagel, P. S., and Warltier, D. C. 2002, Anesthesiology, (In Press).
17. Cason, B. A., Gamperl, A. K., Slocum, R. E., and Hickey, R. F. 1997, Anesthesiology, 87, 1182.
18. Toller, W. G., Kersten, J. R., Pagel, P. S., Hettrick, D. A., and Warltier, D. C. 1999, Anesthesiology, 91, 1437.
19. Cason, B. A., Shubayev, I., and Hickey, R. F. 1994, Anesthesiology, 81, 1245.
20. Crystal, G. J., Gurevicius, J., Salem, M. R., and Zhou, X. 1997, Anesthesiology, 86, 448.
21. Zhou, X., Abboud, W., Manabat, N. C., Salem, M. R., and Crystal, G. J. 1998, Anesthesiology, 89, 182.
22. Crystal, G. J., Zhou, X., Gurevicius, J., Czinn, E. A., Salem, M. R., et al. 2000, Anesthesiology, 92, 1103.
23. Kersten, J. R., Schmeling, T., Tessmer, J., Hettrick, D. A., Pagel, P. S., and Warltier, D. C. 1999, Anesthesiology, 90, 246.
24. Kersten, J. R., and Warltier, D. C. 1997, Anesthesia: Biologic Foundations, TL Yaksh, C Lynch III, WM Zapol, M Maze, JF Biebuyck, LJ Saidman, eds., Lippincott-Raven, Philadelphia, 1169.
25. Smith, G., Rogers, K., and Thorburn, J. 1980, Br. J. Anaesth., 52, 577.
26. Kersten, J. R., Brayer, A. P., Pagel, P. S., Tessmer, J. P., and Warltier, D. C. 1994, Anesthesiology, 81, 995.
27. Bertha, B. G., Folts, J. D., Nugent, M., and Rusy, B. F. 1989, Anesthesiology, 71, 96.
28. Kowalski, C., Zahler, S., Becker, B. F., Flaucher, A., Conzen, P. F., et al. 1997, Anesthesiology, 86, 188.
29. de Klaver, M. J., Manning, L., Palmer, L. A., and Rich, G. F. 2002, Anesthesiology, 97, 24.
30. Schlack, W., Preckel, B., Barthel, H., Obal, D., and Thamer, V. 1997, Br. J. Anaesth., 79, 88.
31. Zaugg, M., Lucchinetti, E., Spahn, D. R., Pasch, T., and Schaub, M. C. 2002, Anesthesiology, 97, 4.
32. An, J., Varadarajan, S. G., Novalija, E., and Stowe, D. F. 2001, Am J Physiol Heart Circ Physiol, 281, H1508.
33. Eskinder, H., Rusch, N. J., Supan, F. D., Kampine, J. P., and Bosnjak, Z. J. 1991,

Anesthesiology, 74, 919.
34. Bosnjak, Z. J., Aggarwal, A., Turner, L. A., Kampine, J. M., and Kampine, J. P. 1992, Anesthesiology, 76, 123.
35. Lynch, C., 3rd. 1988, Anesthesiology, 68, 429.
36. Hatakeyama, N., Momose, Y., and Ito, Y. 1995, Anesthesiology, 82, 559.
37. Inagaki, N., Gonoi, T., Clement, J. P. t., Namba, N., Inazawa, J., et al. 1995, Science, 270, 1166.
38. Noma, A. 1983, Nature, 305, 147.
39. Inoue, I., Nagase, H., Kishi, K., and Higuti, T. 1991, Nature, 352, 244.
40. Liu, Y., Ren, G., O'Rourke, B., Marban, E., and Seharaseyon, J. 2001, Mol. Pharmacol., 59, 225.
41. Yao, Z., and Gross, G. J. 1994, Circulation, 89, 1769.
42. Hamada, K., Yamazaki, J., and Nagao, T. 1998, J. Mol. Cell. Cardiol., 30, 1369.
43. Sato, T., Sasaki, N., Seharaseyon, J., O'Rourke, B., and Marban, E. 2000, Circulation, 101, 2418.
44. Dos Santos, P., Kowaltowski, A. J., Laclau, M. N., Seetharaman, S., Paucek, P., et al. 2002, Am J Physiol Heart Circ Physiol, 283, H284.
45. Dzeja, P. P., Holmuhamedov, E. L., Ozcan, C., Pucar, D., Jahangir, A., and Terzic, A. 2001, Circ. Res., 89, 744.
46. Holmuhamedov, E. L., Wang, L., and Terzic, A. 1999, J Physiol, 519 Pt 2, 347.
47. Holmuhamedov, E. L., Jovanovic, S., Dzeja, P. P., Jovanovic, A., and Terzic, A. 1998, Am. J. Physiol., 275, H1567.
48. Green, D. R., and Reed, J. C. 1998, Science, 281, 1309.
49. Akao, M., Ohler, A., O'Rourke, B., and Marban, E. 2001, Circ. Res., 88, 1267.
50. Ozcan, C., Bienengraeber, M., Dzeja, P. P., and Terzic, A. 2002, Am J Physiol Heart Circ Physiol, 282, H531.
51. Minners, J., Lacerda, L., McCarthy, J., Meiring, J. J., Yellon, D. M., and Sack, M. N. 2001, Circ. Res., 89, 787.
52. Halestrap, A. P. 1989, Biochim. Biophys. Acta, 973, 355.
53. Garlid, K. D. 1996, Biochim. Biophys. Acta, 1275, 123.
54. Garlid, K. D. 1980, J. Biol. Chem., 255, 11273.
55. Gross, G. J., and Auchampach, J. A. 1992, Circ. Res., 70, 223.
56. Kersten, J. R., Gross, G. J., Pagel, P. S., and Warltier, D. C. 1998, Anesthesiology, 88, 495.
57. O'Rourke, B. 2000, Circ. Res., 87, 845.
58. Kersten, J. R., Schmeling, T. J., Hettrick, D. A., Pagel, P. S., Gross, G. J., and Warltier, D. C. 1996, Anesthesiology, 85, 794.
59. Nakayama, M., Fujita, S., Kanaya, N., Tsuchida, H., and Namiki, A. 1997, Acta Anaesthesiol. Scand., 41, 531.
60. Piriou, V., Chiari, P., Knezynski, S., Bastien, O., Loufoua, J., et al. 2000, Anesthesiology, 93, 756.
61. Carroll, R., and Yellon, D. M. 2000, Basic Res. Cardiol., 95, 243.
62. Roscoe, A. K., Christensen, J. D., and Lynch, C., 3rd. 2000, Anesthesiology, 92, 1692.
63. Toller, W. G., Gross, E. R., Kersten, J. R., Pagel, P. S., Gross, G. J., and Warltier, D. C. 2000, Anesthesiology, 92, 1731.
64. Fujimoto, K., Bosnjak, Z. J., and Kwok, W. M. 2002, Anesthesiology, 97, 57.
65. Kwok, W. M., Martinelli, A. T., Fujimoto, K., Suzuki, A., Stadnicka, A., and Bosnjak, Z. J. 2002, Anesthesiology, 97, 50.
66. Han, J., Kim, E., Ho, W. K., and Earm, Y. E. 1996, Biochem. Biophys. Res. Commun., 229, 852.
67. Kohro, S., Hogan, Q. H., Nakae, Y., Yamakage, M., and Bosnjak, Z. J. 2001, Anesthesiology, 95, 1435.
68. Liu, Y., Gao, W. D., O'Rourke, B., and Marban, E. 1997, Am. J. Physiol., 273, H1637.
69. Yao, Z., Mizumura, T., Mei, D. A., and Gross, G. J. 1997, Am. J. Physiol., 272, H334.
70. Piriou, V., Ross, S., Pigott, D., Evans, R., and Foex, P. 1997, Br. J. Anaesth., 79, 68.
71. Patel, H. H., Ludwig, L. M., Fryer, R. M., Hsu, A. K., Warltier, D. C., and Gross, G. J. 2002, FASEB J., 16, 1468.
72. Daut, J., Maier-Rudolph, W., von Beckerath, N., Mehrke, G., Gunther, K., and Goedel-Meinen, L. 1990, Science, 247,1341.

73. Kehl, F., Krolikowski, J. G., Tessmer, J. P., Pagel, P. S., Warltier, D. C., and Kersten, J. R. 2002, Anesthesiology, 97, 725.
74. Toller, W. G., Kersten, J. R., Gross, E. R., Pagel, P. S., and Warltier, D. C. 2000, Anesthesiology, 92, 1400.
75. Kersten, J. R., Orth, K. G., Pagel, P. S., Mei, D. A., Gross, G. J., and Warltier, D. C. 1997, Anesthesiology, 86, 1128.
76. Van Wylen, D. G. 1994, Circulation, 89, 2283.
77. Mizumura, T., Nithipatikom, K., and Gross, G. J. 1995, Circulation, 92, 1236.
78. Ishizawa, Y., Pidikiti, R., Liebman, P. A., and Eckenhoff, R. G. 2002, Mol. Pharmacol., 61, 945.
79. Fryer, R. M., Pratt, P. F., Hsu, A. K., and Gross, G. J. 2001, J. Pharmacol. Exp. Ther., 296, 642.
80. Fryer, R. M., Patel, H. H., Hsu, A. K., and Gross, G. J. 2001, Am J Physiol Heart Circ Physiol, 281, H1184.
81. Fryer, R. M., Schultz, J. E., Hsu, A. K., and Gross, G. J. 1999, Am. J. Physiol., 276, H1229.
82. Liu, H., McPherson, B. C., and Yao, Z. 2001, Am J Physiol Heart Circ Physiol, 281, H404.
83. Puceat, M., and Vassort, G. 1996, Mol. Cell. Biochem., 157, 65.
84. Hemmings, H. C., Jr., and Adamo, A. I. 1996, Anesthesiology, 84, 652.
85. Hemmings, H. C., Jr. 1998, Toxicol. Lett., 100-101, 89.
86. Toller, W. G., Montgomery, M. W., Pagel, P. S., Hettrick, D. A., Warltier, D. C., and Kersten, J. R. 1999, Anesthesiology, 91, 713.
87. Ismaeil, M. S., Tkachenko, I., Hickey, R. F., and Cason, B. A. 1999, Anesthesiology, 91, 1816.
88. Sato, T., O'Rourke, B., and Marban, E. 1998, Circ. Res., 83, 110.
89. Hu, K., Duan, D., Li, G. R., and Nattel, S. 1996, Circ. Res., 78, 492.
90. Liu, Y., Gao, W. D., O'Rourke, B., and Marban, E. 1996, Circ. Res., 78, 443.
91. Sato, T., Sasaki, N., O'Rourke, B., and Marban, E. 2000, Circulation, 102, 800.
92. Light, P. E., Bladen, C., Winkfein, R. J., Walsh, M. P., and French, R. J. 2000, Proc. Natl. Acad. Sci. U. S. A., 97, 9058.
93. Ohnuma, Y., Miura, T., Miki, T., Tanno, M., Kuno, A., et al. 2002, Am J Physiol Heart Circ Physiol, 283, H440.
94. Baines, C. P., Wang, L., Cohen, M. V., and Downey, J. M. 1998, J. Mol. Cell. Cardiol., 30, 383.
95. Ping, P., Zhang, J., Cao, X., Li, R. C., Kong, D., et al. 1999, Am. J. Physiol., 276, H1468.
96. Ambrosio, G., Zweier, J. L., Duilio, C., Kuppusamy, P., Santoro, G., et al. 1993, J. Biol. Chem., 268, 18532.
97. Bolli, R., Patel, B. S., Jeroudi, M. O., Lai, E. K., and McCay, P. B. 1988, J. Clin. Invest., 82, 476.
98. Zweier, J. L., Flaherty, J. T., and Weisfeldt, M. L. 1987, Proc. Natl. Acad. Sci. U. S. A., 84, 1404.
99. Tanguay, M., Blaise, G., Dumont, L., Beique, G., and Hollmann, C. 1991, J. Cardiovasc. Pharmacol., 18, 863.
100. Nakamura, T., Kashimoto, S., Oguchi, T., and Kumazawa, T. 1999, Can. J. Anaesth., 46, 470.
101. Glantz, L., Ginosar, Y., Chevion, M., Gozal, Y., Elami, A., et al. 1997, Anesthesiology, 86, 440.
102. Novalija, E., Varadarajan, S. G., Camara, A. K., An, J., Chen, Q., et al. 2002, Am J Physiol Heart Circ Physiol, 283, H44.
103. Mullenheim, J., Ebel, D., Frassdorf, J., Preckel, B., Thamer, V., and Schlack, W. 2002, Anesthesiology, 96, 934.
104. Tanaka, K., Weihrauch, D., Kehl, F., Ludwig, L. M., LaDisa Jr, J. F., et al. 2002, Anesthesiology, (In Press).
105. Tanaka, M., Fujiwara, H., Yamasaki, K., and Sasayama, S. 1994, Cardiovasc. Res., 28, 980.
106. Baines, C. P., Goto, M., and Downey, J. M. 1997, J. Mol. Cell. Cardiol., 29, 207.
107. Pain, T., Yang, X. M., Critz, S. D., Yue, Y., Nakano, A., et al. 2000, Circ. Res., 87, 460.
108. Tritto, I., D'Andrea, D., Eramo, N., Scognamiglio, A., De Simone, C., et al. 1997, Circ. Res., 80, 743.
109. Obata, T., and Yamanaka, Y. 2000, Arch. Biochem. Biophys., 378, 195.
110. Samavati, L., Monick, M. M., Sanlioglu, S.,

Buettner, G. R., Oberley, L. W., and Hunninghake, G. W. 2002, Am J Physiol Cell Physiol, 283, C273.
111. Forbes, R. A., Steenbergen, C., and Murphy, E. 2001, Circ. Res., 88, 802.
112. McPherson, B. C., and Yao, Z. 2001, Circulation, 103, 290.
113. Paraidathathu, T., de Groot, H., and Kehrer, J. P. 1992, Free Radic. Biol. Med., 13, 289.
114. Vanden Hoek, T. L., Shao, Z., Li, C., Schumacker, P. T., and Becker, L. B. 1997, J. Mol. Cell. Cardiol., 29, 2441.
115. Vanden Hoek, T. L., Becker, L. B., Shao, Z., Li, C., and Schumacker, P. T. 1998, J. Biol. Chem., 273, 18092.
116. Becker, L. B., Vanden Hoek, T. L., Shao, Z. H., Li, C. Q., and Schumacker, P. T. 1999, Am. J. Physiol., 277, H2240.
117. Zhang, D. X., Chen, Y. F., Campbell, W. B., Zou, A. P., Gross, G. J., and Li, P. L. 2001, Circ. Res., 89, 1177.
118. Wang, X. T., McCullough, K. D., Wang, X. J., Carpenter, G., and Holbrook, N. J. 2001, J. Biol. Chem., 276, 28364.
119. Gopalakrishna, R., and Jaken, S. 2000, Free Radic. Biol. Med., 28, 1349.
120. Nishida, M., Schey, K. L., Takagahara, S., Kontani, K., Katada, T., et al. 2002, J. Biol. Chem., 277, 9036.
121. Nishida, M., Maruyama, Y., Tanaka, R., Kontani, K., Nagao, T., and Kurose, H. 2000, Nature, 408, 492.
122. Maulik, N., Watanabe, M., Zu, Y. L., Huang, C. K., Cordis, G. A., et al. 1996, FEBS Lett., 396, 233.
123. Aikawa, R., Komuro, I., Yamazaki, T., Zou, Y., Kudoh, S., et al. 1997, J. Clin. Invest., 100, 1813.
124. Dabrowski, A., Boguslowicz, C., Dabrowska, M., Tribillo, I., and Gabryelewicz, A. 2000, Pancreas, 21, 376.
125. Hanouz, J. L., Yvon, A., Massetti, M., Lepage, O., Babatasi, G., et al. 2002, Anesthesiology, 97, 33.
126. Carroll, R., Gant, V. A., and Yellon, D. M. 2001, Cardiovasc. Res., 51, 691.
127. Tarnow, J., Markschies-Hornung, A., and Schulte-Sasse, U. 1986, Anesthesiology, 64, 147.
128. Belhomme, D., Peynet, J., Louzy, M., Launay, J. M., Kitakaze, M., and Menasche, P. 1999, Circulation, 100, II340.
129. Penta de Peppo, A., Polisca, P., Tomai, F., De Paulis, R., Turani, F., et al. 1999, Ann. Thorac. Surg., 68, 112.
130. De Hert, S. G., ten Broecke, P. W., Mertens, E., Van Sommeren, E. W., De Blier, I. G., et al. 2002, Anesthesiology, 97, 42.
131. Knight, A. A., Hollenberg, M., London, M. J., Tubau, J., Verrier, E., et al. 1988, Anesthesiology, 68, 681.
132. Slogoff, S., and Keats, A. S. 1989, Anesthesiology, 70, 179.
133. Warltier, D. C., Pagel, P. S., and Kersten, J. R. 2000, Anesthesiology, 92, 253.