

Ketamine, but Not S(+)-ketamine, Blocks Ischemic Preconditioning in Rabbit Hearts In Vivo

Jost Müllenheim, M.D., Jan Fräßdorf, M.D.,* Benedikt Preckel, M.D., D.E.A.A.,* Volker Thämer, M.D., Ph.D.,† Wolfgang Schlack, M.D., Ph.D., D.E.A.A.‡*

Background: **Ketamine blocks K_{ATP} channels in isolated cells and abolishes the cardioprotective effect of ischemic preconditioning *in vitro*. The authors investigated the effects of ketamine and *S*(+)-ketamine on ischemic preconditioning in the rabbit heart *in vivo*.**

Methods: **In 46 α-chloralose–anesthetized rabbits, left ventricular pressure (tip manometer), cardiac output (ultrasonic flow probe), and myocardial infarct size (triphenyltetrazolium staining) at the end of the experiment were measured. All rabbits were subjected to 30 min of occlusion of a major coronary artery and 2 h of subsequent reperfusion. The control group underwent the ischemia–reperfusion program without preconditioning. Ischemic preconditioning was elicited by 5-min coronary artery occlusion followed by 10 min of reperfusion before the 30 min period of myocardial ischemia (preconditioning group). To test whether ketamine or *S*(+)-ketamine blocks the preconditioning-induced cardioprotection, each (10 mg kg^{-1}) was administered 5 min before the preconditioning ischemia. To test any effect of ketamine itself, ketamine was also administered without preconditioning at the corresponding time point.**

Results: **Hemodynamic baseline values were not significantly different between groups [left ventricular pressure, 107 ± 13 mmHg (mean ± SD); cardiac output, 183 ± 28 ml/min]. During coronary artery occlusion, left ventricular pressure was reduced to 83 ± 14% of baseline and cardiac output to 84 ± 19%. After 2 h of reperfusion, functional recovery was not significantly different among groups (left ventricular pressure, 77 ± 19%; cardiac output, 86 ± 18%). Infarct size was reduced from 45 ± 16% of the area at risk in controls to 24 ± 17% in the preconditioning group ($P = 0.03$). The administration of ketamine had no effect on infarct size in animals without preconditioning (48 ± 18%), but abolished the cardioprotective effects of ischemic preconditioning (45 ± 19%, $P = 0.03$). *S*(+)-ketamine did not affect ischemic preconditioning (25 ± 11%, $P = 1.0$).**

Conclusions: **Ketamine, but not *S*(+)-ketamine blocks the cardioprotective effect of ischemic preconditioning *in vivo*.**

ISCHEMIC preconditioning, first described by Murry *et al.*,[1] markedly reduces myocyte death during prolonged periods of myocardial ischemia and has been reported in several mammalian species. In addition to animal studies, some research points to the existence of this strongest endogenous protective mechanism against myocardial ischemia in human myocardium.[2] Mitochondrial or sarcolemmal adenosine triphosphate (ATP)–regulated potassium (K_{ATP}) channels have been shown to play an important role in mediating the cardioprotective effects of preconditioning.[3] Ketamine was shown to block K_{ATP} channels in isolated cells.[4] In the preceding study of this issue,[5] we investigated the effects of ketamine and both enantiomers *S*(+)- and *R*(−)-ketamine on preconditioning in isolated rat hearts subjected to global ischemia and reperfusion. In that study, ketamine abolished the cardioprotection induced by preconditioning. The effect was stereoselective and caused by *R*(−)-ketamine administered before the preconditioning periods. However, *in vivo,* the pathophysiology of preconditioning and ischemia–reperfusion becomes more complex. Hemodynamic side effects of ketamine and effects on anesthetic depth and on leucocyte activation might be involved. The current study investigated the effect of a single bolus dose of ketamine or *S*(+)-ketamine on preconditioning in an *in vivo* model of ischemia and reperfusion.

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

Method and Materials

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

General Preparation

After local anesthesia with lidocaine–prilocaine cream, a marginal ear vein was cannulated in 48 male New Zealand White rabbits weighing 2.6–3.6 kg (mean, 3.0 kg). The animals were anesthetized with intravenous propofol (10 mg/kg) followed by a continuous infusion of α-chloralose 40 $mg \cdot kg^{-1} \cdot h^{-1}$. The trachea was intubated (endotracheal tube, 3.0 mm ID) and ventilation was controlled using a Starling pump (Type 874/052; Braun Melsungen AG, Melsungen, Germany). Ventilatory frequency was set at 30–35 breaths/min and tidal volume at 15–20 ml to maintain end-expiratory partial pressure of carbon dioxide (P_{CO_2}) at approximately 35 mmHg (measured by Datex Capnomac Ultima; Division of Instrumentarium Corp., Helsinki, Finland). Arterial blood gas tensions, hemoglobin concentration, and packed cell volume were assessed at regular intervals (before surgery, before the study, and at the end of the

* Scientific Assistant, ‡ Privatdozent of Anesthesiology, Institut für Klinische Anaesthesiologie. † Professor of Physiology, Institut für Herz- und Kreislaufphysiologie.

Received from the Institut für Klinische Anaesthesiologie and the Institut für Herz- und Kreislaufphysiologie, Heinrich-Heine-Universität, Düsseldorf, Germany. Submitted for publication February 29, 2000. Accepted for publication September 26, 2000. Supported in part by Parke-Davis GMBH, Berlin, Germany.

Address reprint requests to Dr. Schlack: Institut für Klinische Anaesthesiologie, Heinrich-Heine-Universität, Postfach 10 10 07, D-40001 Düsseldorf, Germany. Address electronic mail to: wolfgang@herzkreis.uni-duesseldorf.de.

study). Adequacy of the anesthesia regimen was shown by lack of muscle movement and hemodynamic responses during surgery. Additional bolus doses of α-chloralose were administered as needed during surgery.

For measurement of aortic pressure, a 20-gauge catheter was advanced from the left carotid artery into the aortic arch and connected to a Statham transducer (PD23; Gould, Cleveland, OH). After cannulation of the external jugular vein, animals received a continuous infusion of normal saline 15 $ml \cdot kg^{-1} \cdot h^{-1}$ to compensate for fluid loss. After median sternotomy and pericardiotomy, an ultrasonic flow probe was placed around the ascending aorta to measure cardiac output (CO) minus coronary flow volume (4S ultrasonic flow probe, T 208; Transonic Systems Inc., Ithaca, NY). Left ventricular (LV) pressure (LVP) was monitored using a catheter tip manometer (Sensodyn S PO SF-1; Braun Melsungen AG) introduced into the LV *via* the left atrium. A ligature snare was passed around a major coronary artery for later occlusion. Temperature was measured inside the pericardial cradle (GTH 1160; Digital Thermometer, Geisinger Electronic, Germany) and maintained between 38.3 and 38.7°C by adjusting a heating pad and an infrared lamp.

Experimental Procedure

Fifteen minutes after completion of the surgical preparation, baseline measurements were performed. All rabbits were subjected to 30 min of coronary artery occlusion by tightening of the snare. The effectiveness of this maneuver was verified by the appearance of epicardial cyanosis and changes in the surface electrocardiogram. Ventricular fibrillation during coronary artery occlusion was treated by electrical defibrillation (5 J, DCS261 Defibrillator; Piekser, Ratingen, Germany). After 30 min of occlusion, the snare occluder was released and reperfusion was verified by the disappearance of epicardial cyanosis. After 2 h of reperfusion, the heart was arrested by injection of 20 ml potassium chloride solution, 16 mM, into the left atrium, quickly excised, and mounted on a modified Langendorff apparatus for perfusion with ice-cold normal saline *via* the aortic root at a perfusion pressure of 40 cm H_2O to wash away intravascular blood. After 5 min of perfusion, the coronary artery was occluded again and the remaining myocardium was perfused through the aortic root with 0.2% Evans blue in normal saline for 10 min. Intravascular Evans blue was then washed away by perfusion with normal saline for 5 min. This treatment identifies the ischemic-reperfused area (area at risk) as unstained. The heart was then cut into transverse slices, 2 mm thick, and stained for 15 min in buffered 0.75% triphenyltetrazolium chloride solution at 38°C to identify viable and necrotic tissue within the area at risk. The area at risk and the infarcted area were determined by planimetry. The slices were dried and the weight of each slice was measured.

Ten rabbits underwent the ischemia–reperfusion procedure without preconditioning (control group). In the preconditioning group (n = 10), preconditioning was elicited by one 5-min period of coronary artery occlusion followed by 10 min of reperfusion before the 30 min of coronary artery occlusion. In the ketamine groups, ketamine 10 mg/kg (ketamine–preconditioning group, n = 9) or *S*(+)-ketamine 10 mg/kg (*S*(+)-ketamine–preconditioning group, n = 8) were administered by intravenous bolus injection 5 min before the preconditioning ischemia. Rabbits of the ketamine group (n = 9) received ketamine 10 mg/kg without preconditioning, and animals of the control and the preconditioning group received benzethoniumchloride 0.1 mg/kg as the vehicle of ketamine at the corresponding time point, respectively.

Data Analysis

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

Hemodynamic Variables

Global systolic function was measured in terms of LV systolic pressure (LVSP) and maximum rate of pressure increase (dP/dtmax). *Global LV end-systole* was defined as the point of minimum dP/dt (dP/dtmin), and *LV end-diastole* as the beginning of the sharp upslope of the LV dP/dt tracing. The time constant of decrease in LV isovolumic pressure (τ) was used as an index of LV relaxation.[6] CO was calculated from stroke volume and heart rate (HR), rate pressure product (RPP) from HR and LVSP, and systemic vascular resistance (SVR) from mean aortic pressure and CO, assuming a right atrial pressure of 0 mmHg in the open chest preparation.

Statistical Analysis

Data are presented as mean and standard deviation (SD). Statistical analysis was performed by two-way analysis of variance for time and treatment (experimental group) effects. If an overall significance between groups was found, a comparison was performed for each time point using one-way analysis of variance followed by the Dunnett *post hoc* test, with preconditioning as the reference group. If an overall significance within a group (time effect) was found, one-way analysis of variance followed by the Dunnett *post hoc* test, with the baseline value as the reference time point, was used for the assessment of time effects in that group. Changes within and between groups were considered to be statistically significant when the *P* value was less than 0.05.

Results

Forty-eight animals were used. Two animals died of ventricular fibrillation during coronary artery occlusion. In the remaining 46 animals, complete data sets were obtained (control group, n = 10; preconditioning group, n = 10; ketamine–preconditioning group, n = 9; *S*(+)-ketamine–preconditioning group, n = 8; ketamine group, n = 9).

Hemodynamic Function

The hemodynamic variables are summarized in figure 1 and table 1. During baseline recordings, there were no significant differences between groups in LVSP, CO, and HR. Consequently, RPP, as a major determinant of myocardial oxygen consumption, was not significantly different between groups during baseline conditions.

The LVSP and CO were reduced after ketamine (LVSP: by 16 ± 18%, $P = 0.02$ *vs.* baseline; CO: by 11 ± 12%, $P = 0.7$ *vs.* baseline) and *S*(+)-ketamine application (LVSP: by 17 ± 18%, $P = 0.12$ *vs.* baseline; CO: by 13 ± 11%, $P = 0.77$ *vs.* baseline). The preconditioning ischemia led to a further decrease of CO by 8 ± 12% in the ketamine–preconditioning group and by 12 ± 12% in the *S*(+)-ketamine–preconditioning group. In the preconditioning group, the preconditioning ischemia had only a small effect on LVSP (reduced by 4 ± 9%, $P = 1.0$ *vs.* baseline) and CO (reduced by 8 ± 14%, $P = 1.0$ *vs.* baseline). During preconditioning, τ increased (by 21 ± 15% in the preconditioning group [$P = 0.79$ *vs.* baseline], 12 ± 12% in the ketamine–preconditioning group [$P = 0.96$ *vs.* baseline] and 41 ± 18% in the *S*(+)-ketamine–preconditioning group [$P < 0.01$ *vs.* baseline]), indicating an impaired diastolic function.

During the 10 min reperfusion period before prolonged ischemia, LVSP recovered to 92 ± 11% in the ketamine–preconditioning group ($P = 0.84$ *vs.* baseline), to 89 ± 13% in the *S*(+)-ketamine–preconditioning group ($P = 0.73$ *vs.* baseline), and to 96 ± 9% in the preconditioning group ($P = 1.0$ *vs.* baseline).

In all groups, coronary artery occlusion was accompanied by a reduction of LVSP (by a mean of 17 ± 14%, $P < 0.01$ *vs.* baseline), dP/dtmax (by a mean of 24 ± 21%, $P < 0.01$ *vs.* baseline), and CO (by a mean of 16 ± 19%, $P < 0.01$ *vs.* baseline; table 1 and fig. 1). RPP did not significantly differ among groups. With regard to LV relaxation, τ increased by a mean of 18 ± 19% ($P < 0.01$ *vs.* baseline) and LV end-diastolic pressure (LVEDP) by a mean of 75 ± 77% ($P < 0.01$ *vs.* baseline) during coronary artery occlusion (all values at 25 min of coronary artery occlusion). After 2 h of reperfusion, LVSP and dP/dtmax were reduced by 23 ± 19% (LVSP, $P < 0.01$) and 37 ± 20% (dP/dtmax, $P < 0.01$) of baseline values in all groups, still reflecting impaired myocardial contractile function in all groups at the end of the experiments. HR decreased by 11 ± 11% in all groups ($P < 0.01$ *vs.* baseline). As a consequence of the reduction in HR and LVSP, RPP was reduced by a mean of 31 ± 22% ($P < 0.01$ *vs.* baseline), with no significant differences between the preconditioning and other groups. Diastolic function also remained depressed during the reperfusion period. τ was prolonged by a mean of 31 ± 33% ($P < 0.01$ *vs.* baseline) and LVEDP remained increased by a mean of 2.3 ± 2.3 mmHg at the end of the experiment ($P = 0.09$ *vs.* baseline).

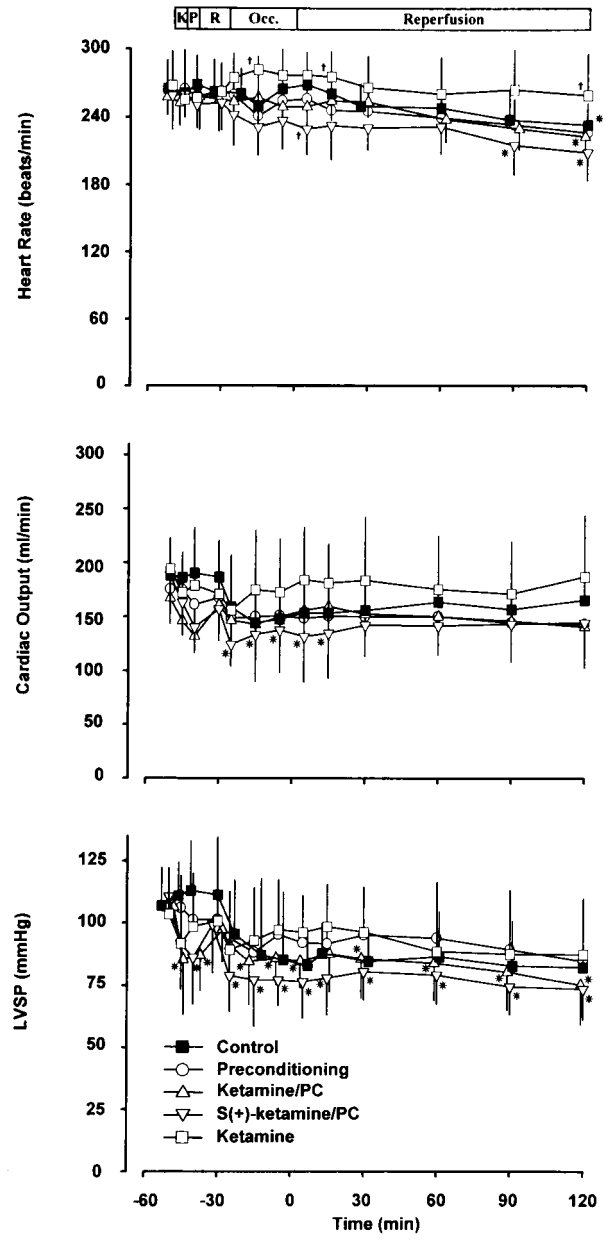

Fig. 1. Line plot showing the time course of heart rate, cardiac output, and left ventricular peak systolic pressure (LVSP) during experiments in the control, preconditioning, ketamine–preconditioning, *S*(+)-ketamine–preconditioning, and ketamine groups. Data are mean ± SD. K = ketamine or vehicle application; P = ischemic preconditioning; R = 10-min reperfusion period after ischemic preconditioning; Occ = time of coronary artery occlusion. *$P < 0.05$ *versus* baseline conditions; †$P < 0.05$ compared with preconditioning group.

Table 1. Hemodynamic Variables

		Intervention Period			Occlusion (25 min)	Reperfusion		
	Baseline	Ket. or Vehic.	PC	Reperfusion		5 min	30 min	120 min
LVEDP (mmHg)								
Control	7.4 ± 1.7	8.2 ± 1.7	9.1 ± 3.8	9.3 ± 4.4	14.0 ± 5.2	14.5 ± 7.3*	12.7 ± 6.7	11.4 ± 3.5
Preconditioning	9.3 ± 2.4	8.9 ± 2.2	11.5 ± 3.2	9.0 ± 3.1	12.3 ± 3.4	12.0 ± 4.9	10.4 ± 3.8	11.2 ± 5.1
Ketamine–PC	8.6 ± 2.8	9.0 ± 2.6	12.2 ± 3.1	10.3 ± 2.6	14.2 ± 3.6	12.6 ± 3.7	12.2 ± 2.5	12.8 ± 4.4
S(+)-ketamine–PC	10.6 ± 5.3	10.5 ± 5.2	13.4 ± 6.7	11.1 ± 6.2	15.2 ± 8.4	13.4 ± 7.7	9.4 ± 1.2	11.4 ± 3.7
Ketamine	7.2 ± 4.3	7.8 ± 4.0	7.3 ± 4.2	7.5 ± 4.1	10.0 ± 3.5	11.9 ± 5.0	8.3 ± 4.5	9.4 ± 6.1
dP/dt_{max} (mmHg/s)								
Control	5,287 ± 1,061	5,501 ± 786	5,548 ± 1,009	5,307 ± 879	3,794 ± 1,106*	2,706 ± 496*†	3,365 ± 803*	3,192 ± 860*
Preconditioning	5,876 ± 1,216	6,047 ± 1,241	5,121 ± 1,079	4,619 ± 889	4,765 ± 1,212	3,842 ± 847*	4,227 ± 1,161*	3,767 ± 1,411*
Ketamine–PC	5,660 ± 603	4,183 ± 1,130*†	4,079 ± 910*	4,183 ± 698*	4,009 ± 931*	3,547 ± 1,041*	3,959 ± 1,174*	3,352 ± 1,103*
S(+)-ketamine–PC	5,289 ± 884	3,801 ± 808*†	3,196 ± 751*†	3,451 ± 541*†	3,029 ± 830*†	2,468 ± 755*†	2,946 ± 422*†	2,715 ± 642*
Ketamine	4,770 ± 999	3,428 ± 1,047†	3,951 ± 1,038	4,190 ± 1,028	4,426 ± 1,197	3,648 ± 1,322	4,205 ± 1,059	3,505 ± 1,522
SVR (mmHg · min · l^{-1})								
Control	467 ± 75	490 ± 72	481 ± 68	475 ± 69	435 ± 102†	427 ± 82	440 ± 73	382 ± 89
Preconditioning	544 ± 130	535 ± 121	581 ± 145	532 ± 127	577 ± 177	544 ± 159	563 ± 158	467 ± 148
Ketamine–PC	570 ± 83	478 ± 134	567 ± 125	527 ± 101	501 ± 78	468 ± 92	482 ± 122	411 ± 84*
S(+)-ketamine–PC	522 ± 70	465 ± 117	521 ± 108	562 ± 79	476 ± 70	474 ± 93	481 ± 115	413 ± 78
Ketamine	489 ± 123	485 ± 186	533 ± 219	571 ± 231	527 ± 177	484 ± 154	488 ± 180	422 ± 163
RPP (mmHg · min^{-1} · 10^3)								
Control	28.4 ± 4.7	29.0 ± 4.2	30.5 ± 6.7	29.4 ± 7.2	20.7 ± 7.8*	21.4 ± 3.4	21.5 ± 3.4	19.2 ± 3.1*
Preconditioning	27.8 ± 6.4	28.1 ± 5.4	26.2 ± 4.7	26.7 ± 4.7	24.4 ± 5.2	23.8 ± 6.0	23.3 ± 4.9	19.4 ± 6.5*
Ketamine–PC	27.5 ± 2.6	21.8 ± 6.9	22.2 ± 4.2	25.0 ± 4.5	21.5 ± 3.2*	21.1 ± 2.9*	21.8 ± 4.4	16.9 ± 4.0*
S(+)-ketamine–PC	28.7 ± 3.6	23.8 ± 5.1*	21.6 ± 4.7*	25.3 ± 6.1	18.4 ± 3.3*	17.6 ± 3.7*†	18.6 ± 3.1*	15.5 ± 3.8*
Ketamine	27.8 ± 5.3	23.8 ± 7.6	25.6 ± 7.2	26.6 ± 6.1	27.1 ± 6.3	26.8 ± 4.7	25.7 ± 5.0	22.8 ± 6.6
τ (ms)								
Control	18.0 ± 3.8	16.4 ± 3.9	17.3 ± 4.9	17.5 ± 4.5	21.3 ± 3.3†	22.2 ± 3.8†	20.8 ± 4.1	21.1 ± 3.5
Preconditioning	14.8 ± 3.7	14.7 ± 3.5	17.8 ± 4.4	15.9 ± 4.0	16.9 ± 4.1	16.9 ± 4.3	17.3 ± 4.5	19.8 ± 7.3
Ketamine–PC	19.4 ± 3.5†	19.4 ± 4.2†	21.6 ± 4.1	19.4 ± 2.6	20.5 ± 2.7	20.5 ± 4.9	21.1 ± 4.4	22.5 ± 4.2
S(+)-ketamine–PC	15.9 ± 1.3	18.4 ± 2.4	22.3 ± 1.9*	18.8 ± 1.9*	20.8 ± 2.5*	21.4 ± 2.3*	21.5 ± 1.9*	25.5 ± 2.8*
Ketamine	14.9 ± 2.3	15.1 ± 3.2	15.5 ± 3.0	15.7 ± 3.3	16.8 ± 3.7	16.0 ± 2.9	15.4 ± 2.8	18.3 ± 5.8

Data are mean ± SD.

* $P < 0.05$ compared with baseline. † $P < 0.05$ compared with preconditioning group.

Ket. or Vehic. = ketamine or vehicle administration; PC = ischemic preconditioning; LVEDP = left ventricular end-diastolic pressure; dP/dt_{max} = maximum rate of increase in left ventricular pressure; SVR = systemic vascular resistance; RPP = rate pressure product; τ = time constant of decrease in isovolumic left ventricular pressure.

Infarct Size

Mean LV dry weight was 0.77 ± 0.15 g, with no significant differences between groups (data from individual groups are shown in table 2). The ischemic-reperfused area (area at risk) was 0.37 ± 0.14 g and the area at risk constituted 49 ± 16% of the LV, with no significant differences between groups. Preconditioning significantly reduced infarct size from 45 ± 16% of the area at risk (control group) to 24 ± 17% ($P = 0.03$ preconditioning *vs.* control group; fig. 2). Pretreatment with ketamine had no effect on infarct size (48 ± 18%) in animals without preconditioning (ketamine group) but blocked preconditioning, as evidenced by an infarct size of 45 ± 19% in the ketamine–preconditioning group ($P = 0.03$ *vs.* preconditioning group). In contrast, *S*(+)-ketamine had no effect on infarct size reduction induced by preconditioning (25 ± 11%; $P = 1.0$ *vs.* preconditioning group).

Discussion

The main finding of our study is that a single dose of ketamine blocks the protective effect of preconditioning in the rabbit heart *in vivo*, but *S*(+)-ketamine does not. Therefore, the influence of ketamine on preconditioning *in vivo* is most likely enantiomer specific.

Critique of Methods

Variables that are important determinants for development of myocardial infarction are duration of ischemia and myocardial oxygen consumption during ischemia, collateral blood flow toward the ischemic area, and myocardial temperature during ischemia. The rabbit has a consistently small collateral circulation[7] and, therefore, it was not necessary to assess collateral blood flow in the ischemic area. Temperature was shown to influence infarct size even in the "normothermic range" to a large extent in the open chest rabbit.[8] Therefore, in this study, temperature was measured inside the pericardium and kept within a narrow range of 0.4°C. All experiments were peformed during anesthesia with α-chloralose. This type of anesthesia maintains near-normal cardiovascular reflexes comparable with the awake state and is a classic anesthetic for physiologic and pharmacologic experiments.[9] An effect of α-chloralose on preconditioning has not been studied so far.

Ketamine or *S*(+)-ketamine 10 mg/kg was administered by intravenous bolus injection 5 min before pre-

Table 2. Weights and Area at Risk Size

	Control	Preconditioning	Ketamine–PC	*S*(+)-ketamine–PC	Ketamine
Body weight (kg)	3.10 ± 0.06	2.82 ± 0.03	2.77 ± 0.03	3.09 ± 0.03	2.90 ± 0.04
LV weight (g)	0.80 ± 0.08	0.79 ± 0.23	0.75 ± 0.16	0.77 ± 0.08	0.71 ± 0.10
Area at risk (g)	0.39 ± 0.10	0.47 ± 0.17	0.32 ± 0.12	0.37 ± 0.17	0.34 ± 0.15
Area at risk/LV (%)	48.8 ± 9.02	60.3 ± 18.1	43.7 ± 16.5	48.7 ± 22.0	48.7 ± 21.3
Infarct size (g)	0.17 ± 0.07	0.11 ± 0.08	0.14 ± 0.07	0.09 ± 0.06	0.16 ± 0.06

Data are mean ± SD.

PC = ischemic preconditioning; LV = left ventricle.

conditioning ischemia. In humans, the peak plasma concentration of ketamine is 3–60 μM after intravenous injection of ketamine 2 mg/kg.[10] Pedraz *et al.*[11] reported a peak plasma concentration of 63 μM after administration of 10 mg/kg ketamine by intravenous bolus injection in the rabbit. Therefore, the plasma levels of ketamine in the current study can be assumed to be in the same range as those achieved in patients after administration of 2 mg/kg ketamine.

We hypothesized that the effect of ketamine and *S*(+)-ketamine on preconditioning is a receptor-mediated mechanism. Therefore, we did not use "equianesthetic" concentrations but the same dose of both drugs to achieve identical plasma concentrations. However, the anesthetic potency of ketamine and *S*(+)-ketamine is different, and differences in anesthetic depth might have influenced the severity of myocardial ischemia.

Interpretation of Results

Brief periods of myocardial ischemia followed by reperfusion provide strong endogenous myocyte protection from a subsequent ischemic injury. This concept, known as "ischemic preconditioning," has been shown to occur in all animals and humans studied.[2] Although the signal transduction pathway of preconditioning is not fully understood, overwhelming evidence suggests that the K_{ATP} channel is an important component of this phenomena and may serve as an end effector.[12–14] Ko *et al.*[4] reported a concentration-dependent inhibitory effect of ketamine on K_{ATP} channel activity in isolated cardiomyocytes. Based on this finding, we hypothesized that ketamine might block preconditioning. A single bolus dose of ketamine was sufficient to block the protective effects of preconditioning in the rabbit heart *in vivo*, whereas the administration of the same dose of ketamine in the absence of preconditioning did not influence infarct size. Ketamine is a 1:1 racemic mixture of two optically active isomers with different effects on cardiac[15] and central nervous system receptors.[16] In general, stereoselectivity is a function of the potency or affinity of the more potent isomer to specific macromolecular receptors, which are themselves stereospecific. Therefore, we hypothesized that the blockade of preconditioning by ketamine may be stereospecific. Indeed, administration of *S*(+)-ketamine before the preconditioning ischemia did not alter the protective effect of preconditioning *in vivo* because infarct size in this group was not different compared with the preconditioning group. Therefore, the blockade of ischemic preconditioning by racemic ketamine is most likely caused by the *R*(−)-isomer. This is in accordance with the results of Molojavyi *et al.* in this issue, where *R*(−)-ketamine blocked the cardioprotective effects of ischemic preconditioning in isolated rat hearts.[5] In the current study, the isolated effects of the *R*(−)-isomer were not tested.

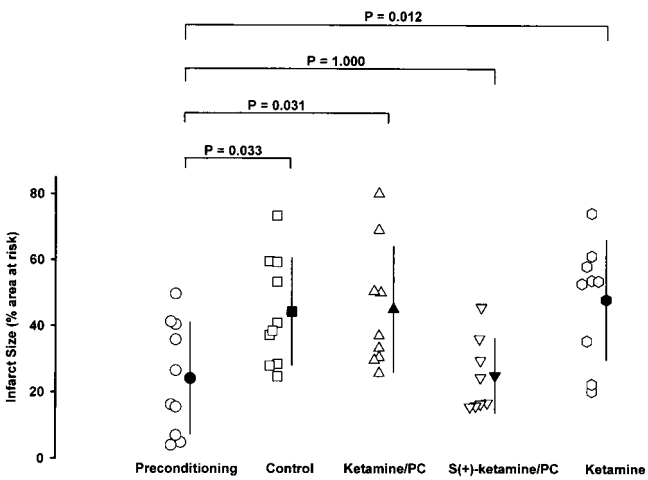

Fig. 2. Infarct size as a percentage of the area at risk in the preconditioning, control, ketamine–preconditioning, *S*(+)-ketamine–preconditioning, and ketamine groups; PC = ischemic preconditioning. Open symbols = single data points, filled symbols = mean ± SD.

Based on our findings, it is not possible to determine the site of action of ketamine on preconditioning. However, the stereoselectivity of this effect suggests a receptor-mediated mechanism. A viable candidate for such a receptor may be the sulfonylurea receptor of the K_{ATP} channel. Binding of an agonist prevents opening of this channel during ischemia.

The result of the current study is in accordance with a recent *in vitro* study from our labaratory.[5] In that study, the effects of ketamine, *R*(−)- and *S*(+)-ketamine on preconditioning were investigated in isolated rat hearts subjected to 30 min of global ischemia. The improved functional recovery and reduced creatine kinase release seen in that model after preconditioning was blocked by

20 μg/ml but not by 2 μg/ml ketamine. The blockade was caused by the *R*(−)-enantiomer, whereas *S*(+)-ketamine had no effect on preconditioning. The current study confirms these findings for a single bolus injection of ketamine before preconditioning.

However, *in vivo*, the pathophysiology of preconditioning and of ischemia–reperfusion is more complex and other effects of ketamine must be considered, *i.e.,* hemodynamic side effects, effects on anesthetic depth, or effects on the mechanism of reperfusion injury.

Hemodynamic Effects. *In vitro* data suggests that ketamine and the two optical isomers exert direct cardiac depressive effects in a dose-dependent manner,[15] with *S*(+)-ketamine inducing less cardiac depression because of an increased availability of catecholamines.[15,17] A reduction of myocardial inotropy increases ischemic tolerance. However, it is unlikely that the cardiodepressive effects of ketamine and *S*(+)-ketamine contributed to differences in infarct size. Administration of both drugs induced a similar decrease in LVSP and CO, but both parameters recovered to values not significantly different from baseline values before the 30-min ischemia. RPP, a major determinant of myocardial oxygen demand, was not different between the groups during the 30 min of ischemia.

Anesthetic Depth. Sympathetic activation caused by surgical trauma may lead to reduced ischemic tolerance. *S*(+)-ketamine is a more potent anesthetic agent,[18] but a less cardiodepressant drug than is ketamine.[17] We observed similar hemodynamic depressant effects caused by *S*(+)-ketamine in comparison with ketamine. Therefore, the more cardiodepressant effect of *S*(+)-ketamine caused by an increase in anesthetic depth might be "counterparted" by a less direct negative inotropic effect. In addition, stimulation of cardiac adrenergic receptors can induce preconditioning because of the potential link of adrenergic receptors to K_{ATP} channels.[19] Therefore, we cannot rule out the possibility that an increased catecholamine level induced by *S*(+)-ketamine might have contributed to the maintained cardioprotection.

Interference with Reperfusion Injury. Reperfusion itself can cause additional cellular damage and may increase the extent of myocardial infarction. Some anesthetics interfere directly with the mechanisms of reperfusion injury at the myocardial level.[20,21] For ketamine, direct effects on myocardial reperfusion injury have not been investigated. However, postischemic endothelial adherence of neutrophils in the coronary system is a major secondary mediator of reperfusion injury.[22] Szekely *et al*. reported a reduction of postischemic adherence of neutrophils in the coronary system of isolated guinea pig hearts by *S*(+)-ketamine, whereas *R*(−)-ketamine worsened coronary vascular leak.[23] Therefore, a reduction in reperfusion injury by *S*(+)-ketamine might have contributed to the infarct size reduction in *S*(+)-ketamine–treated animals *in vivo*.

Several previous studies used ketamine as part of the anesthetic regime for investigating preconditioning, and it is surprising that preconditioning was still possible. Cason *et al*.[24] and Ismaeil *et al*.[25] investigated anesthetic-induced preconditioning in rabbits sedated preoperatively with 70 mg/kg intramuscular ketamine while anesthesia was maintained with intravenous propofol during the remainder of the experiment. Haessler *et al*.[26] used 67 mg/kg intramuscular ketamine for induction of anesthesia followed by a continuous infusion of 20 $mg \cdot kg^{-1} \cdot h^{-1}$ during the experiment. Although the plasma concentrations of ketamine were neither measured in these nor in the current study, it is likely that ketamine plasma concentration was higher in the current study 5 min after intravenous bolus administration of 10 mg/kg ketamine, when preconditioning was elicited.

Walsh *et al.*[13] reported that glibenclamide has anti-preconditioning effects in a rabbit model during combination ketamine (1.5 $mg{\cdot}kg^{-1}{\cdot}min^{-1}$) and xylazine (0.75 $mg{\cdot}kg^{-1}{\cdot}min^{-1}$) anesthesia, but not during pentobarbital anesthesia. Thornton *et al.*[27] found that the K_{ATP} channel blocker glibenclamide was proischemic, but did not eliminate preconditioning in rabbits anesthetized with pentobarbital. In contrast, Toombs *et al.*[28] reported that glibenclamide blocked preconditioning in a nearly identical rabbit model, with the exception that anesthesia was maintained with repeated intramuscular injections of ketamine (10 mg/kg) and xylazine (2 mg/kg). These seemingly conflicting results were explained by the study of Morita *et al.*,[29] who reported that ketamine anesthesia enhanced the blocking effect of glibenclamide on K_{ATP} channels. Taken together, these findings suggest that the glibenclamide dose used in some of the studies only blocked preconditioning in addition with ketamine as a basal anesthetic. The current study showed also a direct blocking effect of ketamine on preconditioning in a similar rabbit model. The role of anesthesia may be critical in determining, evaluating, and reevaluating the results of studies that investigate myocardial preconditioning.

Preconditioning is still a laboratory-based phenomena that has not been conclusively documented in patients. However, some *in vitro* evidence[2] of preconditioning in humans exists, and there are several possible clinical scenarios in which preconditioning might occur, *e.g.*, unstable angina preceding myocardial infarction, percutaneous transluminal coronary angioplasty, and warm-up angina. It is therefore of great practical interest to avoid the use of anesthetic agents that block this strongest endogenous cardioprotective mechanism against myocardial ischemia. In contrast to the detrimental effects of K_{ATP} channel blockade, use of K_{ATP} agonists, including anesthetics such as isoflurane[14] or opiods,[30] is suggested to provide organ protection intraoperatively during cardiac, vascular, or neurologic surgery. However, clinical evidence for the role of anesthetic agents in improving

morbidity and mortality in patients at risk for ischemic or hypoxic injury is lacking.

In summary, the current data indicate that a single bolus dose of ketamine blocks the cardioprotective effect of preconditioning *in vivo*, but *S*(+)-ketamine does not. Therefore, the influence of ketamine on preconditioning is most likely enantiomer specific.

The authors thank T. Wietschorke, cand.med., Institut für Herz- und Kreislaufphysiologie, for excellent technical assistance.

References

1. Murry CE, Jennings RB, Reimer KA: Preconditioning with ischemia: A delay of lethal cell injury in ischemic myocardium. Circulation 1986; 74:1124–36

2. Arstall MA, Zhao YZ, Hornberger L, Kennedy SP, Buchholz RA, Osathanondh R, Kelly RA: Human ventricular myocytes in vitro exhibit both early and delayed preconditioning responses to simulated ischemia. J Mol Cell Cardiol 1998; 30:1019–25

3. Gross GJ, Fryer RM: Sarcolemmal versus mitochondrial ATP-sensitive K^+ channels and myocardial preconditioning. Circ Res 1999; 84:973–9

4. 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

5. 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 2000; 94:623–9

6. Brutsaert DL, Rademakers FE, Sys SU, Gillebert TC, Housmans PR: Analysis of relaxation in the evaluation of ventricular function of the heart. Prog Cardiovasc Dis 1985; 28:143–63

7. Maxwell MP, Hearse DJ, Yellon DM: Species variation in the coronary collateral circulation during regional myocardial ischaemia: A critical determinant of the rate of evolution and extent of myocardail infarction. Cardiovasc Res 1987; 21:737–46

8. Chien GL, Wolff RA, Davis RF, Van Winkle DM: "Normothermic range" temperature affects myocardial infarct size. Cardiovasc Res 1994; 28:1007–14

9. Armstrong, GG, Porter H, Langston JB: Alteration of carotid occlusion response by anesthesia. Am J Physiol 1961; 201:897–900

10. Idvall J, Ahlgren I, Aronsen KF, Stenberg P: Ketamine infusions: Pharmacokinetics and clinical effects. Br J Anaesth 1979; 51:1167–73

11. Pedraz JL, Lanao JM, Dominguez-Gil A: Kinetics of ketamine and its metabolites in rabbits with normal and impaired renal function. Eur J Drug Metab Pharmacokinet 1985; 10(1):35–9

12. Bernardo NL, D'Angelo M, Okubo S, Joy A, Kukreja RC: Delayed PC is mediated by opening of ATP-sensitive potassium channels in the rabbit heart. Am J Physiol 1999; 276:H1323–30

13. Walsh RS, Tsuchida A, Daly JJF, Thornton JD, Cohen MV, Downey JM: Ketamine-xylazine anaesthesia permits a K_{ATP} channel antagonist to attenuate preconditioning in rabbit myocardium. Cardiovasc Res 1994; 28:1337–41

14. Kersten JR, Schmeling TJ, Pagel PS, Gross GJ, Warltier DC: Isoflurane mimics PC via activation of K_{ATP}-channels: Reduction of myocardial infarct size with an acute memory phase. ANESTHESIOLOGY 1997; 87:361–70

15. Graf BM, Vicenzi MN, Martin E, Bosnjak J, Stowe DF: Ketamine has stereospecific effects in the isolated perfused guinea pig heart. ANESTHESIOLOGY 1995; 82:1426–37

16. Zeilhofer HU, Swandulla D, Geisslinger G, Brune K: Differential effects of ketamine enantiomers on NMDA receptor currents in cultured neurons. Eur J Pharmacol 1992; 213:155–8

17. Kunst G, Martin E, Graf BN, Hagl S, Vahl CF: Actions of ketamine and its isomers on contractility and calcium transients in human myocardium. ANESTHESIOLOGY 1999; 90:1363–71

18. White PF, Ham J, Way WL, Trevor A: J. Pharmacology of ketamine isomers in surgical patients. ANESTHESIOLOGY 1980; 52:231–9

19. Bankwala Z, Hale SL, Kloner RA: Alpha-adrenoceptor stimulation with exogenous norepinephrine or release of endogenous catecholamines mimics PC. Circulation 1994; 90(2):1023–8

20. Schlack W, Hollmann M, Stunneck J, Thämer V: Effect of halothane on myocardial reoxygenation injury in the isolated rat heart. Br J Anaesth 1996; 76,860–7

21. Schlack W, Preckel B, Barthel H, Obal D, Thämer V: Halothane reduces reperfusion injury after regional ischaemia in the rabbit heart in vivo. Br J Anaesth 1997; 79:88–96

22. Jennings RB, Yellon DM: Reperfusion injury, The Pathophysiology of Reperfusion and Reperfusion Injury. Edited by Yellon DM, Jennings RB. New York, Raven Press, 1992, pp 1–11

23. Szekely A, Heindl B, Zahler S, Conzen PF, Becker BF: S(+)-ketamine, but not R(-)-ketamine, reduces postischemic adherence of neutrophils in the coronary system of isolated guinea pig hearts. Anesth Analg 1999; 88:1017–24

24. Cason BA, Gamperl AK, Slocum RE, Hickey RF: Anesthetic-induced preconditioning: Previous administration of isoflurane decreases myocardial infarct size in rabbits. ANESTHESIOLOGY 1997; 87:1182–90

25. Ismaeil MS, Tkachenko I, Gamperl AK, Hickey RF, Cason BA: Mechanisms of isoflurane-induced myocardial preconditioning in rabbits. ANESTHESIOLOGY 1999; 90:812–21

26. Haessler R, Kuzume K, Chien GL, Wolff RA, Davis RF, Van Winkle DM: Anaesthetics alter the magnitude of infarct limitation by ischemic preconditioning. Cardiovasc Res 1994; 28:1574–80

27. Thornton JD, Thornton CS, Sterling DL, Downey JM: Blockade of ATP-sensitive potassium channels increases infarct size but does not prevent preconditioning in rabbit hearts. Circ Res 1993; 72:44–9

28. Toombs CF, Moore TL, Shebuski RJ: Limitation of infarct size in the rabbit by PC is reversible with glibenclamide. Cardiovasc Res 1993; 27:617–22

29. Morita Y, Murakami T, Iwase T, Nagai K, Nawada R, Kouchi I, Akao M, Sasayama S: K(ATP) channels contribute to the cardioprotection of preconditioning independent of anaesthetics in rabbit hearts. J Mol Cell Cardiol 1997; 29: 1267–76

30. Miki T, Cohen MV, Downey JM: Opiod receptor contributes to PC through protein kinase C activation in rabbits. Mol Cell Biochem 1998;186:3–12