Anesthesiology 2002; 96:659–66 

# *Ketamine Inhibits Sodium Currents in Identified Cardiac Parasympathetic Neurons in Nucleus Ambiguus*

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***Background:*** **Ketamine increases both blood pressure and heart rate, effects commonly thought of as sympathoexcitatory. The authors investigated the possibility that ketamine increases heart rate by inhibiting the central cardiac parasympathetic mechanisms.**

***Methods:*** **We used a novel *in vitro* approach to study the effect of ketamine on the identified cardiac parasympathetic preganglionic neurons in rat brainstem slices. The cardiac parasympathetic neurons in the nucleus ambiguus were retrogradely prelabeled with the fluorescent tracer by placing rhodamine into the pericardial sac. Dye-labeled neurons were visually identified for patch clamp recording, and ketamine effects on isolated potassium ($K^+$) and sodium ($Na^+$) currents were studied.**

***Results:*** **Cardiac nucleus ambiguus neurons (n = 14) were inherently silent, but depolarization evoked sustained action potential trains with little delay or adaptation. Ketamine (10 μM) reduced this response but had no effect on the voltage threshold for action potentials (n = 14; $P > 0.05$). The current–voltage relations for the transient $K^+$ current and the delayed rectified $K^+$ current (n = 5) were unaltered by ketamine (10 μM–1 mM). Ketamine depressed the total $Na^+$ current dose-dependently (10 μM–1 mM). In addition, ketamine shifted the $Na^+$ current inactivation curves to more negative potentials, thus suggesting the enhancement of the $Na^+$ channel inactivation ($P < 0.05$; n = 7). In the presence of $Cd^{2+}$, ketamine (10 μM) continued to inhibit voltage-gated $Na^+$ currents, which recovered completely within 10 min.**

***Conclusions:*** **Ketamine inhibits $Na^+$ but not $K^+$ channel function in brainstem parasympathetic cardiac neurons, and such actions may mediate the decrease in parasympathetic cardiac activity and increase in heart rate that occurs with ketamine.**

THE intravenous anesthetic ketamine is associated with a hemodynamic profile that typically is characterized by increases in both blood pressure and heart rate. These cardiovascular actions of ketamine are commonly thought of as sympathoexcitatory, and the increases in blood pressure and heart rate have been attributed to "enigmatic" mechanisms of activation of the central sympathetic nervous system.[1] However, this increase in heart rate is paradoxical because increases in blood pressure normally elicit baroreflex-induced decreases in heart rate. Few aspects of the underlying mechanisms responsible for these ketamine responses have been directly examined, particularly at central autonomic sites of action.[1] Although ketamine does not modify baroreceptors,[2] the sensory limb of the arterial baroreflex, it may affect the central nervous system sites, likely autonomic regions below the pons to alter neurons within reflex pathway.[3,4] The heart rate responses could well reflect inhibition of central cardiac parasympathetic mechanisms.

Heart rate is normally dominated by a tonic release of neurotransmitter, acetylcholine, onto cardiac pacemaker cells by cardiac parasympathetic nerves.[5] Thus, a central action of ketamine to depress the discharge of cardiac preganglionic parasympathetic neurons within the brainstem could contribute to the tachycardia associated with ketamine. After parasympathetic blockade with atropine, ketamine did not induce tachycardia, while cardiac sympathetic blockade with propranolol failed to prevent the ketamine-induced increase in heart rate. Such results suggest an important parasympathetic component in ketamine-induced tachycardia.[6–9] To directly examine potential cellular actions of ketamine in the central nervous system, we identified cardiac parasympathetic preganglionic neurons in brainstem slices by a novel retrograde tracing method.[10] With this approach, we selectively recorded rat cardiac parasympathetic preganglionic neurons in the nucleus ambiguus and assessed the effects of ketamine on key membrane characteristics using patch clamp recording. Ketamine depressed the voltage-gated sodium currents and selectively enhanced the inactivation characteristics of voltage-gated sodium channels but did not modulate potassium channels. The combined effects resulted in depressed excitability of cardiac parasympathetic neurons.

## Materials and Methods

All animal procedures were performed in compliance and with the approval of the Institutional Animal Care and Use Committee at George Washington University, and are in accordance with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association and the National Institutes of Health publication "Guide for the Care and Use of Laboratory Animals." Pregnant rats were obtained (Hilltop Lab Animals, Scottdale, PA), and young pups (4–10 days; N = 60) of either sex underwent tracer dye-labeling surgery.

### *Labeling and Identification of Cardiac Parasympathetic Preganglionic Neurons In Nucleus Ambiguus*

Cardiac parasympathetic neurons were identified by fluorescent tracers in an *in vitro* brainstem slice prepa-

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Received from the Department of Pharmacology, George Washington University, Washington, DC; and the Departments of Anesthesiology and Physiology and Pharmacology, Oregon Health Science University, Portland, Oregon. Submitted for publication February 28, 2001. Accepted for publication October 18, 2001. Supported in part by grant No. RO1 HL58769 from the National Institutes of Health, Bethesda, Maryland.

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ration as described previously.[11] For dye implantation, a right thoracotomy was performed to expose the heart under methoxyflurane. A needle was then inserted into the pericardial sac, and the tracer rhodamine (XRITC, 1% solution; Molecular Probes, Eugene, OR) was topically applied to the epicardial surface of cardiac tissue that contains the parasympathetic ganglia. After wound closure, the animals were allowed 2–5 days to recover and for the dye to transport centrally. No postoperative analgesia was necessary. On the day of the recordings, the animals were anesthetized with methoxyflurane and killed by cervical dislocation. The brains were quickly removed and placed in cold (2°C) physiologic buffer that contained 140 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 5 mM glucose, 10 mM HEPES, pH 7.4, equilibrated with 100% $O_2$ and mounted on a vibratome. The medulla was cut in transverse sections 250 $\mu$m thick. Cardiac parasympathetic preganglionic neurons were found predominantly in the nucleus ambiguus, and these neurons were the focus of the studies.[10] Slices that included the nucleus ambiguus were transferred to a recording chamber positioned on the stage of a microscope (Axioskop fixed stage upright microscope with a 40× water submersion objective; Carl Zeiss Inc., Thornwood, NY) equipped with fluorescent filters to visualize rhodamine. The slice recording chamber was perfused at a rate of 3 ml/min with a solution containing 120 mM NaCl, 4.8 mM KCl, 1.2 mM $KH_2PO_4$, 25 mM $NaHCO_3$, 5 mM HEPES, 5.5 mM dextrose, and 2 mM $CaCl_2$, equilibrated with 95% $O_2$–5%$CO_2$, pH 7.4.

Labeled cardiac parasympathetic preganglionic neurons were located under fluorescent illumination. Patch pipettes were then advanced onto the somal membrane of the labeled neurons with visualization provided by differential interference contrast optics under infrared illumination and using a cooled CCD camera (Imagepoint; Roper Scientific, Trenton, NJ). Infrared illumination–differential interference contrast images of the neurons were visualized in real time (30 frames/s). In a limited number of animals (n = 4), we tested the cardiac specificity of the pericardial vagal labeling procedure by injecting fluorescent tracer directly into the chest cavity adjacent to the heart while keeping the pericardial sac intact. The absence of any labeled neurons in the brainstem confirmed that dye leak from the pericardial labeling site did not contribute to labeling of neurons in the medulla (data not shown).

### *Electrophysiologic Recordings from Identified Cardiac Parasympathetic Neurons In Nucleus Ambiguus*

Patch pipettes with input resistances from 1.8 to 3 MΩ were pulled from borosilicate glass capillary tubes (World Precision Instruments, Saratoga, FL) and mounted onto a micromanipulator (Narashige International Inc., East Meadow, NY) *via* a pipette holder and amplifier head stage (Axopatch 200B; Axon Instruments Inc., Union City, CA). The indifferent electrode was an Ag–AgCl plug connected to the bath *via* a 150 mM KCl agar bridge. Pipettes were advanced through the slice under positive pressure, and brief suction promoted formation of a gigaohm seal between the pipette and the cell membrane. Pipette capacitance was canceled at this stage. Intracellular access was obtained by applying a brief period of suction that ruptured the membrane. With this whole cell configuration, the membrane potential was clamped and ionic current measured. In addition to controlling the membrane voltage, intracellular dialysis through the open patch pipette controlled the internal milieu of the cell.

In some experiments, the nystatin or perforated patch configuration was used. When nystatin (258 units/ml) was included in the pipette solution, gigaohm seals were formed without rupturing the membrane, and the input resistances were typically 8–12 MΩ. Such perforated patch recordings offered intracellular access by incorporation of nystatin in the membrane under the pipette tip *via* monovalent ion permeable pores. The nystatin method prevented washout of intracellular calcium ($Ca^{2+}$) and second messengers (such as adenosine triphosphate or guanosine triphosphate) and limited the exchange between cell and pipette to monovalent ions. However, a disadvantage of perforated patch recordings, especially in these neurons with relatively intact dendrites, is that space clamp is not optimal and the kinetics of the rapidly activating and inactivating sodium currents cannot be examined in detail. One option to improve space clamp would be to enzymatically and mechanically dissociate these neurons from surrounding tissue, which also causes the loss of their peripheral processes. However, disadvantages of dissociation could include alterations in the expression and function of sodium channels in response to the loss of peripheral processes or exposure to the enzymes and mechanical pertubations. Patch pipettes were filled with a solution consisting of 130 mM potassium gluconate, 10 mM HEPES, 10 mM EGTA, 1 mM $CaCl_2$, and 1 mM $MgCl_2$ and nystatin, except when $Na^+$ currents were isolated for study, in which case cesium gluconate replaced potassium gluconate.

Action potentials were recorded under current clamp using perforated patch recordings by means of an Axopatch 200A amplifier (Axon Instruments, Foster City, CA). Depolarizing current (beginning at 100 pA and increasing in 100-pA increments until firing was initiated) was injected for 1.5 s. The voltage at which the action potential inflection occurred was recorded as the spike threshold. Voltage-gated ionic currents were studied under voltage clamp with a holding potential of −80 mV and rectangular test command potentials controlled by pClamp software (version 7.0, Axon Instruments). All experiments were performed at room temperature (23–25°C).

*Dissection of Ionic Currents*

Voltage-gated $Na^+$ and $K^+$ currents were isolated in these neurons by pharmacologic or kinetic separation. When cesium was included in the patch pipette, voltage-gated $K^+$ currents were blocked, and the $Na^+$ current remained and was isolated for study. Conversely, to study $K^+$ currents, 1 $\mu$M tetrodotoxin was applied to the bath to block any voltage-gated $Na^+$ current. Analysis of $Na^+$ currents compared the magnitudes of the peak inward currents. Two components of the $K^+$ currents in these rat cardiac parasympathetic neurons have been previously characterized pharmacologically.[11] These studies indicate that the two currents are sufficiently kinetically distinct that early peak $K^+$ current is chiefly the early transient $I_A$ K current, while the late, long-lasting $K^+$ current is a delayed rectifier $K^+$ current. Kinetic separation reflects the subcomponents, which can also be separated pharmacologically.[12] Thus, in the current study, we assessed these two components by measuring the early peak outward current as the $I_A$ $K^+$ current and the average current during the last 10 ms of 350-ms voltage command steps as the delayed rectifier $K^+$ current. For all currents recorded, current–voltage relations were constructed before and after an application of a single bath concentration of ketamine. Specific voltage step protocols are indicated in figures and their legends. $Na^+$ currents were studied in separate groups of neurons from K currents. In all studies after obtaining control responses, neurons were exposed to a single dose of ketamine (10, 100, or 1,000 $\mu$M).

*Drugs*

Ketamine HCl (10–1000 $\mu$M) was diluted with perfusate solution immediately before use. Ketamine was perfused directly onto the neurons from tubing positioned 1.9 mm above the neuron under study. Drug solution was introduced using a syringe pump at a rate of 0.3 ml/min, and the solution was constantly suctioned out of the chamber at a fixed chamber height to keep the fluid within the recording chamber at a constant volume of 1.3 ml. Picrotoxin (100 $\mu$M), strychnine HCl (1 $\mu$M), and prazosin HCl (10 $\mu$M) were added to the bath perfusate to prevent activation of $\gamma$-aminobutyric acid–mediated, glycinergic, and $\alpha_1$-adrenergic currents, respectively. All drugs used in this study were purchased from Sigma-Aldrich (St. Louis, MO).

*Data and Statistical Analysis*

Current amplitudes were measured as described and are presented as mean ± standard error of the mean. Curve fitting of the relations between conditioning voltages and subsequent maximal currents activated depolarizing steps were performed using a logistic sigmoidal function. All of these graphic plots, analyses, and statistical tests were performed using Origin (Origin 5.0; Microcal, Northampton, MA). Paired *t* tests were used to detect differences between control and ketamine treatments. A $P$ value $\leq 0.05$ was accepted as significantly different.

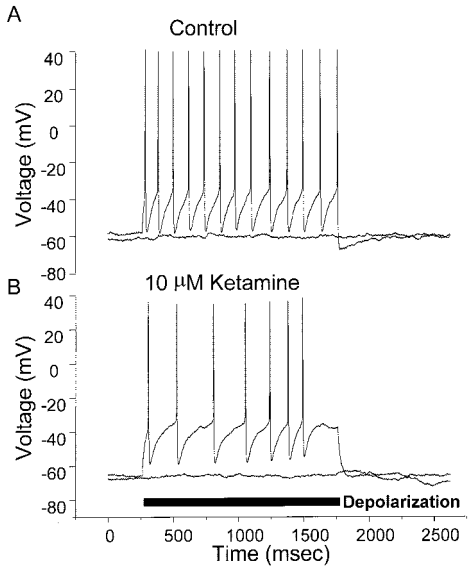


**Fig. 1. Ketamine decreases excitability of cardiac parasympathetic preganglionic neurons. Neurons recorded in current clamp configuration were inherently silent and did not have any spontaneous activity (*A*, lower trace). This representative neuron fired with little delay or spike frequency adaptation in response to injection of a depolarizing current (solid bar) of sufficient intensity to produce sustained discharge (*A*, top trace). Ketamine (*B*, 10 $\mu$M) reduced the firing activity of this cardiac parasympathetic neuron in response to injection of same depolarizing current (top trace, *bottom*). The firing also became less regular and more sporadic. Similar control responses and their alterations in ketamine were observed in all 14 cardiac parasympathetic neurons tested.**

## Results

Cardiac parasympathetic neurons in the nucleus ambiguus (n = 14) were uniformly and inherently silent under resting conditions (fig. 1A). However, injection of current depolarized these neurons and evoked a sustained train of repetitive action potentials (fig. 1A). Typically, action potentials were initiated with little delay and showed little reduction in spike frequency (spike frequency adaptation) during sustained current injection. Application of ketamine (10 $\mu$M) evoked a small but statistically significant ($P < 0.05$) hyperpolarization in membrane potential (control, −62.2 ± 1.7 mV; 10 $\mu$M ketamine, −65.6 ± 1.7 mV; n = 14) in these cardiac parasympathetic neurons (fig. 1B) and also significantly reduced the firing activity of these neurons in response to injection of depolarizing current (control frequency, 9.8 ± 1.0 Hz; with 10 $\mu$M ketamine, 7.3 ± 1.0 Hz; $P < 0.01$; n = 14). In addition, discharge rate during current injection and discharge became more sporadic during depolarization in the presence of ketamine (fig. 1B). These changes were not caused by alterations in the

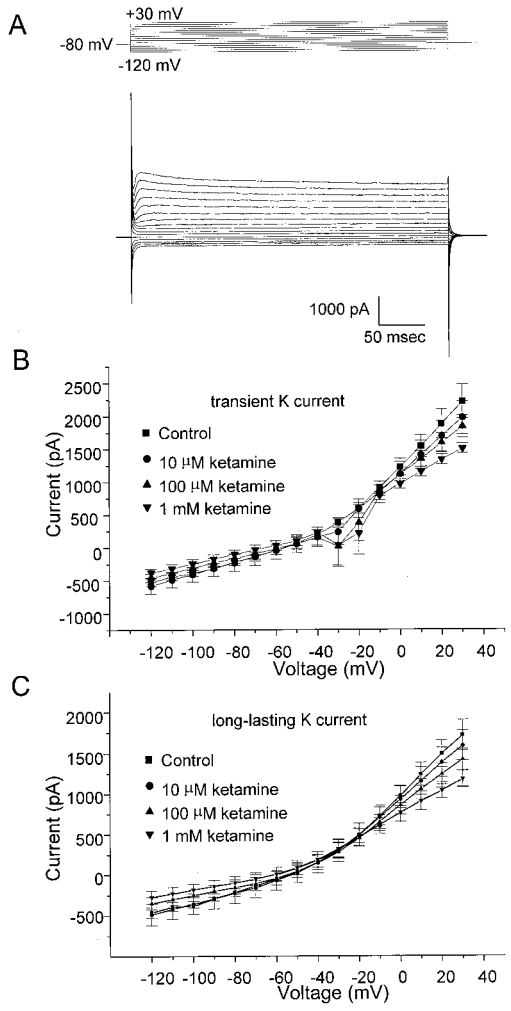


**Fig. 2. Effects of ketamine on isolated $K^+$ currents in cardiac parasympathetic preganglionic neurons in nucleus ambiguus. Neurons were bathed in 1 μM tetrodotoxin to block voltage-gated $Na^+$ currents and to isolate $K^+$ currents for study. From a holding potential of −80 mV, the test potentials were stepped in 10-mV increments between −120 and +30 mV (*A*). The resulting voltage-gated K currents were composed of an initial transient outward followed by a long-lasting outward current. The current–voltage relations of the transient $K^+$ current (*B*) and the long-lasting, rectified $K^+$ current (*C*) were not altered by ketamine (10 μM– mM; n = 5).**

voltage threshold for action potentials (control, −41.4 ± 1.6 mV *vs.* 10 μM ketamine, −41.0 ± 1.3 mV; n = 14; $P > 0.05$).

Action potential generation is dominated by complex interactions of the voltage- and time-dependent properties of primarily $Na^+$ and $K^+$ selective ion channels.[13] To understand the ionic mechanisms responsible for ketamine actions on cardiac parasympathetic neurons, voltage-gated $Na^+$ and $K^+$ currents were recorded under voltage clamp, isolated, and examined during exposure to ketamine. In the presence of 1 μM tetrodotoxin, two types of voltage-gated $K^+$ currents were evident in cardiac parasympathetic neurons.[12] Depolarization to voltages more positive than −40 mV activated two $K^+$ currents (fig. 2A). The early transient current has been identified as the $I_A$ $K^+$ current and can be blocked by 4-aminopyridine.[12] The long-lasting $K^+$ current that follows the early transient $I_A$ $K^+$ develops with a delay in activation and a characteristic rectification of its current–voltage relation (fig. 2B). The rectifying $K^+$ current was inhibited by tetraethyl ammonium in cardiac parasympathetic neurons.[12] Ketamine had little effect on either of the voltage-gated $K^+$ currents (n = 5). The current–voltage relations for the transient $K^+$ current and the delayed rectified $K^+$ current (n = 5) were unaltered ($P > 0.05$) by ketamine concentrations ranging from 10 μM–1 mM (fig. 2C).

$Na^+$ currents in rat cardiac parasympathetic neurons are known to be tetrodotoxin-resistant since relatively high concentrations (1 μM) of tetrodotoxin are necessary to block this $Na^+$ current.[12] In contrast to the $K^+$ currents in these neurons, ketamine substantially inhibited the voltage-gated $Na^+$ currents (fig. 3A; n = 7). Starting at 10 μM, ketamine significantly depressed the total $Na^+$ current in a dose-dependent fashion (fig. 3B). Ketamine progressively reduced the amplitude of these voltage-dependent $Na^+$ currents over the range of concentrations from 10 μM–1 mM.

Voltage-dependent open probability and the number of $Na^+$ channels in the inactivated state critically determines the activity of $Na^+$ channels.[13,14] The $Na^+$ current found in rat cardiac parasympathetic neurons shows a characteristically broad voltage dependence of its inactivation properties.[12] Prolonged steps to voltages more depolarized than approximately −70 mV reduced the peak $Na^+$ current that could be activated (fig. 4A; n = 6). During control conditions, this inactivation of $Na^+$ currents was half maximal (50% inactivation voltage [$IV_{50}$]) at −56.5 ± 3.2 mV and reached 20% ($IV_{20}$) at an average conditioning voltage of −47.8 ± 3.0 mV (n = 7). In addition to depressing the maximal $Na^+$ current, ketamine shifted the inactivation relation for conditioning voltage leftward to more hyperpolarized voltages. Ketamine shifted both the $IV_{50}$ and the $IV_{20}$ with increasing ketamine concentration (fig. 4B and table 1). Thus, in addition to reducing the peak $Na^+$ currents, ketamine enhanced the inactivation state of $Na^+$ channels by shifting their voltage-dependent inactivation to more negative membrane potentials.

The relatively fast inward currents evoked by depolarization might potentially contain contributions from voltage-gated $Ca^{2+}$ channels. To examine this, we tested the ketamine-induced inhibition of the inward currents in additional experiments in the presence of the nonselective blocker of voltage-gated $Ca^{2+}$ channels, $Cd^{2+}$ (100 μM). Ketamine (100 μM) reversibly inhibited the inward $Na^+$ currents in the presence of $Cd^{2+}$ (fig. 5A). To examine the time course and recovery of the ketamine-evoked inhibition of $Na^+$ current, depolarizing steps from −100 to −40 mV were applied every 5 s. Ketamine (100 μM) inhib-

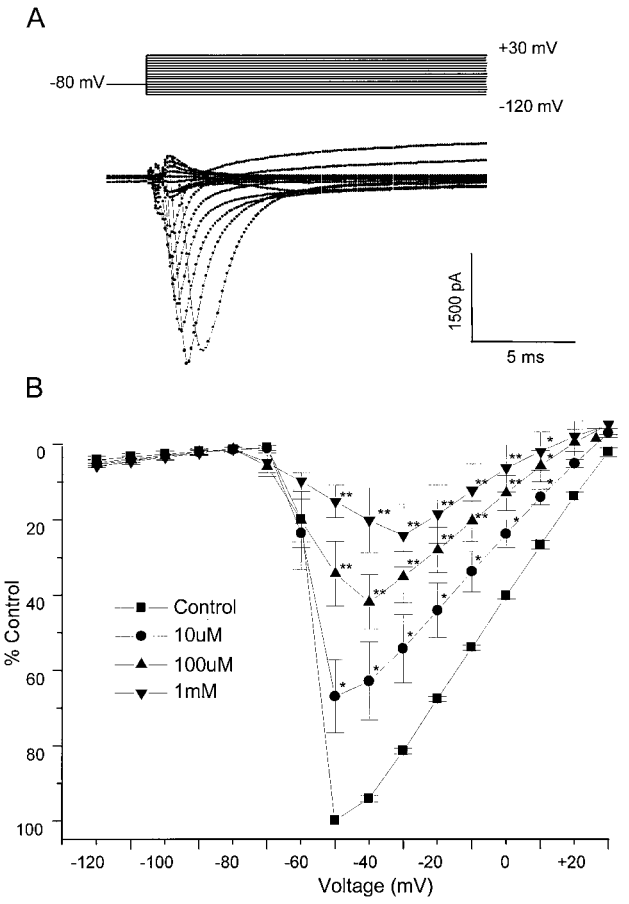


**Fig. 3. Depression of mean peak $Na^+$ currents in cardiac parasympathetic neurons. Cesium was included in the patch pipette to block voltage-gated $K^+$ currents and isolate the $Na^+$ current for study. Depolarizations to potentials more positive than −60 mV activated voltage-gated $Na^+$ currents (*A*). To examine whether ketamine altered these $Na^+$ currents, current–voltage relations were constructed during control and at each ketamine concentration (*B*). Compared with control currents, ketamine significantly inhibited the voltage-gated $Na^+$ currents at potentials from −50 to +10 mV and all concentrations tested (10 μM–1 mM; n = 7; *B*). Currents were normalized to the value of the peak mean current in control, which occurred at −50 mV. *$P < 0.05$ *versus* control. **$P < 0.01$ *versus* control.**

ited the peak $Na^+$ current from control 85.5 ± 3.6 to 31.3 ± 10.4% of maximum (fig. 5B; $P < 0.05$; n = 7). Recovery of the $Na^+$ currents was nearly complete, to 76.9 ± 7.1% (not statistically different from control, 85.5 ± 3.6%; n = 7) and generally required 8–10 min. Experiments performed with application of vehicle instead of ketamine (fig. 5B) indicated there was no time dependent run-down or decrease of the $Na^+$ currents in the course of these experiments (control, 85.0 ± 1.3%; after 23 min, 84.6 ± 0.6% of maximum; n = 7).

## Discussion

The sites of action within the central nervous system and the mechanisms responsible for ketamine-induced alterations of cardiorespiratory regulation are largely unknown. The current work provides two important new findings that implicate specific brainstem neurons responsible for autonomic regulation of heart function. We found that ketamine, at clinically relevant concentrations, directly inhibits parasympathetic cardiac neurons by two mechanisms: a direct inhibition of the voltage-gated $Na^+$ currents and an enhancement of the voltage-dependent inactivation of the $Na^+$ current. The physiologic consequence of the shift of $Na^+$ current inactivation to more negative potentials is that, at the normal resting membrane potential, a smaller portion of $Na^+$ channels is available for activation by excitatory depolarization. With fewer $Na^+$ channels available for activation, excitability and action potential propagation

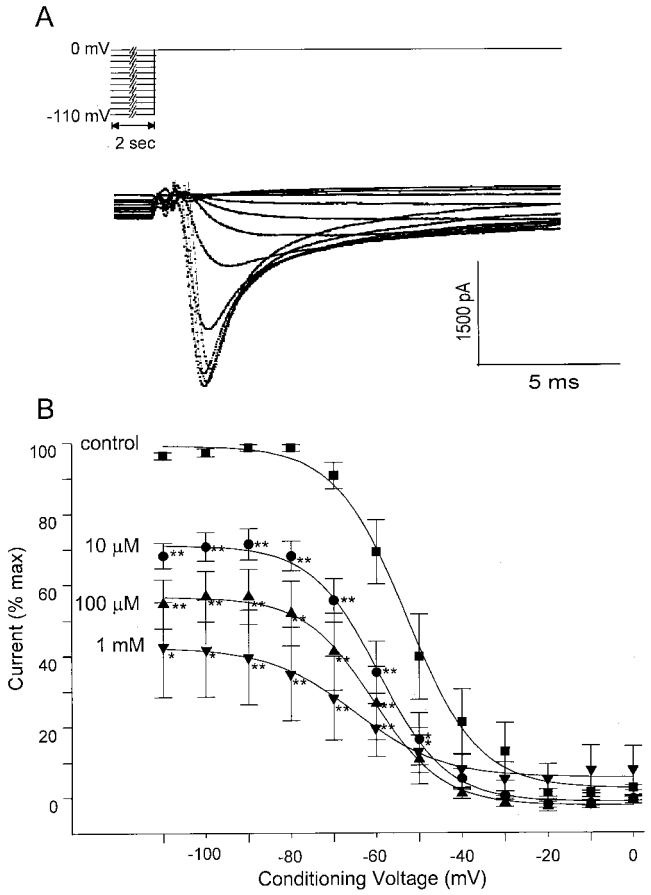


**Fig. 4. Shifts of $Na^+$ current inactivation curves to lower potentials by ketamine. To examine voltage-dependent inactivation, neurons (n = 6) were conditioned (2 s) at voltages from −110 to 0 mV before applying a test command pulse to 0 mV (*A*). Test pulses to 0 mV were applied to open $Na^+$ channels not inactivated by the previous conditioning pulse (note broken time axes). Compared with control conditions, ketamine (10 μM–1 mM) depressed ($P < 0.05$) peak $Na^+$ currents after submaximal, inactivating conditioning voltages. Currents were normalized to the value of the peak mean current in control (*B*). Ketamine shifted the inactivation curves to the left and thus to more hyperpolarized potentials. Ketamine significantly shifted the conditioning voltages, $IV_{20}$ and $IV_{50}$, which are the conditioning voltages required to inactivate 20% and 50% of the peak currents, respectively, at concentrations of 100 μM and 1 mM (table 1). *$P < 0.05$ *versus* control. **$P < 0.01$ *versus* control.**

**Table 1. Sodium Currents: Ketamine Shift in Voltage-dependent Inactivation**

| Ketamine | Control | 10 μM | 100 μM | 1 mM |
|---|---|---|---|---|
| $IV_{20}$ | −47.8 ± 3.0 mV | −51.4 ± 3.9 mV | −55.1 ± 4.8 mV* | −65.1 ± 7.3 mV* |
| $IV_{50}$ | −56.5 ± 3.2 mV | −59.5 ± 4.0 mV | −62.6 ± 4.8 mV* | −73.5 ± 7.4 mV* |

$IV_{20}$ and $IV_{50}$ are, respectively, the conditioning voltages required to inactivate 20% and 50% of the peak currents.
* $P < 0.05$ for paired $t$ test comparison to control. n = 7.

will be depressed. The direct inhibition of these $Na^+$ channels by ketamine also diminished the peak current that can be generated on $Na^+$ channel activation and thus the excitability of the cardiac parasympathetic reflex pathway.

Heart rate is normally strongly influenced by the tonic and reflex control of the cardioinhibitory parasympathetic activity impinging on the heart.[5,15] Increases in blood pressure stimulate arterial baroreceptors and, through brainstem mechanisms, including those in the nucleus tractus solitarius, such pressure increases excite cardiac parasympathetic neurons (nucleus ambiguus), resulting in reflex decreases in heart rate while inhibiting sympathetic pathways to the heart and blood vessels.[5] This baroreceptor reflex is bidirectional, and decreases in arterial pressure evoke increases in heart rate, in part by reductions in activation of cardiac parasympathetic neurons and sympathoexcitation. Normally, the baroreflex offers a rapid and powerful feedback to correct for blood pressure changes through these diverse hemodynamic mechanisms.[5] Thus, ketamine may blunt neuronal responses to baroreceptor stimulation and reduce parasympathetic outflow resulting in reflex tachycardia.

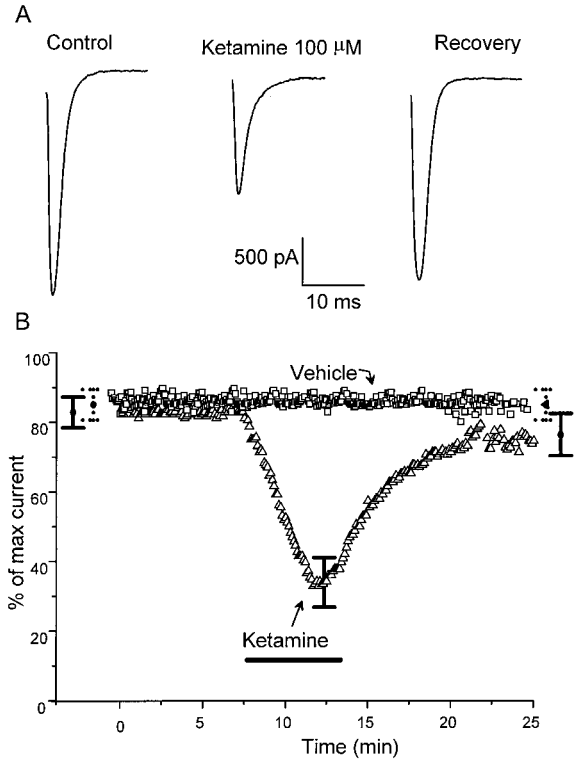


**Fig. 5. Recovery from ketamine inhibition. In the presence of the nonselective $Ca^{2+}$ channel blocker $Cd^{2+}$ (100 μM), ketamine (100 μM) reversibly inhibited the inward $Na^+$ currents (*A*). To examine the time course and recovery of the ketamine-evoked inhibition of $Na^+$ current depolarizing steps from −100 to −40 mV were applied every 5 s. Ketamine (100 μM) inhibited the peak $Na^+$ current from control 85.5 ± 3.6 to 31.3 ± 10.4% of maximum ($P < 0.05$; n = 7) (*B*). Recovery of the $Na^+$ currents was nearly complete, to 76.9 ± 7.1% (not statistically different from control, 85.5 ± 3.6%; n = 7) and generally required 8–10 min (*B*). Experiments performed with application of vehicle (*B*) instead of ketamine indicated there was no time-dependent run-down of the $Na^+$ currents in the course of these experiments (control, 85.0 ± 1.3%; after 23 min, 84.6 ± 0.6% of maximum; n = 7).**

It has been shown that ketamine depresses baroreflex control of heart rate in awake conscious dogs[7,8,16] and rabbits.[3,17] Compared with these studies in brain-intact animals, Blake and Korner[3] demonstrated that ketamine produces essentially identical baroreflex depression in unanesthetized rabbits with infrafollicular decerebration, and the depression is largely caused by a predominant inhibitory effect on the vagal component of the baroreflex control of heart rate. Furthermore, in unanesthetized, midcollicularly transected decerebrate rabbits,[4] ketamine inhibits the decrease in heart rate elicited by stimulation of the aortic depressor nerve (baroreflex activation). Because, in such preparations, ketamine has no effect on the heart rate decreases produced by direct efferent vagal nerve stimulation, postganglionic cardiac neurons and their nerve endings are not likely to be major sites of ketamine action.[4] Together, such studies suggest that sites below the pons are sufficient for ketamine to induce these changes in baroreflex heart rate control and are consistent with our results that ketamine can act on cardiac parasympathetic neurons in the brain stem. In intact animals it is also possible that ketamine may alter the activity of neurons involved in controlling the sympathetic activity to the heart, which may either augment or antagonize the inhibitory effect of ketamine on parasympathetic cardiac neurons.

At least part of the complex and varied responses to ketamine may be a result of differences across experiments in the direct and indirect actions of ketamine. Ketamine is generally thought to stimulate the central sympathetic nervous system.[1] However, experimental evidence suggests that ketamine directly depresses vasomotor and respiratory centers,[1,8] but that this depression is partly compensated by changes in afferent inputs from peripheral receptors. In deafferented rabbits, even low

doses of ketamine depressed the central sympathetic outflow.[18] Thus, ketamine induced sympathetically mediated increases in mean arterial pressure and vascular resistance, which were largely absent after sino-aortic denervation of conscious rabbits.[17] Such studies suggest that much of the sympathoexcitatory reflex effects of ketamine may be secondary and, therefore, indirectly related to other ketamine actions *via* afferent feedback such as that from arterial baroreceptors. Thus, parasympathetic activity is likely a major site of the integrated actions of ketamine.

Clinically, plasma concentration of ketamine peaks generally at approximately 10–60 $\mu$M during general anesthesia after an intravenous administration of 2 mg/kg, and ketamine is bound to plasma proteins variously reported as 12–50%.[19,20] Because animals generally require higher concentrations than humans to induce anesthesia,[3,16,17,21] our experimental concentrations that inhibited peak $Na^+$ currents (10 $\mu$M) and enhanced $Na^+$ channel inactivation (100 $\mu$M) appear likely to be within the clinical range. In rats, plasma concentrations of ketamine greater than 50 $\mu$M were required to produce general anesthesia.[21] When the concentrations of ketamine in brain were compared with those of plasma, the rat brain was shown to have a higher concentration than plasma (brain:plasma ratio of 6.5:1).[21] These actions of ketamine to inhibit cardiac parasympathetic neurons would not only increase heart rate but impair the baroreceptor reflex control of the heart rate.

Ketamine, at very high concentrations, has substantial additional actions at other potential effector cells important in cardiovascular homeostasis. In ventricular myocytes and peripheral nerve, ketamine inhibits voltage-gated $Na^+$ currents. However, these effects require considerably higher concentrations in the range of at least 30–300 $\mu$M.[22,23] Interestingly, in such nerve axons, ketamine does not alter the voltage-dependent inactivation of $Na^+$ currents.[24]

Ketamine had no significant effect on voltage-gated $K^+$ channels in cardiac parasympathetic neurons in the current study. Consistent with an overall lack of effect on $K^+$ channels, ketamine failed to alter the delay before firing during injection of depolarizing current. This delayed excitation is determined primarily by the activity of the $I_A$ $K^+$ current. In addition, action potential repolarization that is dependent on the activity of both the delayed and rectified $K^+$ current was not altered by ketamine. However, in other cell types, ketamine inhibited $K^+$ currents in various experimental cells such as cultured SH-SY5Y cells,[25] frog node of Ranvier,[24] and *Xenopus* axons.[22] In myocytes, ketamine inhibited the inward rectifying $K^+$ current[26] but not the voltage-gated outward current similar to the $K^+$ currents examined in this study. It is likely that ketamine may alter some $K^+$ channel subtypes and not others, and the relevance of anesthetic effects on voltage-gated channels is hard to generalize and needs to be tested in neurons with known cardiorespiratory function.

As suggested in a recent review, control of parasympathetic and sympathetic balance in surgical patients "may have important effects on cardiac mortality in surgical patients intra- and postoperatively."[27] One of the cardiovascular effects of ketamine is an increase in heart rate and blood pressure. Our results are consistent with this increase in heart rate and suggest that, in addition to its sympathomimetic properties, ketamine directly decreases parasympathetic cardiac activity. This increase in heart rate would therefore occur independent of the change in blood pressure. In summary, our study characterizes two mechanisms by which ketamine directly inhibits ion channel function in parasympathetic cardiac neurons in the brainstem, and such actions may mediate the decrease in parasympathetic cardiac activity and increase in heart rate that occurs with ketamine.

## References


1. Reves JG, Glass PSA, Lubarsky DA: Nonbarbiturate intravenous anesthetics, Anesthesia, 5th Edition. Edited by Miller RD. New York, Churchill Livingstone, 2000, pp 228–72
2. Slogoff S, Allen GW: The role of baroreceptors in the cardiovascular response to ketamine. Anesth Analg 1974; 53:704–7
3. Blake DW, Korner PI: Effects of ketamine and althesin anesthesia on baroreceptor–heart rate reflex and hemodynamics of intact and pontine rabbits. J Auton Nerv Syst 1982; 5:145–54
4. McGrath JC, MacKenzie JE, Millar RA: Effects of ketamine on central sympathetic discharge and the baroreceptor reflex during mechanical ventilation. Br J Anaesth 1975; 47:1141–7
5. Loewy AD, Spyer KM: Central Regulation Of Autonomic Functions. Edited by Loewy A, Spyer KM. Oxford, Oxford University Press, 1990, 68–87
6. Inoue K, Arndt JO: Efferent vagal discharge and heart rate in response to methohexitone, althesin, ketamine and etomidate in cats. Br J Anaesth 1982; 54:1105–16
7. Traber DL, Wilson RD, Priano LL: A detailed study of the cardiopulmonary response to ketamine and its blockade by atropine. South Med J 1970; 63: 1077–81
8. Traber DL, Wilson RD, Priano LL: The effect of beta-adrenergic blockade on the cardiopulmonary response to ketamine. Anesth Analg 1970; 49:604–13
9. Tauberger G, Hornchen U: Investigations on the mechanism of the effects of ketamine (Ketanest) on circulation and respiration. Anaesthetist 1980; 29: 547–51
10. Mendelowitz D: Firing properties of identified parasympathetic cardiac neurons in nucleus ambiguus. Am J Physiol Heart Circ Physiol 1996; 271: H2609-14
11. Mendelowitz D, Kunze DL: Identification and dissociation of cardiovascular neurons from the medulla for patch clamp analysis. Neurosci Lett 1991; 132:217–21
12. Mihalevich M, Neff RA, Mendelowitz D: Voltage-gated currents in identified parasympathetic cardiac neurons in the nucleus ambiguus. Brain Res 1996; 739:258–62
13. Hille B: Ionic Channels of Excitable Membranes, 2nd Edition. Sunderland, Sinauer Associates, 1992, pp 411–2
14. Catterall WA: Structure and function of voltage-gated ion channels. Annu Rev Biochem 1995; 64:493–531
15. Stein PK, Kleiger RE: Insights from the study of heart rate variability. Annu Rev Med 1999; 50:249–61
16. Priano LL, Bernards C, Marrone B: Effect of anesthetic induction agents on cardiovascular neuroregulation in dogs. Anesth Analg 1989; 68:344–9
17. Blake DW, Korner PI: Role of baroreceptor reflexes in the hemodynamic and heart rate responses to althesin, ketamine and thiopentone anesthesia. J Auton Nerv Syst 1981; 3:55–70
18. Sasao J, Taneyama C, Kohno N, Goto H: The effects of ketamine on renal sympathetic nerve activity and phrenic nerve activity in rabbits (with vagotomy) with and without afferent inputs from peripheral receptors. Anesth Analg 1996; 82:362–7
19. Benet LZ, Williams RL: Appendix II: Design and optimization of dosage regimens, The Pharmacological Basis of Therapeutics, 9th Edition. Edited by

Goodman LS, Limbird LE, Gilman AG, Molinoff PB, Rall TW. New York, McGraw-Hill, 1996, p 1752

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

21. Cohen ML, Chan S-L, Way WL, Trevor AJ: Distribution in the brain and metabolism of ketamine in the rat after intravenous administration. ANESTHESIOLOGY 1973; 39:370–6

22. Brau ME, Sander F, Vogel W, Hempelmann G: Blocking mechanisms of ketamine and its enantiomers in enzymatically demyelinated peripheral nerve as revealed by single-channel experiments. ANESTHESIOLOGY 1997; 86:394–404

23. Hara Y, Chugun A, Nakaya H, Kondo H: Tonic block of the sodium and calcium currents by ketamine in isolated guinea pig ventricular myocytes. J Vet Med Sci 1998; 60:479–83

24. Benoit E: Effects of intravenous anaesthetics on nerve axons. Eur J Anaesthesiol 1995; 12:59–70

25. Urban BW, Friederich P: Anesthetic mechanisms in-vitro and in general anesthesia. Toxicol Lett 1998; 101:9–16

26. Baum VC: Distinctive effects of three intravenous anesthetics on the inward rectifier (IK1) and the delayed rectifier (IK) potassium currents in myocardium: Implications for the mechanism of action. Anesth Analg 1993; 76:18–23

27. Ebert TJ: Is gaining control of the autonomic nervous system important to our specialty? ANESTHESIOLOGY 1999; 90:651–3