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Signaling Pathways Involved in Desflurane-induced Postconditioning in Human Atrial Myocardium In Vitro

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Background: **Isoflurane and sevoflurane have been shown to elicit myocardial postconditioning, but the effect of desflurane remain unknown. The authors studied the mechanisms involved in desflurane-induced myocardial postconditioning.**

Methods: **Contracting isolated human right atrial trabeculae (34°C, stimulation frequency 1 Hz) were exposed to 30-min hypoxia followed by 60-min reoxygenation. Desflurane at 3%, 6%, and 9% was administered during the first 5-min of reoxygenation. Postconditioning with 6% desflurane was studied in the presence of 1 μM calphostin C, a protein kinase C inhibitor; 800 mM 5-hydroxydecanoate, a mitochondrial adenosine triphosphate–sensitive potassium channels antagonist; 1 μM Akt inhibitor; 20 μM PD89058, an extracellular-regulated kinase 1/2 inhibitor; and 1 μM SB 202190, a p38 mitogen-activated protein kinase inhibitor. The force of contraction at the end of the 60-min reoxygenation period was compared (mean ± SD). The p38 mitogen-activated protein kinase phosphorylation was studied using Western blotting.**

Results: **Desflurane at 3% (77 ± 10% of baseline), 6% (90 ± 14% of baseline), and 9% (86 ± 11% of baseline) enhanced the recovery of force after 60 min of reoxygenation as compared with the control group (51 ± 9% of baseline; $P < 0.001$). Calphostin C (55 ± 3% of baseline), 5-hydroxydecanoate (53 ± 3% of baseline), Akt inhibitor (57 ± 8% of baseline), PD89058 (64 ± 6% of baseline), and SB 202190 (61 ± 3% of baseline) abolished desflurane-induced postconditioning. Western blot analysis showed that 6% desflurane increased p38 mitogen-activated protein kinase phosphorylation.**

***Conclusions: In vitro*, desflurane postconditioned human atrial myocardium through protein kinase C activation, opening of mitochondrial adenosine triphosphate–sensitive potassium channels, Akt and extracellular-regulated kinase 1/2 activation, and p38 mitogen-activated protein kinase phosphorylation.**

SIMILAR to the cardioprotection of volatile anesthetics given as preconditioning stimulus, these drugs can also protect the heart when given during reperfusion. This might result from anesthetic-induced postconditioning after the administration of the anesthetic at the very start of reperfusion. Therefore, isoflurane and sevoflurane administered during the first minute of reperfusion have been shown to reduce myocardial infarct size, *in vivo*, in rabbit and rats.[1–4] The protection afforded was similar to that observed after ischemic postconditioning.[1] Finally, a 15- to 30-min administration of desflurane at the time of reperfusion has been shown to protect myocardium in rabbit *in vivo*[5] and in isolated rat heart.[6]

Mechanisms involved in volatile anesthetic–induced postconditioning remain incompletely studied. The activation of phosphatidylinositol-3-kinase and the opening of mitochondrial adenosine triphosphate–sensitive potassium (mitoK_{ATP}) channels have been identified as important mediators of sevoflurane- and isoflurane-induced postconditioning.[1–3] The phosphatidylinositol-3-kinase pathway results in activation of downstream targets such as protein kinase C (PKC), and survival protein kinases including Akt and extracellular-regulated kinase 1/2 (ERK1/2). PKC has been shown to play a key role in the signaling pathway of ischemic postconditioning, but its role in anesthetic postconditioning has not been studied.[7,8] On the other hand, isoflurane-induced postconditioning has been shown to be mediated, at least in part, by Akt and ERK1/2 activation.[9,10] Nevertheless, the role of Akt and ERK1/2 in desflurane-induced postconditioning remain unknown. p38 mitogen-activated protein kinase (MAPK) activation has been shown to be involved in ischemic postconditioning.[11] In contrast, it has been suggested that p38 MAPK inhibition was involved in attenuated apoptosis after hypoxic postconditioning.[12] The role of p38 MAPK in anesthetic-induced postconditioning remains unknown.

The current study examined the effect of desflurane-induced postconditioning in isolated human atrial myocardium, and studied the role of PKC, mitoK_{ATP} channels, Akt, ERK1/2, and p38 MAPK in desflurane-induced postconditioning.

Materials and Methods

After the approval of local medical ethics committee (Comité de Protection des Personnes Nord Ouest III, Caen, France) and written informed consent, right atrial appendages were obtained during cannulation for cardiopulmonary bypass from patients scheduled for routine coronary artery bypass surgery and aortic valve replacement. All patients received total intravenous an-

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esthesia with propofol, sufentanil, and pancuronium. Patients with chronic atrial arrhythmia and with diabetes mellitus treated with insulin or oral hypoglycemic agents were excluded from the study.

Experimental Conditions

Right atrial trabeculae (one per appendage) were dissected and suspended vertically between an isometric force transducer (MLT0202; ADInstruments, Sydney, Australia) and a stationary stainless clip in a 200-ml jacketed reservoir filled with daily prepared Tyrode's modified solution containing 120 mM NaCl, 3.5 mM KCl, 1.1 mM $MgCl_2$, 1.8 mM NaH_2PO_4, 25.7 mM $NaHCO_3$, 2.0 mM $CaCl_2$, and 5.5 mM glucose. The jacketed reservoir was maintained at 34°C by a thermostatic water circulator (Polystat micropros; Bioblock, Illkirch, France). The bathing solution was insufflated 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 60–90 min to allow stabilization of their optimal mechanical performance at the apex of the length–active isometric tension curve (L_{max}). The force developed was measured continuously, digitized at a sampling frequency of 400 Hz, and stored on a writable compact disc for analysis (MacLab; ADInstruments).

At the end of experiment, 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/total force less than 0.45.

Experimental Protocol

At the end of the stabilization period, the trabeculae were randomly assigned (sealed envelopes) to one of the experimental groups. In all groups, hypoxia–reoxygenation was performed 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 = 10), trabeculae were exposed to the hypoxia reoxygenation protocol alone (fig. 1). In the desflurane treatment groups, desflurane was delivered to the organ bath by the gas flow passing through a specific calibrated vaporizer. Desflurane concentration in the carrier gas phase was measured with an infrared calibrated analyzer (Capnomac; Datex, Helsinki, Finland). Desflurane was administered at 3% (n = 6), 6% (n = 6), and 9% (n = 6) during the first 5 min of reoxygenation (fig. 1). These concentrations correspond to 0.5, 1.0, and 1.5 minimum alveolar concentration (MAC) desflurane in adult humans at 37°C, respectively.

Mechanisms involved in desflurane-induced postconditioning were studied in separate groups exposed to 6% desflurane in the presence of 1 μM calphostin C, a PKC inhibitor (n = 6); 800 μM 5-hydroxydecanoate (5-HD), a mitoK_{ATP} channel antagonist (n = 6); 1 μM Akt inhibitor IV (n = 6); 20 μM PD98059, an ERK1/2 inhibitor (n = 6); 1 μM SB 202190, a p38 MAPK inhibitor (n = 6); and 0.1% dimethyl sulfoxide (DMSO; n = 6). Pharmacologic agents and DMSO were administered 5 min before, throughout, and 10 min after desflurane exposure.

In additional groups, muscles were exposed to 1 μM calphostin C (n = 6), 800 μM 5-HD (n = 6), 1 μM Akt inhibitor IV (n = 6), 20 μM PD98059 (n = 6), 1 μM SB 202190 (n = 6), and 0.1% DMSO (n = 6) 5 min before and in the first 15 min of reoxygenation (fig. 1).

Calphostin C, SB 202190, Akt inhibitor IV, and PD98059 dissolved in DMSO; the volume of DMSO never exceeded 0.1% of the total bath volume. The concentrations of inhibitors used have been validated in previous experimental studies in human myocardium.[13,14]

Chemicals

5-Hydroxydecanoate, calphostin C, and Akt inhibitor IV were purchased from Calbiochem (VWR International, Fontenay sous Bois, France); SB 202190, PD98059, and DMSO were purchased from Sigma Aldrich (Saint Quentin Fallavier, France). Desflurane was purchased from GlaxoWellcome (Marly-le-Roi, France).

Western Blot Analysis

The right atrial appendage was pinned in a chamber (5 ml) containing Tyrode's modified solution, oxygenated with 95% O_2 and 5% CO_2, and maintained at 34° ± 0.5°C (Polystat micropros; Bioblock). The preparation was stimulated at a frequency of 1 Hz.

In all groups, after a 90-min equilibration period, hypoxia was performed by replacing 95% O_2–5% CO_2 with 95% N_2–5% CO_2 in the buffer for 30 min, followed by a 5-min oxygenated recovery period (control; n = 5) or by 5-min exposure to 6% desflurane (desflurane; n = 5) (fig. 1). Then, atrial samples were frozen in liquid nitrogen and stored at −80°C before protein extraction and Western blot analysis. Frozen tissue sample were extracted into extraction buffer containing 20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM σ-glycerophosphate, 1 mM sodium vanadate, 1 mM phenylmethylsulfonyl fluoride, and 10 μg/ml leupeptin-pepstatin A-aprotinin and homogenized with a polytron. Homogenates were centrifuged at 10,000*g* for 30 min, the supernatant was decanted, and protein concentration was determined using the BCA protein assay (Bradford colorimetric method; Bio-Rad, Marnes-la-Coquette, France). Extracted protein samples were reduced with 100 mM DTT

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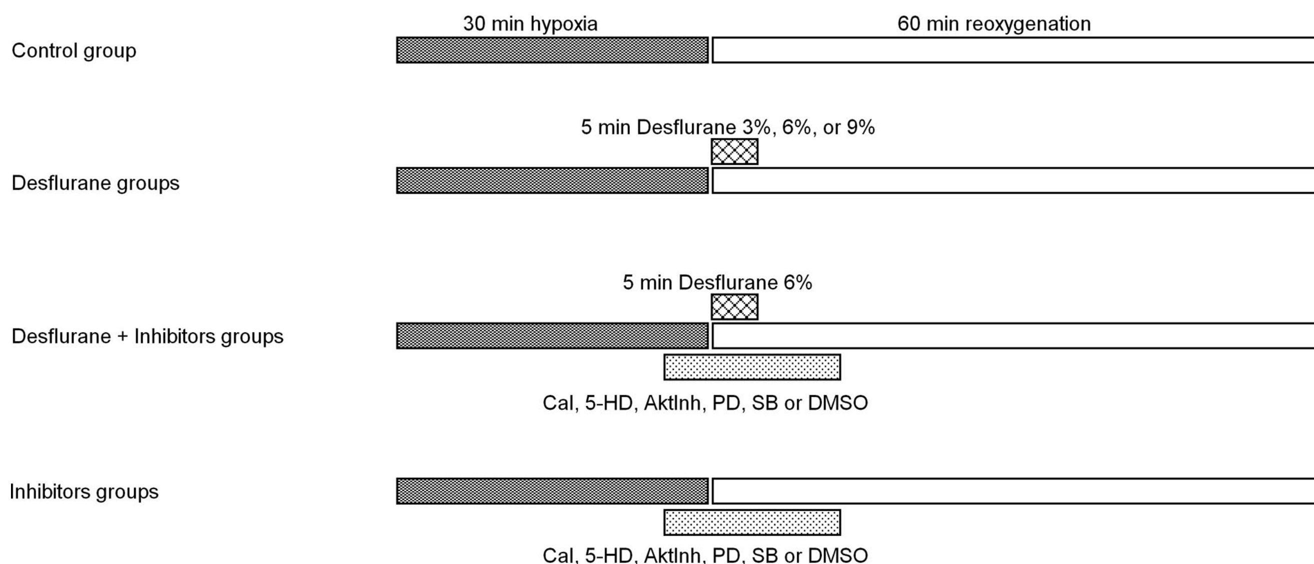
A Contracting muscle experiments



B Western Blot experiments

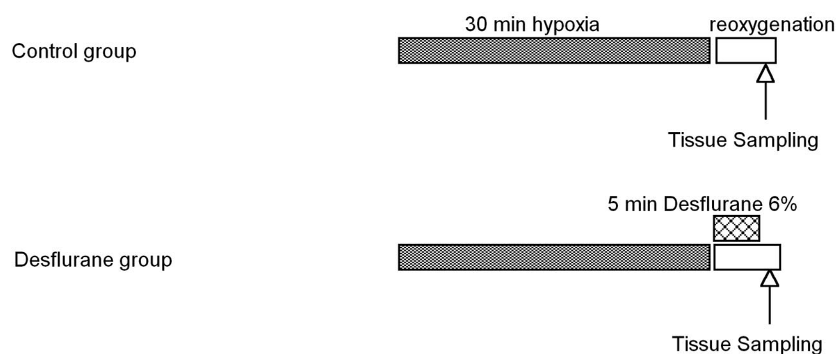


Fig. 1. Schematic diagram depicting the experimental protocol. (*A*) Contracting muscle experimental protocols. In the desflurane plus inhibitor groups and inhibitor groups, Akt inhibitor IV (AktInh) was administered at 1 μM, calphostin C (Cal) was administered at 1 μM, dimethyl sulfoxide (DMSO) was administered at 0.1%, 5-hydroxydecanoate (5-HD) was administered at 800 μM, PD98059 (PD) was administered at 20 μM, and SB 202190 (SB) was administered at 1 μM. (*B*) Western Blot experimental protocols.

and denatured at 95°C for 5 min. Denatured proteins (30 μg/lane) from human atrial tissues were separated on 10% SDS-PAGE and transferred on nitrocellulose. Membranes were blocked for 1 h in TRISS-buffered saline Tween buffer (0.02 M Tris-HCl, pH 7.5, 0.15 M NaCl, and 0.05% Tween 20) containing 10% nonfat dry milk at room temperature.

The membranes were incubated with a rabbit monoclonal antibody recognizing p38 MAPK and phospho-p38 MAPK (Thr 180/Tyr 182, 1/1,000 dilution; Cell Signaling Technology, Ozyme, Saint Quentin Yvelines, France), one night at 4°C. After washing in TRISS-buffered saline Tween, the blots were incubated with a secondary antibody (goat anti-rabbit, 1/1,000 dilution) coupled to peroxidase (Santa Cruz Technology, Tebu-Bio, Le Perray en Yvelines, France) for 1 h at room temperature. The blots were washed again in TRISS-buffered saline Tween, and the bands were detected using chemiluminescence reagent (Pierce Perbio Science, Brebieres, France) before exposure to photography film. The Western blots of each group were stripped and probed again with an antibody against β-tubulin (Santa Cruz Technology) to ensure equivalent loading. The developed films were scanned, and the band densities were quantified using NIH Image J software (Research Service Branch, National Institutes of Mental Health, Bethesda, MD).

Statistical Analysis

The endpoint of the study was the recovery of FoC at 60 min of reoxygenation (FoC_{60}, expressed as percent of baseline). Power analysis calculated a group size of n = 4 to detect a difference of 40% (control and inhibitors group: $FoC_{60} = 50 \pm 9\%$ of baseline; and desflurane 6% group: $FoC_{60} = 90 \pm 9\%$ of baseline) with a power of 0.8 at an α level of 0.05. The number of experiments per group was calculated based on one-way analysis of variance with six control and inhibitor groups and one 6%

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

Group and Heart Disease	Age, yr	Preoperative Drug Treatments	LVEF, %
Control AVR (n = 4); CABG (n = 6)	63 ± 13	ACE (3), βAB (3), BZD (1), CA (1), COR (1), FUR (3), K^+A (2), MOL (1), STA (3), TNT (2)	60 ± 10
3% Desflurane AVR (n = 3); CABG (n = 3)	69 ± 18	ACE (1), βAB (3), BZD (1), CA (0), COR (1), FUR (2), K^+A (2), MOL (0), STA (1), TNT (0)	67 ± 9
6% Desflurane AVR (n = 2); CABG (n = 4)	66 ± 7	βAB (5), ACE (2), BZD (1), CA (0), COR (1), FUR (0), K^+A (2), MOL (2), STA (4), TNT (0)	70 ± 11
9% Desflurane AVR (n = 0); CABG (n = 6)	66 ± 11	ACE (2), βAB (3), BZD (0), CA (0), COR (0), FUR (0), K^+A (3), MOL (0), STA (5), TNT (1)	61 ± 11
Des + AktInh AVR (n = 3); CABG (n = 3)	63 ± 15	ACE (2), βAB (4), BZD (2), CA (0), COR (0), FUR (0), K^+A (2), MOL (0), STA (3), TNT (2)	69 ± 16
Des + Cal AVR (n = 0); CABG (n = 6)	60 ± 9	ACE (4), βAB (6), BZD (0), CA (0), COR (0), FUR (1), K^+A (2), MOL (2), STA (2), TNT (0)	58 ± 9
Des + 5-HD AVR (n = 1); CABG (n = 5)	55 ± 7	ACE (6), βAB (3), BZD (0), CA (1), COR (0), FUR (0), K^+A (1), MOL (0), STA (4), TNT (1)	64 ± 9
Des + PD AVR (n = 4); CABG (n = 2)	71 ± 13	ACE (5), βAB (4), BZD (4), CA (0), COR (0), FUR (0), K^+A (0), MOL (0), STA (5), TNT (0)	53 ± 19
Des + SB AVR (n = 3); CABG (n = 3)	74 ± 6	ACE (2), βAB (4), BZD (2), CA (0), COR (0), FUR (1), K^+A (2), MOL (1), STA (0), TNT (0)	63 ± 5
Des + DMSO AVR (n = 0); CABG (n = 6)	70 ± 9	ACE (3), βAB (5), BZD (0), CA (2), COR (0), FUR (0), K^+A (2), MOL (0), STA (5), TNT (0)	57 ± 19
AktInh AVR (n = 1); CABG (n = 5)	56 ± 11	ACE (4), βAB (5), BZD (0), CA (0), COR (0), FUR (0), K^+A (0), MOL (0), STA (5), TNT (1)	60 ± 3
Cal AVR (n = 5); CABG (n = 1)	61 ± 9	ACE (0), βAB (2), BZD (1), CA (1), COR (0), FUR (2), K^+A (1), MOL (0), STA (4), TNT (1)	74 ± 4
5-HD AVR (n = 6); CABG (n = 0)	72 ± 6	ACE (2), βAB (4), BZD (0), CA (0), COR (0), FUR (2), K^+A (3), MOL (0), STA (4), TNT (0)	67 ± 17
PD AVR (n = 0); CABG (n = 6)	72 ± 10	ACE (0), βAB (5), BZD (2), CA (0), COR (0), FUR (0), K^+A (0), MOL (0), STA (2), TNT (0)	67 ± 6
SB AVR (n = 2); CABG (n = 4)	66 ± 7	ACE (2), βAB (4), BZD (4), CA (2) COR (0), FUR (0), K^+A (1), MOL (2), STA (0), TNT (0)	75 ± 5
DMSO AVR (n = 3); CABG (n = 3)	68 ± 9	ACE (4), βAB (2), BZD (3), CA (0), COR (1), FUR (0), K^+A (0), MOL (0), STA (3), TNT (1)	74 ± 7
Western blot: control AVR (n = 4); CABG (n = 1)	57 ± 18	ACE (2), βAB (5), BZD (1), CA (0), COR (1), FUR (1), K^+A (0), MOL (0), STA (2), TNT (1),	65 ± 11
Western blot: desflurane AVR (n = 3); CABG (n = 2)	62 ± 9	ACE (2), βAB (1), BZD (0), CA (1), COR (1) FUR (1), K^+A (1), MOL (1), STA (5), TNT (0)	57 ± 18

The number in parentheses after heart disease and drug abbreviation indicates the number of patients. Age and preoperative left ventricular ejection fraction (LVEF) are expressed as mean ± SD.

ACE = angiotensin-converting enzyme inhibitor; AktInh = Akt inhibitor IV; AVR = aortic valve replacement; βAB = β-adrenergic blocking drug; BZD = benzodiazepine; CA = calcium channel antagonist; CABG = coronary artery bypass graft; Cal = calphostin C; COR = amiodarone; DMSO = dimethyl sulfoxide; FUR = furosemide; 5-HD = 5-hydroxydecanoate; K^+A = potassium channel agonist; MOL = molsidomine; PD = PD98059; SB = SB 202190; STA = statin; TNT = nitroglycerin.

desflurane group. Data are expressed as mean ± SD. Baseline values of main mechanical parameters, age, preoperative left ventricular ejection fraction, and FoC_{60} were compared by univariate analysis of variance with group factor as the independent variable. If the *P* value was less than 0.05, a Bonferroni *post hoc* analysis was performed. Within-group data were analyzed over time using analysis of variance for repeated measures and

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

Experimental Group	L_{max}, mm	CSA, mm^2	AF, mN/mm^2	RF/TF
Control	7.0 ± 2.2	0.56 ± 0.15	22 ± 13	0.37 ± 0.15
3% Desflurane	7.1 ± 1.0	0.48 ± 0.17	24 ± 8	0.29 ± 0.04
6% Desflurane	7.2 ± 1.2	0.58 ± 0.24	26 ± 12	0.33 ± 0.1
9% Desflurane	6.8 ± 1.2	0.51 ± 0.27	29 ± 15	0.30 ± 0.09
Des + AktInh	5.7 ± 1.2	0.52 ± 0.17	30 ± 9	0.24 ± 0.06
Des + Cal	6.0 ± 1.6	0.48 ± 0.20	24 ± 15	0.33 ± 0.09
Des + 5-HD	5.8 ± 1.2	0.40 ± 0.10	26 ± 9	0.30 ± 0.05
Des + PD	5.8 ± 1.5	0.45 ± 0.13	19 ± 6	0.38 ± 0.07
Des + SB	5.2 ± 1.2	0.45 ± 0.16	19 ± 9	0.39 ± 0.15
Des + DMSO	5.8 ± 1.3	0.58 ± 0.18	27 ± 16	0.31 ± 0.10
AktInh	7.5 ± 2.1	0.46 ± 0.10	21 ± 4	0.39 ± 0.04
Cal	6.2 ± 2.1	0.5 ± 0.13	19 ± 8	0.36 ± 0.09
5-HD	5.7 ± 0.8	0.48 ± 0.11	20 ± 7	0.37 ± 0.04
PD	6.3 ± 1.3	0.60 ± 0.13	19 ± 4	0.28 ± 0.08
SB	7.0 ± 1.7	0.43 ± 0.13	28 ± 6	0.31 ± 0.07
DMSO	6.0 ± 1.1	0.49 ± 0.09	20 ± 6	0.33 ± 0.09

Data are expressed as mean ± SD.

AF = acting isometric force normalized per cross-sectional area; AktInh = Akt inhibitor IV; Cal = calphostin C; CSA = cross-sectional area; DMSO = dimethyl sulfoxide; 5-HD = 5-hydroxydecanoate; L_{max} = maximal length at the apex of the length–active force curve; PD = PD98059; RF/TF = ratio of resting force on total force; SB = SB 202190.

Bonferroni *post hoc* analysis with group factor and time (baseline, hypoxia 5, 10, 20, 30 min, and reoxygenation 5, 10, 20, 30, 40, 50, and 60 min) as independent variables. The preoperative drug treatment repartition was analyzed using a chi-square test.

In Western blotting, band densities for protein of interest were then normalized to that of the band for β-tubulin in the same sample, and then normalized to the mean of control tissues, defined as 1 arbitrary unit ± SD. Statistical comparisons were made by use of analysis of variance for repeated measures and Bonferroni *post hoc* analysis. All *P* values were two-tailed, and a *P* value of less than 0.05 was required to reject the null hypothesis. Statistical analysis was performed using Statview 5 software (Deltasoft, Meylan, France) and PASS 2005 (NCSS Statistical and Power Analysis Software; Kaysville, UT).

Results

There were no statistical differences between groups for patients' demographic data, preoperative treatments, and left ventricular ejection fraction (table 1). One hundred human right atrial trabeculae and 10 right atrial appendages were studied. There were no differences in baseline values for L_{max}, cross-sectional area, ratio of resting force to total force, or FoC between groups (table 2).

Effects of Desflurane on Hypoxia Reoxygenation

In the control group, reoxygenation resulted in a partial recovery of FoC (FoC_{60}: 51 ± 9% of baseline; fig. 2). As compared with the control group, 3% desflurane (77 ± 10% of baseline; $P < 0.0001$), 6% desflurane (90 ± 14% of baseline; $P < 0.0001$), and 9% desflurane (86 ± 11% of baseline; $P < 0.0001$) increased FoC_{60}. There was no difference in FoC_{60} measured in the 3%, 6%, and 9% desflurane groups (fig. 2).

Effects of 5-Hydroxydecanoate, Calphostin C, Akt Inhibitor, PD98059 and SB 202190

As shown in figure 3, the desflurane-induced enhanced recovery of FoC_{60} (6% desflurane: 90 ± 14% of baseline) was abolished in the presence of calphostin C (Des + Cal: 55 ± 3%; $P < 0.0001$), 5-HD (Des + 5-HD: 53 ± 3% of baseline; $P < 0.0001$), Akt inhibitor IV (Des + AktInh: 57 ± 8% of baseline; $P < 0.0001$), PD98059 (Des + PD: 64 ± 6% of baseline; $P < 0.0001$), and SB 202190 (Des + SB: 61 ± 3% of baseline; $P < 0.0001$). DMSO (Des + DMSO: 84 ± 4% of baseline; $P = 0.054$) did not modify FoC as compared with the desflurane group. As compared with the control group (control: 51 ± 9% of baseline), calphostin C (Cal: 54 + 5% of baseline; $P = 0.39$), 5-HD (55 ± 3% of baseline; $P = 0.29$), Akt inhibitor IV (AktInh: 54 ± 5% of baseline; $P = 0.50$), PD98059 (PD: 55 ± 4% of baseline; $P = 0.31$), SB 202190 (SB: 56 ± 5% of baseline; $P = 0.16$), and DMSO (55 ± 7% of baseline; $P = 0.27$) did not significantly modify FoC_{60} (fig. 3).

Phosphorylation of p38 Mitogen-activated Protein Kinase

Desflurane at 6% in the first 5 min of reperfusion significantly increased p38 MAPK phosphorylation (2.8 ± 0.5-fold increase in desflurane *vs.* control; $P < 0.0001$). As compared with the control group, desflurane did not modify protein expression of p38 MAPK (fig. 4).

Discussion

The current study showed that desflurane postconditioned isolated human atrial myocardium exposed to hypoxia–reoxygenation. Furthermore, desflurane-induced postconditioning was dependent on Akt, ERK1/2, PKC, and p38 MAPK activation and opening of $mitoK_{ATP}$ channels. Finally, desflurane-induced postconditioning increased p38 MAPK phosphorylation.

Ischemic postconditioning (*i.e.*, brief cycles of alternating ischemia and reperfusion at the onset of reperfusion) was first established in 2003 by Zhao et *al.*[15] It has been shown that ischemic postconditioning markedly decreased myocardial ischemia–reperfusion injury through recruiting prosurvival signaling pathways.[16] A decade ago, volatile anesthetics administered during the first 30 min of a 1-h reperfusion period were shown to decrease myocardial reperfusion injury.[5,6] We have previously shown that administration of desflurane during the first 15 min of a 3-h reperfusion reduced infarct size to the same extent that desflurane-induced preconditioning in

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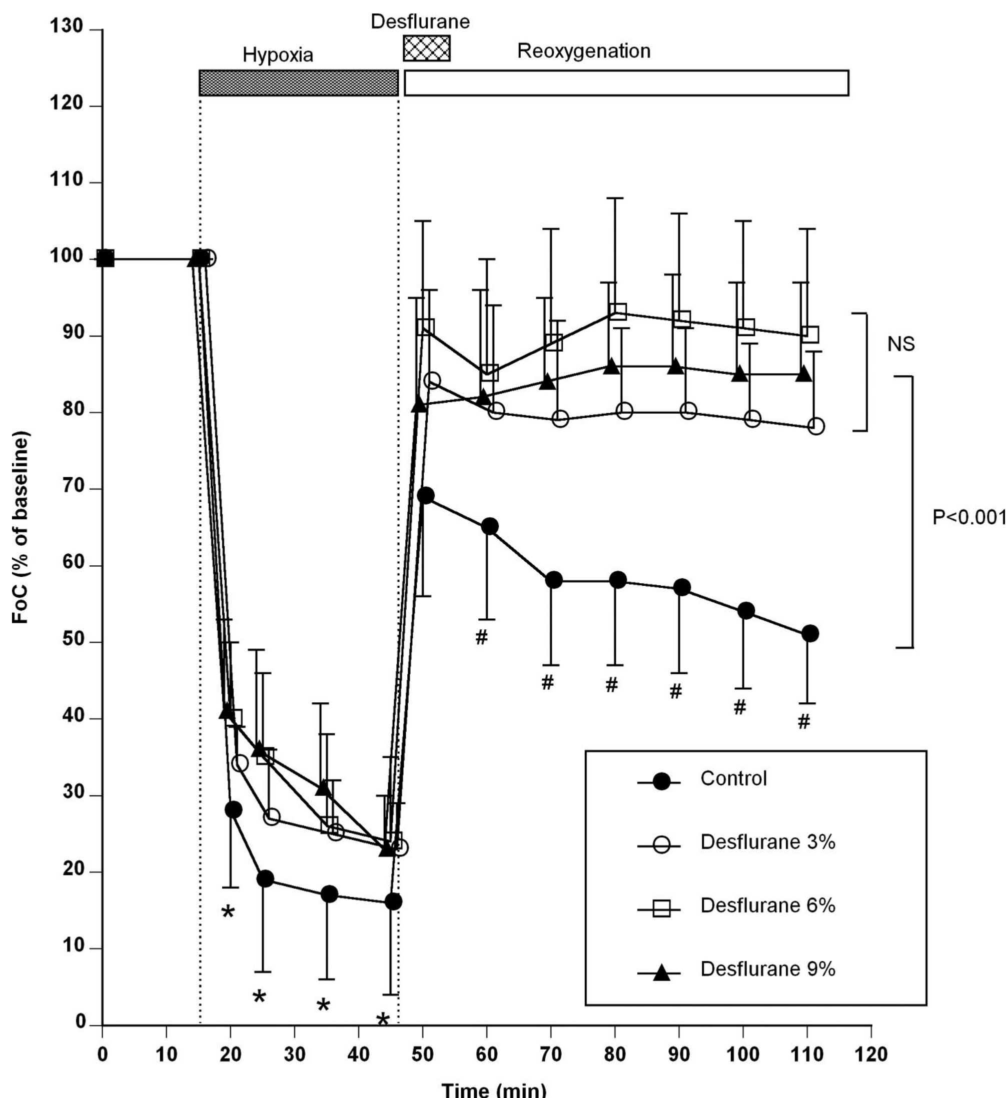

Fig. 2. Time course of force of contraction (FoC; expressed as percent of baseline) of isolated human right atrial trabeculae in the control (n = 10), 3% desflurane (n = 6), 6% desflurane (n = 6), and 9% desflurane (n = 9) groups. Data are mean ± SD. NS = not significant. *P* < 0.0001 denotes between-groups analysis of variance. * *P* < 0.0001 *versus* baseline for all groups. # *P* < 0.0001 *versus* baseline and 3%, 6%, and 9% desflurane groups.

rats *in vivo*.[17] However, this cannot be attributable strictly to pharmacologic postconditioning, which refers to brief interventions at the very start of the reperfusion. On the other hand, increasing evidence supports volatile anesthetic–induced postconditioning during myocardial ischemia–reperfusion injury. Therefore, a brief administration of isoflurane (1.0 MAC) at the beginning of reperfusion has been shown to reduce infarct size in rabbits.[1,2] Sevoflurane administered in early reperfusion was as effective as preconditioning in reducing infarct size in rat heart after ischemia–reperfusion.[3,4] The current study shows that 5-min administration of desflurane (3%, 6%, and 9%) at the onset of reoxygenation postconditioned human myocardium after 30-min hypoxia *in vitro*. The beneficial effect of desflurane-induced postconditioning was observed on the recovery of FoC during the reoxygenation period (fig. 2).

Although the recovery of FoC at the end of the reoxygenation measured in the presence of 3% desflurane was lower than that measured in the presence of 6% and 9% desflurane, the difference was not statistically significant. However, a statistical type II error cannot be ruled out. Previous studies have shown that 1.0 MAC isoflurane and sevoflurane was required to trigger myocardial postconditioning *in vivo*.[1,2,18] Furthermore, increasing concentrations of sevoflurane to 2.0 MAC did not further reduced infarct size.[18] As suggested for myocardial preconditioning, the threshold required to trigger the cardioprotective effect may vary among species, experimental models, and ischemia–reperfusion injury protocols. Further studies are required to determine whether there is a threshold and a concentration-dependent response in volatile anesthetic-induced postconditioning.

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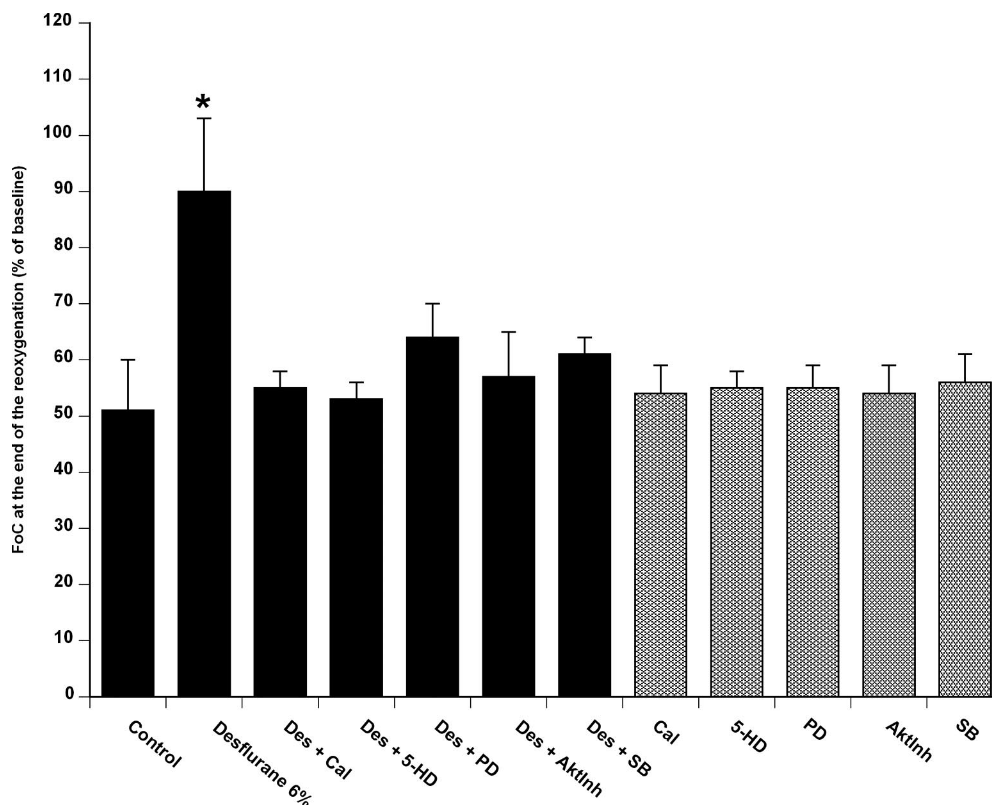

Fig. 3. Recovery of force of contraction (FoC) of isolated human right atrial trabeculae at the end of the 60-min reoxygenation period after 30-min hypoxia. *Gray bars* represent the groups with the inhibitor alone. Data are mean ± SD. * *P* < 0.0001 *versus* control, 6% desflurane (Des) + Akt inhibitor IV (AktInh), Des + calphostin C (Cal), Des + 5-hydroxydecanoate (5-HD), Des + PD98059 (PD), Des + SB 202190 (SB), AktInh, Cal, dimethyl sulfoxide, 5-HD, PD, and SB groups.

A growing body of evidence supports the concept that postconditioning triggers a cardioprotective cascade of molecular signaling events similar to that of ischemic preconditioning. Indeed, many of the triggers/mediators implicated in preconditioning seem to be involved in postconditioning.[16] Therefore, activation and translocation of PKC have been shown to be a key mediator of ischemic and pharmacologic preconditioning[19] and have been suggested to be also a mediator of ischemic postconditioning.[7,8] Zatta *et al.*[7] showed that ischemic postconditioning increased PKC-ε expression and translocation but limited translocation of PKC-δ to mitochondria in rat heart *in vivo*. In addition, activation of PKC just before reperfusion through phorbol 12-myristate 13-acetate decreased infarct size in rabbits *in vivo*.[8] The current investigation showed that desflurane-induced postconditioning was inhibited in the presence of calphostin C, suggesting that PKC was involved in desflurane-induced postconditioning in human myocardium. It has been shown that activation of PKC-ε led to phosphorylation and opening of mitoK_{ATP}[20] and formed a mitochondrial localized signaling complex with MAPKs.[21] Interestingly, the current study also showed that desflurane-induced postconditioning was inhibited by 5-HD and PD98059, suggesting that opening of mitoK_{ATP} channels and ERK1/2 activation were important mediators of desflurane-induced postconditioning. Opening of the mitoK_{ATP} channel has been shown to inhibit the opening of the mitochondrial permeability transition pore (mPTP), which is recognized as an important mediator of reperfusion injury.[16] The current results confirm and extend previous findings showing that mitoK_{ATP} channel opening was involved in isoflurane- and sevoflurane-induced postconditioning in rabbit and rat myocardium.[2,3] The role of mPTP has also been suggested in isoflurane-induced postconditioning because isoflurane-induced postconditioning was inhibited by the mPTP opener atractyloside.[2] Furthermore, it has been shown that 5-HD inhibited the desflurane-induced resistance of the mPTP to calcium-induced opening, suggesting a close link between mitoK_{ATP} and the mPTP.[22] Finally, isoflurane prevented opening of the mPTP, at least in part, through inhibition of glycogen synthase kinase 3β after protein kinase B/Akt phosphorylation.[23]

In addition to PKC-ε, mitoK_{ATP} channel openers have also been shown to activate ERK1/2.[24] In human myocardium, Sivaraman *et al.*[11] showed that ischemic postconditioning enhanced the activity of ERK1/2 and Akt at the time of reperfusion. The current study strongly suggests that ERK1/2 and Akt activation are important mediators of volatile anesthetic–induced postconditioning in human myocardium. To our knowledge, only one study has reported that ERK1/2 activation mediated isoflurane-induced postconditioning in rabbits.[9] Similarly, few studies suggested the involvement of the phos-

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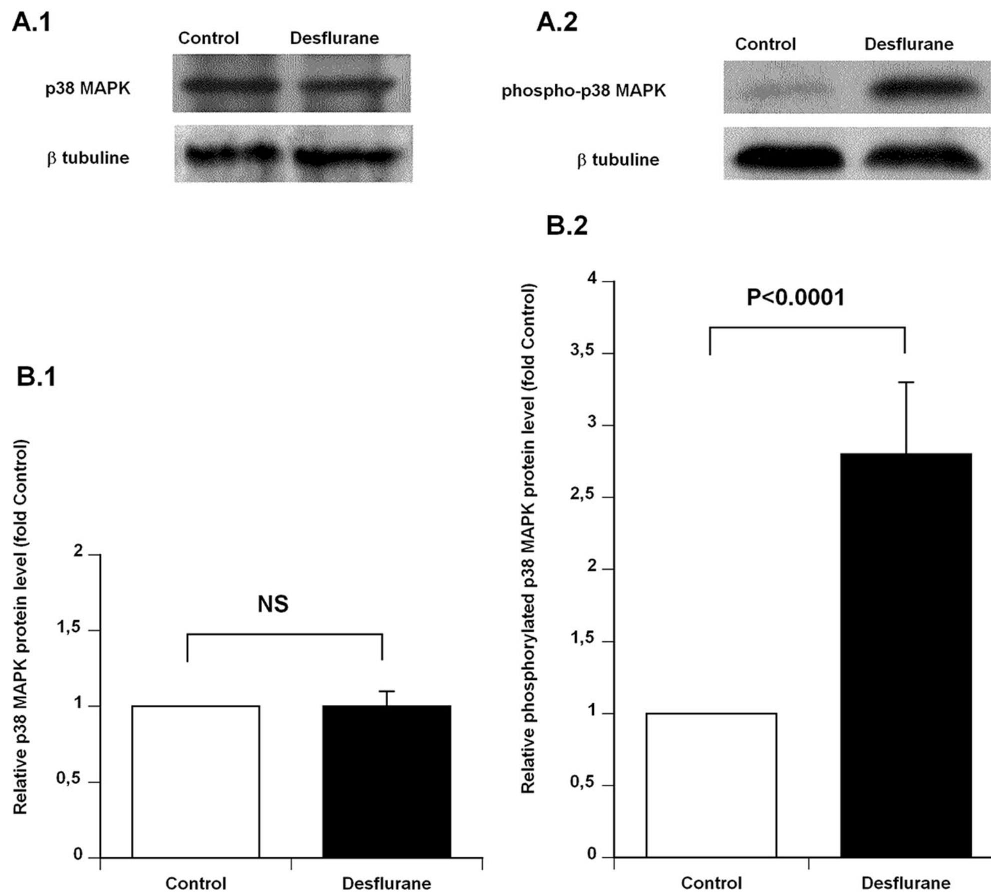

Fig. 4. Western blot analysis showing levels of p38 mitogen-activated protein kinase (MAPK) (*A*.1) and phosphorylated p38 MAPK (*A*.2) after 5 min of reoxygenation alone (control) or in the presence of 5 min of 6% desflurane (desflurane). Western blot analysis was performed using specific antibodies against p38 MAPK, phospho-p38 MAPK, and β-tubulin. Histograms depicting the expression level of p38 MAPK (*B*.1) and the variation of phosphorylation of p38 MAPK (*B*.2) in control and 6% desflurane groups. The relative p38 MAPK protein levels were calculated by averaging the results obtained from five independent experiments and were normalized to the β-tubulin control in each group. Densitometry analysis is expressed in arbitrary units. Data are mean ± SD. NS = not significant.

phatidylinositol-3-kinase/Akt cascade in isoflurane-induced postconditioning.[1,10]

The role of p38 MAPK in ischemic and pharmacologic postconditioning remains poorly studied. Only one study has examined the role of p38 MAPK in ischemic postconditioning. Sun *et al.*[12] showed that hypoxic postconditioning attenuated activation of p38 MAPK and JUN n-terminal kinase concomitantly with a reduction in apoptotic cardiomyocytes. The current study showed that the specific p38 MAPK inhibitor SB 202190 abolished the enhanced recovery of contractile force resulting from desflurane-induced postconditioning. Furthermore, we showed that phosphorylation of p38 MAPK was enhanced after desflurane administration at the onset of the reoxygenation. These results suggest that activation of p38 MAPK was involved in desflurane-induced postconditioning. However, the current study could not determine whether p38 MAPK activation was required during the triggering or mediating phase of desflurane-induced postconditioning. Furthermore, multiple isoforms of p38 MAPK exist. Of these, p38α and β are expressed in the myocardium and may have opposing functions.[25] Further studies are required to determine the precise role of p38 MAPK in desflurane-induced postconditioning both on myocardial infarct size and myocytes apoptosis.

The results of the current study must be interpreted with the knowledge of recent clinical studies suggesting the cardioprotective effects of ischemic postconditioning after coronary angioplasty for ongoing acute myocardial infarction[26] and adult cardiac valve surgery.[27] Importantly, ischemic postconditioning has recently been shown to provide short- and long-term myocardial beneficial effects in patients scheduled for primary angioplasty and stenting of acute myocardial infarction.[28]

Several limitations must be considered in the interpretation of the current results. First the effects of anesthetics drugs, diseases, or medical treatments received by the patients before obtaining the atrial appendages cannot be ruled out.[29] Furthermore, although age has been shown to attenuate volatile anesthetic-induced preconditioning,[30] there was no difference in patients' demographic data between groups. However, the patients included in this study are representative of those pa-

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tients in whom desflurane may be used during anesthesia. Importantly, our investigation included a control group that would be equally affected by any of these potentially modifying factors. Second, our experiments were performed during moderate hypothermia (34°C) to ensure stability of trabeculae over time. Nevertheless, no data exist on the postconditioning effect hypothermia. On the other hand, hypothermia may have decreased mitoK_{ATP} channel sensitivity.[31] However, during surgical procedures, moderate hypothermia may occur. Third, inhibition of PKC by calphostin C has no isoform specificity. Similarly, although SB 202190 and SB 203580 are widely used as p38 MAPK inhibitors, it has been suggested that they may also inhibit JUN n-terminal kinase at high concentration, whereas 1 μM was specific for the inhibition of p38 MAPK.[32] However, this occurred at a higher dose than that administered in the current study. Fourth, we studied isolated contracting human atrial trabeculae but not myocardial ventricular infarct size. Nevertheless, as described in preconditioning, the beneficial effects of postconditioning have also been described on reperfusion-induced arrhythmias[33] and myocardial conctractility.[11]

In conclusion, the current study showed that desflurane postconditioned isolated human myocardium as assessed by recovery of FoC. Furthermore, activation of PKC, Akt, ERK1/2, and p38 MAPK and opening of mitoK_{ATP} channels were involved in desflurane-induced postconditioning.

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