Hemodynamic and renal effects of acute and progressive nitric oxide synthesis inhibition in anesthetized dogs

ALEIX CASES, JOHN HAAS, JOHN C. BURNETT, AND JUAN CARLOS ROMERO

Department of Physiology, Mayo Foundation, Rochester, Minnesota 55905

Received 21 March 2000; accepted in final form 23 August 2000

Cases, Aleix, John Haas, John C. Burnett, and Juan Carlos Romero. Hemodynamic and renal effects of acute and progressive nitric oxide synthesis inhibition in anesthetized dogs. Am J Physiol Regulatory Integrative Comp Physiol 280: R143–R148, 2001.—This study evaluated the effects of progressive nitric oxide (NO) inhibition in the regulation of systemic and regional hemodynamics and renal function in anesthetized dogs. The N⁵-nitro-L-arginine methyl ester group (n = 9) received progressive doses of 0.1, 1, 10, and 50 μg·kg⁻¹·min⁻¹. Renal (RBF), mesenteric (MBF), iliac (IBF) blood flows, mean arterial pressure (MAP), pulmonary pressures, cardiac output (CO), and systemic and pulmonary vascular resistances were measured. During N⁵-nitro-L-arginine methyl ester infusion, MAP and systemic vascular resistances increased in a dose-dependent manner. Mean pulmonary pressure and pulmonary vascular resistances increased in both the N⁵-nitro-L-arginine methyl ester and the control group, but the increase was more marked in the N⁵-nitro-L-arginine methyl ester group during the last two infusion periods. CO decreased progressively, before any significant change in blood pressure was noticeable in the N⁵-nitro-L-arginine methyl ester group. IBF decreased significantly from the first N⁵-nitro-L-arginine methyl ester dose, whereas RBF and MBF only decreased significantly during the highest N⁵-nitro-L-arginine methyl ester dose. Urinary volume and sodium excretion only increased significantly in the time control group during the two last time periods. The pulmonary vasculature was more sensitive than the systemic vasculature, whereas skeletal muscle and renal vasculatures showed a greater sensitivity to the inhibition of NO production than the mesenteric vasculature. NO synthesis inhibition induces a progressive antiuretic and antinatriuretic effect, which is partially offset by the increase in blood pressure.

N⁵-nitro-L-arginine methyl ester; regional blood flows; systemic hemodynamics; renal function; urinary sodium excretion

However, changes of renal blood flow (RBF) relative to any other vascular bed and systemic hemodynamics have not been evaluated. Hence, our interest in knowing if the acute inhibition of NO produced in the dog, by progressively increasing doses of N⁵-nitro-L-arginine methyl ester, is followed by a uniform vasoconstriction in different vascular beds or a more selective influence on some specific vasculature (9, 10). In the identification of selective vascular responses to NO synthesis inhibition, it is important to understand which organ blood flow is dependent on NO synthesis. It was anticipated that this experimental approach would help to define if the renal vascular bed exhibits a highly selective sensitivity to NO inhibition, rendering sodium excretion dependent on glomerular filtration rate (GFR), or if NO affects tubular sodium reabsorption independent of GFR and mean arterial pressure (MAP) levels (11).

This study was therefore undertaken to determine if the acute and progressive inhibition of NO synthesis, induced by the consecutive administration of four increasing doses of N⁵-nitro-L-arginine methyl ester (0.1, 1.0, 10.0, and 50.0 μg·kg⁻¹·min⁻¹), produces 1) a uniform or selective increase in vascular resistance to renal, mesenteric, iliac, and pulmonary vascular beds; 2) a change in MAP; and 3) a change in GFR and renal sodium excretion.

MATeRIAL AND METHODS

The protocol was approved by the Institutional Animal Care and Use Committee. Experiments were conducted in 16 anesthetized mongrel dogs (weight 17–21 kg, either sex, on a standard diet) in two groups: 1) vehicle group (n = 7) and 2) N⁵-nitro-L-arginine methyl ester group (n = 9), which were allowed to drink water ad libitum 16 h before the experiment. Dogs were anesthetized with pentobarbital sodium (30 mg/kg iv), intubated, and mechanically ventilated (Harvard Ventilator, Harvard Apparatus, Millis, MA) with room air at a tidal volume that was determined by the nomogram of Kleinman and Radford (15). To maintain anesthesia during the experiment, pentobarbital sodium (15 mg/ml) was added to the saline infusate. Dogs were placed on a heating pad, and warming lights were adjusted to maintain core temperature between 36 and 38°C.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
The right external jugular vein was cannulated with a 7F Swan-Ganz catheter (Criticath, Ohmeda, Oxnard, CA) and advanced into the pulmonary artery for measuring cardiac output (CO) and cardiac filling pressures. The right femoral artery was cannulated to collect peripheral arterial blood samples and for continuous measurement of MAP with the use of a pressure transducer (Pd23 ID, Statham, Hato Rey, PR) connected to a chart recorder (2600, Gould, Cleveland, OH). The femoral vein was cannulated for infusion of vehicle or N^G^-nitro-L-arginine methyl ester (1 ml/min) and 2% inulin solution at a rate of 1 ml/min with additional anesthetic as necessary.

Transonic flow probes (Transonic #3R, Transonic Systems, Ithaca, NY) were placed through a left flank incision on the left renal, superior mesenteric, and left iliac arteries in segments proximal to the aorta. The left ureter was cannulated with PE-200 tubing for urine collection.

After completion of the surgical procedure, dogs were allowed to stabilize for 60 min without intervention. At the end of this period, a 15-min baseline clearance period was obtained. After this baseline period, vehicle or increasing doses of N^G^-nitro-L-arginine methyl ester (0.1, 1, 10, and 50 μg·kg⁻¹·min⁻¹) were sequentially intravenously administered during 45-min periods. During the last 15 min of each period, a clearance period was obtained. During the clearance periods, serum and urine samples were collected to measure electrolytes, inulin concentration, hemodynamic parameters, and CO.

At the end of the stabilization period and at the end of N^G^-nitro-L-arginine methyl ester infusion, blood samples were also obtained for measuring plasma endothelin (ET) and plasma renin activity (PRA). Plasma and urinary sodium were measured by flame photometry (IL943, Instrumentation Laboratory, Lexington, MA). GFR was calculated by measuring the clearance of inulin. Plasma and urine inulin were measured by a colorimetric method (8). Plasma ET and urinary sodium were also obtained for measuring plasma endothelin (ET) and plasma renin activity (PRA). Plasma and urinary sodium were measured by flame photometry (IL943, Instrumentation Laboratory, Lexington, MA). GFR was calculated by measuring the clearance of inulin. Plasma and urine inulin were measured by a colorimetric method (8). Plasma ET and urinary sodium were also obtained for measuring plasma endothelin (ET) and plasma renin activity (PRA). Plasma and urinary sodium were measured by flame photometry (IL943, Instrumentation Laboratory, Lexington, MA).

CO was measured by a thermodilution technique with a CO computer (model 9520A, Edwards Laboratories, Santa Ana, CA) and considered as the average of three measurements. Calculated hemodynamic parameters included: systemic vascular resistances (SVR) = MAP – right arterial pressure/CO. Pulmonary vascular resistances are equal to pulmonary artery pressure minus pulmonary capillary wedge pressure divided by CO.

Animals were euthanized at the end of each experiment without emerging from anesthesia by an intravenous injection of 200 meq potassium chloride.

Statistics. Data are expressed as means ± SE. The analysis of the data included a repeated-measures ANOVA and a Scheffe’s test to assess differences in the means between periods. To assess differences between control dogs and the N^G^-nitro-L-arginine methyl ester group, ANOVA was performed for each dependent variable, using dog, time, and drug terms as the independent variables. The drug effects were then added to the residuals of this ANOVA model, which created a data set adjusted for dog and time. Two-sample comparisons (t-tests with the Bonferroni correction) were then performed on the adjusted data to determine the significance of N^G^-nitro-L-arginine methyl ester. Differences between baseline and the last infusion period in hormonal variables were assessed by means of a paired t-test. A value of P < 0.05 was considered as statistically significant.

RESULTS

As can be seen in Fig. 1, MAP increased in a dose-dependent manner during the infusion of N^G^-nitro-L-arginine methyl ester [from 125.4 ± 3.7 to 127.7 ± 4.1, 130.1 ± 4.3, 135.8 ± 4.2*, and 144.9 ± 4.91* (P < 0.05 vs. baseline)]. However, these increments did not achieve statistical significance with respect to the baseline period or the control group until the doses of 10 and 50 μg·kg⁻¹·min⁻¹ were administered. At this point, the overall increase of MAP was 16% over the basal period. MAP of the control group did not experience any significant change throughout the experiment [from 129.3 ± 1.85 to 132 ± 2.7, 129.4 ± 3.9, 127.4 ± 4.8, and 126.1 ± 4.4 mmHg, P not significant (NS)].

CO also decreased in a progressive dose-dependent manner in the experimental group [from 3.13 ± 0.14 to 2.95 ± 0.16, 2.74 ± 0.16*, 2.33 ± 0.14*, and 1.98 ± 0.15* l/min (P < 0.05 vs. baseline)], which became statistically significant during the infusion of 1 μg·kg⁻¹·min⁻¹, before MAP was significantly in-

![Fig. 1. Response of mean arterial pressure (MAP), cardiac output (CO), and pulmonary arterial pressure (PAP) after progressive doses (0.1, 1.0, 10, 50 μg·kg⁻¹·min⁻¹) of N^G^-nitro-L-arginine methyl ester (L-NAME) and saline in a time control group. #P < 0.05 vs. baseline; and *P < 0.05 vs. control.](http://ajpregu.physiology.org/)

Downloaded from http://ajpregu.physiology.org/ by 10.220.32.246 on August 28, 2017
increased (Fig. 1). The fall in CO was already significant during the infusion of 1 μg·kg⁻¹·min⁻¹ compared with the control group, in which the CO remained unaltered until the last period (period 4) when it decreased by ~10% [from 3.24 ± 0.27 to 3.2 ± 0.28, 3.1 ± 0.23, 3.03 ± 0.23, and 2.92 ± 0.23* l/min (*P < 0.05 vs. baseline)]. SVR of the experimental group changed in a parallel fashion with respect to MAP [from 40.43 ± 1.9 to 43.65 ± 1.9, 47.7 ± 1.97, 58.1 ± 2.38, and 73.93 ± 4.7* dyn·s·cm⁻² (*P < 0.05 vs. baseline)]. The increments in SVR became significant during the last two periods (10 μg and 50 μg·kg⁻¹·min⁻¹) with respect to both the baseline period and the control group. SVR in the control group remained unchanged throughout the experiment (from 41.6 ± 3.7 to 42.9 ± 3.4, 42.8 ± 2.28, 43.03 ± 2.17, and 44.2 ± 2.35 dyn·s·cm⁻²).

In the experimental group, there was a continuous and progressive increase in pulmonary vascular resistance [from 3.23 ± 0.34 to 3.4 ± 0.34, 3.72 ± 0.33, 3.98 ± 0.38*, and 4.2 ± 0.37* dyn·s·cm⁻² (*P < 0.05 vs. baseline)], which was reflected in parallel elevations of pulmonary arterial pressure (PAP) [from 14.6 ± 0.41 to 15.4 ± 0.51, 17.9 ± 0.7*, 20.8 ± 0.98*, and 23.2 ± 0.98* mmHg (*P < 0.05 vs. baseline)] (Fig. 1). Both of these parameters were significantly increased by the 1-μg·kg⁻¹·min⁻¹ dose. Pulmonary vascular resistances increased in the last period by 2.45-fold, which is greater than the increase recorded in SVR (1.82-fold). In the control group, pulmonary vascular resistance [from 3.23 ± 0.34 to 3.4 ± 0.34, 3.72 ± 0.33, 3.98 ± 0.38*, and 4.2 ± 0.37* (*P < 0.05 vs. baseline)] (30%) and pulmonary artery pressure [from 13.2 ± 0.33 to 13.6 ± 0.4, 14.25 ± 0.35*, 14.9 ± 0.23*, and 15.4 ± 0.28* mmHg (*P < 0.05 vs. baseline)] also experienced a mild increase from the second infusion (Δ2.2 mmHg, P < 0.05). The differences in mean pulmonary pressure and pulmonary vascular resistances between the experimental and control groups reached significance during the last two infusion periods.

As it is shown in Fig. 2, in the experimental group, mesenteric blood flow (MBF) [from 203.8 ± 21.1 to 206.1 ± 21.5, 206.8 ± 20.1, 188.1 ± 17.4, and 153.8 ± 16.8* ml/min (*P < 0.05 vs. baseline)] and RBF [from 185 ± 24.5 to 184.3 ± 23.9, 178.3 ± 22.2, 161 ± 17.3, and 145 ± 16.4* ml/min (*P < 0.05 vs. baseline)] both are decreased by approximately the same proportion, 25 and 22%, respectively. These decrements occur only during the highest dose of N⁵-nitro-L-arginine methyl ester infusion. This contrasted with the decrease in iliac blood flow (IBF) [from 105.7 ± 8.7 to 98.29 ± 7.8, 94 ± 8.7*, 81.43 ± 9.8*, and 60.3 ± 7.7* ml/min (*P < 0.05 vs. baseline)], which occurred earlier and was more marked. In the control group, MBF (from 225.4 ± 13.5 to 222.3 ± 13.0, 227.4 ± 13.4, 233.7 ± 12.8, and 232.9 ± 15.6 ml/min) and IBF (142.6 ± 10, 141.4 ± 9.7, 142 ± 12.3, 132.9 ± 10.7, and 130.9 ± 9.9 ml/min) did not significantly change, whereas RBF [from 159.4 ± 6.21 to 158.9 ± 8.35, 177.7 ± 13.4, 187.7 ± 13.4*, and 185.1 ± 13.6* ml/min (*P < 0.05 vs. baseline)] underwent a progressive elevation of 16%, which became statistically significant from period 3. Furthermore, between the experimental and control groups, there were significant differences in IBF from the dose of 1 μg·kg⁻¹·min⁻¹ and in the MBF and RBF from the dose of 10 μg·kg⁻¹·min⁻¹.

As shown in Fig. 3, GFR did not change significantly in either group (N⁵-nitro-L-arginine methyl ester group from 44.45 ± 5.16 to 38.8 ± 5.1 ml/min and control group from 31.14 ± 1.5 to 33.6 ± 2.2 ml/min at the last infusion period, NS). Urine volume (UVol) (from 0.18 ± 0.05 to 0.21 ± 0.05, 0.29 ± 0.07, 0.29 ± 0.07, and 0.25 ± 0.06, NS), urine sodium excretion (UNa) (from 45.93 ± 6 to 55.3 ± 14.5, 69.1 ± 16.1, 62.73 ± 14.5, and 39.9 ± 11.6 μeq/min), and fractional excretion of sodium (FeNa) (1.11 ± 0.37, 1.67 ± 0.39, and 1.03 ± 0.25%, NS) did not exhibit any significant change in the experimental group. These last three parameters showed a marked tendency toward elevation in the control group, which
became significant during the last two periods for UNa [from 38.12 ± 9.1, 37.58 ± 9.22, 48.25 ± 13.42, 60.47 ± 11.35*, and 70.11 ± 12.7* μeq/min (*P < 0.05 vs. baseline)] and FeNa [from 1.06 ± 0.23 to 1.07 ± 0.24, 1.19 ± 0.23, 1.57 ± 0.29*, and 1.85 ± 0.32% (*P < 0.05 vs. baseline)] and during the last period for UVol [from 0.17 ± 0.03 to 0.17 ± 0.03, 0.19 ± 0.03, 0.25 ± 0.05, and 0.31 ± 0.06* ml/min (*P < 0.05 vs. baseline)]. The differences between the two groups in these three parameters became significant during the last infusion period.

PRA decreased in the N^G-nitro-l-arginine methyl ester group (from 3.01 ± 0.99 to 1.84 ± 0.77 ng·ml⁻¹·h⁻¹, P < 0.05), but it did not change in the vehicle group (from 5.05 ± 0.81 to 4.48 ± 0.87 ng·ml⁻¹·h⁻¹, NS). Plasma ET levels increased after N^G-nitro-l-arginine methyl ester infusion (from 19.35 ± 0.75 to 25.4 ± 1.4 μg/ml, P < 0.05), but not in the control group (from 22.1 ± 2.9 to 23.1 ± 2.9, NS).

**DISCUSSION**

**Changes in systemic and pulmonary circulation.** This study shows that basal release of NO regulates blood pressure and modulates the vasculature of systemic and pulmonary circulation. In systemic circulation, however, the sensitivity of different vascular beds in response to NO synthesis inhibition is not homogeneous. Such findings agree to that reported by many other investigators (9, 10, 25, 33, 34, 38). The IBF exhibited dependency on the synthesis of NO, which was greater than that exhibited by other vascular beds. Similar results have been observed by some authors (9, 10), but not by others (33, 38). In fact, the iliac vasculature developed an early vasoconstriction at doses of N^G-nitro-l-arginine methyl ester (1 μg·kg⁻¹·min⁻¹) that was not seen in the renal or mesenteric vascular beds. At maximal doses of N^G-nitro-l-arginine methyl ester (50 μg·kg⁻¹·min⁻¹), the reduction of IBF was almost twofold higher than that produced in RBF and MBF. This effect is important, because the role of NO in the control of skeletal muscle blood flow has been linked to exercise performance (29) and to the amount of glucose uptake that, when decreased, could lead to situations comparable to those observed during insulin resistance (1). It should be pointed out that the mesenteric vasculature was the least dependent on changes in NO synthesis, because it experienced a 25% decrease only at the highest doses of N^G-nitro-l-arginine methyl ester. Renal vasculature exhibited an intermediate response between the iliac and the mesenteric, considering that RBF increased by 16% in time control animals.

Our results also showed that inhibition of NO synthesis has a major impact on pulmonary circulation, which is an observation previously reported by others (7, 14, 23, 38). In our study, the increase in pulmonary vascular resistance (+146%) and in PAP (+59%) was greater compared with the changes observed in SVR (+83%) and systemic blood pressure (+19.5%), at a dose of 50 μg·kg⁻¹·min⁻¹. Previous studies reported similar results (7, 38), but this is not a universal finding (23).

**Changes in renal hemodynamics and sodium excretion.** In previous studies, intrarenal infusion of NO synthesis inhibitors induced potent renal vasoconstriction and antinatriuresis (19). However, Baylis et al. (2) showed that inhibition of NO synthesis causes a dose-dependent increase in arterial blood pressure and sodium excretion in both conscious and anesthetized rats. In the experimental group of animals, RBF did not decrease significantly until the last period. However, these decrements in blood flow became more apparent if one compares it with the steady increments observed in the control group, which became significant from the third period. This renal vasoconstriction was not accompanied by any change in GFR, thus suggesting that the increase in resistance was taking place simultaneously in glomerular afferent and efferent arterioles.

**Fig. 3.** Response of glomerular filtration rate (GFR), urinary volume, and urinary sodium excretion after progressive doses (0.1, 1.0, 10, 50 μg·kg⁻¹·min⁻¹) of L-NAME and saline in a time control group. *P < 0.05 vs. baseline; and #P < 0.05 vs. control.
Nitro-L-arginine methyl ester induced a dose-dependent mild volume expansion reflected in an increase in RBF, inhibition, but its role in the renal effects in this situation is controversial (6, 22, 24, 37).

Recent studies in rats indicate that ET is partially involved in the hemodynamic changes during NO inhibition. Schnackenberg et al. (30) recently demonstrated, in an elegant experiment in the N\textsubscript{G}-nitro-L-arginine methyl ester that range from 0.1 to 50 mg·kg\textsuperscript{-1}·min\textsuperscript{-1} produces a marked elevation of pulmonary vascular resistance and pulmonary blood pressure, which is almost doubled from that observed in SVR. Systemic vascular territories, on the other hand, do not respond in a uniform manner. The iliac vascular bed is by far the most sensitive, whereas the vascular territories supplied by the mesenteric artery do not seem to be significantly affected. Renal vascular territory exhibits an intermediate response.

In summary, this study shows that in anesthetized dogs, the progressive inhibition of NO synthesis with doses of N\textsubscript{G}-nitro-L-arginine methyl ester correlated with the CO. Finally, inhibition of NO produces a decrease in RBF with no changes in GFR. Under these conditions, pressure natriuresis is not manifested because of the opposing effects of NO inhibition on sodium excretion.

The authors thank Rod Bolterman and Denise Heublein for skillful technical assistance and Kristy Zodrow for skillful secretarial assistance. This work was supported by contract no. DAMD 17–93-C-3116P3 of the United States Army and by National Institutes of Health assistance.

REFERENCES


