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Sevoflurane Mimics Ischemic Preconditioning Effects on Coronary Flow and Nitric Oxide Release in Isolated Hearts

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Background: **Like ischemic preconditioning, certain volatile anesthetics have been shown to reduce the magnitude of ischemia/ reperfusion injury *via* activation of K^+ adenosine triphosphate (ATP)-sensitive (K_{ATP}) channels. The purpose of this study was (1) to determine if ischemic preconditioning (IPC) and sevoflurane preconditioning (SPC) increase nitric oxide release and improve coronary vascular function, as well as mechanical and electrical function, if given for only brief intervals before global ischemia of isolated hearts; and (2) to determine if K_{ATP} channel antagonism by glibenclamide (GLB) blunts the cardioprotective effects of IPC and SPC.**

Methods: **Guinea pig hearts were isolated and perfused with Krebs–Ringer's solution at 55 mmHg and randomly assigned to one of seven groups: (1) two 2-min total coronary occlusions (preconditioning, IPC) interspersed with 5 min of normal perfusion; (2) two 2-min occlusions interspersed with 5 min of perfusion while perfusing with GLB (IPC+GLB); (3) SPC (3.5%) for two 2-min periods; (4) SPC+GLB for two 2-min periods; (5) no treatment before ischemia (control [CON]); (6) CON+GLB; and (7) no ischemia (time control). Six minutes after ending IPC or SPC, hearts of ischemic groups were subjected to 30 min of global ischemia and 75 min of reperfusion. Left-ventricular pressure, coronary flow, and effluent NO concentration ([NO]) were measured. Flow and NO responses to bradykinin, and nitroprusside were tested 20–30 min before ischemia or drug treatment and 30–40 min after reperfusion.**

Results: **After ischemia, compared with before (percentage change), left-ventricular pressure and coronary flow, respectively, recovered to a greater extent ($P < 0.05$) after IPC (42%, 77%), and treatment with SPC (45%, 76%) than after CON (30%, 65%), IPC+GLB (24%, 64%), SPC+GLB (20%, 65%), and CON+GLB (28%, 64%). Bradykinin and nitroprusside increased [NO] by 30 ± 5 (means ± SEM) and 29 ±4 nM, respectively, averaged for all groups before ischemia. [NO] increased by 26 ± 6 and 27 ± 7 nM, respectively, in SPC and IPC groups after ischemia, compared with an average [NO] increase of 8 ± 5 nM ($P < 0.01$) after ischemia in CON and each of the three GLB groups. Flow increases to bradykinin and nitroprusside were also greater after SPC and IPC.**

Conclusions: **Preconditioning with sevoflurane, like IPC, improves not only postischemic contractility, but also basal flow, bradykinin and nitroprusside-induced increases in flow, and effluent [NO] in isolated hearts. The protective effects of both SPC and IPC are reversed by K_{ATP} channel antagonism. (Key words: Anesthetics; bradykinin; cardiac injury; coronary vasculature; myocardial ischemia; nitroprusside.)**

ISCHEMIC preconditioning (IPC) is a phenomenon described as brief periods of ischemia that render the myocardium resistant to a subsequent longer period of ischemia. IPC is most often assessed by observations of reduced infarct size, attenuated mechanical dysfunction, or limited ultrastructural abnormality on reperfusion after prolonged ischemia.[1] This endogenous protective

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mechanism has been shown to occur in all species tested including humans,[2] dogs,[3] rabbits,[4] pigs,[5] and rats.[6] The degree of mechanical and electrical cardioprotection provided by myocardial preconditioning and the mechanisms that mediate its effects have been the focus of intense investigation. Anesthetics may also be preconditioning agents. Kersten *et al.*[7] reported recently that isoflurane mimics IPC, as assessed by reduced infarct size in dogs, and suggested that this protective effect involves K^+ adenosine triphosphate (ATP)-sensitive (K_{ATP}) channel opening.

Several animal studies suggest that IPC can also protect the myocardial vasculature against primary injury[8,9] or subsequent injury[10] induced by regional ischemia and reperfusion, but others do not.[11] No prior studies have been undertaken to demonstrate if preconditioning with ischemia, or a volatile anesthetic, increases nitric oxide release and affords protection against global endothelial and vascular dysfunction that occurs after global ischemia and reperfusion. We have reported that volatile anesthetics improve reperfusion mechanical function and metabolism and reduce dysrhythmias if given 10 min before, during, and 10 min after 30 min of global ischemia[12–14] and similarly if given before, during, and initially after 1-day exposure to hypothermia at 3°C.[15,16]

For this study we tested in isolated hearts if two brief preconditioning periods with sevoflurane or ischemia improve coronary endothelial and vascular function on reperfusion after 30 min of global ischemia. To assess microvascular injury, the endothelium-dependent and -independent vasodilators bradykinin (BK) and nitroprusside (NP) were given to test coronary flow responsiveness, and effluent nitric oxide concentration ([NO]) was measured. Ischemia-induced effects on cardiac electrical, mechanical, and metabolic variables were also examined. The ability of K_{ATP} channel blockade to antagonize the protective effects of sevoflurane and ICP were compared.

Materials and Methods

Preparation and Measurements

This investigation conformed to the guide for care and use of laboratory animals published by the National Institutes of Health[17] and was approved by the institutional animal care committee. English short-haired guinea pigs (250–300 g) were used in all studies. Ketamine (10 mg) and heparin (1,000 units) were injected intraperitoneally and animals were decapitated when unresponsive to noxious stimulation. Hearts were perfused with Krebs-Ringer's solution within 1 min of thoracotomy. Surgical preparation for this model has been described in detail previously.[12–14] Each heart was perfused in a retrograde fashion *via* the aorta with a cold oxygenated, modified Krebs-Ringer's solution equilibrated with 97% oxygen and 3% carbon dioxide (*p*H, 7.38 ± 0.04; PO_2, 588 ± 12 mmHg; and CO_2, 25 ± 1.2 mmHg) and was then rapidly excised. Perfusate was disk-filtered (5 μm bore size) in line and had the following composition (in mM): 137 Na^+, 5 K^+, 1.2 Mg^{2+}, 2.5 Ca^{2+}, 134 Cl^-, 15.5 HCO_3^-, 1.2 $H_2PO_4^-$, 11.5 glucose, 2 pyruvate, 16 mannitol, 0.05 ethylene-diamine-tetraacetic acid, and 5 units/L insulin. Perfusate, bath, oxygen electrode, and nitric oxide electrode temperatures were maintained at 37.2 ± 0.1°C (SEM) using a thermostatically controlled, water circulatory system consisting of glass tubing, bath, and aluminum heat exchangers.

Isovolumetric left-ventricular pressure (LVP) was continuously recorded with a transducer connected to a thin, saline-filled latex balloon (Hugo Sachs Elektronik KG, March-Hugstetten, Germany) inserted into the left ventricle through the mitral valve from a cut in the left atrium. Balloon volume was adjusted to maintain a diastolic LVP of 0 mmHg during the initial control period so that any increase in diastolic LVP reflected an increase in left-ventricular wall stiffness or diastolic contracture. The volume of the balloon was unchanged during the experiment. Two pairs of bipolar electrodes (Teflon-coated silver, diameter 125 μm; Cooner Wire Co., Chatsworth, CA) were placed in the right-atrial appendage and right-ventricular wall to monitor intracardiac electrograms from which spontaneous heart rate was determined from the right-atrial beat-to-beat interval, as detailed previously.[12–14] Coronary (aortic) in-flow was measured continuously at a constant temperature and at a normal aortic perfusion (gravity) pressure of 55 mmHg by a transit-time, self-calibrating, in-line ultrasonic flowmeter (Research Flowmeter Transonic T106X; Transonic System, Ithaca, NY) placed directly into the aortic in-flow line. To determine maximal coronary flow, and possible effect of sevoflurane-induced preconditioning (SPC) on coronary flow reserve, adenosine (0.2 ml of a 200 mM stock solution) was injected directly into the aortic root cannula during the initial control period and after the last control reading. The reactive flow response (maximal flow on initial reperfusion) was measured in all experiments. Coronary out-flow (coronary sinus) O_2 tension and *p*H were measured continuously on-line with a miniature thermostable Clark electrode (model 203B;

Instech Laboratories, Plymouth Meeting, PA) and temperature-compensated *p*H electrode (microcomputer provision *p*H meter, model 05669-20, *p*H electrode PHE 2121; Cole Parmer Instruments, Vernon Hills, IL).

Oxygen delivery was calculated as inflow O_2 tension, in millimeters of mercury, multiplied by O_2 solubility (24 μl/ml Krebs-Ringer's solution at 760 mmHg O_2 and 37°C) and coronary in-flow (in millimeters per minute), and then divided by the wet weight of each heart (1.45 ± 0.03 g). Oxygen extraction was calculated as out-flow O_2 content divided by in-flow O_2 content and was used to assess the vasodilator response independent of any autoregulatory response occurring with a drug-induced change in metabolism. Myocardial oxygen consumption was calculated as O_2 solubility multiplied by the difference between in-flow and out-flow O_2 content times coronary flow per gram of wet heart. In the absence of O_2 debt, an imbalance of oxygen delivery to myocardial oxygen consumption reflects a change in coronary vascular tone. In-flow perfusate O_2 tension was kept constant by maintaining a reservoir container pressure 5 mmHg above atmospheric pressure. Coronary effluent CO_2 tension was calculated from the on-line *p*H signal with HCO_3^- assumed to be constant at 15 mM. These measurements were verified during each maneuver off-line at 37°C with an intermittently self-calibrating analyzer system (Radiometer ABL-2, Medtron Chicago, Des Plaines, IL).

Coronary sinus effluent was collected by placing a small, gas-impermeable cannula into the right ventricle through the pulmonary artery after ligating the superior and inferior vena cavae. [NO] was measured in coronary effluent[18,19] as the change in redox current (in picoamperes) generated by a gas-permeable, water-impermeable Clark type electrode (ISO-NOP 2 mm, World Precision Instruments, Sarasota, FL). The electrode probe measures NO-generated electrical current in aqueous solutions polarographically.[20] Electrical current is generated as NO diffuses through the membrane and becomes oxidized at the platinum electrode and is proportional to diffusion of NO through the membrane based on its partial pressure, which is proportional to [NO] at a probe tip. Current was measured with a sensitive amperometer during zero voltage suppression to expand the response range.[19] NO (picoamperes) and *p*H (millivolts) electrode signals were amplified for continuous display. Because of the very small currents generated by the NO electrode, baseline current stability could not be maintained throughout the experiment, so only the change in current before and after infusion of BK or NP was measured.

The selectivity of the membrane for NO over other gases is determined by the potential applied to the electrode. The presence of NO_2 gas can generate a current, but in aqueous solution NO_2 is highly unstable and at physiologic *p*H it degrades to NO_2^- and NO_3^-, which cannot penetrate the membrane. A possible effect of CO_2 on altering *p*H of the internal electrolyte solution was minimized by using a two-part buffered internal electrolyte solution (#7521; World Precision Instruments, Sarasota, FL) furnished by the supplier. NO calibration curves were generated by graded chemical production of NO from $NaNO_2$, where potassium iodide and H_2SO_4 are in excess.[18,19] The half-life response time was less than 3 s. Calibration was carried out 1 day after changing the gas-permeable membrane sleeve and internal filling solution; two experiments were performed on the same and a subsequent day. There was no significant change in NO electrode sensitivity (in nM) over the 2-day period. Right-ventricle (coronary sinus) cannula to NO electrode transient time, measured by methylene blue, was < 1 s at 10 ml/min.

Electrograms, spontaneous heart rate, AV conduction time, out-flow oxygen (in millimeters of mercury), *p*H (in millivolts), coronary flow, systolic and end-diastolic isovolumetric LVP, and NO electrode current (in picoamperes) were displayed continuously on a fast-writing (3 kHz), high-resolution, eight-channel chart recorder (Dash-8, Astro-Med, West Warwick, RI). Measurements were taken every 5 or 15 min after administering a drug or inducing ischemia.

Experimental Design

There were seven experimental groups consisting of 8 to 15 hearts per group (total n = 76). Each experiment lasted 180 min (fig. 1) beginning after a 30-min period of equilibration. Hearts of each group were infused for 3 min with 10 nM BK and 100 μM NP, given in random order 20 or 30 min before and 20 or 30 min after the 30-min period of global ischemia. Animals were randomized daily into the seven experimental groups; group sizes are not identical because randomization into a group was not tracked on a daily basis. The untreated nonischemic (time control) group (table 1) was not subjected to ischemia or transient preconditioning. There were six ischemia groups that underwent 90 min perfusion followed by a 30-min period of ischemia and 60 min of reperfusion during which hearts were immersed in a water bath maintained at 37°C throughout.

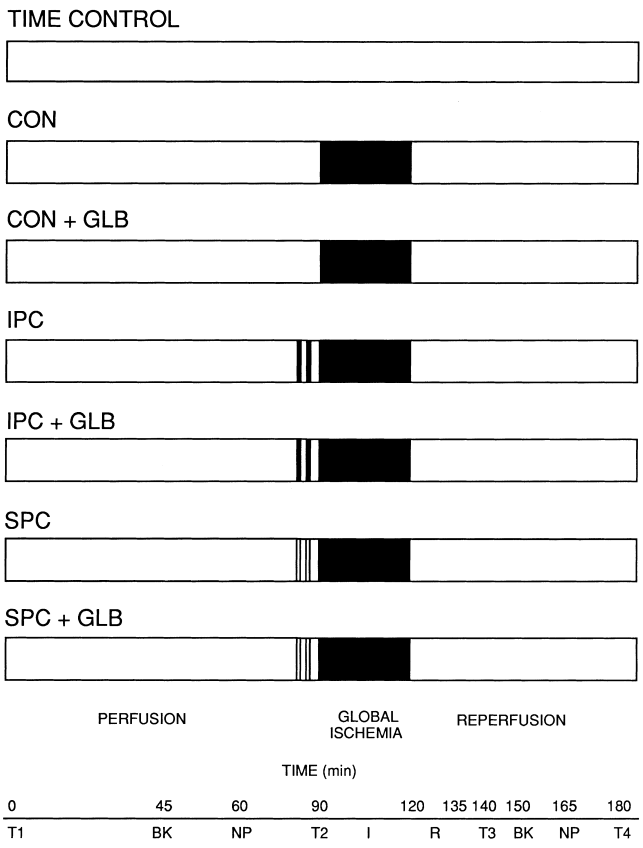

Fig. 1. Schema for preconditioning, ischemia, and reperfusion protocols in seven randomized groups of guinea pig hearts. The two ischemic preconditioning (IPC) pulses were elicited by global coronary occlusion for two 2-min periods. The two sevoflurane preconditioning (SPC) pulses were elicited by exposing the hearts to 3.5% sevoflurane at normal cardiac perfusion pressure for two 2-min periods. Glibenclamide (GLB, 2 μM) was perfused continuously from T1 to T4 time periods after pre-GLB control (CON) values were attained.

The same time protocols were used for both IPC and SPC. One group received no preconditioning treatment (control [CON]), whereas two other pairs received either IPC or SPC. The IPC group had 75 min of perfusion followed by two 2-min periods of transient no-flow global ischemia separated by 5 and 6 min of reperfusion followed by 30 min of global ischemia and 60 min of reperfusion; the SPC preconditioning group had 75 min of perfusion followed by two 2-min periods of perfusion with sevoflurane separated by 5 and 6 min of reperfusion followed by 30 min of ischemia and 60 min of reperfusion. Within each pair of groups studied, one received the K_{ATP} channel antagonist glibenclamide (GLB), 2 μM,[21,22] throughout the study after initial drug-free measurements were taken (CON+GLB, IPC+GLB, SPC+GLB), whereas the other pair did not (CON, IPC,

Table 1. Responses to Vasodilators for Several Variables in the Time Control Group

	Time (min)														
	0	15	45	60	90	120	135	140	150	165	170	180			
	T1	ADE	BK	NP	T2	no	I/R	T3	BK	NP	ADE	T4			
LVDP (mmHg)	84.1 ± 4.2				83 ± 3.6			69.4 ± 3.6*				69.3 ± 3.0*			
CF (ml · g^{-1} · min^{-1})	5.9 ± 0.4	11.3 ± 0.6*	7.3 ± 0.4*	7.0 ± 0.4*	5.9 ± 0.4			5.9 ± 0.3	7.0 ± 0.3*	6.8 ± 0.3*	9.5 ± 0.3*	5.8 ± 0.3			
O_2 Ext (fraction)	0.77 ± 0.03		0.62 ± 0.04*	0.67 ± 0.03*	0.77 ± 0.02			0.75 ± 0.02	0.63 ± 0.03*	0.66 ± 0.03*		0.73 ± 0.02			
ΔNO (nM)			22.9 ± 1.8	17.7 ± 1.5					21.3 ± 3.4	18.9 ± 2.9					
ΔNO release (pmol · g^{-1} · min^{-1})			33.9 ± 5.8	19.0 ± 3.0					23.3 ± 3.9	11.3 ± 3.4					
MV_{O_2} (ml O_2 · g^{-1} · min^{-1})	60.4 ± 2.9		60.1 ± 3.9	63.3 ± 3.4	61.3 ± 3.1			59.7 ± 2.7	59.2 ± 3.0	60.3 ± 3.6		56.9 ± 2.7			

ADE = adenosine; BK = bradykinin; NP = sodium nitroprusside; I = ischemia; R = reperfusion; LVDP = left-ventricular developed pressure; CF = coronary flow; O_2 Ext = oxygen extraction; ΔNO = change in nitric oxide; MV_{O_2} = myocardial oxygen consumption.

* $P < 0.05$ *versus* T1; early and late BK and NP values, and each time control point (T1 to T4), values are not significantly different from each other.

SPC). If ventricular fibrillation (VF) occurred, a 0.25-ml bolus of lidocaine (250 μg) was administered immediately *via* the aortic cannula. All hearts reverted to sinus rhythm and data were collected after stabilization of LVP.

Sevoflurane, 3.5% volume, was administered by placing an agent specific vaporizer in the gas supply line. Samples of coronary perfusate were collected anaerobically from the aortic cannula for measurement of sevoflurane concentration by gas chromatography as reported previously.[12–14] In-flow sevoflurane concentration, measured as 0.53 ± 0.02 mM, was equivalent to equilibration with 3.34 ± 0.22% atmospheres and represents a minimal alveolar concentration of approximately 1.52 ± 0.4. Sevoflurane was not detectable in the effluent during the initial equilibration period, the ischemic period, or the reperfusion period. Only one concentration of sevoflurane was utilized in these experiments for two reasons: It was necessary to design many groups into this study of one anesthetic, and we have not observed a concentration-dependent protective effect of halothane.[13,14]

Statistical Analysis

All results are reported as means ± SEM. Among the seven groups, data were analyzed using two-way analysis of variance at baseline conditions, during administration of BK, NP, and SPC, during IPC, during and after 30 min global ischemia, and during repeat administration of BK and NP. Within-group data were analyzed over time using univariate analysis of variance for repeated measures (Super ANOVA, Abacus Corporation, Berkeley, CA). If F values ($P < 0.05$) were significant, *post hoc* comparisons among means (Student *t* test with Duncan's adjustment for multiplicity) were used to differentiate treatment groups. The incidence of VF *versus* sinus rhythm was determined by chi-square analysis, and differences in VF duration were determined by unpaired *t* tests. Statistical notations not given in the figures are in the tables or text. Statistical symbols used in figures are: *GLB group *versus* corresponding group without GLB; #IPC group *versus* CON; §SPC group *versus* CON; and †IPC ± GLB *versus* SPC ± GLB.

Results

Electrical and Mechanical Effects

Table 1 displays variables obtained for a time-control group (n = 12) not subjected to ischemia, IPC, SPC, or GLB. There was no change in any variable, except LVP at T3 and T4 *versus* T1 ($P < 0.05$), among the time control points (T1–T4) and no difference between initial and final responses to BK and NP. Initial responses (T1, adenosine [ADE], BK, NP) were not different between the time-control group and the other six groups at the same time periods for any variable ($P > 0.1$). LVP was greater in the time-control group compared with all ischemic groups after ischemia (T3, T4) ($P < 0.001$); coronary flow, [NO], and NO release were not different among the time-control, IPC, and SPC groups after ischemia but were different among the remaining ischemia groups ($P < 0.05$).

For all groups there were no differences in heart rate (CON, 256 ± 8; CON+GLB, 251 ± 9; IPC, 248 ± 8; IPC+GLB, 247 ± 8; SPC, 254 ± 11; SPC+GLB, 249 ± 8 beats/min) or atrioventricular conduction time (average 63 ± 2 ms) before ischemia (T2) and after 20 min of reperfusion (T3) ($P > 0.1$). Values before and after GLB for the CON+GLB, IPC+GLB, and SPC+GLB groups, respectively, were as follow: coronary flow 5.3 ±0.4 and 5.2 ± 0.3, 4.6 ± 0.4 and 4.5 ± 0.4, 5.1 ± 0.4 and 5.4 ±0.3 ml · g^{-1} · min^{-1}. Left-ventricular developed (systolic–diastolic) pressure (LVDP) values before and after GLB for the same groups were 101 ± 3 and 96 ± 6, 87 ± 10 and 81 ± 8, and 94 ± 9 and 89 ±7 mmHg. GLB had no significant effect on these variables ($P > 0.1$).

The only dysrhythmia observed on reperfusion was VF, which occurred in all ischemic groups. The incidence of VF for each group (including repeat VF in a given heart) was: CON, 93%; CON+GLB, 50%; IPC, 69%; IPC+GLB, 67%; SPC, 38%; and SPC+GLB, 50%. The incidence of VF was significantly lower for all groups compared with CON and was lower in the SPC group than in all other groups ($P < 0.05$). When VF occurred, its onset occurred within 1 min of reperfusion in CON, CON+GLB, IPC+GLB, and SPC+GLB groups; at 1.8 ± 0.2 min in the IPC group ($P < 0.05$); and much later, at 4.7 ±0.4 min ($P < 0.05$), in the SPC group.

From the protocol described in figure 1, figure 2 shows that each group had a similar initial (T1) LVDP before any treatment, except for the IPC+GLB group, in which LVDP was lower than in all but the SPC+GLB group ($P < 0.05$). During IPC±GLB, LVDP was markedly depressed; during transient sevoflurane with or without GLB, LVDP was only moderately decreased.

After 30 min of global ischemia and 20 min of reperfusion (T3), LVDP was markedly depressed in each ischemic group compared with values obtained before ischemia ($P < 0.05$). However, IPC- and SPC-treated groups exhibited significantly less ($P < 0.05$) depression of LVDP than did CON and CON+GLB groups. Reductions of LVDP on

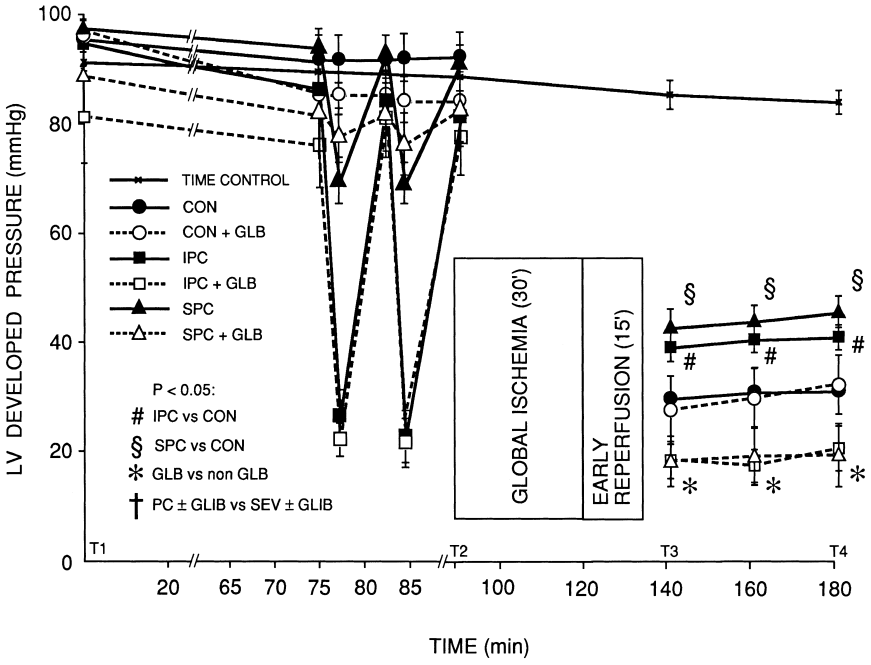

Fig. 2. Time line for left-ventricular (LV) developed pressure before, during, and after global ischemia in control (CON), ischemic preconditioned (IPC), and sevoflurane preconditioned (SPC) groups with and without glibenclamide (GLB). GLB reduced baseline LV developed pressure, and LV developed pressure was least reduced in IPC- and SPC-treated groups after reperfusion. T1, T2, T3, and T4 are control points before and after global ischemia in the graphs that follow. Additional details in text.

reperfusion after ischemia (T3) were 70.0 ± 4.5% (CON), 58.0 ± 2.9% (IPC), 56.5 ± 2.7% (SPC), 72.1 ± 5.6% (CON+GLB), 75.4 ± 3.7% (IPC+GLB), and 80.0 ± 4.1% (SPC+GLB). The improvements in LVDP in IPC and SPC groups were statistically similar. LVDP was significantly lower ($P < 0.05$) after reperfusion in IPC and SPC groups treated with GLB compared with the CON+GLB group. Table 2 shows that left-ventricular end-diastolic pressure was zero before ischemia but was elevated in all groups after global ischemia. During reperfusion, left-ventricular end-diastolic pressure was higher in IPC+GLB and SPC+GLB groups than in IPC and SPC groups and was lower in IPC and SPC groups than in the CON group.

Flow, Metabolic, and NO Effects

Figure 3A shows that basal coronary flow and flow increases to ADE (91 ± 6%), BK (38 ±6%), and NP (31 ± 4%) above initial T1 values ($P < 0.05$) were similar in CON, IPC, and SPC groups (averaged group data) before ischemia. For each group, flow responses to ADE, BK, and NP were greater than at T1 or T2. Sevoflurane before ischemia alone increased flow slightly, by 9.1 ± 2.2%. In CON+GLB and IPC+GLB groups, basal coronary flow (T1) was lower than in corresponding nontreated CON and IPC groups, but this was not dependent on infusion of GLB as noted previously. Figure 3B displays flow responses after ischemia. Basal coronary flow was lower than before ischemia (T3 *vs.* T1, T4 *vs.* T2) in all groups ($P < 0.05$) but the reactive flow response and responses to ADE, BK, and NP were higher than at T1 for each non-GLB group ($P < 0.05$); responses to ADE, BK, and NP after ischemia for all ischemic groups were lower than before ischemia ($P < 0.05$). Figure 3B shows also that basal coronary flow, reactive flow response, and flow increases to ADE were lower in the three GLB-treated groups than in the three corresponding groups not treated with GLB after ischemia, except for responses to BK and NP in CON+GLB and SPC+GLB, *versus* CON and SPC groups, respectively. Moreover, the IPC and SPC groups exhibited greater responses to BK and NP compared with the CON group.

Figure 4A shows that percentage O_2 extraction and responses to BK and NP were similar in CON, IPC, and SPC groups alone and with GLB before ischemia. For all groups prior to ischemia, BK and NP decreased O_2 extraction on average by 25 ± 4% and 16 ± 4%. After ischemia (fig. 4B) O_2 extraction was lower during BK and NP (BK, NP, *vs.* T3 and T4) in IPC and SPC groups ($P < 0.05$). Figure 4B shows that after global ischemia SPC+GLB and IPC+GLB groups exhibited higher O_2 extraction than the corresponding non-GLB groups for T3 and BK responses, respectively. Percent O_2 extraction was reduced more during BK and NP in the SPC group than at the time control points T3 or T4. Myocardial oxygen consumption and effluent *p*H were lower in

Table 2. Effect of Ischemia and Reperfusion on Several Variables in Six Treatment Groups

	Time (min)									
	0	45	60	90	120	135	140	150	165	180
LVEDP (mmHg)	T1	BK	NP	T2	I	R	T3	BK	NP	T4
CON	0			0			30.4 ± 2.8			29.9 ± 3.0
CON + GLB	0			0			36.7 ± 4.6			29.5 ± 4.4
IPC	0			0			20.0 ± 2.3†			18.1 ± 2.0†
IPC + GLB	0			0			32.7 ± 3.4*			30.5 ± 3.8*
SPC	0			0			15.9 ± 2.4‡			14.0 ± 2.4‡
SPC + GLB	0			0			41.5 ± 3.8*			38.8 ± 4.6*
MV_{O_2} (μl $O_2 \cdot g^{-1} \cdot min^{-1}$)	T1	BK	NP	T2	I	R	T3	BK	NP	T4
CON	64.3 ± 4.8			64.8 ± 4.2			37.6 ± 4.3			37.4 ± 4.5
CON + GLB	48.4 ± 2.2*			50.6 ± 3.8*			29.3 ± 4.1			27.6 ± 3.8
IPC	70.2 ± 5.4			68.2 ± 4.2			47.5 ± 3.0			47.8 ± 3.3
IPC + GLB	46.3 ± 3.8*			43.7 ± 3.9*			27.0 ± 4.3*			25.6 ± 4.5*
SPC	63.9 ± 4.0			64.6 ± 3.8			43.4 ± 4.4			43.8 ± 4.1
SPC + GLB	53.1 ± 4.4			57.6 ± 4.2			25.5 ± 9.2*			30.9 ± 5.2*
Effluent *p*H (units)	T1	BK	NP	T2	I	R	T3	BK	NP	T4
CON	7.11 ± 0.04*			7.10 ± 0.01			7.10 ± 0.02			
CON + GLB	7.09 ± 0.01			7.04 ± 0.01*			7.05 ± 0.02			
IPC	7.14 ± 0.02†			7.14 ± 0.02			7.13 ± 0.05			
IPC + GLB	7.04 ± 0.03*			7.04 ± 0.03*			7.05 ± 0.02*			
SPC	7.17 ± 0.02†‡			7.15 ± 0.01‡			7.14 ± 0.02			
SPC + GLB	7.09 ± 0.02*			7.06 ± 0.02*			7.09 ± 0.02			
ΔNO release (pmol $\cdot g^{-1} \cdot min^{-1}$)	T1	BK	NP	T2	I	R	T3	BK	NP	T4
CON		27.6 ± 3.5	19.6 ± 3.1					4.7 ± 1.9	3.9 ± 1.4	
CON + GLB		62.6 ± 17.2*	39.0 ± 10.6*					5.7 ± 1.8	11.1 ± 5.2	
IPC		44.4 ± 7.9	35.4 ± 8.1					34.7 ± 8.0†	31.4 ± 8.5†	
IPC + GLB		66.6 ± 18.0*	57.6 ± 17.8					7.4 ± 1.9*	7.10 ± 1.7*	
SPC		40.8 ± 6.6	34.4 ± 6.7					28.6 ± 10.0‡	21.3 ± 6.7‡	
SPC + GLB		85.1 ± 13.5*	50.0 ± 13.1					11.6 ± 4.3*	10.5 ± 3.4	

LVEDP = left-ventricular end diastolic pressure; SPC = sevoflurane preconditioning; GLB = glibenclamide; IPC = ischemic preconditioning; MV_{O_2} = myocardial oxygen consumption; ΔNO = change in nitric oxide; BK = bradykinin; NP = sodium nitroprusside.

* $P < 0.05$, GLB *versus* non-GLB.

† IPC *versus* CON.

‡ SPC *versus* CON.

the GLB-treated groups than in non–GLB-treated groups both before and after global ischemia (table 2).

Figure 5A shows that [NO] increased similarly and significantly ($P < 0.01$) in response to BK and NP; before ischemia, these increases were not significantly different ($P > 0.1$) among the six treatment groups before preconditioning and the time-control group before and after the T2 time point (table 1). After global ischemia (fig. 5B) there was little increase in [NO] in response to BK and NP in the CON group and in each GLB group, but [NO] increased more in IPC and SPC groups than in the CON group, and these increases in the IPC and SPC groups did not differ statistically from the responses observed prior to ischemia ($P > 0.1$). After global ischemia, increases in [NO] during BK and NP were lower than the preischemic responses in each group, except in the IPC- and SPC-treated groups ($P < 0.05$). NO release increased significantly in response to BK and NP (table 2). Unlike for [NO], NO release before ischemia was higher in GLB-treated groups, except for the NP response in IPC+GLB and SPC+GLB groups, because the increases in flow in response to BK and NP were larger.

Discussion

This is the first study to demonstrate that brief periods of exposure to sevoflurane, like IPC, result in improved coronary vasodilation and greater NO production by endothelium-dependent and -independent vasodilators compared with a nonpreconditioned group. Cardiac contractility and relaxation and electrophysiologic and metabolic function were improved similarly by sevoflurane and IPC in this isolated heart model. Sevoflurane pretreatment also resulted in the lowest incidence and latest onset of VF. Importantly, the vascular and myocar-

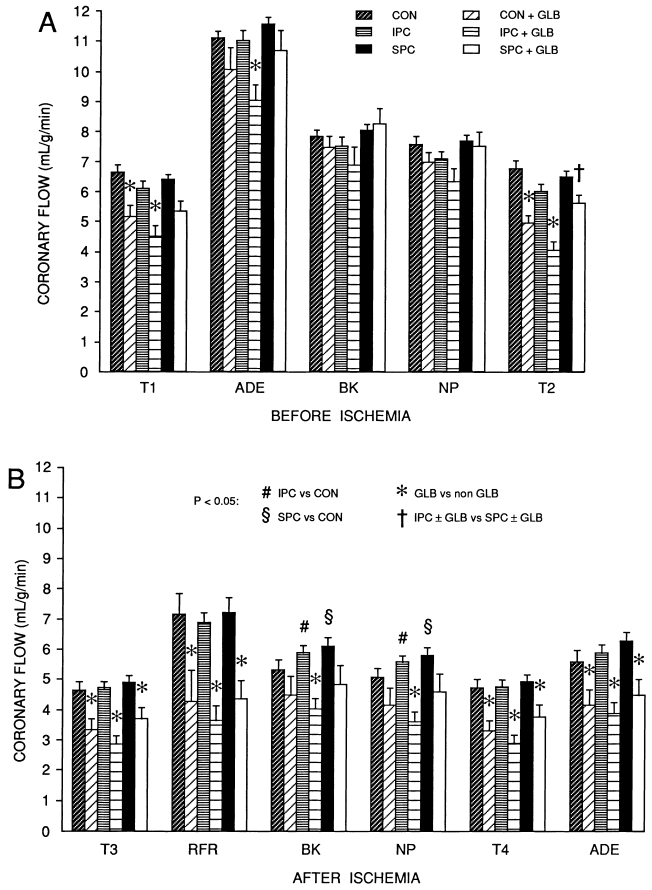

Fig. 3. (*A*) Coronary flow before (T1 and T2) global ischemia in control, ischemic preconditioned (IPC), and sevoflurane preconditioned (SPC) groups with and without glibenclamide (GLB). ADE = the initial response to a bolus of adenosine; BK and NP = responses to 1-min infusions of 10 nM bradykinin and 100 μM nitroprusside, respectively. GLB reduced basal flow (T1) and ADE, BK, and NP each significantly increased flow compared to T1. (*B*) Coronary flow after (T3 and T4) global ischemia in control, IPC, and SPC groups with and without GLB. ADE = the final response to a bolus of adenosine; reactive flow response = is the initial reactive flow response on reperfusion after ischemia. GLB reduced basal flow (T3) and flow responses to ADE, BK, and NP. Flow responses to these drugs were greatest in the IPC and SPC groups.

dial protective effects of sevoflurane and IPC were eliminated by GLB, which suggests that endogenous K_{ATP} channel activation is involved, at least in part, in vascular protective effects of both sevoflurane and IPC.

IPC

Ischemic preconditioning is thought to be mediated primarily *via* sarcolemmal G protein-linked receptors such as ADE (A_1, A_3), BK (BK_2), and opioid (δ_1) receptors[23,24] coupled with protein kinase C. Activation of protein kinase C by IPC has been shown to activate tyrosine kinases in rabbits, which may lead to activation of other kinases, which may in turn result in phosphorylation of sarcolemmal and mitochondrial K_{ATP} channels.[25–28] Biochemical analysis indicates that preconditioning also contributes to conservation of ADE triphosphate during the subsequent ischemic period.[1] When myofibril adenosine triphosphate levels are reduced during ischemia, K_{ATP} channels open and produce K^+ efflux at all depolarized voltages beyond the K^+ equilibrium potential. K^+ channel opening promotes earlier repolarization and earlier inactivation of the Ca^{2+}

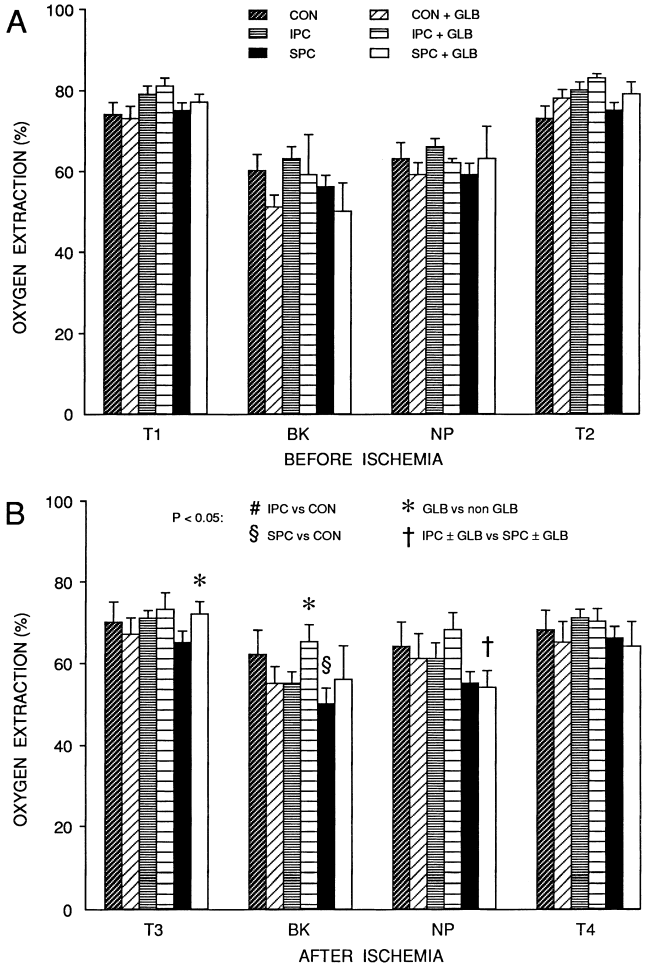

Fig. 4. (*A*) Percentage oxygen extraction before (T1 and T2) global ischemia in CON, ischemic preconditioned (IPC), and sevoflurane preconditioned (SPC) groups with and without glibenclamide (GLB). Bradykinin (BK) and nitroprusside (NP) both reduced percentage oxygen extraction ($P < 0.05$) similarly in each group. (*B*) Percentage oxygen extraction after global ischemia (T3 and T4) in control, IPC, and SPC groups with and without GLB. Percentage oxygen extraction was reduced most by BK and NP in the SPC group.

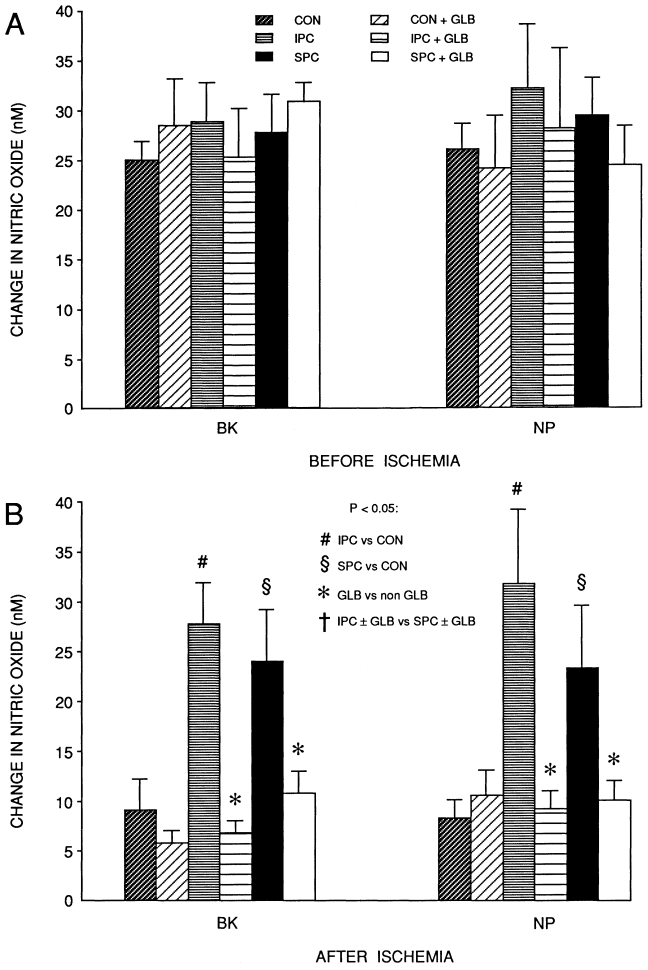

Fig. 5. (*A*) Increase in bradykinin (BK)– and nitroprusside (NP)–induced nitric oxide concentration ([NO]) in coronary effluent before ischemia. Responses to BK and NP were similar in all groups before ischemia. (*B*) Increase in BK- and NP-induced [NO] in coronary effluent after ischemia. Responses to BK and NP were restored statistically to values before ischemia in ischemic preconditioned and sevoflurane preconditioned groups but remained similarly reduced in all other groups.

current that produces a modest negative inotropic effect. K_{ATP} channel agonists enhance functional recovery of postischemic reperfused myocardium *in vivo*, and this effect can be blocked by K_{ATP} channel antagonists such as GLB.[29,30] In the rat model GLB may not block IPC effects unless given at least 30 min prior to IPC.[21,22,31]

There is much controversy regarding the role of NO in preconditioning and ischemia/reperfusion injury. NO release during brief ischemia has been shown to trigger IPC, based on studies that show that its protective effects are blocked by endothelial NO synthase (eNOS) inhibition.[32,33] However, other investigators[10,34,35] suggest that NO, *per se*, does not contribute to IPC. In one of these studies[10] nitrite/nitrate overflow after transient hypoxia in guinea pig hearts was abolished after 30 min of global ischemia but could be restored if hearts were preconditioned by ischemia. Although the IPC effect was mimicked by ADE A_1 and A_3 agonists and blocked by a BK_2 receptor antagonist (HOE 140), it was not blocked by prostaglandin or eNOS inhibitors, suggesting a direct preconditioning effect (possibly mediated by protein kinase C) of BK not mediated *via* NO.[10] In the present study, BK and NP, a direct NO donor, were administered prior to ischemia to elicit endothelium dependent and independent vascular responses. The similar increases in [NO] caused by BK at 45 min and 150 min in the nonischemic control group suggest that the substrate arginine is not depleted during the course of these experiments. BK, whether by a direct vascular smooth muscle effect or as a stimulus for NO production, is also likely to produce preconditioning, because its protective effect is blocked by a BK_2 antagonist.[36] Although BK, and perhaps NP, may themselves have produced a preconditioning effect,[10,36,37] these drugs were administered to all hearts in each group, so it is unlikely that the differential group effects observed were the result of these two agents.

Superoxide radicals, as well as NO radicals, are known to activate a specific protein kinase C,[38,39] so release of these radicals during a brief preconditioning period may confer cardioprotection. However, the release of superoxide radicals during a long period of ischemia may promote production of the toxic compound peroxynitrite if NO is also released in sufficient quantities,[40] which could impair microvascular and myocardial protection afforded by either radical alone. So the increases in [NO] and NO release after IPC and SPC treatments could result from reduced superoxide radical formation and therefore a lesser formation of peroxynitrite. Coronary flow responses to both BK and NP were present but blunted after ischemia, so it is likely that smooth muscle function as well as endothelial function is disturbed by myocardial ischemia and reperfusion injury. The fact that preconditioning improved both endothelial and vascular function implies that at least the overall vascular response was improved. However, we were not able to determine if increases in flow were caused by vasodilatation or by a greater number of patent vascular channels, because 30 min of ischemia causes myocardial infarction in this model. NO and NO donors have been reported to contribute to a late preconditioning effect, as evidenced by abrogation of protection with eNOS

antagonists.[41] Clearly, the relative roles of these free radicals in preconditioning and ischemia reperfusion injury require further investigation.

The relationship between NO release and K_{ATP} channel opening is also not well understood either in cardiac myocytes or in vascular cells. In whole-cell patch-clamped guinea pig myocytes, NO donors have been reported to increase K_{ATP} current, an effect that is blocked by GLB.[42] It is thus possible that because early preconditioning indeed stimulates eNOS activity and release of NO, this may ultimately enhance K_{ATP} channel opening on reperfusion. Our study suggests a link between diminished NO production after ischemia and reperfusion and K_{ATP} channel blockade, but we could not directly determine if K_{ATP} channel opening is a result, in whole or in part, or a cause of the improved NO response after ischemia.

Anesthetic Preconditioning

It is also not known how volatile anesthetics confer effects on the myocardium or its vasculature. We have reported that volatile anesthetics improve reperfusion function and metabolism, and reduce dysrhythmia development, if given 10 min before, during, and 10 min after global ischemia[13,14] or hypoxia[12] in isolated guinea pig hearts. Our studies show additionally that they protect against dysrhythmias and improve mechanical, metabolic, and vascular endothelial function if administered during low-flow perfusion for one day at 3°C.[15,16] Isoflurane administered 45 min before and during 15 min of coronary occlusion in dogs was shown to enhance recovery of regional myocardial contractile function after 5 h of reperfusion.[43] Because this effect was partially blocked by GLB, a role for isoflurane to enhance K_{ATP} channel activation during ischemia and reperfusion was suggested.

There are no other known reports on the effects of a volatile anesthetic on microvascular protection after ischemia. There are recent reports in which isoflurane was given during IPC or was itself used as a preconditioning agent to confer protection by reducing infarct size. Administration of isoflurane for 15 min beginning 30 min before 30 min of global occlusion of rabbit hearts has been shown to partially mimic ischemic IPC by reducing infarct size.[44] Similarly, isoflurane given alone or during four 5-min occlusions before 60 min of regional myocardial ischemia in dogs reduced infarct size, and the protective effect was reversed by GLB, supporting a role for K_{ATP} channel opening.[7] The present *ex vivo* study demonstrates, moreover, that brief exposure to sevoflurane is as effective as IPC on improving coronary perfusion and coronary responsiveness as well as mechanical, electrophysiologic, and metabolic function. Both methods of preconditioning also resulted in a marked enhancement of BK- and NP-induced NO production, and the cardioprotective effects seem to be modulated by K_{ATP} channel activation because GLB blocked these effects. However, neither BK- nor NP-induced flow increases were completely restored after preconditioning with sevoflurane or ischemia. Thus, the vasodilator pathway from guanylyl cyclase to the myofilaments likely remains partially dysfunctional after ischemia. Although NO-dependent endothelial cell function was improved, vascular smooth muscle responsiveness remained attenuated after ischemia. This is also likely caused in part by reduced overall perfusion secondary to areas of interstitial edema and tissue destruction.

Studies with volatile anesthetics are not conclusive concerning their effect on the NO pathway or NO's effector sites.[45–48] Several of these studies suggest that volatile anesthetics alter vascular endothelial function or effects of endothelial factors on vascular smooth muscle activity. However, there is no consensus as to how, or if, anesthetics specifically alter the NO signaling pathway. We reported recently that BK-induced coronary vasodilation and coronary effluent release of NO and L-citrulline were blocked by an eNOS antagonist in intact hearts,[19] but that these effects were not altered by several concentrations of halothane or isoflurane or by sevoflurane.[18] From these studies we concluded that anesthetics do not significantly alter the NO pathway. Therefore, it is unlikely that anesthetics modulate preconditioning by directly stimulating NO generation, as may or may not occur with IPC in our model.

The studies on the effects of anesthetics on the NO pathway, and the present study showing that GLB blocks preservation of NO release and inhibits vascular as well as mechanical function, suggest that the higher [NO] observed after SPC or IPC is a result rather than a cause of preconditioning-induced protection. Improved endothelial and vascular function after IPC or SPC may be elicited by reduced Ca^{2+} loading into endothelial and vascular cells which, in turn, is the link to restoring NO production. Blocking the endothelial and vascular K_{ATP} channel may reduce or slow the effect of K^+ efflux on enhancing depolarization so that the protective effect against Ca^{2+} loading by IPC and SPC is eliminated. Intrinsic activation of K_{ATP} channels during cardiac ischemia has been shown to convey a cardioprotective effect that carries into the reperfusion phase.[49] Thus the pro-

tective effects of SPC and IPC likely have a common final mechanism *via* activation of K_{ATP} channels.

A limitation of this study is that the effect of ischemia and reperfusion injury on regional coronary perfusion secondary to myocardial edema and vessel compression with myocardial necrosis was not examined. We have observed in our model that infarct size is reduced by 24% if a K_{ATP} channel opener is given before 30 min of ischemia (Stowe *et al.*, laboratory observation) so a part of the protective vascular effect may be caused by improved overall coronary perfusion. NO release may be less after ischemia in the CON and GLB groups because total flow is reduced because of a larger volume of nonperfused, infarcted tissue. Another possible limitation is that the drugs used to test microcirculatory responses may have an additive protective effect with sevoflurane or preconditioning. But we have observed that only contractility was slightly improved by about 12% after ischemia if ADE, BK, and NP were given briefly before ischemia.[50] Moreover, we have also observed that both IPC and SPC are cardioprotective without these drugs.[51]

Overall, our study indicates that SPC provides a global cardioprotective effect similar to that of IPC in isolated, crystalloid perfused guinea pig hearts. Not only are mechanical function and oxygen utilization improved, but basal and stimulated coronary flow and NO production are increased. This study demonstrates that volatile anesthetics can diminish not only the degree of cardiac dysfunction, but also the degree of microvascular injury, so that total basal perfusion and vasodilatory reserve are better preserved. The cardioprotective effects of SPC, like IPC, are dependent on K_{ATP} channel activation.

Volatile anesthetics are often selected for patients with coronary artery disease who are at risk for ischemia and infarction during cardiac and noncardiac surgery. Temporary ischemia is often induced during cardiac surgery and angioplasty. Administration of a volatile anesthetic may help to reduce the extent of infarction and to attenuate myocardial dysfunction and improve cardiac perfusion during cardiac and noncardiac procedures in patients with coronary artery disease. Further research, especially clinical studies, is necessary before it can be determined if volatile anesthetics are advantageous in reducing myocardial damage by improving perfusion and contractile function in the perioperative period.

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