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Dual Exposure to Sevoflurane Improves Anesthetic Preconditioning in Intact Hearts

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Background: **Anesthetic preconditioning (APC) with sevoflurane reduces myocardial ischemia–reperfusion injury. The authors tested whether two brief exposures to sevoflurane would lead to a better preconditioning state than would a single longer exposure and whether dual exposure to a lower (L) concentration of sevoflurane would achieve an outcome similar to that associated with a single exposure to a higher (H) concentration.**

Methods: **Langendorff-prepared guinea pig hearts were exposed to 0.4 mM sevoflurane once for 15 min (H1-15; n = 8) or 0.4 mM (H2-5; n = 8) or 0.2 mM sevoflurane (L2-5; n = 8) twice for 5 min, with a 5-min washout period interspersed. Sevoflurane was then washed out for 20 min before 30 min of global no-flow ischemia and 120 min of reperfusion. Control hearts (n = 8) were not subjected to APC. Left ventricular pressure was measured isovolumetrically. Ventricular infarct size was determined by tetrazolium staining and cumulative planimetry. Values are expressed as mean ± SD.**

Results: **The authors found a better functional return and a lesser percentage of infarction on reperfusion in H2-5 (28 ± 9%) than in H1-15 (36 ± 8%; $P < 0.05$), L2-5 (43 ± 6%; $P < 0.05$), or control hearts (52 ± 7%; $P < 0.05$).**

Conclusion: **These results suggest that APC depends not only on the concentration but also on the protocol used for preconditioning. Similarly to ischemic preconditioning, repeated application of the volatile anesthetic seems to be more important than the duration of exposure in initiating the signaling sequence that elicits APC at clinically relevant concentrations. Therefore, repeated cycles of anesthetic exposure followed by volatile anesthetic–free periods may be beneficial for APC in the clinical setting.**

THE intriguing possibility of intraoperative and perioperative cardioprotection *via* the use of volatile anesthetics has triggered an increasing interest in the phenomenon of anesthetic preconditioning (APC) in both basic science and clinical research and may ultimately have an impact on anesthesia practice for cardiac risk patients.[1]

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Volatile anesthetics exert a cardioprotective effect when given before and after ischemia.[2–6] This protection is independent of improved myocardial oxygen supply–demand balance. Volatile anesthetics are also able to "precondition" the myocardium against ischemia–reperfusion (IR) injury. A memory effect is triggered and attenuates IR injury for several hours (first window) even after cessation of anesthetic exposure.[7] Cardioprotection has been reported to return for a second window approximately 24 h later.[8]

Success of APC can be demonstrated by better tissue perfusion,[9,10] improved metabolic and mechanical function,[10,11] and reduced infarction on reperfusion.[7–9,11,12] APC is similarly effective as ischemic preconditioning (IPC)[10,11] and seems to share many of the same signal transduction pathways. APC has the clear advantage of not needing ischemia to initiate preconditioning. Furthermore, volatile anesthetics are routinely used for general anesthesia and, compared with other preconditioning intravenous drugs, their concentration and duration of action in the body are easily controlled.

It has been shown that repeated transient IR periods were more effective in triggering IPC than a single IR period.[13] This suggested greater accumulation of critical IPC mediators with each IR period. Therefore, reperfusion after a transient ischemic period may be as critical in triggering IPC as the triggering stimulus itself. Although cardioprotection by APC has been found to be dose dependent,[10,12] the role of repeated compared with single anesthetic exposure in improving cardioprotection has not been reported.

The aim of this study in guinea pig isolated hearts was to investigate whether dual short exposure to sevoflurane at clinically relevant concentrations is more effective in triggering APC than a single exposure and whether the use of a lower concentration with lesser side effects would achieve an outcome similar to that associated with a single exposure to a higher concentration.

Materials and Methods

The investigation conformed to the *Guide for the Care and Use of Laboratory Animals*[14] and was approved by the Institutional Animal Care and Use Committee (Medical College of Wisconsin, Milwaukee, Wisconsin). Our methods have been described previously.[9–11] Thirty milligrams ketamine and 1,000 U heparin were injected

Table 1. Sevoflurane Concentrations (mM) before and after Ischemia

Group	5 min	10 min	15 min	20 min	35 min	70 min
H1–15	—	—	0.43 ± 0.06	0.11 ± 0.06	0.02 ± 0.03	0.01 ± 0.03
H2–5	0.42 ± 0.07	0.06 ± 0.07	0.43 ± 0.07	0.07 ± 0.03	0.02 ± 0.03	0.01 ± 0.03
L2–5	0.15 ± 0.06†	0.03 ± 0.03	0.16 ± 0.06*†	0.04 ± 0.03*	0.01 ± 0.01	0.00 ± 0.01

Presented are venous sevoflurane concentrations during (time t = 5 and 15 min) and after (t = 10, 20, and 35 min) sevoflurane exposure before ischemia and 5 min after reperfusion (t = 70 min) for the three preconditioned groups as assessed by gas chromatography. H1–15: 0.4 mM (≈2.8 vol% at 37°C) sevoflurane for 15 min; H2–5: 0.4 mM (2.8 vol%) sevoflurane for 2 × 5 min with 5-min washout interspersed; L2–5: 0.2 mM (1.4 vol%) sevoflurane for 2 × 5 min with 5-min washout interspersed.

Data are presented as mean ± SD, n = 8 each.

$P < 0.05$ for * H1–15 *vs.* H2–5 or L2–5; † H2–5 *vs.* L2–5.

intraperitoneally into 32 albino English short-hair guinea pigs (weight, 250–300 g). Animals were decapitated 15 min later when unresponsive to noxious stimulation. After thoracotomy, the aorta was cannulated distal to the aortic valve, and the heart was immediately perfused retrograde with 4°C cold oxygenated Krebs-Ringer's (KR) solution. The inferior and superior venae cavae were ligated, and the heart was rapidly excised. After cannulation of the pulmonary artery to collect the coronary effluent, the heart was placed in the support system, immersed in a 37°C KR bath, and perfused at 55 mmHg and 37°C. The KR perfusate was equilibrated with approximately 95% O_2 and 5% CO_2 to maintain a constant perfusion pH of 7.4. The KR perfusate was filtered (5-μm pore size) in-line and had the following calculated composition (nonionized): 138 mM Na^+, 4.5 mM K^+, 1.2 mM Mg^{2+}, 2.5 mM Ca^{2+}, 134 mM Cl^-, 14.5 mM HCO_3^-, 1.2 mM $H_2PO_4^-$, 11.5 mM glucose, 2 mM pyruvate, 16 mM mannitol, 0.1 mM probenecid, 0.05 mM EDTA, and 5 U/l insulin.

Left ventricular pressure (LVP) was measured isovolumetrically with a saline-filled latex balloon inserted into the left ventricle *via* a cut in the left atrium. At the beginning of the experiment, the balloon volume was adjusted to achieve a diastolic LVP of 0 mmHg so that any subsequent increase in diastolic LVP reflected an increase in left ventricular wall stiffness, or diastolic contracture. Characteristic data from LVP were as follows: systolic, diastolic, and developed (systolic–diastolic) LVP; and the maximal and minimal first derivatives of LVP ($dLVP/dt_{max}$ and $dLVP/dt_{min}$) as indices of contractility and relaxation, respectively. Spontaneous heart rate was monitored with bipolar electrodes placed in the right atrial and ventricular walls. Coronary flow (CF) was measured at constant temperature and perfusion pressure by an ultrasonic flowmeter (Transonic T106X, Ithaca, NY) placed directly into the aortic inflow line.

Coronary inflow (arterial [a]) and outflow (venous [v]) Na^+, K^+, Ca^{2+}, oxygen partial pressure (Po_2), pH, and carbon dioxide partial pressure (Pco_2) were measured off-line with an intermittently self-calibrating analyzer system (Radiometer Copenhagen ABL 505, Copenhagen, Denmark). Pvo_2 tension was also measured continuously on-line with an oxygen Clark type electrode (model 203B; Instech, Plymouth Meeting, PA). Myocardial oxygen consumption was calculated as $(Pao_2 - Pvo_2) \cdot 24\ \mu l\ O_2/ml$ at 760 mmHg · CF · heart wet weight^{-1}.

Sevoflurane (Abbott Laboratories, North Chicago, IL) was bubbled into the KR perfusate using an agent specific vaporizer (Vapor 2000; Dräger Medizintechnik GmbH, Lübeck, Germany) placed in the oxygen–carbon dioxide gas mixture line. Samples of coronary perfusate were collected to measure sevoflurane concentrations by gas chromatography. Venous outflow sevoflurane concentrations are displayed in table 1. If ventricular fibrillation occurred, a bolus of 250 μg lidocaine was immediately injected in the aortic cannula. All data were collected from hearts in sinus rhythm. At the end of 120 min of reperfusion, hearts were removed, and ventricles were cut into six to seven 3-mm-thick transverse sections. These were immediately stained with 0.1% 2,3,5-triphenyltetrazolium chloride in 0.1 M KH_2PO_4 buffer (pH 7.4, 38°C) for 5 min. 2,3,5-Triphenyltetrazolium chloride stains viable tissue red, indicating the presence of a formazan precipitate that results from the reduction of 2,3,5-triphenyltetrazolium chloride by dehydrogenase enzymes present in viable tissue.[15] All slices were digitally imaged by a photoscanner, and the infarcted areas of each slice were measured in a blinded fashion by planimetry using software (Image 1.62) from the National Institutes of Health (Bethesda, MD). Infarcted areas of individual slices were averaged to calculate the total percentage of infarct size of both ventricles. Reproducibility of this method is approximately ±5% based on studies of fresh *ex vivo* but nonperfused Langendorff-prepared hearts (laboratory observation by M. L. Riess, M.D., Medical College of Wisconsin, Milwaukee, Wisconsin, 2002).

All analog signals were digitized (PowerLab/16 SP; ADInstruments, Castle Hills, Australia) and recorded at 200 Hz (Chart & Scope v3.6.3; ADInstruments) on a Power Macintosh Computer G4 (Apple, Cupertino, CA) for later analysis using MATLAB (The MathWorks, Natick, MA) and Microsoft Excel (Microsoft Corporation, Redmond, WA) software. All variables were averaged over the sampling period of 2.5 s.

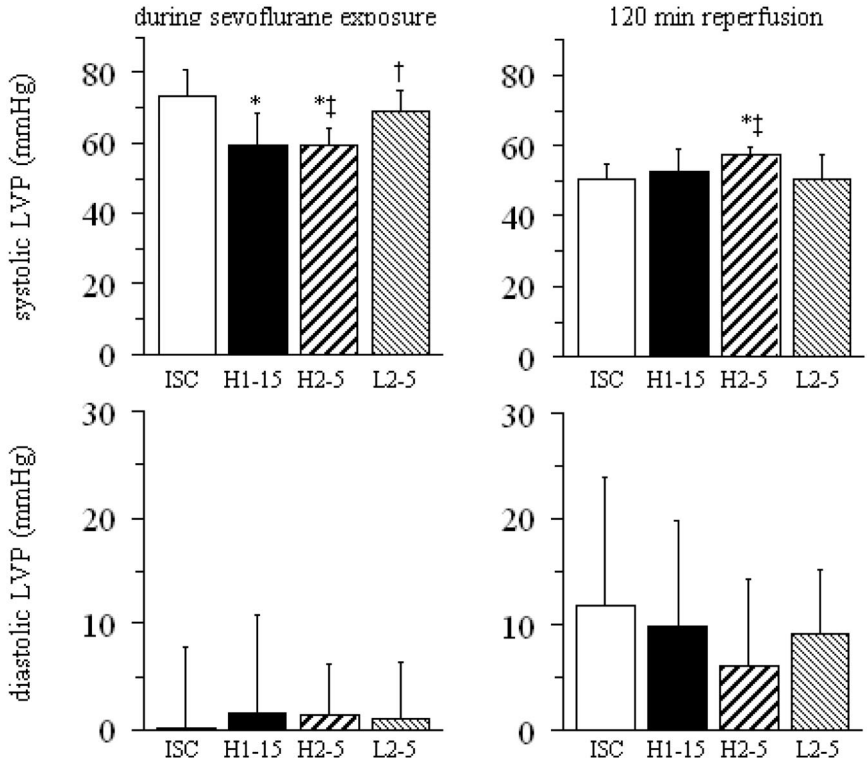

Fig. 1. Systolic and diastolic left ventricular pressure (LVP) during higher (H) and lower (L) anesthetic exposure at time t = 15 min (*left*) and at 120 min of reperfusion (*right*). ISC: no anesthetic exposure; H1-15: 0.4 mM (approximately 2.8 vol% at 37°C) for 15 min; H2-5: 0.4 mM (2.8 vol%) for 2 × 5 min with 5-min washout interspersed; L2-5: 0.2 mM (1.4 vol%) for 2 × 5 min with 5-min washout interspersed. Data are expressed as mean ± SD; n = 8/group. $P < 0.05$ for * ISC *versus* H1-15, H2-5, or L2-5; † H1-15 *versus* H2-5 or L2-5; ‡ H2-5 *versus* L2-5.

Protocol

After stabilization, each experiment lasted 185 min. Hearts were randomly assigned to one of four groups: (1) Untreated ischemic control hearts (n = 8) were not exposed to sevoflurane. (2) H1-15 hearts (n = 8) were exposed to a higher (H) concentration of 0.4 mM (approximately 2.8 vol% at 37°C) sevoflurane once for 15 min. (3) H2-5 hearts (n = 8) were exposed to 0.4 mM (2.8 vol%) sevoflurane twice for 5 min, with a 5-min washout period interspersed. (4) L2-5 hearts (n = 8) were exposed to a lower (L) concentration of 0.2 mM (1.4 vol%) sevoflurane twice for 5 min, with a 5-min washout period interspersed. Sevoflurane exposure was followed by a 20-min washout period before ischemia. All hearts then underwent 30 min of global no-flow ischemia and 120 min of reperfusion. The anesthetic was virtually undetectable immediately before and after ischemia (table 1).

Statistical Analysis

All data were expressed as mean ± SD. Among-group data, control, H1-15, H2-5, and L2-5, were compared by analysis of variance to determine significance (Super ANOVA 1.11 software for Macintosh; Abacus Concepts, Berkeley, CA). If *F* values ($P < 0.05$) were significant, *post hoc* comparisons of means tests (Student-Newman-Keuls) were used to compare the four groups. Differences among means were considered statistically significant when *P* was less than 0.05 (two-tailed).

Results

Exposure to 0.4 mM sevoflurane caused a greater depression in systolic LVP (fig. 1) and $dLVP/dt_{max}$ and $dLVP/dt_{min}$ (fig. 2), indices of contractility and relaxation, respectively, than did exposure to 0.2 mM sevoflurane (t = 15 min; *left*). Myocardial oxygen consumption (fig. 3) was decreased only during the 15 min exposure to 0.4 mM sevoflurane, whereas CF (fig. 3) was increased in all three APC groups during sevoflurane exposure. All changes were reversible on washout of sevoflurane (data not shown).

At 120 min of reperfusion, only H2-5 hearts exhibited higher systolic LVP (fig. 1, *right*), higher developed (systolic–diastolic) LVP (data not shown), greater contractility and relaxation (fig. 2), higher myocardial oxygen consumption, and higher CF (fig. 3) than control hearts. H2-5 hearts were also different from L2-5 hearts with regard to systolic and developed LVP and different from H1-15 hearts with regard to myocardial oxygen consumption and CF.

Infarct size after 120 min of reperfusion was decreased in all three APC groups compared with the nonpreconditioned control group (fig. 4). The dual 5-min exposure to 0.4 mM sevoflurane (H2-5) yielded a smaller infarction than the single 15-min exposure at the same concentration (H1-15). Decreasing the concentration of the dual exposure to 0.2 mM sevoflurane (L2-5) resulted in a greater infarction than for 0.4 mM (H2-5).

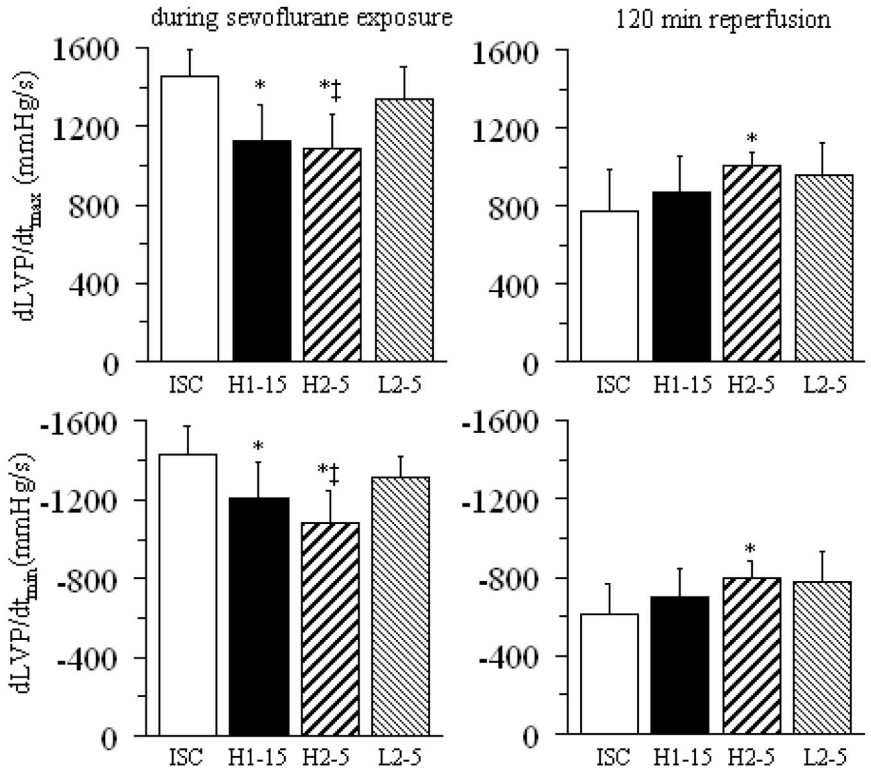

Fig. 2. Maximal and minimal first derivatives of left ventricular pressure (dLVP/dt_{max} and dLVP/dt_{min}) as indices of contractility and relaxation, respectively, during higher (H) and lower (L) anesthetic exposure at time t = 15 min (*left*) and at 120 min of reperfusion (*right*). ISC: no anesthetic exposure; H1-15: 0.4 mM (approximately 2.8 vol% at 37°C) for 15 min; H2-5: 0.4 mM (2.8 vol%) for 2 × 5 min with 5-min washout interspersed; L2-5: 0.2 mM (1.4 vol%) for 2 × 5 min with 5-min washout interspersed. Data are expressed as mean ± SD; n = 8/group. P < 0.05 for * ISC *versus* H1-15, H2-5, or L2-5; † H1-15 *versus* H2-5 or L2-5; ‡ H2-5 *versus* L2-5.

Discussion

Our study furnishes the first direct evidence that APC can be improved by dual exposure compared with a single exposure at the same anesthetic concentration as assessed by reduced infarct size and improved function. Furthermore, dual exposure to a smaller anesthetic concentration led to results similar to those associated with single and longer exposure at a higher concentration.

The phenomenon of cardiac preconditioning against IR injury was first described by Murry *et al.*[16] in 1986.

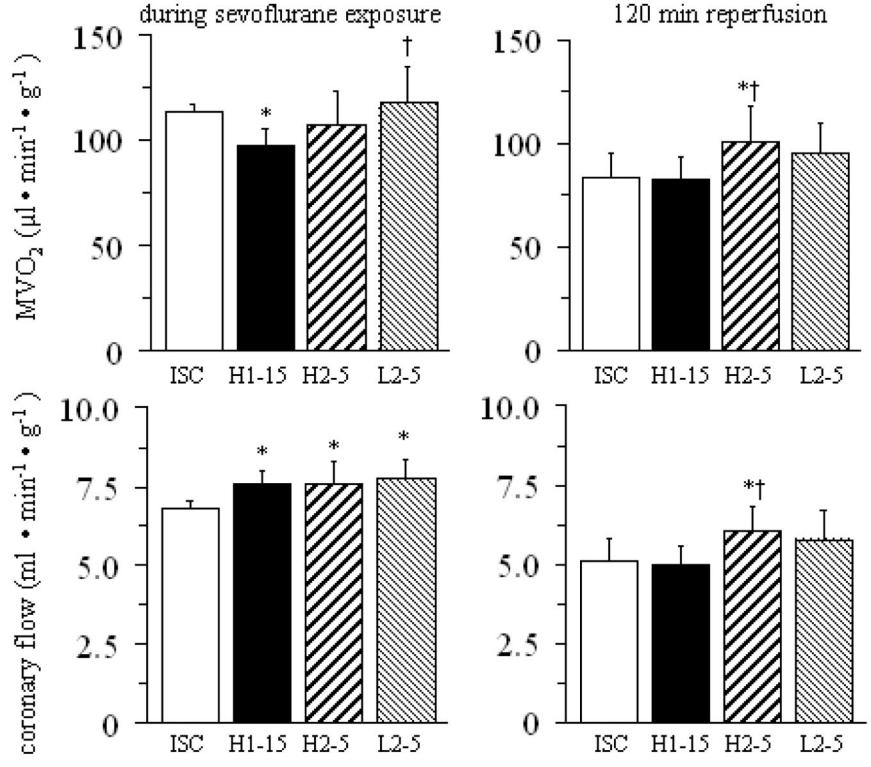

Fig. 3. Myocardial oxygen consumption (MVo_2) and coronary flow during higher (H) and lower (L) anesthetic exposure (*left*) and at 120 min of reperfusion (*right*). ISC: no anesthetic exposure; H1-15: 0.4 mM (approximately 2.8 vol% at 37°C) for 15 min; H2-5: 0.4 mM (2.8 vol%) for 2 × 5 min with 5-min washout interspersed; L2-5: 0.2 mM (1.4 vol%) for 2 × 5 min with 5-min washout interspersed. All data are expressed as mean ± SD; n = 8/group. P < 0.05 for * ISC *versus* H1-15, H2-5, or L2-5; † H1-15 *versus* H2-5 or L2-5; ‡ H2-5 *versus* L2-5.

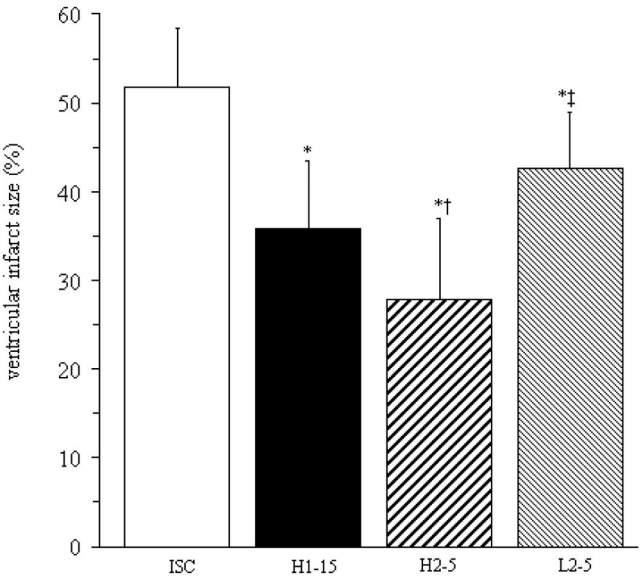

Fig. 4. Ventricular infarct size after 120 min of reperfusion. ISC: no anesthetic exposure; H1-15: 0.4 mM (approximately 2.8 vol% at 37°C) for 15 min; H2-5: 0.4 mM (2.8 vol%) for 2 × 5 min with 5-min washout interspersed; L2-5: 0.2 mM (1.4 vol%) for 2 × 5 min with 5-min washout interspersed. All data are expressed as mean ± SD; n = 8/group. $P < 0.05$ for * ISC *versus* H1-15, H2-5, or L2-5; † H1-15 *versus* H2-5 or L2-5; ‡ H2-5 *versus* L2-5.

Using myocardial infarct size in dogs as an endpoint, they found that IPC with four 5-min circumflex artery occlusions, each separated by 5 min of reperfusion and followed by sustained occlusion for 40 min before reperfusion, were able to attenuate IR injury.

From a clinical standpoint, a desirable goal is to maximize the extent of cardioprotection afforded by preconditioning. Some researchers reported an "all-or-none" effect for IPC,[17,18] whereas others found a significantly decreased infarction zone[13] and lesser norepinephrine[19] and cyclic adenosine monophosphate release[20] during prolonged IR after IPC with repeated cycles compared with only one cycle. However, a maximal effect seems to be reached with three to four IPC cycles,[19,21] after which the benefit disappears.[21,22] Another critical determinant is the duration of transient ischemia to achieve IPC: three 5-min ischemic periods have been found to be more effective than three 2-min cycles,[23] whereas a period of 15 min of ischemia increased infarct size compared with a 5-min period.[24] Despite all of this evidence, there seems to be no single best model or protocol used by researchers to study IPC, so direct comparisons among studies remain difficult.

Similarly, protocols to induce APC by exposure to a volatile anesthetic vary greatly among researchers with regard to the anesthetic chosen, its concentration, the exposure and washout time, and whether applied once or in repeated cycles. We[10] and others[12] have recently reported a concentration-dependent effect in triggering APC with a single anesthetic exposure. The lack of a systemic circulation in our isolated heart model allows for higher anesthetic concentrations with faster washout times to result in both functional improvement and reduced infarct size on reperfusion. In contrast, *in situ* models and clinical studies are usually constrained by the adverse side effects of high anesthetic concentrations on systemic circulation and by longer washout times. Consequently, the success of APC in those studies has often been limited to reduced infarct size[8,12] or release of certain biochemical markers.[25,26]

Our study suggests that, as in IPC, at least two cycles of short exposure to a clinically relevant concentration of a volatile anesthetic followed by its washout enhance cardioprotection afforded by APC. This may decrease the required anesthetic concentrations and thus minimize the adverse cardiovascular effects of anesthetic exposure in patients with coronary artery disease. Clearly, more studies are needed to understand the mechanisms of protection afforded by repeated exposures compared with a single exposure to a volatile anesthetic and to ultimately find an optimal protocol to induce APC. The latter becomes especially relevant when costly multicenter clinical studies are planned to transfer results from basic science research into clinical practice.[1,26]

However, the more complicated the protocol, the less useful it may become in the clinical situation. The success of APC in individuals with risk factors for coronary artery disease has already been challenged by reports that hyperglycemia or use of oral antidiabetics abolish preconditioning,[27] that aged hearts are less sensitive to APC,[28] and that APC may only be effective for a narrow range of ischemia durations.[29]

Potential limitations of our study are that the use of a high-P_{O_2}, blood-free perfusate in this model causes a greater use of CF reserve, although this is balanced in part by decreased oxygen consumption, and that volatile anesthetics may modify neutrophil function related to IR injury in *in vivo* hearts.[30] Moreover, the isolated heart is devoid of autonomic and hormonal input, which could influence IR injury. Systemic storage of the anesthetic *in vivo* likely prolongs washin and washout times compared with our model. Although many investigators use, for example, the rat heart as a model for APC, the guinea pig is believed to be closer in design to the human heart, as we[11] and others[31,32] have observed. Furthermore, we used only one anesthetic agent to induce APC; however, both *in vivo* and *ex vivo* studies have shown that APC can also be induced with other commonly used volatile anesthetics.[7,8,12,33]

In summary, the success of APC as assessed by reduced infarct size and improved function after IR injury in Langendorff-perfused hearts can be improved by dual exposure to sevoflurane at clinically relevant concentrations. Therefore, repeated cycles of anesthetic exposures followed by anesthetic-free periods may be beneficial in maximizing the protective effects of APC at clinically relevant concentrations. However, if adopted

as a standard protocol, repeated cycles may limit the clinical applicability of APC.

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