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Mechanisms of Sevoflurane-induced Myocardial Preconditioning in Isolated Human Right Atria In Vitro

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Background: **The authors examined the role of adenosine triphosphate–sensitive potassium channels and adenosine A_1 receptors in sevoflurane-induced preconditioning on isolated human myocardium.**

Methods: **The authors recorded isometric contraction of human right atrial trabeculae suspended in oxygenated Tyrode's solution (34°C; stimulation frequency, 1 Hz). In all groups, a 30-min hypoxic period was followed by 60 min of reoxygenation. Seven minutes before hypoxia reoxygenation, muscles were exposed to 4 min of hypoxia and 7 min of reoxygenation or 15 min of sevoflurane at concentrations of 1, 2, and 3%. In separate groups, sevoflurane 2% was administered in the presence of 10 μM HMR 1098, a sarcolemmal adenosine triphosphate–sensitive potassium channel antagonist; 800 μM 5-hydroxy-decanoate, a mitochondrial adenosine triphosphate–sensitive potassium channel antagonist; and 100 nM 8-cyclopentyl-1,3-dipropylxanthine, an adenosine A_1 receptor antagonist. Recovery of force at the end of the 60-min reoxygenation period was compared between groups (mean ± SD).**

Results: **Hypoxic preconditioning (90 ± 4% of baseline) and sevoflurane 1% (82 ± 3% of baseline), 2% (92 ± 5% of baseline), and 3% (85 ± 7% of baseline) enhanced the recovery of force after 60 min of reoxygenation compared with the control groups (52 ± 9% of baseline). This effect was abolished in the presence of 5-hydroxy-decanoate (55 ± 14% of baseline) and 8-cyclopentyl-1,3-dipropylxanthine (58 ± 16% of baseline) but was attenuated in the presence of HMR 1098 (73 ± 10% of baseline).**

***Conclusions: In vitro*, sevoflurane preconditions human myocardium against hypoxia through activation of adenosine triphosphate–sensitive potassium channels and stimulation of adenosine A_1 receptors.**

THE beneficial effects of volatile anesthetic–induced preconditioning have been shown on infarct volume,[1–4] postischemic contractile function,[5–7] and metabolic function.[7] Although the mechanisms involved in anesthetic preconditioning remain incompletely understood, protein kinase C, adenosine triphosphate–sensitive potassium (K_{ATP}) channels, and adenosine A_1 receptors seem to play a key role.[1,2,5,6] However, recent studies suggest that volatile anesthetic–induced myocardial preconditioning may be dependent on species and experimental models.[5,8] Thus, halothane has been shown to precondition rabbit myocardium[1] but failed to precondition isolated human myocardium.[5] Similarly, isoflurane failed to precondition isolated rat heart[8] but preconditioned rabbit,[1] dog,[2,3] and human myocardium.[5,9] Finally, sevoflurane failed to precondition rabbit myocardium *in vivo*[10] but has been shown to precondition dog[4] and guinea pig myocardium.[7] Furthermore, it was recently suggested that the myocardial effects of volatile anesthetics[11] and the mechanisms involved in ischemic preconditioning may be different among species.[12]

Sevoflurane has been shown to precondition myocardium *in vivo* in dogs[4] and in isolated guinea pig heart,[7] but its effects on ischemic–reperfused human myocardium remain unknown. Although two recent clinical studies examined this issue in patients undergoing cardiopulmonary bypass surgery, they have led to contradictory results on postoperative cardiac troponin I peak serum concentration.[13,14] Consequently, we performed an experimental study to examine the effect of sevoflurane on isolated human myocardium challenged with simulated ischemia–reperfusion. In addition, because the mechanisms involved in sevoflurane-induced preconditioning have received less attention than those of other volatile anesthetics, the role of sarcolemmal K_{ATP} ($sarcK_{ATP}$) and mitochondrial K_{ATP} ($mitK_{ATP}$) channels and adenosine A_1 receptors were assessed by use of selective antagonists.

This article is featured in "This Month in Anesthesiology." Please see this issue of ANESTHESIOLOGY, page 5A.

Materials and Methods

Experimental Conditions

After the approval of the local medical ethics committee and written informed consent had been obtained, human right atrial trabeculae were obtained from patients scheduled for routine coronary artery bypass surgery or aortic valve replacement. All patients received midazolam or propofol, sufentanil, etomidate, pancuronium, and isoflurane. Patients with atrial dysrhythmia and those taking oral hypoglycemic medications were

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Table 1. Patient Demographic Data, Preoperative Drug Treatments, and Preoperative Left Ventricular Ejection Fraction

Groups and Heart Disease	Age, yr	Preoperative Drug Treatments	Ejection Fraction, %
Control AVR (n = 4); CABG (n = 7)	64 ± 10	βAB (5), ACE (2), ATZ (1), AMI (2), FUR (2), MOL (1), TNT (4)	62 ± 7
PC AVR (n = 3); CABG (n = 3)	67 ± 6	βAB (3), ACE (5), BZD (3), CA (3), K^+A (2), MOL (2), TNT (3)	70 ± 9
Sevoflurane 1% AVR (n = 3); CABG (n = 3)	63 ± 16	βAB (2), ACE (2), AMI (2), BZD (3), FUR (2), STA (4), TNT (3)	71 ± 8
Sevoflurane 2% AVR (n = 3); CABG (n = 5)	67 ± 6	βAB (4), ACE (4), BZD (2), MOL (3), STA (6)	67 ± 11
Sevoflurane 3% AVR (n = 4); CABG (n = 3)	70 ± 9	βAB (6), β_2A (2), ACE (5), BZD (2), STA (3), TNT (1)	71 ± 10
Sevoflurane 2% + 5-HD AVR (n = 3); CABG (n = 3)	61 ± 15	βAB (0), β_2A (1), ACE (4), AMI (1), BZD (2), FUR (2), MOL (2), STA (2)	75 ± 7
Sevoflurane 2% + HMR AVR (n = 4); CABG (n = 3)	71 ± 8	βAB (6), β_2A (2), ACE (3), AMI (2), ATZ (2), MOL (3), STA (4), TNT (4)	66 ± 5
Sevoflurane 2% + DPX AVR (n = 6); CABG (n = 0)	69 ± 5	βAB (0), β_2A (2), ACE (3), AMI (1), ATZ (2), FUR (2), MOL (3), STA (3)	61 ± 8
Control 5-HD AVR (n = 2); CABG (n = 2)	77 ± 5	βAB (0), ACE (1), AMI (1), ATZ (2), BZD (1), FUR (2), MOL (2), STA (1)	73 ± 12
Control HMR AVR (n = 2); CABG (n = 2)	67 ± 9	βAB (0), β_2A (2), ACE (3), BZD (2), FUR (2)	73 ± 3
Control DPX AVR (n = 2); CABG (n = 2)	66 ± 14	βAB (1), ACE (3), BZD (1), FUR (1), STA (3), TNT (2)	70 ± 7

Age and ejection fraction are expressed as mean ± SD. The numbers in brackets after heart disease and drug abbreviations indicate the number of patients. Among the 69 trabeculae, 20 were obtained from 10 patients. When two trabeculae were obtained from one patient, they were never included in the same experimental group.

ACE = angiotensin-converting enzyme inhibitors; AMI = amiodarone; ATZ = acetazolamide; AVR = aortic valve replacement; β_2A = β_2-agonists; βAB = β-adrenergic blocking drugs; BZD = benzodiazepine; CA = calcium channel antagonist; CABG = coronary artery bypass graft; DPX = 8-cyclopentyl-1,3-dipropylxanthine; EF = left ventricular ejection fraction; FUR = furosemide; K^+A = potassium channel agonist; 5-HD = 5-hydroxydecanoate; HMR = HMR 1098; MOL = molsidomine; PC = hypoxic preconditioning; STA = statin.

excluded from the study. Patients' demographic data, preoperative drug treatment, and preoperative left ventricular ejection fraction are reported in table 1. The right atrial appendage was obtained during cannulation for cardiopulmonary bypass as described previously.[6]

Trabeculae were suspended vertically between an isometric force transducer (UC3, Gould, Cleveland, OH) and a stationary stainless steel clip in a 200-ml jacketed reservoir filled with Tyrode's modified solution, prepared daily, containing (mM) 120 NaCl, 3.5 KCl, 1.1 $MgCl_2$, 1.8 NaH_2PO_4, 25.7 $NaHCO_3$, 2.0 $CaCl_2$, and 5.5 glucose. The jacketed reservoir was maintained at 34°C with a thermostatic water circulator (Polystat Micropros, Bioblock, Illkirch, France). The bathing solution was bubbled with carbogen (95% O_2–5% CO_2), resulting in a pH of 7.40 and a partial pressure of oxygen of 600 mmHg. Isolated muscles were field-stimulated at 1 Hz by two platinum electrodes with rectangular wave pulses of 5-ms duration 20% above threshold (CMS 95107, Bionic Instrument, Paris, France).

Trabeculae were equilibrated for 60 to 90 min to allow stabilization of their optimal mechanical performance at the apex of the length-active isometric tension curve and randomly assigned to one experimental group. When several atrial trabeculae were dissected from one appendage, they were included in different experimental groups. The force developed was measured continuously, digitized at a sampling frequency of 400 Hz, and stored on a Writable Compact Disc for analysis (MacLab, AD Instrument, Sydney, Australia).

At the end of each experiment, the length and weight of the muscle were measured. The muscle cross-sectional area was calculated from its weight and length assuming a cylindrical shape and a density of 1. To avoid core hypoxia, trabeculae included in the study should have a cross-sectional area less than 1.0 mm^2, a force of contraction normalized per cross-sectional area (FoC) greater than 5.0 mN/mm^2, and a ratio of resting force to total force less than 0.45. We have previously shown that mechanical parameters of isolated human trabeculae remain stable for at least 2 h.[6]

Experimental Protocol

In all experimental groups, ischemia–reperfusion was simulated by replacing 95% O_2–5% CO_2 with 95% N_2–5% CO_2 in the buffer for 30 min, followed by a 60-min oxygenated recovery period. In the control group (n = 11), muscles were exposed to a 30-min hypoxic period, followed by a 60-min oxygenated recovery period. Hypoxic preconditioning (n = 6) was induced by a 4-min hypoxic period, followed by a 7-min oxygenated period before the simulated ischemia–reperfusion challenge.

In the sevoflurane treatment groups, sevoflurane was delivered to the organ bath by bubbling with 95% O_2–5% CO_2 passing through a specific calibrated vaporizer. To

Table 2. Control Values of Main Mechanical Parameters of Human Right Atrial Trabeculae

Experimental Groups	L_{max}, mm	CSA, mm^2	FoC, $mN \cdot mm^{-2}$	RF/TF
Control (n = 11)	7.6 ± 1.7	0.74 ± 0.29	16 ± 10	0.38 ± 0.17
PC (n = 6)	7.0 ± 1.4	0.75 ± 0.21	16 ± 7	0.32 ± 0.12
Sevoflurane 1% (n = 6)	8.3 ± 2.6	0.88 ± 0.12	15 ± 10	0.39 ± 0.10
Sevoflurane 2% (n = 8)	7.9 ± 1.1	0.59 ± 0.22	15 ± 5	0.37 ± 0.14
Sevoflurane 3% (n = 7)	7.2 ± 1.2	0.84 ± 0.21	16 ± 9	0.33 ± 0.18
Sevoflurane 2% + HMR 1098 (n = 7)	8.2 ± 1.5	0.66 ± 0.19	18 ± 5	0.26 ± 0.05
Sevoflurane 2% + 5-HD (n = 6)	7.2 ± 1.7	0.67 ± 0.23	13 ± 6	0.31 ± 0.12
Sevoflurane 2% + DPX (n = 6)	8.9 ± 2.2	0.73 ± 0.23	14 ± 4	0.31 ± 0.08
Control 5-HD (n = 4)	7.3 ± 1.3	0.71 ± 0.23	23 ± 18	0.34 ± 0.07
Control HMR 1098 (n = 4)	6.6 ± 1.1	0.52 ± 0.18	15 ± 5	0.32 ± 0.10
Control DPX (n = 4)	6.9 ± 1.0	0.50 ± 0.18	18 ± 12	0.33 ± 0.15

Data are mean ± SD.

CSA = cross-sectional area; DPX = 8-cyclopentyl-1,3-dipropylxanthine; FoC = acting isometric force normalized per cross-sectional area; 5-HD = 5-hydroxydecanoate; L_{max} = maximal length at the apex of the length-active force curve; PC = hypoxic preconditioning; RF/TF = ratio of resting force on total force.

minimize evaporation of anesthetic vapors, the jacketed reservoir was nearly hermetically sealed with thin paraffin. Anesthetic concentrations in the gas phase were measured continuously with an infrared calibrated analyzer (Capnomac, Datex, Helsinki, Finland). After a 15-min exposure to 1% (n = 6), 2% (n = 8), and 3% (n = 7) sevoflurane, a 7-min washout period was performed, and muscles underwent ischemia–reperfusion challenge. In additional groups, we studied the mechanism of sevoflurane-induced preconditioning. Thus, 15 min of exposure to 2% sevoflurane was performed after 10 min of pretreatment with 10 μM HMR 1098 (HMR; n = 7), a selective inhibitor of sarcK_{ATP} channels; 800 μM 5-hydroxydecanoate (5-HD; n = 6), a selective inhibitor of mitK_{ATP} channels; or 0.1 μM 8-cyclopentyl-1,3-dipropylxanthine (DPX; n = 6), a selective antagonist of adenosine A_1 receptors.

Additional control groups were established to study the effects of 10 min of administration of 800 μM 5-HD (control + 5-HD; n = 4), 10 μM HMR 1098 (control + HMR; n = 4), and 0.1 μM DPX (control + DPX; n = 4) before the ischemia–reperfusion protocol.

5-HD and DPX were purchased from ICN Pharmaceuticals (Orsay, France), and sevoflurane was purchased from Abbott (Rungis, France). HMR 1098 was a gift from Aventis Pharma (Frankfurt am Main, Germany).

Statistical Analysis

Data are expressed as mean ± SD. Baseline values of the main mechanical parameters and values of FoC at 60 min of reperfusion (end point of the study) were compared by a univariate ANOVA with Newman–Keuls *post hoc* analysis. Within-group data were analyzed over time using univariate ANOVA for repeated measures and Newman–Keuls *post hoc* analysis. All *P* values were two-tailed, and a value of $P < 0.05$ was required to reject the null hypothesis. Statistical analysis was performed on a computer using Statview 5 software (Deltasoft, Meylan, France).

Results

Sixty-nine human right atrial trabeculae were obtained from 59 patients. Ten atrial appendages enabled the dissection of two trabeculae. When two trabeculae were obtained from one appendage, they were included in different experimental groups. There were no differences in control values for length-active isometric tension curve, cross-sectional area, ratio of resting to total force, and FoC between all groups (table 2). Eight experiments (two in the sevoflurane 1% group, one in the sevoflurane 3% group, two in the sevoflurane 2% + 5-HD group, one in the sevoflurane 2% + HMR 1098 group, and two in the sevoflurane 2% + DPX group) were discarded because atrial trabeculae did not meet inclusion criteria.

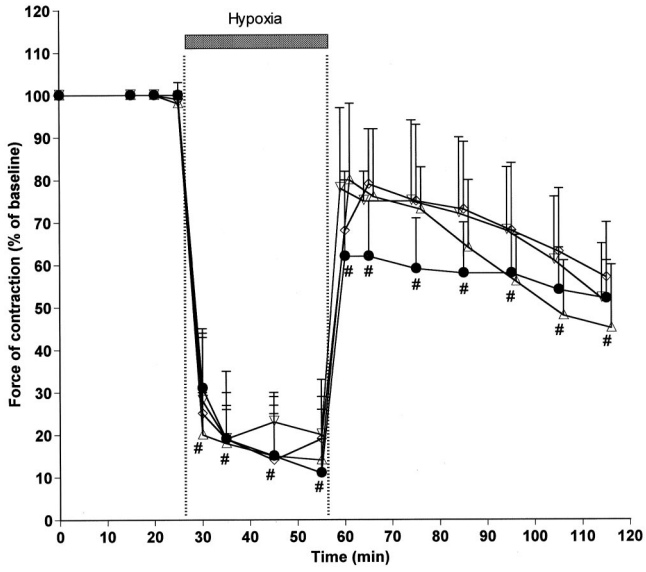

Fig. 1. Time course effects of force of contraction of isolated human right atrial trabeculae during a 30-min hypoxic challenge followed by 60 min of reoxygenation in control (●), control + 5-HD (◇), control + HMR 1098 (△), and control + DPX (▽) groups. 5-HD = 5 hydroxydecanoate; DPX = 8-cyclopentyl-1,3-dipropylxanthine. Data are mean ± SD. #$P < 0.05$ *vs.* baseline value for all groups.

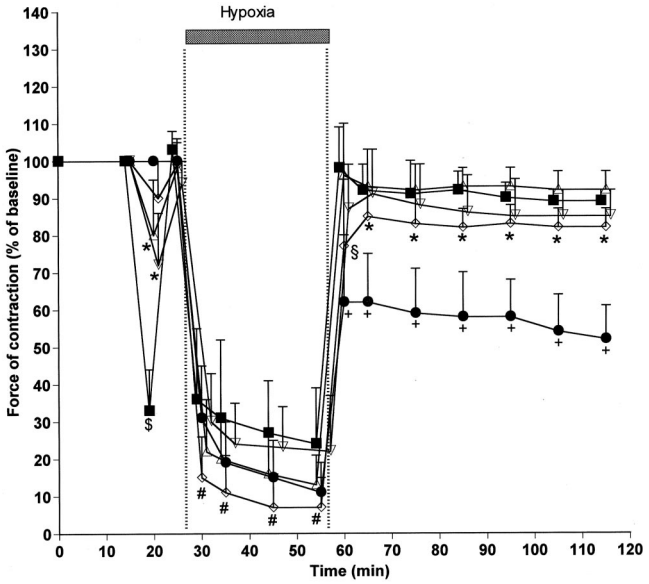

Fig. 2. Time course effects of force of contraction of isolated human right atrial trabeculae during a 30-min hypoxic challenge followed by 60 min of reoxygenation (control; ●). Hypoxic preconditioning (PC; ■) was induced by a 4-min hypoxic period followed by a 7-min reoxygenation period. A 15-min exposure to sevoflurane 1, 2, and 3% was followed by a 7-min washout period before the 30-min hypoxic challenge. Data are mean ± SD. *$P < 0.05$ *vs.* baseline value; #$P < 0.05$ *vs.* baseline value for all groups; +$P < 0.05$ *vs.* baseline, PC, sevoflurane 1%, sevoflurane 2%, and sevoflurane 3% groups; §$P < 0.05$ *vs.* baseline, PC, and sevoflurane 3% groups; $$P < 0.05$ *vs.* control, sevoflurane 1% (◇), sevoflurane 2% (△), and sevoflurane 3% (▽) groups.

Effects of Hypoxia and Reperfusion on Contractile Force of Human Right Atrial Trabeculae

The time course of FoC for the control groups is shown in figure 1. Hypoxia induced a marked decrease in FoC. After 30 min of hypoxia, FoC was 11 ± 8% of baseline. As shown in figure 1, reoxygenation induced a partial recovery of FoC in the control groups. At the end of the 60-min reoxygenation period, the recovery of FoC was 52 ± 9% of baseline. As shown in figure 1, 5-HD (57 ± 13% *vs.* 52 ± 9% of baseline; P = not significant [NS]), HMR 1098 (45 ± 17% *vs.* 52 ± 9% of baseline; P = NS), and DPX (52 ± 15% *vs.* 52 ± 9% of baseline; P = NS) did not modify the recovery of FoC at the end of the 60-min reoxygenation period.

Effect of Hypoxic Preconditioning on Contractile Force of Human Right Atrial Trabeculae

The time course of FoC for the hypoxic preconditioning groups is shown in figure 2. The 4-min hypoxic challenge induced a marked decrease in FoC (33 ± 20% of baseline; $P < 0.05$). At the end of the 7-min reoxygenation period, FoC recovered to 103 ± 5% of baseline (fig. 2).

In hypoxic preconditioning muscles, FoC after 30 min of hypoxia was 24 ± 15% of baseline. As shown in figure 2, at the end of the 60-min reoxygenation period, the recovery of FoC in the hypoxic preconditioning group was significantly greater than that measured in the control group (90 ± 4% *vs.* 52 ± 9% of baseline; $P < 0.05$).

Effects of Sevoflurane on Hypoxia–Reperfusion

The time course of FoC for the sevoflurane groups is shown in figure 2. Fifteen minutes of exposure to 1% (82 ± 3% *vs.* 52 ± 9% of baseline; $P < 0.05$), 2% (92 ± 5% *vs.* 52 ± 9% of baseline; $P < 0.05$), and 3% (85 ± 7% *vs.* 52 ± 9% of baseline; $P < 0.05$) sevoflurane before 30 min of hypoxia resulted in a significant increase in the recovery of FoC at the end of the 60-min reoxygenation period compared with the control group (figs. 2 and 3). The recovery of FoC measured in the sevoflurane groups was not different from that measured in the hypoxic preconditioning group (fig. 3).

The sevoflurane-enhanced recovery of force at the end of the reoxygenation period was abolished by pretreatment with 5-HD (55 ± 14% *vs.* 92 ± 5% of baseline; $P < 0.05$) and DPX (58 ± 16% *vs.* 92 ± 5% of baseline; $P < 0.05$) and was no more different from that measured in the control groups (figs. 4 and 5). Pretreatment with HMR 1098 significantly decreased the sevoflurane-enhanced recovery of FoC at the end of the reoxygenation period (73 ± 10% *vs.* 92 ± 5% of baseline; $P < 0.05$), but the recovery of FoC was significantly different from that measured in the control groups (73 ± 10% *vs.* 52 ± 9% of baseline; $P < 0.05$).

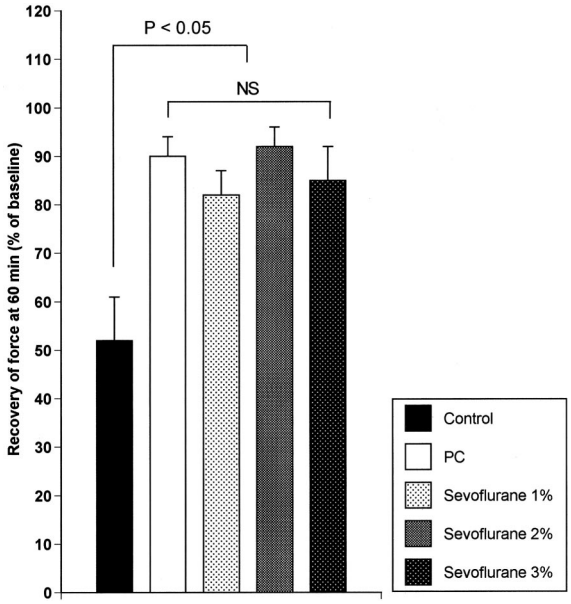

Fig. 3. Recovery of force of contraction of isolated human right atrial trabeculae at the end of the 60-min reoxygenation period after the 30-min hypoxic challenge. These data correspond to the final data point in Fig. 2. PC = hypoxic preconditioning; NS = not significant. Data are mean ± SD.

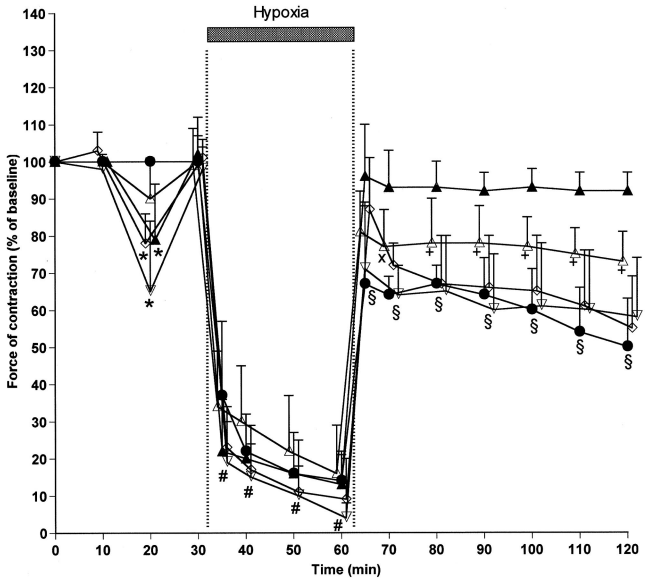

Fig. 4. Time course effects of force of contraction of isolated human right atrial trabeculae during a 30-min hypoxic challenge followed by 60 min of reoxygenation (control; ●). A 15-min exposure to sevoflurane 2% alone (▲) or in the presence of 5-HD (◇), HMR 1098 (△), and DPX (▽) was followed by a 7-min washout period before the 30-min hypoxic challenge. 5-HD = 5 hydroxydecanoate; DPX = 8-cyclopentyl-1,3-dipropylxanthine. Data are mean ± SD. *$P < 0.05$ *vs.* baseline value; #$P < 0.05$ *vs.* baseline value for all groups; §$P < 0.05$ *vs.* baseline value and PC for control, sevoflurane + 5-HD, sevoflurane + HMR 1098, and sevoflurane + DPX groups; +$P < 0.05$ *vs.* baseline, control, PC, sevoflurane + 5-HD, and sevoflurane + DPX; × $P < 0.05$ *vs.* baseline, control, PC, and sevoflurane + DPX.

Direct Inotropic Effects of Sevoflurane, 5-HD, HMR 11098, and DPX on Human Right Atrial Trabeculae

Sevoflurane induced a dose-dependent negative inotropic effect that became significant in the presence of sevoflurane 2% (FoC, 80 ± 15% of baseline; $P < 0.05$) and 3% (FoC, 72 ± 13% of baseline; $P < 0.05$). The negative inotropic effect of sevoflurane 2% was not significantly modified by pretreatment with 5-HD (FoC, 78 ± 8% of baseline; P = NS) and HMR 1098 (FoC, 90 ± 10% of baseline; P = NS) but decreased significantly in the presence of DPX (FoC, 63 ± 19% of baseline).

Discussion

The main results of our study are as follows: (1) brief exposure to 1, 2, and 3% sevoflurane preconditions isolated human right atrial myocardium against 30 min of hypoxia; (2) the main mechanisms involved in sevoflurane-induced preconditioning are opening of $mitK_{ATP}$ channels and stimulation of adenosine A_1 receptors; and (3) $sarcK_{ATP}$ channels do not play a predominant role in this model.

To date, a substantial body of evidence indicates that volatile anesthetic–induced preconditioning decreases myocardial infarct volume[1–4] and stunning[5–7] after an ischemic insult. Furthermore, recent clinical investigations have suggested that volatile anesthetic–induced preconditioning may reduce postoperative serum concentration of cardiac troponin I[9,14] and may improve postischemic left ventricular performance.[15]

Isoflurane- and desflurane-induced myocardial preconditioning have been studied in numerous experimental models and species, including humans,[1–3,5,6,8] but sevoflurane has received less attention. Although sevoflurane has been shown to reduce myocardial infarct size in dogs[4] and stunning in isolated guinea pig heart,[7,16,17] the mechanisms involved in sevoflurane-induced preconditioning remain incompletely studied. The involvement of K_{ATP} channels has been suggested by use of the nonselective antagonist glibenclamide,[4,16] but the specific roles of $mitK_{ATP}$ and $sarcK_{ATP}$ channels and adenosine receptors remain unknown. Importantly, increasing evidence suggests that ischemic and anesthetic preconditioning may be dependent on species and experimental models.[5,6,8,10,12] Finally, Vivien *et al.* showed that the direct myocardial effects of sevoflurane were dependent on species.[11]

Our study shows that 15-min administration of sevoflurane at 1, 2, and 3% before a 30-min hypoxic period triggers myocardial preconditioning in isolated human myocardium (figs. 2 and 4). Our results are in agreement with previous experimental data showing that sevoflurane administered before ischemia improved postischemic contractile function in isolated guinea pig heart and

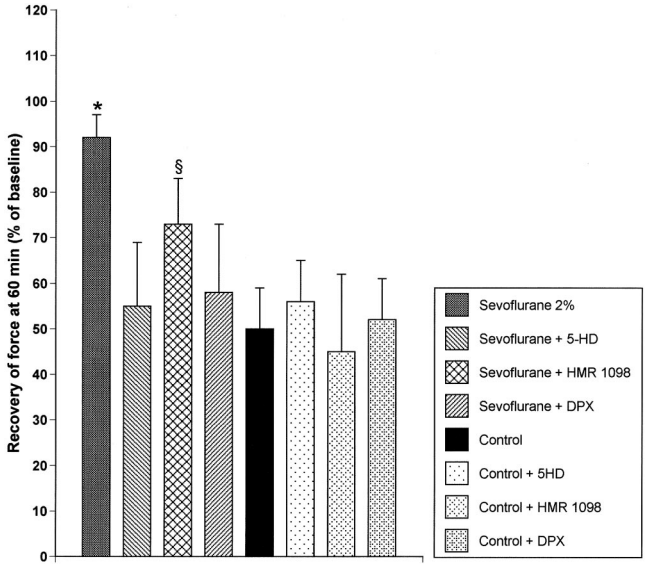

Fig. 5. Recovery of force of contraction of isolated human right atrial trabeculae at the end of the 60-min reoxygenation period after the 30-min hypoxic challenge in groups exposed to sevoflurane 2% alone or in the presence of 5-HD, HMR 1098, and DPX. These data correspond to the final data point in Fig. 3. 5-HD = 5 hydroxydecanoate; DPX = 8-cyclopentyl-1,3-dipropylxanthine. Data are mean ± SD. *$P < 0.05$ *vs.* control groups, sevoflurane + 5-HD, sevoflurane + HMR 1098, and sevoflurane + DPX groups; §$P < 0.05$ *vs.* control groups, sevoflurane 2%, sevoflurane + 5-HD, and sevoflurane + DPX groups.

extend those data to human myocardium.[7,16,17] Furthermore, as previously shown with desflurane,[6] sevoflurane-induced preconditioning is not concentration dependent (fig. 4). In addition, our results show that sevoflurane-induced myocardial preconditioning was completely abolished by pretreatment with 5-HD but was only attenuated by pretreatment with HMR 1098 (fig. 4), whereas in control groups, 5-HD and HMR 1098 alone had no effect on the recovery of force after simulated ischemia–reperfusion. It should be emphasized that HMR 1098 at 10 μM has been shown to be an effective blocker of sarcK_{ATP} channels.[18,19] In contrast, the specificity of 5-HD has recently been questioned, because metabolic effects independent of K_{ATP} channels (*i.e.*, inhibition of respiratory chain complexes) have been shown.[20] Our results suggest that a 5-HD–dependent mechanism (inhibition of mitK_{ATP} channel opening and inhibition of respiratory chain complexes) may be involved in sevoflurane-induced preconditioning. Previous studies have shown that K_{ATP} channels may be implicated in sevoflurane-induced preconditioning.[4,16] However, these studies did not determine specifically the role of sarcK_{ATP} and mitK_{ATP} channels. Although Hara *et al.*[21] showed that the cardioprotective effects of sevoflurane may implicate activation of mitK_{ATP} channels, these authors administered sevoflurane before and during ischemia, which is not strictly a preconditioning stimulus defined by a brief administration of anesthetic before ischemia. Our results are in accordance with recent experimental data showing that in rat ventricular myocytes, protection by volatile anesthetics was selectively inhibited by 5-HD.[22] Strong evidence supports the preeminent role of 5-HD–dependent targets in ischemia-induced[23] and volatile anesthetic–induced myocardial preconditioning.[2,6,18,22,24] Importantly, volatile anesthetic–induced preconditioning may result from the activation of protein kinase C *via* multiple signaling pathways, including G_i-coupled receptors,[22,25] nitric oxide signaling pathway,[16,22] release of reactive oxygen species,[17,26] and adrenoceptor stimulation.[6] Although recent evidence supports the greater role of mitK_{ATP} channels in volatile anesthetic–induced myocardial preconditioning, the contribution of sarcK_{ATP} channels cannot be totally ruled out. Our results show that HMR 1098 significantly attenuated but did not abolish the recovery of force at the end of the 60-min reoxygenation period (fig. 5). In contrast, we have previously reported that desflurane-induced preconditioning was not modified by pretreatment with HMR 1098.[6] However, because the small number of experiments may have led to an insufficient statistical power, further studies would be necessary to precisely determine the role of sarcK_{ATP} and mitK_{ATP} channels in volatile anesthetic–induced preconditioning. Furthermore, preconditioning results from a complex signal cascade involving multiple kinases acting through parallel pathways, which could mask or underestimate the precise role of K_{ATP} channels. To date, several data indicate that sarcK_{ATP} channels may contribute to the beneficial effects of ischemia- and volatile anesthetic–induced preconditioning. Recent experiments using transgenic mice with targeted deletion of Kir6.2, the pore-forming subunit of cardiac sarcK_{ATP} channels, have shown that intact sarcK_{ATP} function is necessary for ischemic preconditioning.[12] Fujimoto *et al.*[27] have shown that isoflurane facilitates the opening of the sarcK_{ATP} channel in the presence of activated protein kinase C. In addition, the cardioprotective effects of desflurane on infarct volume were found to involve both the sarcK_{ATP} and mitK_{ATP} channels.[28] Importantly, it has been suggested that the beneficial effects of ischemic preconditioning on infarct volume and stunning may be mediated *via* separated activation of the mitK_{ATP} and sarcK_{ATP} channels, respectively.[29,30]

Our study shows that sevoflurane-induced preconditioning is inhibited by pretreatment with DPX, a selective adenosine A_1 receptor antagonist (fig. 5). This suggests that sevoflurane may trigger the preconditioning state *via* activation of adenosine A_1 receptors. Although activation of adenosine A_1 receptors in anesthetic myocardial preconditioning has been shown previously for isoflurane[5] and desflurane,[6] the precise mechanism by which volatile anesthetics activate adenosine A_1 receptors remains unknown. This may be interpreted as increasing evidence suggesting that several G-protein–coupled receptors trigger the signaling cascade leading to ischemic preconditioning.

Our results must be interpreted with the knowledge of recent clinical studies dealing with the cardioprotective effects of sevoflurane.[13,14] Pouzet *et al.*[13] suggested that a short exposure to 2.5 minimal alveolar concentration of sevoflurane before aortic cross-clamping and cardioplegia did not modify protein kinase C, p38 mitogen-activated protein kinase, and postoperative troponin I concentration. Nevertheless, this case-matched study enrolled a small number of patients, and the results must be interpreted within the constraints of several methodologic limitations. Importantly, De Hert *et al.*[14] showed that sevoflurane anesthesia not only decreased postoperative cardiac troponin I peak serum concentration but also preserved left ventricular function after cardiopulmonary bypass compared with propofol anesthesia. Nevertheless, this investigation did not study sevoflurane-induced preconditioning alone but rather compared propofol- with sevoflurane-based anesthesia. Thus, further clinical studies are necessary to precisely examine the clinical significance of sevoflurane-induced preconditioning.

Although experiments performed on isolated human myocardium have the benefit of more relevant clinical extrapolations, our results must be interpreted within the constraints of several limitations. First, the effects of anesthetic drugs, diseases, or treatments received by the patients cannot be ruled out. However, the patients

included in this study are representative of the patients in whom sevoflurane-induced preconditioning may be useful. In clinical situations, the preoperative disease and/or treatments would be present. Second, the use of isoflurane and opioids during anesthesia of patients included in this study theoretically could have preconditioned the appendage. However, experimental studies were initiated at least 90 min after removal of the atrial appendage. Most importantly, comparisons have been made with control experiments. Nevertheless, a superimposed effect of opioids or isoflurane used during surgical procedures cannot be ruled out. Third, rather than the true ischemia obtained by coronary occlusion, we used 30 min of hypoxic superfusion to simulate ischemia. However, it has been shown that anoxia is as effective as ischemia in inducing myocardial preconditioning.[31] Furthermore, this model has been shown to be useful to study volatile anesthetic–induced preconditioning in isolated human myocardium.[5,6] Fourth, our experiments were performed at 34°C, which may have decreased the K_{ATP} channel sensitivity[32] and the effect of preconditioning.[33] However, moderate hypothermia may occur during surgical procedures.

In conclusion, sevoflurane induces myocardial preconditioning in isolated human myocardium primarily *via* opening of K_{ATP} channels and stimulation of adenosine A_1 receptors.

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