

## *L-Arginine Attenuates Ketamine-induced Increase in Renal Sympathetic Nerve Activity*

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**Background:** It has been reported that ketamine produces sympathoexcitation by directly stimulating the central nervous system. It also has been shown that nitric oxide (NO) may play a role in signal transduction of the nervous system. Therefore, we hypothesized that the sympathoexcitation of ketamine may be linked to central NO formation. To test this hypothesis, we examined the effects of L-arginine, a substrate of NO formation, on renal sympathetic nerve activity (RSNA) during ketamine anesthesia.

**Methods:** Using 45 rabbits given basal anesthesia with  $\alpha$ -chloralose, we measured changes in heart rate, mean arterial pressure, and RSNA in response to intravenous ketamine (1 mg/kg) and investigated the effect of intravenous L-arginine and D-arginine (bolus 30 mg/kg followed by continuous 30 mg·kg<sup>-1</sup>·min<sup>-1</sup>). The animals were divided into intact, sinoaortic- and vagal-deafferented, and spinal cord-transected groups.

**Results:** Ketamine caused significant increases in RSNA (172 ± 16%), heart rate (12 ± 2 beats/min), and mean arterial pressure (8 ± 1 mmHg) in the intact rabbits. Ketamine also increased RSNA in sinoaortic- and vagal-deafferented rabbits, but not in spinal cord-transected rabbits. L-Arginine attenuated the ketamine-induced increase in RSNA in intact and deafferented rabbits, whereas D-arginine had no effect on RSNA. In addition, N<sup>G</sup>-nitro-L-arginine methyl ester, a NO synthase inhibitor, increased RSNA and the increase was attenuated by L-arginine.

**Conclusions:** Ketamine may act centrally to increase sympathetic outflow, and the sympathoexcitation may be attenuated by increasing NO formation with L-arginine in the central nervous system. (Key words: Anesthetics, intravenous: ketamine. Endothelium: endothelium-derived relaxing factor;

nitric oxide. Sympathetic nervous system: renal sympathetic nerve.)

It is well known that blood pressure and heart rate are increased during ketamine anesthesia in humans and experimental animals.<sup>1,2</sup> The pressor response is considered to originate primarily from direct stimulation of the central sympathetic nervous system.<sup>3</sup> The mechanism of this central sympathoexcitation by ketamine is not known.

Glutamate is a major excitatory neurotransmitter in the brain and mediates its effects by activating three distinct receptor subtypes (N-methyl-D-aspartate [NMDA], quisqualate, and kainate).<sup>4</sup> It has been shown that ketamine selectively reduces the activation of NMDA receptors in the mammalian brain.<sup>5</sup> The discovery and isolation of nitric oxide (NO) synthase from neural tissue suggests that NMDA receptor activation and subsequent activation of NO synthase increases the production of NO from free L-arginine.<sup>6</sup> Therefore, it seems likely that the antagonistic effect of ketamine on NMDA receptors may exert an inhibitory influence on NO formation.

It has recently been reported that the intravenous administration of NO synthase inhibitor elicited an increase in central sympathetic outflow in the rat with total baroreceptor deafferentation,<sup>7</sup> and that the central administration of NO synthase inhibitor also elicited an increase in sympathetic outflow.<sup>8</sup> These findings suggested that centrally synthesized NO plays a role as a second messenger, a neurotransmitter, or both in the central regulation of sympathetic outflow by exerting tonic sympathoinhibition.

From these findings, we hypothesized that the sympathoexcitation of ketamine may be linked to the suppression of central NO formation. It has been reported that L-arginine is a substrate of NO formation and may increase NO formation even when NO synthase is inhibited.<sup>9</sup> Therefore, we tested the hypothesis by examining the effects of L-arginine on the ketamine-

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induced increase in renal sympathetic nerve activity (RSNA) in intact, baroreceptor-deafferented, and spinal cord-transected rabbits.

## Materials and Methods

### General Procedures

This study was approved by our institutional animal care committee. Experiments were performed on 50 Japanese white rabbits weighing 2.7–3.6 kg. The animals were anesthetized initially with pentobarbital sodium (30 mg/kg iv).  $\alpha$ -Chloralose (10-mg/kg bolus and 10-mg  $\cdot$  kg<sup>-1</sup>  $\cdot$  h<sup>-1</sup> infusion) was given intravenously as basal anesthesia because it has a minor effect on hemodynamics.<sup>10</sup> The anesthetic level was confirmed as appropriate by the absence of circulatory or neural responses to pinching the ear. After tracheostomy, the lungs were mechanically ventilated with a Harvard respirator (Mills, MA). Arterial blood gases and pH were measured periodically and ventilation was adjusted within normal levels. Body temperature was maintained above 37°C by a heating pad and a heating lamp. A 16-G double-lumen catheter was inserted *via* the right femoral vein to the inferior vena cava as a route for drug administration. An 18-G catheter was inserted into the right femoral artery to monitor arterial pressure. The arterial catheter was connected to a pressure transducer (Uniflow, Baxter, CA) and a carrier amplifier (AP 601G, Nihon Kohden, Tokyo, Japan). Heart rate was determined from the arterial pressure wave with a heart rate counter (AT 601G, Nihon Kohden).

### Nerve Recording

The method for nerve recording has been previously reported.<sup>11</sup> Briefly, the left kidney was exposed retroperitoneally with a left flank incision. The left renal sympathetic nerves were separated from the surrounding connective tissue under a microscope, cut distally, and covered with warm mineral oil. The efferent RSNA was recorded with a bipolar stainless steel electrode. Impulses were amplified by a band pass amplifier (MEG-1200, Nihon Kohden). The amplified signals were fed into a dual beam oscilloscope (VC-10, Nihon Kohden) and into a nerve spike counter (MET-1100, Nihon Kohden) with a window discriminator set just above the noise level. Spikes were counted and integrated for 4-s intervals. The raw electroneurogram, the output from the spike counter, heart rate, and arterial pressure were displayed on a Macintosh-IISi computer (Apple, Cu-

pertino, CA) through an analogue-to-digital converter (Mac-Lab, Analog Digital Instruments, Castle Hill, Australia). Changes in RSNA were expressed as percent RSNA, which was obtained by the changes from the baseline level regarding the baseline level as 100%.

### Denervation

The vagi were exposed and cut through a midcervical incision. Sinoaortic denervation was performed by making a bilateral section of the aortic nerves and cervical sympathetic chains, by interrupting all nerve fibers between the internal and external carotid artery, and by stripping the adjacent adventitia in the region of the carotid sinus. The effectiveness of sinoaortic denervation was confirmed by the absence of a decrease in heart rate and RSNA after a phenylephrine-induced increase in arterial pressure.

### Spinal Cord Transection

The rabbits were placed in a frame with their heads inclined downward. A skin incision was made at the back of the neck between the ears, and muscles were dissected to expose the vertebral bone. The dura was opened through the intervertebral space between the second and third cervical vertebrae. Spinal cord transection was performed with a surgical knife using a microscope and electrical cautery. The effectiveness of spinal cord transection was confirmed by the absence of a reflex increase in heart rate and RSNA after nitroprusside-induced hypotension. This was reconfirmed by examining the spinal cord at the end of the experiment.

### Experimental Protocols

After the completion of the surgical procedures, at least 1 h was allowed for the stabilization of hemodynamic variables and nerve activity. The study was divided into seven groups.

**Group 1.** Effects of ketamine on RSNA, heart rate, and mean arterial pressure (MAP) in intact rabbits ( $n = 7$ ). Changes in RSNA, heart rate, and MAP in response to a bolus administration of ketamine (1 mg/kg) were examined in intact rabbits.

**Group 2.** Effects of L-arginine and D-arginine on ketamine-induced changes in RSNA in intact rabbits ( $n = 12$ ). Two minutes after ketamine administration (1 mg/kg), L-arginine ( $n = 7$ ) or D-arginine ( $n = 5$ ) in a dose of bolus 30 mg/kg followed by continuous 30 mg  $\cdot$  kg<sup>-1</sup>  $\cdot$  min<sup>-1</sup> were infused in intact rabbits.

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**Group 3.** Effects of ketamine on RSNA in intact rabbits pretreated with L-arginine or D-arginine ( $n = 7$ ). Ketamine (1 mg/kg) was administered in intact rabbits pretreated with L-arginine ( $n = 4$ ) or D-arginine ( $n = 3$ ) in a dose of bolus 30 mg/kg followed by continuous  $30 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ .

**Group 4.** Effects of L-arginine and D-arginine on ketamine-induced changes in RSNA in sinoaortic- and vagal-deafferented rabbits ( $n = 9$ ). In sinoaortic- and vagal-deafferented rabbits, L-arginine ( $n = 5$ ) or D-arginine ( $n = 4$ ) were infused with the same dose as group 2, 2 min after ketamine administration (1 mg/kg).

**Group 5.** Effects of L-arginine on  $\text{N}^G$ -nitro-L-arginine methyl ester (L-NAME)-induced changes in RSNA in sinoaortic- and vagal-deafferented rabbits ( $n = 4$ ). L-NAME, a NO synthase inhibitor, was administered intravenously (30 mg/kg) in sinoaortic- and vagal-deafferented rabbits. Two minutes after the administration, L-arginine was infused with the same dose as group 2.

**Group 6.** Effects of ketamine on RSNA in sinoaortic- and vagal-deafferented rabbits pretreated with L-NAME ( $n = 5$ ). Two minutes after the administration of L-NAME (30 mg/kg) in sinoaortic- and vagal-deafferented rabbits, ketamine (1 mg/kg) was administered intravenously.

**Group 7.** Effects of ketamine on RSNA, heart rate, and MAP in spinal cord-transected rabbits ( $n = 6$ ). Ketamine was administered intravenously (1 mg/kg) to rabbits with a spinal cord transection between the second and third cervical vertebrae.

### Drugs

The following drugs were used: ketamine hydrochloride (Sankyo, Tokyo, Japan), pentobarbital sodium (Abbot, North Chicago, IL),  $\alpha$ -chloralose and sodium nitroprusside (Nacalai Tesque, Kyoto, Japan), phenylephrine (Kowa, Nagoya, Japan), L-arginine, D-arginine, and L-NAME (Sigma Chemical, St. Louis, MO).

### Statistical Analysis

All data were expressed as mean  $\pm$  SEM (standard error of the mean). Changes from baseline values were compared by one-way analysis of variance with repeated measures followed by Bonferroni's modification of paired  $t$  test. A two-way analysis of variance was performed to compare the responses to L-arginine with those to D-arginine. If the  $F$  test was significant, an unpaired  $t$  test was performed. A value of  $P < 0.05$  was considered statistically significant.

## Results

Table 1 shows the baseline values of heart rate, MAP, and RSNA in each group. The heart rate and RSNA in rabbits with sinoaortic and vagal deafferentation were significantly higher than in intact rabbits, whereas the heart rate, MAP, and RSNA in the spinal cord-transected rabbits were significantly lower than in the intact rabbits.

### Intact Rabbits

**Effect of Ketamine on Renal Sympathetic Nerve Activity, Heart Rate, and Mean Arterial Pressure.** Figure 1 shows the effects of intravenous administration of ketamine 1 mg/kg on RSNA, heart rate, and MAP in intact rabbits. Ketamine caused a significant increase in RSNA, reaching a plateau of  $172 \pm 16\%$  of the preketamine level within 2 min after the administration. The increase in RSNA lasted for approximately 10 min. The heart rate and MAP increased by  $12 \pm 2$  beats/min and  $8 \pm 1$  mmHg, respectively, at 2 min after ketamine administration. The increases also continued for approximately 10 min.

**Effect of L-Arginine and D-Arginine on the Ketamine-induced Increases in Renal Sympathetic Nerve Activity.** Figure 2 shows the effect of intrave-

**Table 1. Baseline Values for Heart Rate, Mean Arterial Pressure, and Renal Sympathetic Nerve Activity in Intact (Groups 1, 2, and 3), Sinoaortic- and Vagal-deafferented (Groups 4, 5, and 6), and Spinal Cord-transected Rabbits (Group 7)**

	Heart Rate (beats/min)	MAP (mmHg)	RSNA (spikes/s)
Group 1 ( $n = 7$ )	$250 \pm 13$	$112 \pm 2$	$50 \pm 7$
Group 2			
L-Arginine ( $n = 7$ )	$255 \pm 12$	$102 \pm 4$	$43 \pm 4$
D-Arginine ( $n = 5$ )	$253 \pm 17$	$106 \pm 4$	$44 \pm 7$
Group 3			
L-Arginine ( $n = 4$ )	$260 \pm 14$	$102 \pm 5$	$49 \pm 7$
D-Arginine ( $n = 3$ )	$253 \pm 20$	$102 \pm 3$	$44 \pm 4$
Group 4			
L-Arginine ( $n = 5$ )	$289 \pm 12^*$	$108 \pm 7$	$145 \pm 14^\dagger$
D-Arginine ( $n = 4$ )	$276 \pm 5^*$	$103 \pm 7$	$157 \pm 6^\dagger$
Group 5 ( $n = 4$ )	$283 \pm 7^*$	$111 \pm 6$	$123 \pm 5^\dagger$
Group 6 ( $n = 5$ )	$278 \pm 12^*$	$115 \pm 4$	$120 \pm 9^\dagger$
Group 7 ( $n = 6$ )	$190 \pm 11^*$	$65 \pm 3^\dagger$	$20 \pm 2^*$

Values are mean  $\pm$  SEM.

MAP = mean arterial pressure; RSNA = renal sympathetic nerve activity.

\*  $P < 0.05$  versus group 1.

†  $P < 0.01$  versus group 1.

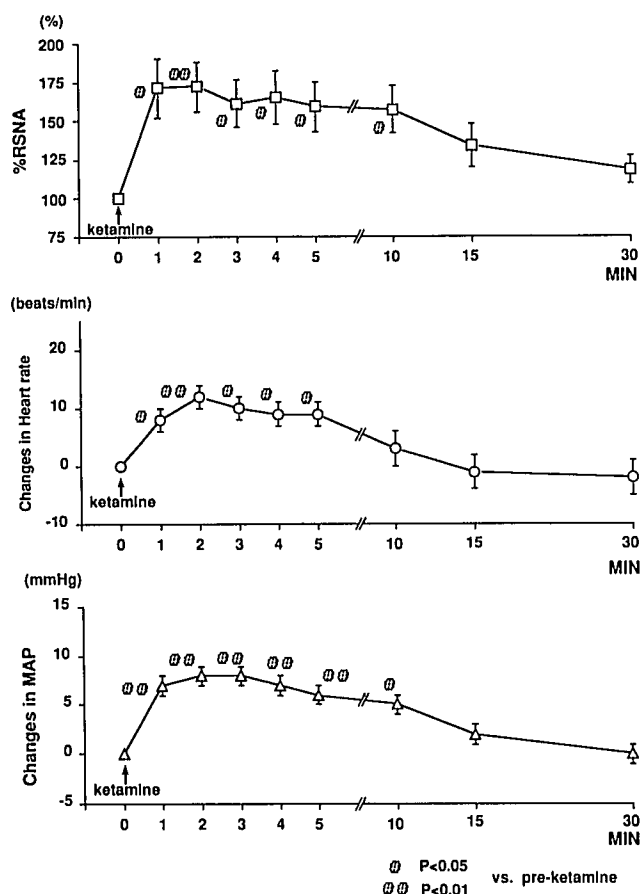


Fig. 1. Effect of intravenous administration of ketamine 1 mg/kg on renal sympathetic nerve activity (RSNA, *top*), heart rate (*middle*), and mean arterial pressure (MAP, *bottom*) in intact rabbits ( $n = 7$ ). The increases of RSNA, heart rate, and MAP after ketamine administration reached plateaus within 2 min and continued for approximately 10 min. Values are mean  $\pm$  SEM.

nous L-arginine and D-arginine on the ketamine-induced increases in RSNA, heart rate, and MAP. L-Arginine attenuated the ketamine-induced increase in RSNA from  $157 \pm 10\%$  to  $84 \pm 7\%$ , although D-arginine did not. L-Arginine also attenuated the heart rate change from  $14 \pm 3$  to  $0 \pm 4$  beats/min. The attenuated responses of RSNA and the heart rate during L-arginine infusion disappeared several minutes after discontinuation of the infusion.

**Effects of Ketamine on Renal Sympathetic Nerve Activity after Pretreatment with L-Arginine or D-Arginine.** Figure 3 shows the effect of ketamine on RSNA, heart rate, and MAP in intact rabbits pretreated with L-arginine or D-arginine. Intravenous infusions of

both L-arginine and D-arginine did not significantly alter RSNA ( $89 \pm 5\%$  of baseline level with L-arginine and  $97 \pm 3\%$  with D-arginine) and heart rate (by  $3 \pm 2$  beats/min with L-arginine and by  $3 \pm 3$  beats/min with D-arginine). L-Arginine did not significantly change MAP, but D-arginine slightly increased MAP by  $6 \pm 2$  mmHg.

Ketamine did not significantly change RSNA in rabbits pretreated with L-arginine ( $92 \pm 7\%$  at 2 min after ketamine), whereas ketamine significantly increased RSNA in rabbits pretreated with D-arginine ( $175 \pm 12\%$  at 2 min after ketamine). Ketamine increased heart rate and

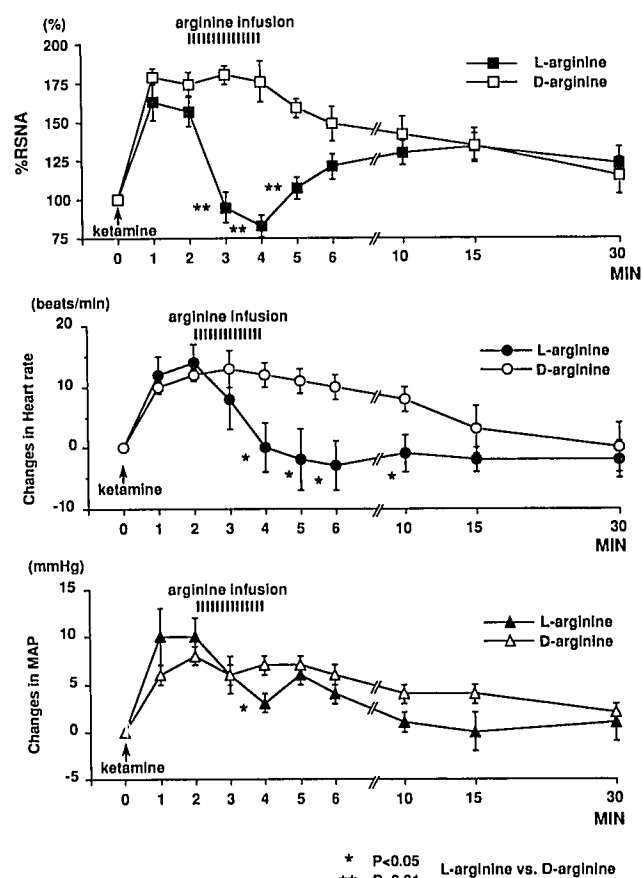


Fig. 2. Effect of L-arginine ( $n = 7$ ) and D-arginine ( $n = 5$ ) on the ketamine-induced increase in renal sympathetic nerve activity (RSNA, *top*), heart rate (*middle*), and mean arterial pressure (MAP, *bottom*) in intact rabbits. L-Arginine attenuated the ketamine-induced increase in RSNA to the baseline level, whereas D-arginine did not. L-Arginine also attenuated the ketamine-induced increase in heart rate. The responses of heart rate and MAP during L-arginine infusion were significantly different from those during D-arginine infusion. Values are mean  $\pm$  SEM.

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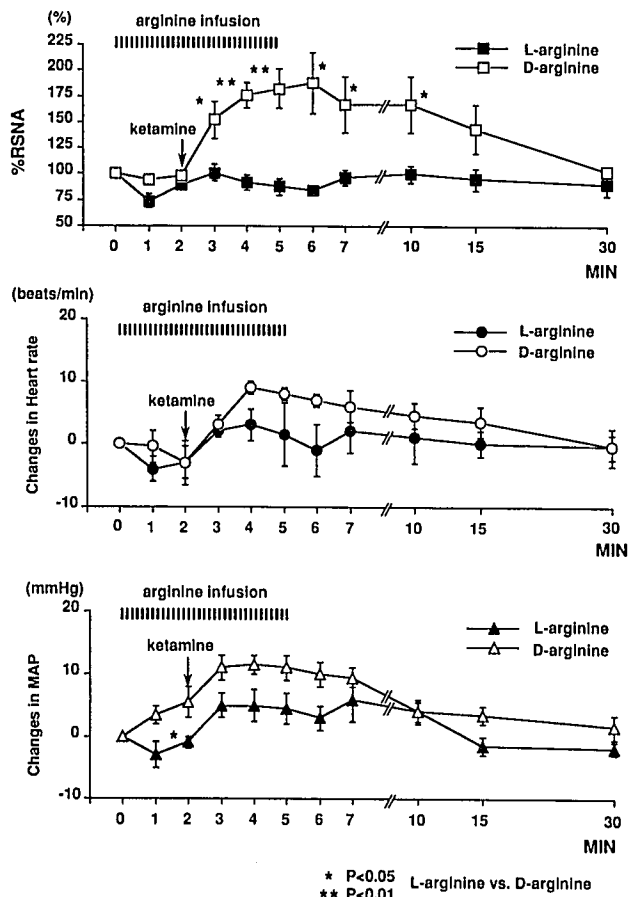


Fig. 3. Effect of ketamine in intact rabbits pretreated with L-arginine ( $n = 4$ ) or D-arginine ( $n = 3$ ) on renal sympathetic nerve activity (RSNA, top), heart rate (middle), and mean arterial pressure (MAP, bottom). Intravenous infusions of both L-arginine and D-arginine did not significantly alter RSNA. Ketamine did not significantly change RSNA in rabbits pretreated with L-arginine, whereas it significantly increased RSNA in those with D-arginine. Ketamine increased heart rate and MAP in rabbits pretreated with D-arginine and tended to increase in those with L-arginine. Values are mean  $\pm$  SEM.

MAP in rabbits pretreated with D-arginine and tended to increase in those with L-arginine.

#### Sinoaortic- and Vagal-deafferented Rabbits

**Effect of L-arginine on the Ketamine-induced Increases in Renal Sympathetic Nerve Activity.** Figure 4 shows recordings of responses of RSNA to L-arginine and D-arginine, infused at 2 min after the administration of ketamine in sinoaortic- and vagal-deafferented rabbits. The increase in RSNA produced by ketamine was reduced by L-arginine, but it was unal-

tered by D-arginine. As figure 5 shows, ketamine caused a significant increase in RSNA in rabbits with sinoaortic and vagal deafferentation. Subsequent administrations of L-arginine attenuated the augmented RSNA from  $145 \pm 11\%$  to  $93 \pm 5\%$ , whereas the administration of D-arginine had no effect. Two minutes after discontinuation of L-arginine, RSNA returned to its level before L-arginine infusion ( $140 \pm 13\%$ ). Ketamine failed to increase the heart rate and MAP in these deafferented rabbits. Subsequent infusions of L-arginine caused significantly greater decreases in heart rate and MAP, whereas D-arginine had no effect.

**Effects of L-arginine on L-NAME-Induced Changes in Renal Sympathetic Nerve Activity.** As shown in figure 6, L-NAME caused a significant increase in RSNA ( $133 \pm 2\%$  at 2 min after the administration) and subsequent administration of L-arginine attenuated the augmented RSNA nearly to the baseline level ( $97 \pm 4\%$ ). L-NAME elicited an increase in MAP by  $19 \pm 5$  mmHg from baseline, and subsequent administration of L-arginine attenuated it. As far as heart rate was concerned, L-NAME caused no significant changes.

**Effects of Ketamine on Renal Sympathetic Nerve Activity after Pretreatment with L-NAME.** L-NAME, which was infused in sinoaortic- and vagal-deafferented rabbits before ketamine, caused an increase in

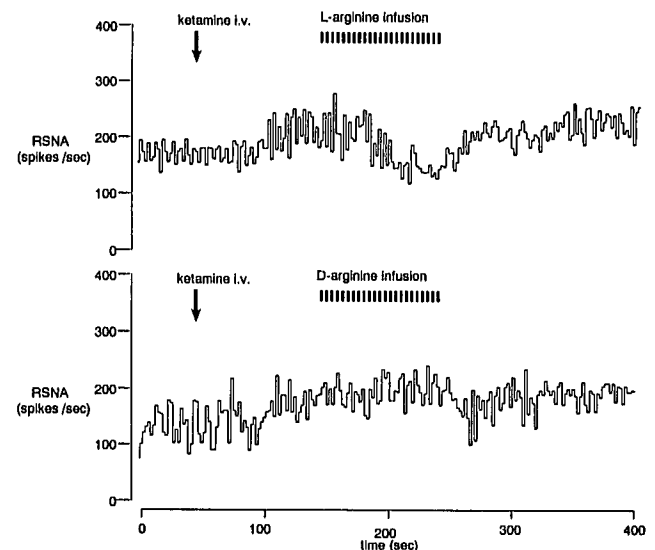


Fig. 4. Recordings of renal sympathetic nerve activity (RSNA) in response to intravenous infusion of L-arginine (top) and D-arginine (bottom) after the administration of ketamine in rabbits with sinoaortic and vagal deafferentation. Note that the infusion of L-arginine reduced the ketamine-induced increase in RSNA, but D-arginine did not.

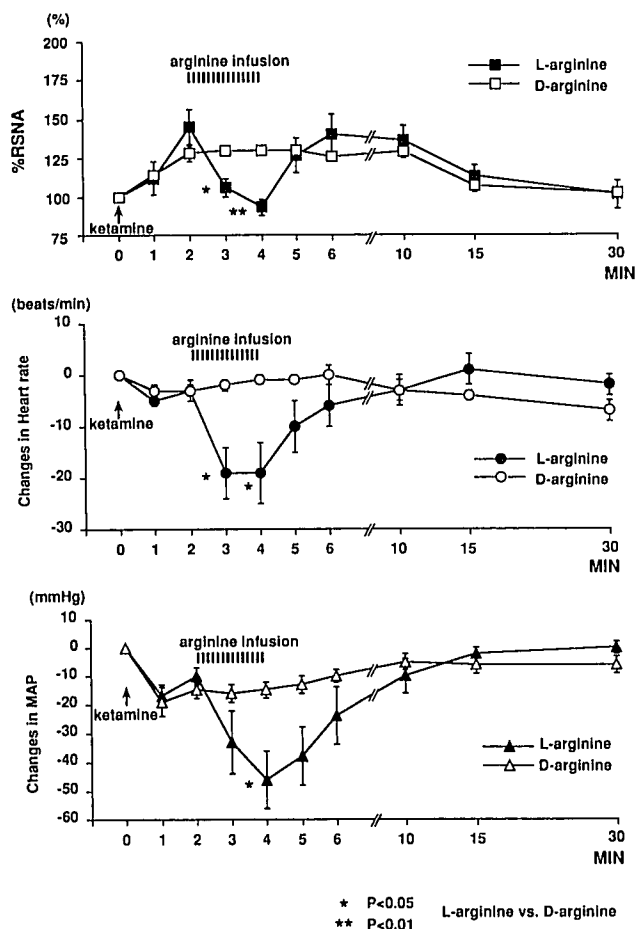


Fig. 5. Effect of L-arginine ( $n = 5$ ) and D-arginine ( $n = 4$ ) on the ketamine-induced changes in renal sympathetic nerve activity (RSNA, *top*), heart rate (*middle*), and mean arterial pressure (MAP, *bottom*) in rabbits with sinoaortic and vagal deafferentation. L-Arginine reduced the ketamine-induced increase in RSNA almost to the baseline level, whereas D-arginine did not. Ketamine failed to increase the heart rate and MAP in these deafferented rabbits. Subsequent infusions of L-arginine caused significantly greater decreases in the heart rate and MAP, whereas D-arginine had no effect. Values are mean  $\pm$  SEM.

RSNA by  $135 \pm 8\%$  (fig. 7). The subsequent administration of ketamine caused a further increase in RSNA by  $147 \pm 9\%$  at maximum (fig. 7).

#### Spinal Cord-Transected Rabbits

**Effect of Ketamine on Renal Sympathetic Nerve Activity, Heart Rate, and Mean Arterial Pressure.** In rabbits with spinal cord transection, ketamine was only observed to decrease RSNA and MAP (fig. 8).

## Discussion

This study provides two major findings. First, intravenous ketamine increased efferent RSNA in intact as well as in sinoaortic- and vagal-deafferented rabbits, but not in spinal cord-transected rabbits. Second, intravenous L-arginine attenuated the increase in RSNA produced by ketamine in the intact as well as the sinoaortic- and vagal-deafferented rabbits, whereas D-arginine did not. These findings suggest that ketamine increased sympathetic outflow by directly stimulating the central nervous system, independently of the baroreceptor reflex, and that the increase in sympathetic outflow might be linked to the reduced formation of NO in the central nervous system.

The sympathoexcitatory effects of ketamine in humans and animals have been reported by a number of investigators.<sup>1,2</sup> However, direct evidence of the increase in efferent sympathetic nerve discharge has not been demonstrated in animals whose neural inputs from visceral organs, such as arterial baroreceptors or cardiopulmonary baroreceptors, have been eliminated. The central stimulation of sympathetic outflow has been shown by an increase in plasma norepinephrine concentrations after ketamine administration,<sup>12</sup> and its likelihood is supported by the finding that cardiovascular augmentation during ketamine anesthesia was attenuated with a ganglion blocker<sup>13</sup> or epidural anesthesia.<sup>14</sup> However, because plasma norepinephrine concentrations are themselves an indirect and insensitive index of sympathetic traffic,<sup>15</sup> such observations cannot be considered definitive evidence for sympathoexcitation by ketamine. By recording sympathetic nerve traffic, it is possible to gain clearer insights into how ketamine influences the nervous system. This study presents direct evidence of the increase in efferent sympathetic nerve discharge by ketamine.

Dowdy and Kaya<sup>16</sup> reported that activation by ketamine of the sympathetic nervous system resulted from the depression of baroreceptor reflex activity. However, our results indicated that the effect of ketamine on sympathetic efferent nerve activity was independent of baroreflex mechanisms, because they were observed in rabbits with sinoaortic denervation and bilateral vagotomy. The failure of ketamine to produce an increase in RSNA in rabbits with a spinal cord transection (fig. 8) suggests that ganglionic stimulation does not contribute to the increase in RSNA. Therefore, we suggest that ketamine activates sympathetic outflow by acting directly on the central nervous system.

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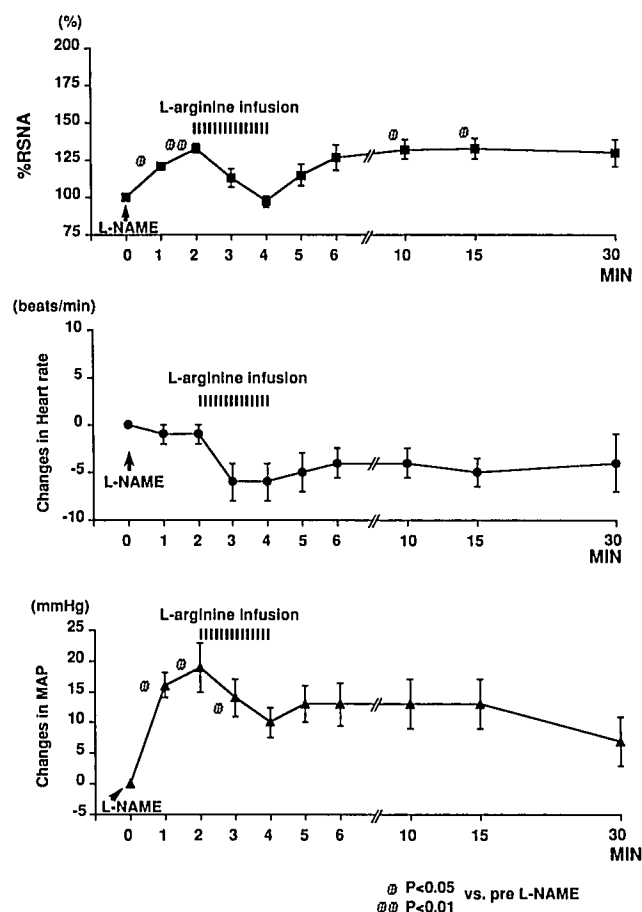


Fig. 6. Effect of L-arginine ( $n = 4$ ) on the L-NAME-induced changes in renal sympathetic nerve activity (RSNA, top), heart rate (middle), and mean arterial pressure (MAP, bottom) in rabbits with sinoaortic and vagal deafferentation. L-NAME increased RSNA and mean arterial pressure (MAP), and subsequent infusions of L-arginine attenuated the L-NAME-induced increase in RSNA and MAP. Values are mean  $\pm$  SEM.

We attempted to examine the mechanisms by which ketamine exerted its sympathoexcitatory effect in the central nervous system. We then hypothesized that the sympathoexcitatory effect is linked to suppression of central NO formation, based on the following findings. Ketamine has been shown to suppress neural excitation derived from NMDA as a noncompetitive NMDA antagonist.<sup>6</sup> Ogawa *et al.*<sup>17</sup> recently demonstrated that the effect of ketamine on cardiovascular responses was mediated by the suppression of the NMDA receptor in the nucleus tractus solitarius (NTS). NMDA belongs to the analogues of excitatory amino acids, such as L-glutamate.<sup>4</sup> Garthwaite *et al.*<sup>6</sup> reported that NO was released to elevate cyclic guanosine monophosphate, in re-

sponse to the stimulation of NMDA receptors in the brain. It has also been shown that an intravenous and intracisternal administration of a NO synthase inhibitor, N<sup>G</sup>-monomethyl-L-arginine, increased RSNA with totally deafferented rats, and that this increase in RSNA was blocked by intravenous L-arginine.<sup>7,8</sup> Thus, a decrease in NO formation in the brain may be linked to the increase in RSNA, and vice versa. These findings suggest that ketamine may act to decrease NO formation by suppressing NMDA receptors, thereby increasing sympathetic outflow (fig. 9).

To test the hypothesis, we examined whether the increase in RSNA was attenuated by an administration of L-arginine, a substrate of NO formation. NO is synthesized from L-arginine in the central nervous system<sup>18</sup> as well as in other tissues, including vascular endothelial cells,<sup>19</sup> murine macrophages, platelets, and neutrophils.<sup>20</sup> Our results show that an intravenous administration of L-arginine attenuated the ketamine-in-

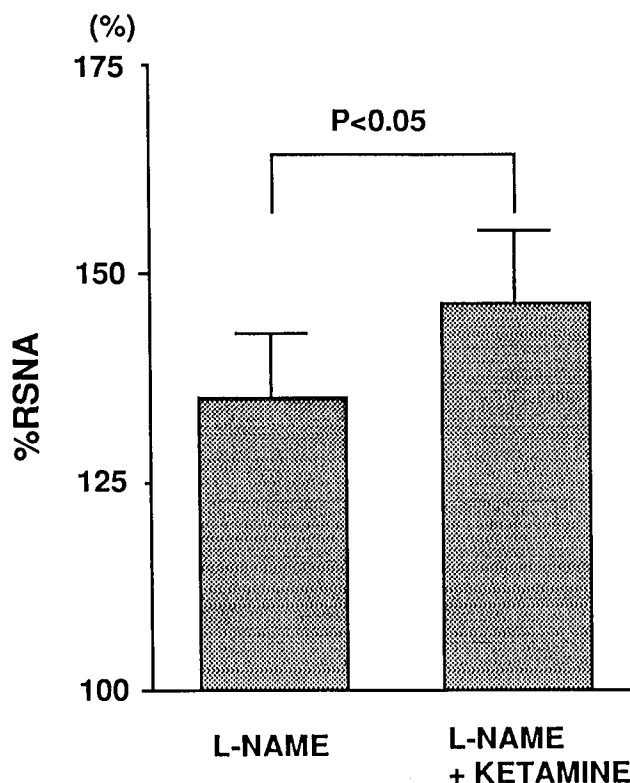


Fig. 7. Effect of ketamine on renal sympathetic nerve activity (RSNA) in rabbits pretreated with L-NAME ( $n = 5$ ). L-NAME elicited an increase in RSNA, and the subsequent administration of ketamine caused a further increase in RSNA. Values are mean  $\pm$  SEM.

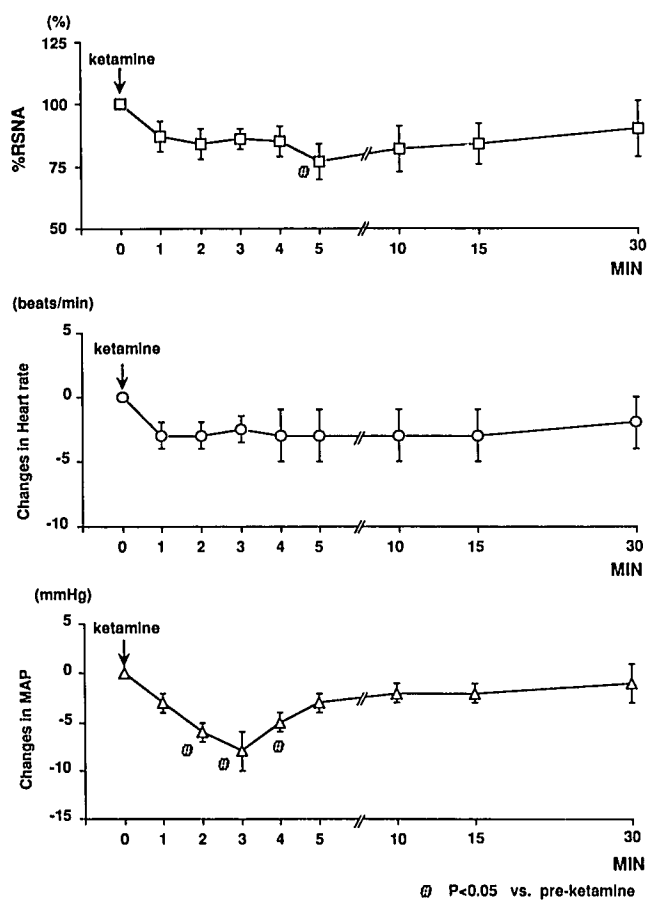


Fig. 8. Effect of ketamine on renal sympathetic nerve activity (RSNA, *top*), heart rate (*middle*), and mean arterial pressure (MAP, *bottom*) in spinal cord-transected rabbits ( $n = 6$ ). Ketamine did not increase RSNA, heart rate, and MAP. Values are mean  $\pm$  SEM.

duced increase in RSNA (figs. 2 and 3). We should consider the possibility that certain mechanisms, other than the formation of NO in the central nervous system, influenced the attenuation of RSNA during the administration of L-arginine. In particular, we should consider the effects of NO formation in the endothelium of systemic vascular beds, the effects of sensory afferent inputs, and other effects. Vascular endothelial NO formation may cause systemic vasodilatation and a decrease in arterial blood pressure, which may cause baroreflex mechanisms to increase RSNA, rather than to decrease it. Therefore, it is unlikely that L-arginine decreased RSNA by increasing vascular endothelial NO formation. It is possible that L-arginine may influence

the visceral organs to change the afferent input to the central nervous system, and thereby decrease RSNA. However, this also is unlikely, because the effect of L-arginine was observed after the interruption of visceral sensory afferents by sinoaortic denervation and bilateral vagotomy. In addition, the findings that D-arginine (an enantiomer of L-arginine), which is not a substrate for NO and is inactive in the central nervous system, did not affect the ketamine-induced increases in RSNA in baroreceptor deafferented rabbits (fig. 5), suggest that the attenuation of ketamine-induced increases in RSNA with L-arginine may be by way of increasing NO formation in the central nervous system. In addition, L-NAME, an inhibitor of NO synthase, mimicked the effect of ketamine on RSNA and the effect was also abolished by L-arginine (fig. 6). This result also suggests that the ketamine-induced increase in RSNA and its attenuation with L-arginine may be linked to central NO formation. However, nonspecific effect of large doses of L-arginine

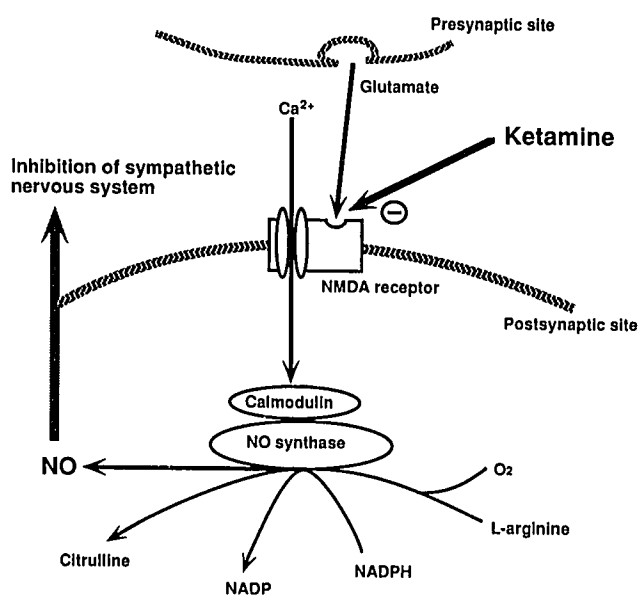


Fig. 9. A model for the operation of the nitric oxide (NO) system and our postulated mechanism by which ketamine may act in the central nervous system. In response to the N-methyl-D-aspartate (NMDA) receptor activation by glutamate, an increased calcium ion influx activates NO synthase *via* calmodulin, and NO is formed from L-arginine. Thus formed, NO may act as a second messenger, a neurotransmitter, or both and may have various effects including an inhibition of the sympathetic nervous system. Ketamine is a noncompetitive NMDA receptor antagonist. Therefore, ketamine may act in a direction of suppression of NO formation, resulting in the facilitation of the sympathetic nervous system.



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could not be ruled out in the current study. L-Arginine also releases growth hormone,<sup>21</sup> insulin,<sup>22</sup> and prolactin.<sup>23</sup> These hormones are less likely to be responsible for the decrease in RSNA. Further, the contribution of increased NO formation in the macrophages, platelets, or neutrophils to the decrease in RSNA could not be determined in this study. Therefore, we believe that the observed attenuation of ketamine-induced sympathoexcitation by L-arginine may have resulted from increased NO formation in the central nervous system.

L-NAME increased RSNA by  $135 \pm 8\%$  and the subsequent administration of ketamine caused a further but small increase in RSNA by  $147 \pm 9\%$  (fig. 7). This result suggests that the sites of action where ketamine increases RSNA may not be completely the same as those where L-NAME does. Ketamine might act *via* more pathways than NO inhibition, or L-NAME itself might act on various sites or pathways in the central nervous system other than our hypothesized "NMDA-NO" pathway.

Recently Paola *et al.*<sup>24</sup> demonstrated that N<sup>G</sup>-monomethyl-L-arginine microinjected in the NTS in rats attenuated the depressor effect evoked by glutamate microinjected in the NTS. Because glutamate acts as a neurotransmitter at the NTS,<sup>25</sup> this findings suggest the possibility that NO is involved in neural transmission in the NTS. The NTS relays primary sensory afferents, including baroreceptors, and regulates sympathetic outflow.<sup>25</sup> Ogawa *et al.*<sup>17</sup> demonstrated that ketamine exerted its cardiovascular effect in rats by suppressing NMDA receptors in the NTS. Harada *et al.*<sup>26</sup> also demonstrated that N<sup>G</sup>-monomethyl-L-arginine microinjected in the NTS of rabbits increased RSNA. These findings may support our hypothesis that the sympathoexcitatory effect of ketamine is linked to suppression of central NO formation, and suggest that NTS may be the site where ketamine produces its sympathoexcitatory effect.

Our results show that a ketamine-induced increase in RSNA can be attenuated by an intravenous infusion of L-arginine, but not by an infusion of D-arginine. Because L-arginine is the sole substrate of NO, it is possible that the increase in RSNA produced by ketamine may result from the inhibition of NO formation in the central nervous system.

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## References

- Domino EF, Chodoff P, Corssen G: Pharmacologic effects of CI-581, a new dissociative anesthetic in man. *Clin Pharmacol Ther* 6: 279-291, 1965
- Tweed WA, Minuck MS, Mymin D: Circulatory response to ketamine anesthesia. *ANESTHESIOLOGY* 37:613-619, 1972
- Ivankovich AD, Miletich DJ, Reimann C, Albrecht RF, Zahed B: Cardiovascular effect of centrally administered ketamine in goats. *Anesth Analg* 53:924-933, 1974
- Watkins JC, Evans RH: Excitatory amino acid transmitters. *Annu Rev Pharmacol Toxicol* 21:165-204, 1981
- Thomson AM, West DC, Lodge D: An N-methylaspartate receptor-mediated synapse in rat cerebral cortex: A site of action of ketamine? *Nature* 313:479-481, 1985
- Garthwaite J, Charles SL, Chess-Williams R: Endothelium-derived relaxing factor release on activation of NMDA receptors suggests role as intercellular messenger in the brain. *Nature* 336:385-8, 1988
- Sakuma I, Togashi H, Yoshioka M, Saito H, Yanagida M, Tamura M, Kobayashi T, Yasuda H, Gross SS, Nathan C, Levi R: N<sup>G</sup>-methyl-L-arginine, an inhibitor of L-arginine-derived nitric oxide synthesis, stimulates renal sympathetic nerve activity in vivo: A role for nitric oxide in the central regulation of sympathetic tone? *Circ Res* 70: 607-611, 1992
- Togashi H, Sakuma I, Yoshioka M, Kobayashi T, Yasuda H, Kitabatake A, Tamura M, Saito H, Gross SS, Levi R: A central nervous system action of nitric oxide in blood pressure regulation. *J Pharmacol Exp Ther* 262:343-347, 1992
- Rees DD, Palmer RMJ, Moncada S: Role of endothelium-derived nitric oxide in the regulation of blood pressure. *Proc Natl Acad Sci USA* 86:3375-3378, 1989
- Greisheimer EM: The circulatory effects of anesthetics, *Handbook of Physiology*. Volume 3. Baltimore, Waveley Press, 1965, pp 2477-2510
- Okamoto H, Hoka S, Kawasaki T, Sato M, Yoshitake J: Effects of CGRP on baroreflex control of heart rate and renal sympathetic nerve activity in rabbits. *Am J Physiol* 263:R874-R879, 1992
- Takki S, Bikki P, Jaattela A: Ketamine and plasma catecholamines. *Br J Anaesth* 44:1318-1322, 1972
- Traber DL, Wilson RD, Priano LL: Blockade of the hypertensive responses to ketamine. *Anesth Analg* 49:420-426, 1970
- Traber DL, Wilson RD: Involvement of the sympathetic nervous system in the pressor response to ketamine. *Anesth Analg* 48:248-252, 1969
- Floras J, Jones JV, Hassan MO, Osikowska BA, Sever PS, Sleight P: Failure of plasma norepinephrine to consistently reflect sympathetic activity in humans. *Hypertension* 8:641-649, 1986
- Dowdy EG, Kaya K: Studies of the mechanism of cardiovascular responses to CI-581. *ANESTHESIOLOGY* 29:931-943, 1968
- Ogawa A, Uemura M, Kataoka Y, Oi K, Inokuchi T: Effects of ketamine on cardiovascular responses mediated by N-methyl-D-aspartate receptor in the rat nucleus tractus solitarius. *ANESTHESIOLOGY* 78:163-167, 1993
- Knowles RG, Palacios M, Palmer RMJ, Moncada S: Formation of nitric oxide from L-arginine in the central nervous system: A transduction system mechanism for stimulation of the soluble guanylate cyclase. *Proc Natl Acad Sci USA* 86:5159-5162, 1989
- Palmer RMJ, Rees DD, Ashton DS, Moncada S: L-Arginine is the precursor for the formation of nitric oxide in endothelium-de-

pendent relaxation. *Biochem Biophys Res Commun* 153:1251–1256, 1988

20. Moncada S, Palmer RMJ, Higgs A: Nitric oxide: Physiology, pathophysiology, pharmacology. *Pharmacol Rev* 43:109–142, 1991

21. Merimee TJ, Lillicap DA, Racobinowitz D: Effect of arginine on serum-levels of human growth hormone. *Lancet* 2:668–670, 1965

22. Palmer JP, Walter RM, Ensink JW: Arginine-stimulated acute phase of insulin and glucagon secretion: I. In normal man. *Diabetes* 24:735–740, 1975

23. Rakoff JS, Silter TM, Sinha YN, Yen SSC: Prolactin and growth

hormone release in response to sequential stimulation by arginine and TRF. *J Clin Endocrinol Metab* 37:641–644, 1973

24. Paola EDD, Vidal MJ, Nistico G: L-Glutamate evokes the release of an endothelium-derived relaxing factor-like substance from the rat nucleus tractus solitarius. *J Cardiovasc Pharmacol* 17(suppl 3): S269–S272, 1991

25. Spyer KM: Neural organization and control of baroreceptor reflex. *Rev Physiol Biochem Pharmacol* 88:23–124, 1981

26. Harada S, Tokunaga S, Momohara M, Masaki H, Tagawa T, Imaizumi T, Takesita A: Inhibition of nitric oxide formation in the nucleus tractus solitarius increases renal sympathetic nerve activity in rabbits. *Circ Res* 72:511–516, 1993