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Sevoflurane Preconditioning Limits Intracellular/ Mitochondrial Ca^{2+} in Ischemic Newborn Myocardium

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Sevoflurane preconditioning (SPC) in adult hearts reduces myocardial ischemia/reperfusion (I/R) injury, an effect that may be mediated by reductions in intracellular Ca^{2+} ($[Ca^{2+}]_i$) and/or mitochondrial Ca^{2+} ($[Ca^{2+}]_m$) accumulation during ischemia and reperfusion. Because the physiology, pharmacology, and metabolic responses of the newborn differ from adults, we tested the hypothesis that SPC protects newborn myocardium by limiting $[Ca^{2+}]_i$ and $[Ca^{2+}]_m$ by a K_{ATP} channel-dependent mechanism. Fluorescence spectrofluorometry and nuclear magnetic resonance spectroscopy were used to measure $[Ca^{2+}]_i$, $[Ca^{2+}]_m$, and adenosine triphosphate (ATP) in 4- to 7-day-old Langendorff-perfused rabbit hearts. Three experimental groups were used to study the effect of SPC on $[Ca^{2+}]_m/[Ca^{2+}]_i$, ATP, as well as hemodynamics and ischemic injury. The role of mitochondrial K_{ATP} channels was assessed by exposing the SPC hearts to the mitochondrial K_{ATP} channel blocker 5-hydroxydecanoic acid. Our results show that SPC significantly decreased $[Ca^{2+}]_i$ and $[Ca^{2+}]_m$ during I/R, as well as decreased creatine kinase release during reperfusion and resulted in higher ATP. 5-Hydroxydecanoic acid abolished the effect of SPC on $[Ca^{2+}]$, hemodynamics, ATP, and creatine kinase release. In conclusion, decreased $[Ca^{2+}]_i$ and $[Ca^{2+}]_m$ observed with SPC is associated with greater ATP recovery as well as diminished cell injury. Mitochondrial K_{ATP} channel blockade attenuates the SPC effect during I/R, suggesting that these channels are involved in the protective effects of SPC in the newborn.

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Preconditioning with sevoflurane (SPC) or other inhaled anesthetics has been reported to provide myocardial protection against ischemia/reperfusion (I/R) injury in adult hearts (1). Many pathophysiological processes in cardiac I/R are associated with derangement of cellular ion homeostasis, with calcium overload likely having a key role in the impairment of ischemic and reperfused tissue (2,3). Given the important role of calcium overload, some have suggested that inhaled anesthetic preconditioning reduces I/R injury by activating adenosine triphosphate (ATP)-sensitive potassium channels (K_{ATP}), thereby decreasing intracellular calcium ($[Ca^{2+}]_i$) and mitochondrial Ca ($[Ca^{2+}]_m$) in adult hearts (1,4,5). However, the effect of inhaled anesthetic preconditioning on $[Ca^{2+}]_m$, as well as its efficacy in intact newborn hearts, has not been addressed. Because the physiology, pharmacology, and metabolic responses of the newborn heart differ from those of the adult heart (6), extrapolation of results from the adult heart is not necessarily warranted. To better understand the mechanism of I/R injury in the newborn heart, we tested the hypothesis that SPC opens mitochondrial K_{ATP} channels and decreases the driving force for Ca^{2+} influx, thus attenuating calcium overload during I/R in newborn myocardium.

Methods

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

New Zealand white rabbits (4–7 days old) were anesthetized with sodium pentobarbital (65 mg/kg) and heparinized (1000 USP U/kg). A total of 52 rabbits were used for this study. Hearts were removed, not paced, and perfusion pressure was set at 100 cm H_2O at 37° ± 0.5°C. Left ventricular end-diastolic pressure

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(LVEDP) and left ventricular developed pressure (LVDP measured by isovolumic balloon) were measured continuously. LVEDPs were set between 7 to 10 cm H_2O before ischemia. Ischemia started at time 0 (t = 0 min), lasted for 40 min, followed by 40 min of reperfusion. Control perfusate contained (mmole/liter): 133 NaCl, 4.75 KCl, 1.25 $MgCl_2$, 1.82 $CaCl_2$, 25 $NaHCO_3$, 11.1 dextrose. Perfusates were equilibrated with 95% O_2/5% CO_2 which provided a pH of 7.35–7.45.

Three experiment protocols were used in this study. All the hearts were perfused for 30 min before acquiring any data. Control hearts were subjected to 30 min perfusion followed by 40 min of ischemia, and 40 min of reperfusion without pharmacologic interventions. Hearts in the SPC group were perfused for 10 min, then exposed to 2.5% sevoflurane for 10 min, followed by 10 min washout before 40 min of ischemia, and 40 min of reperfusion. Finally, hearts in the third group followed the SPC protocol and, in addition, were treated with the selective mitochondrial K_{ATP} channel inhibitor, 5-hydroxydecanoic acid (5-HD) (Sigma, St. Louis, MO), 100 μM, before ischemia. Hemodynamic variables, [ATP], creatine kinase (CK) release, $[Ca^{2+}]_i$ and $[Ca^{2+}]_m$, were measured in all three groups as described below.

Fluorescence measurements were performed, as previously described in detail, using a modified spectrofluorometer (model SLM8100; SLM Instruments, Rochester, NY) (7). After a 30-min equilibration period, baseline background fluorescence was measured and subtracted. Hearts were then loaded for 10 min by perfusion with Krebs-Henseleit buffer containing indo 1, acetoxymethyl ester (indo 1-AM) 6 μM, dissolved in dimethyl sulfoxide and Pluronic F-127, 20% wt/vol (Molecular Probes, Johnston City, OR) and 1% fetal bovine serum. Probenecid 0.1 mM (Sigma) was added to all perfusates to slow the extrusion of indo 1 from the myocytes (7). Residual indo 1-AM was washed out by perfusing with standard buffer for 25 min. To determine mitochondrial fluorescence, cytosolic fluorescence was quenched by adding $MnCl_2$ at a final concentration of 17.5 μM to the perfusate 10 min before the 40-min ischemic period. Adequate quenching was verified by the loss of calcium transients after manganese loading. The addition of $MnCl_2$ did not alter cardiac function (heart rate or developed pressure) (7).

$[Ca^{2+}]_i$ was calculated using the standard equation for fluorescent calcium indicators, where R is the ratio of fluorescence at 385 and 456 nm and R_{min} and R_{max} are the fluorescence ratios at zero and saturating $[Ca^{2+}]_i$, respectively (7). $[Ca^{2+}]_m$ was calculated using the same equation used to calculate total $[Ca^{2+}]_i$ from the fluorescent intensity after manganese quenching.

Sevoflurane (Abbott Laboratories, North Chicago, IL) was delivered at 2.5% to the gas mixture via a standard Sevotec5 variable bypass vaporizer (Datex-Ohmeda, Milwaukee, WI). Samples of perfusate at coronary run-off were collected for measurement of sevoflurane concentration by gas chromatography (Varian, Walnut Creek, CA). The sevoflurane concentration was 0.4 ± 0.02 mM. Sevoflurane was not detected at the end of the washout period immediately before ischemia or during reperfusion.

A Bruker AMX400 nuclear magnetic resonance spectrometer (Bruker, Rheinstetten, Germany) was used for ATP measurement. ^{31}P spectra were generated from the summed free induction decays of 148 excitation pulses (60°) using 4K word data files and ±4000 Hz sweep widths (Fig. 1). The β-ATP peak height was used as an indicator for myocardial ATP (3,8).

Perfusates were collected during the baseline and reperfusion intervals, and a Shimadzu UV-VIS Recording Spectrophotometer (Shimadzu, Columbia, MD) was used to measure the time-integrated total CK release from the myocardium using a CK-10 kit (Sigma Diagnostics, St. Louis, MO). Units are expressed as international unit/gram dry weight (3,8).

The animals were randomly assigned to each group. Data presented are mean ± SEM. Differences in data between groups were analyzed using analysis of variance. Two-tailed Student-Newman-Keuls post-test was used if the analysis of variance was significant. A value of $P < 0.05$ was considered statistically significant.

Results

Consistent with previous measurements in adult hearts (7), SPC attenuated the increases in $[Ca^{2+}]_i$ and $[Ca^{2+}]_m$ during I/R. $[Ca^{2+}]_i$ increased in both systole and diastole during I/R in control neonatal hearts (Figs. 2 and 3), with systolic $[Ca^{2+}]_i$ (nanomolar) increasing significantly from 567 ± 63 before ischemia to 1304 ± 179 at the end of ischemia and recovering to 901 ± 136 at the end of reperfusion ($P < 0.05$). Diastolic $[Ca^{2+}]_i$ was 256 ± 9 before ischemia, increased to 489 ± 25 at the end of ischemia ($P < 0.05$ versus baseline), and recovered to 291 ± 33 at the end of reperfusion. The increases in systolic $[Ca^{2+}]_i$ in SPC-treated hearts were modest and significantly less than the control group (456 ± 12 before ischemia, 516 ± 18 at the end of ischemia, and 447 ± 2 at the end of reperfusion). The changes in diastolic Ca^{2+} were similar to those of systolic Ca^{2+}, with increases in SPC-treated hearts significantly less than that of control hearts: 270 ± 11 before ischemia, 356 ± 14 at the end of ischemia ($P < 0.05$ versus control group), and 284 ± 6 at the end of reperfusion.

Ischemia increased $[Ca^{2+}]_m$ in control hearts from 360 ± 28 before ischemia to 955 ± 104 at the end of ischemia, and to 623 ± 48 by the end of reperfusion ($P > 0.05$, compared with the baseline before ischemia) (Fig. 4). As with $[Ca^{2+}]_i$, SPC limited the increases in neonatal $[Ca^{2+}]_m$ calcium during I/R: $[Ca^{2+}]_m$ increased from 305 ± 25 before ischemia to 462 ± 32 at the end of ischemia and recovered to 353 ± 26 at the end of reperfusion, values which were significantly less than control

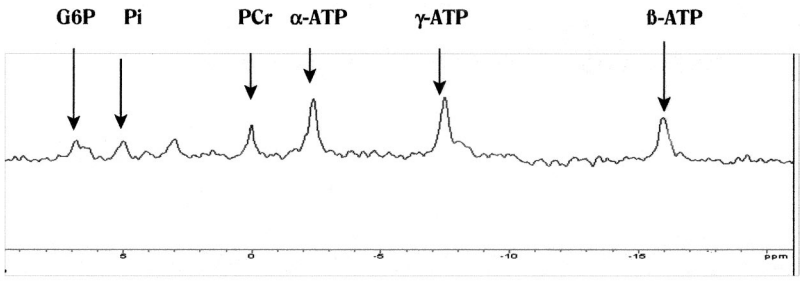

Figure 1. Representative ^{31}P spectra from neonatal heart. G6P = glucose-6-phosphate; Pi = inorganic phosphorus; PCr = phosphocreatine; α-, β-, γ-ATP = α, β, γ phosphates of adenosine triphosphate (ATP). The β-ATP peak height was used for ATP calculation.

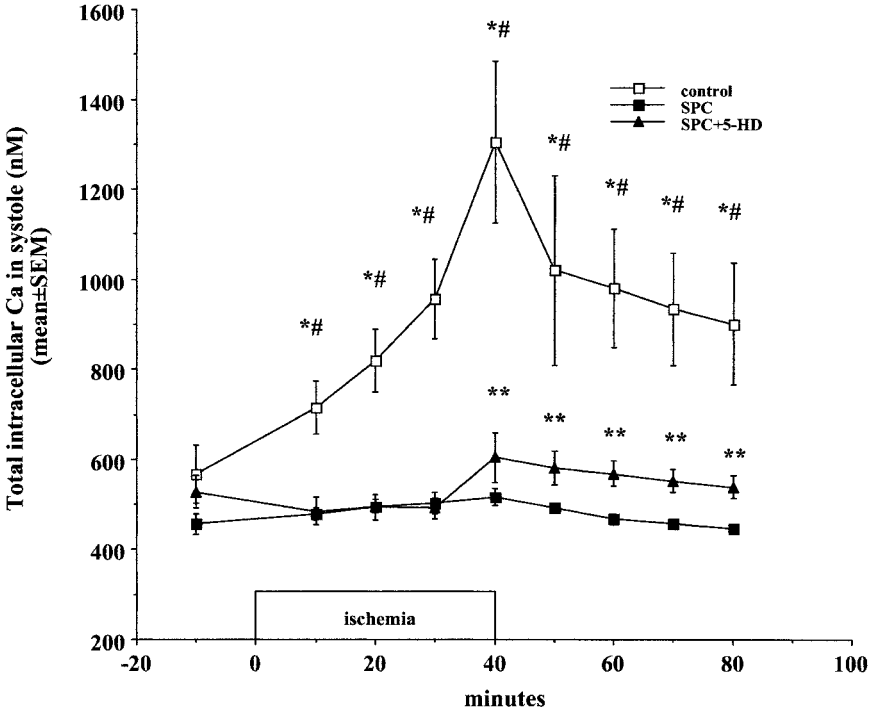

Figure 2. Total intracellular calcium $[Ca^{2+}]$ in newborn rabbit myocardium increased in systole during ischemia and reperfusion in control hearts (□). Sevoflurane preconditioning (SPC) significantly limited the increase in total calcium (■). *SPC versus control, $P < 0.05$. The mitochondrial K_{ATP} channel inhibitor, 5-hydroxydecanoic acid (5-HD), partially blunted the effect of SPC during reperfusion (▲). **SPC + 5-HD versus SPC and #SPC + 5-HD versus control, each $P < 0.05$.

group ($P < 0.05$). Thus, in comparison to control hearts, SPC significantly limited the increase in both $[Ca^{2+}]_i$ and $[Ca^{2+}]_m$ during I/R.

Administration of the mitochondrial K_{ATP} channel antagonist, 5-HD, blunted the protective effect of SPC on $[Ca^{2+}]_m$ and diastolic $[Ca^{2+}]_i$, during I/R (Figs. 2 and 3). With the addition of 5-HD to the SPC-treated hearts, $[Ca^{2+}]_i$ in systole increased from 528 ± 37 before ischemia to 604 ± 55 at the end of ischemia, and recovered to 539 ± 26 at the end of reperfusion, a value significantly more than the SPC group ($P < 0.05$). $[Ca^{2+}]_i$ in diastole increased from 272 ± 10 before ischemia, to 433 ± 20 ($P < 0.05$ compared with SPC) at the end of ischemia, and recovered to 268 ± 13 ($P < 0.05$) at the end of reperfusion. After adding 5-HD to the SPC-treated hearts, $[Ca^{2+}]_m$ increased from 349 ± 7 before ischemia, to 779 ± 73 at the end of ischemia, and 460 ± 18 at the end of reperfusion, with the latter values significantly more than the SPC hearts ($P < 0.05$).

Figure 5 summarizes the results of experiments to determine the effects of SPC on high-energy phosphate metabolism and ischemic injury in newborn ischemic myocardium. After 40 min of reperfusion, ATP recovered to 36% ± 5% of baseline in control hearts and 59% ± 9% in the SPC hearts ($P < 0.05$). SPC decreased myocardial CK release during the first 10 min of reperfusion (Fig. 6) from 330 ± 47 (IU/g dry weight) in the control group to 10 ± 4 (IU/g dry weight) in the SPC-treated group ($P < 0.05$).

Blockade of mitochondrial K_{ATP} channels using 5-HD effectively eliminated the protective effects of SPC on ATP and CK release. ATP recovery in SPC + 5-HD hearts was 33% ± 1% of baseline, compared with 36% ± 5% in control hearts and 59% ± 9% in the SPC hearts ($P < 0.05$). 5-HD partially blocked the protective effect of SPC on myocardial CK release during reperfusion (60 ± 23 IU/g dry weight) in the SPC + 5-HD group ($P < 0.05$ compared with SPC group).

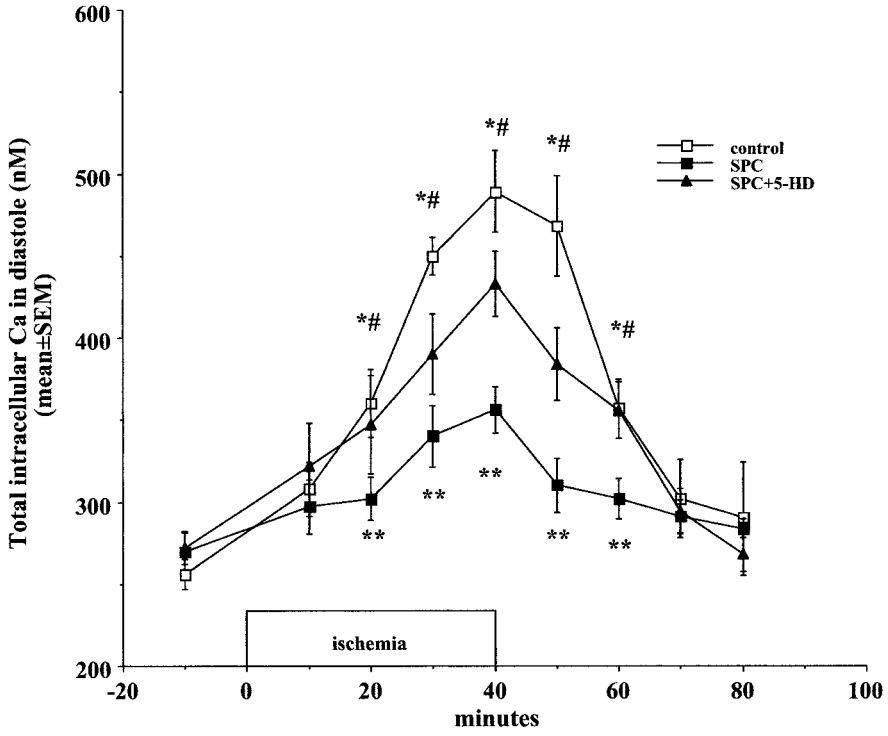

Figure 3. Total intracellular calcium [Ca^{2+}] increased in diastole during ischemia and early reperfusion (□). Sevoflurane preconditioning (SPC) significantly limited the increase (■, *SPC versus control, $P < 0.05$). 5-Hydroxydecanoic acid (5-HD) (▲) partially blunted the effect, resulting in values that were significantly different than both SPC and control ($P < 0.05$). (**SPC + 5-HD versus SPC; #SPG + 5-HD versus control.)

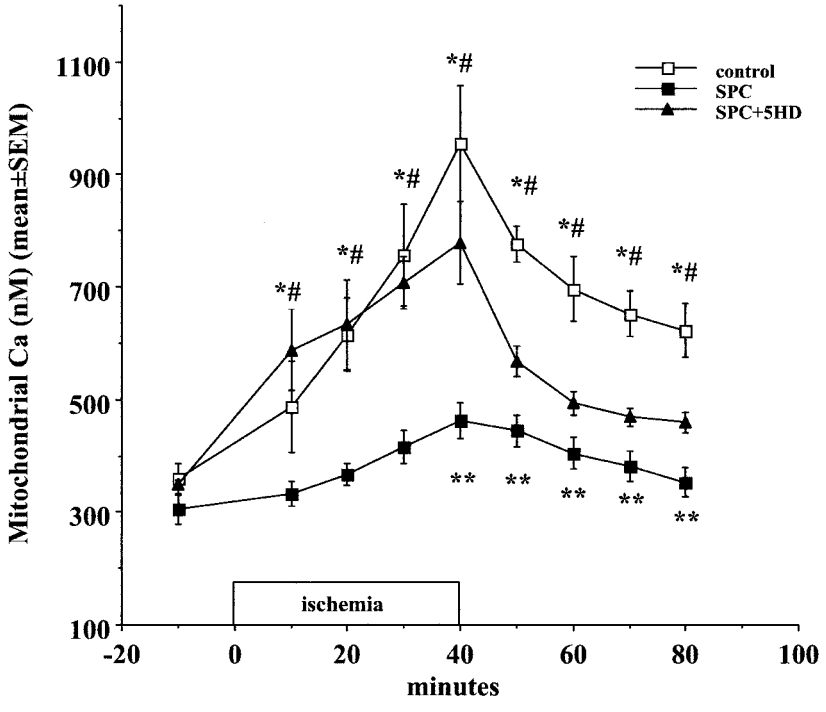

Figure 4. Mitochondrial calcium [Ca^{2+}] increased during ischemia and reperfusion (□). Sevoflurane preconditioning (SPC) significantly limited the increase of mitochondrial [Ca^{2+}] (■, *SPC versus control, $P < 0.05$). The mitochondrial K_{ATP} channel inhibitor, 5-hydroxydecanoic acid (5-HD), completely abolished the effect of SPC during ischemia and partially blunted the effect during reperfusion (▲, **SPC + 5-HD versus SPC, $P < 0.05$) (#SPC + 5-HD versus control, $P < 0.05$).

In parallel with the protective effects of SPC on [Ca^{2+}] and ATP recovery, SPC significantly improved left ventricular functional recovery (LVDP and LVEDP) during reperfusion compared with the control group (Table 1). Specifically, SPC improved reperfusion LVDP to 83% of the baseline value. As with its effects on [Ca^{2+}] and ATP, the addition of 5-HD completely eliminated these beneficial effects.

Discussion

It has been demonstrated that ischemia and reperfusion cause increases in $[Ca^{2+}]_i$ and $[Ca^{2+}]_m$ resulting in further Ca-dependent events leading to cell damage (3,7–11). Several studies have shown that a brief exposure to inhaled anesthetics before ischemia results in improved myocardial function and decreased infarct

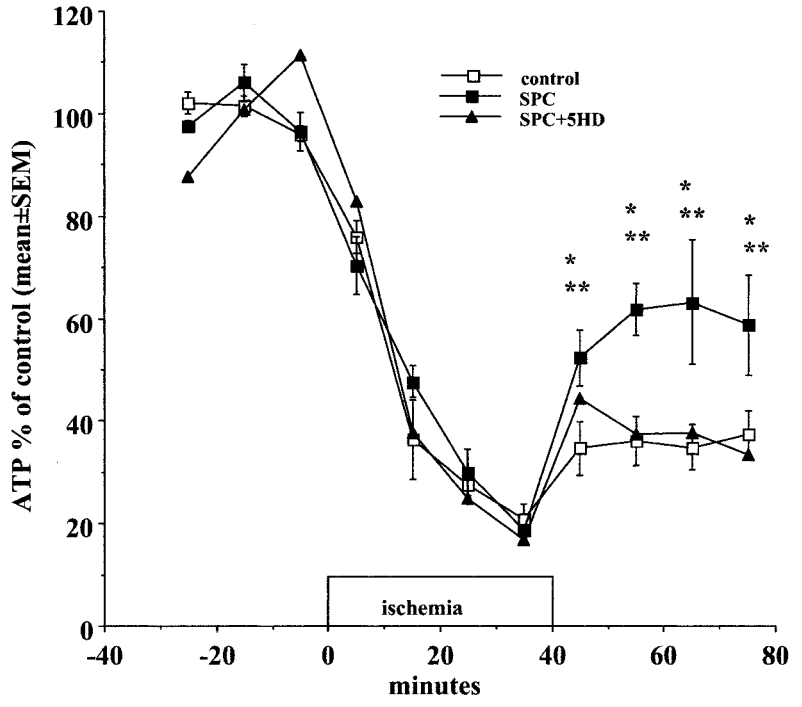

Figure 5. Myocardial adenosine triphosphate (ATP) decreased significantly and equally during ischemia in all groups. Sevoflurane preconditioning (SPC) significantly improved the recovery of ATP during reperfusion (■, *SPC versus control, $P < 0.05$), an effect abolished by 5-hydroxydecanoic acid (5-HD) (▲, **SPC + 5-HD versus SPC, $P < 0.05$).

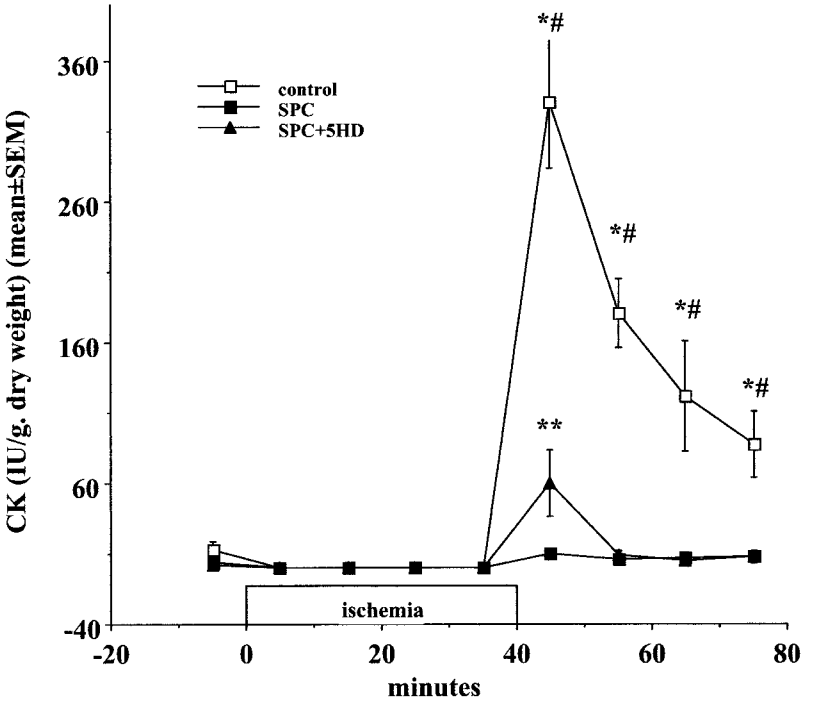

Figure 6. Sevoflurane preconditioning (SPC) essentially eliminated cell injury as indicated by the minimal release of creatine kinase (CK) (*SPC versus control, $P < 0.05$) during reperfusion. The mitochondrial K_{ATP} channel inhibitor, 5-hydroxydecanoic acid (5-HD), partially blocked the protective effect of SPC at 10 min of reperfusion (**SPC + 5-HD versus SPC, $P < 0.05$), but still resulted in CK release that was significantly less than control (#SPC + 5-HD versus control, $P < 0.05$).

size in a manner similar to ischemic preconditioning (IPC) in adult hearts (1,4,5,12,13). One of the proposed mechanisms of IPC is the opening of mitochondrial K_{ATP} channels which results in K influx, expansion of the mitochondrial matrix volume, and a reduction of the inner mitochondrial membrane potential established by the proton pump. This change is expected to decrease the driving force for Ca^{2+} influx, therefore attenuating $[Ca^{2+}]_m$ overload under conditions (such as I/R) in which cytosolic calcium is increased (14,15). A study in isolated rat mitochondria and intact rat cardiomyocytes supports this hypothesis, because K_{ATP} channel openers prevented $[Ca^{2+}]_m$ overload by both reducing the driving force for Ca^{2+} uptake and by increasing Ca^{2+} efflux (16). Further evidence is provided in isolated perfused adult rat hearts, in which opening of K_{ATP} channels with

Table 1. Results of LVEDP and LVDP Before Ischemia and at the End of Reperfusion in Three Treatment Groups

	Group					
	Control		SPC		SPC + 5-HD	
	LVEDP	LVDP	LVEDP	LVDP	LVEDP	LVDP
Before ischemia	8 ± 1	95 ± 10	7 ± 2	98 ± 4	8 ± 2	96 ± 11
End of reperfusion	25 ± 2	42 ± 3	16 ± 2*	83 ± 5*	30 ± 2†	41 ± 6†

The unit for LVEDP is mm Hg and the unit for LVDP is % of baseline. Data are presented as mean ± SEM.
LVEDP = left ventricular end-diastolic pressure, LVDP = left ventricular developed pressure, SPC = sevoflurane preconditioning, 5-HD = 5-hydroxydecanoic acid.
* SPC versus control, † SPC versus SPC/5-HD; $P < 0.05$ is considered significant. $n = 6$ in each group.

diazoxide before ischemia reduced $[Ca^{2+}]_m$ during I/R in a manner similar to IPC (7).

It is well documented that the newborn is not simply a smaller version of an adult. The physiology, pharmacology, and metabolic responses of the newborn heart differ from those of an adult. Although numerous studies have demonstrated that volatile anesthetic preconditioning is effective in protecting against ischemic damage in adult hearts (1,5,17), no studies have addressed the effects of inhaled anesthetic preconditioning in an intact newborn heart. The current study is the first to assess the effect of SPC on $[Ca^{2+}]_i$, $[Ca^{2+}]_m$, and high-energy phosphates in intact newborn hearts.

SPC Decreases $[Ca^{2+}]_i$ and $[Ca^{2+}]_m$ During I/R

$[Ca^{2+}]_i$ overload during myocardial I/R comes largely from extracellular Ca^{2+} via Na^+-Ca^{2+} exchanger (3,10,11,18,19). However, mitochondria have distinct pathways for Ca^{2+} influx and efflux (7). The mitochondrial membrane potential driven uniporter is the primary influx pathway for calcium, whereas the mitochondrial permeability transition pore and Na^+-Ca^{2+} exchanger are the main efflux pathways in mitochondria. Studies in adult hearts have shown that $[Ca^{2+}]_i$ overload is closely correlated with myocardial damage and cell death (3,8,21). The results of the current study demonstrate similar findings in newborn hearts, namely that SPC diminishes the increase of $[Ca^{2+}]_i/[Ca^{2+}]_m$ otherwise observed in newborn rabbit hearts during I/R (22,23).

SPC Preserves High-Energy Phosphates and Contractile Function and Decreases Myocardial Injury During Reperfusion

Although the above-described alterations in ion homeostasis are vitally important, changes in energy metabolism may also have an important role in the development of irreversible myocyte injury during I/R. It is generally accepted that I/R injury profoundly disrupts mitochondrial energy metabolism, and numerous studies have shown that mitochondria isolated from I/R hearts manifest reduced function, decreased membrane potential, and respiratory impairment (15).

As in adult rat hearts (13), exposure of newborn hearts to a volatile anesthetic preserved ATP during reperfusion. One postulate is that SPC could diminish use of ATP during reperfusion as a result of the relative decrease in $[Ca^{2+}]_i$ and $[Ca^{2+}]_m$ by decreasing Ca^{2+}-dependent energy-consuming processes (e.g., Na/K adenosine triphosphatase [ATPase] and Ca^{2+} ATPase) in the cytosol and mitochondria.

A consequence of SPC decreasing $[Ca^{2+}]_i$ and preserving high-energy phosphates would be preservation of myocardial function and reduction in myocardial injury secondary to less mitochondrial calcium and, hence, preservation of mitochondrial function. This postulate is supported by the findings in SPC hearts that LVDP recovered significantly in reperfusion, and myocardial compliance improved as reflected by lower LVEDP during reperfusion. Similarly, total CK release was much less in the SPC-treated group compared with the control group.

Inhibition of Mitochondrial K_{ATP} Channels Blunts the Protective Effects of SPC on $[Ca^{2+}]_i/[Ca2^+]_m$ During I/R

It has been suggested that the activation of the mitochondrial K_{ATP} channels has an important role in myocardial protection during IPC and SPC (7,12,20). One mechanism could be the opening of mitochondrial K_{ATP} channels which reduces the driving force for Ca^{2+} influx, thus attenuating $[Ca^{2+}]_m$ overload and preserving mitochondrial respiration and ATP synthesis (15,20,24,25). These studies in newborn hearts support the previous findings in adult hearts using either IPC or SPC (1,4,5,15), indicating that similar mechanisms are operative, independent of age.

One interesting finding from these studies is that 5-HD had its greatest effect on mitochondrial $[Ca^{2+}]$ during ischemia, essentially abolishing the effect of SPC (Fig. 4), whereas the effect on cytosolic $[Ca^{2+}]$ was only partial. The concurrence of the effect of 5-HD on mitochondrial $[Ca^{2+}]$ with changes in ATP, CK release, and function suggest that the accumulation of calcium by mitochondria, rather than in the cytosol, is

a key event in determining cellular injury and survival (7,26). However, there is no proof of a causal role of higher $[Ca^{2+}]_m$ in mediating injury in this setting.

Several limitations of this study should be noted. First, whereas these experiments focused on the pathway outlined in the hypothesis, there is more than one mechanism involved in anesthetic preconditioning (27). Second, only newborn rabbit hearts, equivalent to 21-day-old human hearts, were used and no adult hearts were studied for comparison. Thus, these data cannot be extrapolated to other species or ages. However, the literature shows significant consistency, suggesting common elements of protective mechanisms in mammals. Third, the sevoflurane concentration used in these studies was 2.5%, a concentration equivalent to 1 minimum alveolar concentration in a young adult animal; other concentrations may have greater or lesser effects. Fourth, measurements of ATP, $[Ca^{2+}]_i$, and $[Ca^{2+}]_m$ were done in separate experiments.

These data provide further support for previous findings that newborn hearts respond to ischemia and reperfusion with an increase in $[Ca^{2+}]_i$ and $[Ca^{2+}]_m$ associated with myocardial cell damage and dysfunction. SPC in newborn rabbit hearts limits the increases in $[Ca^{2+}]_i$ and $[Ca^{2+}]_m$ during 40 minutes of warm I/R, a protective effect partially mediated by K_{ATP} channel opening. Finally, the observed decreases in $[Ca^{2+}]_i$ and $[Ca^{2+}]_m$ accumulation are associated with greater ATP recovery, decreased CK release, and improved recovery of left ventricular function during reperfusion.

References

1. Sniecinski R, Liu H. Reduced efficacy of volatile anesthetic preconditioning with advanced age in the isolated rat myocardium. Anesthesiology 2004;100:589–97.
2. Dennis SC, Gevers W, Opie LH. Protons in ischemia: where do they come from; where do they go to? J Mol Cell Cardiol 1991;23:1077–86.
3. Liu H, Cala P, Anderson S. Ethyl-isopropylamiloride diminishes changes in intracellular Na, Ca, and pH in ischemic newborn myocardium. J Mol Cell Cardiol 1997;29:2077–86.
4. Kersten JR, Schmeling TJ, Pagel PS, et al. 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.
5. 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.
6. Imura H, Caputo M, Parry A, et al. Age-dependent and hypoxia-related differences in myocardial protection during pediatric open heart surgery. Circulation 2001;103:1551–6.
7. Wang L, Cherednichenko G, Hernandez L, et al. Preconditioning limits mitochondrial Ca(2+) during ischemia in rat hearts: role of K(ATP) channels. Am J Physiol Heart Circ Physiol 2001; 280:H2321–8.
8. 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.
9. Anderson SE, Murphy E, Steenbergen C, et al. Na/H exchange in myocardium: effects of hypoxia and acidification on Na and Ca. Am J Physiol 1990;259:C940–8.
10. Pike MM, Luo C, Clark M, et al. NMR measurements of Na^+ and cellular energy in ischemic rat heart: role of Na^+-H^+ exchange. Am J Physiol 1993;265:H2017–26.
11. Tani M, Neely J. Role of intracellular Na^+ and Ca^{2+} overload and depressed recovery of ventricular function of reperfused ischemic rat hearts. Circ Res 1989;65:1045–56.
12. An J, Varadarajan SG, Novalija E, Stowe DF. Ischemic and anesthetic preconditioning reduces cytosolic [Ca2+] and improves Ca(2+) responses in intact hearts. Am J Physiol Heart Circ Physiol 2001;281:H1508–23.
13. Boutros A, Wang J, Capuano C. Isoflurane and halothane increase adenosine triphosphate preservation, but do not provide additive recovery of function after ischemia, in preconditioned rat hearts. Anesthesiology 1997;86:109–17.
14. Ferrari R. The role of mitochondria in ischemic heart disease. J Cardiovasc Pharmacol 1996;28:S1–10.
15. Fryer RM, Eells JT, Hsu AK, et al. Ischemic preconditioning in rats: role of mitochondrial K(ATP) channel in preservation of mitochondrial function. Am J Physiol Heart Circ Physiol 2000; 278:H305–12.
16. Holmuhamedov EL, Wang L, Terzic A. ATP-sensitive K+ channel openers prevent Ca2+ overload in rat cardiac mitochondria. J Physiol 1999;519(Pt 2):347–60.
17. Roscoe AK, Christensen JD, Lynch C III. Isoflurane, but not halothane, induces protection of human myocardium via adenosine A1 receptors and adenosine triphosphate-sensitive potassium channels. Anesthesiology 2000;92:1692–701.
18. Anderson S, Dickinson C, Liu H, Cala P. Effects of Na-K-2Cl cotransport inhibition on myocardial Na and Ca during ischemia and reperfusion. Am J Physiol 1996;270(2 Pt 1):C608–18.
19. Steenbergen C, Murphy E, Levy L, London R. Elevation in cytosolic free calcium concentration early in myocardial ischemia in perfused rat heart. Circ Res 1987;60:700–7.
20. Gross GJ, Fryer RM. Sarcolemmal versus mitochondrial ATP-sensitive K+ channels and myocardial preconditioning. Circ Res 1999;84:973–9.
21. Varadarajan SG, An J, Novalija E, Stowe DF. Sevoflurane before or after ischemia improves contractile and metabolic function while reducing myoplasmic Ca2+ loading in intact hearts. Anesthesiology 2002;96:125–33.
22. Anderson SE, Liu H, Ho HS, et al. Age-related differences in Na+-dependent Ca2+ accumulation in rabbit hearts exposed to hypoxia and acidification. Am J Physiol 2003;284:C1123–32.
23. Ho HS, Liu H, Cala PM, Anderson SE. Hypertonic perfusion inhibits intracellular Na and Ca accumulation in hypoxic myocardium. Am J Physiol Cell Physiol 2000;278:C953–64.
24. Iwai T, Tanonaka K, Koshimizu M, Takeo S. Preservation of mitochondrial function by diazoxide during sustained ischaemia in the rat heart. Br J Pharmacol 2000;129:1219–27.
25. Park JW, Chun YS, Kim YH, et al. Ischemic preconditioning reduces Op6 generation and prevents respiratory impairment in the mitochondria of post-ischemic reperfused heart of rat. Life Sci 1997;60:2207–19.
26. Miyamae M, Camacho SA, Weiner MW, Figueredo VM. Attenuation of postischemic reperfusion injury is related to prevention of [Ca2+]m overload in rat hearts. Am J Physiol 1996;271: H2145–53.
27. Zhong C, Zhou Y, Liu H. Nuclear factor-kappa B activation and anesthetic preconditioning with sevoflurane during myocardial ischemia/reperfusion. Anesthesiology 2004;100:540–6.