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Authors

Deyhimy, David I

Fleming, Neal W

Brodkin, Ian G

et al.

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Anesthetic Preconditioning Combined with Postconditioning Offers No Additional Benefit Over Preconditioning or Postconditioning Alone

David I. Deyhimy, MD*

Neal W. Fleming, MD, PhD*

Ian G. Brodkin, MD†

Hong Liu, MD*

BACKGROUND: Recent investigations demonstrate that anesthetic preconditioning and postconditioning reduce myocardial infarct size to a degree comparable to that achieved with ischemic preconditioning. We hypothesized that the combination of sevoflurane preconditioning and postconditioning would result in greater preservation of myocardium.

METHODS: Langendorff perfused rat hearts were divided into four groups: control, preconditioning, postconditioning, and preconditioning plus postconditioning. During reperfusion, left ventricular function (left ventricular developed pressure, left ventricular end diastolic pressure, and dp/dt) were measured. At the end of reperfusion, the infarct sizes were measured with 2,3,5 triphenyltetrazolium chloride staining. Nuclear magnetic resonance was used to measure intracellular pH, Na^+, and Ca^{2+}.

RESULTS: Left ventricular developed pressure, left ventricular end diastolic pressure, left ventricular dp/dt_{max} and dp/dt_{min} were significantly improved in the treatment groups when compared with those in the controls. Myocardial infarct size (24% ± 7%, 16% ± 8%, and 22% ± 7% in preconditioning, postconditioning, and pre-plus postconditioning groups versus 44% ± 8% in the control group, $P < 0.05$) and intracellular Na^+ and Ca^{2+} were significantly decreased in all experimental groups at the end of reperfusion when compared with those in control. However, there were no differences between these variables in each treatment group.

CONCLUSION: Sevoflurane postconditioning is as effective as preconditioning in protecting myocardial function after global ischemia. The combination of sevoflurane preconditioning and postconditioning offered no additional benefit over either intervention alone.

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Since Murry et al. (1) first described ischemic preconditioning (IPC) in dogs, numerous studies have confirmed that periods of myocardial ischemia limit the extent of subsequent myocardial infarction in various animal models. Other forms of preconditioning (pharmacologic) including anesthetic preconditioning (exposure to volatile anesthetics known as APC) have also been described, and studies have demonstrated similar efficacy of APC and IPC in protecting the myocardium against prolonged ischemia (2–4). Preconditioning the myocardium results in improved recovery of ventricular function, decreased incidence of arrhythmias and, most importantly, decreased infarct size (IS) (3,5). There are numerous underlying cellular mechanisms through which preconditioning confers protection to the myocardium (2), but one of the key end effects is prevention of intracellular calcium ($[Ca^{2+}]_i$) accumulation. Calcium accumulation has been repeatedly shown to play a major role in cellular dysfunction and death after ischemia and reperfusion (I/R) (6,7). Preconditioning prevents $[Ca^{2+}]_i$ accumulation by activating protective cellular mechanisms, some of which ultimately result in the activation of mitochondrial and sarcolemmal ATP sensitive potassium channels (K_{ATP}), and Na/H exchangers within myocytes (2,3,8,9).

Although preconditioning is an effective mechanism for protection against myocardial ischemic damage in a laboratory setting, it has significant limitations in a clinical setting, as ischemic episodes in humans are often unpredictable and precipitous. One would have to know in advance that a patient was about to

From the *Department of Anesthesiology and Pain Medicine, University of California, Davis, California; and †Department of Anesthesiology, Vancouver General Hospital, Vancouver, British Columbia, Canada.

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Address correspondence and reprint requests to Hong Liu, MD, Department of Anesthesiology and Pain Medicine, University of California Davis Health System, 4150 v. Street, Suite 1200, Sacramento, CA 95817. Address e-mail to hualiu@ucdavis.edu.

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have a myocardial infarction in order to provide appropriately timed preconditioning. Recently, the concept of postconditioning, whereby ischemia or volatile anesthetics are introduced immediately upon reperfusion in an effort to attenuate myocardial I/R injury, has garnered increased attention (10–14). Postconditioning could have significant clinical applications for patients with continuing myocardial ischemia who arrive in the operating room or cardiac catheterization laboratory for revascularization as it can be implemented at the time of reperfusion.

The goal of this study was to investigate, in an experimental model, whether the combination of sevoflurane preconditioning followed by sevoflurane postconditioning would result in decreasing intracellular Na^+ and Ca^{2+} concentrations, and decreased myocardial IS by attenuating reperfusion injury, and thus provide greater preservation of myocardial function than either modality alone.

METHODS

This study was approved by the Animal Care Committee of the University of California, Davis (Davis, CA), and all of the experiments were performed within the guidelines for animal care from the National Institutes of Health (Bethesda, MD).

Preparation of Isolated Rat Hearts

Male Fischer 344 rats, aged 2–4 mo, were anesthetized with intraperitoneal pentobarbital (50 mg/kg) plus heparin (1000 IU/kg). Once surgical anesthesia was achieved (no response to tail clamp), the hearts were excised via thoracotomy and rapidly transferred to a nonrecirculating Langendorff perfusion system. The aorta was cannulated and the hearts perfused with a Krebs–Henselet bicarbonate solution (127 mM NaCl, 4.75 mM KCl, 1.25 mM $MgCl_2$, 2.5 mM $CaCl_2$, 25 mM $NaHCO_3$, and 10 mM glucose). Perfusion pressure was maintained between 120 and140 cm H_2O, and the perfusate was continuously oxygenated with 95% O_2/5% CO_2 maintaining a pH of 7.40 ± 0.05. Temperature was kept constant at 37°C ± 0.5°C with a water-jacketed column. A balloon-tipped pressure transducer was placed into the left ventricle (LV) via an incision in the left atrial appendage, and LV end diastolic pressure (LVEDP) was set at 10 cm H_2O. The hearts were paced at 5 Hz via right atrial pacing wires. All preparations were allowed a 20-min period for equilibration. Global ischemia was achieved by interrupting perfusion to the heart. Pacing was discontinued during the ischemic period.

Experimental Design

The rats were randomly divided into four groups consisting of six hearts per group. Three of the groups underwent treatment and one group served as a control (Fig. 1). Rat hearts in the treatment groups were exposed to sevoflurane delivered to the gas mixture at a concentration of 2.5% via a sevotec5 variable bypass vaporizer (Datex-Ohmeda, Milwaukee, WI). After the 20-min equilibration period, hearts in the preconditioning group were exposed to sevoflurane (2.5%) for 10 min, followed by a 5-min washout period before 25 min of global ischemia and a 60-min reperfusion. Hearts in the postconditioning group underwent equilibration, then 25 min of global ischemia, followed immediately by sevoflurane (2.5%) exposure for the first 10 min of the 60-min reperfusion period. The preconditioning plus postconditioning group incorporated both protocols. The control group received no treatment before 25 min of global ischemia and none during the 60-min reperfusion period.

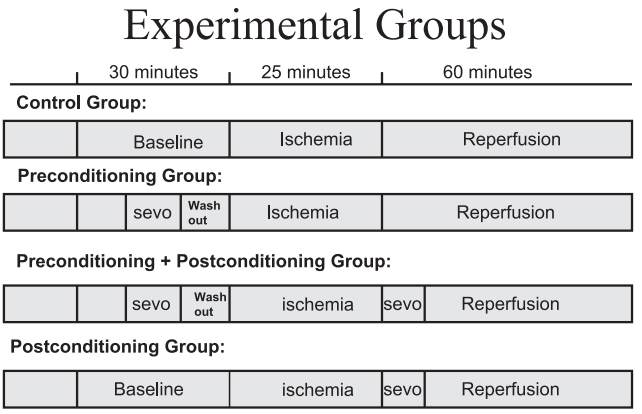

Figure 1. Schematic illustration of the experimental protocols used in this study. sevo = sevoflurane.

Myocardial IS

At the end of reperfusion, hearts were removed from the Langendorff apparatus and quickly sectioned into 2-mm slices. The slices were immersed in 2% 2,3,5 triphenyltetrazolium chloride staining solution and placed in a 37°C incubator for 20 min. After incubation, slices were washed with water, placed on petri dishes and then scanned into a computer using Adobe Photoshop software (Adobe, San Jose, CA). Standard computer plainmeteric analysis, using NIH image 1.62 (National Institutes of Health, Bethesda, MD), was performed to determine infarct area. The total area of infarction was divided by the total area of myocardium to yield the percent area of infarction (15).

LV Function

LV pressures were measured using a latex balloon filled with water inserted via an incision in the left atrial appendage through the mitral valve. Pressures were recorded using Powerlab 4/20 (ADInstruments, CO Springs, CO). During the equilibration period, LVEDP was set by adjusting the balloon volume to yield a left end diastolic pressure of approximately 10 cm H_2O. LV pressure measurements included: LV developed pressure (LVDP), calculated as LV end systolic pressure minus LVEDP and LVDP over time, LV dp/dt_{max} and dp/dt_{min}.

Table 1. Results of HR, LVESP, pH, Infarct Size, and CK Before Ischemia and at the End of Reperfusion in All Four Groups

	Before ischemia				End of reperfusion			
	Control	Precond.	Postcond.	Pre + postcond.	Control	Precond.	Postcond.	Pre + postcond.
HR	328 ± 17	320 ± 21	321 ± 45	318 ± 32	322 ± 12	304 ± 33	305 ± 64	299 ± 52
LVESP	115 ± 38	124 ± 46	114 ± 14	118 ± 20	75 ± 25	67 ± 8	66 ± 24	81 ± 17
pH	7.16 ± 0.06	7.23 ± 0.1	7.12 ± 0.02	7.16 ± 0.04	7.13 ± 0.1	7.21 ± 0.15	7.09 ± 0.05	7.12 ± 0.09
IS					44 ± 8	24 ± 7*	16 ± 8*	22 ± 7*
CK	15 ± 4	13 ± 5	14 ± 4	12 ± 6	1367 ± 562	341 ± 51*	378 ± 67*	422 ± 89*

The unit for left ventricular end systolic pressure (LVESP) data is cm H_2O. Heart rate (HR) is bpm. Infarct size (IS) expressed as % area of necrosis and the creatine kinase (CK) unit is IU/g dry weight. Data are presented as mean ± SD.

* Treatment groups versus. Control, $P < 0.05$ is considered significant. $n = 6$ in each group.

Creatine Kinase Analysis

Coronary sinus effluent was collected in 10-min aliquots throughout each experiment for measurement of creatine kinase (CK) as an indicator of myocardial cell damage. The amount of CK released was determined using a CK-10 kit (SIGMA Diagnostics, St. Louis, MO) and a Shimadzu UV-VIS recording photospectrometer (Shimadzu, Columbia, MD). Units are expressed as U/gm dry weight (15).

Nuclear Magnetic Resonance Spectroscopy

^{19}F NMR spectroscopy was performed to measure $[Ca^{2+}]_i$. The hearts were loaded for 30 min with perfusate containing the acetoxymethyl ester of 5F-1, 2-bis (2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (FBAPTA) at 2.5 μM. FBAPTA was washed out of the extracellular space with control solution before measurement of $[Ca^{2+}]_i$. ^{19}F spectra were generated from the summed free induction decays of 1500, 45° excitation pulses using 2K word data files and ±5000 Hz sweep width. $[Ca^{2+}]_i$ was calculated by using: $[Ca^{2+}]_i$ = Kd [bound]/[free], where the ratio of Ca^{2+}-bound and free FBAPTA concentrations was equal to the ratio of their corresponding peak areas and Kd is 300 nM.

^{31}P NMR spectroscopy was assessed from the summed free induction decays of 148, 60° excitation pulses using 4K word data files and ±4000 Hz sweep width. Intracellular pH (pH_i) was calculated from the shift in inorganic phosphorus resonance calibrated at 37°C with reference to control phosphocreatine. High-energy phosphates were determined as percent of control peak intensity (15).

^{23}Na NMR spectroscopy was performed to measure intracellular sodium concentrations ($[Na_i]$) by substituting 7.5 mM dysprosium triethylenetetramine-hexaacetic acid for NaCl and by adding and Ca^{2+} 2.5 mM (measured by a calcium electrode) to the perfusate. ^{23}Na spectra were generated from the summed free induction decays of 1000 with excitation pulses at 90° using 2K word data files and ±4000 Hz sweep widths. The $[Na_i^+]$ in mEq/kg dry weight was calculated from the calibrated area under the unshifted peak of the ^{23}Na spectra after subtracting the extracellular peak. At the end of the experiment, the hearts were weighed wet and then dried (at least 48 h) at 65°C to determine dry weight (15).

Statistical Analysis

Analysis of variance for repeated measures was used to test differences among groups. When differences among groups were found, a *post hoc* Tukey *t*-test was used to determine where these differences occurred. For all comparisons, differences were considered significant when $P < 0.05$. Data are reported as mean ± SD.

RESULTS

IS

In all three groups exposed to sevoflurane (preconditioning, postconditioning, and pre-plus postconditioning), IS was significantly decreased versus control (Table 1). The preconditioning, postconditioning, and pre-plus postconditioning groups had infarct areas of 24% ± 7%, 16% ± 8%, and 22% ± 7% respectively, compared with the control group of 44% ± 8% ($P < 0.05$). Differences among the treatment groups were not statistically significant.

CK

The CK results are listed in Table 1 as the total CK (U/gm dry weight) released during the 60-min reperfusion. APC rat hearts released significantly less CK than control rats: 341 ± 51 vs 1367 ± 562 ($P < 0.05$). Postconditioning hearts released significantly less CK than control hearts: 378 ± 67 vs 1367 ± 562 ($P < 0.05$). Combined pre-plus postconditioning hearts also released significantly less CK (422 ± 89) than control hearts ($P < 0.05$). There were no statistical differences for CK release among the three sevoflurane treatment groups.

LV Function

All LV function parameters measured (LVDP% recovery, LVEDP, LV dp/dt_{max} and dp/dt_{min}) were better ($P < 0.05$) in the sevoflurane treatment groups (preconditioning, postconditioning, and pre-plus postconditioning) compared with the control group. LVDP is expressed as the percentage of baseline

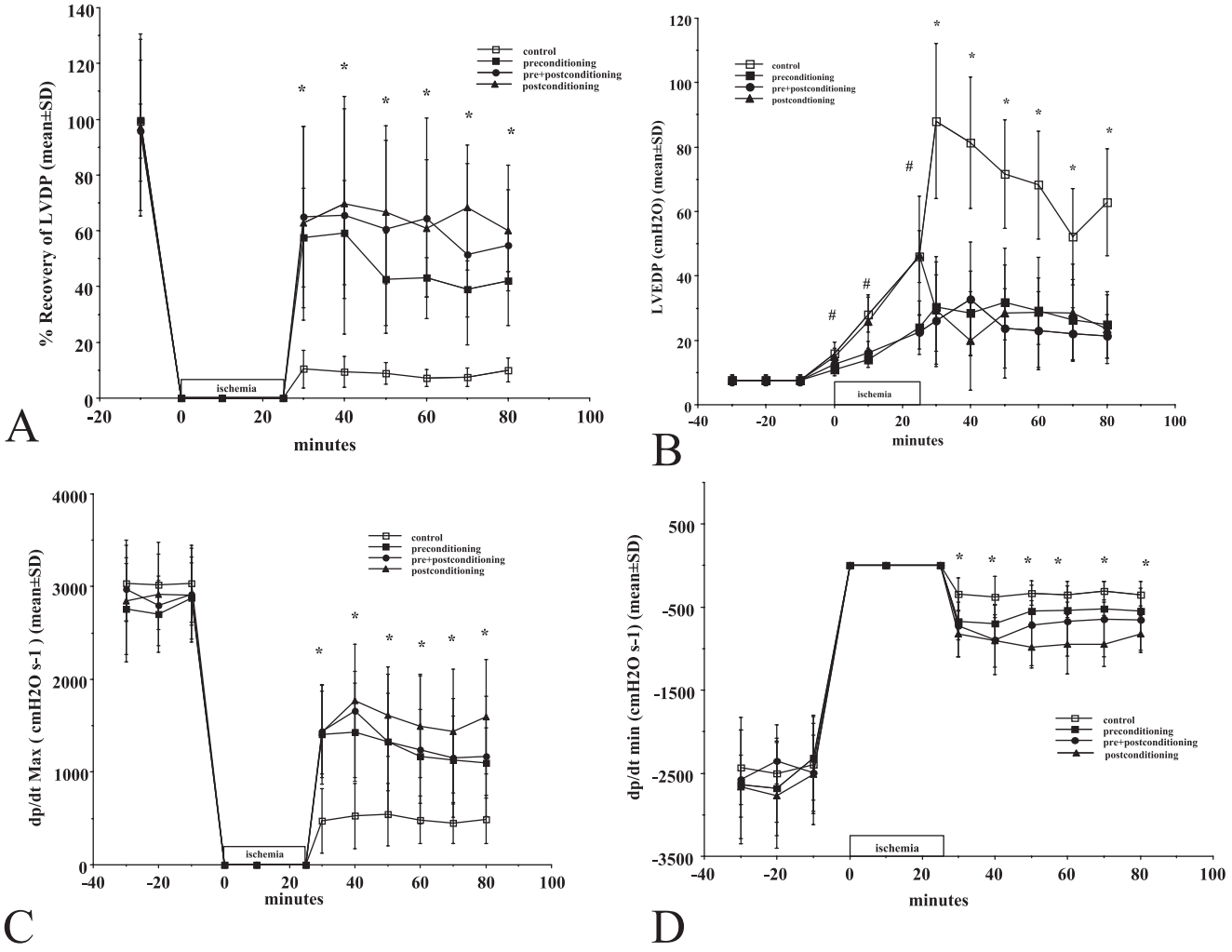

Figure 2. A. Recovery of left ventricular developed pressure (LVDP) during reperfusion. The LVDP was measured throughout reperfusion as a percentage compared to the baseline LVDP during the equilibration phase prior to ischemia. All experimental groups (preconditioning: closed square; postconditioning: closed triangle; and pre-plus postconditioning: closed circle) had significant ($P < 0.05$) recoveries of LVDP versus control (open square). There were no statistical differences among the three experimental groups. B. Results of left ventricular end diastolic pressure (LVEDP). Units are in cm H_2O. There were significantly lower diastolic pressures in the preconditioning and pre-plus postconditioning groups during ischemia ($P < 0.05$) compared with those in the postconditioning and control groups. There were significantly lower diastolic pressures in the experimental groups during reperfusion ($P < 0.05$) compared with those in the control. There were no statistical differences among the three experimental groups during reperfusion. C. Recovery of left ventricular systolic function. LV dp/dt_{max} values (in cm $H_2O\ s^{-1}$) were measured throughout reperfusion. All experimental groups had significant ($P < 0.05$) recoveries of LV dp/dt_{max} versus control. There were no statistical differences among the three experimental groups. D. Recovery of left ventricular diastolic function. LV dp/dt_{min} values (in cm $H_2O \cdot s^{-1}$) were measured throughout reperfusion. The LV dp/dt_{min} values were significantly lower in all the experimental groups ($P < 0.05$) versus control. There were no statistical differences among the three experimental groups. *Experimental groups versus control ($P < 0.05$). #Preconditioning and pre-plus postconditioning versus control and postconditioning ($P < 0.05$). Data presented as mean ± SD. $n = 6$ in each group. pre-plus postconditioning: combined preconditioning and postconditioning.

function recovery after 60 min of reperfusion. All other values reflect function after 60 min of reperfusion. There were no statistically significant differences among the sevoflurane treatment groups for any of the measured LV function parameters. LVDP recovery (% of baseline) was 42% ± 8%, 60% ± 14%, and 55% ± 28% for pre-, post-, and pre-plus postconditioning groups, respectively, compared with the control group 10% ± 4% recovery (Fig. 2A). LVEDP values (in cm H_2O) after reperfusion were 25 ± 10, 24 ± 10, and 21 ± 6 respectively for the sevoflurane treatment groups versus 63 ± 16 for the control group (Fig. 2B). LV dp/dt_{max} values (in cm $H_2O \cdot s^{-1}$) were 1102 ± 378, 1598 ± 614, and 1170 ± 649, respectively, for the sevoflurane treatment groups versus 491 ± 259 for the control group (Fig. 2C). LV dp/dt_{min} values (in cm $H_2O \cdot s^{-1}$) were −543 ± 54, −822 ± 201, and −656 ± 388, respectively, for the sevoflurane treatment groups versus −355 ± 161 for the control group (Fig. 2D). There were no statistically significant differences in heart rate and LV end systolic pressure among all four groups (Table 1).

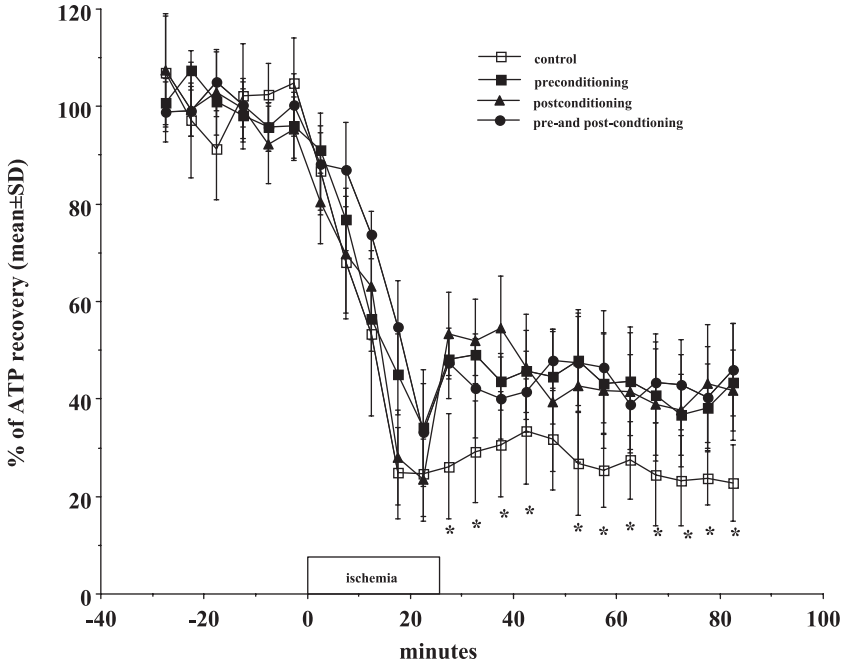

Figure 3. Ischemia decreased ATP levels during the experiment. The ATP was better preserved in all the experimental groups (preconditioning: closed square; postconditioning: closed triangle; and pre-plus postconditioning: closed circle) ($P < 0.05$) versus control (open square). There were no statistical differences among the three experimental groups. *Experimental groups versus control ($P < 0.05$). Data presented as mean ± SD. $n = 6$ in each group. pre-plus postconditioning: combined preconditioning and postconditioning. ATP = adenosine triphosphate.

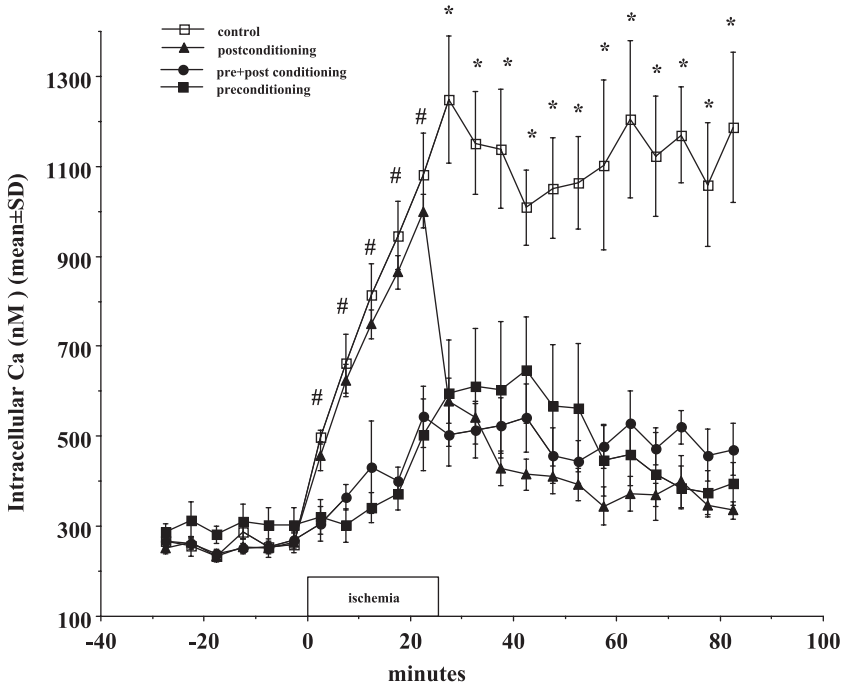

Figure 4. Ischemia causes an increase in intracellular Ca $[Ca^{2+}]_i$ in control hearts (open square) during ischemia and reperfusion and in the postconditioning heart (closed triangle) during ischemia. The increases in $[Ca^{2+}]_i$ were significantly limited in preconditioning hearts (closed square) and pre-plus postconditioning hearts (closed circle) during ischemia and in all the experimental groups (preconditioning: closed square; postconditioning: closed triangle; and pre-plus postconditioning: closed circle) ($P < 0.05$) versus control (open square) during reperfusion. There were no statistical differences among the three experimental groups during reperfusion. *Experimental groups versus control ($P < 0.05$). #Preconditioning and pre-plus postconditioning versus control and postconditioning ($P < 0.05$). Data presented as mean ± SD. $n = 6$ in each group. pre-plus postconditioning: combined preconditioning and postconditioning.

ATP Preservation

Results for ATP measurements are shown in Figure 3. Myocardial ischemia led to a significant ($P < 0.05$) decrease in the ATP levels in all groups. The preconditioning and pre-plus postconditioning groups had higher ATP levels at the end of ischemia (34 ± 12 and 33 ± 10, respectively) than the control and postconditioning groups (24 ± 8 and 23 ± 8 respectively). At the end of reperfusion, all sevoflurane treatment groups had significantly better recovery of ATP levels than the control group. The preconditioning, postconditioning, pre-plus postconditioning and control group ATP values were 44 ± 10, 42 ± 8, 46 ± 9, and 23 ± 7, respectively. Although the treatment groups had statistically significant recovery of ATP versus the control group, there were no significant differences among the treatment groups.

$[Ca^{2+}]_i$

The effects of I/R on $[Ca^{2+}]_i$ are shown in Figure 4. In the control hearts, $[Ca^{2+}]_i$ increased from 260 ± 16 at baseline to 1081 ± 177 at the end of ischemia, and to 1187 ± 324 at the end of reperfusion. Exposure to sevoflurane attenuated the increases in $[Ca^{2+}]_i$ in preconditioning, postconditioning, and pre-plus postconditioning groups. By the end of reperfusion $[Ca^{2+}]_i$

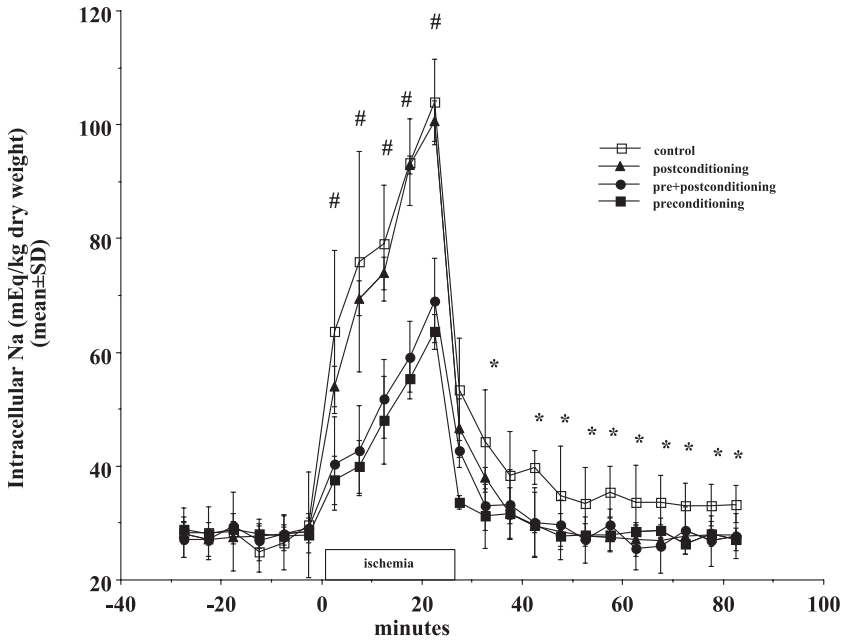

Figure 5. Ischemia causes an increase in intracellular Na (Na^+_i) in control hearts (open square) during ischemia and reperfusion and in the postconditioning heart (closed triangle) during ischemia. The increases in Na^+_i were significantly limited in all the experimental groups (preconditioning: closed square; postconditioning: closed triangle; and pre-plus postconditioning: closed circle) ($P < 0.05$) versus control (open square) during reperfusion. There is no statistical difference between the control and postconditioning groups during ischemia. There were no statistical differences among the three experimental groups during reperfusion. *Experimental groups versus control ($P < 0.05$). #Preconditioning and pre-plus postconditioning versus control and postconditioning ($P < 0.05$). Data presented as mean ± SD. $n = 6$ in each group. pre-plus postconditioning: combined preconditioning and postconditioning. "We only used one sevoflurane concentration, a higher concentration (1.5 MAC) experiment could show a different result when used in a combined pre- and postconditioning protocol."

levels were 395 ± 94 in the preconditioning group, 336 ± 40 in the postconditioning group, and 470 ± 111 in the pre-plus postconditioning group. The decreases in $[Ca^{2+}]_i$ seen in the three treatment groups were statistically significant compared with those seen in the control group ($P < 0.05$), but there were no statistical differences among the three treatment groups.

Na^+_i Accumulation

Changes in Na^+_i accumulation that occurred during I/R are shown in Figure 5. In the control hearts, baseline Na^+_i increased from 30 ± 9 to 104 ± 8 at the end of ischemia and declined to 33 ± 3 at the end of reperfusion. Exposure to sevoflurane attenuated Na^+_i accumulation in the preconditioning, postconditioning, and pre-plus postconditioning groups. Na^+_i levels at the end of ischemia and the end of reperfusion were 64 ± 3 and 27 ± 2 in the preconditioning group, 101 ± 4 and 28 ± 2 in the postconditioning group, and 69 ± 7 and 28 ± 4 in the pre-plus postconditioning group. The decreased Na^+_i accumulation at the end of ischemia seen in the three treatment groups was statistically significant when compared with that in the control group ($P < 0.05$), but there were no statistical differences among the three treatment groups.

Intracellular Proton Accumulation

At the end of 25 min of sustained ischemia pH_i decreased in all groups: control (5.91 ± 0.06), preconditioning (6.19 ± 0.10), postconditioning (5.96 ± 0.06), and pre-plus postconditioning group (6.17 ± 0.10). After 60 min of reperfusion, all groups had a similar recovery of pH_i:control (7.13 ± 0.10), preconditioning (7.21 ± 0.15), postconditioning (7.09 ± 0.05), and pre-plus postconditioning (7.12 ± 0.09) (Table 1). There were no statistical differences among any of the groups at the end of ischemia or at the end of reperfusion.

DISCUSSION

In this study, APC and postconditioning with sevoflurane, alone or in combination, was shown to be equally effective in preventing the increases in Na^+_i and $[Ca^{2+}]_i$, decreasing IS and preserving ATP and LV function after I/R.

Myocardial ischemia during the perioperative period is a serious risk for patients undergoing both cardiac and noncardiac operations. About 30–40% of patients with coronary artery disease experience perioperative myocardial ischemia during noncardiac surgery, increasing the risk for myocardial infarction (16). Anesthetic postconditioning is a potentially useful clinical intervention, as it can be implemented in patients who arrive at the hospital with ischemia and myocardial injury already in progress (17). Anesthetic postconditioning is easy to implement and may be used in combination with a variety of revascularization procedures, such as percutaneous transluminal

coronary angioplasty and coronary artery bypass grafting.

Reduction in myocardial IS is the "gold standard" in determining efficacy of preconditioning or postconditioning protocols for myocardial protection against I/R injury (18,19). Numerous studies have demonstrated APC to be an effective intervention against I/R injury (3–5). This study demonstrated that sevoflurane preconditioning, postconditioning, and pre-plus postconditioning all provided similar significant reductions (approximately 50%) in IS when compared with a control group. The combination of sevoflurane preconditioning and postconditioning provided no significant benefit over either intervention alone for any of the measured parameters. We also demonstrated that sevoflurane preconditioning, postconditioning, and pre-plus postconditioning provided similar, significant, reductions in CK release when compared with a control group, correlated with the reduction in IS seen in the treatment groups.

Previous studies have demonstrated that preconditioning not only decreases IS, but also better preserves LV function after I/R (2,20,21). We demonstrated that both sevoflurane preconditioning and postconditioning preserve LV function after I/R to a similar degree. Across all measures of LV function (systolic and diastolic), the sevoflurane-treated groups had significantly better postischemic function when compared with controls. The improved functional recovery correlates with the decreased IS discussed previously. The sevoflurane pre-plus postconditioning group showed no additional improvement over either intervention alone.

Loss of calcium homeostasis with subsequent $[Ca^{2+}]_i$ accumulation results in cell damage and death (2,7,22–24). Ischemia results in a diminished ATP supply and increases in H^+ production, which stimulates the Na^+/H^+ exchanger (NHE-1) to transport H^+ out of the cell in exchange for Na^+, leading to $Na^+{}_i$ accumulation. This results in an increased $[Ca^{2+}]_i$ via Na^+/Ca^{2+} exchanger. In all three of our treatment groups, $[Ca^{2+}]_i$ was substantially lower at the end of reperfusion when compared with controls. The lower $[Ca^{2+}]_i$ correlated with the improved LV function and decreased IS seen in the treatment groups. There were no statistical differences among the treatment groups, suggesting that similar protective mechanisms may be responsible for the decreased $[Ca^{2+}]_i$ accumulation observed for both pre- and postconditioning. Similarly, $Na^+{}_i$ levels were substantially lower in the three treatment groups compared with that in the control. A lower level of $Na^+{}_i$ results in less $[Ca^{2+}]_i$ accumulation and presumably improved LV function and decreased IS, all of which were observed in all three treatment groups. Again, there were no statistical differences among the treatment groups suggesting similar protective mechanisms for both preconditioning and postconditioning.

As expected, pH_i decreased for all groups during ischemia. During reperfusion, all groups showed similar recovery of pH_i to near normal preischemic levels. The recovery of pH_i likely involves the NHE-1 and Na^+/Ca^{2+} exchanger discussed previously. The cell is able to improve pH_i by transferring H^+ out of the cell at the expense of Na^+ and Ca^{2+} accumulation. However, other mechanisms may also be involved in maintaining pH_i and may explain why there was no significant difference among the treatment and control groups. Some studies indicate that preconditioning does not impact certain cellular mechanisms responsible for maintenance of normal pH_i during I/R (25,26).

A multitude of vital cellular mechanisms depend on constant production of ATP by the mitochondria including maintenance of low $[Ca^{2+}]_i$ levels via the ATP-dependant ion exchangers discussed above. A number of studies have demonstrated that mitochondrial function is often profoundly disrupted by I/R injury, resulting in impairment of cellular respiration and a tremendous decrease in ATP production (2,8,27). APC has been shown to be protective by preserving mitochondrial function and maintaining ATP levels after I/R injury (2,3,5). Our results are consistent with previous findings demonstrating that APC results in preservation of mitochondrial function and maintenance of ATP stores. Both of the preconditioned groups (preconditioning alone and pre-plus postconditioning) had significantly higher ATP levels at the end of ischemia than the control and postconditioning groups. However, by the end of the reperfusion period, all three treatment groups had similar recovery of ATP levels relative to the control. Again, there were no statistically significant differences among the three treatment groups, indicating that the combination of anesthetic pre-plus postconditioning does not provide additional protection over either intervention alone. These findings demonstrate that anesthetic postconditioning preserves mitochondrial function and ATP production to a similar degree as APC alone. Interestingly, the postconditioning group showed similar ATP levels during ischemia to the control group, but recovered ATP levels to those seen in the preconditioned groups by the end of the reperfusion period. This suggests that attenuation or prevention of reperfusion injury is vital for the preservation of mitochondrial function. In addition, the postconditioning intervention, whether ischemic or anesthetic, needs to be implemented immediately upon reperfusion if reperfusion injury is to be prevented (10,28).

Although we are uncertain as to why APC and postconditioning are not additive, we surmise that similar end cellular effectors are induced by either intervention to a comparable degree. Similar studies have been performed with mixed results (13,14,19). Halkos et al. (19) combined IPC and postconditioning without benefit over either intervention alone. Chiari

et al. (14) combined IPC and anesthetic postconditioning with isoflurane and found an additive effect versus either intervention alone, suggesting differing mechanisms of myocardial protection between ischemic and anesthetic postconditioning. Finally, Obal et al. (13) were able to show an additive benefit by combining anesthetic pre- and postconditioning with an *in vivo* rat model, which differed from our findings with an *in vitro* rat model.

Both IPC and APC are effective in preserving myocardial function and decreasing IS in numerous species (10,17,18) and volatile anesthetics are powerful mediators of preconditioning and postconditioning (2,11–14,28–30). Although definitive end-points, such as IS reduction, are difficult to measure in humans, studies using markers of myocardial injury (troponins and CK) indicate less myocardial damage in patients who underwent preconditioning or postconditioning (17,18,20). However, the obvious drawback to preconditioning is lack of foreknowledge of an impending ischemic event. In contrast, postconditioning can be implemented in patients with continuing ischemia at the onset of reperfusion after revascularization procedures, such as coronary angioplasty and stenting, coronary artery bypass grafting, and possibly even organ transplantation.

Our study has several significant limitations that should be considered in interpreting the data. We did not specifically evaluate the mechanisms through which our preconditioning or postconditioning groups were able to provide myocardial protection, so it is difficult to draw definitive conclusions as to what they have in common or how they differ. In addition, a number of studies have demonstrated that delayed APC (24 or 48 h) before ischemia is particularly effective in providing myocardial protection as inducible cytoprotective proteins likely provide additional protection against ischemic injury (6,29). Perhaps, if we had a delayed APC in combination with the postconditioning group, we may have seen an additive benefit over preconditioning or postconditioning alone. We did not measure $[Ca^{2+}]_m$ in this study; however, studies have showed that increased $[Ca^{2+}]_i$ correlates with an increased $[Ca^{2+}]_m$ (6,31) Finally, our results are specific to our experimental design with a 25-min period of ischemia and a 60-min reperfusion. Whether these findings can be extrapolated to humans is not known. We did not assess long-term outcome, which may be completely different despite initial similar IS. Perhaps, a more prolonged ischemic insult would show that there is no difference between sevoflurane pre- and postconditioning and that a longer perfusion time, such as 90 min or longer will have a clear demarcation of necrosis. We only used one sevoflurane concentration; a higher concentration (1.5 MAC) experiment could show a different result when used in a combined pre-and postconditioining protocol.

In conclusion, this study demonstrates that sevoflurane administration during the early minutes of reperfusion results in preservation of myocardial function and decreased IS by attenuating I/R injury. Our data show that no additional benefit is obtained when sevoflurane preconditioning and postconditioning are combined over either intervention alone. Sevoflurane postconditioning may be clinically applicable in situations where the potential for I/R injury is of concern.

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