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Forum Review

Cardiac Preconditioning by Volatile Anesthetic Agents: A Defining Role for Altered Mitochondrial Bioenergetics

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ABSTRACT

Volatile anesthetic agents, such as halothane, isoflurane, and sevoflurane, are the drugs most commonly used to maintain the state of general anesthesia. They have long been known to provide some protection against the effects of cardiac ischemia and reperfusion. Several mechanisms likely contribute to this cardioprotection, including coronary vasodilation, reduced contractility with corresponding decreased metabolic demand, and a direct effect to decrease myocardial Ca^{2+} entry through L-type Ca^{2+} channels. Recently, a memory phase to cardioprotection has been observed by these agents, which is inhibited by ATP-sensitive potassium channel inhibition. These features suggest a pathway that shares components with those required for ischemic preconditioning, despite the remarkable differences between these two stimuli, and the term anesthetic preconditioning (APC) has been adopted. Scavengers of reactive oxygen species (ROS) abrogate APC, suggesting an effect of anesthetic agents to cause ROS formation. Such an effect has recently been directly demonstrated. The mechanism by which these drugs induce ROS formation is unclear. However, direct inhibition of mitochondrial electron transport system enzymes, and altered mitochondrial bioeneregtics in hearts preconditioned by volatile anesthetics, strongly implicate the mitochondria as the target for these effects. Furthermore, decreased mitochondrial ROS formation during ischemia and reperfusion in hearts preconditioned by volatile anesthetics might underlie the improved postischemic structure and function. APC presents a safe mode to apply preconditioning to human hearts. This review summarizes the major developments in a field that is exciting to clinicians and basic scientists alike. *Antioxid. Redox Signal.* 6, 439–448.

CARDIAC PRECONDITIONING

In 1997, Cope *et al.* (13), Kersten *et al.* (42), and Cason *et al.* (12) demonstrated that transient exposure of animal hearts to potent volatile anesthetics led to a state of partial protection from the effects of ischemia and reperfusion. This protection was characterized by a memory phase (*i.e.,* was intact after discontinuation of the anesthetic) and was inhibited by the ATP-sensitive potassium (K_{ATP}) channel inhibitor glibenclamide. These features, and the magnitude of the protection obtained, suggested a commonality with ischemic preconditioning (IPC), and the term anesthetic preconditioning (APC) was adopted. APC soon became a focus of intense interest for the anesthesia research community.

The ability of volatile anesthetic agents to decrease infarction in animal models, and the long record of safety of these drugs in skilled hands, place APC as a potential prophylactic therapy for use in the perioperative period. The majority of perioperative deaths occur as the result of an acute myocardial infarction in patients with preexisting ischemic heart dis-

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ease. With a rapidly aging national demography and a steadily increasing incidence of diabetes mellitus, the population presenting for major surgery will increasingly feature patients at high risk for such adverse cardiac events. Further spurring the interest of anesthesiologists was the finding that this phenomenon was confirmed in human myocardium (5, 33, 71), and evidence was provided of meaningful clinical benefits, albeit in trials involving small numbers of patients, when comparing volatile anesthetics with alternative anesthetic agents that do not induce preconditioning (16).

Several essential mediators of APC signaling have been identified, including K_{ATP} channels (42), protein kinase C (PKC) (58, 84), and more recently, reactive oxygen species (ROS) (44, 53, 59, 79). Several anesthetic agents in common use, including barbiturates and the phenol propofol, act as free radical scavengers, a feature long presumed by clinicians to be universally beneficial. Interesting new evidence directly demonstrates increased generation of ROS in intact hearts during exposure to volatile anesthetics (44) and in cardiac tissue slices following exposure (79). The mechanism by which these agents generate ROS is just becoming unraveled, but remarkably, over 30 years ago, a direct effect of anesthetic agents on mitochondrial bioenergetics was first described (54). The possible implications of this discovery are now becoming apparent. In this discussion, we briefly review the evidence supporting cardioprotective effects of volatile anesthetics and we attempt to place in perspective the potential for clinical application of this phenomenon. Particular emphasis will be placed on the mechanisms by which anesthetic agents are postulated to trigger ROS formation to induce APC, and on emerging evidence that links altered mitochondrial bioenergetics with induction of cardioprotection by anesthetic agents. Initially, background information on these drugs is provided for the nonanesthesiologist.

VOLATILE ANESTHETICS AND CARDIAC ISCHEMIA/REPERFUSION

The potent volatile anesthetic agents are a class of nonflammable halogenated hydrocarbons that induce unconsciousness following inhalation. Since the introduction of the prototype, halothane, in 1956, they have remained by far the most widely used agents for maintenance of the state of general anesthesia, offering several advantages over intravenous agents, including low cost, easy titratability, and predictability of hemodynamic effects. The volatile anesthetic agents currently available include halothane, isoflurane, enflurane, desflurane, and sevoflurane (Fig. 1). Although broadly similar in clinical effects, these agents differ in a number of respects, primarily in their pharmacokinetic profiles. Despite almost 50 years of investigation, the mechanism of general anesthetic action of these drugs remains unclear. There is evidence of action at protein receptors (22) causing release of inhibitory neurotransmitters (50). But an extremely close relationship between lipid solubility and anesthetic potency of these agents (67), and lack of stereospecific effect of different isomers (28), are proposed to support a nonspecific effect on neuronal and all other cell membranes. The

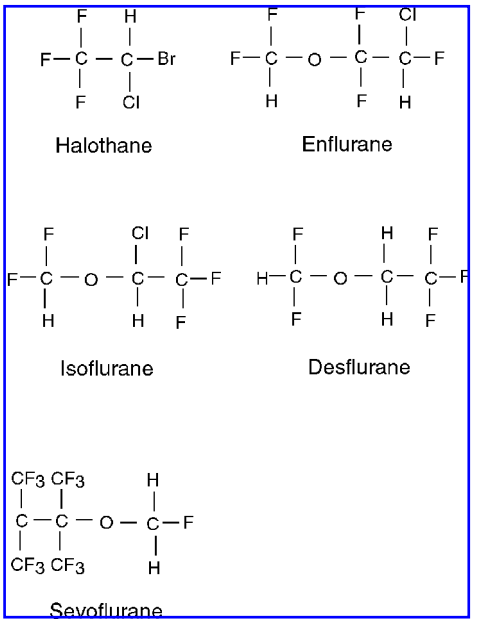

FIG. 1. Chemical structures of volatile anesthetic agents in use today. Each has moderately different pharmacokinetic and pharmacodynamic characteristics.

detailed pharmacology of these agents is reviewed in standard textbooks on anesthesiology (51).

Volatile anesthetics have a generally predictable and dose-dependent inhibitory effect on cardiac function, but the magnitude of cardiac effect does not necessarily parallel potency to induce unconsciousness. They each cause dose-dependent myocardial depression, particularly halothane, but the newer agents do so to a lesser extent. This occurs because of cellular effects at multiple levels. Ca^{2+} entry through L-type Ca^{2+} channels is decreased (8), thus attenuating Ca^{2+}-dependent Ca^{2+} release and decreasing Ca^{2+} storage in the sarcoplasmic reticulum. Myofilament Ca^{2+} sensitivity is reduced although this effect appears to be small (14), and there is a direct inhibitory effect on formed actin–myosin cross-bridges (3). Although these effects lead to negative inotropy and decreased cardiac output, diastolic function is not directly impaired (62).

Volatile anesthetics directly dilate coronary arteries due to attenuation of Ca^{2+} entry through voltage-gated Ca^{2+} channels on vascular smooth muscle (9). In addition, it was recently reported that halothane and desflurane, but not isoflurane, increased release of nitric oxide ($NO^{\bullet}$) in rabbit coronary arteries (4). Despite these effects, coronary flow tends to decrease because the decreased myocardial oxygen consumption, secondary to negative inotropy, leads to coronary vasoconstriction by innate autoregulatory mechanisms. Automaticity is decreased at all levels of the conducting system due to complex effects on sarcolemmal ion channels (19); the cardiac action potential is shortened and the atrioventricular refractory period is prolonged, and a variety of arrhythmias are seen during general anesthesia with these agents.

Reports of protective effects of volatile anesthetics against myocardial ischemia and reperfusion injury predate those of

IPC, or of any pharmacologic agents now known to induce preconditioning (10, 23, 74). The possibility of a memory phase to this cardioprotection was, however, not recognized at the time. Indeed, it is not possible to discern from those early reports if cardioprotection was due to activation of signaling pathways now postulated to induce APC, or if it was due to effects of anesthetics to directly improve myocardial oxygen supply/demand ratios, direct effects on sarcolemmal Ca^{2+} entry, or other direct effects that might attenuate ischemia and reperfusion injury. Decreased myocardial oxygen consumption is proposed to account, at least in part, for cardioprotection due to volatile anesthetics (10, 76), although Oguchi *et al.* (60) found no effect in ischemic hearts. In any event, delivery of anesthetic or other drug to myocardium will necessarily cease during ischemia. Moreover, the high vapor pressure of these anesthetics makes it is highly unlikely that they can remain in the myocardium in effective doses during ischemia to significantly improve oxygen supply/demand ratios or to have other effects postulated to be protective.

In those early experimental studies, volatile anesthetics were administered up to the initiation of ischemia, but anesthetics were also frequently administered to residually perfused myocardium during the ischemic period in regional ischemia (coronary ligation) models. In addition, it was presumed necessary to deliver the agent at reperfusion to attenuate reperfusion-induced injury; indeed, delivery of halothane (73) or sevoflurane (87) only at reperfusion was found to be protective. In support of the belief that anesthetic exposure during reperfusion was essential for cardioprotection, one of the few studies that did not find a protective effect involved administration of halothane or isoflurane prior to ischemia but not at reperfusion in a rabbit model (49). However, the duration of ischemia, 15 min, may not have induced sufficient injury to demonstrate a difference. We have recently demonstrated that protection by sevoflurane cannot be demonstrated in guinea pig hearts if ischemia is less than 25 min duration (43).

ANESTHETIC PRECONDITIONING

In 1997, Cope *et al.* (13), Kersten *et al.* (42), and Cason *et al.* (12) administered a volatile anesthetic to animal hearts followed by washout of the anesthetic prior to initiation of ischemia. These investigators found reduced infarction after ischemia in isolated rabbit hearts, intact dogs, and intact rabbits, respectively. In addition, abrogation of the protective effects of volatile anesthetics occurred when an adenosine receptor antagonist (13), a PKC inhibitor (13), or a K_{ATP} channel blocker (42) was administered with the anesthetic. These studies established that the signaling of cardioprotection due to these agents bore important similarities to IPC, despite the marked difference of these two initiating stimuli.

Requirement for PKC during APC was confirmed by subsequent studies (39, 58, 84); the specific PKC isoform involved in APC appears to be ε, but not δ (58). It was also proposed that mitochondrial, rather than or in addition to sarcolemmal, K_{ATP} channels are required for APC (33, 34, 44, 66, 69, 70). All these studies utilized 5-hydroxydecanoate (5-HD) to inhibit the mitochondrial K_{ATP} channel, but the specificity of 5-HD for this channel has been questioned (30, 32). Nonetheless, there is evidence that both forms of the channel may be involved (27, 85).

APC holds an obvious advantage over IPC from the practical standpoint that volatile anesthetics can be easily and safely administered. This is in contrast to the brief application of ischemia, which is either impossible or perilous in most clinical circumstances. Animal studies suggest effective APC can be achieved at anesthetic doses commensurate with those used clinically, although the degree of protection may increase at higher doses (41). Several studies have demonstrated that APC can occur in human cardiac tissue. Hanouz *et al.* (33) exposed human atrial trabeculae to desflurane in a tissue bath prior to anoxia; recovery of contractile force was improved by desflurane pretreatment, and this effect was abrogated by glibenclamide or 5-HD. Roscoe *et al.* (71) demonstrated protective effects of sevoflurane, but not halothane, in human atrial trabeculae; glibenclamide or the adenosine-receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) abrogated this effect of sevoflurane. Human *in vivo* studies also support the contention that APC may find practical application. Belhomme *et al.* (5) randomized 10 patients undergoing coronary artery bypass grafting to isoflurane pretreatment, before aortic cross-clamping, and compared per-operative release of myocardial-bound fraction of creatine kinase and troponin I with those of 10 control patients. Anesthesia was provided throughout with intravenous agents. In the isoflurane-treated patients, release of cardiac enzymes was significantly decreased compared with control patients. However, the study was insufficiently powered to demonstrate differences in survival or postoperative contractile function. Haroun-Bizri *et al.* (35) performed a similar study in 49 patients undergoing coronary artery bypass grafting. Patients treated with isoflurane prior to aortic cross-clamping were found to have increased cardiac index and fewer episodes of ischemia by electrocardiographic criteria than control patients. Larger trials will be necessary, however, to ascertain if APC is sufficiently protective in human patients.

APC IS INITIATED BY ROS AND OPENING OF K_{ATP} CHANNELS

Recent studies support a role for ROS in APC, both in the signaling pathways during the exposure phase through generation of ROS, and in mediating protection during the ischemia and reperfusion phase through a decrease in ROS formation. Müllenheim *et al.* (53) investigated the requirement for ROS in APC in instrumented rabbits and probed for a suspected commonality of APC as noted for IPC (2). APC was achieved with isoflurane pretreatment before 30 min of occlusion of a major coronary artery and 2 h of reperfusion; in two additional groups, the ROS scavengers, *N*-(2-mercaptopropionyl)glycine (MPG) and Mn(III)tetrakis(4-benzoic acid)porphyrin chloride (MnTBAP), were given during the isoflurane pretreatment phase. Whereas isoflurane alone produced a reduction in infarct size compared with controls (29 ± 19% versus 49 ± 17%, $p = 0.04$), both scavengers abrogated protection, leading to

infarct sizes similar to controls (isoflurane + MPG, 50 ± 24%, $p = 0.02$; isoflurane + MnTBAP, 55 ± 10%, $p = 0.001$). ROS production, however, was not measured.

Novalija *et al.* (57) preconditioned isolated guinea pig hearts with sevoflurane prior to 30 min of global ischemia and 2 h of reperfusion. APC was manifested by improved contractile function and coronary flow, and by a decreased infarct size compared with controls. When a mixture of ROS scavengers, superoxide dismutase (SOD), catalase, and glutathione, or the nitric oxide synthase inhibitor *N*ω-nitro-L-arginine methyl ester (L-NAME) was given during the sevoflurane pretreatment phase, protection was abrogated in all respects. Kevin *et al.* (44) similarly preconditioned guinea pig isolated hearts with sevoflurane, and showed that APC was abrogated by MnTBAP. Tanaka *et al.* (79) found similarly that MPG or *N*-acetylcysteine abrogated isoflurane-induced APC in intact rabbits. They used oxidation of dihydroethidium (DHE) to fluorescent ethidium as a measure of ROS in instrumented rabbits exposed to 30 min of isoflurane (79); 30 min later, the rabbits were euthanized, the hearts were excised and frozen in liquid N_2, and cryostat sections were examined using a laser fluorescence system. Fluorescent intensity was significantly greater in myocardial nuclei of isoflurane-pretreated hearts compared with controls. Each of the above studies provides direct or indirect evidence that ROS are generated in response to volatile anesthetics and suggests that ROS are a component of the triggering process of preconditioning by anesthetics.

The evidence is compelling that both ROS and K_{ATP} channel opening are required for cardiac preconditioning. But how ROS and K_{ATP} channels interact to produce either IPC or APC is not clear. Some investigators contend that ROS activate K_{ATP} channel opening (21, 82, 83, 90), whereas others support the idea that K_{ATP} channel opening causes ROS generation (11, 27, 63). The question remains then whether volatile anesthetics first induce ROS generation, which then leads to K_{ATP} channel opening, or the opposite. Tanaka *et al.* (80), in a more recent study, suggested that K_{ATP} channel opening precedes ROS generation because 5-HD, given as a bolus to dogs 10 min before 30 min of isoflurane and 60 min of washout, prevented the isoflurane-induced increase in DHE fluorescence in tissue frozen for later analysis; 5-HD also blocked APC. However, it is possible that this protocol allowed enough time for initiation of APC by ROS, mediation by kinase cascades, and opening of K_{ATP} channels. 5-HD may not have been washed out.

Temporal and more direct evidence for ROS as a trigger for APC in guinea pig intact hearts was provided by our laboratory (44). DHE was used to measure ROS, primarily $O_2^{\bullet -}$, spectrophotometrically in real time by placing a fiber-optic probe against the free wall of the left ventricle. Sevoflurane itself caused an immediate and reversible increase in ethidium fluorescence that was ~10% of that produced by 5 min of ischemia and reperfusion. MnTBAP obliterated the sevoflurane-induced increase in fluorescence, and prevented preconditioning. 5-HD did not prevent the sevoflurane-induced increase in fluorescence, but did prevent preconditioning. We concluded that although the mitochondrial K_{ATP} channel appears to be involved in manifesting APC, it is not required for the generation of ROS during anesthetic exposure. Recent patch-clamp data also suggest that volatile anesthetics cannot directly activate the K_{ATP} channel if cellular ATP levels are normal (25, 47). Rather, they sensitize the K_{ATP} channel to decreased ATP (27, 77), an effect that is enhanced at low intracellular pH (75).

In contrast to the many studies on IPC, there are few published investigations of the involvement of so-called reactive nitrogen species in the triggering pathway of APC. Novalija *et al.* (57) found attenuated cardioprotection when the nitric oxide synthase inhibitor L-NAME was given during sevoflurane pulses to guinea pig isolated hearts. Ischemia was initiated 20 min after sevoflurane/L-NAME exposure. The study suggested that $NO^{\cdot}$ is involved in initiating early development of the APC state. For IPC, $NO^{\bullet}$ appears only involved in the late, "delayed," phase of preconditioning (15). Nonetheless, further work is required to clarify whether there truly is a difference in the role of $NO^{\bullet}$ in APC and IPC. The source of $NO^{\bullet}$ during anesthetic exposure also needs to be determined.

It is worth observing that other physiologic effects of volatile anesthetics appear to be mediated by ROS. Yoshida and Okabe (89) were the first to demonstrate that ROS might be responsible for some effects of volatile anesthetics. SOD or deferoxamine abrogated the effect of sevoflurane to antagonize endothelium-dependent vasodilation of isolated mesenteric vessels from the rat. Park *et al.* (64) later demonstrated that isoflurane-induced vasoconstriction of isolated coronary resistance vessels from rabbit was abrogated by manganese-SOD or mannitol. Teppema *et al.* (81) found that ascorbic acid and α-tocopherol prevented the effect of halothane to depress human ventilatory responses to hypoxia, suggesting that halothane produced ROS in the carotid body. Recent reports describe a preconditioning effect of volatile anesthetics in neural tissue (7, 40), but it is not reported if ROS are involved in this phenomenon in a manner similar to cardiac preconditioning. There is also evidence that endothelial $NO^{\bullet}$ is modulated by halothane (6), although vascular responses to $NO^{\bullet}$ are not altered by volatile anesthetics (77). Recent reports describe APC of rat brain; this effect is K_{ATP} channel-dependent (88) and requires activation of inducible nitric oxide synthase (40).

APC REDUCES FORMATION OF ROS DURING ISCHEMIA AND REPERFUSION

In contrast to the apparent requirement that ROS must be generated to initiate APC, the phenomenon of APC is characterized by *decreased* ROS formation during ischemia and reperfusion. In a study from our laboratory (57), we observed that release of peroxynitrite ($ONOO^-$), measured using formation of dityrosine from the oxidation of tyrosine, was decreased on reperfusion after ischemia if hearts were anesthetic-preconditioned. Nakamura *et al.* (56) reported that hydroxyl radical ($OH^{\bullet}$), identified by its reaction with salicylic acid to yield dihydroxybenzoic acids, was decreased during reperfusion of isolated rat hearts after exposure to halothane or isoflurane (although a similar effect of sevoflurane was not found). After sevoflurane exposure, we found a decrease in $O_2^{\bullet -}$, assessed using DHE, not only during reperfusion after ischemia,

but also during ischemia (44). We have also provided recent evidence that APC specifically decreases ROS generation after ischemia/reperfusion in isolated mitochondria (58). Whether decreased oxidant formation fundamentally underlies the cardioprotection of APC, contributes in part to cardioprotection, or is merely an accompaniment to APC in the resulting decrease in ischemic injury is unclear. Certainly, APC attenuates other intracellular events known to be contributory to ischemia and reperfusion injury, most importantly cytoplasmic (1, 87) and mitochondrial Ca^{2+} loading (69). Potent inhibitors of complex I, such as rotenone, have also been reported to increase the rate of production of ROS by cardiac submitochondrial particles (38). It is important to note that anesthetics inhibit ROS formation by neutrophils (24, 55), suggesting that NAD(P)H oxidase is an unlikely candidate for the observed ROS formation by heart tissue. Neutrophils may yet play a role in anesthetic-induced cardioprotection, however, as suppression of ROS formation by neutrophils exposed briefly to anesthetics decreased cardiac injury during later ischemia and reperfusion (37).

VOLATILE ANESTHETICS, ALTERED BIOENERGETICS, GENERATION OF ROS, AND MITOCHONDRIAL K_{ATP} (mK_{ATP}) CHANNELS

How do anesthetics alter mitochondrial function to induce ROS formation? All hydrophobic agents, which include volatile anesthetics, pass easily through membranes and are known to attenuate mitochondrial respiration (52). It is possible that anesthetics reversibly slow respiration, particularly at complex III, which is diffusely located within the mitochondrial lipid membrane, and/or complex I, which transverses the membrane, enough to cause a small electron leak leading to augmented $O_2^{\bullet -}$ generation. We have summarized a postulated mechanism for APC in Fig. 2 with feed forward loops. Similar pathways may exist for IPC as well. Although many of the factors involved in APC are known, the sequence, amplification, and inhibition of these factors remain to be worked out, including the critical roles of ROS and K_{ATP} channels in initiating APC.

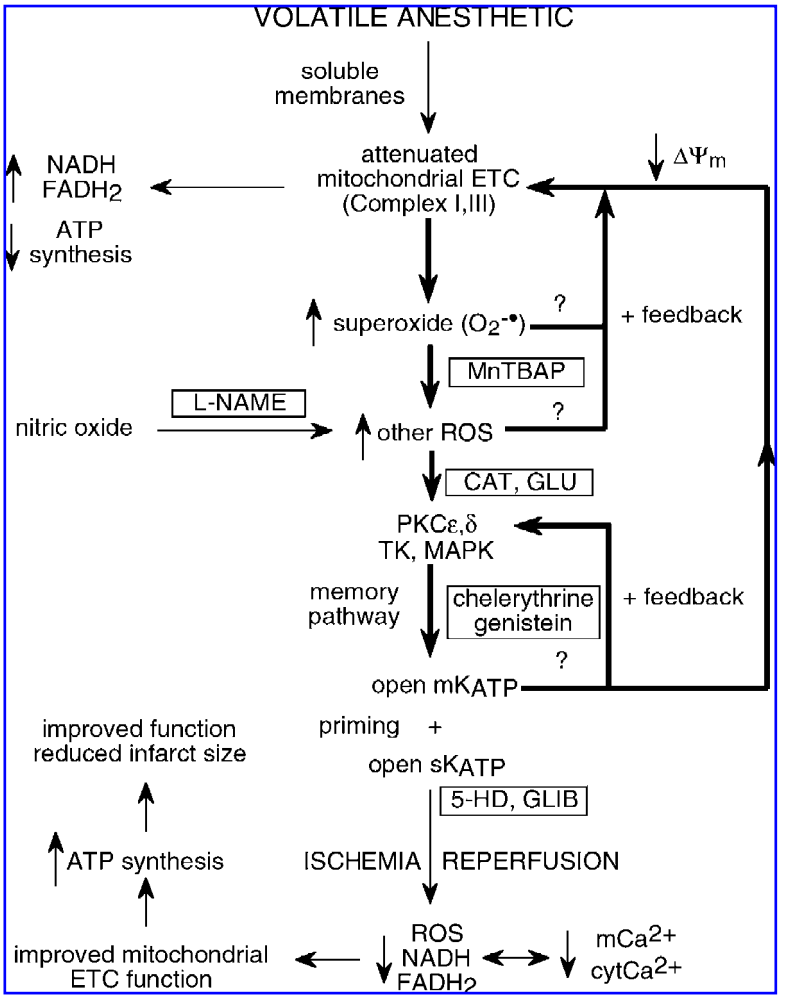

FIG. 2. Suggested pathways by which volatile anesthetics induce cardioprotection. Because of their high lipoid solubility, volatile anesthetics likely have differential effects on all lipophilic cell structures, including membranes, structural proteins (*e.g.,* L-type Ca^{2+} channel), and enzymes. The anesthetic-induced effect to reduce mitochondrial NADH oxidoreductase activity (complex I and/or complex III) may lead to greater generation of $O_2^{\bullet -}$, a ROS, and accumulation of NADH. ROS ($O_2^{\bullet -}$, H_2O_2, $OH^{\bullet}$), a reactive nitrogen species (RNS), or a reactant, *e.g.,* $ONOO^-$, may in turn activate intracellular PKC and tyrosine kinase (TK) pathways that result in a lower threshold for K_{ATP} channel opening (memory effect of APC); ROS and K_{ATP} channel opening may operate in a feed-forward loop. K_{ATP} channel opening may cause a small decrease in mitochondrial membrane potential ($\Delta\psi_m$) to slow electron chain transport and oxidative phosphorylation during ischemia and initial reperfusion. Thus, protection of mitochondrial bioenergetics leads to reduced generation of damaging ROS/RNS, better preservation of NADH, and reduced mitochondrial and cytosolic Ca^{2+} loading. Protection results in improved function and reduced infarct size on reperfusion after ischemia. Drugs in blocks are ROS scavengers or pathway inhibitors. CAT, catalase; GLIB, glibenclamide; MAPK, mitogen-activated protein kinase; ETC, electron transport chain.

In the early 1970s, Hall *et al.* (29) reported a decrease in mitochondrial respiration in rat liver exposed to halothane, and Nahrwold *et al.* (54) reported decreased hepatocyte mitochondrial respiration in the presence of halothane, isoflurane, and enflurane. In 1983, Kissin *et al.* (45) reported that volatile anesthetics increase NADH in isolated rat hearts. They reported that halothane, isoflurane, and enflurane increased NADH by 10% of the maximum produced by anoxia when given in concentrations used clinically, and that the potency of NADH effect paralleled their potencies as general anesthetics. Riess *et al.* (68, 70) recently reported a similar effect of sevoflurane to increase NADH in isolated guinea pig hearts, and suggested an effect of volatile anesthetics to inhibit complex I (NADH: ubiquinone oxidoreductase) of the electron transport system (ETS). This finding was confirmed and extended on by Hanley *et al.* (31), who reported that NADH oxidation was inhibited in the order halothane > isoflurane = sevoflurane. The order of potency for general anesthetic action of these agents is halothane > isoflurane > sevoflurane in guinea pig ventricular myocytes during exposure to the volatile anesthetics. They tested if complex I was the site of action of anesthetics leading to an increase in NADH by measuring the effect of these agents on activity of NADH:ubiquinone oxidoreductase using the ubiquinone analogue decylubiquinone as substrate. At equivalent anesthetic concentrations, halothane and isoflurane inhibited NADH:ubiquinone activity by 20% and sevoflurane inhibited NADH:ubiquinone activity by 10%. Succinate oxidation was not affected by isoflurane or sevoflurane, suggesting complexes II and IV were unaffected by these agents, whereas halothane inhibited succinate dehydrogenase. The significance of these differential effects remains unknown.

The actual reactive signaling molecule(s) that initiates APC is not known. Although volatile anesthetics can cause generation of $O_2^{\bullet -}$, a product or reactant of $O_2^{\bullet -}$, such as hydrogen peroxide (H_2O_2), $OH^{\bullet}$, or $ONOO^-$, may induce translocation or phosphorylation of intracellular second messengers (PKC, tyrosine kinase) that precede opening of K_{ATP} channels (58, 61). Because $NO^{\bullet}$ synthase exists in proximity to cytochrome *c* (72), mitochondrial $NO^{\bullet}$ might modulate ROS activity and mitochondrial respiration to initiate APC. On the other hand, endothelial-derived $NO^{\cdot}$ may participate in APC by reacting with $O_2^{\bullet -}$ to form $ONOO^-$ (57). Overall, these findings provide a plausible model whereby exposure to volatile anesthetics causes generation of ROS by the mitochondrial ETS. We propose that volatile anesthetics induce cardioprotection through initial generation of ROS at complex I and/or III and that this leads, via phosphorylation cascades, to K_{ATP} channel opening and augmented ROS generation by a feed forward mechanism (Fig. 2.)

How does K_{ATP} channel opening lead to cardioprotection? Contradictory mechanisms for cardioprotection afforded by mK_{ATP} channel opening have been proposed. It was found that K_{ATP} channel openers produce a 20–30 mV depolarization of the mitochondrial membrane potential ($\Delta\psi_m$) that causes uncoupling of oxidative phosphorylation and inhibition of mCa^{2+} uptake (36, 48). This led these investigators to suggest that endogenous activation of K_{ATP} channels preserved mitochondrial function during ischemia and reperfusion by attenuating mCa^{2+} loading that in turn disrupts mitochondrial function by inducing the mitochondrial Ca^{2+} permeability transition. However, the laboratory of Garlid and co-workers countered that the concentrations of K_{ATP} channel openers (pinacidil and diazoxide) used were too high and that the effects of these drugs on $\Delta\psi_m$ were not due to K_{ATP} channel opening (26, 46); smaller concentrations, which opened the channels, caused only minor changes in $\Delta\psi_m$. They furnished data to offer an alternative explanation for mitochondrial protection (46). They observed that mK_{ATP} opening significantly increased mitochondrial matrix volume by ~20% while changing $\Delta\psi_m$ only 1–3 mV. They proposed that matrix K^+ uptake (along with H_2O and P_i) through mK_{ATP} channels is an important mechanism to maintain mitochondrial matrix volume when $\Delta\psi_m$ falls and the matrix contracts due to increased oxidative phosphorylation (increased work load) or accelerated ATP hydrolysis due to dysfunctional creatine kinase and its reduced coupling with adenine nucleotide translocase (hypoxia, ischemia). Matrix contraction results in expansion of the cytosolic side and disrupts structure and function of key enzymes, *e.g.,* creatine kinase. They propose that as cytosolic P_i concentration decreases and the phosphorylation potential increases, this slows respiration and may allow leaking of electrons to form $O_2^{\bullet -}$. Thus, the function of the mK_{ATP} channel may be to maintain mitochondrial matrix volume and efficiency during increased work load and with restoration of substrates and O_2 after ischemia.

IMPORTANCE OF APC

The effect of brief exposure of the heart to a volatile anesthetic induces a state of protection against the effects of ischemia and reperfusion; this has been demonstrated with each of these agents and in multiple animal models and in human tissue. Several aspects of the phenomenon appear to be the same as for IPC, suggesting that at least part of the signaling sequence is likely common for these otherwise highly different stimuli. ROS is a prerequisite for IPC, and also appears to be such for APC, but neither the specific reactive species nor the role it plays in inducing preconditioning is known. The molecular mechanism by which anesthetics induce ROS formation is also unknown, as is the precise cell-signaling pathway that produces the cardiac protected state. However, the reported effect of volatile anesthetic exposure to inhibit mitochondrial respiration strongly implicates the ETS as the likely source of ROS generation that initiates APC, just as the K_{ATP} channel may be a downstream mediator and effector of APC.

Knowledge of the mechanism of APC and its similarity to IPC are important in understanding how healthy cells and organs react to ischemic stress. It is also important to know if APC occurs in animal models of aged or diseased hearts and in elderly humans with heart disease. It is unclear if IPC can be successfully induced in aged animal models, and studies of IPC in cardiac models of disease (*e.g.,* diabetes) are few (18). Multiple clinical studies have shown that IPC can be induced in patients; however, IPC appears not to occur in patients with diabetes mellitus or advanced age, in patients taking the sulfonylurea glibenclamide (K_{ATP} channel blockers), and with the concomitant use of cold cardioplegic solutions during bypass surgery. Nevertheless, a few studies indicate that IPC is preserved in older patients.

As for IPC, few studies on APC have been conducted in animal models with disease states. One study reported that cardiac APC was attenuated in drug-induced diabetes in dogs (78). In contrast, volatile anesthetics are routinely administered to heart disease patients before they undergo cardiopulmonary bypass and cardiac surgery. Several studies have now reported improved ventricular function (5, 17, 65) and reduced release of markers of ischemia after APC (5, 20, 86).

Whether preconditioning becomes adopted as an accepted clinical therapy remains to be seen, but of known triggers of preconditioning, volatile anesthetics may have the best profile for this purpose, with their ease of administration and long history of safety in patients monitored for cardiac ischemia and dysfunction. Nonetheless, for almost 50 years, these agents have been routinely applied to patients at risk of cardiac ischemia, so it seems that we have been inadvertently preconditioning all along. The discovery of the phenomenon of APC may simply constitute an interesting pharmacologic mimicker of an endogenous cellular protective mechanism that would not necessarily change standard anesthetic management. One could argue, however, that the use of a volatile agent rather than a nonvolatile anesthetic agent may be favorable for patients with cardiac disease.

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ABBREVIATIONS

APC, anesthetic preconditioning; CAT, catalase; DHE, dihydroethidium; ETS, electron transport system; GLUT, glutathione; 5-HD, 5-hydroxydecanoic acid; H_2O_2, hydrogen peroxide; IPC, ischemic preconditioning; K_{ATP}, ATP-sensitive potassium channel; MnTBAP, Mn(III)tetrakis(4-benzoic acid)porphyrin chloride; MPG, *N*-(2-mercaptopropionyl)glycine; L-NAME, N^{ω}-nitro-L-arginine methyl ester; $NO^{\bullet}$, nitric oxide; $O_2^{\bullet -}$, superoxide anion; $OH^{\bullet}$, hydroxyl radical; $ONOO^-$, peroxynitrite; PKC, protein kinase C; ROS, reactaive oxygen species; SOD, superoxide dismutase; $\Delta\psi_m$, mitochondrial membrane potential.

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