Ketamine Has Stereospecific Effects in the Isolated Perfused Guinea Pig Heart

Bernhard M. Graf, M.D.,* Martin N. Vicenzi, M.D.,† Elke Martin, M.D.,‡ Zeljko J. Bosnjak, Ph.D.,§ David F. Stowe, M.D., Ph.D.§

Background: S(+)Ketamine is judged to produce more potent anesthesia than either the racemate or the R(−) ketamine isomer because of differential activation of specific cerebral receptors. Other than central nervous system effects, the most important side effects of ketamine occur in the cardiovascular system. We examined the direct cardiac effects of the isomers and the racemate of ketamine in the isolated perfused guinea pig heart.

Methods: Twenty-three guinea pig hearts were perfused by the Langendorff technique with modified 37°C Krebs–Ringer's solution (97% oxygen and 5% carbon dioxide) at a constant perfusion pressure. Eight animals were pretreated with reserpine to deplete hearts of catecholamines. These pretreated hearts were also perfused with Krebs–Ringer's solution containing propranolol, phenoxycyanazine, and atropine to block any remaining effects of catecholamines and of acetylcholine. Five additional hearts were perfused with naloxone to block cardiac opioid receptors. Ten hearts were not treated.

All 23 hearts were then exposed to four increasing equimolar concentrations of each isomer and the racemate of ketamine for 10 min. Heart rate, atrioventricular conduction time (AVCT), left ventricular pressure, coronary flow, and inflow and outflow oxygen tensions were measured. Percentage oxygen extraction, oxygen delivery, and oxygen consumption were calculated.

Results: Both isomers and the racemate caused a concentration-dependent depression of systolic left ventricular pressure and an increase in AVCT. In the untreated hearts, S(+)ketamine decreased heart rate and left ventricular pressure and, at higher concentrations, oxygen consumption and percentage oxygen extraction significantly less than R(−)ketamine independent of blocked or unblocked opioid receptors. Racemic ketamine depressed cardiac function to a degree intermediate to that produced by the isomers. Coronary flow and AVCT were equally affected by the isomers and by the racemic mixture. In the catecholamine-depleted hearts both isomers and the racemate caused equitropic depression of all variables. In these hearts cardiac depression was greater, and AVCT, coronary flow, and oxygen delivery were significantly greater than in untreated and opioid receptor–blocked hearts.

Conclusions: Lesser cardiac depression by the S(+) isomer is attributable to an increased availability of catecholamines, because previous depletion of catecholamine stores and autonomic blockade completely inhibited these differences. The inability of cardiac tissue to reuptake released catecholamines into neuronal or extraneuronal sites during exposure to ketamine is stereoselective and caused predominantly by the S(+) isomer. Cardiac opioid receptors are apparently not involved in this phenomenon. (Key words: Anesthetics, intravenous; ketamine; stereoisomers. Animals: guinea pig heart; autonomic blockade; catecholamine depletion; coronary flow; electrophysiology; isolated perfused; left ventricular pressure; opioid blockade; oxygen consumption.)

KETAMINE is used clinically as a 1:1 racemic mixture of both optically active isomers that differ in their analgesic and psychomimetic effects. It is well established that individual optical isomers of a number of psychomimetic compounds including hallucinogens,1 amphetamines,2 opioids,3 and sedative–hypnotics7 differ in their pharmacologic properties. Both forms of ketamine, R(−)-ketamine and S(+)ketamine, are known to be effective analgesics and hypnotics. However, S(+)ketamine is about four times more potent in these

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Received from the Anesthesiology Research Laboratory, Department of Anesthesiology, and the Department of Physiology, Cardiovascular Research Center, The Medical College of Wisconsin and Department of Veterans Affairs Medical Center, Milwaukee, Wisconsin, and from the Clinic of Anesthesiology, Universität Heidelberg, Heidelberg, Germany. Submitted for publication September 6, 1994. Accepted for publication February 28, 1995. Supported by a fellowship grant from the American Heart Association of Wisconsin (to Dr. Graf) and by Department of Veterans Affairs Merit grant 8204-04P (to Dr. Stowe). Presented in part at the annual meeting of the American Society of Anesthesiologists, San Francisco, California, October 1994.

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respects than R(-)-ketamine. The finding that these effects are stereoselective is consistent with a receptor-mediated, pharmacologic mechanism. It has been shown that ketamine analogs result from non-competitive blockade of the N-methyl-D-aspartate (NMDA) receptor, a ligand-gated ion channel composed of at least two different protein subunits (NMR 1 and NMR 2). The phenylcyclidine (PCP)-like dissociative anesthetic ketamine acts by occupying specifically a site (the PCP binding site) within the NMDA receptor, so that entry of Na+ and Ca2+ into target cells is blocked. The affinity of ketamine isomers to the PCP binding site within the NMDA receptor correlates well with their analgesic potency. In contrast, psychomimetic emergence reactions and agitated behavior are far less common with S(+)-ketamine than with its R(-) isomer or with the racemate of ketamine due, at least in part, to a higher affinity of the R(-) isomer to the sigma opiate binding site.

Numerous studies in animals and humans confirm that there are both quantitative and qualitative differences between central nervous system effects of ketamine isomers. Stereoselective differences have been observed in the affinity of ketamine for NMDA receptors, and in effects of ketamine on NMDA receptor currents, on catecholamine reuptake and on acetyl cholinesterase activity. After central nervous system effects, the most important side effects of anesthetics are those on the cardiovascular system. Ketamine administered to humans and animals has been reported to increase heart rate, blood pressure and cardiac output, but these findings in vivo are not consistent with those reported in several models in vitro. Direct facilitory actions of racemic ketamine on myocardial contractility have been repeatedly discounted because of the many reports from isolated preparations consistently failing to demonstrate a direct positive effect of ketamine on contractile function. In fact, previous investigations with heart muscle strips or with whole cardiac preparations exposed to autonomic blocking agents have demonstrated primarily the opposite, a depressant effect of ketamine on myocardial contractility. In studies of ketamine in which there was no autonomic blockade it has been occasionally observed that there is a small positive inotropic and chronotropic effect at lesser concentrations of ketamine. These effects, however, were not seen after beta-adrenergic receptor blockade or after depletion of endogenous catecholamine stores. The current consensus, therefore, is that ketamine stimulates cardiac function indirectly by altering autonomic nervous system activity but that it directly depresses cardiac function.

Although the stereospecific, receptor-mediated, central and psychomimetic effects of ketamine isomers have been well investigated, the question of direct cardiovascular stereoselectivity has not been addressed. Therefore, the purpose of this study was to compare the direct actions of the isomers and the racemate of ketamine on mechanical, electric, and metabolic activity in the isolated, perfused heart devoid of influences by autonomic reflexes, hormones, and metabolites of ketamine. The use of the isolated perfused heart preparation as a useful model in vitro for the assessment of anesthetic effects has been documented frequently in our laboratory.

Materials and Methods

After approval was obtained from the Animal Studies Committee of the Medical College of Wisconsin, 10 mg ketamine and 1,000 U heparin were injected intraperitoneally into each of 23 English short-haired, albino guinea pigs (300–350 g). Eight of the guinea pigs were pretreated with reserpine injections (2 mg·kg 1·day 1, intraperitoneal) for 2 days before the experiments to deplete endogenous catecholamine stores. Each animal was decapitated, and the heart was rapidly excised during continuous retrograde aortic perfusion with cold, oxygenated, modified Krebs-Ringer's solution (equilibrated with 97% oxygen and 3% carbon dioxide). A description of the surgical preparation for this model has been reported in detail previously.

After placement in the Langendorff apparatus, the hearts were perfused through the aortic cannula with nonrecirculated and oxygenated Krebs-Ringer's solution at a constant perfusion pressure of 55 mmHg (75 cm fluid column). The perfusion solution had the following composition (millimolar): NaCl 137, KCl 4.5, MgCl 1.2, CaCl 2.5, Cl 134, HCO3 15.5, H2PO4 1.2, glucose 11.5, pyruvate 2, mannitol 16, and ethylene-diamine tetraacetic acid 0.05, and insulin 5 U/L. Perfusion and bath temperature were maintained at 36.5 ± 0.2°C by a thermostatically controlled water circulator.

Left ventricular pressure was continuously recorded isovolumetrically with a transducer (Gould-Statham P23, Gould Electronics, Elk Grove, IL), connected to a thin, saline-filled latex balloon (Hugo Sachs Electronic KG, March, Germany) that was inserted into the
left ventricle through the mitral valve from a cut in the left atrium. The balloon volume was adjusted to maintain an initial diastolic left ventricular pressure (DLVP) of 0 mmHg during the control period so that any increase in DLVP reflects an increase in left ventricular wall stiffness or diastolic contracture. The volume of the balloon was unchanged during the experimental period. Two pairs of bipolar silver electrodes (polytetrafluoroethylene-coated silver, diameter 125 μm, Cooner Wire, Chatsworth, CA) were placed on the right atrium and the pulmonary conus to monitor atrioatrial and atrioventricular time. Spontaneous atrial heart rate was determined from the right atrial beat-to-beat interval. Atrioventricular conduction time (AVCT) was determined from the right atrial to the right ventricular pulmonary conus beat-to-beat interval. Coronary inflow was measured under constant pressure and at constant temperature by a transit-time in-line ultrasound flow meter (Research Flowmeter T106, Transonic Systems, Ithaca, NY). To determine maximal coronary flow, adenosine (0.2 ml of a 200 μM stock solution) was injected directly into the aortic root cannula during the initial control period and after the last control reading. Coronary sinus effluent was collected by placing a small catheter into the right ventricle through the pulmonary artery after ligating both venae cavae.

Oxygen tensions of the coronary inflow and outflow were measured continuously on-line by temperature-controlled miniature Clark electrodes (203B, Instech Laboratories, Plymouth Meeting, PA) calibrated periodically with 21% and 97% oxygen to adjust oxygen tension to 150 and 650 mmHg, respectively. These measurements were verified off-line with an intermittently self-calibrating gas analyzer (Radiometer ABL-2, Metcon Chicago, Des Plaines, IL). Oxygen delivery (DO₂) was calculated as inflow oxygen tension in mmHg multiplied by oxygen solubility (24 μM per ml Krebs–Ringer’s solution at 760 mmHg oxygen and 37°C), multiplied by coronary inflow (milliliters per minute), and then divided by the wet weight of each heart (1.90 ± 0.06 g). Oxygen tension of the inflow perfusate was kept constant. Percentage oxygen extraction was calculated as the difference between inflow and outflow oxygen tensions multiplied by 100, divided by inflow oxygen tension. Similarly, myocardial oxygen consumption (MVO₂) was calculated as oxygen solubility multiplied by the difference between inflow and outflow oxygen tensions times coronary inflow per gram of wet heart tissue.

Atrial and ventricular electrograms, heart rate, AVCT (both spontaneous and paced from the atrium at a constant frequency of 240 impulses/min), outflow oxygen tension, coronary flow, systolic left ventricular pressure (SLVP) and DLVP, and perfusion pressure were displayed on a fast-writing (3 kHz), high resolution, eight channel chart recorder (AstroMed, West Warwick, RI).

Experimental Protocol

Ketamine isomers had chemical purities of greater than 99.0%. The optical purities for S(+) and R(−) isomers were 95.4% and 99.5%, respectively. The isomers and the racemate were prepared directly into the preoxygenated Krebs–Ringer’s solution (pH 7.41 ± 0.07, oxygen tension 689 ± 28 mmHg, and carbon dioxide tension 25 ± 0.9 mmHg) to obtain 25, 50, 100 and 200 μM solution concentrations.

Adenosine was injected (0.2 ml of 200 μM stock) into the aortic cannula and at least 30 min was allowed for stabilization before initial control measurements were obtained. Subsequently, in randomized order, the S(+) or R(−) isomer of ketamine or the racemic mixture was administered to each of 23 hearts at increasing concentrations for 10 min periods. Each of these experimental intervals was followed by a 15 min drug-free washout period, during which the ketamine concentration was incrementally increased. Ten hearts served as the nonpretreated control group. Five hearts were perfused with solution containing 25 μM naloxone hydrochloride (Sigma Chemical, St. Louis, MO) to block any cardiac opioid receptors. Hearts of the eight reserpine pretreated animals were perfused with solution containing 1 μM phenoxybenzamine, 2 μM phentolamine, and 1 μM atropine to block any effects mediated by acetylcholine and any remaining catecholamines. Measurements were obtained during the last minute of exposure to each isomer or the racemate at each concentration, and during the last minute of each control (washout) period. After the last control period, adenosine was again injected at the same concentration into the aortic root to observe any change in the maximal coronary flow response. The hearts of the reserpine pretreated animals were finally perfused with 100 μM tyramine to test for completeness of the depletion of catecholamine stores. Hearts of this group were used for statistical analyses, only if spontaneous atrial heart rate or systolic left ventricular pressure did not change by more than 10% from control during the washout period.

Statistical Analyses

All data were analyzed using the mean ± SD. Repetitive measurements of the same group were performed on the same hearts. As the untreated group versus the pretreated group at the same concentration did not differ significantly, the data were collapsed into one value for the post hoc analysis of variance by the method of Abacus Concepts, Inc. Cupertine, CA. The F and t analyses of variance were used to test the equality of slopes and intercepts.

Fig. 1. Effects of optical isomers of ketamine on coronary blood flow in 8 nonpretreated (UT) and 8 pretreated (R) hearts. Data were performed in duplicate using 5 hearts in each group. R = 8 cyclically depleted (CD) hearts. Two hearts were perfused containing controls; and CD hearts were pretreated previously with phenoxybenzamine and phentolamine. A flow control and duplicate measurements were performed at each concentration. The data were analyzed with the Student-Newman-Keuls test. Means with bars not sharing a common symbol were significantly different. *P<0.05, **P<0.01.
by more than $\pm 10\%$ from control during the tyramine perfusion.

**Statistical Analysis**

All data are expressed as means $\pm$ standard errors of the means (SEM). The following comparisons were made using analysis of variance for repeated measures, increasing concentrations of either the isomers or the racemate versus the controls; subsequent controls during washout versus initial control values; the S(+) isomer, the R(−) isomer, and the racemate of ketamine all at equimolar concentrations versus each other in the same group, and the same isomer or racemate in the untreated control group versus the opioid receptor–blocked group versus the reserpine pretreated group at equimolar concentrations. A P value of less than or equal to 0.05 was accepted as indicating a significant difference. Bonferroni’s method was used for post hoc multiple comparison testing when analysis of variance was significant (Super Anova 1.11 software [Abacus Concepts, Berkeley, CA] for Macintosh [Apple, Cupertino, CA]). For several variables linear regression analyses of the concentration–response coordinates were used to test for differences in y-intercepts and slopes and their significances.

**Results**

**Heart Rate and Atrioventricular Conduction Time**

Heart rate (fig. 1) was $172 \pm 5$ beats/min during the initial and final control periods in the catecholamine-depleted group and was initially $218 \pm 5$ beats/min during the initial control period and $213 \pm 6$ beats/min in the final control period in the untreated and opioid-blocked groups. In all groups heart rate was decreased significantly in a concentration-dependent manner by ketamine. At 25, 50, 100, and 200 $\mu$m in the untreated and opioid-blocked groups, the R(−) isomer of ketamine decreased heart rate more than the S(+) isomer. The heart rate values for the racemate at these concentrations were intermediate and were not different from those of the S(+) and R(−) isomers of ketamine. In catecholamine-depleted hearts there were no differences between isomers for heart rate at each concentration. During washout control periods heart rate and AVCT returned to initial control values. y-Intercepts for S(+), S(−/+), and R(−) isomers of the untreated group were 218, 215, and 214 beats/min, and slopes were 0.29, 0.34, and 0.37, respectively. The S(+) slope was significantly different from the S(−) slope. There were no significant differences in y-inter-

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Fig. 1. Effect of four concentrations of the optical isomers and the racemate of ketamine on spontaneous heart rate in untreated (UT) ($n = 10$), opioid receptor–blocked (OB) ($n = 5$), and catecholamine-depleted (CD) autonomic blocked ($n = 8$) isolated guinea pig hearts. UT hearts constituted the control group; OB hearts were perfused continuously with solution containing naflexon to block opioid receptors, and CD hearts taken from reserpine-pretreated animals were perfused continuously with atropine, propranolol, and phenoxybenzamine to block autonomic receptors. Absolute values at the initial control and during exposure to individual concentrations of ketamine are shown. Control values between the individual concentrations are not displayed. All values are significantly different from the preceding control values except for 25 $\mu$m S(−)-ketamine in the UT group. Values for the CD group are significantly less than those for the UT and OB groups (significance not shown). There is no difference between the UT group and the OB group at equal concentrations. *$P < 0.05$ versus S(+)–ketamine at equal concentrations for the same group.

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Table 1. Effects of Isomers and Racemate of Ketamine on Atroventricular Conduction Time (AVCT, ms) in Untreated (n = 10), Opioid Receptor Blocked (n = 5), and Catecholamine Depleted and Autonomically Blocked (n = 8) Hearts

<table>
<thead>
<tr>
<th></th>
<th>Untreated</th>
<th>Opioid Blocked</th>
<th>Catecholamine Depleted</th>
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<tr>
<td></td>
<td>S(+) Isomer</td>
<td>Racemate</td>
<td>R(−) Isomer</td>
</tr>
<tr>
<td>Control 1</td>
<td>60.7 ± 1.4</td>
<td>60.5 ± 1.5</td>
<td>61.2 ± 1.5</td>
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<tr>
<td>25 µM</td>
<td>61.6 ± 1.6</td>
<td>61.8 ± 1.4</td>
<td>62.2 ± 1.6</td>
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<tr>
<td>50 µM</td>
<td>62.4 ± 1.5</td>
<td>62.3 ± 1.6</td>
<td>62.5 ± 1.6</td>
</tr>
<tr>
<td>100 µM</td>
<td>63.5 ± 1.5</td>
<td>63.9 ± 1.6</td>
<td>63.9 ± 1.6</td>
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<tr>
<td>200 µM</td>
<td>66.5 ± 1.4</td>
<td>67.4 ± 1.6</td>
<td>66.5 ± 1.7</td>
</tr>
<tr>
<td>Control 2</td>
<td>61.1 ± 1.3</td>
<td>61.2 ± 1.5</td>
<td>61.2 ± 1.6</td>
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</table>

Opioid receptor blocked hearts were perfused with solution containing naloxone hydrochloride. Catecholamine depleted hearts were prepared by pretreating animals with reserpine and perfusing hearts with perfusate containing atropine, propranolol, and phenoxynbenzamine. All data are expressed as mean ± SEM. Control washout data between individual concentrations are not shown.

* P < 0.05 versus control. AVCT in the catecholamine depleted group is significantly higher than in untreated and opioid receptor blocked groups (significance is not shown) during control and ketamine exposure. There is no significant difference between the untreated group and the opioid receptor blocked group. At equimolar concentrations within the same group significant differences were not observed between ketamine isomers and racemate.

cents or slopes for each isomeric form of ketamine in the catecholamine-depleted group.

There were different electrophysiologic effects of the individual isomers and of the racemate of ketamine on untreated hearts, opioid-blocked hearts, and hearts depleted of catecholamines. AVCT (table 1) was significantly prolonged with increasing concentrations of isomers and racemate in the untreated, opioid-blocked, and catecholamine-depleted groups. In each group there were no differences between isomers and racemate at the same concentration. However, AVCT was significantly longer in the catecholamine-depleted group than in the untreated and opioid-blocked groups at each concentration. During atrial pacing at 240 beats/min AVCT increased in a concentration-dependent manner from 72.4 ± 1.7 to 87.8 ± 2.7 ms at 200 µM in the untreated group, 71.9 ± 1.6 to 86.8 ± 3.1 ms in the opioid-blocked group, and from 86.7 ± 2.7 to 118 ± 3.2 ms in the catecholamine-depleted group, indicating that conduction delay was independent of changes in heart rate. AVCT in the opioid-blocked group was identical to that of the untreated group during sinus rhythm.

Left Ventricular Systolic and Diastolic Pressures

Isometric SLVP decreased significantly in all groups in a concentration-dependent manner for both isomers and racemate (fig. 2). SLVP was significantly higher in the untreated group (101 ± 5 mmHg, initial control, and 95 ± 5 mmHg, final control) than in the catecholamine-depleted group (71 ± 4 mmHg, initial control and 64 ± 4 mmHg, final control). In untreated and opioid-blocked groups the S(+) isomer of ketamine decreased SLVP significantly less than the R(−) isomer at 50, 100 µM (P ≤ 0.05) and 200 µM (P ≤ 0.01), and additionally for the racemate at 25 µM (P ≤ 0.05). The values for the racemate at equal concentrations were intermediate to those of the isomers. SLVP in catecholamine-depleted hearts was equivalent at equimolar concentrations for ketamine isomers and for the racemate ketamine. During ketamine-free control periods, SLVP returned to initial control levels (C1) for both isomeric and racemic forms of ketamine. y-Intercepts for S(+), S(+/−), and R(−) isomers of the untreated group were 99.97, and 100 mmHg, and slopes were 0.23, 0.24, and 0.29, respectively. The S(+) slope was significantly different from the S(−) slope. There were no significant differences in y-intercepts or slopes for each isomeric form of ketamine in the catecholamine-depleted group.

DLVP, initially set to 0 mmHg, increased slightly in all groups in a concentration-dependent manner up to a maximum of 3 ± 1 mmHg at 200 µM ketamine (data not shown). During washout control periods DLVP returned to 0 mmHg.

Oxygen Consumption and Percentage Oxygen Extraction

\[ MV_{O_2}, \text{ initially } 77.3 \pm 4.7 \, \mu l \cdot g^{-1} \cdot min^{-1} \text{ (untreated group), } 73.5 \pm 3.9 \, \mu l \cdot g^{-1} \cdot min^{-1} \text{ (opioid-blocked group), and } 65.3 \pm 3.4 \, \mu l \cdot g^{-1} \cdot min^{-1} \text{ (catecholamine-depleted group), decreased in a concentration-dependent manner with each isomer and the racemate of ketamine (fig. 3). Percentage oxygen extraction also de-
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Fig. 2. Effect of four concentrations of the optical isomers and the racemate of ketamine on systolic left ventricular pressure (LVP) in untreated (UT) (n = 10), opioid receptor-blocked (OB) (n = 5), and catecholamine-depleted (CD) and autonomically blocked (n = 8) isolated hearts. Control values between the individual groups are not displayed. All values are significantly different from the preceding control values except for 25 μM S(-)-ketamine in the UT and OB groups, and of both isomers and the racemate at 25 μM in the CD group. Values for SLVP in the CD and autonomically blocked hearts are significantly lower than those of the UT and OB groups during control and at all ketamine concentrations (significance not shown). There is no difference between the UT group and the OB group at equal concentrations. See figure 1 for additional details. *P < 0.05 versus S(+) ketamine at equal concentrations for the same group.

Fig. 3. Effect of four concentrations of the optical isomers and the racemate of ketamine on myocardial oxygen consumption in untreated (UT) (n = 10), opioid receptor-blocked (OB) (n = 5), and catecholamine-depleted (CD) (n = 8) hearts. Control values between the individual groups are not displayed. All values are significantly different from the preceding control values for ketamine concentrations greater than or equal to 25 μM. Absolute values in CD and autonomically blocked hearts are significantly lower than those of the UT and OB groups during control and during exposure to the S(+) isomer and the racemate at 10 and 200 μM (significance not shown). There is no difference between the UT group and the OB group at equal concentrations. See figure 1 for additional details. *P < 0.05 versus S(+) ketamine at equal concentrations for the same group.

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was given as well as during exposure to ketamine compared with the untreated and opioid-blocked groups. For the catecholamine treated group there were no differences for MV\textsubscript{O\textsubscript{2}} and percentage oxygen extraction among isomers and racemate at equimolar concentrations. y-Intercepts for MV\textsubscript{O\textsubscript{2}} with S(+) S(+-), and R(-) isomers of the untreated group were 73, 74, and 71 \mu l g\textsuperscript{-1} min\textsuperscript{-1}, and slopes were 5.5, 9.2, and 9.8, respectively. The S(+) slope was significantly different from S(-) and S(+-) slopes. There were no significant differences in y-intercepts or slopes for each isomeric form of ketamine in the catecholamine-depleted group. y-Intercepts for percentage oxygen extraction with S(+) S(+-), and R(-) isomers of the untreated group were 66, 67, and 67%, and slopes were 0.09, 0.11, and 0.13, respectively. The S(+) slope was significantly different from the S(-) slope. There were no significant differences in y-intercepts or slopes for each isomeric form of ketamine in the catecholamine-depleted group.

**Coronary Flow and Oxygen Supply-to-Demand Ratio**

Initial and final maximal coronary flow responses to a bolus injection of adenosine were similar in untreated, opioid-blocked, and catecholamine-depleted groups (11.8 ± 0.7 ml g\textsuperscript{-1} min\textsuperscript{-1}, 11.8 ± 0.7 ml g\textsuperscript{-1} min\textsuperscript{-1}, and 10.6 ± 0.8 ml g\textsuperscript{-1} min\textsuperscript{-1}, respectively). Within each group there was no significant difference in coronary flow with equimolar concentrations of racemic and isomeric forms of ketamine. Basal flow was greater in the catecholamine-depleted group than in the untreated and opioid-blocked groups (table 2). In no group was there a significant change in coronary flow during exposure to 25 and 50 \mu M ketamine (each form), but coronary flow increased at higher concentrations in a concentration-dependent manner in each group, such that the two isomeric and racemic forms exhibited no differences in their effect on coronary flow.

Oxygen delivery (D\textsubscript{O\textsubscript{2}}), relative to oxygen demand (MV\textsubscript{O\textsubscript{2}}), increased in a concentration-dependent manner for isomers and racemate in each group (fig. 5). Similarly, there was a significant difference in D\textsubscript{O\textsubscript{2}}/MV\textsubscript{O\textsubscript{2}} between ketamine isomers at 200 \mu M in untreated and opioid-blocked groups, whereas there was no significant difference in the catecholamine depleted group. There were no significant differences in D\textsubscript{O\textsubscript{2}}/MV\textsubscript{O\textsubscript{2}} between untreated and opioid-blocked groups, but these groups were significantly different from the catecholamine-depleted group without ketamine as well as during exposure to equimolar concentrations of isomers and racemate. The initial ratio for D\textsubscript{O\textsubscript{2}}/MV\textsubscript{O\textsubscript{2}} was lower (1.4 ± 0.1) in the untreated than in the catecholamine-depleted group (1.7 ± 0.1). The D\textsubscript{O\textsubscript{2}}/MV\textsubscript{O\textsubscript{2}} ratio increased in a concentration-dependent manner by 60 ± 12% in the untreated and opioid-blocked groups, and by 64 ± 8% in the catecholamine-depleted group at 200 \mu M for ketamine isomers and racemate. There was no significant difference in D\textsubscript{O\textsubscript{2}}/MV\textsubscript{O\textsubscript{2}} between untreated and opioid-blocked groups during exposure to equimolar concentrations of identical concentrations of S(+), and S(+-), and R(-) isomers and racemate.

**Discussion**

This is the first study to examine the effects of ketamine isomers on the myocardium of identical concentrations of S(+), and S(+-), and R(-) isomers and racemate. We found that the S(+) isomer produced a greater increase in isovolumetric myocardial blood flow (MV\textsubscript{O\textsubscript{2}}). Further studies need to be conducted to determine the optimal concentration of isomers and racemate for clinical use.

**Central and Peripheral Effects of Ketamine**

Steroselective effects of ketamine have been observed in both animals and patients. 25-28 The use of ketamine in clinical practice is limited by its potential for adverse effects, including sedation, nausea, and hypotension. To avoid these side effects, ketamine is often administered in combination with other medications. 27-29

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Table 2. Effects of Isomers and Racemate of Ketamine on Coronary Flow (ml·g⁻¹·min⁻¹) in Untreated (n = 10), Opioid Receptor Blocked (n = 5), and Catecholamine Depleted and Autonomically Blocked (n = 8) Hearts

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<td>25 µM</td>
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<tr>
<td>100 µM</td>
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<tr>
<td>200 µM</td>
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<td>8.7 ± 0.7*</td>
<td>8.3 ± 0.4*</td>
</tr>
<tr>
<td>Control 2</td>
<td>7.0 ± 0.3</td>
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*P < 0.05 versus control 1. At equimolar concentrations of ketamine and during intermediate controls coronary flow in the catecholamine-depleted group is significantly higher than in the untreated control group and in the opioid receptor blocked group (significance is not shown). There is no significant difference between the control group and the opioid receptor blocked group. At equimolar concentrations within the same group significant differences were not observed between the isomers and the racemate.

Discussion

This is the first known report of direct effects of optical isomers of ketamine on the isolated perfused heart. We found that greater equimolar concentrations of the S(+) isomer, the R(−) isomer, and the racemate of ketamine produced significantly different decreases in heart rate, isovolumetric SLVP, percentage oxygen extraction, and MVCO₂. Furthermore, the data show that S(+) ketamine caused less cardiac depression than either the racemate or the R(−) isomer of ketamine, although both forms and the racemate produced cardiac depression in a concentration-dependent fashion.

Central Nervous System Stereoselective Effects of Ketamine

Stereoselectivity does not occur randomly. In general stereoselectivity is a function of the potency or affinity of the more potent isomer to its selective binding site. As a rule, optically active isomers bind stereoselectively to specific macromolecular receptors, which are themselves stereospecific. Studies of isomers of ketamine in animals, and in healthy volunteers reveal a consistent picture: the S(+) isomer is a more potent anesthetic, has a more favorable therapeutic index, and gives rise to less psychic emergence reactions in the postanesthetic period than the R(−) isomers and the racemate of ketamine. This finding of central stereoselectivity for ketamine is consistent with a receptor-mediated pharmacologic mechanism. Klepsstad et al. demonstrated that the relative order of anagliptic potency for ketamine isomers correlates positively with the PCP binding site of ketamine. PCP binding site ligands block the NMDA receptor–operated ion channel in a noncompetitive way and inhibit the action of excitatory amino acids at NMDA receptors. S(+) Ketamine has a four times greater affinity for the PCP site than R(−) ketamine, which is the same as the ratio for the anagliptic effects of its isomers. These results are consistent with the theory that ketamine anesthetia results from PCP binding site–mediated inhibition of the NMDA receptor–operated ion channel. In contrast, White et al. found that greater concentrations of R(−) ketamine caused more psychomimetic side effects than S(+) ketamine, even though S(+) ketamine was the most potent anesthetic. So far, the sigma opiate binding site is the only recognition site for ketamine known to have a higher affinity for R(−) ketamine than S(+) ketamine. This site is therefore a plausible candidate for a receptor mediated, excitatory psychomimetic effect of ketamine.

Cardiac Stereoselective Effects of Ketamine

Besides its psychomimetic effects, the major side effect of ketamine is its effect on the cardiovascular system. Even though it is well known that ketamine binds stereoselectively to specific receptors in the central nervous system, stereoselective effects of ketamine in the
heart and circulation have not been examined. To infer if there are really specific binding sites for anesthetics in a given tissue three levels of interactions of stereoselectivity must be considered: penetration, recognition, and activation. Use of the isolated perfused heart model confines the different levels of stereoselective interaction to recognition and activation, as discussed and shown earlier for volatile anesthetics.22 Determination of the precise mechanism by which isomers of ketamine exert differential chronotropic and inotropic effects is complicated because there are multiple sites in the excitation-contracture process where intervention may occur. However, our findings indicate that complete blockade of the autonomic nervous system nullifies these differences in isomers compared with the racemate. This, in turn, suggests that the differential indirect effects of ketamine isomers and the racemate are the result of stereoselective modulation of the autonomic nervous system.

**Dose Effects of Racemic Ketamine**

In humans, ketamine appears to stimulate heart function by modifying autonomic nervous system discharge and by influencing the activity of endogenous substances, but in other studies in vivo, ketamine effects are variable and appear to be species related.20 Compared with many previous studies, our study differs in the evaluated dose range of ketamine. Some studies have used excessively high doses. Several authors have demonstrated a negative inotropic effect at higher concentrations (>500 μM). Other authors have shown a biphasic effect, with an early positive inotropic effect followed by a negative effect.31 The concentrations of isomers and racemate selected for our study approximate concentrations in blood after induction (100 μM) and maintenance (25 μM) of anesthesia with racemic ketamine.32 The free unbound concentration of ketamine in Krebs–Ringer’s perfusate was not measured. There is a wide discrepancy in values for protein binding of ketamine in plasma or blood. They range from 12%33 to 47%34 binding, probably because pH, albumin concentration, and albumin fraction, each of which affects binding, may differ among studies. Because of the discrepancy among reports of plasma binding at various ketamine concentrations and because the anesthetic potencies of the isomers differ, we used a wide range of ketamine concentrations. Experiments in vitro show that racemic ketamine depresses myocardial contractile force and automaticity at higher concentrations15-19,35 and, in the presence of autonomic blocking agents, also at lower concentrations.15,16,19,36

**Lack of Opioid Effect of Ketamine Isomers**

Based on evidence that there may be opioid receptors on cardiac ventricular cells37 and that opioid peptides can be produced and secreted by cardiac myocytes,58 we examined effects of the ketamine isomers in hearts treated with naloxone to block any cardiac opioid receptors. Our findings show that blockade of cardiac opioid receptors had no added effect beyond that of the ketamine isomers, thereby suggesting that the stereoselectivity of ketamine isolectotypes is responsible for the observed effects. Although the opioid effects are relevant, the exact role of opioids in mediating myocardial depression is not clear. Caution in using PCP binders with nearly all drug classes is clearly indicated.

**Mechanism of Ketamine Depression of Myocardial Contractility**

The direct and indirect effects of ketamine concentrations on myocardial contractility in the guinea pig ventricle have been extensively studied in several species.39-41 Although the mechanisms of the contractile action of ketamine are not completely clear, rabbit papillary muscle preparations have provided a new insight.42-44 In these experiments, Ca2+ influx into the sarcoplasmic reticulum was decreased and the transsarcolemma calcium exchange was clamped, which suggests that ketamine decreases calcium sequestration.43-45 This increase in intracellular calcium does not occur when the membrane is blocked by adenosine.45-47 These data suggest that a mechanism exists by which the isomers of ketamine depress myocardial contractility, and a mechanism is likely to exist for the direct effect of ketamine

**Cardiac Myocardium**

There is also an indirect mechanism by which ketamine inhibits central neural activity and can inhibit cardiac muscle. As with other muscle11 and other organs, it is unclear whether ketamine binds and acts on cardiac contractile proteins directly or through a modulatory mechanism.
the ketamine isomers as presented here. This indicates that the stereoselective cardiovascular effects of ketamine isomers are not likely to be attributable to any significant effect on an opioid receptor site in the heart. Although central naloxone sensitive opioid receptors are involved in overall cardiovascular reflexes, the exact role of cardiac opioid receptors in directly regulating myocardial function requires further investigation. Certain other cardiac receptors, such as the PCP binding site, could also be involved, because nearly all receptors are stereoselective.

**Mechanism of Cardiac Depression by Ketamine Stereoisomers**

The direct cardiac depressive effects of ketamine are well known. Ketamine decreases contractile force in a concentration related manner as observed in isolated guinea pig hearts and in cardiac preparations of other species. Ketamine may reduce the transsarcolemmal influx of Ca2+ into the sarcoplasmic reticulum. Baum and Tocco documented an inhibitory effect of racemic ketamine on transsarcolemmal Ca2+ influx rather than an effect on Ca2+ release from the sarcoplasmic reticulum. Baum and Tocco documented an inhibitory effect of racemic ketamine on transsarcolemmal Ca2+ entry in whole cell, voltage clamped, adult guinea pig ventricular myocytes. They observed that any direct positive inotropic effect of ketamine seen in vivo does not involve a directly mediated increase in transsarcolemmal Ca2+ entry. This however does not exclude a secondary effect on Ca2+ entry mediated by catecholamines, by other mediators of cyclic adenosine monophosphate activation, or by additional mechanisms. Hence, a direct, selective effect of the isomers of ketamine on Ca2+ entry mechanisms appears unlikely.

There is a strong evidence, however, that indirect mechanisms are involved in the specific cardiac effects of ketamine isomers. Ketamine is known to increase central nervous system sympathetic outflow and to inhibit catecholamine uptake in smooth and skeletal muscle as well as in cardiac muscle. Lundy et al. showed that both ketamine isomers are capable of inhibiting neuronal or extraneuronal catecholamine uptake in vitro. Depending on the tissue examined, they found that either the S(+) isomer or the R(-) isomer of ketamine is a more potent uptake inhibitor of catecholamines.

Our results in isolated guinea pig hearts suggest that ketamine increases the availability of catecholamines in the adrenergic neuroeffector function, resulting primarily from inhibition of catecholamine reuptake by the S(+) ketamine. Catecholamines, released continuously from neuronal tissues, persist for longer periods and at higher concentrations in the region of the postsynaptic β-adrenergic receptor. The result is an enhancement of both intensity and duration of the adrenergic response. This interpretation is consistent with the observation in the present study that when the myocardial tissues are depleted of catecholamines by reserpine and by additional blockade of adrenergic (and acetylcholine) receptors, the effects of the isomers and the racemate of ketamine are identical.

In the course of the studies reported, we attempted to determine whether the differential effects of ketamine isomers were attributable to blockade of neuronal or extraneuronal catecholamine uptake. Progesterone, a classical inhibitor of extraneuronal reuptake, and desmethyl-imipramine, a potent inhibitor of neuronal uptake, were administered in a few trial hearts. However, both agents reduced SLVP and heart rate rather than potentiating these variables as anticipated. Perfusion with ketamine isomers additionally reduced SLVP and heart rate and we could not distinguish a difference in effects between the two isomers. Therefore, we cannot determine whether S(+) ketamine blocks the neuronal or extraneuronal catecholamine uptake in the isolated heart because of the cardiac depressant effects of these drugs.

**Limitations and Clinical Implications**

Our assessment of changes in global cardiac electrophysiologic, mechanical and metabolic function by isomers of ketamine in the presence of other pharmacologic tools does not allow a more detailed analysis of mechanical contraction and relaxation parameters as described in isolated muscle strips. Another potential limitation of our model is that hearts were perfused with a crystalloid solution devoid of blood cells. Decreased oxygen carrying capacity induces a relative increase in basal coronary flow and a decrease in coronary reserve. This may limit interpretation of drug effects on the vasculature. However, a criteria for inclusion...
of hearts in our study is a doubling of flow with adenosine. Moreover, crystalloid perfused hearts are not ischemic as they do not produce lactate or release adenosine and do not exhibit maximal extraction of oxygen. Because all hearts were perfused similarly and the treatments were randomized, we do not expect the use of crystalloid perfusate to alter the major conclusions of our study.

Data obtained with our model may be controversial when compared with models in vitro and other models in vitro of heart tissue as well as noncardiac tissue and other species. Other investigators\(^{15,16}\) observed in similar models in vitro a positive isotropic effect by ketamine even after pretreatment with reserpine and after \(\alpha\)- and \(\beta\)-adrenergic receptor blockade. Because both groups performed their studies using rat hearts, there may exist a species difference. In a canine heart model in vitro\(^{10}\) the positive isotropic effect was abolished by propranolol or pretreatment with reserpine as is the guinea pig heart used for our study.

Because S(+)-ketamine is a more potent anesthetic than racemic ketamine and R(−)-ketamine at equivalent concentrations, S(+)-ketamine would appear to have an advantage because of its lesser cardiac depressant effect. Clinically, these results would add to other advantages of the S(+) isomer compared with the R(−) isomer and the racemate. However, caution would need to be exercised during administration of the S(+)ketamine form because its lesser cardiac depressant effects appear to be caused by a delayed reuptake of catecholamine, thereby possibly intensifying sympathetic activation centrally, a response that may be undesirable in patients with cardiovascular disease. Moreover, in patients with long term sympathetic activation or blockade, administration of either isomer of ketamine might result in cardiac depression.

In summary, our study indicates that in the isolated guinea pig heart the two optical isomers of ketamine exhibit stereoselective depressant effects that are independent of cardiac opioid receptor activation but dependent on neuronal or extraneuronal uptake of catecholamines. Although both isomers and the racemate directly depress cardiac function in a dose-dependent fashion, the S(+)-ketamine has a significantly less cardiac depressant action at higher concentrations than does either the R(−)-ketamine or the racemic mixture at equimolar concentrations. Importantly, after depletion of catecholamine stores, there is no differences in cardiac effects between the isomers and the racemate. We conclude that S(+)-ketamine is a more potent inhibitor of catecholamine uptake and infer that it increases availability of catecholamines at neuroeffector junctions. This mechanism could also contribute to the positive isotropic and chronotropic effects of ketamine observed in some studies in vivo.\(^{8,9,12}\)

The authors thank Dr. B. Gebhardt (Parke Davis, Freiburg, Germany) for testing purity and for providing the isomers and racemate of ketamine and thank James Heiser for technical assistance.

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Anesthesiology, V 82, No 6, Jun 1995