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Effects of Ketamine on Cardiovascular Responses Mediated by N-methyl-D-aspartate Receptor in the Rat Nucleus Tractus Solitarius

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Background: **Ketamine is often administered to patients in whom hemodynamic instability is suspected. This study was designed to investigate the effect and possible site of action of ketamine on baroreflex response in rats. The site focused upon was the N-methyl-D-aspartate (NMDA) receptor of the nucleus tractus solitarius (NTS).**

Methods: **The effect of ketamine upon the baroreflex response was examined in urethan-anesthetized rats. The baroreflex was elicited by traction of the left carotid artery. Additional studies tested the acute hypotension and effect of ketamine on the hemodynamic response elicited by microinjection of NMDA into the NTS.**

Results: **Upon traction, the mean arterial pressure (MAP) decreased by 28.0 ± 1.0 (mean ± SE) mmHg and the heart rate (HR) decreased by 7.5 ± 0.8 beats/min. Ketamine (9 mg/kg iv) attenuated these responses with a resultant decrease in MAP and HR of 3.6 ± 1.3 mmHg and 1.6 ± 0.2 beats/min, respectively ($P < .01$). It also suppressed the NMDA-induced decrease in MAP from 47 ± 5 to 6 ± 0.7 mmHg and delayed the decrease in HR. D-2-amino-5-phosphonovalerate, a competitive NMDA antagonist, blocked the NMDA-induced decrease in MAP from 47 ± 5 to 18 ± 6 mmHg and delayed the decrease in HR.**

Conclusions: **These findings support the view that ketamine might attenuate cardiovascular responses such as baroreflex by interacting, at least in part, with the NMDA receptor in the NTS. (Key words: Anesthetics, intravenous: ketamine. Brain: nucleus tractus solitarius. Receptors: N-methyl-D-aspartate.)**

KETAMINE has been described to have effects on the autonomic cardiovascular system[1] and to block baroreflex from the carotid sinus.[2]

Recent electrophysiologic experiments have shown that ketamine suppresses neural excitation derived from N-methyl-D-aspartate (NMDA)[3–7] as a noncompetitive NMDA antagonist.[7] NMDA belongs to the analogs of excitatory amino acids, L-glutamate.[8] NMDA receptor is proven to exist in neural cells throughout the brain regions.[9] NMDA receptors in the hippocampus are related to learning,[10,11] and its functions in the ventrobasal thalamus involve somatosensory signal reception.[12] As to the autonomic circulatory system, glutamatergic neurons in the nucleus tactus solitarii (NTS) integrate cardiovascular responses.#[13,14] NMDA receptors have been thought to play an important role in the NTS,[15] where the primary afferent fibers terminate from the carotid sinus exclusively.[16–20] The present study was, therefore, aimed at elucidating the effect and the possible mechanism of action of ketamine on baroreflex, with special reference to NMDA receptor in the NTS.

Materials and Methods

Following institutional approval, 41 male Wistar rats, weighing 280–320 g, were anesthetized with urethan (1.2–1.3 g/kg ip). A cannula was inserted into the right femoral artery for monitoring and recording arterial blood pressure (BP) and heart rate (HR) by a polygraph (Nihonkohden, Japan), another cannula was placed in the femoral vein of the same side for administration of the agents. Following tracheostomy, the rats were paralyzed by pancuronium bromide (0.2 mg/kg iv) and their lungs mechanically ventilated (1.2 ml/100 g body

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weight, 70–80 strokes/min) with room air *via* the tracheal tube.

A standard baroreflex from the carotid sinus was obtained in 16 rats utilizing a procedure described previously.[21] The left common carotid artery was exposed, and a silk tie was looped around the artery. The rat was placed in a prone position on a stereotaxic frame (Narishige, Japan). The reflex was produced by pulling the tie toward the heart for 12 s. The pulling force loaded the artery with almost maximum tension within tolerance in each traction. First, the reflex was examined to discover whether it is reproducible for several times under background anesthesia using one rat. Then, in the 15 rats, the traction was performed twice at an interval of 2 min prior to the iv ketamine as control. The traction was repeated 3, 5, 15, and 25 min after iv ketamine at a dose of 3, 6, or 9 mg/kg (n = 5, for each dose).

The remaining 25 rats were placed on a stereotaxic apparatus, and the dorsal surface of the medulla was exposed by occipital craniotomy and retraction of the posterior vermis of the cerebellum for microinjection into the NTS or the dorsal nucleus of the vagus nerve. Microinjection was made with a volume of 0.05 μl for 12 s manually in each injection. NMDA (Sigma) or D-AP5 (Cambridge Research Biochemicals, UK) was dissolved in 40 mM phosphate buffer (pH 7.4), containing 2% methylene blue (Sigma) as injection marker. A fine glass micropipette (30–50-μm tip diameter) was glued to Hamilton 1 μl-microsyringe, a syringe that was mounted to a manipulator. The glass micropipette was introduced into the NTS (0.1 mm rostral to the calamus scriptorius, 0.1 mm lateral to the midline, and 0.3 mm deep from the dorsal surface of the medulla oblongata). In 3 of the 25 rats, a solution without agent was injected into the NTS. In 5 of the 25 rats, 2 ng NMDA was injected into the NTS. In 5 of the 25 rats, NMDA injection was performed 3 min after 9 mg/kg iv ketamine (Sankyo, Japan). In 2 of the 25 rats, cardiovascular responses were examined by microinjection of NMDA into the dorsal nucleus of the vagus nerve, located just ventral to the NTS (0.1 mm rostral to the calamus scriptorius, 0.2 mm lateral to the midline, and 0.6 mm deep from the surface of the medulla). In the remaining 10 rats, D-AP5 (0.5, 5, and 50 ng for n = 3, 3, and 4, respectively) was co-injected with 2 ng NMDA into the NTS. When the experiments were over, the rats were perfused through the ascending aorta with 0.9% saline, followed by 10% formalin saline. The brain was immediately removed and placed in 0.9% saline containing 30% sucrose and 10% formalin overnight. Transverse frozen sections through the medulla were made serially at 50-μm thickness. The sections were mounted onto slide glasses and stained with 1% neutral red as background of methylene blue. Injection sites were traced with methylene blue by microscopic observation.

Statistical analysis was performed by *t* test or ANOVA to evaluate overall significance, with subsequent individual comparisons using Duncan's test.

Results

Blood pressure and HR during traction of the carotid artery are presented in figure 1. This reflex was reproducible (fig. 1A). Traction on the artery was performed twice, before iv ketamine, to serve as control. Although the traction was performed manually, standard errors of the decrease in mean arterial pressure (MAP) and HR were less than 2 mmHg and 2 beats/min, respectively. Inhibitory effect of ketamine on this reflex reached the peak 3 min after iv ketamine (figs. 1B–1D), and was dose-dependent (table 1).

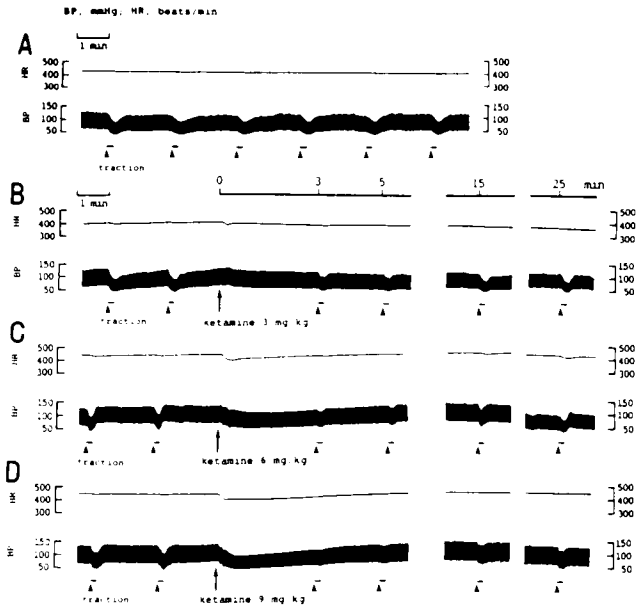

Fig. 1. Response to traction of the left common carotid artery and effects of ketamine. The response was reproducible without attenuation (*A*). In the experiment with administration of ketamine, traction was performed twice before iv ketamine, and succeeding tractions were done 3, 5, 15, and 25 min after the administration of ketamine with a dose of 3 mg/kg (*B*), 6 mg/kg (*C*), and 9 mg/kg (*D*). The baroreflex was suppressed at peak 3 min after the intravenous ketamine, and the suppression was dose-dependent, particularly in blood pressure.

Table 1. Maximum Reduction in Mean Arterial Pressure and Heart Rate Produced by Traction of a Left Common Carotid Artery 3 min after iv Ketamine Administration

Ketamine	Mean Arterial Pressure (mmHg)	Heart Rate (beats/min)
Control (n = 15)	27.9 ± 1.0	7.5 ± 0.8
3 mg/kg (n = 5)	15.8 ± 4.0*	4.8 ± 2.0
6 mg/kg (n = 5)	8.0 ± 1.0*†	3.0 ± 1.3‡
9 mg/kg (n = 5)	3.6 ± 0.6*†	1.6 ± 0.2*

Values represent mean ± SE.

* $P < .01$ *versus* control, using Duncan's test.

† $P < .01$ significant differences between 3 mg/kg and 6 or 9 mg/kg of ketamine.

‡ $P < .05$ *versus* control, using Duncan's test.

The injection sites of the 26 rats that received microinjections into the NTS were localized within the medial part of the NTS. A diagram is shown in figure 2 with a transverse section at the level of 0.6–0.9 mm caudal to the obex. Microinjections of 2% methylene blue dissolved in buffered solution led to a slight decrease in MAP of 10 mmHg for a short duration but had no effect on HR (fig. 3A).

Injections of NMDA into the NTS resulted in an obvious decrease in both BP and HR in all five cases. These responses were sometimes recovered rapidly (fig. 3B), but sometimes continued for several minutes (fig. 3C). Arrhythmia was elicited after NMDA injection in four of five cases (double arrow in fig. 3C).

The responses elicited by the injections of NMDA into the NTS were attenuated after iv ketamine. A case is shown in figure 3D, in which the response to NMDA is

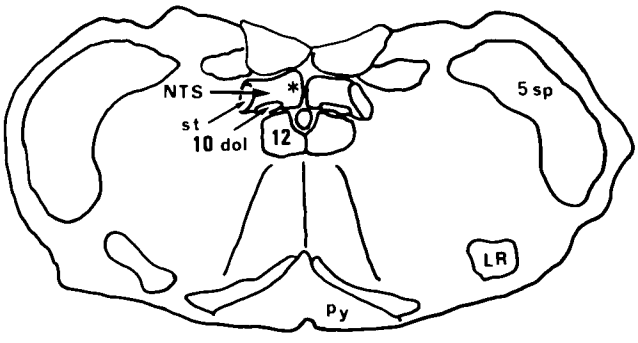

Fig. 2. A diagram of a full transverse section of a rat at the level of 0.5 mm caudal to obex. An asterisk in the left nucleus tractus solitarius (NTS) indicates the injection site. Scale bar = 0.5 mm. 10 dol = dorsal nucleus of the vagal nerve; 12 = hypoglossal nucleus; 5sp = nucleus of the spinal tract of the trigeminal nerve; LR = lateral reticular nucleus; py = pyramidal tract; st = solitary tract.

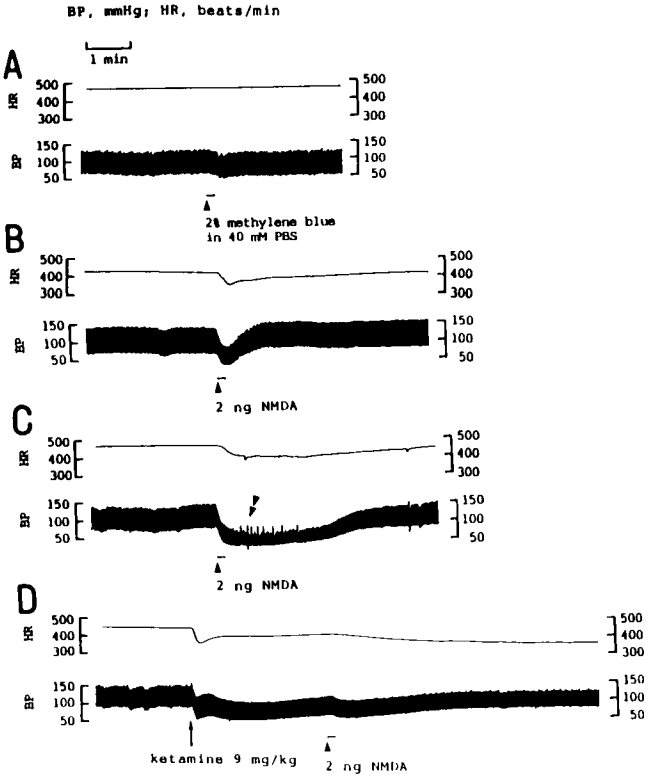

Fig. 3. Effects of microinjection into the nucleus of tractus solitarius. (*A*) Injection of 40 mM phosphate buffered solution containing 2% methylene blue. NMDA was dissolved in this solution in B, C, and D. (*B* and *C*) Injection of 2 ng NMDA. All cases presented drastic hypotension and bradycardia. Some rats revealed arrhythmia (with double arrow) and long-lasting hypotension as seen in C. (*D*) Response in blood pressure elicited by injection of 2 ng NDMA was attenuated, and that in heart rate was delayed and dulled by 9 mg/kg iv ketamine.

prevented, of the five rats administered with 9 mg/kg ketamine. A reduction in MAP by injection of NMDA into the NTS was significantly attenuated by iv ketamine (table 2, $P < .001$). Although effects of ketamine on HR were not significant, reduction in HR evoked by

Table 2. Maximum Reduction in Mean Arterial Pressure and Heart Rate by 2-ng NMDA Injection into the NTS 3 min after 9 mg/kg iv Ketamine Administration

	Mean Arterial Pressure (mmHg)	Heart Rate (beats/min)
Control (n = 5)	47.2 ± 4.9	42.4 ± 9.5
Ketamine (n = 5)	5.8 ± 0.7*	32.4 ± 7.0

Values are mean ± SE.

NMDA = N-methyl-D-aspartate; NTS = nucleus tractus solitarius.

* $P < .001$ *versus* control using *t* test.

Table 3. Influence of D-AP5 When Co-injected with 2-ng NMDA into the NTS on Mean Arterial Pressure and Heart Rate

D-AP5	Mean Arterial Pressure (mmHg)	Heart Rate (beats/min)
Control (n = 5)	47.2 ± 4.9	42.4 ± 9.5
0.5 ng (n = 3)	30.7 ± 1.8*	51.7 ± 10.9
5 ng (n = 3)	24.0 ± 3.2†	23.3 ± 11.6
50 ng (n = 4)	16.7 ± 5.1†‡	17.5 ± 6.6

Values are mean ± SE of maximum reduction.

NMDA = N-methyl-D-aspartate; NTS = nucleus tractus solitarius.

* $P < .05$ *versus* control, using Duncan's test.

† $P < .01$ *versus* control, using Duncan's test.

‡ $P < .05$ significant difference between 0.5 ng and 50 ng of D-AP5.

NMDA occurred much more slowly under the administration of ketamine when compared with control; it took several minutes to reach the maximum bradycardic state (fig. 3D). Ketamine blocked the arrhythmia elicited by the NMDA injection into the NTS in all rats.

The injection of NMDA into the dorsal nucleus of the vagus nerve of the other two rats induced frequent arrhythmias and reduction in HR, but elevated BP in contrast to injection into the NTS.

NMDA-elicited responses were inhibited by co-injection with D-AP5 (table 3). NMDA-induced hypotension was weakened by D-AP5 (0.05 ng; $P < .05$, 5 ng and 50 ng; $P < .01$). D-AP5 at a dose of 50 ng inhibited the response to NMDA much more effectively than did 0.5 ng D-AP5 ($P < .05$). Reduction in HR was not significantly changed by D-AP5. However, D-AP5 delayed remarkably the onset of action of NMDA on HR. These effects of D-AP5 on the NMDA-elicited responses in MAP and HR were the same as those of iv ketamine.

Discussion

Ketamine is used clinically as a short-acting anesthetic or an induction agent and has various effects on cardiovascular function.[1,22] The present observation that an iv injection of ketamine dose-dependently blocked hypotension and bradycardia induced by a traction of left common carotid artery was consistent with a previous study.[2] Slogoff and Allen[23] have proposed that the site of cardiovascular action of ketamine exists in the central nervous system rather than in the baroreceptor, since the baroreceptor had not been affected by this anesthetic. In this study, not only a standard baroreflex but also a response produced by a direct injection of NMDA, an analog of L-glutamate, into the NTS are examined under ketamine anesthesia. The response by NMDA injection into the NTS is elicited by an excitatory synaptic mediation in the NTS in a manner similar to that of a standard baroreflex, excluding an involvement of peripheral baroreceptors.

The NTS is proven to be the projection sites of the primary afferent fibers from the carotid sinus by electrophysiologic[16,17] and morphologic[18–20] experiments. The neurons of the NTS directly project to the rostral ventrolateral medulla oblongata, which contains neurons projecting to the sympathetic preganglionic neuron of the spinal cord, and this pathway is necessary for the expression of the vasodepressor reflexes from arterial baroreceptors.[21–24] The NTS also projects to the vagal efferent column.[24] Chemical mediation for cardiovascular responses in the NTS was noted to be a function of the glutamatergic system.#[13,14] L-glutamate and its analogs, NMDA, kainate, and quisqualate, could all induce hypotension bradycardia by microinjections into the NTS.[15,25] Our findings also demonstrate direct stimulation to the NTS with NMDA, which resulted in a decrease in MAP and HR. All descriptions above suggest that NMDA receptors in the NTS may be involved in mediation of inhibitory action of ketamine on baroreflex response. The present study was designed to confirm this and described that an iv administration of ketamine dose-dependently attenuated the cardiovascular response induced by NMDA injected into the NTS.

In the present study, methylene blue was used to identify the injection sites. Methylene blue has been known to prevent activation of guanylate cyclase by nitric oxide, which is operative for a part, in signal transduction of glutamate through NMDA receptor.[26,27] However, NMDA injection without methylene blue resulted in the same effect on the MAP and HR as with the dye. In addition, the cells containing nitric oxide synthase stained by an enzyme histochemical technique for NADPH-diaphorase[28] were scarcely detected in the NTS (data not shown). This evidence would allow us to use methylene blue as an injection marker in this experiment and also suggests that the nitric oxide system may not mediate a cardiovascular response of NMDA in the NTS.

Based on electrophysiologic findings, ketamine depresses neural depolarization[4,6,7] or firing rate[5] in response to the application of NMDA *in vitro*, and the frequency of neural firing activated by NMDA is reduced after iv administration of 5 mg/kg ketamine injection in the rat ventrobasal thalamus.[5] These observations support the view that iv ketamine blocked the baro-

reflex response, especially hypotension, by interacting with NMDA receptor in the NTS.

Although the effects of modulation by the NMDA receptor in a site other than the NTS cannot be ruled out, the NTS is the only candidate that involves NMDA transduction in this reflex pathway described above.

Ketamine is a noncompetitive NMDA antagonist probably acting within the ion channel gated by NMDA receptor, whereas D-AP5 is a competitive NMDA antagonist.[29] D-AP5 inhibited NMDA-induced hypotension in a dose-dependent manner and delayed the onset of action of NMDA on HR, as seen after iv ketamine. This finding provides additional evidence favoring our view that attenuation of the baroreflex by ketamine largely results from the interruption of neural mediation, probably *via* NMDA receptors in the NTS.

The present investigation shows that activity of NMDA receptor in the NTS was suppressed by ketamine and could lead to a blockade of cardiovascular responses mediated by NMDA receptors in the NTS, such as a baroreflex from carotid sinus. The results obtained may lead to a better understanding of action of ketamine involving glutamatergic transmission.

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