

Sevoflurane Confers Additional Cardioprotection after Ischemic Late Preconditioning in Rabbits

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Background: Sevoflurane exerts cardioprotective effects that mimic the early ischemic preconditioning phenomenon (EPC) by activating adenosine triphosphate-sensitive potassium (K_{ATP}) channels. Ischemic late preconditioning (LPC) is an important cardioprotective mechanism in patients with coronary artery disease. The authors investigated whether the combination of LPC and sevoflurane-induced preconditioning results in enhanced cardioprotection and whether opening of K_{ATP} channels plays a role in this new setting.

Methods: Seventy-three rabbits were instrumented with a coronary artery occluder. After recovery for 10 days, they were subjected to 30 min of coronary artery occlusion and 120 min of reperfusion (I/R). Controls ($n = 14$) were not preconditioned. LPC was induced in conscious animals by a 5-min period of coronary artery occlusion 24 h before I/R (LPC, $n = 15$). Additional EPC was induced by a 5-min period of myocardial ischemia 10 min before I/R (LPC+EPC, $n = 9$). Animals of the sevoflurane (SEVO) groups inhaled 1 minimum alveolar concentration of sevoflurane for 5 min at 10 min before I/R with (LPC+SEVO, $n = 10$) or without (SEVO, $n = 15$) additional LPC. The K_{ATP} channel blocker 5-hydroxydecanoate (5-HD, 5 mg/kg) was given intravenously 10 min before sevoflurane administration (LPC+SEVO+5-HD, $n = 10$).

Results: Infarct size of the area at risk (triphenyltetrazolium staining) was reduced from $45 \pm 16\%$ (mean \pm SD, control) to $27 \pm 11\%$ by LPC ($P < 0.001$) and to $27 \pm 17\%$ by sevoflurane ($P = 0.001$). Additional sevoflurane administration after LPC led to a further infarct size reduction to $14 \pm 8\%$ (LPC+SEVO, $P = 0.003$ vs. LPC; $P = 0.032$ vs. SEVO), similar to the combination of LPC and EPC ($12 \pm 8\%$; $P = 0.55$ vs. LPC+SEVO). Cardioprotection induced by LPC+SEVO was abolished by 5-HD (LPC+SEVO+5-HD, $41 \pm 19\%$, $P = 0.001$ vs. LPC+SEVO).

Conclusions: Sevoflurane administration confers additional cardioprotection after LPC by opening of K_{ATP} channels.

ISCHEMIC preconditioning, first described by Murry *et al.*,¹ markedly reduces myocyte death during prolonged periods of myocardial ischemia and has been reported in several mammalian species. The initial protective effect of classic or early preconditioning (EPC) is transient, disappearing between 1 and 2 h after the preconditioning ischemia. Recent studies demonstrated that cardioprotection from preconditioning reappears 24 h after the initial stimulus and lasts for approximately 2–3 days.² This phenomenon is known as late preconditioning

(LPC). EPC and LPC are suggested to be important endogenous cardioprotective mechanisms of patients with coronary artery disease.^{3,4} Both EPC and LPC can be induced by a variety of stimuli, *e.g.*, brief ischemia, or by pharmacologic agents such as adenosine,⁵ opioids,^{6,7} or nitric oxide (NO) donors.⁸ Previous investigations have demonstrated that EPC can also be mimicked by administration of several halogenated inhalational anesthetics,^{9–12} including sevoflurane.^{13–15}

The signal transduction cascades of both LPC and EPC differ considerably, but activation of mitochondrial and/or sarcolemmal adenosine triphosphate (ATP)-regulated potassium (K_{ATP}) channels has been shown to be a key mechanism triggering and/or mediating the protective effects of both ischemic and pharmacologically induced EPC¹⁶ and LPC.¹⁷

Although both phases of preconditioning are well described, little is known about a potential interaction between the two protective processes and the underlying mechanisms. A recent investigation from our laboratory demonstrated additive cardioprotective effects of ischemic LPC and EPC mediated by opening of K_{ATP} channels.¹⁷

However, it remains elusive whether pharmacologically induced EPC by volatile anesthetics confers additional cardioprotection when the heart is already in a protected state after ischemic LPC. Therefore, the objective of the present study was (1) to determine a possible interaction between LPC and sevoflurane-induced preconditioning and (2) to investigate the potential role of mitochondrial K_{ATP} channels in this new setting by using the K_{ATP} channel blocker 5-hydroxydecanoate (5-HD) in the rabbit heart *in vivo*.

Materials and Methods

The present study conforms to the Guiding Principles in the Care and Use of Animals as approved by the Council of the American Physiologic Society and was approved by the local institutional Animal Care Committee.

Surgical Preparation

Surgical preparation and animal care have been described in detail previously.¹⁷ Briefly, rabbits underwent a left thoracotomy in the fourth intercostal space under sterile conditions, and the left anterior descending coronary artery was encircled with two 5-0 prolene sutures

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with atraumatic needles (Ethicon 5/0, 1-metric, TF, Johnson-Johnson, Brussels, Belgium). A reinforced tube (2.5-mm internal diameter; Mallinckrodt Medical, Athlone, Ireland) was tunneled subcutaneously and externalized between the scapulae. The internal end was placed close to the sutures around the coronary artery and fixed at the pericardium. The two sutures were then externalized through this tube. The chest wound was closed in layers and covered by a vest (Rabbit jacket size M; Byron, Grand Island, NY) to protect the tube and the externalized sutures until the end of the experiments. Postoperative analgesia and antibiotic prophylaxis were provided by subcutaneous injection of piritramide (2 mg/kg) and amoxicillin (15 mg/kg), respectively.

LPC Protocol

Rabbits were allowed to recover for 7 to 10 days. Then one pair of sutures was tightened for 5 min to induce occlusion of the coronary artery. This was verified by the immediate occurrence of ST-segment elevations in the electrocardiogram (SC 9000; Siemens AG, Düsseldorf, Germany). At the end of the 5-min period of coronary artery occlusion, the suture was released and removed to ensure proper reperfusion, which was verified by the disappearance of the electrocardiogram changes within 5 min in each animal.

EPC and Myocardial Infarction Protocol

The EPC and myocardial infarction protocol has been described in detail previously.¹⁸ Briefly, α -chloralose-anesthetized New Zealand White rabbits weighing a mean of 2.8 ± 0.16 kg were instrumented for measurement of aortic pressure (Statham transducer PD23; Gould, Cleveland, OH), cardiac output (CO; 4S ultrasonic flow probe, T 208; Transonic Systems Inc., Ithaca, NY), and left ventricular (LV) pressure (Millar tip catheter 5F, SPC-340; Millar, Houston, TX). The remaining second suture around the coronary artery was dissected free, and a plastic tube was placed around this suture for later coronary artery occlusion. The effectiveness of coronary artery occlusion was verified by the appearance of epicardial cyanosis and changes in the surface electrocardiogram. Ventricular fibrillation during coronary artery occlusion was treated by electrical defibrillation (5 J; DCS261 Defibrillator; Piekser, Ratingen, Germany). After coronary artery occlusion, the snare occluder was released and reperfusion was verified by the disappearance of epicardial cyanosis and disappearance of the electrocardiogram changes within 5 min in each animal. Temperature was measured inside the pericardial cradle and maintained between 38.3° and 38.7°C by adjusting a heating pad and an infrared lamp.

Infarct Size Assessment

After 2 h of reperfusion, the heart was arrested by injection of potassium chloride solution into the left

atrium and quickly excised. The size of the area at risk was then determined by Evans blue staining of the non-ischemic area, and infarct size within the area at risk was determined by triphenyltetrazolium chloride staining as described previously.¹⁸

Study Protocol

The study protocol is shown in figure 1. Eighty-two rabbits were assigned to one of the six groups, and all animals were subjected to 30 min of sustained ischemia followed by 120 min of reperfusion. In the control group, rabbits were not preconditioned. In the LPC group, LPC was elicited in awake animals by one 5-min period of coronary artery occlusion 24 h before the sustained ischemia. Rabbits of the sevoflurane (SEVO) group received 1 minimum alveolar concentration of sevoflurane (end-tidal concentration) for 5 min, followed by a 10-min washout period before the 30 min of ischemia without LPC. In the LPC+SEVO group, animals were subjected to LPC 24 h before additional sevoflurane treatment. To test the role of K_{ATP} channels in this setting, the K_{ATP} channel blocker 5-hydroxydecanoate (5-HD, 5 mg/kg) was given intravenously 10 min before sevoflurane administration in additional experiments (LPC+SEVO+5-HD). In the LPC+EPC group, EPC was induced 24 h after LPC by a 5-min period of myocardial ischemia 10 min before the infarct-inducing ischemia.

Data Analysis

LV pressure, its first derivative dP/dt , aortic pressure, and CO were recorded continuously on an ink recorder (Recorder 2800; Gould Inc., Cleveland, OH). The data were digitized using an analog-to-digital converter (Data Translation, Marlboro, MA) at a sampling rate of 500 Hz and processed later on a personal computer.

Hemodynamic Variables

Global systolic function was measured in terms of LV peak systolic pressure (LVSP) and maximum rate of pressure increase (dP/dt_{\max}). Global LV end systole was defined as the point of minimum dP/dt and LV end diastole as the beginning of the sharp upslope of the LV dP/dt tracing. The time constant of decrease in LV isovolumic pressure (τ) was used as an index of LV diastolic function. The rate-pressure product (RPP) was calculated from heart rate and LVSP, and systemic vascular resistance from mean aortic pressure and CO, assuming a right atrial pressure of 0 mmHg in the open-chest preparation.

Statistical Analysis

Data are presented as mean and SD. Statistical analysis of infarct size measurements was performed by the Student t test if variances were equal or by the alternate Welch t test if variances were different, with Bonferroni-Holmes correction for multiple comparisons.

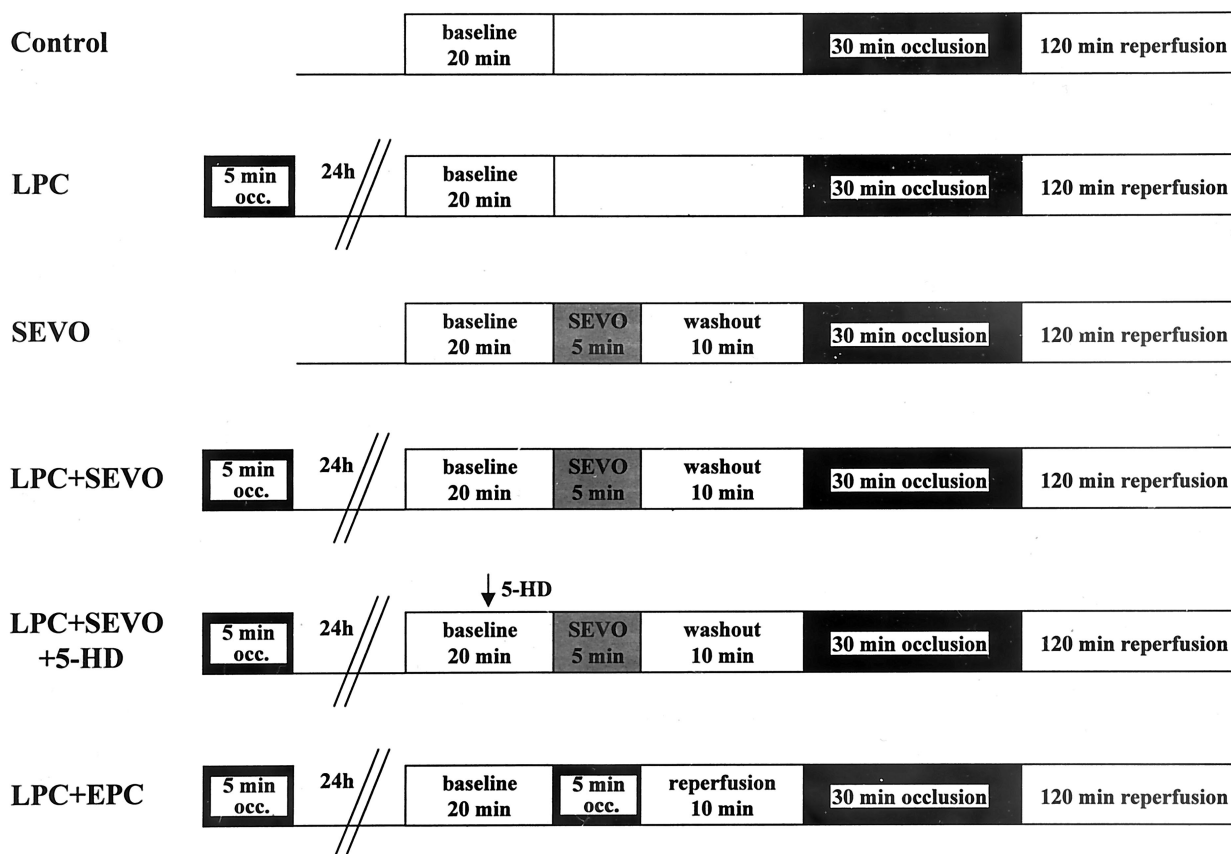


Fig. 1. Experimental protocol. EPC = ischemic early preconditioning; 5-HD = 5-hydroxydecanoate (5 mg/kg); LPC = ischemic late preconditioning; occ. = coronary artery occlusion; SEVO = sevoflurane application.

Statistical analysis of hemodynamic measurements was performed by two-way repeated measures ANOVA for time and treatment (experimental group) effects. If an overall significance between groups was found, comparison was done for each time point using one-way ANOVA followed by the Student *t* test with Bonferroni-Holmes correction for multiple comparisons. Time effects within each group were analyzed by repeated-measures ANOVA followed by the Dunnett *post hoc* test with the baseline value as the reference time point. Changes were considered significant at a value of $P < 0.05$.

Results

A total of 82 animals were used. Nine animals died of ventricular fibrillation during coronary artery occlusion. In the remaining 73 animals, complete data sets were obtained (control group, $n = 14$; LPC, $n = 15$; SEVO, $n = 15$; LPC+SEVO, $n = 10$; LPC+SEVO+5-HD, $n = 10$; LPC+EPC, $n = 9$).

Hemodynamic Function

The hemodynamic variables are summarized in table 1 and figure 2. During baseline recordings, there were no

significant differences between groups in LVSP, CO, or heart rate. Consequently, RPP, a major determinant of myocardial oxygen consumption, was not significantly different between groups during baseline. The intravenous bolus administration of 5-HD did not change hemodynamics. The 5-min period of ischemia for induction of EPC led to a decrease of LVSP by 8% and CO by 10%. Sevoflurane inhalation reduced LVSP by a mean of 6% and led to an increase of CO by 12%. Systemic vascular resistance was reduced by 17% in the three sevoflurane-treated groups during sevoflurane inhalation. During the 10-min washout of sevoflurane and during the 10-min reperfusion after EPC, all hemodynamic variables recovered and were not significantly different from baseline. Coronary artery occlusion was accompanied by a reduction of LVSP (by a mean of 7%), dp/dt_{max} (by a mean of 16%), and CO (by a mean of 10%) in all groups (table 1 and fig. 2). RPP did not differ significantly between groups. With regard to LV relaxation, τ increased by 24% and LV end-diastolic pressure by 6 ± 5 mmHg (all values at 25 min of ischemia). After 2 h of reperfusion, LVSP and dp/dt_{max} were reduced in all groups, still reflecting impaired myocardial contractile function at the end of the experiments. RPP was reduced by a

Table 1. Hemodynamic Variables

| | Baseline | SEVO/EPC | Washout/ Reperfusion | Coronary Occlusion | | Reperfusion | | |
|---|-------------|-------------|-------------------------|--------------------|-------------|-------------|-------------|--------------|
| | 0 | 5 | 15 | 5 | 25 | 5 | 30 | 120 |
| LVEDP, mmHg | | | | | | | | |
| Control | 5.4 ± 5.2 | 5.5 ± 5.7 | 6.7 ± 5.9 | 10.0 ± 7.6 | 9.1 ± 5.2 | 9.6 ± 5.6 | 8.4 ± 5.2 | 8.1 ± 5.1 |
| LPC | 5.1 ± 3.2 | 5.2 ± 3.1 | 6.6 ± 2.9 | 9.5 ± 7.7 | 13.7 ± 8.2 | 13.2 ± 8.3 | 12.9 ± 10.4 | 13.2 ± 8.9 |
| SEVO | 3.9 ± 3.5 | 4.4 ± 4.0 | 5.1 ± 4.9 | 5.9 ± 5.0 | 7.3 ± 6.1 | 7.1 ± 5.8 | 6.5 ± 5.4 | 5.9 ± 4.4 |
| LPC+SEVO | 2.7 ± 1.8 | 7.4 ± 6.4 | 8.4 ± 4.7 | 7.5 ± 4.6 | 7.3 ± 4.6 | 8.8 ± 8.2 | 4.8 ± 4.3 | 6.2 ± 4.5 |
| LPC+SEVO+5-HD | 5.2 ± 3.7 | 7.0 ± 4.7 | 7.0 ± 4.2 | 9.1 ± 6.0 | 7.7 ± 4.2 | 9.3 ± 3.9 | 7.7 ± 3.8 | 10.0 ± 6.9 |
| LPC+EPC | 3.1 ± 1.8 | 6.6 ± 5.7 | 4.3 ± 2.5 | 8.5 ± 7.7 | 9.2 ± 7.7 | 10.3 ± 6.6 | 7.7 ± 5.0 | 6.8 ± 4.4 |
| dP/dt _{max} , mmHg s ⁻¹ | | | | | | | | |
| Control | 4538 ± 1588 | 4671 ± 1536 | 4956 ± 1555 | 4075 ± 1348 | 3907 ± 1580 | 3339 ± 1557 | 4003 ± 1621 | 2995 ± 1243 |
| LPC | 3906 ± 1370 | 4025 ± 1406 | 4087 ± 1250 | 3514 ± 1104 | 3269 ± 984 | 2729 ± 1082 | 2720 ± 1015 | 2257 ± 976* |
| SEVO | 4228 ± 1730 | 3633 ± 1744 | 4385 ± 1694 | 3424 ± 1197 | 3528 ± 1332 | 2989 ± 1271 | 3300 ± 1413 | 2255 ± 1025* |
| LPC+SEVO | 4231 ± 1269 | 3347 ± 1500 | 4579 ± 1119 | 3480 ± 1322 | 3278 ± 1284 | 3004 ± 1276 | 3006 ± 1193 | 2391 ± 1141* |
| LPC+SEVO+5-HD | 4599 ± 1540 | 3887 ± 1420 | 4942 ± 1516 | 3314 ± 1517 | 3352 ± 1451 | 3180 ± 1246 | 3534 ± 1121 | 2710 ± 972 |
| LPC+EPC | 4122 ± 1031 | 3351 ± 1262 | 3982 ± 1364 | 3673 ± 1403 | 3484 ± 1420 | 3402 ± 1331 | 4267 ± 959 | 3792 ± 1056 |
| SVR, mmHg min ⁻¹ l | | | | | | | | |
| Control | 402 ± 117 | 403 ± 110 | 405 ± 107 | 445 ± 159 | 413 ± 110 | 406 ± 118 | 394 ± 93 | 376 ± 141 |
| LPC | 347 ± 94 | 355 ± 89 | 365 ± 87 | 396 ± 84 | 414 ± 141 | 343 ± 76 | 358 ± 101 | 331 ± 115 |
| SEVO | 412 ± 156 | 347 ± 161 | 404 ± 171 | 415 ± 126 | 420 ± 175 | 383 ± 139 | 386 ± 150 | 328 ± 115 |
| LPC+SEVO | 407 ± 116 | 345 ± 104 | 437 ± 122 | 438 ± 127 | 391 ± 83 | 347 ± 51 | 334 ± 64 | 327 ± 77 |
| LPC+SEVO+5-HD | 367 ± 99 | 293 ± 64 | 373 ± 85 | 384 ± 179 | 310 ± 40 | 305 ± 55 | 304 ± 48 | 305 ± 91 |
| LPC+EPC | 364 ± 96 | 379 ± 123 | 366 ± 78 | 396 ± 103 | 425 ± 190 | 356 ± 104 | 408 ± 144 | 379 ± 91 |

Data are mean ± SD.

dP/dt_{max} = maximum rate of increase in left ventricular pressure; EPC = early preconditioning; 5-HD = 5-hydroxydecanoate; LPC = late preconditioning; LVEDP = left ventricular end-diastolic pressure; RPP = rate pressure product; SEVO = sevoflurane; SVR = systemic vascular resistance; τ = time constant of decrease in isovolumic left ventricular pressure.

$P < 0.05$ compared with baseline.

mean of 21%. LV end-diastolic pressure remained increased by a mean of 4.4 ± 3.0 mmHg at the end of the experiments.

Infarct Size

Mean LV dry weight was 0.79 ± 0.19 g, with no significant differences between groups (data from individual groups are given in table 2 and fig. 3). The ischemic-reperfused area (area at risk) was 0.39 ± 0.18 g and constituted $50.6 \pm 21.2\%$ of the left ventricle, with no significant differences between groups.

Infarct size in percentage of the size of the area at risk was reduced from $45 \pm 16\%$ (mean ± SD, control) to $27 \pm 11\%$ by LPC ($P < 0.001$) and to $27 \pm 17\%$ by sevoflurane ($P = 0.001$). Additional sevoflurane administration after LPC (LPC+SEVO) led to a further infarct size reduction to $14 \pm 8\%$ ($P = 0.003$ vs. LPC; $P = 0.032$ vs. SEVO), similar to the combination of LPC and EPC ($12 \pm 8\%$; $P = \text{NS}$ vs. LPC+SEVO). Enhanced cardioprotection induced by LPC+SEVO was abolished by the K_{ATP} blocker 5-HD (LPC+SEVO+5-HD, $41 \pm 19\%$, $P = 0.001$ vs. LPC+SEVO).

Discussion

The main finding of our study is that sevoflurane-induced preconditioning confers additional cardiopro-

tection after LPC in the rabbit heart *in vivo*. Opening of K_{ATP} channels is a key mechanism in this new setting.

In the present investigation, pretreatment with 1 minimum alveolar concentration of end-tidal sevoflurane for 5 min before the prolonged ischemia reduces infarct size by 40% in comparison with controls. In a former study performed in our laboratory using the same experimental animal model, anesthetic regimen, duration of ischemia (30 min), and reperfusion (2 h), one 5-min period of ischemic preconditioning reduced infarct size by 47%.¹⁸ Thus, we could confirm the results of previous studies that pretreatment with sevoflurane,^{13-15,19,20} as with other volatile anesthetics,⁹⁻¹² mimics the cardioprotective effects of EPC. However, nothing was known about the potential interaction between anesthetic-induced EPC and LPC. For patients with coronary artery disease, LPC might be the more important mechanism, because LPC-induced cardioprotection is long-lasting (2 to 3 days).² LPC may contribute to the better outcome of patients with repetitive angina.³ In this context, it would be interesting to know whether anesthetic-induced preconditioning may confer additional cardioprotection when the myocardium is already in a protected state after LPC.

Therefore, the present study investigated a possible interaction of LPC and sevoflurane-induced preconditioning and the involvement of K_{ATP} channel opening in the rabbit heart *in vivo*. All experiments were per-

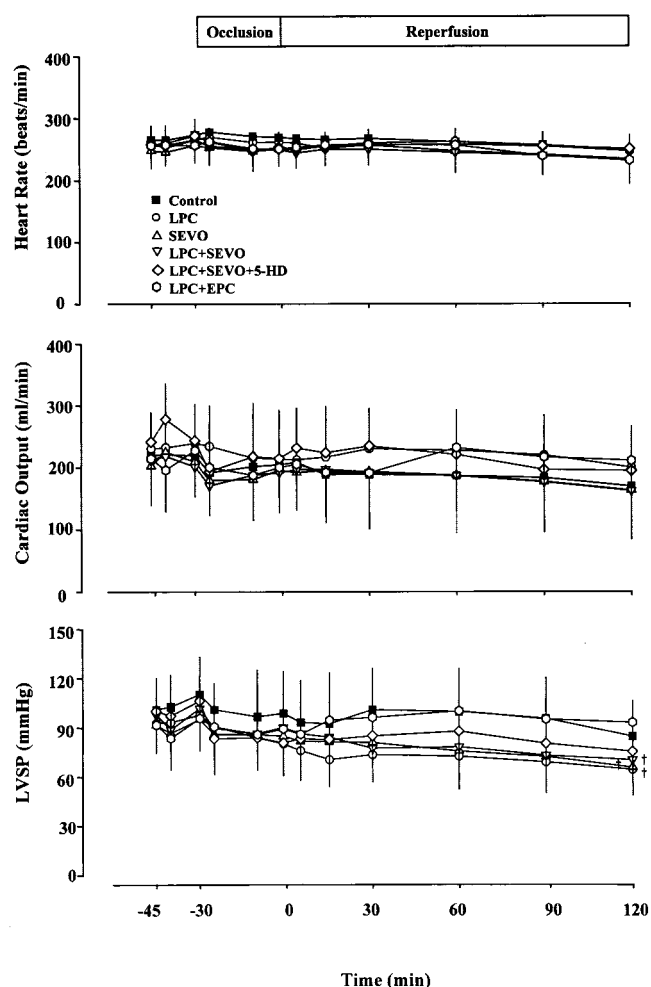


Fig. 2. Line plot showing the time course of heart rate, cardiac output, and left ventricular peak systolic pressure (LVSP) during experiments in the control, late preconditioning (LPC), sevoflurane (SEVO), LPC+SEVO, LPC+SEVO+5-hydroxydecanoate (LPC+SEVO+5-HD), and LPC+early ischemic preconditioning (LPC+EPC) groups. Data are mean \pm SD. $\dagger P < 0.05$ versus baseline conditions.

formed during anesthesia with α -chloralose. This type of anesthesia maintains near-normal cardiovascular reflexes comparable to the awake state and is a classic anesthetic for physiologic and pharmacologic experiments.²¹ A recent study by Zaugg *et al.*²² reported that the main metabolite of α -chloralose, 2,2,2-trichloroethanol, potentiates the mitochondrial K_{ATP} channel-opening effect of

diazoxide. However, in contrast to volatile or intravenous anesthetics, which may induce^{9,13} or block EPC¹⁸ and LPC,²³ a preconditioning effect of α -chloralose *in vivo* has not been described thus far.

One potential mechanism for the additive cardioprotection of LPC and sevoflurane-induced preconditioning could have been an incomplete activation of the LPC mechanism. However, this simple explanation seems highly unlikely: first, in the present study, LPC led to a similar strong infarct size reduction, as in a previous study performed in our laboratory¹⁷ and in studies by others.^{24,25} Second, Baxter *et al.* demonstrated that one 5-min period of coronary artery occlusion already induces maximal cardioprotection.²⁴ We have recently confirmed this finding using the same animal preparation and experimental conditions as in this study by demonstrating that repeated cycles of preconditioning ischemia (5×4 min) do not further increase LPC-mediated cardioprotection compared with one 5-min cycle.²⁶ Thus, the interaction of LPC and sevoflurane-induced preconditioning seems to be more complex. To explain it, one might first consider the key steps in the signal transduction pathways of both preconditioning mechanisms. Although the exact mechanisms underlying the cardioprotective effects of sevoflurane-induced preconditioning are unknown, opening of K_{ATP} channels is a key step in the signal transduction cascade. Administration of a K_{ATP} channel blocker before volatile anesthetic exposure is reported to block preconditioning induced not only by sevoflurane^{13,14,19} but also by isoflurane^{9,27-30} and desflurane.¹¹ Opening of K_{ATP} channels is important not only for the cardioprotective effects of sevoflurane but also for its cerebral³¹ and coronary³² vasodilatory effects. Furthermore, sevoflurane preconditioning has been shown to reduce Ca^{2+} loading while augmenting contractile responsiveness to Ca^{2+} and improving postischemic cardiac function. These effects were also blocked by previous administration of a K_{ATP} channel blocker.¹⁵ Two studies recently investigated the effects of sevoflurane on mitochondrial K_{ATP} channel opening using the autofluorescence of flavoproteins as an index of mitochondrial K_{ATP} channel activity: Kohro *et al.*³³ reported that sevoflurane induces a dose-dependent increase of flavoprotein oxidation, indicating in-

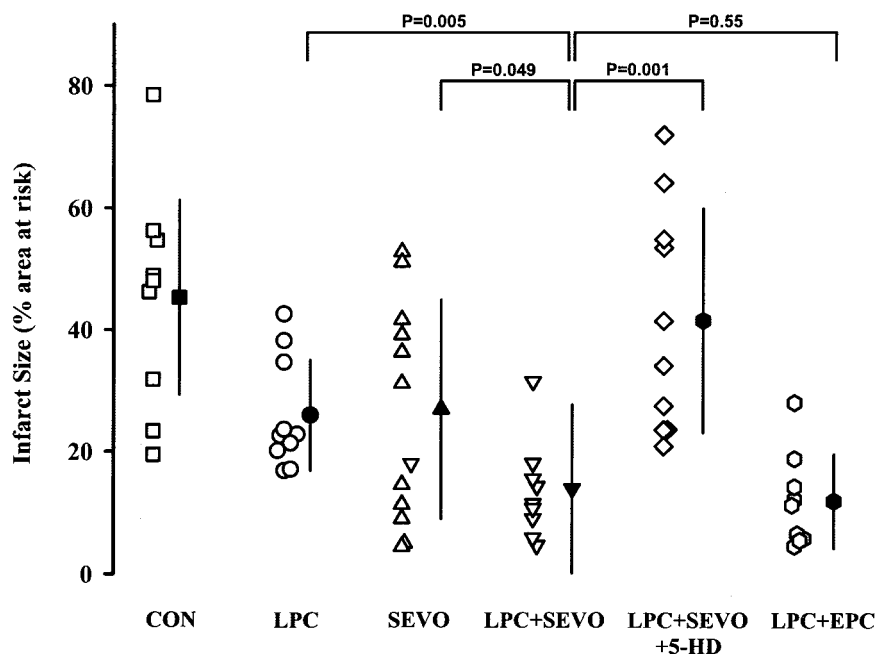
Table 2. Weights and Size of Area at Risk

| | Control | LPC | SEVO | LPC+SEVO | LPC+SEVO+5-HD | LPC+EPC |
|--------------------|-----------------|---------------------------|------------------|------------------|----------------------------|-----------------|
| Body weight, g | 2789 \pm 191 | 2778 \pm 120 | 2777 \pm 204 | 2827 \pm 119 | 2811 \pm 127 | 2771 \pm 259 |
| LV weight, g | 0.76 \pm 0.09 | 0.80 \pm 0.28 | 0.82 \pm 0.16 | 0.87 \pm 0.26 | 0.80 \pm 0.11 | 0.71 \pm 0.07 |
| Area at risk, g | 0.36 \pm 0.10 | 0.41 \pm 0.17 | 0.38 \pm 0.2 | 0.40 \pm 0.24 | 0.39 \pm 0.20 | 0.45 \pm 0.19 |
| Area at risk/LV, % | 48.0 \pm 15.2 | 54.4 \pm 25.3 | 46.9 \pm 21.6 | 45.6 \pm 16.3 | 47.7 \pm 21.3 | 63.2 \pm 25.2 |
| Infarct size, g | 0.18 \pm 0.08 | 0.11 \pm 0.07 \dagger | 0.10 \pm 0.10* | 0.06 \pm 0.05§ | 0.16 \pm 0.12 \ddagger | 0.05 \pm 0.05 |

Data are mean \pm SD. EPC = early preconditioning; 5-HD = 5-hydroxydecanoate; LPC = late preconditioning; LV = left ventricle; SEVO = sevoflurane.

* $P = 0.039$ compared with Control group; $\dagger P = 0.026$ compared with Control group; $\ddagger P = 0.024$ compared with LPC+SEVO group; § $P = 0.046$ compared with LPC group.

Fig. 3. Infarct size as a percentage of the area at risk in the control, late preconditioning (LPC), sevoflurane (SEVO), LPC+SEVO, LPC+SEVO+5-hydroxydecanoate (LPC+SEVO+5-HD), and LPC+early ischemic preconditioning (LPC+EPC) groups. Open symbols = single data points, filled symbols = mean \pm SD.



creased opening of mitochondrial K_{ATP} channels. Zaugg *et al.*³⁴ demonstrated that sevoflurane enhances diazoxide-mediated activation of mitochondrial K_{ATP} channels.

The signal transduction cascade of LPC is also not fully understood, but the NO hypothesis proposed by Bolli *et al.*⁸ is now well accepted: the preconditioning ischemia induces increased production of NO by the endothelial NO synthase and reactive oxygen species, which in turn activate a signal transduction cascade consisting of protein kinase C and tyrosine kinase, finally resulting in increased transcription of the inducible NO synthase gene and increased NO production during the sustained ischemia. Sasaki *et al.*³⁵ provided direct evidence that NO enhances K_{ATP} channel activity in cardiac myocytes. In fact, opening of K_{ATP} channels is a key mechanism not only in ischemia- and anesthetic-induced EPC but also in LPC.¹⁷

In the present investigation, the enhanced cardioprotection induced by the combination of LPC and sevoflurane-induced preconditioning was not different compared with the combination of LPC and EPC. The differences in infarct size were not caused by differences in area at risk sizes, temperature, or hemodynamic parameters during ischemia and reperfusion. Our results show that K_{ATP} channel opening is also critically important in the interaction of LPC and sevoflurane-induced preconditioning. Administration of the K_{ATP} channel blocker 5-HD before sevoflurane exposure blocks the additive cardioprotective effects of LPC and sevoflurane-induced preconditioning. In a previous study performed in our laboratory using the same experimental model and infarct size protocol, 5-HD itself did not affect infarct size.¹⁷ To explain the positive interaction, an attractive hypothesis is that LPC increases NO release during the

infarct-inducing ischemia.⁸ This would lead to an increased open-state probability of K_{ATP} channels,³⁵ which could be further augmented by sevoflurane-induced preconditioning. Although the relative contribution of mitochondrial *versus* sarcolemmal K_{ATP} channels during anesthetic- and ischemia-induced preconditioning is currently still a matter of debate, there is considerable evidence that 5-HD is a selective inhibitor of the mitochondrial K_{ATP} channel.³⁶ Thus, we propose that the additive protective effects may be triggered and/or mediated by mitochondrial rather than sarcolemmal K_{ATP} channel opening. At present, there is still no clear indication of how mitochondrial K_{ATP} channel opening may protect ischemic tissue. However, opening of K_{ATP} channels is no longer believed to be the end effector mediating the cardioprotective effects of preconditioning but rather may act as a trigger by activating different downstream kinases, such as protein kinase C and tyrosine kinases.³⁷ Thus, another possible explanation for the observed additive cardioprotection of LPC and sevoflurane-induced preconditioning in the present study may be the interaction of phosphorylating enzymes, such as protein kinase C, which may regulate K_{ATP} channel activity³⁸ or is activated by K_{ATP} channel opening.³⁹ In fact, activation of protein kinase C is involved in the signal transduction cascade of both LPC²⁵ and volatile anesthetic-induced preconditioning with isoflurane,^{34,40} sevoflurane,³⁴ and halothane.¹⁰ Although there is no direct evidence that sevoflurane preconditioning enhances protein kinase C activity, sevoflurane has been shown to stimulate inositol triphosphate-3 production in skeletal muscle.⁴¹ Inositol triphosphate-3 release has been shown to activate protein kinase C.⁴²

The cardioprotection induced by sevoflurane preconditioning not only occurs in cardiac myocytes but also extends to the endothelium of the coronary vasculature.¹⁴ Sevoflurane pretreatment improves coronary vasodilation and increases NO production depending on the opening of K_{ATP} channels.¹⁴ NO inhibits leukocyte adhesion and migration into reperfused tissues,⁴³ thereby preventing the postischemic "no reflow" phenomenon. Although a protective effect of LPC on endothelial injury has not been described so far, it is possible that the increase of NO release caused by the combination of LPC and sevoflurane-induced preconditioning leads to enhanced endothelial protection with consecutive reduced myocardial necrosis.

In the present investigation, the significant reduction in infarct size induced by the combination of LPC and sevoflurane preconditioning was not accompanied by a better functional recovery, suggesting that stunning occurred in a much larger area of the LV than the infarcted area. This is in contrast to other studies in which a protective effect of sevoflurane-induced preconditioning or LPC against myocardial stunning was observed.^{15,20,44} However, our result is in line with a study performed by Cohen *et al.*⁴⁵ in which the enhanced functional recovery resulting from infarct size reduction by preconditioning becomes apparent as early as 1 to 3 days later. Furthermore, the absolute difference in infarct size (in grams) between the LPC+SEVO and the other groups is small in comparison with total LV mass, thereby reducing the influence of infarct size reduction on global myocardial function.

Myocardial ischemic preconditioning is still a laboratory-based phenomenon that has not been documented conclusively in patients. However, some *in vitro* and *in vivo* evidence of ischemic preconditioning in humans exists,^{3,4,46} and there are several possible clinical scenarios in which ischemic preconditioning might occur, *e.g.*, unstable angina preceding myocardial infarction³ or percutaneous transluminal coronary angioplasty.⁴⁷ There is also strong evidence that anesthetic-induced preconditioning also exists in human myocardium,³⁰ even under clinical conditions.¹²

Our findings of additive cardioprotective effects of LPC and anesthetic-induced EPC suggest that the pre-existing cardioprotection after angina³ may be further enhanced by the use of routinely administered volatile anesthetics.

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