N^{ω}-Amino-L-Arginine, an Inhibitor of Nitric Oxide Synthase, Raises Vascular Resistance but Increases Mortality Rates in Awake Canines Challenged with Endotoxin*

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Summary

Inhibitors of nitric oxide synthase (NOS) have been reported to increase mean arterial pressure in animal models of sepsis and recently have been given to patients in septic shock. However, controlled studies to determine the effects of these agents on cardiovascular function and survival in awake animal models of sepsis have not been reported. To examine the therapeutic potential of NOS inhibition in septic shock, we challenged canines with endotoxin (2 or 4 mg/kg i.v.) and treated them with either normal saline or N^{ω}-amino-L-arginine (10 or 1 mg/kg/h), the most specific inhibitor available for the isoform of NOS implicated in septic shock. Endotoxemic animals treated with N^{ω}-amino-L-arginine (n = 11) had higher systemic and pulmonary vascular resistance indices (SVRI and PVRI, $p \leq 0.033$) and decreased heart rates (p = 0.009), cardiac indices (CI, p = 0.01), oxygen delivery indices (p = 0.027), and oxygen consumption indices (p = 0.046) compared with controls (n = 6). Moreover, N^{ω}-amino-L-arginine increased mortality rates after endotoxin challenge (10 of 11 vs. 1 of 6 controls, p = 0.005). Administration of L-arginine did not improve survival or alter the cardiopulmonary effects of N^{ω}-amino-L-arginine, which suggests that inhibition of NOS may not have been competitive. In normal animals, N^{ω}-amino-L-arginine alone (n = 3) increased SVRI (p = 0.0008) and mean arterial pressure (p = 0.016), and decreased CI (p = 0.01) compared with saline-treated controls (n = 3), but, at the high dose, also produced neuromuscular rigidity and seizure-like activity that was not apparent in the endotoxemic model. Thus, the mortality rate from endotoxemia increased either because of NOS inhibition per se or because of properties unique to N^{ω}-amino-L-arginine, or both.

Despite antibiotic therapy and advances in critical care, septic shock is associated with a high mortality rate (1, 2). Approximately 50% of patients who die of septic shock have persistent hypotension and low systemic vascular resistance refractory to vasopressor therapy (3–5). New evidence suggests that overproduction of endothelium-derived relaxing factor (EDRF)[1], recently identified as nitric oxide (NO) (6) or a closely related nitrosothiol (7, 8), contributes to the development of sepsis-induced hypotension (9). A calcium-independent isoform of nitric oxide synthase (NOS) can be induced in cultured endothelial cells by interferon-γ combined with bacterial LPS (endotoxin), TNF, or IL-1 (10–12), and in vascular smooth muscle cells in vitro after stimulation

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[1] *Abbreviations used in this paper:* CBC, complete blood count; CI, cardiac index; DO_2I, oxygen delivery index; EDRF, endothelium-derived relaxing factor; ER, extraction ratio; HR, heart rate; L-ARG, L-arginine; LVEF, left ventricular ejection fraction, MAP, mean arterial pressure; NAA, N^{ω}-amino-L-arginine; NOS, nitric oxide synthase; PCWP, pulmonary capillary wedge pressure; PVRI, pulmonary vascular resistance index; SVI, stroke volume index; SVRI, systemic vascular resistance index; VO_2I, oxygen consumption index.

with endotoxin (13). It is believed that this inducible isoform of NOS is responsible for the excess production of NO in sepsis, which leads to the development of shock (9, 14, 15).

Some L-arginine analogs reversibly inhibit NOS, restore endotoxin-induced loss of catecholamine vasomotor responsiveness in vivo (16, 17), and reverse hypotension in animal models of septic shock. N^{ω}-methyl-L-arginine (18, 19) has been shown in anesthetized animals to reverse endotoxin and TNF-induced hypotension (9, 20–22). Most recently, N^{ω}-methyl-L-arginine and another NOS inhibitor, N^{ω}-nitro-L-arginine methyl ester, were reported to increase systemic vascular resistance and blood pressure in three patients with septic shock (23, 24). Together, these studies support the hypothesis that production of excess NO is a contributor to the hypotension of septic shock, and suggest a potential therapeutic role for inhibitors of NOS as antihypotensive agents in this condition.

However, inhibition of endogenous NO production may be harmful. Administration of N^{ω}-methyl-L-arginine to anesthetized rats and canines has been shown to increase renal vascular resistance (25) and decrease renal blood flow (26, 27). The use of this NOS inhibitor in awake canines results in dose-related increases in basal epicardial coronary artery tone (28). Further, N^{ω}-methyl-L-arginine increases capillary leak and enhances intestinal damage in rats and depresses cardiac output in anesthetized canines given endotoxin (21, 29). High doses of N^{ω}-methyl-L-arginine (300 mg/kg) administered after endotoxin challenge can precipitate cardiovascular collapse and death in anesthetized rats (22).

Most previous investigations have examined the cardiovascular effects of short-term infusions of N^{ω}-methyl-L-arginine in the presence of general anesthetics, which may also produce significant hemodynamic changes (30). Further, studies using NOS inhibitors in large animal models of sepsis and in humans have not been designed to determine the effect of these agents on survival. In this study, we evaluated the therapeutic value of a continuously infused NOS inhibitor by serially following cardiopulmonary function, laboratory parameters, and survival in awake canines challenged with intravenous endotoxin. N^{ω}-amino-L-arginine was used in these experiments because it is a potent NOS inhibitor in vivo (31, 32), is the most specific inhibitor available for the induced isoform of NOS (15), has not been reported to be significantly metabolized to the substrate L-arginine (unlike N^{ω}-methyl-L-arginine (33), and is readily water soluble (unlike N^{ω}-nitro-L-arginine).

Materials and Methods

Reagents. N^{ω}-amino-L-arginine was prepared as the lyophilized hydrochloride salt by Dr. Owen W. Griffith at Cornell University Medical College (32, 34). The drug was reconstituted with pyrogen-free normal saline and passed through a 22-μm filter (Millex-GV; Millipore, Bedford, MA) before intravenous administration. *Escherichia coli* 0111:B4 endotoxin and L-arginine (Sigma Chemical Co., St. Louis, MO) were suspended in pyrogen-free normal saline for intravenous injection. Ceftriaxone (Rocephin®; Hoffman LaRoche Inc., Nutley, NJ) was reconstituted with pyrogen-free sterile water.

Experimental Groups (Table 1) and Study Design (Fig. 1): 23 2-yr-old, purpose-bred, 8–13 kg beagles were studied. A baseline comprehensive hemodynamic evaluation was performed for each animal at least 3 d before endotoxin challenge, as described previously (35, 36). Briefly, arterial and thermodilution, balloon-tipped, pulmonary artery catheters were inserted in awake animals to obtain serial hemodynamic measurements (monitor model 90603; Spacelabs Inc., Redmond, WA) before and after an intravenous volume infusion (40 ml/kg Ringer's solution over 30 min). Left ventricular ejection fractions (LVEF) were determined by radionuclide ventriculography (35).

At time 0 h (Fig. 1), 17 animals received a 1- or 2-h intravenous infusion of endotoxin, 2 mg/kg/h, delivered using a micropump (Infu-Med™ 300; Medfusion, Inc., Duluth, Georgia). 11 of these 17 animals were given a 22-h, continuous, N^{ω}-amino-L-arginine intravenous infusion at either 1 or 10 mg/kg/h after a loading dose (see Table 1). L-arginine was administered to 4 of the 11 N^{ω}-amino-L-arginine-treated animals at 1, 10, 20 or 50 mg/kg/h after a loading dose (Table 1) to evaluate its ability to competitively reverse NOS inhibition (9, 19, 20). The remaining six endotoxin-challenged animals served as controls and received only normal saline at a rate equivalent in ml/h to that of N^{ω}-amino-L-arginine infusion (0.9–1.2 ml/h). Six normal animals not challenged with endotoxin received N^{ω}-amino-L-arginine alone at 1 mg/kg/h (n = 1), or 10 mg/kg/h (n = 2), or an equal volume of normal saline (n = 3). Ceftriaxone, 100 mg/kg, was injected intravenously immediately before and 22 h after endotoxin challenge to prevent catheter-related infection. All animals received 10 ml/kg/h Ringer's solution intravenously continuously for 6 h after endotoxin challenge. 40 ml/kg of Ringer's solution was infused intravenously in all animals over 30 min immediately before time 0, 10, 14, and 22 h. Hemodynamic measurements and blood for laboratory analysis were obtained at 0, 2, 6, 10, 14, 18, and 22 h. Simultaneous radionuclide LVEFs were determined at 6 and 22 h. All catheters were removed at 24 h and the animals were returned to individual cages for 3–10 d of close observation depending upon their clinical status.

Laboratory Analysis. Blood samples for quantitative bacterial culture were collected into 1.5-ml isolator tubes (DuPont Medical Products Department, Wilmington, DE) at 0 and 22 h after endotoxin challenge just before the dose of ceftriaxone. Serial dilutions of the lysed samples were plated for bacterial colony quantitation. Serum and whole blood were analyzed by an outside source (MetPath Mid-Atlantic Regional Laboratory, Rockville, MD) for serum chemistry and complete blood count (CBC) using standard automated methods. Arterial and mixed venous blood gases were determined using an automated system (288 Blood Gas System and 2500 Co-oximeter; Ciba-Corning Diagnostic Corp., Medifield, MA). The N^{ω}-amino-L-arginine solution was assayed for endotoxin contamination by Dr. H. Donald Hochstein (Division of Product Quality Control, Food and Drug Administration, Bethesda, MD) using a limulus amebocyte lysate gel test (Associates of Cape Cod, Woods Hole, MA). Plasma endotoxin concentrations were determined as previously described using a kinetic, chromogenic limulus lysate assay (MA Bioproducts, Walkersville, MD) (37).

Animal Care. The protocol used in this study was approved by the Clinical Center Animal Care and Use Committee of the National Institutes of Health. Every effort was made by a team of experienced veterinarians, physicians, and research technicians to keep the animals comfortable within the constraints of the protocol. Animals were euthanized if they appeared to be suffering in the judgment of the veterinarians or the investigators.

Cardiopulmonary Calculations and Statistics. The methods used for the measurement and calculation of hemodynamic and LVEF

data have been described (35, 38). Oxygen delivery (DO_2) and consumption (VO_2) were calculated from measured values using standard formulae. Extraction ratio (ER) was calculated as VO_2/DO_2. The following parameters were either measured directly from stripchart recordings or calculated using standard formulae: mean arterial pressure (MAP, mm Hg), systemic and pulmonary vascular resistance indices (SVRI and PVRI, respectively, dyn·sec/cm^5·kg), cardiac index (CI, ml/min·kg), heart rate (HR, beats/min), LVEF, pulmonary capillary wedge pressure (PCWP, mm Hg), stroke volume index (SVI, ml/kg), VO_2 index (VO_2I, ml/min·kg), and DO_2 index (DO_2I, ml/min·kg).

Serial effects of endotoxin alone and serial effects of N$^\omega$-amino-L-arginine in endotoxin-challenged animals were analyzed by analysis of variance (ANOVA) (39). A three-way ANOVA was constructed with treatment group, dog nested within group, time, and group–time interaction effects extracted. A Tukey multiple comparisons procedure (39) or a Dunnett test (40) for comparing to a common baseline was used to adjust p values. Survival data were analyzed using Fisher's exact test.

Results

Survival and Clinical Manifestations. Continuous intravenous N$^\omega$-amino-L-arginine administration decreased survival after intravenous endotoxin challenge (Table 1 and Fig. 2). The survival rates of canines that received either 2 or 4 mg/kg of endotoxin plus normal saline did not differ significantly. This was also true for the endotoxin-challenged animals treated with either 1 or 10 mg/kg/h of N$^\omega$-amino-L-arginine. 10-d survival rates of the combined control groups (n = 6) and the treatment groups (n = 7) were 83 and 14%, respectively (Fig. 2, p = 0.029). Two dogs in the low dose N$^\omega$-amino-L-arginine group were euthanized at 24 and 144 h and considered nonsurvivors in keeping with the experimental protocol (see Materials and Methods). One was preterminal and the other had bilateral hind limb paralysis as a result of the experiment. The addition of a continuous L-arginine infusion at 1, 10, 20, or 50 mg/kg/h did not improve survival (Fig. 2). Two animals (nonsurvivors) in this group were euthanized in preterminal states. The animal that received L-arginine at 10 mg/kg/h was euthanized at 24 h, and the animal that received L-arginine at 50 mg/kg was euthanized at 36 h. Combining data from all animals that received endotoxin plus N$^\omega$-amino-L-arginine, with or without L-arginine (n = 11), revealed a 10-d survival rate of 9.1% (p = 0.005 vs. saline-treated controls). During the study, no clinical differences were noted between these groups.

Because N$^\omega$-amino-L-arginine increased mortality after endotoxin challenge in this model, normal animals were studied during an infusion of either N$^\omega$-amino-L-arginine or normal saline. The infusion of N$^\omega$-amino-L-arginine at 10 mg/kg/h

Table 1. *Experimental Groups*

	Number of dogs	Dose: Endotoxin	Dose: NAA‡	10 d Survival (%)
	n	*mg/kg*	*mg/Kg/h*	
Endotoxin challenged				
NS*	2	2		2 (100)
	4	4		3 (75)
NAA	2	2	10	0 (0)
	5	4	1	1 (20)
NAA + L-arginine§	4	4	1	0 (0)
Normal				
NS	3			3 (100)
NAA	2		10	0 (0)
	1		1	1 (100)

* NS normal saline.
‡ N$^\omega$-amino-L-arginine (NAA) at 1 mg/kg/h or 10 mg/kg/h preceded by a 10 or 20 mg/kg NAA loading dose, respectively.
§ L-arginine at 1, 10, 20, or 50 mg/kg/h preceded by a 1, 10, 200, or 200 mg/kg IV loading dose, respectively.

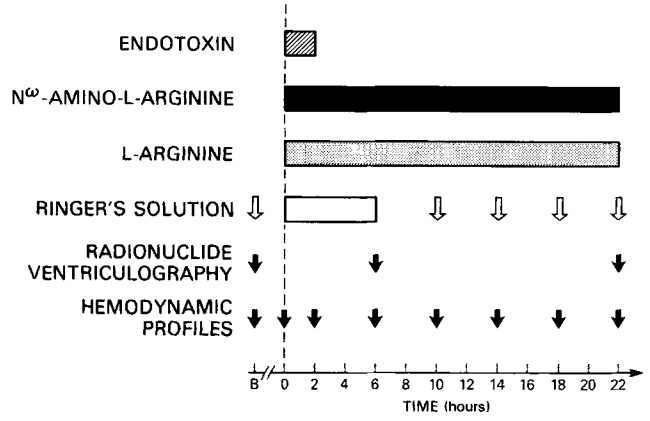

Figure 1. Experimental design. (*Bars*) Duration of infusions of endotoxin, N$^\omega$-amino-L-arginine, L-arginine, and Ringer's solution as indicated. (*Open arrows*) Times when a 30-min intravenous infusion of 40 ml/kg Ringer's solution was administered. (*Solid arrows*) Times when physiologic measurements were performed. Baseline (*B*) measurements were obtained at least 3 d before endotoxin challenge (0 h).

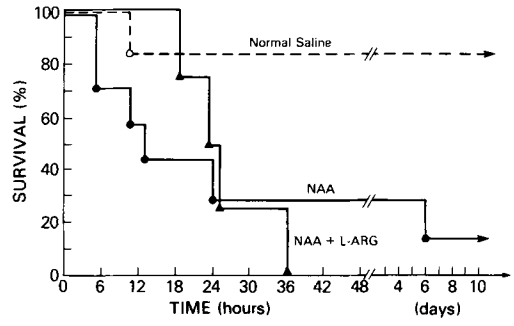

Figure 2. Survival vs. time in endotoxin-challenged canines treated with either normal saline (controls, O—O), N$^\omega$-amino-L-arginine (*NAA*, ●—●), or N$^\omega$-amino-L-arginine plus L-arginine (*NAA + L-ARG*, ▲—▲).

in two animals was stopped at 6 and 14 h because of the onset of muscular hypertonicity, myoclonus, and seizure-like activity. Because of persistent hypertonicity, the animals were euthanized 9 and 10 h, respectively, after discontinuation of N$^{\omega}$-amino-L-arginine. The animal that received N$^{\omega}$-amino-L-arginine at 1 mg/kg/h and those that received only normal saline experienced no untoward sequelae.

Hemodynamic and Blood Gas Analysis. Differences (p values) between groups are presented in this and the following section based upon ANOVA. Means (±SE) and time points at which these differences were significant are shown in Figs. 3, 4, and 5.

Data from the normal animals that received N$^{\omega}$-amino-L-arginine alone (n = 3) were pooled and compared with values from animals that received only normal saline (n = 3). N$^{\omega}$-amino-L-arginine increased SVRI (p = 0.0008) and MAP (p = 0.016) and decreased CI (p = 0.01) and SVI (0.014) compared with normal saline (Fig. 3). Decreases in DO_2I and VO_2I in N$^{\omega}$-amino-L-arginine–treated animals approached, but did not reach, statistical significance (p = 0.07 and 0.08, respectively, data not shown). N$^{\omega}$-amino-L-arginine did not have significant effects on HR, PCWP, or LVEF ($p \geq 0.30$ for each parameter compared with normal saline, data not shown). Blood gas analysis revealed a decrease in arterial bicarbonate concentrations (16.5 ± 1.5 vs. 21.0 ± 0.6 mM/L, p = 0.004) and an increase in arterial lactate (1.90 ± 0.09 vs. 0.33 ± 0.07 mM/liter, p = 0.002) in normal animals treated with N$^{\omega}$-amino-L-arginine compared with normal saline at 22 h. N$^{\omega}$-amino-L-arginine–treated animals also had a lower mixed venous oxygen (P_vO_2) at 2 h compared with those that received normal saline (32 ± 1 vs. 45 ± 3 mm Hg, p = 0.002).

Because there were no significant differences in any hemodynamic variable between groups of animals that received either the low (n = 2) or high dose (n = 4) of endotoxin,

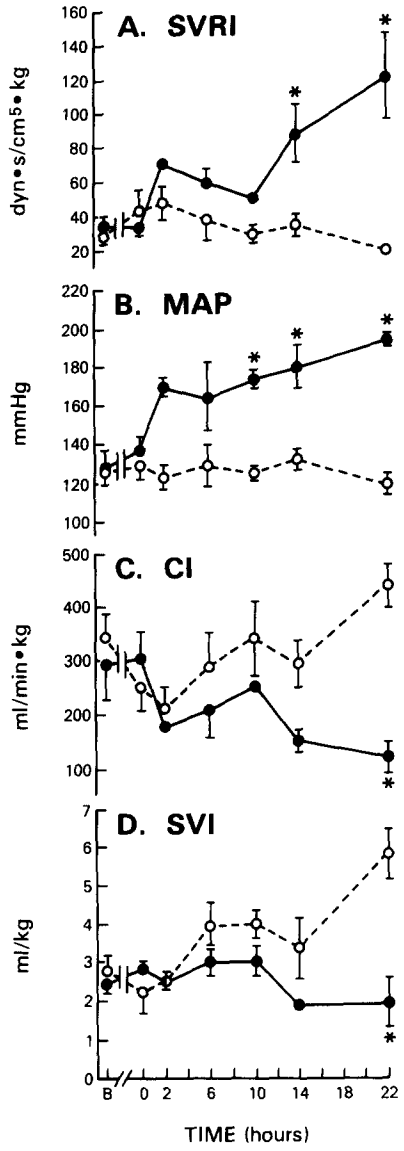

Figure 3. Serial changes (mean ± SE) in (*A*) SVRI; (*B*) MAP; (*C*) CI; and (*D*) SVI in normal dogs given a 22-h infusion of normal saline (○—○) or N$^{\omega}$-amino-L-arginine (●—●). *$p < 0.05$, comparing N$^{\omega}$-amino-L-arginine to normal saline treatment at same time point.

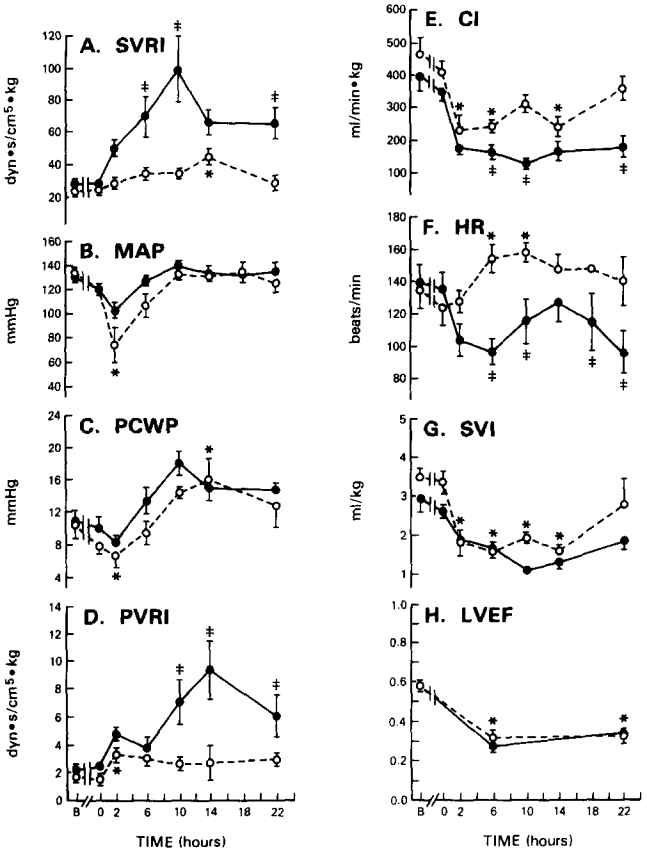

Figure 4. Serial changes (mean ± SE) in (*A*) SVRI; (*B*) MAP; (*C*) PCWP; (*D*) PVRI; (*E*) CI; (*F*) HR; (*G*) SVI; and (*H*) LVEF in dogs challenged with endotoxin (time 0 h) and treated with a 22-h infusion of normal saline (○—○) or N$^{\omega}$-amino-L-arginine (●—●). *$p < 0.05$, comparing the value at the indicated time point to t = 0 h in normal saline-treated canines to examine the effect of endotoxin. ‡$p < 0.05$, comparing N$^{\omega}$-amino-L-arginine to normal saline treatment at the same time point.

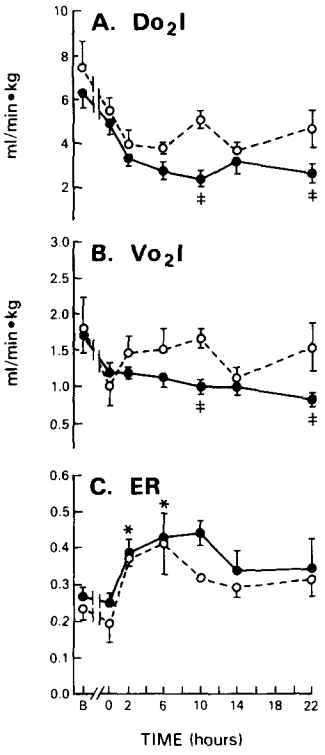

Figure 5. Serial changes in (*A*) DO_2I; (*B*) VO_2I; and (*C*) ER in dogs challenged with endotoxin (time 0 h) and treated with a 22-h infusion of normal saline (○—○) or N$^\omega$-amino-L-arginine (●—●). *$p < 0.05$ comparing the value at the indicated time point to $t = 0$ h in normal saline-treated canines to examine the effect of endotoxin. ‡$p < 0.05$, comparing N$^\omega$-amino-L-arginine to normal saline treatment at same time point.

values from these animals were pooled for analysis ($n = 6$). Compared to values at 0 h, endotoxin infusion decreased MAP, CI, SVI, and LVEF and increased SVRI, PVRI, HR, and PCWP ($p < 0.05$ for each parameter, Fig. 4). Endotoxin challenge also led to significant decreases in arterial pH ($p = 0.0001$), bicarbonate concentrations ($p = 0.0001$), and base excess ($p = 0.0001$), compared to values at 0 h (Table 2). Additionally, there was a significant increase in ER ($p = 0.01$), but no statistically significant effect on DO_2I or VO_2I (Fig. 5).

There were no statistically significant differences in any of the cardiopulmonary parameters measured in endotoxin-challenged dogs that were treated with N$^\omega$-amino-L-arginine at either the low or high dose, with ($n = 4$) or without ($n = 7$) L-arginine. Thus, in the absence of significant differences, data from all animals treated with N$^\omega$-amino-L-arginine were pooled to maximize the ability to detect any N$^\omega$-amino-L-arginine effect. Combined data ($n = 11$) demonstrated that treatment of endotoxin challenged animals with N$^\omega$-amino-L-arginine increased SVRI ($p = 0.008$), PVRI ($p = 0.047$), and arterial lactate levels ($p = 0.046$) and decreased HR ($p = 0.009$), CI ($p = 0.01$), arterial pH ($p = 0.04$), DO_2I ($p = 0.027$), and VO_2I ($p = 0.046$) compared with saline-treated controls (Figs. 4 and 5, Table 2). Differences between N$^\omega$-amino-L-arginine and saline-treated groups for other cardiopulmonary values were not significant.

Laboratory Analysis. Endotoxin infusion resulted in a significant rise in plasma endotoxin levels from undetectable at 0 h to 1330 ± 405 endotoxin units (EU)/ml and 3682 ± 1462 EU/ml at 2 h in the saline and N$^\omega$-amino-L-arginine–treated groups, respectively. Differences between these groups were not significant. Treatment with N$^\omega$-amino-L-arginine either with or without L-arginine had no effect on any laboratory values in endotoxemic animals (data not shown, $p > 0.05$). Blood culture data were available at 0 and 22 h for all but two of the animals studied (one received endotoxin alone and the other endotoxin plus N$^\omega$-amino-L-arginine); none of the 21 animals tested was bacteremic. The endotoxin concentrations of the N$^\omega$-amino-L-arginine preparations were all ≤0.036 EU/ml (≤3.6 pg/ml reference endotoxin). Animals thus received <10 pg/kg/d of reference endotoxin equivalent by way of the N$^\omega$-amino-L-arginine infusion.

Discussion

N$^\omega$-amino-L-arginine unexpectedly increased mortality in this canine model of endotoxic shock. The drug increased SVRI but decreased HR, CI, DO_2I, and VO_2I during en-

Table 2. *Arterial Blood Gas Analysis in Endotoxin-challenged Canines*

	Normal saline			N$^\omega$-amino-L-arginine‖		
Time	0 h	10 h	22 h	0 h	10 h	22
pH_a	7.41 ± 0.02*	7.31 ± 0.01‡	7.22 ± 0.01‡	7.36 ± 0.01	7.29 ± 0.03	7.09 ± 0.04§
P_aCO_2 (mmHg)	32 ± 2	28 ± 2	29 ± 2	34 ± 1	21 ± 2§	37 ± 5
P_aO_2 (mmHg)	115 ± 6	105 ± 5	97 ± 7	103 ± 3	99 ± 7	80 ± 8
P_vO_2 (mmHg)	57 ± 10	44 ± 2	44 ± 0	45 ± 3	38 ± 2	42 ± 4
$_aHCO_3$(mM/L)	19.7 ± 0.7	13.4 ± 1.0‡	12.0 ± 0.8‡	19.1 ± 0.4	9.8 ± 0.9	11.0 ± 0.7
$_a$BE (mM/L)	−3.4 ± 0.5	−10.4 ± 1.0‡	−14.2 ± 0.8‡	−4.8 ± 0.4	−14.0 ± 0.8	−18.2 ± 1.4
$_a$lactate (mM/L)	0.47 ± 0.06	0.74 ± 0.12	0.48 ± 0.06	0.49 ± 0.08	1.3 ± 0.2§	0.52 ± 0.17

* Values shown as mean ± SE.
‡ $p < 0.05$ compared within the same group to values at time 0 h.
§ $p < 0.05$ compared with normal saline-treated group at the same time point.
‖ Pooled data from endotoxin-challenged animals that received N$^\omega$-amino-L-arginine with and without L-arginine.

dotoxemia. Administration of L-arginine, a substrate reported to competitively reverse the effects of NOS inhibitors, including N^{ω}-amino-L-arginine (9, 31, 34), failed to improve survival or to alter the hemodynamic profile of endotoxic shock in animals treated concomitantly with N^{ω}-amino-L-arginine. In normal (nonendotoxemic) dogs, N^{ω}-amino-L-arginine increased SVRI and MAP and decreased CI and SVI. Further, this compound caused a previously unreported toxicity manifested as muscular hypertonicity, myoclonus, and seizure-like activity that was apparent in the two normal animals given 10 mg/kg/h N^{ω}-amino-L-arginine.

The increase in SVRI and decrease in CI associated with N^{ω}-methyl-L-arginine infusion are consistent with previous findings on the use of NOS inhibitors at similar doses in anesthetized animals given endotoxin (9, 21). Notably, N^{ω}-amino-L-arginine, a related NOS inhibitor, has been reported to decrease CI by depressing SVI in both normal and endotoxin-challenged animals under pentobarbital anesthesia (21). However, we observed a difference in the N^{ω}-amino-L-arginine-associated decrease in CI between our normal and endotoxemic dogs. Normal dogs treated with N^{ω}-amino-L-arginine developed a depressed CI because of a decrease in SVI without significant change in HR. In contrast, endotoxemic dogs treated with N^{ω}-amino-L-arginine developed a depressed CI due to a decrease in HR without significant change in SVI. There was no evidence that N^{ω}-amino-L-arginine had a direct effect on myocardial performance (as measured by LVEF) in normal dogs, and it did not alter the fall in LVEF characteristic of endotoxic shock (41). These results are not consistent with a direct role for NO in the pathogenesis of myocardial depression during sepsis (42). Further, the lack of an effect on LVEF would argue against N^{ω}-amino-L-arginine-induced global myocardial ischemia.

Most previous laboratory studies have used NOS inhibitors to treat endotoxin or cytokine-induced shock in anesthetized canines (9, 20, 21). In our study, general anesthesia was not used because these agents have marked effects on cardiovascular function and autonomic reflexes that could mask or augment the effect of either endotoxin or NOS inhibition. Anesthesia itself can produce hypotension, splanic vasoconstriction, dose-dependent cardiac depression, and blunting of normal autonomic reflexes (30). Pentobarbital, the anesthetic used in some previous studies of NOS inhibition (9, 21), is a myocardial depressant that causes an increase in HR and a decrease in SVI (30, 43). In addition, positive pressure ventilation is often used in conjunction with anesthetics and may further alter cardiopulmonary function, thus making data interpretation difficult. Notably, in our study in awake canines, N^{ω}-amino-L-arginine had only modest effects on endotoxin-induced hypotension compared with the results obtained in anesthetized models (9). The role, if any, of nitric oxide in anesthetic-induced hypotension has not been investigated.

L-arginine was not found to improve survival or affect cardiopulmonary parameters in endotoxemic dogs given N^{ω}-amino-L-arginine. It has been demonstrated in vitro that inhibition of NOS by L-arginine analogs may not be reversible under certain conditions (44, 45). Most previous studies using L-arginine in vivo to reverse the effects of NOS inhibitors, in particular N^{ω}-methyl-L-arginine, measured hemodynamic changes immediately after rapid intravenous infusions of NOS inhibitors and L-arginine (9, 19, 20, 36). It seems likely in the present investigation that inhibition of NOS by N^{ω}-amino-L-arginine was not reversed by the continuous administration of L-arginine, based on the lack of a cardiovascular effect. The continuous infusion of N^{ω}-amino-L-arginine in our study may have resulted in irreversible, rather than competitive, inhibition of NOS. This hypothesis is supported by a recent report on the use of these agents to treat human septic shock (23), as the duration of action of 1 mg/kg N^{ω}-methyl-L-arginine appeared to increase after successive intravenous bolus infusions.

The adverse impact of N^{ω}-amino-L-arginine on survival after endotoxin challenge was unexpected and the exact mechanism for this enhanced mortality remains unknown. The continuous infusion of N^{ω}-amino-L-arginine may have raised serum or tissue concentrations of this agent to levels sufficient to cause toxicity. The relatively short-lived hemodynamic effects of NOS inhibitors in animal models (9, 20, 23, 31) have led to the recommendation (22) and use (23, 27) of these agents as continuous infusions. However, as mentioned above, the pharmacologic profile and metabolic fate of these agents have not been thoroughly characterized. Further, endotoxemia may alter the metabolism of NOS inhibitors. N^{ω}-amino-L-arginine alone demonstrated a previously unreported neuromuscular toxicity in normal animals, manifested by an increase in neuromuscular excitability. It is possible that N^{ω}-amino-L-arginine or one of its metabolites is epileptogenic or might have disrupted normal guanidine metabolism, as abnormal serum levels of other guanidine compounds have been linked to seizure activity (46, 47). We cannot determine from this study whether the neuromuscular toxicity of N^{ω}-amino-L-arginine contributed to the increased mortality of endotoxin challenge. N^{ω}-amino-L-arginine at 1 mg/kg/h did not cause obvious toxicity in the one normal animal tested, but did increase the mortality of endotoxemia.

It is notable that N^{ω}-amino-L-arginine did have potentially harmful effects during endotoxemia, as demonstrated by N^{ω}-amino-L-arginine–induced decreases in CI, DO_2I, and VO_2I. It is possible that DO_2I may have become inadequate to meet metabolic demands, leading to the observed fall in VO_2I and an increase in mortality (48). This relationship, however, cannot be confirmed from the present investigation, though it is supported by the higher arterial lactate levels and lower pH measured in the endotoxemic animals treated with N^{ω}-amino-L-arginine. Other investigators have reported that NOS inhibitors worsen endotoxin-induced capillary leak and gastrointestinal damage in rodents, which suggests that NO may be important in maintaining vascular integrity and organ blood flow in sepsis (29). However, we did not observe enhanced endotoxin-induced hepatic damage (reflected by serial measures of liver function tests) in our N^{ω}-amino-L-arginine–treated canines, as has been reported in mice given N^{ω}-methyl-L-arginine (49). This may represent species-related differences, NOS inhibitor differences, or both.

It is important to consider the potentially harmful hemo-

dynamic effects of N^{ω}-amino-L-arginine in the context of the limitations of this particular model of septic shock. The hemodynamic profile of human septic shock, characterized by a high CI and a low SVRI, was not simulated by the endotoxin model used in this study, despite the large volumes of intravenous fluids (200 ml/kg/d) used for resuscitation. It is not clear that an NOS inhibitor-induced increase in SVRI would be harmful in septic shock patients with a high CI and low SVRI. The ability of NOS inhibitors to reverse the catecholamine hyporesponsiveness of the septic vasculature (16, 17, 50) may be useful clinically for the treatment of refractory septic shock, especially in patients who cannot tolerate high doses of vasopressors. However, based upon the results of this investigation, we conclude that prolonged administration of N^{ω}-amino-L-arginine is harmful to normal and endotoxin-challenged canines, and that it should not be used in patients. Clearly, the neurotoxic potential of some NOS inhibitors must be appreciated and more fully studied, ideally in unanesthetized animals. In addition, the pharmacokinetic and pharmacodynamic profiles of NOS inhibitors need to be determined in vivo before the optimal route and timing of administration can be established.

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