Comparative effects of vasopressin, norepinephrine, and L-canavanine, a selective inhibitor of inducible nitric oxide synthase, in endotoxic shock

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Levy, Bruno, Chantal Vallée, François Lauzier, Gérard E. Plante, Arnaud Mansart, Jean-Pierre Maille, and Olivier Lesur. Comparative effects of vasopressin, norepinephrine, and L-canavanine, a selective inhibitor of inducible nitric oxide synthase, in endotoxic shock. Am J Physiol Heart Circ Physiol 287: H209–H215, 2004. First published February 26, 2004; 10.1152/ajpheart.00946.2003.—Norepinephrine (NE), a standard of care, AVP, an alternative candidate, and L-canavanine (LC), a selective inhibitor of inducible nitric oxide synthase, were compared for efficacy and innocuousness on global and regional hemodynamics, plasmatic and tissue lactate-to-pyruvate ratio (L/P), tissue high-energy phosphates, renal function, and tissue capillary permeability in a rat model of endotoxic normokinetic shock. Mean arterial pressure (MAP) decreased (~35%) but aortic blood flow increased during endotoxin infusion (P < 0.05 vs. control). Additionally, there was a decrease in mesenteric MBF and renal (RBF) blood flows along with regional-to-systemic ratio (P < 0.05 vs. control). All tested drugs restored MAP to basal levels but slightly decreased abdominal aortic flow; however, RBF and MBF remained unchanged. Endotoxin significantly decreased diuresis and inulin clearance (~3- to 4-fold), whereas AVP or LC attenuated this drop (P < 0.05 vs. control). In contrast, NE did not improve endotoxin-induced renal dysfunction. Endotoxin induced gut and lung hyperpermeability (P < 0.05 vs. control). Endotoxin-induced gut hyperpermeability was inhibited by AVP, LC, and NE. Endotoxin-induced lung hyperpermeability was further worsened by NE (~2-fold increase) but not AVP infusion (P < 0.05 vs. endotoxin). LC significantly improved endotoxin-induced pulmonary hyperpermeability. Endotoxin increased renal lactate and decreased renal ATP. NE did not change renal lactate or renal ATP. AVP and LC decreased renal lactate and normalized renal ATP. Finally, endotoxin was associated with increased lactate levels and L/P (~2- and 1.5-fold increases vs. control, respectively), whereas AVP and LC, but not NE, normalized both parameters after endotoxin challenge. These results suggest that, in a short-term endotoxic shock model, AVP improves systemic hemodynamics without side effects and has particular beneficial effects on renal function.

The human response to septic challenge usually includes increased cardiac output, decreased peripheral resistance, low arterial pressure, and increased vascular permeability. AVP, also known as “the” antidiuretic hormone (ADH), is an emerging alternative candidate for management of redistributive shock (7). Indeed, AVP exhibits strong vasoconstrictor effects through stimulation of the V1 receptor leading to arterial pressure improvement (28). Moreover, low concentrations of exogenous AVP mediate vasodilatation in coronary, cerebral, and pulmonary arterial circulation (20). The recognized effects of AVP on renal function are complex and include association of an antidiuretic effect through stimulation of V2 receptors, an increase of diuresis/natriuresis through V1 receptors by effe-
t arteriolar vasoconstriction, and oxytocin receptor-mediated stimulation (23). In severe sepsis as well as in septic shock, low doses of exogenously infused AVP have been reported to induce diuresis (21). The effects of AVP on intestinal blood flow and gut mucosa integrity remain largely unknown. On the other hand, norepinephrine (NE) is currently used to increase arterial pressure after volume loading and is generally considered the standard of care in septic shock (3). Nevertheless, high doses of NE have been reported to increase tissue oxygen demand, to decrease renal and mesenteric blood flow, and to enhance pulmonary vascular resistances (24). Furthermore, incremental doses of NE infusion can sometimes be ineffective in maintaining arterial pressure in septic shock. A rational, but still experimental, approach to increasing mean arterial pressure (MAP) while preserving cardiac output is to selectively inhibit inducible nitric oxide (NO) synthase (iNOS) (14). In sepsis patients, pulmonary capillary permeability for water and protein is increased early. In a number of cases, this results in Acute Respiratory Distress Syndrome (ARDS). The comparative effects of a vasoconstrictor on vascular permeability in endotoxic shock have not been studied previously. In light of this knowledge, we conducted an experimental study to compare the effects of AVP with those of NE and L-canavanine (LC), a selective inhibitor of iNOS, with an endotoxic shock model. The efficacy and innocuousness of these drugs in the above context were evaluated on 1) global and regional hemodynamics, 2) systemic and tissue lactate and pyruvate levels, 3) tissue high-energy phosphates, 4) renal function, 5) tissue capillary permeability, and 6) gut mucosal injury.

Materials and Methods

Experiments were conducted in adherence to the “Guiding Principles for Research Involving Animals and Human Beings” of the American Physiological Society.

Animal Preparation and Monitoring

Wistar rats (300–320 g) fasted overnight were anesthetized with ketamine-xylazine (50 and 20 mg/kg, respectively). A tracheostomy was performed, and the animals were mechanically ventilated. 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.
right jugular vein and carotid artery were cannulated. Rectal temperature was maintained at 37°C. Abdominal aortic blood flow (transducer placed just distal to the diaphragm) along with renal (RBF) and mesenteric (MBF) blood flows were measured with perivascular probes and a small-animal flowmeter (T206, Transonic Systems, Ithaca, NY).

Experimental Protocol

Five groups of 10 animals each were set up. Four groups were infused intravenously with endotoxin as the experimental challenge and were subsequently randomized to receive a continuous infusion of saline alone (5 ml/h; Endotoxin-S group), AVP (20 U/ml; Ferring, Toronto, ON, Canada; Endotoxin-AVP group), NE (NE bitartrates, Sabex, Boucherville, QC, Canada; Endotoxin-NE group), or LC (Sigma, St. Constant, QC, Canada; Endotoxin-LC group) titrated to maintain MAP at baseline values (±10%) from 90 (T90) to 180 (T180) min. The same amount of fluid was continuously infused in all rats, i.e., 5 ml/h. The assignment of treatment was randomized, and the investigators were blinded to the treatment. The fifth group was the Control group and was designed to receive a similar amount of fluid infusion (±10%). The adequacy of vascular loading was assessed by respiratory changes in pulse pressure every 10 min in all groups. Systolic and diastolic arterial pressures were measured on a beat-to-beat basis, and pulse pressure (Pp) was calculated as the difference between systolic and diastolic pressure. Maximal and minimal values ratio 0.45; 5 ml/20 min) during the endotoxin infusion, followed by rats also received hydroxypentastarch (mol wt 260, molar substitution min. The same amount of

injected with 20 ml/kg of a 25 mg/ml solution of Evans blue dye (EB) reader (International Equipment, Needham, MA).

Determination of Evans blue dye extravasation.

The mucosal structure of villi from both

Morphological analysis. The mucosal structure of villi from both the duodenum and the terminal ileum was analyzed by light microscopy as follows. After death, a segment of intestine and ileum was immediately fixed in 10% buffered formalin. After processing was completed, semithin (2–4 μm) sections were cut and stained with 1% toluidine blue. The degree of mucosal damage was graded in a blinded fashion with the grading system of Chiu et al. (2). Under this classification system, normal villi are graded as 0. As the extent of mucosal injury increases, the numerical grade increases. Thus mucosal edema limited to the villus tip is graded 1, whereas necrosis of the entire villus is graded 5. Five random fields from each animal were evaluated at ×100 magnification.

Measurement of renal function. Glomerular filtration rate was estimated from the clearance of labeled inulin. Urinary flow was measured through a suprapubic cesion and PE catheter. A bolus injection of inulin (2 mg/kg) was administered, followed by a constant infusion of saline containing inulin (0.03 mg·kg⁻¹·min⁻¹) to maintain plasma concentrations. After a 45-min equilibration period, urine was collected and a blood sample was drawn. Urine was collected from T160 to T180, and a blood sample was drawn at T180. The ³H activity in plasma and urine was measured by the liquid scintillation technique.

Tissue adenosine nucleotide and lactate/pyruvate measurements. At the end of the experiments, with the animal still alive, tissue samples from the right kidney, jejunum, liver, and heart were rapidly taken with stainless steel tongs precooled in liquid nitrogen, immediately frozen in liquid nitrogen, and stored at −80°C. Two experimenters took part in the sampling procedure to keep its total duration below 2 s. ATP, ADP, AMP, and phosphocreatine (PCr) were determined by HPLC after disruption of the cell membrane with an Ultraturrax in a chloroform-acetic acid mixture (1:2) at −20°C. After neutralization (potassium hydroxide) and centrifugation, 25 μl of the supernatant was then used for HPLC analysis with a Supercosil LC column. ATP, ADP, AMP, and PCr were simultaneously detected by a photodiode array detector at 254 and 210 nm. Tissue pyruvate and lactate were assayed enzymatically as previously described (13). Additional samples were taken for the determination of the dry-to-wet weight ratio in the different organs. Furthermore, from these data, we calculated the adenyate energy charge [([ATP + 0.5 ADP)/(ATP + ADP + AMP)]).

Data presentation and statistics. Results are expressed as means ± SD. A repeated-measures one-way analysis of variance was used to evaluate within-group differences. Difference between groups was tested with a two-way analysis of variance (repeated time measurements and treatment as independent variables). When relevant, F values were significant at the 5% level, further pairwise comparisons were made with Dunnett’s test for the effect of time and with the Bonferroni correction for the effects of treatment at specific times.

RESULTS

Systemic and Regional Hemodynamics: Comparison with Baseline Values

ΔPp remained above 10% during the study in all investigated groups (Table 1 and Fig. 1).

Control-saline group. MAP, heart rate, aortic blood flow, MBF, and RBF all remained unchanged during the experimentation period (Figs. 1 and 2).

Endotoxin-S group. During endotoxin infusion, heart rate increased from 356 ± 9 to 420 ± 14 beats/min (P < 0.05 vs. Control group), whereas MAP decreased from 115 ± 11 to 78 ± 8 mmHg (P < 0.01 vs. Control group) (Fig. 1). Although aortic blood flow increased (P < 0.01 vs. Control group), both MBF and RBF along with regional-to-systemic ratio (data not shown) decreased (P < 0.05 vs. Control group) (Fig. 1).

Endotoxin-NE group. The mean efficient dose to maintain MAP was observed at 1.8 ± 0.4 μg·kg⁻¹·min⁻¹. Heart rate increased from 362 ± 10 to 412 ± 11 beats/min (P < 0.05 vs. Endotoxin-AVP and Control groups), whereas aortic blood flow slightly decreased. RBF, aortic blood flow/RBF, MBF,
and MBF/aortic blood flow remained unchanged relative to the Endotoxin-S group.

Endotoxin-AVP group. The mean efficient dose was observed at 0.02 ± 0.01 U·kg⁻¹·h⁻¹. Heart rate did not change throughout the endotoxin-AVP infusion (342 ± 10 to 372 ± 12 beats/min; P < 0.05 vs. all groups). AVP infusion normalized MAP and induced a slight decrease in aortic blood flow. RBF, MBF-to-aortic blood flow ratio, MBF, and MBF-to-aortic blood flow ratio did not change compared with the Endotoxin-LC group.

Endotoxin-LC group. According to a previous study (14), a fixed dose of 100 mg·kg⁻¹·h⁻¹ was used. Heart rate increased from 340 ± 9 to 395 ± 11 beats/min (P < 0.05 vs. Control and AVP groups). LC normalized MAP, whereas aortic blood flow was slightly decreased. RBF, aortic blood flow/RBF, MBF, and MBF/aortic blood flow remained unchanged relative to the Endotoxin-S group.

Renal Function: Comparison with Baseline Values

Decreases of 20 ± 5% and 15 ± 3% in diuresis and inulin clearance, respectively, were observed in the Control group at the end of the experimental protocol. Endotoxin infusion alone induced a further decrease in diuresis and inulin clearance (80 ± 10% and 62 ± 8%, respectively; P < 0.05 vs. Control, Endotoxin-AVP, and Endotoxin-LC groups). NE administration after endotoxin infusion did not change the effects of endotoxin on diuresis or inulin clearance (70 ± 7%; P < 0.05 vs. Control, Endotoxin-AVP, and Endotoxin-LC groups). AVP and LC administration after endotoxin infusion were especially efficient in attenuating these declines in diuresis (55 ± 7% and 45 ± 5%, respectively; P < 0.05 vs. Control group and) and in inulin clearance (20 ± 2% and 25 ± 6%; P < 0.05 vs. Control group). T₁₈₀ fractional excretion of sodium values significantly decreased in Endotoxin-S, Endotoxin-AVP, and Endotoxin-NE groups but doubled in the Endotoxin-LC group (P < 0.05 vs. Control group; Table 1).

Tissue Permeability: Comparison with Control Group

Endotoxin induced an increase in gut and lung permeability of 22 ± 5% and 300 ± 80%, respectively (P < 0.05 vs. Control group; Fig. 3). Endotoxin-induced gut hyperpermeability was inhibited by AVP and NE. In contrast, endotoxin-induced lung hyperpermeability was further enhanced by NE.

Table 1. Hemodynamic, biological, and acid-base values at T₁₈₀

<table>
<thead>
<tr>
<th></th>
<th>C-Saline</th>
<th>Endotoxin-S</th>
<th>Endotoxin-NE</th>
<th>Endotoxin-AVP</th>
<th>Endotoxin-LC</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mmHg</td>
<td>105±10</td>
<td>68±9</td>
<td>101±9</td>
<td>97±8</td>
<td>108±9</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>356±9</td>
<td>420±14*</td>
<td>412±11*</td>
<td>372±12</td>
<td>395±11*</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>40±3</td>
<td>40±4</td>
<td>42±6</td>
<td>43±1</td>
<td>39±3</td>
</tr>
<tr>
<td>Abdominal aortic blood flow, ml/min</td>
<td>52±8</td>
<td>58±7*</td>
<td>49±7*</td>
<td>48±9*</td>
<td>48±9*</td>
</tr>
<tr>
<td>MBF, ml/min</td>
<td>5±0.2</td>
<td>3.6±0.4*</td>
<td>3.5±0.3*</td>
<td>3±0.2*</td>
<td>3.6±0.3*</td>
</tr>
<tr>
<td>RBF, ml/min</td>
<td>5±0.4</td>
<td>3.1±0.3*</td>
<td>3.1±0.4*</td>
<td>3.1±0.5*</td>
<td>3±0.3*</td>
</tr>
<tr>
<td>Arterial bicarbonate, meq/l</td>
<td>7.39±0.02</td>
<td>7.31±0.05†</td>
<td>7.30±0.05†</td>
<td>7.33±0.04*</td>
<td>7.35±0.02*</td>
</tr>
<tr>
<td>Plasma Na⁺, meq/l</td>
<td>22±2</td>
<td>16±4*</td>
<td>16±3*</td>
<td>19±3*</td>
<td>20±2</td>
</tr>
<tr>
<td>FeNa, % vs. basal value</td>
<td>+20±3</td>
<td>0±4*</td>
<td>+4±2*</td>
<td>-13±4*</td>
<td>+100±12*</td>
</tr>
<tr>
<td>Arterial lactate, mmol/l</td>
<td>1.2±0.3</td>
<td>3±1.0*</td>
<td>2.3±1.0*</td>
<td>1.6±0.7</td>
<td>1.4±0.7</td>
</tr>
<tr>
<td>L/P</td>
<td>12±2</td>
<td>19±2*</td>
<td>19±3*</td>
<td>13±2</td>
<td>11±1</td>
</tr>
</tbody>
</table>

Values are means ± SD in control rats (C-Saline) and endotoxemic rats without treatment (Endotoxin-S) or treated from 90 to 180 min (T₁₈₀) with AVP (Endotoxin-AVP), norepinephrine (Endotoxin-NE), or l-canavanine (Endotoxin-LC). MAP, mean arterial pressure; HR, heart rate; MBF, mesentric blood flow; RBF, renal blood flow; FeNa, fractional excretion of sodium; L/P, arterial lactate-to-pyruvate ratio. *P < 0.05 vs. Control group; †P < 0.05 vs. Control, Endotoxin-AVP, and Endotoxin-LC groups.

Fig. 1. Regional hemodynamics. Adult rats (n = 10/group) were prepared as described in MATERIALS AND METHODS. Aortic, kidney, and mesenteric blood flows at 180 min (T₁₈₀) are expressed as % of basal value for rats treated with endotoxin-saline (Endotoxin-S group; filled bars), endotoxin-norepinephrine (Endotoxin-NE group; hatched bars), endotoxin-AVP (Endotoxin-AVP group; open bars), and endotoxin-l-canavanine (Endotoxin-LC; gray bars). *P < 0.05 vs. Control group.

Fig. 2. Renal function. Adult rats (n = 10 per group) were prepared as described in MATERIALS AND METHODS. Diuresis and inulin clearance (at T₁₈₀) are expressed as % of basal value (T₀) in rats treated with saline (Control group; striped bars) and endotoxin-S (filled bars), endotoxin-NE (hatched bars), endotoxin-AVP (open bars), and endotoxin-LC (gray bars) rats. *P < 0.05 vs. Control, Endotoxin-AVP, and Endotoxin-LC groups.
infusion but not by AVP infusion. LC significantly decreased endotoxin-induced gut and pulmonary permeability compared with all treated groups. Renal permeability was increased by AVP but remained unchanged for all other treatments.

**Acid-Base and Lactate Metabolism**

Arterial pH decreased significantly in all treated groups (Table 1), whereas a stable PaCO₂ was maintained (data not shown). Endotoxin was associated with an increase in lactate levels and a slight increase in lactate-to-pyruvate ratio (L/P; 0.2 1.20), whereas a stable PaCO₂ was maintained (data not shown). Endotoxin was associated with an increase in lactate levels and a slight increase in lactate-to-pyruvate ratio (L/P; 0.2 1.20). AVP and LC blunted the increase in lactate levels and 0.05 vs. all groups.

**Tissue Lactate and L/P**

In endotoxin-treated rats, lactate and L/P increased in gut, heart, liver, and kidney (P < 0.05 vs. Control group; Table 2). NE did not change the endotoxin-induced increase in tissue lactate and L/P. AVP and LC blunted the increase in lactate and L/P in kidney.

**Adenine Nucleotide, PCr, and Energy Charge**

Gut and heart ATP and energy charge did not change in any treated group (Table 3). In the kidney, endotoxin and NE decreased ATP concentrations and energy charge. ATP concentration and energy charge in liver decreased in all treated groups. PCr decreased in all organs during endotoxemia (P < 0.05) and did not change during treatments.

**Gut Histology**

In this model, endotoxin and/or treatments did not induce any particular histological or inflammatory lesions compared with the Control group.

**DISCUSSION**

The major finding of this study is that AVP, compared with NE, exhibits a favorable impact on renal function, lactate levels, and lung permeability without deleterious effect on the gut in a model of acute endotoxic shock.

**Characterization of Endotoxic Model**

The experimental design selected here was both a hypokinetic model (12), which led to major gut mucosa injury.

**Effects of AVP, NE, and LC on Hemodynamics**

All drugs increased MAP to near-baseline values and decreased aortic blood flow, pronounced lactic acidosis, and major gut mucosa injury.

**Table 2. Tissue lactate and L/P**

<table>
<thead>
<tr>
<th></th>
<th>Gut</th>
<th>Heart</th>
<th>Liver</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lactate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-Saline</td>
<td>2.2±0.7</td>
<td>1.2±0.3</td>
<td>1.2±0.7</td>
<td>0.8±0.3</td>
</tr>
<tr>
<td>Endotoxin-S</td>
<td>3.1±0.6*</td>
<td>2.0±0.9*</td>
<td>4.2±1.2*</td>
<td>3.8±0.6*</td>
</tr>
<tr>
<td>Endotoxin-NE</td>
<td>3.3±0.5*</td>
<td>6.4±1.2*</td>
<td>4.2±1.2*</td>
<td>4.2±1.0*</td>
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<tr>
<td>Endotoxin-AVP</td>
<td>2.9±0.8*</td>
<td>5.8±1.0*</td>
<td>4.4±1.1*</td>
<td>1.5±0.3*</td>
</tr>
<tr>
<td>Endotoxin-LC</td>
<td>2.6±1.5*</td>
<td>5.9±1.1*</td>
<td>3.9±1.1*</td>
<td>1.7±0.6*</td>
</tr>
<tr>
<td>L/P</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-Saline</td>
<td>15±5</td>
<td>17±9</td>
<td>22±6</td>
<td>17±10</td>
</tr>
<tr>
<td>Endotoxin-S</td>
<td>25±7*</td>
<td>29±7*</td>
<td>42±9*</td>
<td>52±8*</td>
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<tr>
<td>Endotoxin-NE</td>
<td>29±9*</td>
<td>32±7*</td>
<td>44±9*</td>
<td>60±12*</td>
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<td>Endotoxin-AVP</td>
<td>17±5</td>
<td>25±6*</td>
<td>40±8*</td>
<td>24±8*</td>
</tr>
<tr>
<td>Endotoxin-LC</td>
<td>19±5</td>
<td>26±8*</td>
<td>39±7*</td>
<td>25±5*</td>
</tr>
</tbody>
</table>

Values are means ± SD. *P < 0.05 vs. C-Saline group; †P < 0.05 vs. C-Saline, Endotoxin-S, and Endotoxin-NE groups.

**Table 3. High-energy phosphate concentration**

<table>
<thead>
<tr>
<th></th>
<th>Gut</th>
<th>Heart</th>
<th>Liver</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ATP</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>C-Saline</td>
<td>1.3±0.2</td>
<td>3.5±0.6</td>
<td>2.6±0.2</td>
<td>1.20±0.3</td>
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<tr>
<td>Endotoxin-S</td>
<td>1.2±0.4</td>
<td>3.0±0.4</td>
<td>2.1±0.4*</td>
<td>0.89±0.2*</td>
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<tr>
<td>Endotoxin-NE</td>
<td>1.2±0.2</td>
<td>2.9±0.4</td>
<td>1.9±0.2*</td>
<td>0.70±0.4*</td>
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<tr>
<td>Endotoxin-AVP</td>
<td>1.4±0.4</td>
<td>3.1±0.5</td>
<td>2.0±0.3*</td>
<td>1.20±0.3</td>
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<tr>
<td>Endotoxin-LC</td>
<td>1.6±0.5</td>
<td>3.2±0.5</td>
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<td>1.17±1.1</td>
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<tr>
<td><strong>Energy charge</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>C-Saline</td>
<td>0.85±0.01</td>
<td>0.85±0.02</td>
<td>0.87±0.01</td>
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<tr>
<td>Endotoxin-S</td>
<td>0.80±0.01</td>
<td>0.79±0.01</td>
<td>0.78±0.01*</td>
<td>0.74±0.01*</td>
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<td>Endotoxin-NE</td>
<td>0.83±0.01</td>
<td>0.81±0.02</td>
<td>0.76±0.01*</td>
<td>0.70±0.01*</td>
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<tr>
<td>Endotoxin-AVP</td>
<td>0.81±0.01</td>
<td>0.80±0.01</td>
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<td>Endotoxin-LC</td>
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<td>0.81±0.02</td>
<td>0.79±0.01*</td>
<td>0.81±0.01</td>
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</tbody>
</table>

Values are means ± SD. *P < 0.05 vs. C-Saline group.
The lack of effects of NE on RBF confirmed previous observations (35), whereas other authors, using a canine endotoxic shock model, described an NE-induced increase in RBF (1). The latter effect was related to increased perfusion pressure and to complex effects on renal vascular resistances, both of which can be shared by almost all powerful vasoconstrictive agents (1). In our model, all treatments had similar effects on RBF despite large differences in pharmacological effects. Nevertheless, blood flow partition between the cortex and the medulla was not measured. In addition, improvement of systemic hemodynamics was paralleled by a decrease in arterial lactate levels with, the exception of NE-treated rats, in which putative metabolic effects of β1-stimulation could be postulated (13).

**Effects of Drug Treatment on Diuresis and Renal Function**

Despite identical effects on MAP, cardiac output, and RBF, AVP, LC, and NE exhibited differential effects on diuresis and renal function. A putative explanation is that endotoxemia leads to a redistribution of renal microperfusion without any alteration in total RBF or systemic hemodynamics.

AVP infusion blunted endotoxemia-induced drop in diuresis, and different mechanisms can be argued to explain this effect. First, renal efferent arteriolar vasoconstriction, relatively sparing the afferent renal arterioles, increases renal perfusion pressure per se. Second, V2 receptor-associated natriuretic effects, as well as the release of the atrial natriuretic factor, can also be involved. On the other hand, AVP infusion stimulates aquaporin-2 apical membrane expression in renal collecting ducts under physiological conditions, although modulation of this process after endotoxin challenge is unknown. “Vasopressin escape” from antidiuresis has been described in animals after several days of drug infusion and is related (6) to a marked AVP-independent decrease in kidney aquaporin-2 mRNA and protein expression as well as AVP V2 receptor binding (18, 29). In this study natriuresis did not increase after endotoxin challenge and AVP infusion, but the experimental period was very short.

The renal effects of LC were similar to those of AVP, although the mechanisms of action involved are most likely different. Renal effects of NO inhibition extend beyond its effects on arterial pressure and renal perfusion pressure. Selective NO inhibition increases arterial pressure without affecting renal function (26). Interestingly, recent evidence suggests that iNOS-dependent high NO levels could lead to renal vasoconstriction and reduction in glomerular filtration rate by inhibiting endothelial NO synthase activity (25) and that NO scavenging inhibits the increase in medullary perfusion, thus maintaining glomerular hydrostatic pressure and glomerular filtration rate (16).

The renal effects of NE are surprising because this drug usually improves diuresis without changing renal function in human septic shock and in other experimental designs (22). Despite an increase in perfusion pressure, NE did not change diuresis in the present model, suggesting that the effect on renal vascular resistances was more pronounced than on systemic resistances.

**Effects of Endotoxin and Drugs on High-Energy Phosphates**

Data relating to the effects of sepsis on tissue levels of adenine nucleotides are plentiful but conflicting and thus difficult to interpret. One reason for the variance in results may depend on the septic models that were employed, i.e., the species, doses of LPS, intensity of resuscitation, early or late sepsis, and the level of hypotension. Therefore, our results should be interpreted cautiously with regard to our model. Endotoxin decreased ATP levels in the kidney and had no effects on gut ATP. In these organs, ATP contents found in our control and endotoxemic rats perfectly matched those reported in these studies (33). In our resuscitated model of endotoxiaemia, MBF decreased and jejunal L/P increased without alteration of jejunal ATP levels. A possible explanation is that gut ATP levels do not distinguish mucosal from other layer levels (i.e., submucosa, muscularis). Thus normal whole tissue ATP levels did not preclude selective decrease in mucosal ATP levels.

In the gut drugs had no effect on blood flow, lactate, and ATP levels, whereas in the kidney the increase in lactate levels and L/P was associated with a decrease in ATP in the NE group. Thus, in this model, NE had detrimental effects on renal perfusion and energetic state whereas AVP improved renal function and energetics.

**Effects on Capillary Permeability**

It is well demonstrated that sepsis has a significant but tissue-dependent effect on transvascular albumin exchange, leading to enhanced microvascular permeability and interstitial edema that could contribute to the development of multiple organ failure (19). EB extravasation was the method selected in the present study to examine regional capillary permeability, and it has been validated previously (30). Endothelial resistance is organ- and/or tissue dependent and is associated with blood redistribution (4). The most striking result observed is that NE infusion further increased endotoxemia-induced pulmonary permeability compared with AVP treatment. These data are difficult to explain, although NE may have influenced alveolar permeability through modification of hydrostatic pressures (31). Increase in lung vascular permeability has dramatic effects on the relationship between transvascular fluid filtration and vascular pressure (15), with small changes in pressure inducing huge fluid accumulation. Infusion of NE does not increase lung vascular permeability to proteins in nonseptic conditions; however, it increases fluid filtration, suggesting that microvascular pressure is increased because of postcapillary vessel preferential constriction (17). High doses of NE are known to increase pulmonary pressure and resistances, whereas AVP tends to decrease these parameters. Gest et al. (8), using a nonseptic model, observed that AVP decreases cardiac index without affecting pulmonary artery pressure and resistance and that the rate of lung lymph flow was not affected, whereas lymph-to-plasma protein ratio decreased significantly. In our septic model AVP did not further alter endotoxin-induced lung permeability, and one might assume that vasoconstrictor effects were likely overwhelmed by NO-mediated pulmonary vasodilatation. LC normalized lung permeability, confirming previous studies (11).

Gut permeability was also increased by endotoxin challenge, and all drugs were preventive. Interestingly, in this normokinetnic and volume-resuscitated model, AVP neither further
induced gut lesions nor decreased liver ATP, in contrast to the hypokinetic model (34). This underlies the major role of hemodynamic treatment in modulating endotoxin-induced splanchnic alterations. Recently, Dunser et al. (5) demonstrated that AVP increased MAP in 48 patients with catecholamine-resistant vasodilatory shock without deleterious side effects on gastric perfusion. On the other hand, van Haren et al. (32) demonstrated an increase in PCO₂ gap during vasopressin infusion in catecholamine-dependent patients exhibiting septic shock. As for renal alterations, Kang et al. (10) demonstrated that LPS causes structural damage and calcium accumulation in the mitochondria. In our study, AVP, but not NE and LC, further increased endotoxin-induced renal permeability. This could be due to increased glomerular filtration through increased perfusion or extravasation of albumin, although not affecting AVP’s beneficial effect on renal function.

Limitations of Study

Study monitoring was limited to 90 min, and this does not preclude that the effects may have been different for a longer period. Sun et al. (27) demonstrated that some advantages of AVP use are mainly observed after 16 h. Our data clearly demonstrate the acute effects of AVP in hypotensive normodynamic shock but cannot be extrapolated for a longer period. This limitation could also explain the lack of histological lesions. Despite a generous fluid infusion and the normality of pulse pressure variation, decreased diuresis and inulin clearance observed in the control group do not preclude that some hypovolemia or other factors such as anesthesia or laparotomy per se could have been contributive at least in several animals. Nevertheless, this should also have affected the other experimental groups.

In conclusion, our data demonstrate that, in contrast to NE, exogenous AVP and LC administration effectively improves renal function and renal energetics without deleterious effects on lung permeability and with comparable systemic and splanchnic hemodynamic and metabolic effects in endotoxin-induced circulatory normokinetic shock. These findings suggest that AVP can help in clinical management of acute renal and respiratory failures induced by septic shock. Selective iNOS inhibition, although not available for clinical use, remains an attractive area of research.

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