

Role of nitric oxide in regulation of renal sympathetic nerve activity during hemorrhage in conscious rats

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Fujisawa, Yoshihide, Naoko Mori, Kouichi Yube, Hiroshi Miyanaka, Akira Miyatake, and Youichi Abe. Role of nitric oxide in regulation of renal sympathetic nerve activity during hemorrhage in conscious rats. *Am. J. Physiol. 277 (Heart Circ. Physiol. 46): H8–H14, 1999.*—The effect of inhibition of nitric oxide (NO) synthesis on the responses of blood pressure (BP), heart rate (HR), and renal sympathetic nerve activity (RSNA) during hemorrhaging was examined with the use of an NO synthase inhibitor, *N*^G-nitro-L-arginine methyl ester (L-NAME), in conscious rats. In the 0.9% saline group, hemorrhage (10 ml/kg body wt) did not alter BP but significantly increased HR and RSNA by 88 ± 12 beats/min and $67 \pm 12\%$, respectively. Intravenous infusion of L-NAME ($50 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) significantly attenuated these tachycardic and sympathoexcitatory responses to hemorrhage (14 ± 7 beats/min and $26 \pm 12\%$, respectively). Pretreatment of L-arginine (87 mg/kg) recovered the attenuation of HR and RSNA responses induced by L-NAME (92 ± 6 beats/min and $64 \pm 10\%$, respectively). L-NAME by itself did not alter the baroreceptor reflex control of HR and RSNA. Hemorrhage increased the plasma vasopressin concentration, and its increment in the L-NAME-treated group was significantly higher than that in the 0.9% saline group. Pretreatment with the vascular arginine vasopressin V_1 -receptor antagonist OPC-21268 (5 mg/kg) recovered the attenuation of RSNA response induced by L-NAME ($54 \pm 7\%$). These results indicate that NO modulated HR and RSNA responses to hemorrhage but did not directly affect the baroreceptor reflex arch. It can be assumed that NO modulated the baroreflex function by altering the secretion of vasopressin induced by

heart rate; vasopressin

NITRIC OXIDE (NO) plays an important role in the control of blood pressure (BP) by its direct action to dilate vascular smooth muscle (7, 14, 15). Apart from the direct action of NO on vascular smooth muscle, additional roles of NO in the regulation of cardiovascular function including the sympathetic nervous system have been proposed recently. Kumagai et al. (12) reported that the intravenous administration of an inhibitor for NO synthesis increases baseline BP and decreases renal sympathetic nerve activity (RSNA) in conscious spontaneously hypertensive rats. On the contrary, the blockade of NO synthesis in the central nervous system has been reported to increase sympathetic nerve activity in rabbits (10) and rats (29). NO

participates in the modulation of RSNA through its action on the central nervous system. Zanzinger et al. (28) provided evidence that NO inhibits peripheral sympathetic vasoconstriction that is independent of the central sympathetic tone, because baseline sympathetic activity was not altered by L-NAME in the baroreceptor-denervated cats. Thus there is a close relationship between central or peripheral NO and the sympathetic nervous system; however, no agreement on its relationship has been reached.

The aim of the present experiment is to define the role of NO on the control of the autonomic nervous system by examining the effects of NO synthesis inhibition on the responses of BP, heart rate (HR), and RSNA to hemorrhage. Hemorrhage was chosen because it is a situation in which peripheral sympathetic outflow contributes significantly to maintain BP (3, 16, 30). During the nonhypotensive phase of hemorrhage, BP is well maintained by increases in HR, vascular resistance, and sympathetic outflow via the baroreflex arch. In addition, hemorrhage is a potent stimulus for the secretion of vasoconstrictive humoral factors, i.e., norepinephrine, renin, and vasopressin. These hormones not only contribute to BP regulation but also affect sympathetic outflow. Previously, we demonstrated that an extended release of vasopressin during hemorrhaging influences sympathetic nerve activity (6). Recently, NO has been implicated in the regulation of the secretion of vasopressin (1, 2, 8, 9, 17, 20, 26), raising the possibility that it may influence the sympathetic nervous system by altering the secretion of this hormone. Thus in the present study we examined how the inhibition of NO synthesis modifies the responses of HR, RSNA, and vasopressin secretion to nonhypotensive hemorrhage. Moreover, the effects of a V_1 -receptor antagonist on the HR and RSNA responses to hemorrhage were also examined. These experiments were performed in conscious animals to avoid the confounding effects of anesthesia and surgical stress.

MATERIALS AND METHODS

The experiment was performed with 9- to 11-wk-old male Sprague-Dawley rats weighing 270–350 g (Japan SLC, Hamamatsu, Japan). The rats were housed in separate cages in a temperature-controlled room with a 12:12-h light-dark cycle. They were fed a standard laboratory diet and water ad libitum. All surgical and experimental procedures were approved by the Animal Care and Use Committee, Kagawa Medical University, and conformed to the Guidelines for Animal Experimentation, Kagawa Medical University. Under pentobarbital sodium anesthesia, one polyethylene catheter (PE-60) was inserted into the abdominal aorta via the right femoral artery for measurement of BP and arterial blood

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withdrawal, and another catheter (PE-50) was inserted into the inferior vena cava via the right femoral vein for administration of fluids and drugs. A third catheter (PE-50) was inserted into the right femoral vein in the experiments on baroreceptor reflex and vasopressin measurement. All catheters were filled with heparinized saline (100 U/ml) and tunneled under the skin to be exteriorized at the nape of the neck. The electrodes for RSNA recording were implanted as described in *Renal nerve recording and signal processing*. After the catheterization and implantation procedures were completed, the rats were put into a small plastic chamber (25 × 15 × 15 cm) and allowed to recover for 48 h. The rats behaved normally and exhibited normal food and water intake at the time of the experiment.

Renal nerve recording and signal processing. RSNA was recorded from the left renal nerve branch as previously described (6). The nerve was isolated near the aortic-renal arterial junction through a left flank incision and placed on a Teflon-coated stainless steel bipolar electrode with the aid of an operation microscope. The renal nerve and electrode were covered with silicone rubber (semicosil A and B). The lead wire from the recording electrode was exteriorized at the nape of the neck. The renal nerve discharge was amplified using a differential amplifier (Nihon Kohden AVB-10) with a band-pass filter (low frequency 50 Hz, high frequency 1 kHz). The amplified and filtered signal was visualized on a dual-beam oscilloscope (Nihon Kohden VC-10) and monitored by an audio speaker. The output from the amplifier was integrated by an integrator (Nihon Denki Sanei 1322) with 1-s resetting. The baseline noise was subtracted from the integrated RSNA. The baseline noise was determined when nerve activity was eliminated by increasing BP with phenylephrine (PE; 4 µg/animal iv). The output from the integrator was displayed on a polygraph system recorder (Nihon Denki Sanei 8M14). For the quantification of RSNA, the height of integrated nerve discharge was measured for 30 s in each experiment. The changes in nerve activity were expressed as percentages of control resting spontaneous nerve activity.

Experimental protocols. During the experiment, the femoral arterial catheter was connected to a Statham pressure transducer (P23 ID). The systemic BP and HR were continuously recorded in a multichannel polygraph (Nihon Denki Sanei 360). HR triggered by the BP pulse was measured with a cardiometer (Nihon Denki Sanei 1321).

Effects of inhibition of NO synthesis on BP, HR, and RSNA responses to hemorrhage. Rats were divided into 4 groups: 0.9% saline ($n = 9$), L-NAME (50 µg·kg⁻¹·min⁻¹; $n = 9$), L-arginine (L-Arg; 87 mg/kg) + L-NAME ($n = 8$), and L-Arg ($n = 5$). L-NAME and L-Arg were dissolved in 0.9% saline. The infusion of 0.9% saline was started at a rate of 20 µl·min⁻¹·300 g body weight⁻¹ and continued for the entire experiment. After a 60-min stabilization period, control values of BP, HR, and RSNA were measured for 5 min, and then the rats were subjected to arterial hemorrhage. Blood was withdrawn from the femoral arterial catheter at a rate of 10 ml·min⁻¹·kg body weight⁻¹ for 1 min. Thereafter, BP, HR, and RSNA were continuously recorded for 30 min. The same procedures were performed in the second group, except that the saline infusion was replaced by L-NAME at 50 µg·kg⁻¹·min⁻¹. When a higher dose of L-NAME such as 500 µg·kg⁻¹·min⁻¹ was used, RSNA decreased to nearly zero and the RSNA response to hemorrhage could not be observed; therefore a dose of 50 µg·kg⁻¹·min⁻¹ was chosen to observe the RSNA response. L-NAME infusion was started after the control values were measured, and hemorrhage was performed 20 min after the L-NAME infusion was started. In the third group, L-Arg (87 mg/kg) was injected, and L-NAME infusion was started 5 min

after the L-Arg injection. In the fourth group, the same procedures used for the third group were performed, except that the L-NAME infusion was replaced by 0.9% saline infusion. The hemorrhage was performed 20 min after the start of L-NAME infusion as described above. All experiments were performed with only one hemorrhage procedure for each rat.

Effects of NO synthesis inhibition on the baroreceptor reflex control of HR and RSNA. Baroreceptor reflex curves (HR or RSNA vs. mean BP) were generated by measuring HR or RSNA in response to the changes in BP induced by intravenous infusion of PE and nitroprusside (NP) through the catheter in other rats. Rats were divided into two groups: 0.9% saline infusion (20 µl·min⁻¹·300 g body weight⁻¹; $n = 7$) and L-NAME infusion (50 µg·kg⁻¹·min⁻¹; $n = 7$). The doses of PE and NP were 1, 2, 5, and 10 µg·kg⁻¹·min⁻¹ and 1.7, 3.4, 8.5, and 17 µg·kg⁻¹·min⁻¹, respectively. Each infusion lasted for 3 min, and ~10 min elapsed between each infusion. Rats were infused with either PE or NP. BP, HR, and RSNA data collected during the last minute of each infusion were averaged and used to generate baroreceptor reflex curves.

Effects of NO synthesis inhibition on vasopressin secretion during hemorrhage. The time course of plasma vasopressin levels after hemorrhage was examined in conscious rats with sham operation of RSNA recording. The rats were divided into two groups: 0.9% saline (20 µl/min, $n = 5$) and L-NAME (50 µg·kg⁻¹·min⁻¹, $n = 6$). The first blood sample as a control was an initial portion of hemorrhaged blood. The second and third blood samples were taken at 2.5 and 30 min after bleeding. The second blood sample was replaced by the simultaneous intravenous injection through the catheter with an equal volume of rat donor blood that was obtained by decapitation of an animal on the day of the experiment. Donor blood was warmed to 37°C just before use. Plasma was prepared by Sep-Pak C18 extraction (27), and vasopressin concentration was measured by radioimmunoassay using a kit from Mitsubishi Yuka Biochemical Laboratory (Tokyo, Japan).

Effects of V₁-receptor antagonist on BP, HR, and RSNA responses to hemorrhage during inhibition of NO synthesis. In this experiment, the V₁-receptor antagonist OPC-21268 (Otsuka Pharmaceutical, Tokyo, Japan) was used in another seven rats. OPC-21268 was dissolved in 20% ethanol saline solution. OPC-21268 (5 mg/kg) was injected and followed by the L-NAME infusion, and the previous hemorrhagic procedure was performed.

Statistical analysis. Results are expressed as means ± SE. Basal values and the effects of L-NAME and L-Arg were analyzed by Student's paired or unpaired *t*-test. All responses to hemorrhage over time were analyzed by analysis of variance for repeated measurements (25). When significant differences were detected by analysis of variance, multiple comparisons were performed using Scheffé's *F*-test. *P* values <0.05 were accepted as statistically significant.

RESULTS

The basal values of mean BP, HR, and RSNA and the effects of L-NAME and L-Arg + L-NAME on these parameters are tabulated in Table 1. There were no significant differences in those basal values among the different groups. Intravenous administration of L-NAME significantly increased BP but did not change HR and RSNA. L-Arg did not affect any of those values, and subsequent infusion of L-NAME did not affect BP but significantly increased HR and RSNA.

Table 1. Basal values of MBP, HR, and RSNA and effects of L-NAME and L-Arg on these parameters in conscious rats

	MBP, mmHg	HR, beats/min	RSNA, %
0.9% Saline (n=9)			
Control	124 ± 2	401 ± 20	100
0.9% Saline	124 ± 2	413 ± 21	101 ± 2
L-NAME (50 µg · kg ⁻¹ · min ⁻¹) (n=9)			
Control	122 ± 2	442 ± 12	100
L-NAME	132 ± 2*†	438 ± 11	104 ± 9
L-Arg (87 mg/kg) + L-NAME (n=8)			
Control	126 ± 3	412 ± 12	100
L-Arg	125 ± 3	412 ± 11	99 ± 2
L-NAME	127 ± 3	445 ± 15*	113 ± 5*†

Values are means ± SE; n = no. of rats. MBP, mean blood pressure; HR, heart rate; RSNA, renal sympathetic nerve activity; L-NAME, N^G-nitro-L-arginine methyl ester; L-Arg, L-arginine. RSNA in control period was normalized at 100%. *P < 0.05, significant difference from 0.9% saline value. †P < 0.05, significant difference from 0.9% saline value.

Responses to hemorrhage. BP, HR, and RSNA responses to hemorrhage are shown in Figs. 1 and 2. In the 0.9% saline group, withdrawal of 10 ml/kg body weight did not affect BP but significantly increased HR and RSNA by 88 ± 12 beats/min and 67 ± 12%, respectively, at 2.5 min after bleeding, and these increments continued for 30 min. L-Arg alone did not affect HR and RSNA, and hemorrhage after the administration of L-Arg increased HR and RSNA by more than 50

beats/min and 40%, respectively (data not shown). Thus L-Arg did not affect HR and RSNA responses to hemorrhage.

Effects of L-NAME on responses to hemorrhage. In the L-NAME group, BP decreased slightly at 10 min after bleeding and was then restored to the prehemorrhagic level at 20 min. HR and RSNA increased by 14 ± 7 beats/min and 26 ± 12% at 2.5 min after bleeding, respectively, and these values were maintained during hemorrhage. However, the increases were significantly lower than those in the 0.9% saline group (Figs. 1 and 2). These results indicate that the infusion of L-NAME significantly attenuated tachycardic and sympathoexcitatory responses to hemorrhage.

Effects of L-NAME after pretreatment with L-Arg on responses to hemorrhage. In the L-Arg + L-NAME group, BP did not change after bleeding except for the value at 20 min after bleeding. HR and RSNA increased by more than 50 beats/min and 50% at 20 min, respectively, but these increases were reduced at 30 min after bleeding (Figs. 1 and 2). The results indicate that the pretreatment of L-Arg recovered the attenuation of HR and RSNA responses induced by the infusion of L-NAME.

Effects of L-NAME on baroreceptor reflex control of HR and RSNA. The effects of L-NAME on the baroreceptor reflex control of HR and RSNA are shown in Fig. 3. Infusion of L-NAME did not alter the baroreceptor reflex curve of RSNA but reduced the upper and lower plateau of the curve of HR. However, within the range of BP response (110–130 mmHg) in the hemorrhage

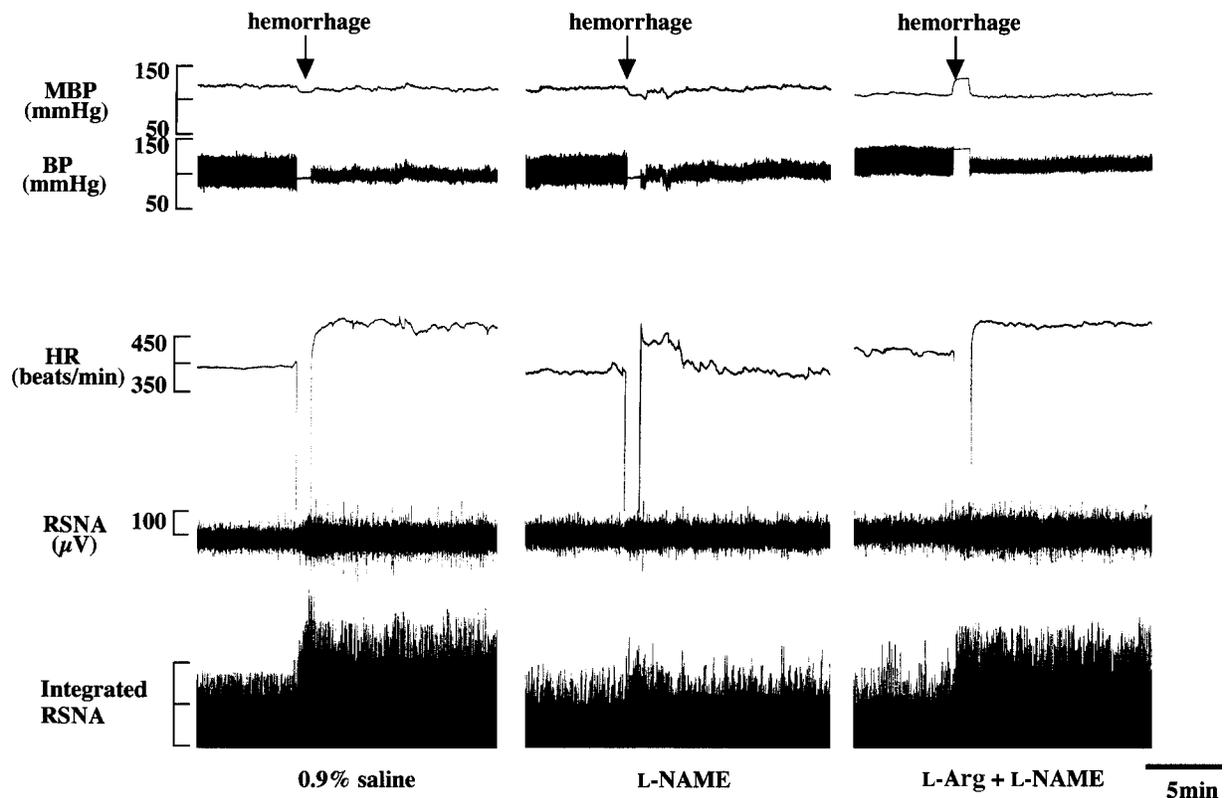


Fig. 1. Original recording showing effects of 0.9% saline, N^G-nitro-L-arginine methyl ester (L-NAME), and L-arginine (L-Arg) + L-NAME on responses of mean blood pressure (MBP), blood pressure (BP), heart rate (HR), renal sympathetic nerve activity (RSNA), and integrated RSNA to hemorrhage.

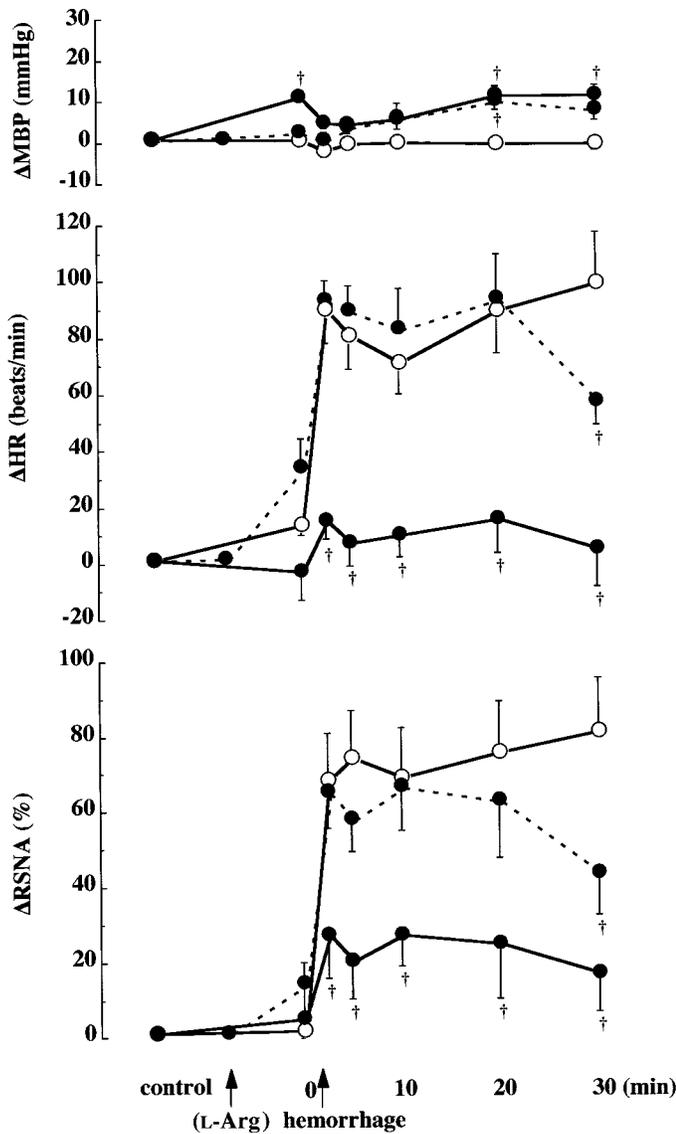


Fig. 2. Comparison of effects of 0.9% saline (○ and solid line), L-NAME (● and solid line), and L-Arg + L-NAME (● and dashed line) on MBP, HR, and RSNA responses to hemorrhage. †*P* < 0.05, significant difference from 0.9% saline value.

experiment, L-NAME did not affect the baroreceptor reflex control of HR and RSNA.

Effects of L-NAME on vasopressin secretion during hemorrhage. The effects of L-NAME on vasopressin secretion during hemorrhage are shown in Table 2. In

Table 2. Effect of hemorrhage on plasma vasopressin concentration

	<i>n</i>	Plasma Vasopressin Concentration, pg/ml		
		Control	2.5 min	30 min
0.9% Saline	5	4.8 ± 3.2	69.7 ± 20.3*	23.1 ± 3.8*
L-NAME	6	8.2 ± 1.5	170.7 ± 30.7*†	23.3 ± 2.3*

Values are means ± SE; *n* = no. of rats. Responses were measured 2.5 and 30 min after hemorrhage. **P* < 0.05, significant difference from control value. †*P* < 0.05, significant difference from 0.9% saline value.

the control period, there was no significant difference between groups. Hemorrhaging increased plasma vasopressin concentration in both groups. Infusion of L-NAME increased plasma vasopressin concentration much higher compared with the 0.9% saline group (170.7 ± 30.7 vs. 69.7 ± 20.3 pg/ml, respectively) at 2.5 min after bleeding.

Effects of V₁-receptor antagonist on responses to hemorrhage. The infusion of OPC-21268 did not change basal cardiovascular parameters (mean BP, 122 ± 1 to 123 ± 1 mmHg; HR, 417 ± 14 to 413 ± 16 beats/min; RSNA, 100 to 93 ± 3%). The effects of the V₁-receptor antagonist on BP, HR, and RSNA responses to hemorrhage are shown in Fig. 4. In the OPC-21268 + L-NAME group, BP did not change after bleeding. HR increased more than 20 beats/min for 10 min, and the increase was reduced at 20 min after bleeding. However, RSNA increased by >50% for 20 min. The increase of RSNA was nearly the same as with that in the 0.9% saline group.

DISCUSSION

The main finding of this study is that NO synthase inhibition by intravenous administration of L-NAME attenuated the hemorrhage-induced tachycardia and sympathoexcitation in conscious rats. The attenuation induced by L-NAME infusion was recovered by pretreatment of L-Arg. These findings suggest that NO played an important role in the control of sympathetic nerve activity during acute hemorrhaging as well as the direct vasodilatory action on vascular smooth muscle.

In the present study, hemorrhage was chosen to evaluate the possible role of NO in the regulation of cardiovascular function and sympathetic nerve tone

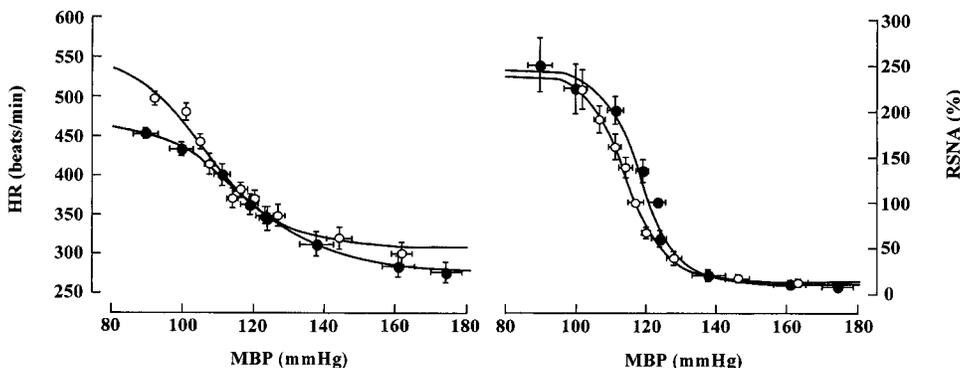


Fig. 3. Composite baroreflex curves. Left: relationship between HR and MBP. Right: relationship between RSNA and MBP. ○, 0.9% saline; ●, L-NAME.

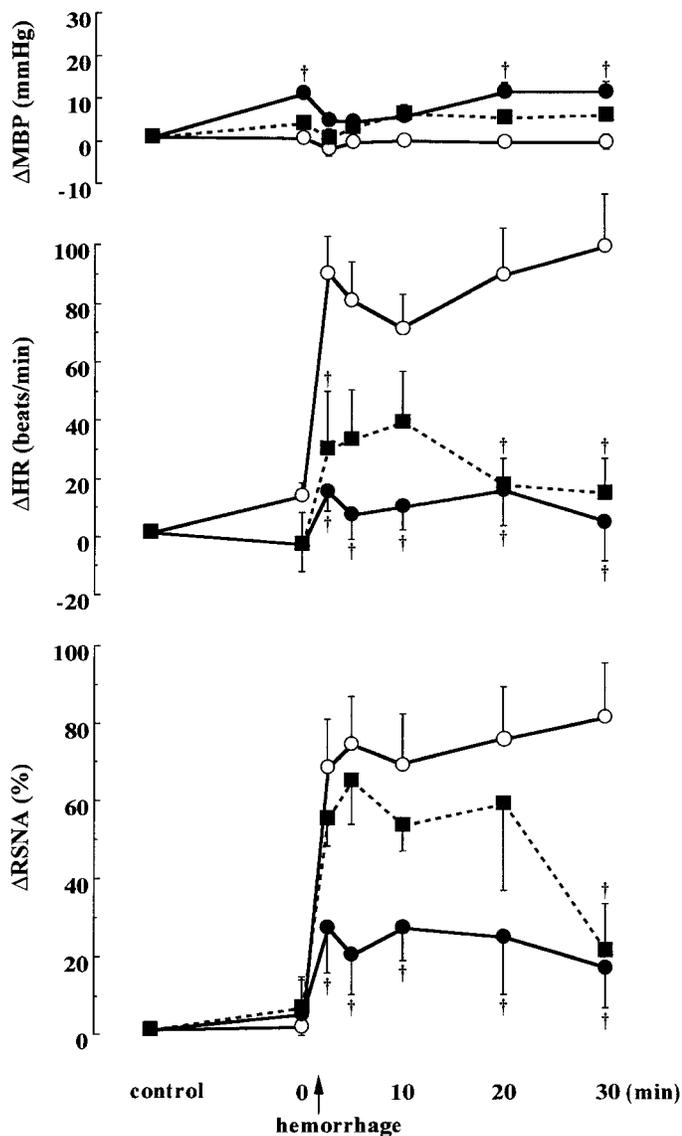


Fig. 4. Comparison of effects of 0.9% saline (○ and solid line), L-NAME (● and solid line), and V₁-receptor antagonist + L-NAME (■ and dashed line) on MBP, HR, and RSNA responses to hemorrhage. † $P < 0.05$, significant difference from 0.9% saline value.

because hemorrhage is a typical stimulation for sympathoexcitation (3, 16, 30). Generally, BP is maintained in the early stage of hemorrhage with reflex increases in HR, vascular resistance, and peripheral sympathetic nerve activity. In fact, the removal of blood (10 ml/kg) did not affect BP but significantly increased HR and RSNA. However, L-NAME attenuated hemorrhage-induced tachycardia and sympathoexcitation. As has been reported previously (10, 12, 21, 23), central or peripheral NO affects the sympathetic nerve activity. Lacolley et al. (13) indicated that pretreatment with a ganglion-blocking agent eliminates NO inhibitor-induced hypertension in urethan-anesthetized rats. Also, other reports have indicated that hypertension induced by the inhibition of NO synthesis decreases HR and sympathetic outflow in rats (4, 12, 19). On the contrary, Sakuma et al. (21) reported that the systemic

inhibition of NO increases RSNA in anesthetized and immobilized rats. On the other hand, some reports indicated that administration of NO synthase inhibitor into the central nervous system increases sympathetic outflow. Harada et al. (10) and Zhang et al. (29) have reported that microinjection of L-NAME into the nucleus tractus solitarius or paraventricular nucleus elicits an increase in RSNA, HR, and BP but that its injection into the area postrema does not. Taken together, these findings suggest that the inhibition of central NO may increase the sympathetic outflow; however, the peripheral inhibition of NO synthesis may decrease the sympathetic nerve activity via a baroreflex arch. The present results show that intravenous administration of L-NAME at a dose of $50 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ did not change basal HR and RSNA. However, when this dose of L-NAME was increased tenfold, HR significantly decreased along with a marked rise of BP, and RSNA decreased to almost zero. In this case, hemorrhage did not induce any tachycardic and sympathoexcitatory responses (data not shown). Thus we chose the present dose of NO synthase inhibitor to have the least possible affect on basal HR and RSNA.

We examined whether the attenuation of hemorrhage-induced sympathoexcitatory and tachycardic responses by L-NAME might be mediated via the changes in baroreflex function or the altered secretion of humoral factors such as vasopressin. First, we examined whether L-NAME affects the baroreceptor reflex control of HR and RSNA. Our results indicate that L-NAME did not affect the baroreceptor reflex control of HR and RSNA; it only reduced the upper and lower plateau of the curve in HR response. Thus L-NAME did not alter the baroreceptor reflex control of HR and RSNA within the range of BP response (110–130 mmHg) in the present study. The present results are consistent with the results of other investigators. Du et al. (5) reported that NO synthase inhibitor reduces the lower plateau but does not affect HR sensitivity. Jimbo et al. (11) reported that NO does not modulate the baroreceptor reflex arch, and Harada et al. (10) also reported that NO synthase inhibitor administered in the nucleus tractus solitarius does not alter the baroreceptor reflex function. Therefore, it is suggested that the attenuation of hemorrhage-induced tachycardia and sympathoexcitation by L-NAME does not depend on a functioning baroreceptor reflex mechanism.

Second, we examined the possibility that the secretion of vasopressin during hemorrhage is altered by the inhibition of NO synthesis and whether its alteration influences the sympathetic nerve activity. Recently, Goyer et al. (9) reported that a blockade of NO synthesis increases resting plasma vasopressin concentration in conscious rabbits, suggesting an inhibitory role of NO in the control of vasopressin secretion. Chiu and Reid (2) also reported that L-NAME increases resting plasma vasopressin concentration but did not alter the vasopressin secretion to hemorrhage. In the present study, L-NAME did not alter the resting plasma vasopressin concentration but increased the subsequent

vasopressin secretion to hemorrhage. Moreover, treatment of the V_1 -receptor antagonist recovered the attenuation of RSNA response induced by L-NAME. Therefore, the increased circulating vasopressin played a role in HR and RSNA responses to hemorrhage during inhibition of NO synthesis. Our previous report showed that vasopressin exerts inhibitory action on HR and RSNA via the V_1 receptor during hypotensive hemorrhage (6). In other reports, Patel and Schmid (18) reported that a greater bradycardia induced by vasopressin is abolished with lesion of the median preoptic area. Undesser et al. (24) indicated that a greater sympathoinhibition induced by intravenous infusion of vasopressin was attenuated with lesion of the area postrema. Vasopressin-induced renal sympathoinhibition with and without functioning arterial and cardiopulmonary baroreflex is attenuated with lesion of the nucleus tractus solitarius or the area postrema (22). Therefore, it can be assumed that increased circulating vasopressin due to hemorrhaging suppressed HR and RSNA responses via these above-described central areas devoid of blood-brain barrier during inhibition of NO synthesis. However, we cannot explain at present why L-NAME administered intravenously stimulates vasopressin secretion to hemorrhage and where the action site of L-NAME for arginine vasopressin secretion is located. Further studies are needed to determine the action sites and mechanisms of L-NAME for vasopressin secretion.

In summary, it can be concluded that NO synthesis inhibition attenuates the hemorrhage-induced tachycardia and sympathoexcitation and that this attenuation may be mediated by vasopressin, which is significantly augmented after the inhibition of NO synthesis.

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