Anesthesiology 2005; 102:1190–6

Ketamine Preconditions Isolated Human Right Atrial Myocardium

Roles of Adenosine Triphosphate–sensitive Potassium Channels and Adrenoceptors

Jean-Luc Hanouz, M.D., Ph.D., Lan Zhu, M.D.,† Emmanuel Persehaye, M.D.,* Massimo Massetti, M.D.,‡ Gerard Babatasi, M.D., Ph.D.,‡ André Khayat, M.D.,§ Pierre Ducouret, Ph.D.,‖ Benoit Plaud, M.D., Ph.D.,# Jean-Louis Gérard, M.D., Ph.D.***

Background: **The authors examined the effect of ketamine and its *S*(+) isomer on isolated human myocardium submitted to hypoxia–reoxygenation *in vitro*.**

Methods: **The authors studied isometric contraction of human right atrial trabeculae suspended in an oxygenated Tyrode's modified solution at 34°C. Ten minutes before a 30-min hypoxic period followed by a 60-min reoxygenation, muscles were exposed for 15 min to racemic ketamine and its *S*(+) isomer at 10^{-6}, 10^{-5}, and 10^{-4} M alone or in the presence of 8.10^{-4} M 5-hydroxydecanoate, 10^{-5} M HMR 1098 (sarcolemmal adenosine triphosphate–sensitive potassium channel antagonist), 10^{-6} M phentolamine (α-adrenoceptor antagonist), and 10^{-6} M propranolol (β-adrenoceptor antagonist). Force of contraction at the end of the 60-min reoxygenation period was compared between groups (mean ± SD).**

Results: **Ketamine (10^{-6} M: 85 ± 4%; 10^{-5} M: 95 ± 10%; 10^{-4} M: 94 ± 14% of baseline) and *S*(+)-ketamine (10−6 M: 85 ± 4%; 10^{-5} M: 91 ± 16%; 10^{-4} M: 93 ± 14% of baseline) enhanced recovery of force of contraction at the end of the reoxygenation period as compared with the control group (47 ± 10% of baseline; $P < 0.001$). Ketamine-induced preconditioning at 10^{-4} M was inhibited by 5-hydroxydecanoate (60 ± 16%; $P < 0.001$), HMR 1098 (60 ± 14%; $P < 0.001$), phentolamine (56 ± 12%; $P < 0.001$), and propranolol (60 ± 7%; $P < 0.001$).**

Conclusions: ***In vitro*****, ketamine preconditions isolated human myocardium, at least in part, *via* activation of adenosine triphosphate–sensitive potassium channels and stimulation of α- and β-adrenergic receptors.**

KETAMINE, especially the *R*(−) isomer, has been reported to block ischemic preconditioning in isolated rat heart and rabbit myocardium.[1,2] In addition, in isolated rat myocytes, it has been shown that *R*(−)- but not *S*(+)-ketamine abolished diazoxide-induced flavoprotein oxidation, suggesting that it may inhibit mitochondrial adenosine triphosphate–sensitive potassium ($mitoK_{ATP}$) channel activity.[3] Importantly, these studies also suggested that racemic and *S*(+)-ketamine did not trigger myocardial preconditioning based on infarct size in rabbit myocardium[2] and rat myocyte survival.[3]

However, the direct myocardial effects of ketamine are dependent on species. Ketamine induced a negative inotropic effect in guinea pig hearts[4] and a positive inotropic effect independent of adrenoceptors stimulation in isolated rat myocardium[5] but related to an increase in catecholamine availability in ferret myocardium.[6] In human myocardium, we have shown that the direct inotropic effect of clinically relevant concentrations of racemic ketamine was related, at least in part, to the stimulation of β adrenoceptors.[7] Furthermore, some discrepancies between experimental studies may suggest that anesthetic preconditioning also depends on experimental conditions and species.[8] Increasing evidence suggests that there are multiple branching and converging signaling pathways involved in initiating ischemic and pharmacologic preconditioning.[9] The different pathways may predominate under different conditions but seem to converge on mitochondrial targets. Finally, although ischemic and anesthetic preconditioning may share several mechanisms, distinct cardioprotective phenotypes have been pointed out.[10] Several signaling molecules may trigger the biochemical cascade of myocardial preconditioning such as G protein–coupled receptors,[9] including α and β adrenoceptors.[11,12] Interestingly, we have previously shown that stimulation of α and β adrenoceptors was involved in desflurane-induced preconditioning of human myocardium.[13] This raises the question of whether ketamine might precondition human myocardium through adrenoceptor stimulation. Therefore, we examined the effect of ketamine on isolated human atrial myocardium submitted to hypoxia–reoxygenation.

Materials and Methods

Experimental Conditions

After the approval of the local medical ethics committee (Centre Hospitalier Universitaire Côte de Nacre, Caen, France), right atrial appendages were obtained during cannulation for cardiopulmonary bypass from

* Assistant Professor, # Professor of Anesthesiology, Department of Anesthesiology, † Research Fellow, ‖ Staff Scientist, Laboratory of Experimental Anesthesiology and Cellular Physiology, ‡ Assistant Professor, § Professor of Cardiothoracic Surgery, Department of Cardiac and Thoracic Surgery, ** Professor of Anesthesiology and Chairman, Director of the Laboratory of Experimental Anesthesiology and Cellular Physiology, Department of Anesthesiology, Centre Hospitalier Universitaire Caen.

Received from Laboratoire d' Anesthésiologie Expérimentale et de Physiologie Cellulaire, Unité Propre Enseignement Supérieur–Equipe d'Acceuil 3212, University of Caen, Caen, France, and Département d' Anesthésie-Réanimation, Centre Hospitalier Universitaire Côte de Nacre, Caen, France. Submitted for publication October 25, 2004. Accepted for publication March 3, 2005. Support was provided solely from institutional and/or departmental sources.

Address reprint requests to Dr. Hanouz: Département d' Anesthésie-Réanimation (niveau 6), Centre Hospitalier Universitaire de Caen, Avenue Côte de Nacre, 14033 Caen Cedex, France. Address electronic mail to: hanouz-jl@chu-caen.fr.

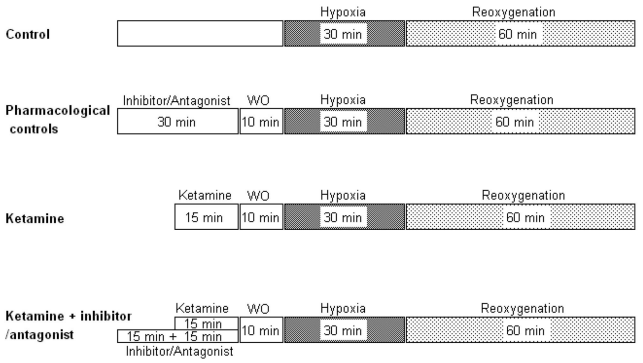

Fig. 1. Schematic representation of experimental protocols. WO = washout.

patients scheduled to undergo routine coronary artery bypass surgery or aortic valve replacement. In all patients, anesthesia was induced with a target-controlled infusion of propofol, sufentanil or remifentanil, and pancuronium. Patients with atrial arrhythmia and those who were taking oral hypoglycemic medications were excluded from the study.

Right atrial trabeculae (one to two 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 reservoir was maintained at 34°C using 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 in 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}). At the end of the stabilization period, trabeculae were randomly assigned to one of the experimental groups summarized in figure 1. The force developed was measured continuously, digitized online at a sampling frequency of 400 Hz (PowerLab 4SP; ADInstruments), and stored on a Writable Compact Disc for analysis (Chart version 5.0.1; ADInstruments).

At the end of each experiment, the length and the weight of the muscle were measured. The muscle cross-sectional area was calculated from its weight and length assuming a cylindric 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, an active isometric force normalized per cross-sectional area (FoC) greater than 5.0 mN/mm^2, and a ratio of resting force/total force less than 0.50.

Experimental Protocol

Experimental protocols are summarized in figure 1. In all groups, hypoxia was induced 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 reoxygenation period (HR protocol). In the control group (n = 10), muscles were exposed to the HR protocol alone. In all other groups, a 10-min washout period preceded the HR protocol. Racemic ketamine and its *S*(+) isomer were administered for 15 min at 10^{-6}, 10^{-5}, and 10_{-4} M (n = 6 in each group). Mechanisms involved in ketamine-induced preconditioning were studied at 10^{-4} M of racemic ketamine in the presence of 10^{-5} M HMR 1098 (specific inhibitor of the sarcolemmal K_{ATP} ($sarcK_{ATP}$) channel opening; n = 6), 8.10^{-4} M 5-hydroxydecanoate (5-HD; inhibitor of $mitoK_{ATP}$ channel opening; n = 6), 10^{-6} M propranolol (β-adrenoceptor antagonist; n = 6) and phentolamine (α-adrenoceptor antagonist; n = 6). The effects propranolol (n = 6), phentolamine (n = 6), 5-HD (n = 6), and HMR 1098 (n = 6) alone were examined in separate pharmacologic control groups.

Propranolol, phentolamine, and 5-HD were purchased from ICN Pharmaceuticals (Orsay, France), and racemic ketamine and its *S*(+) isomer were purchased from Sigma Aldrich (St. Quentin Fallavier, France). HMR 1098 was a gift from Aventis Pharma (Frankfurt am Main, Germany).

Statistical Analysis

Data are expressed as mean ± SD. Baseline values of main mechanical parameters and values of FoC at 60 min of reperfusion were compared by univariate analysis of variance. If the *F* value was significant ($P < 0.05$), Newman-Keuls *post hoc* analysis was performed. Within-group data were analyzed over time using univariate analysis of variance for repeated measures and Newman-Keuls *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).

Results

Patients' demographic data, preoperative treatments, and left ventricular ejection fractions are reported in table 1. Ninety-four human right atrial trabeculae were studied. There were no significant differences in control values for L_{max}, cross-sectional area, or FoC (table 2). Although differences were found between groups for ratio of resting force/total force, there was no muscle with a ratio of resting force/total force greater than 0.50 according to our selection criteria.

Table 1. Patients' Preoperative Drug Treatment, Preoperative Left Ventricular Ejection Fraction, and Age

Experimental Group	Age, yr	Preoperative Drug Treatment (No. of Patient)	EF, %
Control (n = 10)	67 ± 6	ACE (5), BAB (6), BZD (3), COR (1), FUR (3), MOL (2), STA (3), TNT (4)	67 ± 6
10^{-6} M Ketamine (n = 6)	62 ± 5	ACE (1), BAB (3), BZD (2), CA (2), FUR (1), K^+A (2), STA (1), TNT (1)	59 ± 6
10^{-5} M Ketamine (n = 6)	62 ± 11	ACE (4), BAB (5), FUR (2), K^+A (1), STA (2), TNT (2)	71 ± 12
10^{-4} M Ketamine (n = 6)	73 ± 2	BAB (2), BZD (1), CA (1), FUR (3), K^+A (1), MOL (1), STA (2), TNT (4)	65 ± 12
10^{-6} M *S*-ketamine (n = 6)	64 ± 12	ACE (1), BAB (3), BZD (2), CA (2), FUR (1), K^+A (2), STA (1), TNT (1)	59 ± 11
10^{-5} M *S*-ketamine (n = 6)	69 ± 6	ACE (4), BAB (6), BZD (2), CA (1), FUR (2), K^+A (1), STA (3), TNT (3)	74 ± 2
10^{-4} M *S*-ketamine (n = 6)	76 ± 7	ACE (1), BAB (2), BZD (1), CA (1), FUR (4), MOL (1), STA (2), TNT (3)	67 ± 8
Ketamine + 5-HD (n = 6)	72 ± 6	BAB (2), BZD (1), CA (1), COR (1), FUR (3), STA (2), TNT (3)	64 ± 13
Ketamine + HMR (n = 6)	67 ± 7	ACE (4), BAB (5), BZD (4), CA (2), COR (1), FUR (2), STA (4), TNT (2)	72 ± 4
Ketamine + propranolol (n = 6)	71 ± 8	ACE (2), BAB (4), BZD (2), FUR (1), K^+A (2), STA (3), TNT (2)	67 ± 10
Ketamine + phentolamine (n = 6)	70 ± 5	ACE (1), BAB (4), BZD (2), CA (1), FUR (2), K^+A (0), STA (4), TNT (1)	66 ± 5
Control 5-HD (n = 6)	75 ± 6	ACE (2), BAB (4), BZD (3), COR (1), CA (2), FUR (2), STA (4), TNT (3)	71 ± 11
Control HMR (n = 6)	67 ± 9	ACE (2), BAB (3), BZD (2), CA (1), FUR (1), K^+A (1), STA (3), TNT (4)	73 ± 3
Control propranolol (n = 6)	61 ± 8	ACE (2), BAB (4), BZD (2), FUR (1), K^+A (1), STA (3), TNT (3)	68 ± 5
Control phentolamine (n = 6)	64 ± 4	ACE (1), BAB (3), BZD (1), COR (1), FUR (1), K^+A (1), STA (3), TNT (2)	63 ± 5

Data are presented as mean ± SD.

ACE = angiotensin-converting enzyme inhibitors; BAB = β-adrenergic blocking drugs; BZD = benzodiazepine; CA = calcium channel antagonists; COR = amiodarone; EF = left ventricular ejection fraction; FUR = furosemide; K^+A = potassium channel agonists; MOL = molsidomine; STA = statins; TNT = nitroglycerin.

Effect of Hypoxia Reoxygenation

The time course of FoC for the control group and pharmacologic controls is shown in figure 2. Hypoxia induced a marked decrease in FoC (15 ± 14% of baseline after 30 min of hypoxia). In the control group, reoxygenation resulted in a partial recovery of FoC (FoC = 47 ± 10% of baseline at the end of the reoxygenation period; fig. 2).

As compared with the control group, the time course and recovery of FoC at the end of the reoxygenation period were not modified by 5-HD (51 ± 15% of baseline; not significant [NS]), HMR 1098 (49 ± 17% of baseline; NS), propranolol (46 ± 12% of baseline; NS), or phentolamine (50 ± 9% of baseline; NS).

Direct Inotropic Effects of Ketamine on Human Right Atrial Trabeculae

Racemic ketamine at 10^{-6} M (99 ± 2% of baseline; NS) at 10^{-5} M (104 ± 6% of baseline; NS) and at 10^{-4} M (99 ± 6% of baseline; NS) did not modify the FoC of atrial trabeculae. In contrast, *S*(+)-ketamine induced a dose-dependent increase in FoC (*S*(+)-ketamine at 10^{-6} M: 103 ± 5% of baseline; NS; *S*(+)-ketamine at 10^{-5} M: 104 ± 3% of baseline; $P = 0.04$; *S*(+)-ketamine at 10^{-4} M: 108 ± 6% of baseline; $P = 0.02$). Phentolamine at 10^{-6} M (99 ± 2% of baseline; NS) and propranolol at 10^{-6} M (92 ± 4% of baseline; NS) did not modify FoC of atrial trabeculae. Racemic ketamine in the presence of propranolol (64 ± 14% of FoC value after propranolol;

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

Experimental Group	L_{max}, mm	CSA, mm^2	FoC, mN/mm^2	RF, mN/mm^{-2}	RF/TF
Control (n = 10)	6.7 ± 1.0	0.67 ± 0.25	20 ± 12	8 ± 7	0.29 ± 0.15
10^{-6} M Ketamine (n = 6)	9.0 ± 1.6	0.59 ± 0.26	19 ± 9	9 ± 9	0.38 ± 0.08
10^{-5} M Ketamine (n = 6)	6.9 ± 0.8	0.69 ± 0.34	18 ± 11	11 ± 6	0.29 ± 0.07*
10^{-4} M Ketamine (n = 6)	7.6 ± 1.4	0.67 ± 0.25	19 ± 6	8 ± 2	0.46 ± 0.04
10^{-6} M *S*-ketamine (n = 6)	7.8 ± 1.8	0.57 ± 0.19	17 ± 11	14 ± 8	0.35 ± 0.09*
10^{-5} M *S*-ketamine (n = 6)	7.2 ± 1.5	0.53 ± 0.22	25 ± 15	14 ± 8	0.44 ± 0.07*
10^{-4} M *S*-ketamine (n = 6)	7.1 ± 0.8	0.54 ± 0.19	17 ± 7	13 ± 5	0.67 ± 0.25*
Ketamine + 5-HD (n = 6)	7.6 ± 1.2	0.70 ± 0.23	22 ± 8	8 ± 5	0.26 ± 0.12
Ketamine + HMR (n = 6)	8.3 ± 0.9	0.83 ± 0.24	23 ± 7	6 ± 2	0.18 ± 0.03
Ketamine + propranolol (n = 6)	8.0 ± 2.5	0.66 ± 0.26	17 ± 5	8 ± 5	0.29 ± 0.11*
Ketamine + phentolamine (n = 6)	7.3 ± 0.6	0.76 ± 0.36	14 ± 10	7 ± 5	0.34 ± 0.04*
Control 5-HD (n = 6)	7.7 ± 2.5	0.76 ± 0.27	19 ± 10	9 ± 5	0.38 ± 0.08*
Control HMR (n = 6)	7.0 ± 1.1	0.50 ± 0.14	15 ± 5	10 ± 5	0.39 ± 0.09*
Control propranolol (n = 6)	7.7 ± 2.1	0.52 ± 0.27	15 ± 7	11 ± 7	0.43 ± 0.09
Control phentolamine (n = 6)	5.7 ± 1.0	0.65 ± 0.23	14 ± 6	10 ± 7	0.36 ± 0.09

Data are presented as mean ± SD.

* $P < 0.05$ *vs.* ketamine + HMR 1098 (HMR) group.

CSA = cross-sectional area; FoC = acting isometric force normalized per cross-sectional area; L_{max} = maximal length at the apex of the length–active force curve; RF = resting force normalized per cross-sectional area; RF/TF = ratio of resting force on total force of contraction.

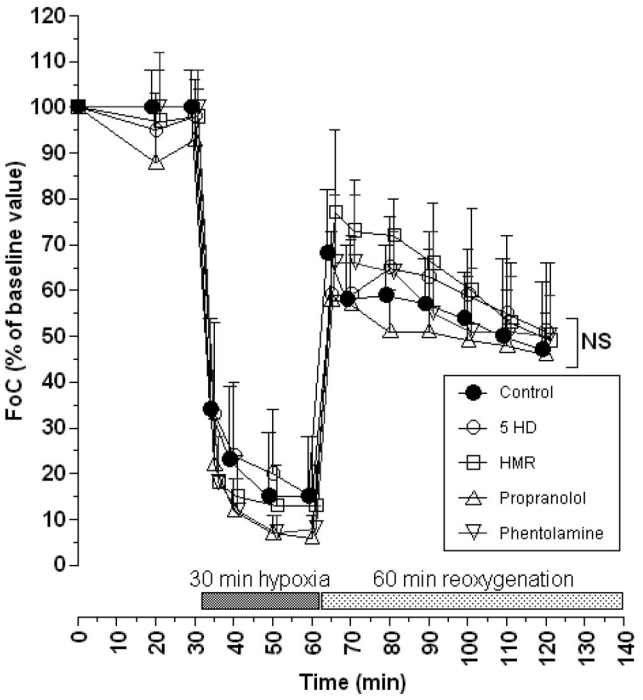

Fig. 2. Time course of force of contraction (FoC) of isolated human right atrial trabeculae during a 30-min hypoxic challenge followed by a 60-min reoxygenation period in control (n = 10), 5-hydroxydecanoate (5-HD; n = 6), HMR 1098 (HMR; n = 6), propranolol (n = 6), and phentolamine (n = 6) groups. In pharmacologic groups, hypoxia–reoxygenation was preceded by a 10-min washout period. Data are presented as mean ± SD. NS = not significant.

$P = 0.002$) but not phentolamine (92 ± 14% of FoC value after phentolamine; NS) induced a significant decrease in FoC reversed after a 10-min washout period (FoC: 90 ± 12% of baseline value).

Effects of Ketamine on Hypoxia Reoxygenation

Ketamine at 10^{-6}, 10^{-5}, and 10^{-4} M, and its *S*(+) isomer at 10^{-6}, 10^{-5}, and 10^{-4} M enhanced the recovery of FoC at the end of the 60-min reoxygenation period as compared with the control group (table 3 and fig. 3).

Role of K_{ATP} Channels and α and β Adrenoceptors in the Ketamine-induced Preconditioning

The enhanced recovery of FoC at the end of the reoxygenation period induced by racemic ketamine at 10^{-4} M was abolished in the presence of 10^{-5} M HMR 1098, 8.10^{-4} M 5-HD, 10^{-6} M propranolol, and 10^{-6} M phentolamine (table 3 and fig. 4).

Discussion

Our study shows that a brief exposure to clinically relevant concentrations of racemic and *S*(+)-ketamine preconditions isolated human myocardium as shown by the enhanced recovery of force after hypoxia–reoxygenation. Furthermore, we show that a 5-HD–dependent

Table 3. Force of Contraction of Isolated Human Right Atrial Trabeculae at the End of the Reoxygenation Period

Experimental Group	FoC at End of Reoxygenation, % of Baseline
Control (n = 10)	47 ± 10
10^{-6} M Ketamine (n = 6)	84 ± 5*
10^{-5} M Ketamine (n = 6)	95 ± 10*
10^{-4} M Ketamine (n = 6)	94 ± 14*
10^{-6} M *S*-ketamine (n = 6)	85 ± 4*
10^{-5} M *S*-ketamine (n = 6)	91 ± 16*
10^{-4} M *S*-ketamine (n = 6)	93 ± 14*
Ketamine + 5-HD (n = 6)	60 ± 16
Ketamine + HMR (n = 6)	60 ± 16
Ketamine + propranolol (n = 6)	60 ± 7
Ketamine + phentolamine (n = 6)	56 ± 12
Control 5-HD (n = 6)	51 ± 15
Control HMR (n = 6)	49 ± 17
Control propranolol (n = 6)	46 ± 12
Control phentolamine (n = 6)	50 ± 9

Data are presented as mean ± SD.

* $P < 0.001$ *vs.* control, ketamine + 5-hydroxydecanoate (5-HD), ketamine + HMR 1098 (HMR), ketamine + propranolol, ketamine + phentolamine, control 5-HD, control HMR, control propranolol, control phentolamine.

FoC = active isometric force normalized per cross-sectional area.

mechanism, the inhibition of $sarcK_{ATP}$ channel opening, and the specific blockade of α and β adrenoceptors abolished the preconditioning effect of ketamine.

Numerous studies have shown that volatile anesthetics precondition myocardium against ischemia. In clinical practice, anesthetic preconditioning has been shown to be associated with improved cardiac outcome after coronary artery bypass graft surgery.[14] Currently, there are few data about intravenous anesthetic preconditioning.[9,15] However, this could help to identify the best

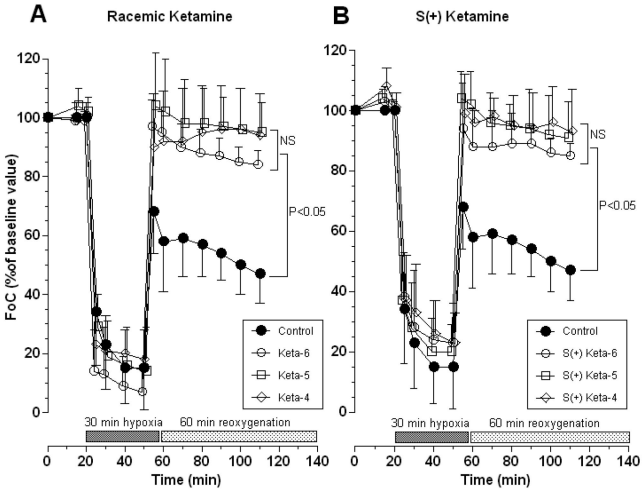

Fig. 3. Effect of racemic ketamine (*A*) and *S*(+)-ketamine (*B*) on the time course of force of contraction (FoC) of isolated human right atrial trabeculae during a 30-min hypoxic challenge followed by a 60-min reoxygenation period. Fifteen minutes of exposure to ketamine and *S*(+)-ketamine (10^{-6} M [*S*(+) Keta-6], 10^{-5} M [*S*(+) Keta-5], and 10^{-4} M [*S*(+) Keta-4]; n = 6 in each group) was followed by a 10-min washout period before the 30-min hypoxic challenge. Data are presented as mean ± SD. NS = not significant.

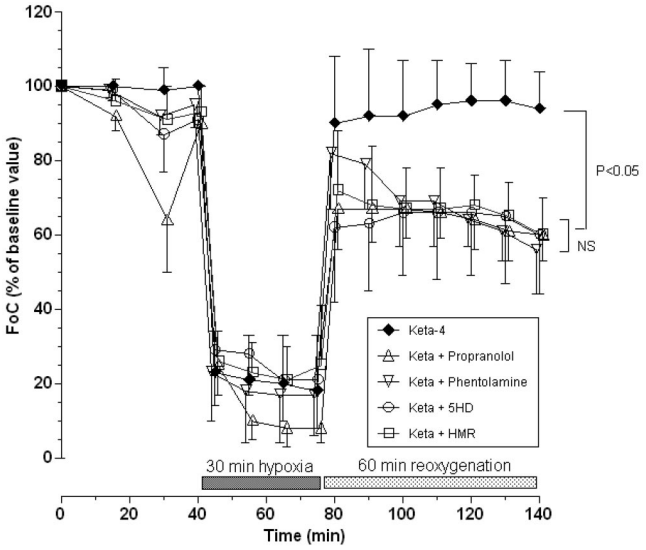

Fig. 4. Effect of 5-hydroxydecanoate (5-HD; n = 6), HMR 1098 (HMR; n = 6), propranolol (n = 6), and phentolamine (n = 6) on the time course of force of contraction (FoC) of isolated human right atrial trabeculae during a 30-min hypoxic challenge followed by a 60-min reoxygenation period. Hypoxia–reoxygenation was preceded by a 10-min washout period. Data are presented as mean ± SD. Keta = ketamine; NS = not significant.

anesthetic association leading to the greatest cardioprotective effect against ischemia. This may be of importance in the perioperative period, when myocardial ischemia frequently occurs in patients with ischemic heart disease, and in patients scheduled to undergo cardiac surgery with cardiopulmonary bypass.

Although ketamine may be used for induction of anesthesia in high-risk patients because it maintains hemodynamic variables, its effect on ischemia–reperfusion remains poorly studied and has led to controversial results. Haessler *et al.*[16] have shown that ketamine/xylazine-based anesthesia resulted in an infarct volume comparable to that of isoflurane- or pentobarbitone-based anesthesia in rabbits undergoing 30 min of coronary occlusion followed by 3 h of reperfusion. In contrast, Cope *et al.*[17] showed that ketamine/xylazine-based anesthesia resulted in an infarct volume comparable to that measured with pentobarbital- or propofol-based anesthesia but twice as large as that measured with volatile anesthetic–based anesthesia. Two recent studies suggested that ketamine may have a stereoselective inhibitory effect on ischemic preconditioning.[1,2] These authors also showed that a bolus administration of racemic ketamine or its *R*(−) isomer before 30 min of myocardial ischemia did not modify infarct volume after 2 h of reperfusion in rabbit heart[2] or contractile function after 1 h of reperfusion in isolated rat heart.[1] In contrast, in rabbits anesthetized with a single injection of ketamine/xylazine followed by a continuous infusion of propofol, ischemic preconditioning was more effective in reducing the infarct volume than a 15-min administration of isoflurane at 0.5 minimal alveolar concentration.[18] Finally, Zaugg *et al.*[3] showed that ketamine did not modify rat cardiomyocyte viability after simulated ischemia–reperfusion but that ketamine stereoselectively affected diazoxide-induced mitoK_{ATP} channel opening. Several hypothesis may explain the discrepancies observed between these studies. First, Haessler *et al.* studied rabbits anesthetized with α-chloralose, the main metabolite of which has been shown to precondition isolated rat myocytes and to potentiate the diazoxide-induced opening of mitoK_{ATP} channels.[3] Second, xylazine associated with ketamine could be a confounding factor because it may limit preconditioning through xylazine-induced hyperglycemia and decrease in postsynaptic sympathetic activity.[19,20] Third, Molojavyi *et al.*[1] anesthetized rats with halothane, the effect of which could not be totally excluded. Furthermore, these authors also suggested that isolated rat heart is not a suitable model to study anesthetic preconditioning because they did not observe isoflurane-induced preconditioning.[8] Fourth, the mode and duration of administration of ketamine and the concentration tested are not comparable between studies. Our results showing that racemic and *S*(+)-ketamine precondition isolated human myocardium are in contrast with those previously reported in rat[1,3] and rabbit[2] myocardium. However, as discussed above, differences between species, experimental model, and concentrations of ketamine tested could explain this discrepancy.

Increasing evidence suggests that adrenoceptors are important triggers of ischemic and pharmacologic myocardial preconditioning.[11,12,15] We have previously shown that desflurane preconditioning was mediated, at least in part, *via* α- and β-adrenoceptor stimulation.[13] The current study also shows that ketamine may precondition isolated human myocardium, at least in part, *via* α- and β-adrenoceptor stimulation. It should be noted that the blockade either of α or β adrenoceptors completely abolished ketamine-induced preconditioning. The main hypothesis that could explain our result is that the preconditioning phenomenon is a graded response, not an "all-or-none" response. A threshold must be reached by a stimulus to trigger the preconditioning cascade.[21] Therefore, the blockade of α or β adrenoceptors may be sufficient to decrease the stimulus below the threshold required to trigger the preconditioning cascade. The stimulation of adrenoceptors by ketamine is dependent on species[4–7] but participates in the direct inotropic effect of ketamine in isolated human myocardium.[7] The origin of ketamine-induced adrenoceptor stimulation remains incompletely understood but has been related to inhibition of neuronal (*i.e.*, extracardiac and intrinsic cardiac neurons) and extraneuronal (nonneuronal cardiac adrenergic cells) catecholamine uptake.[22]

The preconditioning signal triggered by α and β adrenoceptors may involve multiple actors of the adrenoceptor signal transduction pathway. Therefore, β-adreno-

ceptor stimulation leads to the activation of cyclic adenosine monophosphate-protein kinase A–dependent pathway, which has recently been shown to mediate ischemic preconditioning in dog myocardium.[23] α-Adrenoceptor stimulation activates phospholipases C and D, leading to the formation of inositol triphosphate, which induces release of Ca^{2+} from the sarcoplasmic reticulum and diacylglycerol, which, in turn, activates protein kinase C, the crucial role of which is well known in ischemic and anesthetic preconditioning.[10,15] Finally, it has been suggested that a diversity of signaling pathways all seem to converge to inhibit glycogen synthase kinase 3β, regulating the mitochondrial permeability transition pore complex activity, which is a key component of cardioprotection.[24]

Our results suggest that sarcK_{ATP} channel opening is involved in ketamine-induced preconditioning because pretreatment with HMR1098 abolished the protective effect of ketamine. First, it should be emphasized that HMR 1098 is a specific blocker of sarcK_{ATP} channels (IC_{50} = 0.8 μM).[25] An HMR 1098 concentration of 10 μM has been used to block more than 90% of sarcK_{ATP} channels in various experimental models. Our result is in contrast with previous results showing that racemic ketamine may inhibit sarcK_{ATP} channel activity in rat ventricular myocytes.[26] However, in that study, the activity of sarcK_{ATP} channels was measured in nonphysiologic conditions, *i.e.*, adenosine triphosphate–free bath solution, pinacidil-induced activation of K_{ATP} channels, isolated nonbeating myocytes, and patch clamp techniques that modify K_{ATP} channel environment. Our study also showed that a 5-HD–dependent mechanism (inhibition of mitoK_{ATP} channel opening, inhibition of respiratory chain complexes) was involved in ketamine-induced preconditioning. Zaugg *et al.*[3] reported that ketamine did not alter flavoprotein oxidation in rat myocytes and that *R*(−)- but not *S*(+)-ketamine abolished diazoxide-induced flavoprotein oxidation. However, it has been suggested that it is no longer possible to assume that diazoxide and flavoprotein oxidation specifically indicate the involvement of mitoK_{ATP}.[27] Further studies are needed to determine the precise effects of ketamine on cardiac sarcK_{ATP} and mitoK_{ATP}.

Mitochondrial K_{ATP} channels play a major role in ischemic preconditioning, but recent studies have also shown that sarcK_{ATP} channels are essential. Therefore, ischemic preconditioning cannot be conferred in transgenic mice with targeted deletion of Kir6.2, the pore-forming subunit of cardiac sarcK_{ATP} channel.[28] Similarly, Toller *et al.*[29] have shown that, in dogs, desflurane-induced preconditioning was dependent on both sarcK_{ATP} and mitoK_{ATP} channels. However, in isolated human myocardium submitted to hypoxia–reoxygenation, we have shown that inhibition of sarcK_{ATP} channels opening attenuated desflurane-induced recovery of contractile force.[14] Two main hypothesis may explain, at least in part, these discrepancies. First, the relative importance of sarcK_{ATP} or mitoK_{ATP} channels during preconditioning may depend on species. The rapid heart rate of small animals may magnify the importance of sarcK_{ATP} channels.[28] Second, recent findings suggest distinct roles of sarcK_{ATP} and mitoK_{ATP} channels in myocardial ischemic preconditioning benefits on infarct volume and contractile recovery.[30] Third, different signaling pathways may be initiated depending on conditions and molecules tested. Further studies are required to determine the relative participation and the precise timing of activation of sarcK_{ATP} and mitoK_{ATP} channels during ischemic and pharmacologic preconditioning.

Our results must be interpreted within the constraints of several possible limitations. First, the effects of anesthetics drugs, diseases, or treatments received by the patients cannot be totally ruled out. Therefore, patients taking oral hypoglycemic medications were excluded from the study. Importantly, we have previously reported that preoperative treatment such as β-adrenergic blocking drugs do not mask desflurane-induced adrenoceptor stimulation.[13] Theoretically, the use of opioids during anesthesia of patients included in this study could have preconditioned the appendage. However, comparisons have been made with control experiments. Nevertheless, a superimposed effect of opioids used during surgical procedures cannot be ruled out.[31] Second, rather than the true ischemia obtained by coronary occlusion, we used a 30-min anoxic superfusion to simulate ischemia. However, it has been shown, in various experimental models, that anoxia is as effective as ischemia in inducing preconditioning.[32] Third, we measured postischemic contractile function recovery but not the infarct size. However, it has been shown that the improved recovery of contractile function produced by preconditioning was proportional to reduced infarct size.[33] In addition, our results, as well as previous ones, showed that this model provides a useful tool to study the mechanisms involved in ischemic preconditioning in human myocardium.[13] Fourth, our results could have been affected by advanced age of the patients. Aging has been shown to impair ischemic and sevoflurane myocardial preconditioning.[34] Nevertheless, there was no difference in age between groups, and several studies have shown that ischemic preconditioning may be restored in aged patients and animals by food restriction or physical activity.[35] Fifth, our experiments were performed at 34°C, which may decrease the K_{ATP} channel sensitivity[36] and the effect of preconditioning.[37] However, studies in isolated human right atrial myocardium must be performed at 34°C to obtain a stable isometric contraction during several hours. Furthermore, during surgical procedures, moderate hypothermia may occur in patients. Finally, because there is no protein in Tyrode's solution, the concentrations of ketamine tested in our study are free concentrations. However, taking into account the

20% protein binding of ketamine, the range of concentrations tested included the free plasmatic concentrations of ketamine encountered in clinical anesthesia.[38]

In conclusion, ketamine and its *S*(+) isomer precondition isolated human right atrial myocardium. Ketamine-induced preconditioning involves, at least in part, K_{ATP} channels and stimulation of α and β adrenoceptors.

References

1. Molojavyi A, Preckel B, Comfère T, Müllenheim J, Thämer V, Schlack W: Effects of ketamine and its isomers on ischemic preconditioning in the isolated rat heart. ANESTHESIOLOGY 2001; 94:623–9
2. Müllenheim J, Fräßdorf J, Preckel B, Thämer V, Schlack W: Ketamine, but not *S*(+)-ketamine, blocks ischemic preconditioning in rabbit hearts in vivo. ANESTHESIOLOGY 2001; 94:630–6
3. Zaugg M, Lucchinetti E, Spahn DR, Pasch T, Garcia C, Schaub MC: Differential effects of anesthetics on mitochondrial K_{ATP} channel activity and cardiomyocyte protection. ANESTHESIOLOGY 2002; 97:15–23
4. Graf BM, Vicenzi MN, Martin E, Bosnjak ZJ, Stowe DF: Ketamine has stereospecific effects in the isolated perfused guinea pig heart. ANESTHESIOLOGY 1995; 82:1426–37
5. Riou B, Lecarpentier Y, Viars P: Inotropic effect of ketamine on rat cardiac papillary muscle. ANESTHESIOLOGY 1989; 71:116–25
6. Cook DJ, Carton EG, Housmans PR: Mechanism of the positive inotropic effect of ketamine in isolated ferret ventricular papillary muscle. ANESTHESIOLOGY 1991; 74:880–8
7. Hanouz JL, Persehaye E, Zhu L, Lammens S, Lepage O, Massetti M, Babatasi G, Khayat A, Bricard H, Gerard JL: Inotropic and lusitropic effects of ketamine in isolated human atrial myocardium: Effect of adrenoceptor blockade. Anesth Analg 2004; 99:1689–95
8. Martini N, Preckel B, Thamer V, Schlack W: Can isoflurane mimic ischaemic preconditioning in isolated rat heart? Br J Anaesth 2001; 86:269–71
9. Tanaka K, Ludwig LM, Kersten JR, Pagel PS, Warltier DC: Mechanisms of cardioprotection by volatile anesthetics. ANESTHESIOLOGY 2004; 100:707–21
10. Sergeev P, da Silva R, Lucchinetti E, Zaugg K, Pasch T, Schaub MC, Zaugg M: Trigger-dependent gene expression profiles in cardiac preconditioning: Evidence for distinct genetic programs in ischemic and anesthetic preconditioning. ANESTHESIOLOGY 2004; 100:474–88
11. Kitakaze M, Hori M, Morioka T, Minamino T, Takashima S, Sato H, Shinozaki Y, Chujo M, Mori H, Inoue M, Kamada T: Alpha 1-adrenoceptor activation mediates the infarct size-limiting effect of ischemic preconditioning through augmentation of 5'-nucleotidase activity. J Clin Invest 1994; 93:2197–205
12. Miyawaki H, Ashraf M: Isoproterenol mimics calcium preconditioning-induced protection against ischemia. Am J Physiol 1997; 272:H927–36
13. Hanouz JL, Yvon A, Massetti M, Lepage O, Babatasi G, Khayat A, Bricard H, Gerard JL: Mechanisms of desflurane-induced preconditioning in isolated human right atria *in vitro*. ANESTHESIOLOGY 2002; 97:33–41
14. Garcia C, Julier K, Bestmann L, Zollinger A, von Segesser LK, Pasch T, Spahn DR, Zaugg M: Preconditioning with sevoflurane decreases PECAM-1 expression and improves one-year cardiovascular outcome in coronary artery bypass graft surgery. Br J Anaesth 2005; 94:159–65
15. Zaugg M, Lucchinetti E, Uecker M, Pasch T, Schaub MC Anaesthetics and cardiac preconditioning: I. Signalling and cytoprotective mechanisms. Br J Anaesth 2003; 91:551–65
16. Haessler R, Kuzume K, Chien GL, Wolff RA, Davis RF, Van Winkle DM: Anaesthetics alter the magnitude of infarct limitation by ischaemic preconditioning. Cardiovasc Res 1994; 28:1574–80
17. Cope DK, Impastato WK Cohen MV, Downey JM: Volatile anesthetics protect the ischemic rabbit myocardium from infarction. ANESTHESIOLOGY 1997; 86:699–709
18. Cason BA, Gamperl KA, Slocum RE, Hickey RF: Anesthetic-induced preconditioning: Previous administration of isoflurane decreases myocardial infarct size in rabbits. ANESTHESIOLOGY 1997; 87:1182–90
19. Tranquilli WJ, Maze M: Clinical pharmacology of and use of α_2-adrenergic agonists in veterinary anaesthesia. Anesth Pharmacol Rev 1993; 1:297–309
20. Illera JC, Gonzalez Gil A, Silvan G, Illera M: The effects of different anaesthetic treatments on the adreno-cortical functions and glucose levels in NZW rabbits. J Physiol Biochem 2000; 56:329–36
21. Jaberansari MT, Baxter GF, Muller CA, Latouf SE, Roth E, Opie LH, Yellon DM: Angiotensin-converting enzyme inhibition enhances a subthreshold stimulus to elicit delayed preconditioning in pig myocardium. J Am Coll Cardiol 2001; 37:1996–2001
22. Lundy PM, Lockwood PA, Thompson G, Frew R: Differential effects of ketamine isomers on neuronal and extraneuronal catecholamine uptake mechanisms. ANESTHESIOLOGY 1986; 64:359–63
23. Sanada S, Asanuma H, Tsukamoto O, Minamino T, Node K, Takashima S, Fukushima T, Ogai A, Shinozaki Y, Fujita M, Hirata A, Okuda H, Shimokawa H, Tomoike H, Hori M, Kitakaze M: Protein kinase A as another mediator of ischemic preconditioning independent of protein kinase C. Circulation 2004; 110:51–57
24. Juhaszova M, Zorov DB, Kim SH, Pepe S, Fu Q, Fishbein KW, Ziman BD, Wang S, Ytrehus K, Antos CL, Olson EN, Sollott SJ: Glycogen synthase kinase-3β mediates convergence of protection signaling to inhibit the mitochondrial permeability transition pore. J Clin Invest 2004; 113:1535–49
25. Light PE, Kanji HD, Fox JE, French RJ: Distinct myoprotective roles of cardiac sarcolemmal and mitochondrial K_{ATP} channels during metabolic inhibition and recovery. FASEB J 2001; 15:2586–94
26. Ko SH, Lee SK, Han YJ, Choe H, Kwak YG, Chae SW, Cho KP, Song HS: Blockade of myocardial ATP-sensitive potassium channels by ketamine. ANESTHESIOLOGY 1997; 87:68–74
27. Hanley PJ, Mickel M, Löffler M, Brandt U, Daut J: K_{ATP} channel-independent targets of diazoxide and 5-hydroxydecanoate in the heart. J Physiol (London) 2002; 542:735–41
28. Suzuki M, Sasaki N, Miki T, Sakamoto N, Ohmoto-Sekine Y, Tamagawa M, Seino S, Marban E, Nakaya H: Role of sarcolemmal K_{ATP} channels in cardioprotection against ischemia/reperfusion injury in mice. J Clin Invest 2002; 109:509–16
29. Toller WG, Gross ER, Kersten JR, Pagel PS, Gross GJ, Warltier DC: Sarcolemmal and mitochondrial adenosine triphosphate–dependent potassium channels: Mechanism of desflurane-induced cardioprotection. ANESTHESIOLOGY 2000; 92:1731–9
30. Toyoda Y, Friehs I, Parker RA, Levitsky S, McCully JD: Differential role of sarcolemmal and mitochondrial KATP channels in adenosine-enhanced ischemic preconditioning. Am J Physiol 2000; 279:H2694–703
31. Ludwig LM, Patel HH, Gross GJ, Kersten JR, Pagel PS, Warltier DC: Morphine enhances pharmacological preconditioning by isoflurane: Role of mitochondrial K_{ATP} channels and opioid receptors. ANESTHESIOLOGY 2003; 98:705–11
32. Lasley RD, Anderson GM, Mentzer RM: Ischemic and hypoxic preconditioning enhance postischemic recovery of function in the rat heart. Cardiovasc Res 1993; 27:565–70
33. Cohen MV, Liu GS, Downey JM: Preconditioning causes improved wall motion as well as smaller infarcts after transient coronary occlusion in rabbits. Circulation 1991; 84:341–9
34. Sniecinski R, Liu H: Reduced efficacy of volatile anesthetic preconditioning with advanced age in isolated rat myocardium. ANESTHESIOLOGY 2004; 100:589–97
35. Abete P, Testa G, Galizia G, Mazzella F, Della Morte D, de Santis D, Calabrese C, Cacciatore F, Gargiulo G, Ferrara N, Rengo G, Sica V, Napoli C, Rengo F: Tandem action of exercise training and food restriction completely preserves ischemic preconditioning in the aging heart. Exp Gerontol 2005; 40:43–50
36. Saito W, Noguchi K, Okazaki K, Matsuda T, Kato Y, Tanaka H, Shigenobu K: Temperature-sensitive effects of potassium channel openers on isolated guinea pig myocardium and aorta. J Cardiovasc Pharmacol 1998; 31:327–9
37. Dote K, Wolff RA, Van Winkle DM: Hypothermia increases the threshold for ischemic preconditioning. J Thorac Cardiovasc Surg 1998; 116:319–26
38. Idvall J, Ahlgren I, Aronsen KR, Stenberg P: Ketamine infusions: Pharmacokinetics and clinical effects. Br J Anaesth 1979; 51:1167–73