

UC Davis

UC Davis Previously Published Works

Title

Reduced Efficacy of Volatile Anesthetic Preconditioning with Advanced Age in Isolated Rat Myocardium

Permalink

<https://escholarship.org/uc/item/7jk1x3sr>

Journal

Anesthesiology, 100(3)

ISSN

0003-3022

Authors

Sniecinski, Roman

Liu, Hong

Publication Date

2004-03-01

DOI

10.1097/00000542-200403000-00019

Peer reviewed

Reduced Efficacy of Volatile Anesthetic Preconditioning with Advanced Age in Isolated Rat Myocardium

Roman Sniecinski, M.D.,* Hong Liu, M.D.†

Background: Ischemic preconditioning and anesthetic preconditioning (APC) are reported to decrease myocardial infarct size during ischemia–reperfusion injury. However, the beneficial effects of ischemic preconditioning have been shown to decrease with advancing age. Although the mechanisms of ischemic preconditioning and APC are thought to be similar, it is not known whether the beneficial effects of APC are also reduced in the aged myocardium.

Methods: Male Fischer 344 rats of three age groups (2–4, 10–12, and 20–24 months) were used. Hearts were Langendorff perfused. Six hearts in each age group were pretreated with 10 min of sevoflurane and a 5-min washout before 25 min of ischemia and 60 min of reperfusion. Six control hearts in each age group received no treatment before ischemia. Nuclear magnetic resonance was used to measure intracellular Na, intracellular Ca, and intracellular pH, respectively. Left ventricular developed pressure, creatine kinase, and infarct size were measured.

Results: Ischemia decreases intracellular pH and increases intracellular Na and intracellular Ca in all age groups. APC blunts the pH decreases in young adult and middle-aged rats, but not in aged rats. APC decreased intracellular Na and intracellular Ca accumulation during ischemia in young adult and middle-aged hearts. APC improved adenosine triphosphate recovery in young rats but not in aged rats. Creatine kinase and infarct sizes were significantly reduced and left ventricular developed pressure was improved with APC in the young adult and middle-aged groups but not the aged group.

Conclusions: The benefits of APC are significantly reduced with advanced age in an isolated rat heart model.

BRIEF periods of sublethal ischemia before prolonged ischemia help to protect the heart against extensive infarction. Since 1986 when Murry *et al.*¹ demonstrated the phenomenon in dogs, ischemic preconditioning (IPC) has been shown to occur in every mammalian species in which it has been investigated. Although infarction size is not an available endpoint in human studies, patients with “preinfarction angina” are believed to represent a type of IPC. These patients have fewer complications and a reduced mortality compared with those who have myocardial infarction without it.²

One of the questions about IPC has been its efficacy in aged myocardium. Recent studies in the isolated perfused rat heart model have shown that IPC failed to induce cardioprotection in the senescent myocardium.^{3,4} Clinically, it has been suggested that the benefit of “preinfarction angina” in elderly patients seems to be reduced.⁵ Age may play a significant role in the IPC phenomenon.

Volatile anesthetics have been shown to elicit cardioprotective properties similar to those of IPC.^{6,7} The mechanism of volatile anesthetic preconditioning (APC), although not fully understood, is believed to share similarities with IPC by activation of adenosine triphosphate (ATP)-sensitive potassium (K_{ATP}) channels, particularly in the mitochondria.^{8–11} Volatile anesthetics are now being investigated as pharmacologic agents to precondition the heart during coronary artery bypass surgery, with both positive^{12–14} and negative¹⁵ results. The effect of different ages on APC has not been investigated. Given its similarities to IPC, it seems reasonable to hypothesize that the beneficial effects of APC may also be reduced in aged myocardium.

The goal of the current study was to determine the effect of age on APC. The volatile anesthetic sevoflurane has been previously shown to decrease infarct size in the isolated perfused rat heart model by a preconditioning mechanism.¹⁶ In this study, we present the results of APC with sevoflurane when applied to isolated rat hearts of three different age groups.

Materials and Methods

The study protocol was approved by the Animal Care Committee of the University of California, Davis (Davis, California), and all experiments were conducted in accordance with guidelines of animal care from the National Institutes of Health.

Preparation of Isolated Hearts

Rat hearts were obtained from male Fischer 344 rats (National Institute on Aging, National Institutes of Health, Bethesda, Maryland) of the following age groups: 2–4, 10–12, and 20–24 months. Anesthesia was first obtained with an intraperitoneal injection of sodium thiopental (65 mg/kg) along with 1,000 U heparin. Sodium thiopental was chosen for initial anesthesia because this drug has been shown not to influence preconditioning.¹⁷ When no response to tail clamping was obtained, the heart was excised *via* thoracotomy and placed in an iced solution of Krebs-Henselet buffer

* Resident. Current position: Cardiac Anesthesia Fellow, Department of Anesthesia, Emory University Hospital, Atlanta, Georgia. † Assistant Professor.

Received from the Department of Anesthesiology and Pain Medicine, University of California, Davis, Davis, California. Submitted for publication September 4, 2003. Accepted for publication November 24, 2003. Supported in part by the New Investigator Award from the Foundation for Anesthesia Education Research, Mayo Clinic, Rochester, Minnesota, and the University of California Davis Health System Research Award, Davis, California. Funding for spectrometers was provided by grant No. RR08206 from the National Institutes of Health, Bethesda, Maryland. Presented in part at the 16th Annual Meeting of the American Society of Anesthesiologists, San Francisco, California, October 11–15, 2003.

Address reprint requests to Dr. Liu: Department of Anesthesiology and Pain Medicine, University of California Davis Medical Center, 4150 V Street, Suite 1200, Sacramento, California 95817. Address electronic mail to: hualiu@ucdavis.edu. Individual article reprints may be purchased through the Journal Web site, www.anesthesiology.org.

(127 mM NaCl, 4.7 mM KCl, 1.25 mM MgCl₂, 2.5 mM CaCl₂, 25 mM NaHCO₃, and 10 mM glucose). The aorta was quickly cannulated and aerobically perfused with Krebs-Henselet buffer on a nonrecirculating Langendorff apparatus at a perfusion pressure of 140 ± 10 cm H₂O. The perfusate was continuously oxygenated with a 95% O₂-5% CO₂ mixture, and its temperature was kept constant at 37 ± 0.5°C with a water-jacketed column. The time from excision to cannulation was less than 60 s in all hearts. After cannulation, pacing wires were placed in the right atrium.

Experimental Design

Twelve rats from each age group were randomly selected. Six hearts in each age group served as controls and underwent a 30-min equilibration period, followed by 15 min of perfusion, 25 min of global ischemia, and finally, 60 min of reperfusion. The other six hearts in each age group underwent a 30-min equilibration period, 10 min of sevoflurane exposure, and a 5-min wash-out period before the 25 min of global ischemia and 60 min of reperfusion. The sevoflurane was delivered at 2.5% to the gas mixture *via* a standard Sevotec5 variable bypass vaporizer (Datex-Ohmeda, Milwaukee, WI) at a concentration of 0.4 ± 0.02 mM measured in the liquid phase by gas chromatography (Varian, Walnut Creek, CA). The concentration is approximately 0.9 minimum alveolar concentration (MAC) for young rats and 1.1 MAC for middle-aged rats. Global ischemia was induced by stopping all flow of the Krebs-Henselet buffer perfusate to the heart. Atrial pacing at 5 Hz was used during all phases of the experiment, except during the period of global ischemia. Any episodes of ventricular fibrillation were mechanically converted when they occurred.

Nuclear Magnetic Resonance Spectroscopy

²³Na, ¹⁹F, and ³¹P nuclear magnetic resonance were used to measure intracellular Na (Na_i), intracellular Ca ([Ca]_i), and intracellular pH (pH_i) and high-energy phosphates, respectively.^{18,19} All three nuclei were measured in separate hearts. To measure Na_i, 7.5 mM dysprosium triethylenetetraminehexaacetic acid (DyTTHA) was substituted iso-osmotically for NaCl in the perfusate, and Ca was added to reach a perfusate concentration of 2.5 mM as measured by Ca electrode. To measure [Ca]_i, hearts were perfused with a 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 then washed out of the extracellular space with control solution for 15 min before measurement of [Ca]_i. ²³Na, ¹⁹F and ³¹P experiments were conducted using a Bruker AMX400 spectrometer (Bruker, Rheinstetten, Germany). ²³Na, ¹⁹F, and ³¹P spectra were generated from the summed free induction decays of 1,000, 1,500, and 148 excitation pulses (90, 45, and 60°) using 2,000-, 2,000-, and 4,000-word data files and ±4,000-, ±5,000-

and ±4,000-Hz sweep widths, respectively. For all nuclei, data files were collected over 5-min intervals. Because the nuclear magnetic resonance signal intensity reflects the time average for the interval over which data are collected, data are represented in time as corresponding to the midpoint of the 5-min acquisition interval.

Intracellular Na (in mEq/kg dry weight) was calculated from the calibrated area under the unshifted peak of the ²³Na spectra after subtracting out the extracellular peak.^{18,19} At the end of the experiment, hearts were weighed wet and dried to a constant weight (at least 48 h) at 65°C to determine the dry weight. [Ca]_i (in nmol/l cell water) was calculated as the product of the ratio of the areas of the Ca-bound and Ca-free peaks in the FBAPTA spectrum and the 500 nM Ca-FBAPTA dissociation constant.^{18,19} pH_i was determined from the chemical shift of the inorganic phosphate resonance (with reference to control phosphocreatine) calibrated at 37°C. High-energy phosphates are reported as percent of control peak height.^{18,19}

Hemodynamic Measurements

To measure left ventricular pressures, a latex balloon was filled with water and connected to a pressure transducer (Medex, Dublin, CA) using PE40 tubing. The balloon was inserted into the left ventricle *via* the left atrial appendage through the mitral valve. The balloon volume was adjusted during the equilibration period to yield a left ventricular end-diastolic pressure (LVEDP) of 5–10 cm H₂O. Pressures were recorded using Powerlab 4/20 hardware with amplifiers (ADInstruments, Colorado Springs, CO) and Chart for Windows v4.0.4 software (ADInstruments). Hemodynamic measurements included LVEDP, left ventricular end-systolic pressure (LVESP), and left ventricular developed pressure (LVDP). LVDP was defined as LVESP minus LVEDP.

Creatine Kinase Analysis

The run-off from the coronary sinus was collected for the first 10 min of reperfusion. This was placed in aliquots and stored at -80°C until analysis could take place. After thawing, the amount of creatine kinase (CK) was determined using a CK-10 kit (Sigma Diagnostics, St. Louis, MO) and a Shimadzu UV-VIS recording photometer (Shimadzu, Columbia, MD). Units are expressed as U/g dry weight.¹⁹

Determination of Infarct Size

At the end of reperfusion, the hearts were quickly taken down from the Langendorff apparatus and sliced into 2-mm sections. The sections were immersed in 2% 2,3,5-triphenyltetrazolium chloride staining solution and placed in a 37°C incubator for 20 min. Noninfarcted myocardium stains a bright red that is caused by reduction of 2,3,5-triphenyltetrazolium chloride by dehydro-

Table 1. Results of LVEDP, LVESP, LVDP, Infarct Size, and CK before Ischemia and at the End of Reperfusion in Three Age Groups

	Control					APC				
	LVEDP	LVESP	LVDP	Infarct Size	CK	LVEDP	LVESP	LVDP	Infarct Size	CK
Young adult										
Before ischemia	7 ± 1	79 ± 10	72 ± 10			7 ± 1	84 ± 13	76 ± 13		
End of reperfusion	14 ± 4	26 ± 4	12 ± 2	33 ± 2	295 ± 38	7 ± 2*	32 ± 2	25 ± 2*	16 ± 2*	21 ± 8*
Middle-aged										
Before ischemia	7 ± 1	104 ± 9	97.9 ± 9			8 ± 1	102 ± 9	95 ± 9		
End of reperfusion	26 ± 5	36 ± 2	9 ± 1	28 ± 3	254 ± 34	12 ± 2*	29 ± 3*	17 ± 1*	17 ± 2*	21 ± 4*
Aged										
Before ischemia	7 ± 1	156 ± 20	149 ± 20			6 ± 1	147 ± 19	139 ± 19		
End of reperfusion	27 ± 4	40 ± 4	11 ± 2	24 ± 3	304 ± 14	24 ± 3	40 ± 5	16 ± 3	25 ± 2	299 ± 14

The unit for hemodynamic data is cm H₂O. Infarct size is expressed as percent area of necrosis, and the creatine kinase (CK) unit is U/g dry weight. Data are presented as mean ± SEM. n = 6 in each group.

* Anesthetic preconditioning vs. control; $P < 0.05$ is considered significant.

LVDP = left ventricular developed pressure; LVEDP = left ventricular end-diastolic pressure; LVESP = left ventricular end-systolic pressure.

genes present in viable tissue.^{20,21} After 20 min, the sections were rinsed off with water and placed on transparent Petri dishes. They were then scanned into a computer using Adobe Photoshop 5.0LE software (Adobe, San Jose, CA). Standard computer planimetric analysis, using NIH image 1.62 (National Institutes of Health, Bethesda, MD), was used to determine the infarct area. Because the entire heart was at risk from global ischemia, the infarct size was expressed by dividing the necrotic area by the total slice area to obtain the percent necrosis.

Analysis of variance for repeated measures was used to test for differences between treatments. When differences between treatments were found, the unpaired *t* test was used to determine the times at which differences between treatments occurred. The *t* test was used only across treatments for a particular time. Under these conditions, the *t* test and multiple-comparison tests provide identical results for two treatments. Data are reported as mean ± SEM. For all comparisons, differences were considered significant when *P* was less than 0.05.

Results

Hemodynamic Results

Average hemodynamic variables during the preischemic period are presented in table 1. There was no significant difference in preischemic hemodynamic variables between the APC hearts and their respective controls for any of the three age groups. Hemodynamic measurements after 60 min of reperfusion are presented in table 1. The percentage of LVDP recovery was calculated by dividing the measurement after 60 min of reperfusion by the average LVDP during the preischemic period. There was a significant difference between control and APC hearts in the young adult (16 ± 2 vs. 38 ± 7%) and middle-aged (10 ± 1 vs. 18 ± 1%; $P < 0.05$) groups but not the aged hearts (9 ± 1 vs. 12 ± 2%; $P < 0.05$).

Figure 1 displays the percent LVDP recovery of each age group at 10-min intervals for the entire 60 min of reperfusion. The other observation to reach statistical significance was LVESP in the middle-aged groups (table 1).

Creatine Kinase and Infarct Size Measurements

Creatine kinase and infarct size measurements are summarized in table 1. Significant differences between control and APC hearts were found in the young adult and middle-aged groups only. Likewise, the difference in infarct size was only significant in the young adult and middle-aged groups. No differences between control and APC hearts were found in the aged group for CK measurements and infarct size.

High-energy Phosphate Metabolism

Figure 2 demonstrates a significant difference in ATP between control and APC hearts in the young adult group but not the middle-aged or aged group. Similarly, phosphocreatine, a substrate of ATP synthesis, recovered better in young adult hearts but not in middle-aged or aged hearts in APC treated groups after reperfusion (fig. 3). Inorganic phosphate, an ATP breakdown product, was significantly lower at the end of ischemia and reperfusion when compared with control groups and in young adult hearts of the APC groups but not in middle-aged adult or aged hearts (fig. 4).

Intracellular Ca Changes

Figure 5 shows that ischemia causes increases in [Ca]_i during ischemia and reperfusion in all three age groups. APC attenuates the increases in [Ca]_i in young adult and middle-aged hearts, but not in aged hearts. [Ca]_i levels were significantly different between control and APC hearts in the young adult group at the end of ischemia (1,505 ± 242 vs. 502 ± 79 nM; $P < 0.05$) and reperfusion (1,339 ± 145 vs. 385 ± 43 nM; $P < 0.05$). Middle-aged hearts also showed less [Ca]_i accumulation at the end of

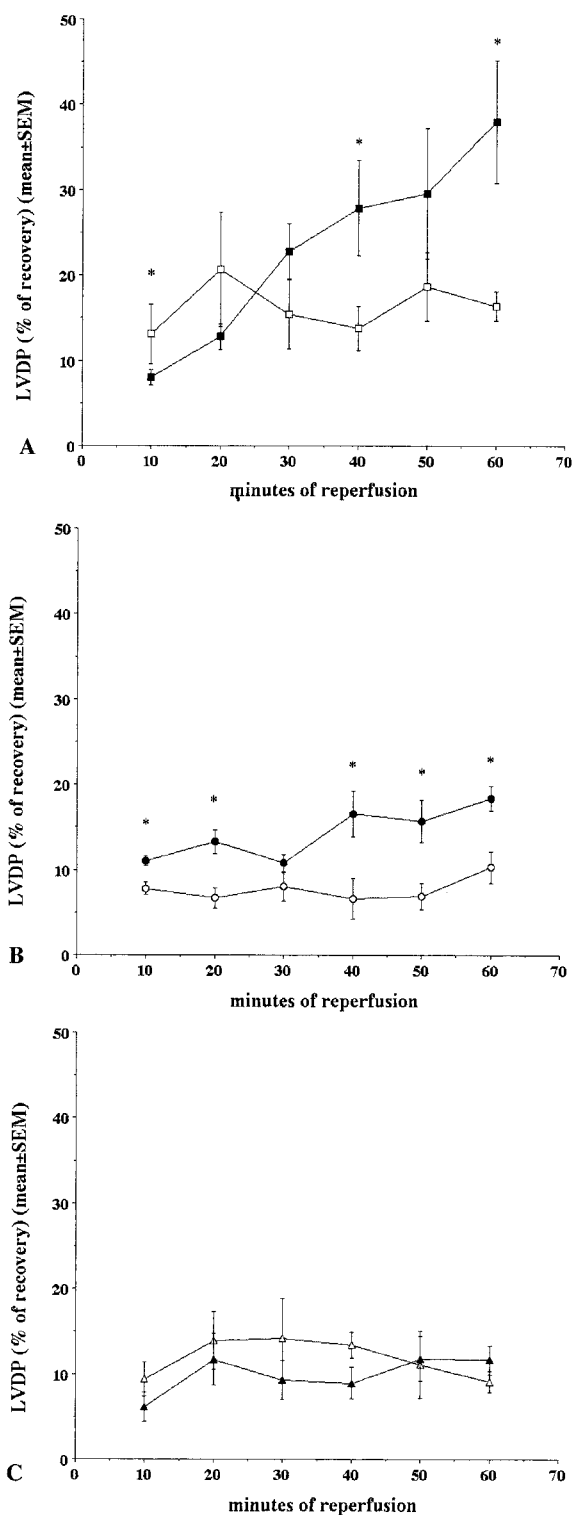


Fig. 1. (A) In young adult hearts, left ventricular developed pressure (LVDP) recovery during the 60-min reperfusion is better in the anesthetic preconditioning (APC)-treated hearts (closed squares) than in the control hearts (open squares) ($* P < 0.05$). (B) In middle-aged hearts, LVDP recovery during the 60-min reperfusion is better in the APC-treated hearts (closed circles) than in the control hearts (open circles) ($* P < 0.05$). (C) There are no differences in LVDP recovery in aged hearts between the control (open triangles) and APC (closed triangles) groups. $n = 6/\text{group}$. $*$ APC versus control.

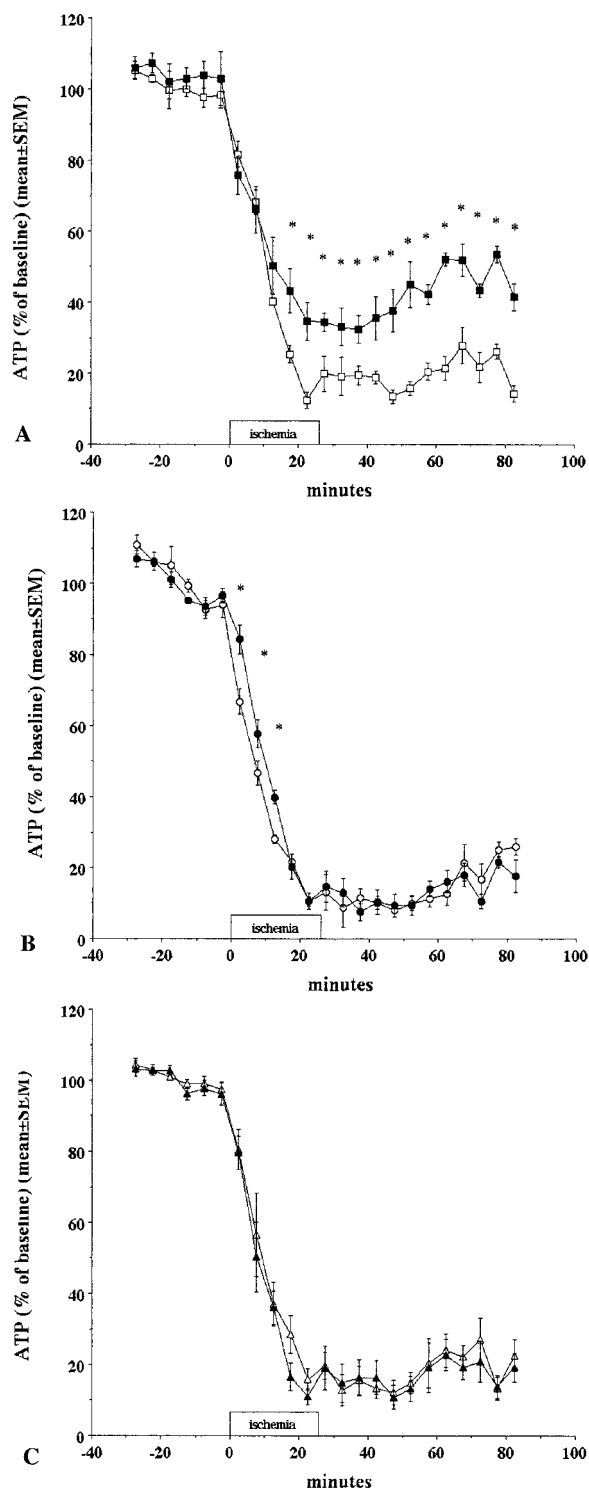


Fig. 2. (A) Ischemia causes a decrease in adenosine triphosphate (ATP) (open squares) and anesthetic preconditioning (APC) limits the decrease and significantly improves the recovery of ATP (closed squares) during ischemia and reperfusion in young adult hearts ($* P < 0.05$). (B) Ischemia causes a significant decrease in ATP (open circles) and APC slows the decrease of ATP (closed circles) during ischemia in middle-aged hearts. (C) Ischemia causes a significant decrease in ATP (open triangles) and APC does not limit the decrease of ATP (closed triangles) during ischemia and reperfusion in aged hearts. $n = 6/\text{group}$. $*$ APC versus control.

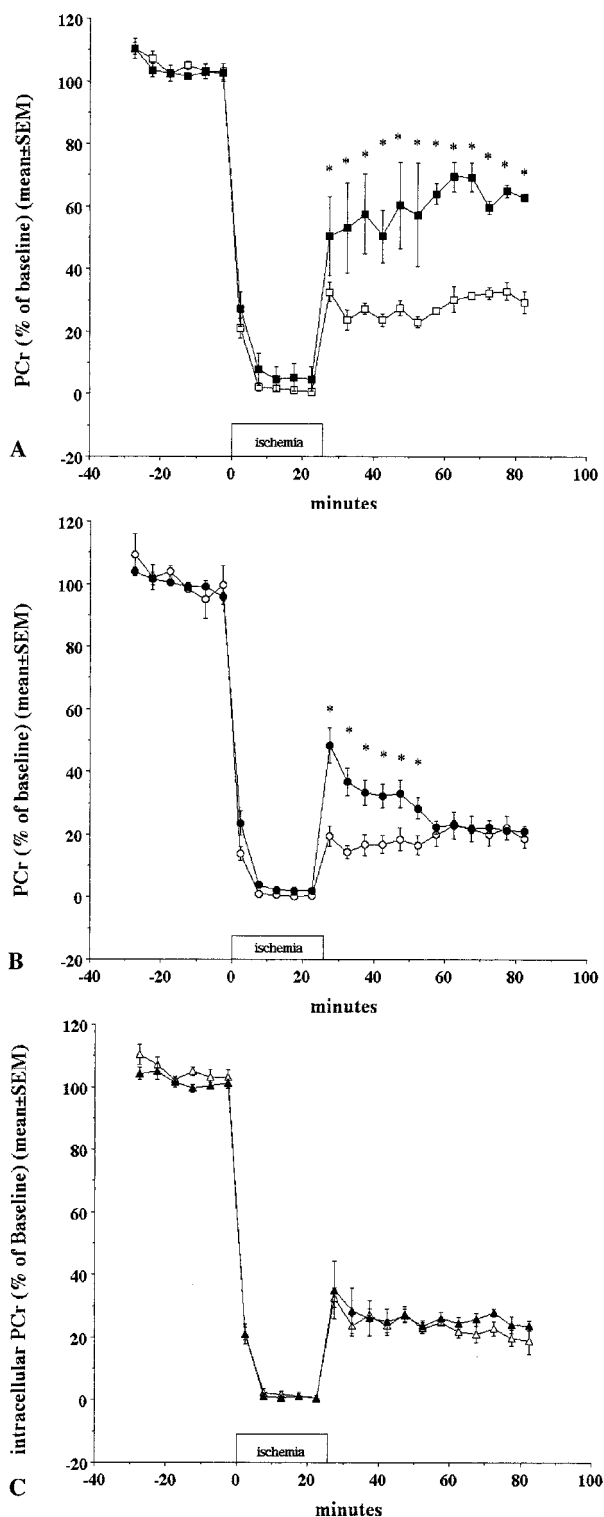


Fig. 3. (A) Ischemia limits recovery in phosphocreatine (PCr) (open squares) and anesthetic preconditioning (APC) significantly improves recovery of PCr (closed squares) during reperfusion in young adult hearts (* $P < 0.05$). (B) Ischemia limits recovery in PCr (open circles) and APC significantly improves recovery of PCr (closed circles) during early reperfusion in middle-aged hearts (* $P < 0.05$). (C) Ischemia causes a significant decrease in PCr (open triangles) and APC does not limit the decrease of PCr (closed triangles) during ischemia and reperfusion in aged hearts. $n = 6/\text{group}$. * APC versus control.

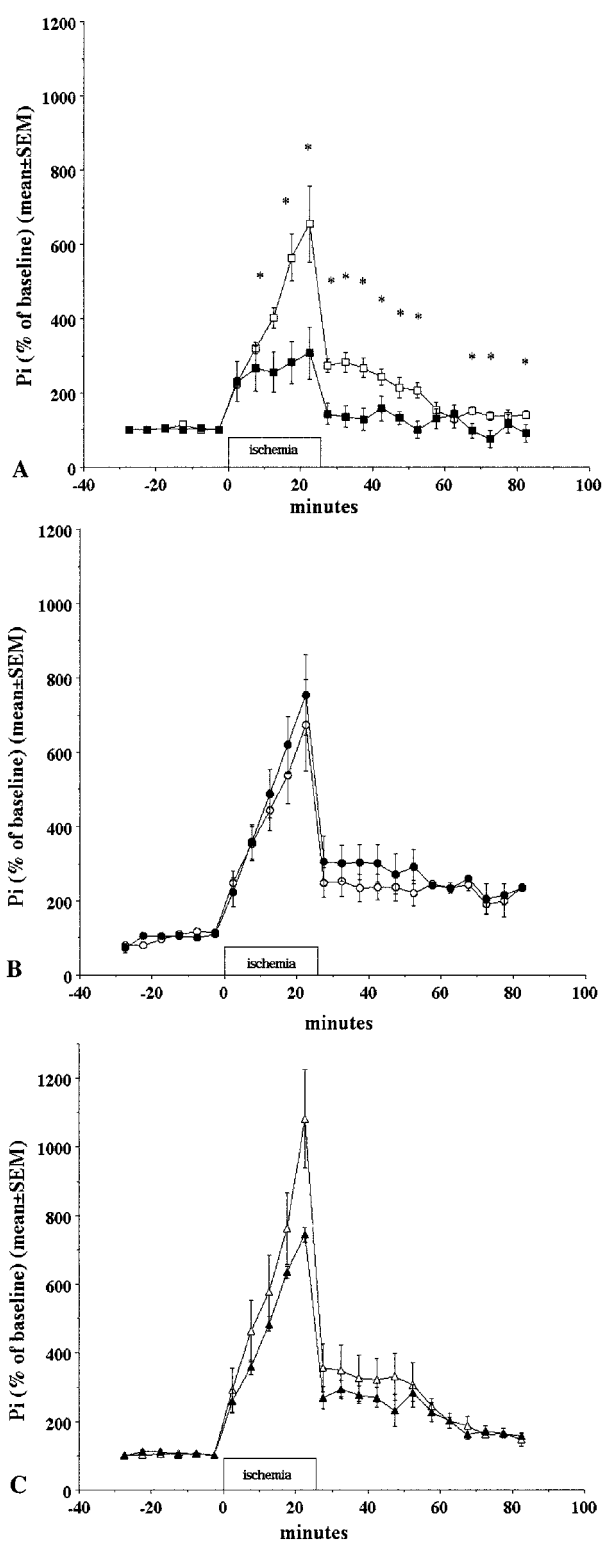


Fig. 4. (A) Ischemia causes an increase in inorganic phosphate (Pi) (open squares) and anesthetic preconditioning (APC) limits the Pi increases (closed squares) during ischemia and improves the recovery during reperfusion in young adult hearts (* $P < 0.05$). (B) Ischemia causes an increase in Pi (open circles) and APC does not limit the Pi increases (closed circles) in middle-aged hearts. (C) Ischemia causes an increase in Pi (open triangles) and APC does not limit the Pi increase (closed triangles) during ischemia in aged hearts. $n = 6/\text{group}$. * APC versus control.

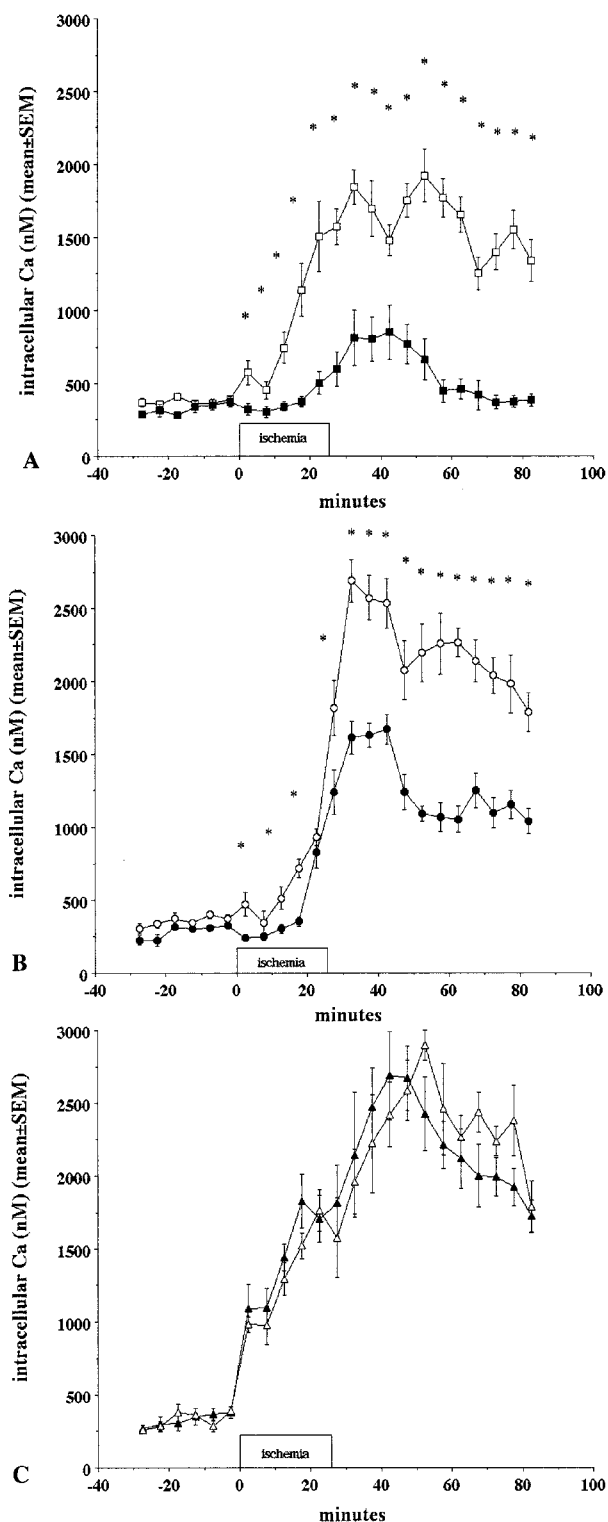


Fig. 5. (A) Ischemia causes an increase in intracellular Ca ($[Ca]_i$) (open squares) and anesthetic preconditioning (APC) limits the $[Ca]_i$ (closed squares) during ischemia and reperfusion in young adult hearts (* $P < 0.05$). (B) Ischemia causes an increase in $[Ca]_i$ (open circles) and APC limits the $[Ca]_i$ (closed circles) during ischemia and reperfusion in middle-aged hearts (* $P < 0.05$). (C) Ischemia causes an increase in $[Ca]_i$ (open triangles) and APC does not limit the increase in $[Ca]_i$ (closed triangles) during ischemia and reperfusion in aged hearts. $n = 6/\text{group}$. * APC versus control.

reperfusion when exposed to sevoflurane ($1,786 \pm 133$ vs. $1,039 \pm 87$ nM for control and APC, respectively; $P < 0.05$). No differences were found between control and APC hearts in the aged group.

Intracellular Proton Accumulation

Intracellular pH was measured during ischemia with and without APC as one variable required to assess the effect of APC on pH regulation during and after ischemia. Figure 6 shows that at the end of 25 min of sustained ischemia, the pH_i decreased in both control and APC hearts in all the three age groups, but the pH_i decreased less at the end of ischemia in young adult and middle-aged hearts than in aged hearts. The recovery of pH_i after prolonged ischemia and 60 min of reperfusion was not different in any of the three age groups between control and APC hearts.

Intracellular Na Uptake

Figure 7 shows that APC blunts the increases in Na_i in young adult and middle-aged hearts during ischemia. We also demonstrated that APC does not prevent the increase in Na_i in aged myocardium. Significant differences between control and APC hearts were found in the young adult and middle-aged groups only at the end of ischemia. There was no significant difference between control and APC in aged hearts during ischemia and reperfusion.

Discussion

The percentage of the world's population that is elderly is increasing. As a result, the number of elderly patients undergoing surgical procedures is increasing.²² This has important implications for intraoperative cardioprotection because there are metabolic and structural differences in the senescent myocardium.²³ IPC is one protection strategy that has been shown to have decreased effectiveness in the aged heart.^{3,4} APC shares similarities with IPC, but the effects of age on this phenomenon have yet to be studied.

We investigated the effects of APC with sevoflurane using isolated perfused rat hearts from three different age groups: young adult, 3–4 months old; middle-aged, 10–12 months old (12 months for a rat is approximately equal to a 50 yr for a human); and aged, 20–24 months old (24 months for a rat is approximately equal to 75 yr for a human). Benefits of APC have been previously demonstrated in this model using young rats. Specifically, Li *et al.*¹⁶ and Mathur *et al.*²⁴ showed a greater recovery of systolic function, as measured by LVDP, and diastolic relaxation, as measured by LVEDP, in APC hearts after global ischemia. In addition, Li *et al.*¹⁶ examined myocardial tissue under light microscopy and

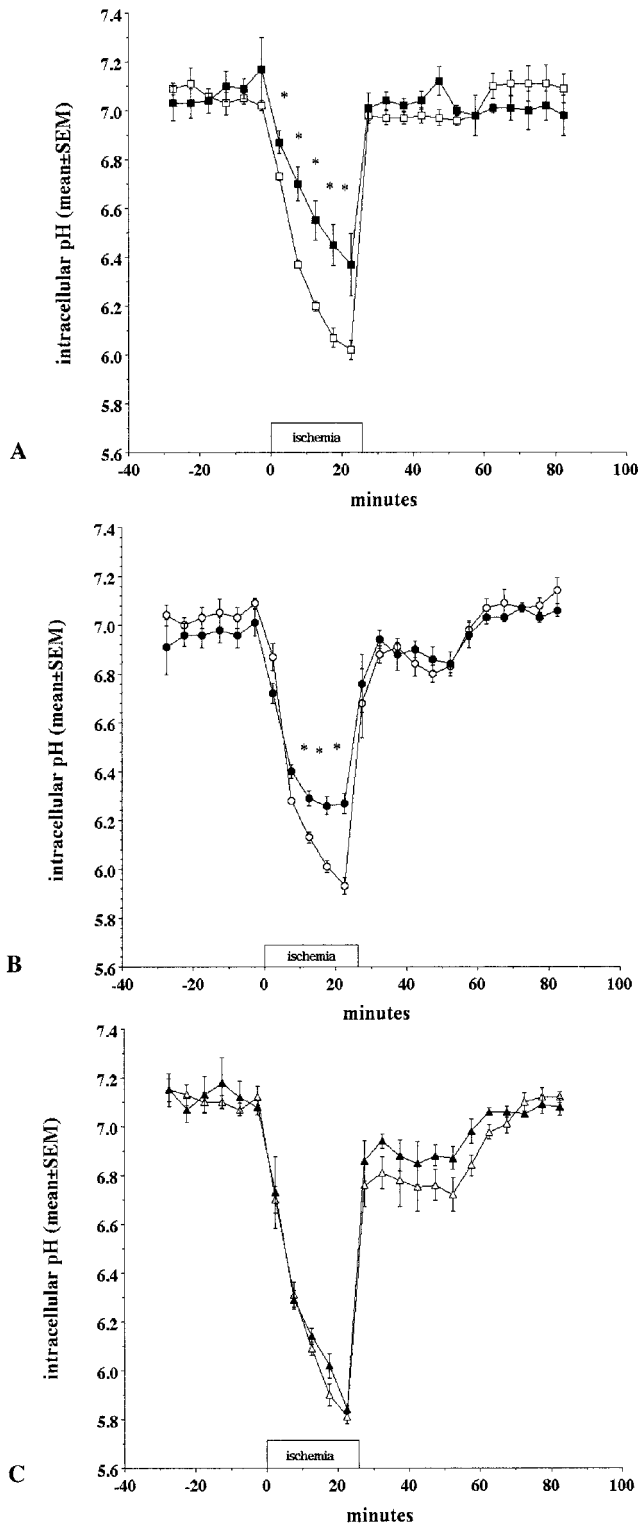


Fig. 6. (A) Ischemia causes a decrease in intracellular pH (pH_i) (open squares) and APC limits the decrease of pH_i (closed squares) during ischemia in young adult hearts (* $P < 0.05$). (B) Ischemia causes a decrease in pH_i (open circles) and APC limits the decrease of pH_i (closed circles) during ischemia in middle-aged hearts (* $P < 0.05$). (C) Ischemia causes a decrease in pH_i (open triangles) and APC does not limit the decrease of pH_i (closed triangles) during ischemia and reperfusion in aged hearts. $n = 6/\text{group}$. * APC versus control.

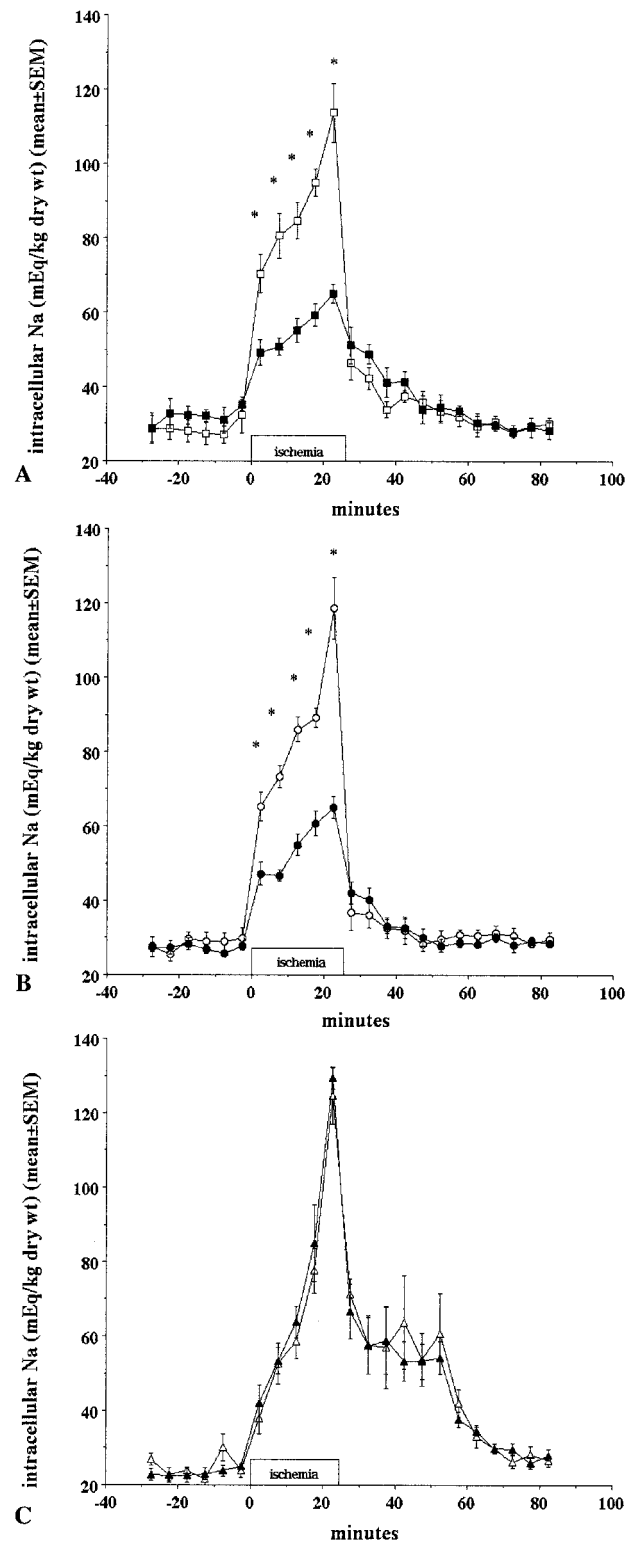


Fig. 7. (A) Ischemia causes an increase in intracellular Na_i (open squares) and anesthetic preconditioning (APC) limits the Na_i (closed squares) during ischemia in young adult hearts (* $P < 0.05$). (B) Ischemia causes an increase in Na_i (open circles) and APC limits the Na_i (closed circles) during ischemia and reperfusion in middle-aged hearts (* $P < 0.05$). (C) Ischemia causes an increase in Na_i (open triangles) and APC does not limit the increase in Na_i (closed triangles) during ischemia and reperfusion in aged hearts. $n = 6/\text{group}$. * APC versus control.

found a 50% reduction in the infarction area with APC hearts.

Our hemodynamic measurements in the young adult and middle-aged rats were consistent with these two studies. Systolic function, as measured by LVDP, recovered to a greater extent in the APC hearts after 60 min of reperfusion. Diastolic relaxation, as measured by LVEDP, tended to be improved in the young adult and middle-aged APC hearts, but it did not reach statistical significance in aged hearts. One difference between our study and those of Li *et al.*¹⁶ and Mathur *et al.*²⁴ is that our protocol included a 5-min washout period before global ischemia. Although this may have decreased the overall benefit of sevoflurane administration, it created a true preconditioning model. Cope *et al.*²⁵ first demonstrated decreased infarct size in rabbit hearts exposed to volatile anesthetics using a washout period. This correlates with the "early phase" of preconditioning originally shown by Murry *et al.*¹

In addition to hemodynamic recovery, we also compared tissue damage among groups. CK release was used as an indirect measurement of cellular injury. APC hearts in the young adult and middle-aged groups had significantly less CK releases than controls, presumably because of less myocardial damage. We confirmed this by using the accepted standard for measuring tissue damage, infarct size analysis. APC hearts in the young adult and middle-aged groups had almost 50% and 30% reduced infarct sizes, respectively, compared with controls.

None of the benefits of APC seen in the young adult and middle-aged groups could be demonstrated in the aged group. There was no difference between APC and control hearts in any hemodynamic measurement, CK release, or infarct size. Schulman *et al.*⁴ exposed rats from three different age groups (3, 12, and 18–20 months) to an IPC protocol before prolonged regional ischemia and failed to demonstrate any benefit in the oldest rats. Fenton *et al.*³ also failed to show IPC benefit in rats aged 22 months. If the mechanisms of IPC and APC are similar, their effects on the senescent myocardium would likely be comparable.

Although the exact mechanism of APC is unknown, its protective benefits may be related to decreased $[Ca]_i$. Increased $[Ca]_i$ has been identified as a causal factor in reperfusion injury.^{18,19,26} The ischemic chain of events leading to cell damage or necrosis is presumed to begin with increased anaerobic metabolism, leading to intracellular acidosis and decreased pH_i . This activates an Na-H exchanger (NHE1) to maintain pH balance and causes increased Na_i .^{27,28} $[Ca]_i$ then increases as a result of impaired or reversed Na-Ca exchange.^{18,19,26} A cascade of events is then initiated by a prolonged increase in $[Ca]_i$, leading to increased ATP consumption, inefficient ATP synthesis, cytoskeletal damage, and cell death.^{29–35} In two articles, Varadarajan *et al.*,^{30,32}

showed that previous exposure to sevoflurane in intact guinea pig hearts resulted in decreased cytosolic and mitochondrial calcium loading and improved indices of left ventricular function.

The results of this study are consistent with the above hypothesis. We demonstrated that anaerobic metabolism causes an increase in H_i and a decrease in pH_i . This causes Na influx *via* NHE1. The increased Na_i then activates the Na-Ca exchanger and causes $[Ca]_i$ accumulation. The whole cascade consumes high-energy phosphates, which was demonstrated here by decreasing phosphocreatine and ATP, at the same time increasing inorganic phosphate production at the end of ischemia.^{18,19,28,29} The increased Na influx from the extracellular space and the accumulation of Na_i can independently inhibit the mitochondria state 3 respiration, cause irreversible damage to mitochondria during ischemia, and further decrease ATP synthesis.³⁶ Our results show that APC blunts the decrease in pH_i , as well as the increase in Na_i in young adult hearts. High-energy phosphates are better preserved in younger hearts using APC, with significantly less $[Ca]_i$ accumulation during ischemia and reperfusion.

Together with our functional and histologic data, our results support the hypothesis that increased $[Ca]_i$ plays a major role in myocardial cell damage. APC, like IPC, failed to protect aged myocardium from ischemia-reperfusion injury. This further supports the hypothesis that one of the cardioprotective mechanisms of APC is to maintain intracellular calcium homeostasis. Our results are also consistent with the report by Ataka *et al.*³³ that cytoplasmic calcium accumulates to a greater extent in aged rabbit hearts. This may be because of a decreased ability of the cardiac sarcoplasmic reticulum to uptake excess calcium.^{34,35} Na-Ca exchanger activity may also be altered with aging.³⁷ This reduced ability of the aged cells to handle calcium may contribute to the failure of APC in the senescent rat heart. Findings of Tricarico *et al.*³⁸ indicate that K_{ATP} channels from aged rats show decreases in open state probability and in the number of functional channels. K_{ATP} channels from aged rats also exhibit greater sensitivity to inhibitors, an effect that is redox sensitive. Therefore, it is possible that changes in K_{ATP} channel activation are responsible for the observed results in senescent myocardium.³⁸

One of the limitations of our study was the difference in baseline pressures between the age groups. Compared with the young adult and middle-aged groups, the aged hearts had a significantly higher LVESP. The failure of APC could have been due to this relative hypertension. However, in two studies of IPC in hypertensive rats, IPC was still effective in one³⁹ and had an increased effect in the other.⁴⁰ Therefore, it seems unlikely that the failure of APC in the aged rats was due solely to their increased baseline pressures.

Our study was also limited by applying the same dose

of sevoflurane to all age groups even though the MAC value decreases with age. Although the most effective dose to elicit APC protective mechanisms is still debatable, it is possible that this dose may vary with age. Further studies would be needed to determine whether higher, or possibly lower, doses could activate APC pathways in aged hearts. Also, we only used sevoflurane in this study, and we do not know whether all the volatile anesthetics are equal regarding APC.

Finally, we were unable to measure the mitochondrial calcium concentrations with the nuclear magnetic resonance spectroscopy we used. Because APC may act at the mitochondrial level, this could be an important measurement. However, numerous studies have reported that increased $[Ca]_i$ has a positive correlation with increased mitochondrial calcium.

In conclusion, we have demonstrated improved myocardial functional recovery; less cellular damage; decreased infarct size; minimized H_i , Na_i and $[Ca]_i$ increases; and better-preserved high-energy phosphates with APC in young adult and middle-aged isolated rat hearts. However, these beneficial effects of APC could not be reproduced in aged rat hearts. This is consistent with the observed decrease in efficacy of IPC in the senescent myocardium.

References

- Murry CE, Jennings RB, Reimer KA: Preconditioning with ischemia: A delay in lethal cell injury in ischemic myocardium. *Circulation* 1986; 74:1124-36
- Anzai T, Yoshikawa T, Asakura Y, Abe S, Akaishi M, Mitamura H, Handa S, Ogawa S: Preinfarction angina as a major predictor of left ventricular function and long-term prognosis after a first Q wave myocardial infarction. *J Am Coll Cardiol* 1995; 26:319-27
- Fenton RA, Dickson EW, Meyer TE, Dobson JG: Aging reduces the cardioprotective effect of ischemic preconditioning in the rat heart. *J Mol Cell Cardiol* 2000; 32:1371-9
- Schulman D, Latchman DS, Yellon DM: Effect of aging on the ability of preconditioning to protect rat hearts from ischemia-reperfusion injury. *Am J Physiol* 2001; 281:H1630-6
- Abete P, Ferrara N, Cacciatore F, Madrid A, Bianco S, Calabrese C, Napoli C, Scognamiglio P, Bollella O, Cioppa A, Longobardi G, Rengo F: Angina-induced protection against myocardial infarction in adult and elderly patients: A loss of preconditioning mechanism in the aging heart? *J Am Coll Cardiol* 1997; 30:947-54
- Kersten JR, Schmeling TJ, Pagel PS, Gross GJ, Wartler DC: Isoflurane mimics ischemic preconditioning *via* activation of K_{ATP} channels: Reduction of myocardial infarct size with an acute memory phase. *ANESTHESIOLOGY* 1997; 87:361-70
- Cason BA, Gamperl AK, Slocum RE, Hickey RF: Anesthetic induced preconditioning: Previous administration of isoflurane decreases myocardial infarct size in rabbits. *ANESTHESIOLOGY* 1997; 87:1182-90
- Toller WG, Kersten JR, Pagel PS, Hettrick DA, Wartler DC: Sevoflurane reduces myocardial infarct size and decreases the time threshold for ischemic preconditioning in dogs. *ANESTHESIOLOGY* 1999; 91:1437-46
- Kohro S, Hogan QH, Nakae Y, Yamakage M, Bosnjak ZJ: Anesthetic effects on mitochondrial ATP-sensitive K channel. *ANESTHESIOLOGY* 2001; 95:1435-40
- Toller WG, Gross ER, Kersten JR, Pagel PS, Gross GJ, Wartler DC: Sarcolemmal and mitochondrial adenosine triphosphate-dependent potassium channels: Mechanism of desflurane-induced cardioprotection. *ANESTHESIOLOGY* 2000; 92:1731-9
- Zaugg M, Lucchinetti E, Spahn DR, Pasch T, Schaub MC: Volatile anesthetics mimic cardiac preconditioning by priming the activation of mitochondrial K_{ATP} channels *via* multiple signaling pathways. *ANESTHESIOLOGY* 2002; 97:4-14
- Belhomme D, Peynet J, Louzy M, Launay JM, Kitakaze M, Menasche P: Evidence for preconditioning by isoflurane in coronary artery bypass graft surgery. *Circulation* 1999; 100(suppl II):II-340-4
- Haroun-Bizri S, Khoury SS, Chehab IR, Kassas CM, Baraka A: Does isoflurane optimize myocardial protection during cardiopulmonary bypass? *J Cardiothorac Vasc Anesth* 2001; 15:418-21
- DeHert SG, Broecke PW, Mertens E, Van Sommeren EW, De Blier IG, Stockman BA, Rogrigus IE: Sevoflurane but not propofol preserves myocardial function in coronary surgery patients. *ANESTHESIOLOGY* 2002; 97:42-9
- Pouzet B, Lechary JB, Dehoux M, Paquin S, Kitakaze M, Mantz J, Menasche P: Is there a place for preconditioning during cardiac operations in humans? *Ann Thorac Surg* 2002; 73:843-8
- Li F, Hayes JK, Wong KC, Szakacs J: Administration of sevoflurane and isoflurane prior to prolonged global ischemia improves heart function in isolated rat heart. *Acta Anaesthesiol Sin* 2000; 38:113-21
- Mullenheim J, Molojavji A, Preckel B, Thamer V, Schlack W: Thiopentone does not block ischemic preconditioning in the isolated rat heart. *Can J Anesth* 2001; 48:784-9
- Anderson SE, Liu H, Ho HS, Lewis EJ, and Cala PM: Age-related differences in Na^+ -dependent Ca^{2+} accumulation in rabbit hearts exposed to hypoxia and acidification. *Am J Physiol* 2003; 284:C1123-32
- Liu H, Cala PM, Anderson SE: Ischemic preconditioning: effects on pH, Na and Ca in newborn rabbit hearts during ischemia/reperfusion. *J Mol Cell Cardiol* 1998; 30:685-97
- Liu GS, Thornton J, Van Winkle DM, Stanley AW, Olsson RA, Downey JM: Protection against infarction afforded by preconditioning is mediated by A_1 receptors in the rabbit heart. *Circulation* 1991; 84:350-6
- Lott FD, Guo P, Toombs CF: Reduction in infarct size by ischemic preconditioning persists in a chronic rat model of myocardial ischemia-reperfusion injury. *Pharmacology* 1996; 52:113-8
- Winker MA: Aging in the 21st century: A call for papers. *Arch Neurol* 2002; 59:518-9
- Besse S, Moalic JM, Delcayre C, Chevalier B, Assayag P, Swynghedauw B: The senescent heart: Clinical and biological characteristics. *Facts Res Gerontol* 1993; 7:95-102
- Mathur S, Farhangkhgoee P, Karmazyn M: Cardioprotective effects of propofol and sevoflurane in ischemic and reperfused rat hearts: Role of K_{ATP} channels and interaction with the sodium-hydrogen exchange inhibitor HOE 642 (cariporide). *ANESTHESIOLOGY* 1999; 91:1349-60
- Cope DK, Impastato WK, Cohen MV, Downey JM: Volatile anesthetics protect the ischemic rabbit myocardium from infarction. *ANESTHESIOLOGY* 1997; 86:1128-39
- Anderson SE, Murphy E, Steenbergen C, London RE, Cala PM: Na/H exchange in myocardium: Effects of hypoxia and acidification on Na and Ca . *Am J Physiol* 1990; 259:C940-8
- Ramasamy R, Liu H, Anderson S, Lundmark J, Schaefer S: Ischemic preconditioning stimulates sodium and proton transport in the isolated rat heart. *J Clin Invest* 1995; 96:1464-72
- Pike MM, Luo CS, Clark MD, Kirk KA, Kitakaze M, Madden MC, Cragoe EJ Jr, Pohost GM: NMR measurements of Na and cellular energy in ischemic rat heart: Role of Na/H exchange. *Am J Physiol* 1993; 265:H2017-26
- Steenbergen C, Fralix TA, Murphy E: Role of increased cytosolic free calcium concentration in myocardial ischemic injury. *Basic Res Cardiol* 1993; 88:456-70
- Varadarajan SG, An JZ, Novalija E, Smart SC, Stowe EF: Changes in $[Na]_i$ compartmental $[Ca]_i$ and NADH with dysfunction after global ischemia in intact hearts. *Am J Physiol* 2001; 280:H280-93
- An J, Varadarajan SG, Novalija E, Stowe DF: Ischemic and anesthetic preconditioning reduces cytosolic $[Ca]_i$ and improves Ca responses in intact hearts. *Am J Physiol* 2001; 281:H1508-23
- Varadarajan SG, An J, Novalija E, Stowe DF: Sevoflurane before or after ischemia improves contractile and metabolic function while reducing myoplasmic Ca loading in intact hearts. *ANESTHESIOLOGY* 2002; 96:125-33
- Ataka K, Chen D, Levitsky S, Jimenez E, Feinberg H: Effect of aging on intracellular $[Ca]_i$, pH_i , and contractility during ischemia and reperfusion. *Circulation* 1992; 86(suppl II):II371-6
- Frolkis VV, Frolkis RA, Mkhitarian LS, Fraifeld VE: Age dependent effects of ischemia and reperfusion on cardiac function and Ca transport in myocardium. *Gerontology* 1991; 37:233-7
- Narayann N: Comparison of ATP-dependent calcium transport and calcium activated ATP-ase activities of cardiac sarcoplasmic reticulum and sarcolemma from rats of various ages. *Mech Ageing Dev* 1987; 38:127-43
- Iwai T, Tanonaka K, Inoue R, Kasahara S, Motegi K, Nagaya S, Takeo S: Sodium accumulation during ischemia induces mitochondrial damage in perfused rat hearts. *Cardiovasc Res* 2002; 55:141-9
- Heyliger CE, Prakash AR, McNeil JH: Alterations in membrane Na/Ca exchange in the aging myocardium. *Age* 1988; 11:1-6
- Tricarico D, Camerino DC: ATP-sensitive K^+ channels of skeletal muscle fibers from young adult and aged rats: Possible involvement of thiol-dependent redox mechanisms in the age-related modifications of their biophysical and pharmacological properties. *Mol Pharmacol* 1994; 46:754-61
- Boutros A, Wang J: Ischemic preconditioning, adenosine and betanachol protect spontaneously hypertensive isolated rat hearts. *J Pharmacol Exp Ther* 1995; 275:1148-56
- Randall MD, Gurdiner SM, Bennett J: Enhanced cardiac preconditioning in the isolated heart of the transgenic (mREN-2)27 hypertensive rat. *Cardiovasc Res* 1997; 33:400-9