

# Ketamine Inhibits Presynaptic and Postsynaptic Nicotinic Excitation of 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 possible central nervous system actions of ketamine to inhibit cardiac parasympathetic neurons in the brainstem by inhibiting multiple nicotinic excitatory mechanisms.

**Methods:** The authors used a novel *in vitro* approach to study the effect of ketamine on 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. The effects of ketamine were tested on nicotine-evoked ligand-gated currents and spontaneous glutamatergic miniature synaptic currents (mini) in cardiac parasympathetic preganglionic neurons.

**Results:** Ketamine (10  $\mu$ M) inhibited (1) the nicotine (1  $\mu$ M)-evoked presynaptic facilitation of glutamate release (mini frequency,  $18 \pm 7\%$  of control;  $n = 9$ ), and (2) the direct postsynaptic ligand-gated current ( $27 \pm 8\%$  of control;  $n = 9$ ), but ketamine did not alter the amplitude of postsynaptic miniature non-*N*-methyl-D-aspartate currents.  $\alpha$  Bungarotoxin, an antagonist of  $\alpha 7$  containing nicotinic presynaptic receptors, blocked ketamine actions on mini frequency ( $n = 10$ ) but not mini amplitude.

**Conclusions:** Ketamine inhibits the presynaptic nicotinic receptors responsible for facilitating neurotransmitter release, as well as the direct ligand-gated inward current, but does not alter the nicotinic augmentation of non-*N*-methyl-D-aspartate currents in brainstem parasympathetic cardiac neurons. Such actions may mediate the decrease in parasympathetic cardiac activity and increase in heart rate that occurs with ketamine.

THE intravenous anesthetic ketamine normally produces cardiovascular activation by increasing both blood pressure and heart rate. The increase in blood pressure with ketamine is anticipated because the action of ketamine is commonly regarded as sympathoexcitatory.<sup>1</sup> However, the tachycardia with ketamine is enigmatic in mechanism<sup>1</sup> because an increase in heart rate is unexpected. Normally, increases in blood pressure would evoke baroreflex-induced decreases in heart rate. In cardiac

baroreflex responses, blood pressure increases activate arterial baroreceptors that then excite second-order neurons in the nucleus of the solitary tract, and these neurons in turn activate cardioinhibitory parasympathetic preganglionic neurons located primarily in the nucleus ambiguus.<sup>2</sup> In principle, compromise of any of these sites within the baroreflex pathway could increase heart rate. Baroreceptor sensors of the arterial baroreflex appear not to contribute to ketamine-induced tachycardia,<sup>3</sup> but ketamine likely affects components of the reflex within autonomic regions below the pons.<sup>4,5</sup> If cardiac muscarinic receptors are blocked with atropine, ketamine no longer induces tachycardia and, conversely, propranolol block of the cardiac sympathetic pathway does not prevent ketamine-evoked increases in heart rate. Such results indicated the importance of the contribution of parasympathetic control of heart rate as a mechanism for ketamine-induced tachycardia.<sup>6-9</sup> However, little is known concerning the mechanisms of action of ketamine at relevant central autonomic sites.<sup>9</sup>

Heart rate is determined primarily by the activity of cardiac preganglionic parasympathetic neurons within the brainstem.<sup>2</sup> Previous studies from this laboratory have described the activation of cardiac preganglionic parasympathetic neurons by nicotine and acetylcholine.<sup>10-11</sup> Such nicotinic receptors may be potential clinically relevant targets of ketamine. Ketamine inhibits nicotinic cholinergic receptors exogenously expressed in *Xenopus* oocytes or endogenous nicotinic receptors in PC12 and SH-SY5Y cells.<sup>12-16</sup> In the brainstem, this cholinergic activation may involve at least two general classes of nicotinic receptor sites that lead to excitation: presynaptic receptors that increase the probability of excitatory neurotransmitter release and postsynaptic receptors that alter other postsynaptic receptors or that directly evoke cholinergic-gated inward current.<sup>10-11</sup> Thus, ketamine could lead to inhibition by reducing these nicotinic responses.

In the current study, we directly examined the cellular actions of ketamine on the nicotinic-mediated excitation of cardiac parasympathetic preganglionic neurons in brainstem slices. We selectively recorded from rat cardiac parasympathetic preganglionic neurons in the nucleus ambiguus identified by a novel retrograde tracing method and assessed nicotinic excitation using patch clamp recording. Ketamine inhibited the presynaptic nicotinic receptors responsible for facilitating neurotransmitter release, as well as the direct ligand-gated

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nicotinic-evoked inward current, but did not alter the nicotinic augmentation of glutamate evoked non-*N*-methyl-D-aspartate (NMDA) currents in brainstem parasympathetic cardiac neurons. Such actions may contribute to the decrease in parasympathetic cardiac activity and increase in heart rate that occurs with ketamine.

## Materials and Methods

All animal procedures were performed with the approval of the Institutional Animal Care and Use Committee at George Washington University and 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, Scottsdale, 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 preparation using a two-stage procedure.<sup>17–19</sup> In an initial surgery 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 as determined by the veterinary staff at George Washington University. On the day of the recordings, the animals were anesthetized with methoxyflurane and killed by cervical dislocation. The brains were quickly removed, placed in cold (2°C) physiologic buffer (containing 140 mM NaCl, 5 mM KCl, 2 mM CaCl<sub>2</sub>, 5 mM glucose, 10 mM HEPES, pH 7.4), equilibrated with 100% O<sub>2</sub>, and mounted on a vibratome. The medulla was cut in transverse sections 250 μm thick. Slices that included the nucleus ambiguus were transferred to a recording chamber positioned on the stage of a fixed-stage upright microscope (Carl Zeiss Inc., Thornwood, NY) using a 40× water submersion objective equipped with fluorescent filters to visualize rhodamine. Cardiac parasympathetic preganglionic neurons were visualized and identified by the presence of the fluorescent tracer rhodamine in their cell bodies.

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<sub>2</sub>PO<sub>4</sub>, 25 mM NaHCO<sub>3</sub>, 5 mM

HEPES, 5.5 mM dextrose, 2 mM and CaCl<sub>2</sub>, equilibrated with 95% O<sub>2</sub>–5% CO<sub>2</sub>, pH 7.4. Picrotoxin (100 μM), strychnine (1 μM), prazosin (10 μM), D-2-amino-5-phosphonovalerate (50 μM), and tetrodotoxin (1 μM) were infused into the recording chamber to prevent γ-aminobutyric acid, glycinergic, α<sub>1</sub>-adrenergic, and glutamatergic NMDA postsynaptic currents, respectively. α-Bungarotoxin (αBgTx, 100 nM) was used to block α<sub>7</sub> subunit containing nicotinic receptors. All drugs used in this study were purchased from Sigma Aldrich (St. Louis, MO).

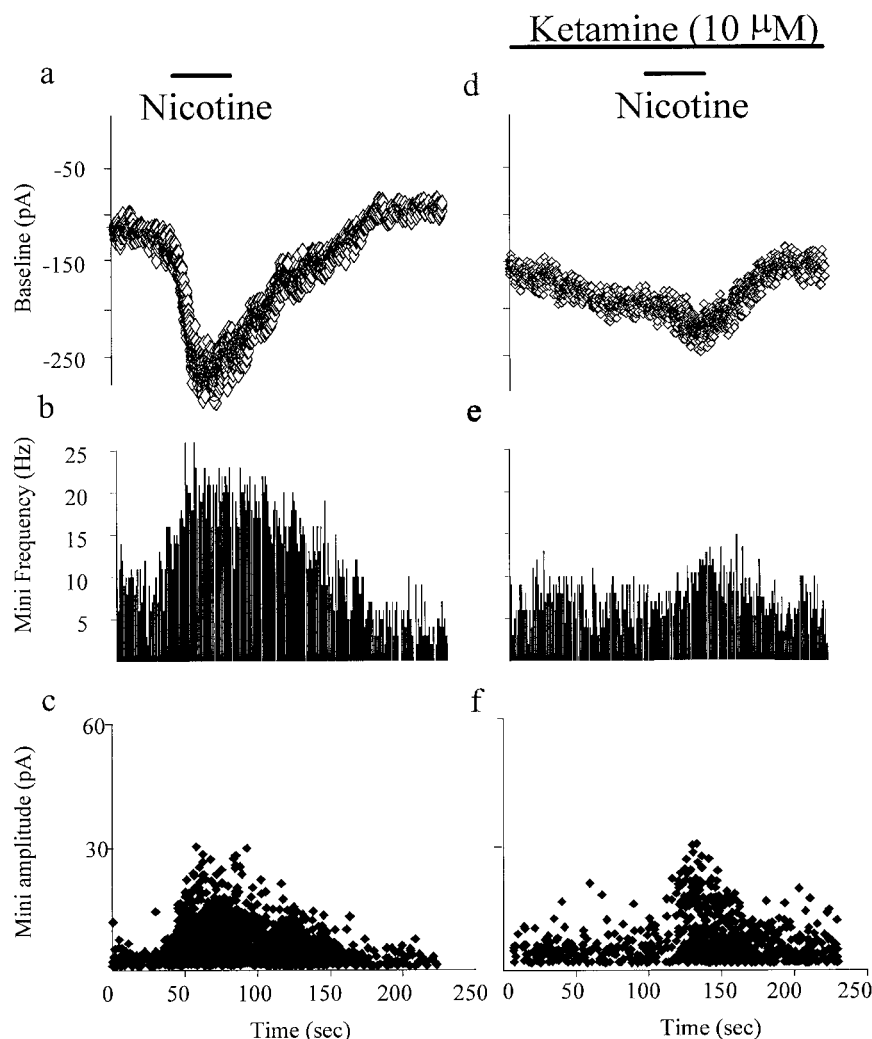
Patch pipettes were then advanced onto the somal membrane of the labeled neurons with visualization provided by differential interference contrast optics under infrared illumination and using a cooled charge-coupled device camera (Imagepoint; Roper Scientific, Trenton, NJ). Infrared-differential interference contrast images of the neurons were visualized in real time (30 frames/s).

### *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, Sarasota, FL) and mounted onto a micromanipulator (Narishige 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. Patch pipettes were filled with a solution consisting of 130 mM potassium gluconate, 10 mM HEPES, 10 mM EGTA, 1 mM CaCl<sub>2</sub>, and 1 mM MgCl<sub>2</sub>. Ligand-gated inward currents and glutamatergic synaptic events were studied under voltage clamp with a holding potential of –80 mV controlled by pClamp software (version 7.0; Axon Instruments, Foster City, CA). All experiments were performed at room temperature (23–25°C).

Nicotine (1 μM) was delivered by pressure ejection directly onto the neuron by a micropipette positioned directly above the neuron for 20–40 s. After application of nicotine, a brief (approximately 10-s) negative pressure pulse was applied to limit any diffusion of nicotine out of the micropipette. After a 1–2-min period, the slice was then perfused with ketamine (0.1, 1.0, or 10.0 μM) for 20 min. At the end of this 20-min period, nicotine was reapplied in the continued presence of ketamine. A 20-min delay was used to minimize any desensitization of the cell by nicotine. αBgTx (100 nM) was also included in

**Fig. 1.** Cholinergic nicotinic actions on cardiac parasympathetic neurons. Nicotine evokes an inward current in these neurons (A). Nicotine also elicits an increase in the frequency of miniature glutamatergic synaptic events (minis) (B) and an increase in mini amplitude (C). After a 20-min delay, nicotine was reapplied to the cardiac parasympathetic neuron, but now in the presence of ketamine (10  $\mu$ M). Administration of ketamine (10  $\mu$ M) significantly inhibited the nicotine-evoked inward current (D) and increase in mini frequency (E) but did not significantly alter the nicotine-evoked increase in mini amplitude (F).



the perfusate for 20 min before application of nicotine in the experiments examining the role of  $\alpha_7$  subunit containing nicotinic receptors. A slice was only used for one experiment and only one concentration of ketamine. Analysis of spontaneous postsynaptic events was performed using MiniAnalysis (version 4.3.1; Synaptosoft, Decatur, GA) with an amplitude threshold of 6 pA. Responses to nicotine were averaged from a 1-s period at the peak of the nicotine-induced inward current. The nicotine-evoked responses with ketamine were normalized to the control responses evoked by nicotine alone for each neuron. Previous work has shown that nicotine can be applied repetitively to cardiac parasympathetic neurons using these protocols, with no attenuation of responses.<sup>10</sup> Different groups of neurons were examined at each concentration of ketamine.

#### Data and Statistical Analysis

Current amplitudes were measured as described and presented as the mean  $\pm$  standard error of the mean. All graphical plots, analyses, and statistical tests were per-

formed 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.

#### Results

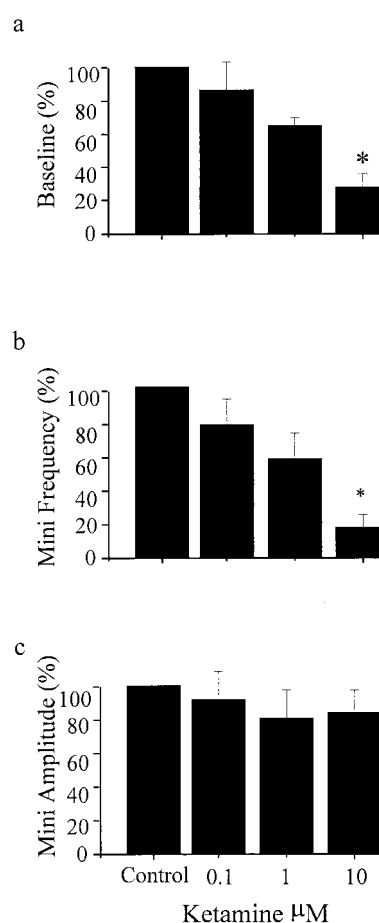
Cholinergic synaptic inputs to preganglionic cardiac parasympathetic neurons are excitatory and are mediated through nicotinic receptors.<sup>10,11</sup> Thus, application of nicotine (fig. 1A) evoked a substantial inward current in these neurons. These experiments were conducted in the presence of tetrodotoxin to block action potential generation. Tetrodotoxin thus eliminates contributions of local active processes as well as possible interference from activation of remote neurons that might subsequently activate synaptic contacts on the recorded neuron. During these conditions, the miniature synaptic events (minis) recorded are a result of spontaneous release of neurotransmitter from the presynaptic endings. Minis are thought to be the postsynaptic responses

evoked by the spontaneous (not action potential-evoked) release of transmitter from a single presynaptic vesicle. Mini activity is tetrodotoxin-insensitive and is analogous to miniature end-plate potentials observed at neuromuscular junctions.

During nicotine ( $1 \mu\text{M}$ ) application, spontaneous synaptic minis increased substantially both in frequency (fig. 1B) and amplitude (fig. 1C). Such results indicate that nicotinic receptors mediate multiple response mechanisms at cardiac parasympathetic neurons: (1) a direct nicotine-evoked inward current in the postsynaptic neuron; (2) a presynaptic facilitation of the probability of transmitter release (increased mini frequency); and (3) a facilitation of the postsynaptic response to released transmitter (increased mini amplitude). Our previous work during these pharmacologic and recording conditions identified these minis as resulting from glutamate release activating non-NMDA receptors.<sup>10</sup>

To examine whether ketamine alters these nicotinic mechanisms in cardiac parasympathetic neurons, we analyzed the nicotine-evoked changes in mini frequency and amplitude before and during anesthetic application. In the presence of ketamine ( $10 \mu\text{M}$ ), nicotine responses were significantly inhibited. The nicotine-evoked inward current (fig. 1D) was strongly depressed. Although ketamine eliminated the increase in mini frequency (fig. 1E), the nicotine-evoked increase in mini amplitude remained (fig. 1F). The summary data from nine cells show that ketamine produced significant depression of these nicotinic responses at  $10\text{-}\mu\text{M}$  concentrations (fig. 2). The basal mini frequency, mini amplitudes, and baseline currents were not changed by ketamine (control  $22.8 \pm 2.2$  Hz, ketamine  $18.8 \pm 1.4$  Hz,  $P > 0.05$ ; control  $111.8 \pm 26.7$  pA, ketamine  $119.7 \pm 5.5$  pA,  $P > 0.05$ ; control  $-207.0 \pm 7.1$  pA, ketamine  $-217.0 \pm 14.4$  pA,  $P > 0.05$ , respectively), suggesting that the basic release process for glutamate and glutamate receptors were unaffected by ketamine at these concentrations, but ketamine does specifically alter the nicotinic modulation of this neurotransmission.

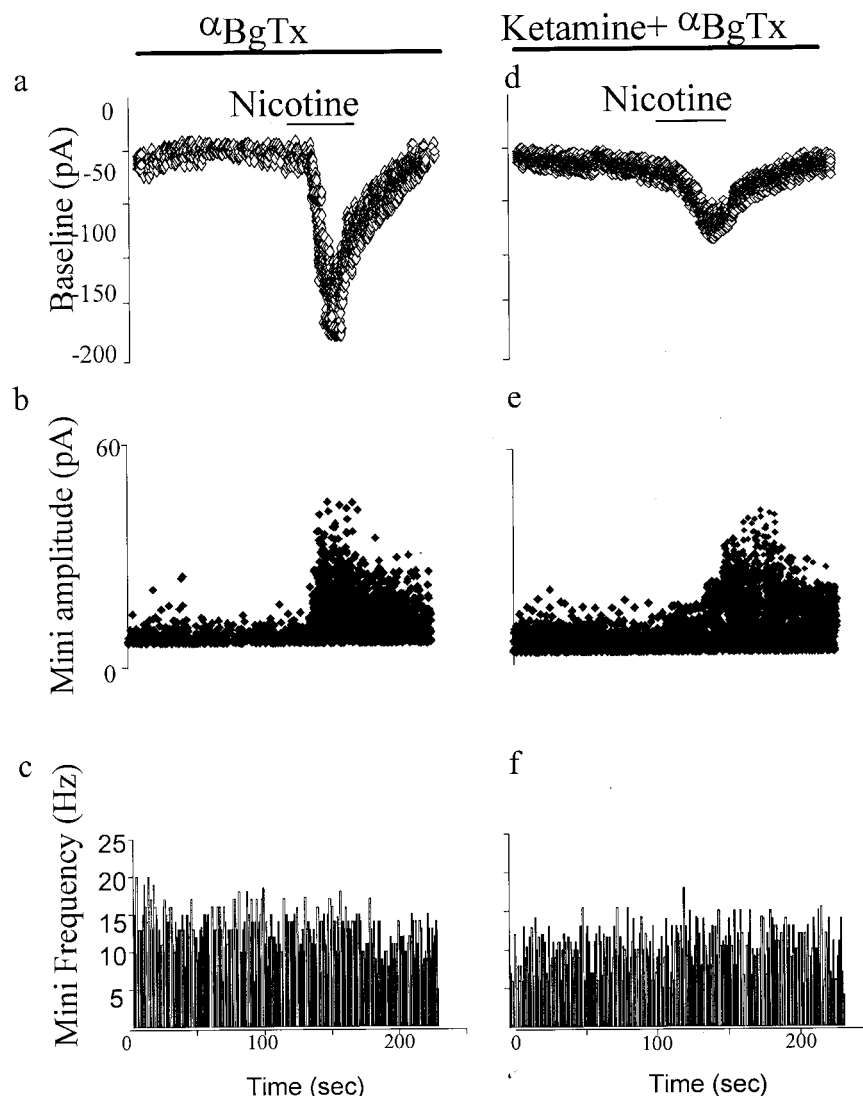
Nicotinic receptors mediate multiple actions at cardiac parasympathetic neurons in nucleus ambiguus. Ketamine appears to alter some but not all of these nicotinic sites. To better test this separation of action, we used the neurotoxin  $\alpha\text{BgTx}$  to specifically block nicotinic receptors containing the  $\alpha_7$  gene product.<sup>20</sup> Nicotinic receptors with  $\alpha_7$  subunits are often selectively localized to presynaptic sites and, as shown in previous work, modulate the presynaptic release of glutamate onto cardiac parasympathetic neurons.<sup>20</sup> In the presence of  $\alpha\text{BgTx}$ , the postsynaptic actions of nicotine to increase the baseline current (fig. 3A) and mini amplitude (fig. 3B) persisted.  $\alpha\text{BgTx}$  selectively eliminated the nicotine-evoked increase in mini frequency in cardiac parasympathetic neurons (fig. 3C), which is caused by nicotine acting at presynaptic receptors to increase the probability of neu-



**Fig. 2.** Summary ketamine dose-response relations for cardiac parasympathetic neurons ( $n = 9$ ). The nicotine-evoked inward current (normalized to control, A) was inhibited by increasing concentrations of ketamine, and this inhibition was statistically significant at a ketamine concentration of  $10 \mu\text{M}$ . Ketamine also significantly ( $P < 0.05$ ) inhibited the nicotine-evoked increase in miniature glutamatergic synaptic event (mini) amplitude (B), and this inhibition was significant at a ketamine concentration of  $10 \mu\text{M}$ . Ketamine, at all concentrations examined ( $0.1\text{--}10 \mu\text{M}$ ), had no significant effect on mini amplitude (C).

rotransmitter release.<sup>10</sup> In the presence of  $\alpha\text{BgTx}$ , ketamine continued to inhibit the nicotine-evoked postsynaptic direct ligand-gated inward current (fig. 3D). The nicotine-evoked increase in mini amplitude was unaffected by ketamine (fig. 3E). Ketamine did not affect the  $\alpha\text{BgTx}$  blockade of nicotine actions on mini frequency (fig. 3F). On average ( $n = 10$ ), the nicotine-evoked inward current responses were significantly depressed by  $10 \mu\text{M}$  ketamine in the presence of  $\alpha\text{BgTx}$  (fig. 4). Although the increase in mini amplitude occurred during increases in mini frequency, summation of nearly simultaneous minis cannot be solely responsible for the increase in mini amplitude. The  $\alpha\text{BgTx}$  results (fig. 3) indicate that the increase in mini amplitude is independent of changes in mini frequency, since the increase in mini amplitude persisted even when the increase in mini frequency was prevented with  $\alpha\text{BgTx}$ . Thus, summation is very unlikely to be responsible for the observed in-

**Fig. 3.** Role of  $\alpha_7$  subunit-containing nicotinic receptors in ketamine responses.  $\alpha$  Bungarotoxin ( $\alpha$ BgTx), a selective antagonist of nicotinic receptors containing the  $\alpha_7$  subunit, did not alter the nicotine-evoked inward current (*A*) or increase in miniature glutamatergic synaptic event (mini) amplitude (*B*), but did block the nicotine-evoked alteration in mini frequency (*C*). These results are consistent with the findings that nicotinic receptors containing  $\alpha_7$  subunits are predominantly located presynaptically and alter the probability of transmitter release. Inclusion of ketamine in the perfusate continued to inhibit the nicotine-evoked inward current (*D*), but the effect of ketamine on the nicotine-evoked increase in mini frequency was occluded by the presence of  $\alpha$ BgTx (*F*). Neither ketamine nor  $\alpha$ BgTx had an effect on mini amplitude (*E*).



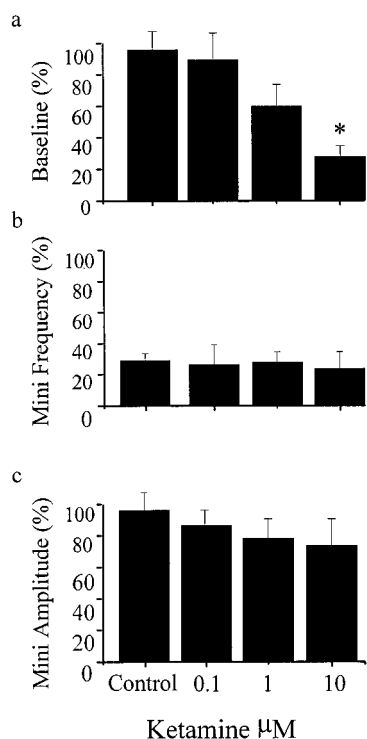
crease in mini amplitude (figs. 3B and E). These experiments also suggest that the increase in mini amplitude is likely caused by postsynaptic mechanisms that facilitate non-NMDA receptor-mediated currents, and that these nicotinic responses are insensitive to ketamine.

## Discussion

Although central cardiorespiratory regulation is altered by ketamine, little is known about the mechanisms or responsible sites of action within the central nervous system. The current work provides several important new findings that illustrate actions at specific brainstem neurons within the autonomic regulatory network controlling heart function. We have identified new specific mechanisms of ketamine action involving alterations of both presynaptic and postsynaptic nicotinic receptor responses to inhibit these neurons.

We found that, at clinically relevant concentrations, ketamine modulates parasympathetic cardiac neurons by

inhibition of two key nicotinic cholinergic excitatory mechanisms: reductions in postsynaptic nicotinic cholinergic excitatory inward currents and elimination of presynaptic nicotinic enhancement of the presynaptic release of glutamate. As illustrated in figure 5, a major excitatory projection to cardiac parasympathetic neurons originates from neurons in the nucleus tractus solitarius. This pathway likely provides an essential link in the baroreflex control of heart rate.<sup>2</sup> For example, during increases in blood pressure, there is increased activity in arterial baroreceptors that evokes an increase in activity of neurons in the nucleus tractus solitarius. The subsequent increased activity in the pathway from the nucleus tractus solitarius to cardiac parasympathetic neurons would increase the activity of the cardioinhibitory parasympathetic neurons, reducing heart rate, cardiac output, and blood pressure.<sup>2</sup> Cholinergic projections to cardiac parasympathetic neurons are likely involved in the respiratory modulation of heart rate.<sup>2</sup> One such cholinergic pathway to cardiac parasympa-



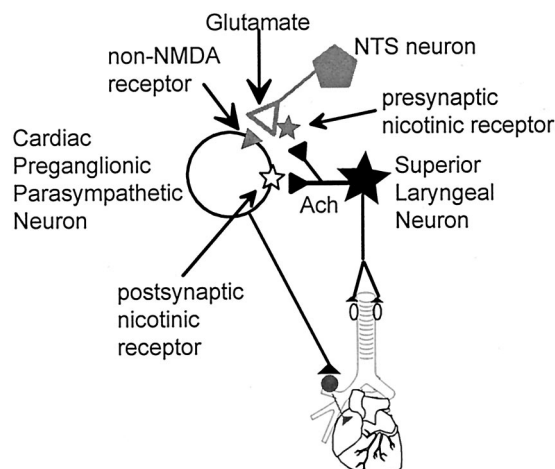
**Fig. 4.** Summary ketamine dose-response relations for cardiac parasympathetic neurons and the role of  $\alpha_7$  subunit-containing nicotinic receptors ( $n = 10$ ). Ketamine, at increasing concentrations, continued to inhibit the nicotine-evoked inward current (normalized to control, *A*) in the presence of  $\alpha$  bungarotoxin ( $\alpha$ BgTx), and this inhibition was statistically significant at a ketamine concentration of  $10 \mu\text{M}$ . However, in the presence of  $\alpha$ BgTx, nicotine did not evoke an increase in miniature glutamatergic synaptic event (mini) frequency (*B*), and ketamine at all concentrations examined ( $0.1$ – $10 \mu\text{M}$ ) had no further effect on mini frequency. Ketamine, at all concentrations examined ( $0.1$ – $10 \mu\text{M}$ ), had no significant effect on mini amplitude (*C*) in the presence of  $\alpha$ BgTx.

thetic neurons has been shown to originate from cholinergic superior laryngeal neurons,<sup>21</sup> as shown in figure 5. This cholinergic modulation of cardiac parasympathetic activity can occur *via* nicotinic activation of direct postsynaptic inward currents, as well as presynaptic nicotinic receptors that modulate the release of glutamate, which are both sensitive to ketamine. The third mechanism of action of nicotine, to increase glutamatergic non-NMDA synaptic responses, is insensitive to ketamine.

Clinically, plasma concentration of ketamine is generally 2–5 mM at emergence and peaks at approximately 10–60  $\mu\text{M}$  during general anesthesia after an intravenous administration of 2 mg/kg, and ketamine is bound to plasma proteins variously reported as 12–50%.<sup>22,23</sup> Animals have generally been found to require considerably higher concentrations of ketamine than humans to induce anesthesia.<sup>4,15,16,22</sup> In rats, plasma concentrations of ketamine greater than 50  $\mu\text{M}$  were required to produce general anesthesia.<sup>24</sup> 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).<sup>24</sup> This suggests that, relative to their respective anesthetic doses, the experimental concentrations in isolated slices that inhibited presynaptic and postsynaptic nicotinic responses ( $10 \mu\text{M}$ ) in this study are within the clinically effective range for ketamine.

Very similar to the ketamine-induced inhibition of the nicotinic depolarizing current in cardiac parasympathetic neurons, ketamine has also been recently shown in two studies to inhibit a nicotine-elicited inward current in PC12 cells.<sup>14–15</sup> Similar to the results in this study, the half-maximal concentration for this inhibition in these studies were shown to be 5.2 and 5.4  $\mu\text{M}$  for *S*(+) and *R*(–) ketamine and 2.8–21.4  $\mu\text{M}$  for racemic ketamine.<sup>14–15</sup> Although PC12 cells contain the mRNA for many different nicotinic subunits, ( $\alpha_3$ ,  $\alpha_5$ ,  $\alpha_7$ ,  $\beta_2$ ,  $\beta_3$ , and  $\beta_4$ ), the  $\alpha_3\beta_4$ -containing receptors are thought to be predominant in PC12 cells.<sup>14–15</sup> Ketamine has also been shown to inhibit human neuronal nicotinic acetylcholine receptors expressed in *Xenopus* oocytes.<sup>12–13</sup> Nicotinic receptors expressed in *Xenopus* oocytes with  $\beta_4$  subunits were more sensitive to block with ketamine than  $\beta_2$ -containing subunits, with half-maximal concentra-



**Fig. 5.** Glutamatergic and cholinergic pathways to cardiac parasympathetic neurons. Stimulation of the nucleus tractus solitarius (NTS) evokes a glutamatergic pathway that activates non-N-methyl-D-aspartate (NMDA) postsynaptic currents in cardiac parasympathetic neurons.<sup>2</sup> This pathway may constitute the essential link between increases in blood pressure and afferent baroreceptor activity, which activates neurons in the NTS, and the reflex compensatory decrease in heart rate caused by increases in efferent cardioinhibitory cardiac parasympathetic activity. Acetylcholine (ACh) is likely involved in mediating cardiorespiratory interactions and excites cardiac parasympathetic neurons *via* three mechanisms: activating a direct ligand-gated postsynaptic nicotinic receptor, enhancing postsynaptic non-NMDA currents, and presynaptically (*via*  $\alpha_7$  subunit-containing nicotinic receptors) facilitating transmitter release.<sup>10,11</sup> At least one cholinergic pathway to cardiac parasympathetic neurons originates from superior laryngeal neurons.<sup>21</sup> Ketamine inhibits both the postsynaptic nicotinic receptors that evoke an inward current, as well as the presynaptic nicotinic receptors that facilitate glutamatergic neurotransmission.

tions for inhibition of 9.5–29  $\mu\text{M}$  and 50–92  $\mu\text{M}$ , respectively.<sup>12</sup>  $\alpha_3$  subunit-containing receptors were more sensitive to ketamine inhibition than other  $\alpha$ -containing receptors.<sup>12</sup> This study is the first to demonstrate that native presynaptic  $\alpha_7$  subunit-containing nicotinic receptors in the brain are sensitive to ketamine.

The subunit composition of the postsynaptic nicotinic receptors responsible for the inward current and increase in mini amplitude in cardiac parasympathetic neurons is currently unknown. The subtype of nicotinic receptors responsible for the increase in mini amplitude are unaffected by ketamine, whereas the nicotinic receptors responsible for the inward current are inhibited by ketamine. Both the nicotine-evoked inward current and increase in mini amplitude are unaltered by  $\alpha\text{BgTx}$ , indicating that these postsynaptic nicotinic receptors do not contain the  $\alpha_7$  nicotinic subunit. However, the presynaptic receptors that, when activated, increase the frequency of glutamatergic minis are selectively blocked by  $\alpha\text{BgTx}$  and depend on activation of P- and L-type voltage-gated calcium currents.<sup>10,11</sup>  $\alpha\text{BgTx}$  is known to be a selective antagonist to nicotinic receptors composed of  $\alpha_7$  subunits.<sup>20</sup> Because ketamine inhibited the nicotine-evoked increase in mini frequency and such increases could be blocked by  $\alpha\text{BgTx}$ , leaving no ketamine-sensitive mini frequency component, this result is consistent with ketamine acting on presynaptic nicotinic receptors containing the  $\alpha_7$  subunit.

Ketamine depresses cardiac baroreflexes in conscious animals whether they are brain intact<sup>7,8,25,26</sup> or after infrafollicular decerebration.<sup>4</sup> Ketamine appears to inhibit the baroreflex largely through effects on the parasympathetic component.<sup>4</sup> The predominance of central actions of ketamine were demonstrated by observations in unanesthetized, midcollicularly transected decerebrate rabbits in which the heart rate baroreflex responses evoked by electrical stimulation of arterial baroreceptor axons in the aortic depressor nerve were inhibited by ketamine, but not heart rate decreases produced by direct efferent vagal nerve stimulation.<sup>5</sup> Together, such studies suggest that ketamine can act at sites below the pons to induce these changes in baroreflex heart rate control—a conclusion consistent with the ketamine actions we found on cardiac parasympathetic neurons in the brain stem.

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.”<sup>27</sup> 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 occurs *via* at least two mechanisms. Recent work has shown that ketamine, at clinically relevant concentrations, inhibits

the magnitude and enhances the inactivation of voltage-gated sodium currents in cardiac parasympathetic neurons.<sup>28</sup> Ketamine did not alter the voltage-gated potassium currents.<sup>28</sup> The ketamine-induced inhibition of voltage-gated sodium currents would reduce the response of cardiac parasympathetic neurons to excitatory inputs. The work in this study extends these observations and demonstrates that ketamine also inhibits excitatory synaptic inputs to cardiac parasympathetic neurons. Ketamine inhibits both presynaptic nicotinic cholinergic receptors that play a facilitatory role in excitatory glutamatergic neurotransmission, and also inhibits the responses of postsynaptic nicotinic receptors that act to directly depolarize cardiac parasympathetic neurons. Inhibitory actions both on voltage-gated sodium currents and cholinergic modulation of glutamatergic neurotransmission may contribute to the decrease in parasympathetic cardiac activity and increase in heart rate that occurs with ketamine, although other mechanisms and central autonomic sites are also likely to contribute.

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