Noradrenaline and $N^{\omega}$-monomethyl-L-arginine (L-NMMA): effects on haemodynamics and regional blood flow in healthy and septic sheep

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ABSTRACT

This prospective, non-randomized, controlled experimental study looks at the effects of $N^{\omega}$-monomethyl-L-arginine (L-NMMA) on haemodynamics, oxygen transport and regional blood flow in healthy and septic sheep, and compares these effects with those of noradrenaline (NA; norepinephrine). All sheep were chronically instrumented. Six sheep received L-NMMA (7 mg [kg$^{-1}$ [h$^{-1}$]), six sheep received NA, and seven sheep received the carrier alone (0.9% NaCl). The NA dosage was continuously and individually adjusted to achieve the same increase in blood pressure as observed in matched sheep of the L-NMMA group (non-septic phase). Treatment was discontinued after 3 h. Sepsis was initiated and maintained by a continuous infusion of live Pseudomonas aeruginosa. After 24 h of sepsis, the sheep were again challenged over a treatment period of 3 h with their previously assigned drug (septic phase). During the non-septic phase of the experiment, NA and L-NMMA both caused an increase in mean arterial pressure (MAP) through vasoconstriction. After 24 h of sepsis, all sheep developed a hyperdynamic circulatory state. While L-NMMA caused an increase in MAP through intense vasoconstriction, NA caused MAP to increase through a further elevation of the cardiac index. The NA dosage needed was significantly higher in the septic phase compared with the non-septic phase, reflecting a reduced vascular responsiveness to catecholamines during sepsis. Renal blood flow remained unchanged during either treatment in both the non-septic and the septic phases. Nevertheless, urine output increased during NA treatment in both the non-septic and the septic phases, while L-NMMA caused urine output to increase only under septic conditions.

INTRODUCTION

Despite modern intensive care medicine, the mortality caused by sepsis remains unchanged, and a majority of patients die during the course of their illness [1]. Although some patients die because of refractory hypotension, others die after successful initial treatment of this hypotension because of the development of multi-organ failure. The pathogenesis of multi-organ failure is not completely understood. Most probably, multi-organ failure is the result of reperfusion injury or irreversible damage sustained either during the period of hypotension [2,3] or during the period of treatment, especially when vasoconstrictive compounds are used to restore systemic blood pressure. Adverse effects of vasoconstrictors on tissue perfusion have frequently been suggested to cause...

Key words: methylinarginine, nitric oxide, noradrenaline, oxygen transport, regional blood flow, sepsis.

Abbreviations: CI, cardiac index; $D_{O_{2}}$, oxygen delivery; MAP, mean arterial pressure; NA, noradrenaline (norepinephrine); L-NMMA, $N^{\omega}$-monomethyl-L-arginine; NOS, nitric oxide synthase; SVRI, systemic vascular resistance index; $V_{O_{2}}$, oxygen consumption.

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organ damage [4,5]. While this certainly is a matter of dosing, it may also depend on the haemodynamic condition at the time when vasoconstrictive treatment is initiated [6].

In previous studies, we have focused on the effects of noradrenaline (NA; norepinephrine) and inhibition of nitric oxide synthase (NOS) on global [7] and regional [8] haemodynamics in sheep with sepsis. However, the effects may be different in non-septic, healthy sheep. In the case of NA, the $\alpha$-receptors are not down-regulated under non-septic conditions; in the case of NOS inhibition, the inducible isoform of NOS may not be up-regulated. Therefore results obtained under healthy conditions may not be applicable under septic conditions, and vice versa [9,10]. However, the effects of these vasoconstrictors under both conditions need to be known, since the patient’s condition may vary between these two states.

In the present study, we have focused on the vasoconstrictive properties of $N^\omega$-monomethyl-$l$-arginine ($l$-NMMA), a competitive inhibitor of NOS, and NA, a mainly $\alpha$-agonistic catecholamine, under healthy and septic conditions. We used an established model of ovine sepsis which mimics the hyperdynamic circulation typically found in septic patients [11,12]. Besides global haemodynamics, we were interested in examining regional blood flows to determine whether organ dysfunction may be caused by tissue hypoperfusion during the vasoconstrictive treatment.

**METHODS**

The experiments adhered to National Institutes of Health guidelines on the use of experimental animals. Approval of the Animal Use Committee of the University of Texas Medical Branch at Galveston was obtained before the experiments began.

In this study, 19 female range ewes of the Merino breed were instrumented for chronic study. After a 24-h fasting period, the animals were anaesthetized with halothane (2.5–3.5 % in oxygen) through an animal anaesthesia mask, until the depth of anesthesia allowed endotracheal intubation to be performed (tubing internal diam. 10 mm; Mallinckrodt, Glen Falls, NY, U.S.A.). The sheep were then mechanically ventilated with 1.5–2.5 % halothane in oxygen. The respiratory frequency was adjusted to maintain arterial $CO_2$ levels within the normal range; the tidal volume was fixed at 12 ml/kg. Under sterile conditions, a femoral arterial catheter and a venous catheter were positioned, and a Swan–Ganz catheter (model 93A-131-7F; American Edwards Laboratories, Irvine, CA, U.S.A.) was positioned through the jugular vein into the pulmonary artery. Through a left-sided thoracotomy (fifth intercostal space), a Silastic catheter was placed into the left atrium. Ultrasonic flow probes (Transonic System Inc., Ithaca, NY, U.S.A.) were positioned on the carotid, left renal and superior mesenteric arteries, and the infrarenal aorta. The abdominal flow probes were fixed to the psoas muscle, preventing rotation and thus ensuring good readings in a standing animal. After closure of all wounds, the animals were weaned from mechanical ventilation, allowed to awaken and allowed to recover for at least 5 days.

When the animals showed no signs of post-operative inflammation, such as elevated temperature or white blood cell count, the catheters were connected to pressure transducers (Statham Gould P23 ID, Oxnard, CA, U.S.A.) and a physiological recorder (Honeywell OMJ9; Electronics for Medicine, Pleasantville, NY, U.S.A.). A cardiac output computer (Model 9529; American Edwards Laboratory) served for cardiac output measurements by the thermodilution technique. Regional blood flows were measured with the ultrasonic flow probes and a flow meter (Model T 101; Transonic Systems Inc.). Mixed-venous and arterial blood gases were analysed for each measurement of cardiac output (model 1302 pH/blood gas analyser and model 282 CO-Oximeter; Instrumentation Laboratory, Lexington, MA, U.S.A.).

Values for systemic vascular resistance index (SVRI), oxygen delivery ($DO_2$), oxygen consumption ($VO_2$) and oxygen extraction were calculated using standard formulae.

**Experimental protocol**

The awake sheep were held and studied in metabolic cages with free access to food and water. A continuous intravenous maintenance infusion of Ringer’s lactate (2 ml·kg$^{-1}$·h$^{-1}$) was begun 24 h before starting the experiment.

**Non-septic phase**

After baseline measurements of the non-septic phase had been taken, the sheep were assigned to one of the following three groups. (1) $l$-NMMA: sheep ($n = 6$) received a continuous infusion of $l$-NMMA (7 mg·kg$^{-1}$·h$^{-1}$) for 3 h. (2) NA: sheep ($n = 6$) received a continuous infusion of NA over 3 h. The dosage was adjusted continuously to achieve the same increase in mean arterial pressure (MAP) as observed in a corresponding sheep that received $l$-NMMA. NA was administered by means of a Harvard pump at a concentration of 0.1 mg/ml. (3) Control: sheep ($n = 7$) received the carrier alone (0.9 % NaCl). During the non-septic phase, haemodynamic measurements, regional blood flows, and blood gases were taken every 1 h.

**Septic phase**

After 2 days of recovery, the animals entered the septic phase of the protocol. After baseline measurements of the septic phase had been taken, live *Pseudomonas aeruginosa*
Noradrenaline and $N^\omega$-monomethyl-$L$-arginine in sheep

Figure 1 Haemodynamic effects of the NOS inhibitor L-NMMA and the catecholamine NA in healthy sheep (left panels) and septic sheep (right panels)

While the effects obtained during L-NMMA treatment were comparable between healthy and septic sheep, NA ($=NE$) caused a vasoconstriction only in the non-septic phase of the experiment. Significance of differences: * $P < 0.05$ compared with baseline. PVRI, peripheral vascular resistance index.

(2.5 × 10^9 colony-forming units·kg$^{-1}$·min$^{-1}$) was infused continuously through the femoral venous catheter. This infusion was maintained for the remainder of the experiment. Throughout the entire experiment, the left atrial pressure was kept at baseline level ± 3 mmHg by adjusting the amount of infused Ringer’s lactate solution. Haemodynamic readings were taken every 4 h. After 24 h of bacterial infusion, the animals again received their previously assigned treatment, as in the non-septic phase. The NA dose again was adjusted continuously to achieve the same increase in MAP as observed in a corresponding septic sheep that received L-NMMA. During the 3 h...
treatment period, haemodynamics, regional blood flows and blood gases were obtained every 1 h.

**Statistics**

For statistical analysis, analysis of variance with post hoc Scheffé F-test was used (Statview® II, Version 1.04; Abacus Concepts Inc., Berkeley, CA, U.S.A.). Significance was defined as $P \leq 0.05$. Data are expressed as means ± S.E.M.

**RESULTS**

In the non-septic phase of this experiment, both L-NMMA and NA caused significant systemic vasoconstriction, as shown by the increase in the SVRI (Figure 1). This vasoconstriction was accompanied by a significant decrease in the cardiac index (CI). $V_{O_2}$ remained unchanged, although a significant decrease in $D_{O_2}$ was seen in the L-NMMA group (Table 1). In the pulmonary circulation, only L-NMMA caused a vasoconstriction, as reflected by an increase in the pulmonary vascular resistance index. No changes were found in the control group.

The increase in MAP was achieved using a constant dose of L-NMMA in the L-NMMA group, and using an individually adjusted dose of NA in the NA group. As can be seen from Figure 2, the dose of NA needed to keep the MAP elevated for the 3-h study period of the non-septic phase was constant.

The reduction in overall blood flow due to L-NMMA administration caused a parallel decrease in blood flow in the carotid artery and in the infrarenal aorta (Figure 3). Blood flow to the gut and kidneys remained unchanged in all three groups.

![Figure 2](image)

**Figure 2** Dose of NA needed over a 3-h period to achieve the same increase in MAP as obtained by the administration of L-NMMA in matched sheep

The dose needed was significantly higher during sepsis. Further, the dose had to be elevated during the course of sepsis, while a constant dose was sufficient in healthy sheep. Significance of differences: *$P < 0.05$ compared with non-septic; †$P < 0.05$ compared with 1 h time point.

After 2 days of recovery, the septic phase was initiated and then maintained by a continuous infusion of live *Pseudomonas aeruginosa* (2.5 x 10⁶ colony-forming units·kg⁻¹·min⁻¹). During the first 24 h of sepsis, all sheep developed a hyperdynamic hypotensive circulation, characterized by an increase in CI, a reduction in MAP and a reduction in SVRI. After 24 h of sepsis, the sheep were again challenged with their initial treatment. L-NMMA caused the similar haemodynamic alterations as in the previous non-septic phase: a significant decrease in CI and a significant increase in MAP, SVRI and the pulmonary vascular resistance index (see Figure 1). However, in contrast with the non-septic phase, NA did

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<th>Table 1 Parameters of oxygen transport and urine production in the non-septic and septic phases of the experiment</th>
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Figure 3 Effects of the NOS inhibitor L-NMMA and the catecholamine NA on regional perfusion in healthy sheep (left panels) and septic sheep (right panels)

Blood flow (BF) was measured using ultrasonic flow probes. Treatment with L-NMMA caused a reduction in carotid and aortic blood flow in both the non-septic and septic phases. NA (NE) had no significant effect on blood flow during the non-septic phase. However, during sepsis, the increase in CI was paralleled by a significant increase in aortic blood flow. SMA, superior mesenteric artery. Significance of differences: *P < 0.05 compared with baseline.

not cause vasoconstriction in the septic phase. Instead, the increase in MAP was related to a significant increase in CI, while SVRI remained unchanged.

As in the non-septic phase, regional blood flows were not altered by NA or saline (control). L-NMMA caused a significant reduction in carotid and infrarenal aortic blood flow during the septic phase, a phenomenon that had also be seen in the non-septic phase (Figure 3).

Urine production was significantly elevated by NA in both the non-septic and the septic phases (Table 1).
NMMA, however, caused urine production to increase in the septic phase only, while urine output remained unchanged in the non-septic phase. In the control group there was no change in urine production.

DISCUSSION

Both NA and L-NMMA increased MAP in the non-septic and septic phases of the experiment. However, the dose of NA needed to achieve the desired increase in MAP was significantly higher in the septic phase (see Figure 2). Further, to keep the blood pressure elevated, the NA dose had to be increased during the 3-h period of sepsis, while this was not the case during the non-septic period. In a previous investigation, we found fading vasoconstrictive properties when NA was given continuously during a prolonged course of sepsis (48 h) [7]. These results are in accordance with in vitro experiments using isolated vessel rings harvested from healthy and septic sheep [13]. The mechanisms of the underlying down-regulation are not completely understood, but NO seems to play a dominating role: blocking NO in endotoxaemic sheep restored the vascular responsiveness to catecholamines immediately [14,15]. Due to the down-regulation of α-receptors during sepsis, the vasoconstrictive properties of NA seen in the non-septic phase could not be reproduced under septic conditions (see Figure 1). Although NA caused an increase in SVRI in the non-septic phase, SVRI remained unchanged during sepsis. In the septic phase, the increase in MAP was mainly achieved by an increase in CI, a phenomenon not seen in the non-septic phase.

L-NMMA caused a comparable vasoconstriction in both parts of the experiment, although it works through the blockade of two different enzymes. In the non-septic phase, L-NMMA blocks the constitutive isoform of NOS. During sepsis, however, inducible NOS is up-regulated. L-NMMA thus causes vasoconstriction in the septic phase through blocking this inducible NOS. The constitutive isoform is down-regulated during sepsis [16–19], and therefore cannot be blocked by L-NMMA. Although the down-regulation of the constitutive isoform of NOS has been shown by several investigators in vitro, no in vivo data are available. Thus, although unlikely, we cannot rule out the possibility that, even after prolonged ovine sepsis, constitutive NOS is still active and may have been blocked by L-NMMA, rather than being down-regulated during the course of sepsis. During the septic phase, treatment with L-NMMA resulted in a restoration of MAP and SVRI to pre-septic levels (Figure 1). However, the values for MAP and SVRI were lower than those observed after L-NMMA treatment in the non-septic phase. This finding suggests that first, NO plays a major role in septic vasodilation and, secondly, other vasodilators must contribute to septic vasodilation. We found in the same model of hyperdynamic sepsis that atrial natriuretic peptide is a potent vasodilator in sepsis [20]. Also, vascular hyporesponsiveness not only to catecholamines, as shown in the present study, but also to other vasoconstrictors (such as endothelin) should be noted in this regard [21].

Despite the fact that the constitutive NOS isoform is down-regulated during sepsis, no reduced vasoconstrictive efficacy of L-NMMA could be observed in the septic phase. This is related to the up-regulation of the inducible NOS. Further, the efficacy of L-NMMA remained constant over time. Compared with NA, the vasoconstrictive properties of which are hard to predict during sepsis, especially when given over a prolonged period of time, L-NMMA seems to be the more reliable and predictable vasoconstrictor.

Although L-NMMA caused intense vasoconstriction with a concomitant reduction in $D_O_{2p}$, $V_O_{2p}$ remained unchanged due to an increase in oxygen extraction. This increase in oxygen extraction may be related to a redistribution of blood flow to metabolically active tissue, away from metabolically less active tissue [11,22]. However, during L-NMMA administration, $D_O_{2p}$ never fell below baseline. This certainly is different from other models of sepsis, where large quantities of endotoxin or bacteria are used to induce a hypodynamic state of septic shock [6]. Under these conditions, typically characterized by a low CI and an elevated SVRI, the administration of a vasoconstrictor to increase MAP is not indicated, and may be detrimental [4,5]. Instead, vasodilators and inotropes are indicated under such haemodynamic conditions.

In contrast with L-NMMA, NA caused an increase in oxygen extraction only in the non-septic phase of the experiment. During sepsis, however, $V_O_{2p}$ also remained unchanged, due to an increase in $D_O_{2p}$. A further increase in systemic blood flow during NA treatment in the septic phase, leading to a subsequent increase in $D_O_{2p}$, did not enhance $V_O_{2p}$. Obviously, during hyperdynamic sepsis oxygen transport is sufficient, as suggested previously by us [23]. This is also in accordance with a study on supranormal oxygen delivery in septic patients that showed no beneficial effect on mortality [24].

Although global hypoxia of the organism can be excluded by the measurement of $D_O_{2p}$ and $V_O_{2p}$, this does not necessarily hold true for possibly malperfused regions or certain organs. Therefore we also examined regional blood flow using ultrasonic flow probes. During treatment with L-NMMA, blood flow in the carotid artery decreased significantly. This fall was comparable in both the non-septic and septic phases, and paralleled the drop in CI. The increase in CI during NA treatment in the septic phase was paralleled by an increase in infrarenal aortic blood flow, while perfusion to the brain, gut and kidney remained unchanged (Figure 3). Even treatment with L-NMMA did not cause a reduction in
gut perfusion in the septic phase, despite a significant fall in CI. Obviously, vasoconstriction through NOS blockade causes a redistribution of blood flow to the gut, keeping VO₂ constant despite a decrease in DO₂. Further, a redistribution of blood flow within the gut may have occurred [25]; however, this could not be measured with ultrasonic flow probes in the present study. The usefulness of vasoconstrictors has aroused controversy, since their effects on tissue perfusion have been suggested to cause organ damage, leading to (multi-)organ failure [4,5]. Our data do not support such theories.

Kidney perfusion was not significantly affected by either L-NMMA or NA. At the same time, urine output increased in both treatment groups during the septic phase. The increase in MAP, with a subsequent increase in renal perfusion pressure, is probably responsible for this phenomenon. During the non-septic phase, however, L-NMMA caused no increase in urine output, despite an elevation in MAP. This is in contrast with NA, which caused a significant increase in urine output even in the non-septic phase. The difference between these two agents is surprising, since they caused comparable increases in MAP combined with comparable decreases in CI. Perhaps the difference is related to a non-significant decrease in renal blood flow under L-NMMA treatment (see Figure 3), which may be related to the extreme sensitivity of the renal microvasculature to NO [26]. Millar and Thiemermann [28] suggested that NO produced extensively during sepsis has a direct toxic renal effect. L-NMMA could then cause improved renal function during sepsis by preventing NO-related renal toxicity, but has no renal effect during the non-septic phase.

In conclusion, L-NMMA and NA were both capable of increasing blood pressure in the non-septic phase and in the septic phase. However, during sepsis, higher NA doses were required to achieve the desired increase in blood pressure when compared with the non-septic phase. Further, the haemodynamic effect of NA ‘faded’ during the 3 h treatment period in the septic phase, leading to increasing NA doses. This is not necessarily a downside of NA. However, septic individuals treated with NA should be continuously monitored, and the NA dose should be carefully and frequently adjusted to the individual needs of the septic subject. In contrast with NA, the vasoconstrictive efficacy of L-NMMA was not affected by sepsis, and nor did it fade away during sepsis. The haemodynamic effects of L-NMMA thus were more predictable than those of NA. In addition, neither compound caused tissue hypoperfusion. Even the necessary increase of the administered NA dose did not cause adverse vasoconstriction in this model of hyperdynamic sepsis. Therefore NA, which is clinically available, should be considered as a suitable alternative to adrenaline for increasing blood pressure in septic individuals [29–31]. Inhibitors of NO are not currently available for clinical use. More studies are needed to investigate the safety profile of these compounds during hyperdynamic sepsis.

REFERENCES


Received 20 April 1999; 2 September 1999; accepted 27 October 1999