Angiotensin II in Septic Shock: Effects on Tissue Perfusion, Organ Function, and Mitochondrial Respiration in a Porcine Model of Fecal Peritonitis*

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Objectives: To compare effects of norepinephrine and angiotensin II in experimental sepsis on hemodynamics, organ function, and mitochondrial respiration.

Design: Randomized, controlled, study.

Setting: University experimental laboratory.

Subjects: Twenty-eight anesthetized, mechanically ventilated pigs.

Interventions: Sixteen pigs were randomized to receive after 12 hours of fecal peritonitis fluid resuscitation and either norepinephrine (group NE; $n = 8$) or angiotensin II (group AT-II; $n = 8$) for 48 hours. A separate group ($n = 8$), treated with enalapril for 1 week before peritonitis and until study end, received fluids and norepinephrine (group E). The blood pressure dose-response to angiotensin II was evaluated in additional four nonseptic pigs.

Measurements and Main Results: Blood pressure target (75–85 mm Hg) was reached in both NE and AT-II, and cardiac output increased similarly (NE: from 64 mL/kg/min [60–79 mL/kg/min] to 139 mL/kg/min [126–157 mL/kg/min]; AT-II from 79 mL/kg/min [65–86 mL/kg/min] to 145 mL/kg/min [126–147 mL/kg/min]; median, interquartile range). Renal plasma flow, prevalence of acute kidney injury, inflammation and coagulation patterns, and mitochondrial respiration did not differ between NE and AT-II. In group E, blood pressure targets were not achieved (mean arterial pressure at study end: NE: 81 mm Hg [76–85 mm Hg]; AT-II: 80 mm Hg [77–84 mm Hg]; E: 53 mm Hg [49–66 mm Hg], $p = 0.002$, compared to NE), whereas skeletal muscle adenosine triphosphate concentrations were increased. During resuscitation one animal died in groups AT-II and E.

Conclusions: Angiotensin II reversed sepsis-induced hypotension with systemic and regional hemodynamic effects similar to those of norepinephrine. Inhibition of angiotensin-converting enzyme before sepsis worsened the hypotension but enhanced skeletal muscle adenosine triphosphate. Modifying the renin-angiotensin system in sepsis should be further evaluated. (*Crit Care Med* 2014; 42:e550–e559)

Key Words: angiotensin II; enalapril; mitochondrial respiration; multiple organ failure; norepinephrine; septic shock

***See also p. 1961.**

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Norepinephrine is the standard vasopressor to treat septic shock (1, 2). High doses of catecholamines may contribute to organ dysfunction and mortality in sepsis (3, 4). Vasopressin has been proposed as an additional treatment of septic hypotension in order to reduce the need for catecholamine vasopressors and their potential adverse effects. In a large multicenter trial, vasopressin failed to reduce mortality in septic shock (5). Angiotensin II, a strong physiologic

vasoconstrictor, might be able to reverse the vasoplegia associated with septic shock. Some reports of its clinical use to treat severe hypotension in septic shock have been published (6–8). In short-term experimental sepsis, angiotensin II preserved renal function and bioenergetics despite reduction of renal blood flow (9, 10). Angiotensin II may modify tissue energy metabolism either directly or via its effects on tissue perfusion. The effect of angiotensin II on tissue energy metabolism of extrarenal tissues in sepsis is not known. Based on these observations, we hypothesized that angiotensin II can be used to stabilize hemodynamics in experimental sepsis without adverse effects on renal function or mitochondrial respiration. To test this hypothesis, we compared the effects of angiotensin II and norepinephrine on systemic and regional hemodynamics, renal function, and renal, hepatic, and cardiac mitochondrial respiration in a clinically relevant porcine abdominal sepsis model. In order to assess the role of endogenous angiotensin, a separate group with inhibition of angiotensin-converting enzyme prior and during sepsis was studied.

MATERIALS AND METHODS

This study was performed in accordance with the National Institutes of Health guidelines for the care and use of experimental animals and with the approval of the Animal Care Committee of the Canton of Bern, Switzerland.

Twenty domestic male pigs (weight: 41 kg [40–43 kg]; median, interquartile range [IQR]) were randomized to fecal peritonitis ($n = 16$) or controls ($n = 4$). Peritonitis animals were further randomized to fluid resuscitation and norepinephrine (maximum dose: 5,000 μg/hr) or angiotensin II infusion (maximum dose: 1,000 ng/kg/min) ($n = 8$, each) to maintain a mean arterial blood pressure of 75–85 mm Hg. This randomization occurred only at the end of the observation period, just before sepsis treatment started (**Fig. 1**). Block randomization with sealed envelopes was used (2 × 8 envelopes). In the four controls, angiotensin II (maximum dose, 23–27 ng/kg/min) was infused 18 hours after instrumentation to evaluate the dose effect in nonseptic animals (described in the **supplemental data**, Supplemental Digital Content 1, http://links.lww.com/CCM/A965).

In a separate group ($n = 8$; weight: 39 kg [38–40 kg]), the angiotensin-converting enzyme was inhibited with enalapril 20 mg/d orally 1 week before the experiment. To sustain the inhibition of angiotensin-converting enzyme, a low dose of enalapril (0.02 mg/kg/hr, reduced if hypotensive despite resuscitation) was infused during treatment of septic shock with fluids and norepinephrine (maximum dose: 5,000 μg/hr). A septic control group without treatment was not included because such a group was part of a previous study using the same experimental model (11).

Surgical Preparation

A pulmonary artery catheter was inserted via the right jugular vein, and femoral veins were catheterized. A catheter was placed into the right kidney vein via right femoral vein under fluoroscopy. An arterial catheter for blood pressure measurement and blood sampling was placed into the right carotid artery. A 4-mm ultrasound Doppler flow probe (Transonic Systems, Ithaca, NY) was placed around the left carotid and femoral arteries. A cystostomy for urine drainage was performed. After preparation, the abdominal incision was closed. Ringer's lactate (RL) was infused at 3 mL/kg/hr during surgery.

Study Protocol, Monitoring, and Blood Sampling

Surgical preparation was followed by 6 hours of stabilization. RL for hydration and glucose 50% (G50%) to keep blood glucose at 3.5–5.0 mmol/L were infused (total 1.5 mL/kg/hr). Then, baseline measurements were performed, and fecal peritonitis was induced by peritoneal instillation of 2 g/kg body weight of autologous feces dissolved in 200 mL of warmed glucose 5% solution (Fig. 1).

After 12 hours of peritonitis, 48 hours of protocolized resuscitation was started (resuscitation period) (**Fig. S1**, Supplemental Digital Content 1, http://links.lww.com/CCM/A965). Resuscitation was conducted by trained intensivists as published before (11, 12). The volume status was evaluated clinically every hour. If signs of hypovolemia became evident, alternating boluses of 150 mL RL and 6% hydroxyethyl starch (130/0.4) were given. If mean arterial blood pressure was lower than 75 mm Hg, vasopressors (norepinephrine or angiotensin II) were given. For further details, see supplemental data (Supplemental Digital Content 1, http://links.lww.com/CCM/A965). Following 48 hours of protocolized resuscitation, the animals were deeply sedated and euthanized with an overdose of potassium chloride. Monitoring and blood sampling are described in the supplemental data (Supplemental Digital Content 1, http://links.lww.com/CCM/A965).

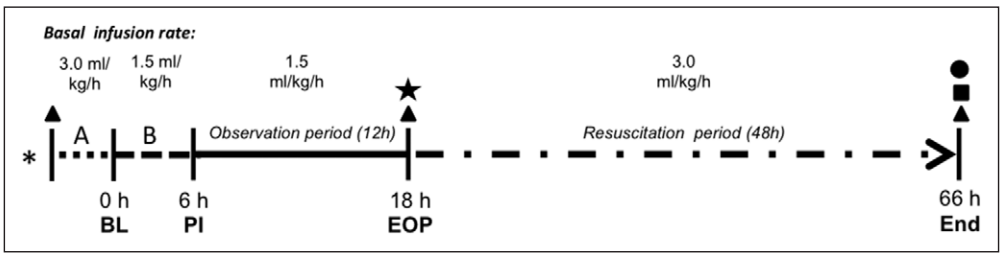

Figure 1. Study design and basal fluid infusion rates. BL = baseline, PI = peritonitis induction, EOP = end of observation period (before starting resuscitation), End = end of the experiment (at 48 hr of resuscitation or before death if earlier). *Administration of enalapril for 7 days before the study for enalapril group, A: surgery period (1–2 hr), B: stabilization period (6 hr), *black triangles*: thromboelastography analysis, *black star*: first dose of IV antibiotic (piperacillin/tazobactam, 2.25 g, 8-hourly), *black circle*: samples from the right quadriceps muscle to assess skeletal muscle ATP content, *black square*: tissue samples from the liver, heart, and kidney for mitochondrial function analysis at the end of the experiment (66 hr or before death, if earlier).

Estimation of Renal Blood Flow and Function

Renal blood flow was estimated using primed, continuous infusion of para-aminohippurate (PAH; Aminohippurate sodium; Merck, Whitehouse

Station, NJ). Acute kidney injury (AKI) was defined according to the Acute Kidney Injury Network criteria (11, 13). For details, see supplemental data (Supplemental Digital Content 1, http://links.lww.com/CCM/A965).

Mitochondrial Function Analysis and Skeletal Muscle Adenosine Triphosphate Content

Tissue samples were taken from the kidney, liver, and heart sequentially and immediately put on ice at the end of the experiment, before euthanasia. In animals which died earlier, the final samples were taken when the animals were still alive and receiving the maximal vasopressor dose and when mean arterial blood pressure approached 30 mm Hg. Mitochondria of the various tissues were analyzed as previously described (11, 14, 15). For details, see supplemental data (Supplemental Digital Content 1, http://links.lww.com/CCM/A965).

Complex I-, II-, and IV-dependent respiration rates were measured using high-resolution respirometry (Oxygraph-2k; Oroboros Instruments, Innsbruck, Austria). Further details and measurement of muscle adenosine triphosphate (ATP) content are indicated in supplemental data (Supplemental Digital Content 1, http://links.lww.com/CCM/A965).

Statistical Analysis

The primary and main analysis was performed between the two randomized groups AT-II (angiotensin II) and NE (norepinephrine). In addition, post hoc comparisons were made between (nonrandomized) groups E (enalapril + norepinephrine) and NE. Since randomization between AT-II and NE occurred only at the end of the observation period (EOP; before sepsis treatment started), baseline measurements were not included in the within-group analysis in order to avoid more than one prerandomization measurement. Mann-Whitney *U* test or Wilcoxon signed rank test and Friedman test were used for between- and within-group comparisons, respectively, and Fisher exact test for categorical data. The effect of untreated peritonitis was assessed in the whole cohort (i.e., all the animals subsequently randomized) by comparing the baseline with the EOP in the pooled NE and AT-II groups. To compare the effects of peritonitis between NE/AT-II groups and enalapril animals, the differences between EOP and baseline were compared using Mann-Whitney *U* test. Statistical analyses were performed using IBM SPSS Statistics version 20.0 for Windows (SPSS Inc., Armonk, NY).

RESULTS

Hemodynamic Response to Angiotensin in Healthy Controls

In controls, the highest dose of angiotensin II (23–27 ng/kg/min) resulted in an increase in blood pressure from 76 mm Hg (74–77 mm Hg, median, range) to 131 mm Hg (96–154 mm Hg). Although pulmonary artery pressure increased, cardiac output did not change. Further details are indicated in **Table S1** and **Figure S2** (Supplemental Digital Content 1, http://links.lww.com/CCM/A965).

Clinical Impact of Untreated Fecal Peritonitis

Twelve hours of peritonitis without treatment in groups NE and AT-II resulted in decreased cardiac output and renal blood flow and increased arterial lactate concentration. Systemic oxygen consumption was maintained (**Table S2**, Supplemental Digital Content 1, http://links.lww.com/CCM/A965). Signs of AKI were observed in nine of 16 animals (56%) (**Table 1**), and fractional excretion of sodium and potassium decreased (Table S2, Supplemental Digital Content 1, http://links.lww.com/CCM/A965).

Norepinephrine Versus Angiotensin II

One animal in the AT-II group died 55 hours after peritonitis induction, whereas all in group NE survived. Gas exchange (**Table S3**, Supplemental Digital Content 1, http://links.lww.com/CCM/A965) and fluid requirements during resuscitation (**Table 2**) were similar in NE and AT-II groups. The mean arterial blood pressure goal of 75–85 mm Hg was reached using 0.58 μg/kg/min (0.26–1.31 μg/kg/min) (median [IQR]) of norepinephrine in group NE and 309 ng/kg/min (120–582 ng/kg/min) of angiotensin II in group AT-II (Table 2 and **Fig. 2**). Individual blood pressure evolution and angiotensin/norepinephrine infusion rates are displayed in **Figure S3** (Supplemental Digital Content 1, http://links.lww.com/CCM/A965). In both groups, approximately half of the animals needed continuously increasing or maximal doses of the vasoconstrictor. Cardiac output, stroke volume, and mean pulmonary artery blood pressure increased comparably (**Fig. 3**).

Arterial lactate recovered to baseline values in both groups (**Table S4**, Supplemental Digital Content 1, http://links.lww.com/CCM/A965). Systemic and renal oxygen consumption did not change (Table 1) (Table S4, Supplemental Digital Content 1, http://links.lww.com/CCM/A965). Arterial PAH concentrations were stable after 30 minutes of infusion (**Fig. S4**, Supplemental Digital Content 1, http://links.lww.com/CCM/A965). PAH clearance, which had decreased during untreated peritonitis, increased to baseline levels in both groups during the resuscitation period, and signs of AKI were attenuated during resuscitation (Table 1).

Variables reflecting inflammation, body biochemistry, and coagulation were similar in both groups (**Table 3**) (**Tables S5 and S6**, Supplemental Digital Content 1, http://links.lww.com/CCM/A965). All animals from both groups required glucose infusion to maintain normal blood glucose concentrations (Table 2).

Mitochondrial Function and Skeletal Muscle ATP Content

Mitochondrial respiration rates of complex I-dependent state 4 respiration (resting state) of isolated kidney mitochondria were slightly lower in the angiotensin II group in comparison to norepinephrine group (**Fig. 4**), whereas heart and liver mitochondrial respiration did not differ between both groups (**Figs. S5 and S6**, Supplemental Digital Content 1, http://links.lww.com/CCM/A965). Oxygen consumption of

TABLE 1. Kidney Function Tests

Variables	Group	Baseline	End of the Observation Period	End[a]	*p*[b]
Creatinine (mg/dL)	NE	1.1 (1.0–1.2)	1.6 (1.5–2.1)	1.0 (0.9–1.3)	0.148[c]
	AT-II	1.1 (1.1–1.2)	1.2 (1.1–1.9)	1.2 (0.9–1.3)	0.641[d]
	E	1.1 (1.1–1.1)	1.8 (1.2–2.0)	2.2 (1.6–3.7)	0.109[e]
Para-aminohippurate clearance (mL/kg/min)	NE	19.3 (14.9–24.2) (6)	12.8 (10.8–21.6) (5)	25.2 (20.5–29.1) (7)	0.063[c]
	AT-II	18.6 (13.1–20.7)	11.7 (10.7–16.0) (7)	20.0 (17.9–26.3)	0.016[d]
	E	15.9 (13.7–20.6)	14.4 (12.7–18.5)	25.6 (21.8–30.7) (7)	0.016[e]
FE sodium (%)	NE	0.26 (0.16–0.51)	0.05 (0.03–0.06)	0.04 (0.04–0.05)	0.719[c]
	AT-II	0.10 (0.07–0.15)	0.04 (0.03–0.05)	0.04 (0.03–0.06)	1.000[d]
	E	0.09 (0.06–0.11)	0.05 (0.04–0.06)	0.12 (0.05–0.22)	0.031[e]
FE potassium (%)	NE	25.1 (18.9–27.6)	5.6 (4.2–6.0)	5.8 (3.9–6.3)	0.844[c]
	AT-II	13.3 (11.7–16.6)	5.8 (4.6–10.3)	2.6 (2.0–4.6)	0.047[d]
	E	17.3 (14.8–19.9)	9.8 (6.1–10.7)	11.4 (8.3–12.6)	0.148[e]
Kidney Vo_2 (mL/min)	NE	20 (16–40) (6)	18 (15–29) (4)	17 (16–21) (6)	0.875[c]
	AT-II	19 (15–28) (8)	20 (15–26) (7)	19 (12–25) (7)	0.578[d]
	E	16 (15–18) (8)	21 (18–25) (8)	17 (13–39) (7)	0.938[e]
Kidney Vo_2/Do_2	NE	0.22 (0.21–0.29) (6)	0.20 (0.16–0.23) (4)	0.16 (0.14–0.17) (6)	0.250[c]
	AT-II	0.25 (0.22–0.27) (8)	0.21 (0.17–0.27) (7)	0.19 (0.14–0.24) (7)	0.688[d]
	E	0.22 (0.18–0.22) (8)	0.18 (0.17–0.22) (8)	0.13 (0.09–0.26) (7)	0.469[e]
Acute kidney injury, *n* (%)	NE		7 (88%)	2 (25%)	0.041[f]
	AT-II		2 (25%)	2 (25%)	0.569[g]
	E		5 (63%)	6 (75%)	1.000[h]
					0.066[i]

NE = norepinephrine, AT-II = angiotensin II group, E = enalapril + norepinephrine group, FE = fractional excretion.

[a]End of the experiment (at 48 hr of resuscitation or before death if earlier).

[b]Time effect with Wilcoxon signed rank test including end of the observation period (EOP) and end of the experiment (END) for: [c]NE, [d]AT-II, and [e]E.

[f]Fisher exact test at EOP comparing AT-II versus NE.

[g]Fisher exact test at EOP comparing E versus NE.

[h]Fisher exact test at END comparing AT-II versus NE.

[i]Fisher exact test at END comparing E versus NE.

Values are medians with interquartile ranges.

Due to technical problems, few measurements of the kidney vein had to be excluded from analysis, and the remaining *n* of each group is indicated in brackets.

permeabilized myocardial fibers was not affected (**Fig. S7**, Supplemental Digital Content 1, http://links.lww.com/CCM/A965), and skeletal muscle ATP content was not different between groups (**Fig. 5**).

Effects of Angiotensin-Converting Enzyme Inhibition

Two animals in the enalapril group died during the 12-hour observation before resuscitation and were subsequently replaced. One animal died during the resuscitation period 21 hours after peritonitis induction. At the end of the 12-hour observation period, group E had higher cardiac output and stroke volume than group NE (Fig. 3).

Fluid requirements during resuscitation in group E were similar to those in group NE (Table 2). Group E failed to reach the blood pressure targets although all animals received the maximum dose of norepinephrine (Figs. 2 and 3) (Fig. S3, Supplemental Digital Content 1, http://links.lww.com/CCM/A965).

Renal Function

Six animals in group E had signs of AKI at the end of the experiment (vs two in group NE; $p = 0.066$) (Table 1) despite increased renal plasma flow (14.4 mL/kg/min [12.7–18.5 mL/kg/min] at EOP vs 25.6 mL/kg/min [21.8–30.7 mL/kg/min] at study end; $p = 0.016$), stable renal oxygen consumption, and recovery of

TABLE 2. Administered Treatments, Fluid Output, and Balance During the Resuscitation Period

Variables/Study group	NE	AT-II	E	*p* AT-II Versus NE	*p* E Versus NE
Propofol (mg/kg/hr)	5.5 (4.9–6.1)	6.0 (4.3–6.6)	5.2 (4.7–5.4)	0.878[a]	0.574[a]
Fentanyl (μg/kg/hr)[b]	4.1 (3.0–5.0)	4.4 (3.3–5.9)	8.2 (7.5–8.9)	0.798[a]	< 0.001[a]
Midazolam (mg/kg/hr)	0.03 (0.02–0.06)	0.04 (0.02–0.07)	0.04 (0.03–0.04)	0.959[a]	0.572[a]
Glucose 50% (mL/kg/hr)	0.9 (0.6–1.2)	0.9 (0.5–1.1)	0.3 (0.2–0.4)	0.442[a]	< 0.001[a]
Fluid bolus (mL/kg/hr)[c]	1.6 (1.2–2.0)	1.1 (0.9–1.8)	1.9 (1.7–2.8)	0.442[a]	0.130[a]
Ringer's lactate bolus (mL/kg/hr)	1.1 (1.0–1.4)	0.9 (0.8–1.3)	1.3 (1.3–2.2)	0.442[a]	0.195[a]
Hydroxyethyl starch bolus (mL/kg/hr)	0.4 (0.2–0.6)	0.2 (0.1–0.4)	0.5 (0.4–0.6)	0.525[a]	0.290[a]
Norepinephrine					
n/*n* group (%)	8/8 (100%)		8/8 (100%)		
μg/kg/min	0.58 (0.26–1.31)		1.96 (1.62–2.02)	0.002[a]	
Angiotensin II					
n/*n* group (%)		8/8 (100%)			
ng/kg/min		309 (120–582)			
Dobutamine					
n/*n* group (%)	2/8 (25%)	1/8 (12.5%)	2/8 (25%)		
μg/kg/min	0.00 (0.00–0.06)	0.00 (0.00–0.00)	0.00 (0.00–0.02)	1.000[d]	1.000[d]
Total volume received (mL/kg/hr)	4.6 (4.2–5.0)	4.1 (3.9–4.8)	4.9 (4.7–5.9)	0.442[a]	0.161[a]
Urine output (mL/kg/hr)	1.0 (0.9–1.0)	1.0 (0.8–1.2)	0.6 (0.5–1.0)	0.798[a]	0.038[a]
Gastric tube output (mL/kg/hr)[e]	0.3 (0.2–0.6)	0.3 (0.2–0.5)	0.5 (0.1–0.7)	0.742[a]	0.856[a]
Final balance (mL/kg/hr)[f]	3.2 (2.7–3.8)	2.7 (2.3–3.7)	4.0 (3.4–4.9)	0.505[a]	0.083[a]

NE = norepinephrine group, AT-II = angiotensin II group, E = enalapril + norepinephrine group.
[a]Mann-Whitney *U* test.
[b]Sum of basic infusion with additional boli.
[c]Sum of Ringer's lactate (RL) and hydroxyethyl starch (HES, 6% [130/0.4]).
[d]Fisher exact test.
[e]Collected during the entire study period.
[f]Final balance: (RL basal infusion + glucose 50% basal infusion + RL bolus + HES bolus) − (urine output + gastric tube output).
Values are medians with interquartile ranges.

fractional sodium and potassium excretion during resuscitation (Table 1). Further results of enalapril-pretreated animals can be found in the supplemental data (Supplemental Digital Content 1, http://links.lww.com/CCM/A965).

DISCUSSION

The main finding of the study was that exogenous angiotensin II reversed sepsis-induced hypotension similarly as norepinephrine. Likewise, cardiac output and stroke volume increased to the same extent, and renal perfusion and function were restored in both groups.

The cardiovascular effects of exogenous angiotensin II appear to be modified by the underlying clinical condition and experimental model. In healthy volunteers, angiotensin II infusion decreased cardiac output and renal blood flow in a dose-dependent manner (16). In ovine gram-negative sepsis without fluid resuscitation, 2–6 hours of angiotensin II infusion at doses up to 600 ng/kg/min had no major effect on cardiac output and had either no effect on renal blood flow or reversed an increased flow (9, 10). The increase of cardiac output in our study can be explained by the combination of enhanced preload with fluids and restoration of vascular tone by angiotensin II. We suggest that the differences between the present and previous studies (9, 10) on cardiac output and renal blood flow effects of angiotensin II are most likely explained by fluid resuscitation. Renin and angiotensin II concentrations are elevated in clinical sepsis (17). AT-1 receptors are down-regulated in septic mice and rats and in mice after cytokine injection (18, 19). It is conceivable that the increasing

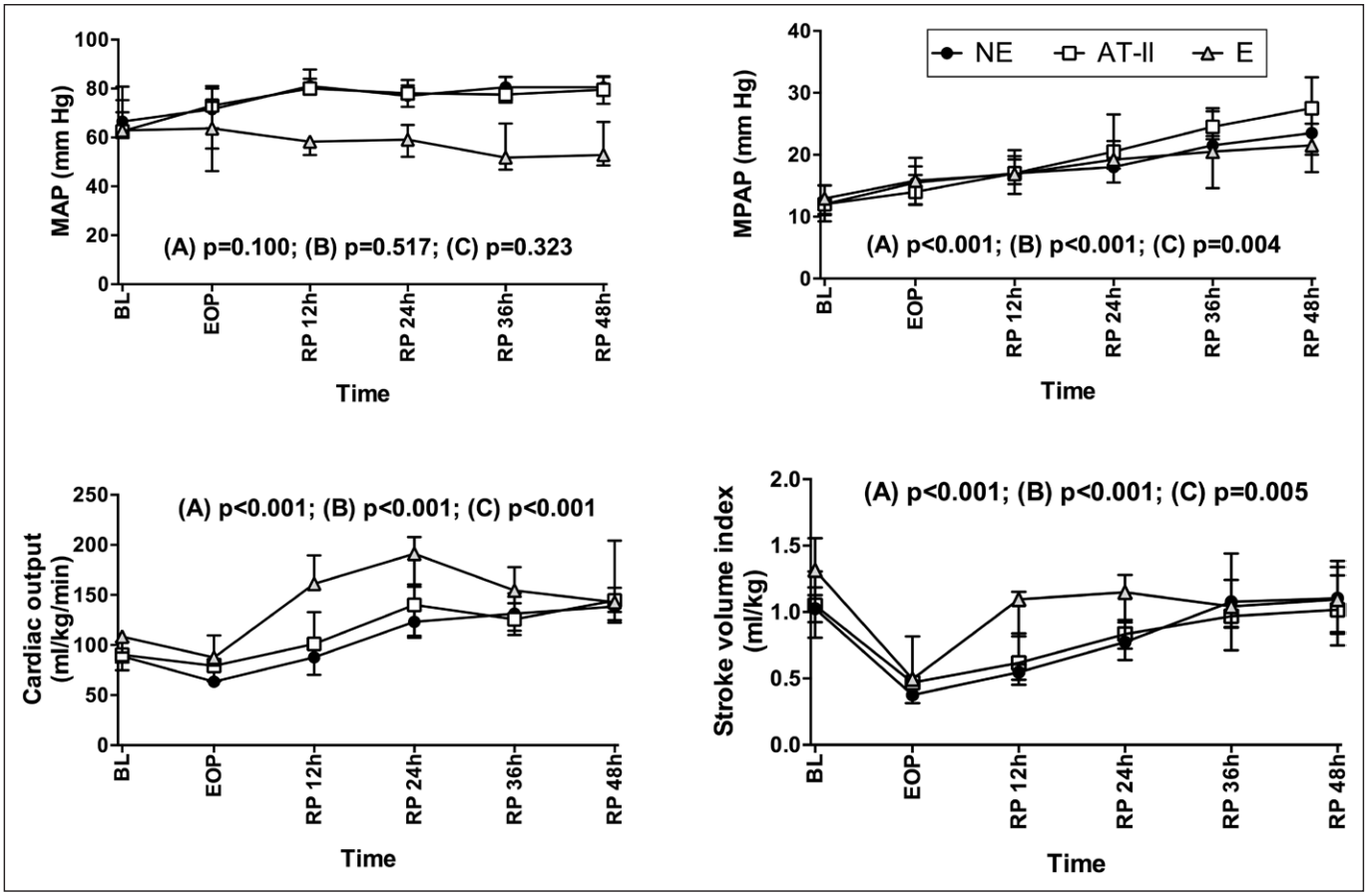

Figure 2. Systemic hemodynamics. Values are medians with interquartile ranges. NE = norepinephrine group, AT-II = angiotensin II group, E = enalapril + norepinephrine group, BL = baseline, EOP = end of observation period, RP = resuscitation period, MAP = mean arterial blood pressure, MPAP = mean pulmonary artery pressure. p: time effect with Friedman's test including EOP, RP 12 hr, RP 24 hr, RP 36 hr, and RP 48 hr for NE (A), AT-II (B), and E (C).

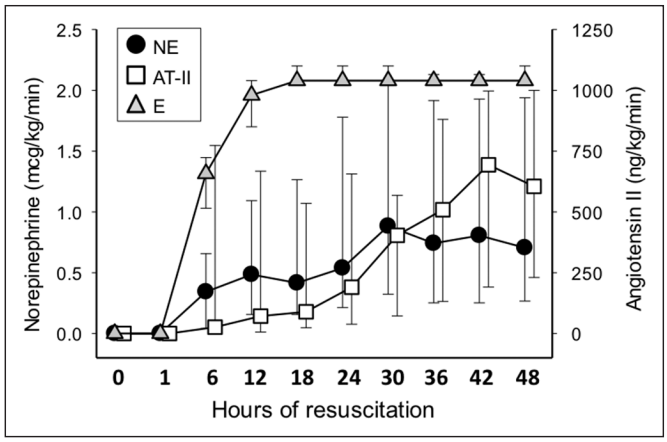

Figure 3. Administered vasopressors during the 48 hr of protocolized resuscitation. Values indicate medians with interquartile ranges. NE = norepinephrine group, AT-II = angiotensin II group, E = enalapril + norepinephrine group.

needs of angiotensin II over time to maintain blood pressure in our and other studies (9, 10) was due to angiotensin receptor down-regulation.

Classically, acute renal dysfunction during sepsis has been attributed to reduced kidney blood flow as a result of systemic vasodilation with low blood pressure in combination with renal vasoconstriction due to increased renal sympathetic activity and angiotensin plasma concentrations (20). Recently, this view has been challenged by experimental evidence of increased renal blood flow and renal vasodilation during continuous lipopolysaccharide (LPS) infusion (21). This may suggest that low intraglomerular perfusion pressure is an important contributor to sepsis-induced acute kidney dysfunction. Angiotensin II with its predominant effects on efferent renal arterioles has therefore the potential to restore intrarenal hemodynamics and improve kidney function (9).

The reduced mortality in patients with community-acquired pneumonia (22) and multiple organ failure (23) treated with angiotensin-converting enzyme inhibitors has been related to anti-inflammatory properties of angiotensin converting enzyme inhibitors and their ability to restore deteriorated autonomic function (24–26). In our study, we found that inhibition of angiotensin-converting enzyme before and during sepsis worsened the severity of hypotension and reduced the response to norepinephrine. Accordingly, in this clinically relevant sepsis model, the endogenous renin-angiotensin system appeared to be relevant for restoring hemodynamic stability during the first 48 hours of resuscitation. The protective effects of angiotensin-converting enzyme inhibition in sepsis, if any, are therefore unlikely to be related to hemodynamics.

TABLE 3. Inflammatory Markers and Hematology

Variables	Group	Baseline	End of the Observation Period	End[a]	*p*[b]
Interleukin-6 (pg/mL)	NE	14 (7–21)	521 (391–1,059)	85 (71–138)	0.008[c]
	AT-II	18 (14–39)	486 (255–721)	104 (95–142)	0.008[d]
	E	17 (17–27)	480 (284–624)	72 (57–217)	0.039[e]
Tumor necrosis factor-α (pg/mL)	NE	76 (65–88)	194 (176–310)	110 (85–130)	0.008[c]
	AT-II	86 (70–94)	198 (168–290)	124 (103–151)	0.016[d]
	E	94 (72–118)	209 (176–277)	154 (110–183)	0.055[e]
Hemoglobin (g/dL)	NE	9.1 (9.0–9.8)	14.3 (14.1–14.9)	9.5 (9.1–9.8)	0.008[c]
	AT-II	8.8 (8.5–9.1)	13.7 (12.8–14.3)	8.7 (7.7–9.1)	0.008[d]
	E	9.6 (9.1–9.9)	14.5 (13.5–14.6)	9.6 (9.3–10.9)	0.008[e]
Platelet ($\times 10^9$/L)	NE	307 (261–356)	164 (122–183)	111 (99–132)	0.008[c]
	AT-II	290 (217–356)	182 (124–155)	94 (65–149)	0.008[d]
	E	371 (266–428)	247 (189–321)	139 (129–173)	0.008[e]
WBC count ($\times 10^9$/L)	NE	20.8 (15.5–22.7)	13.6 (12.0–19.5)	21.1 (17.9–34.9)	0.008[c]
	AT-II	19.0 (15.4–22.0)	10.1 (8.4–11.7)	23.8 (19.8–25.6)	0.008[d]
	E	18.8 (17.8–21.2)	13.4 (10.8–15.9)	15.4 (13.0–18.6)	0.148[e]
Bands (%)	NE	2.5 (0.8–6.0)	58.0 (47.3–65.5)	34.5 (25.5–47.3)	0.148[c]
	AT-II	5.5 (2.5–8.5)	54.5 (46.3–65.8)	26.5 (22.0–60.8)	0.148[d]
	E	1.0 (0.5–2.8)	58.3 (54.5–61.3)	22.8 (14.8–31.8)	0.008[e]
Metamyelocytes (%)	NE	0.0 (0.0–0.0)	9.5 (5.5–11.5)	0.3 (0.0–4.0)	0.008[c]
	AT-II	0.0 (0.0–0.0)	7.8 (6.0–12.5)	1.0 (0.3–4.3)	0.008[d]
	E	0.0 (0.0–0.0)	5.8 (3.3–8.0)	0.5 (0.0–3.0)	0.008[e]

NE = norepinephrine group, AT-II = angiotensin II group, E = enalapril + norepinephrine group.

[a]End of the experiment (at 48 hr of resuscitation or before death if earlier).

[b]Time effect with Wilcoxon signed rank test including end of the observation period and end of the experiment for [c]NE, [d]AT-II, and [e]E.

Values are medians with interquartile ranges.

In our study, renal plasma flow was similar in all groups at the end of the experiment, but the prevalence of AKI and fractional excretion of sodium and potassium were higher in enalapril-treated animals. Similarly, in a fluid-resuscitated ovine model of peritonitis with norepinephrine to treat hypotension, a single dose of enalapril after peritonitis induction resulted in increased serum creatinine levels compared with placebo despite similar blood pressure levels (21). This suggests an important role for intraglomerular perfusion pressure in preventing acute renal dysfunction in sepsis.

Administration of enalapril in our study was associated with increased skeletal muscle ATP content and improved renal mitochondrial respiration efficiency. It has been shown that enalapril can attenuate mitochondrial dysfunction in aging rats (27). On the other hand, incubation of isolated kidney cortex mitochondria with enalapril inhibited mitochondrial respiration, indicating a direct effect of enalapril on nicotinamide adenine dinucleotide/cytochrome c oxidoreductase (28). How the high doses of norepinephrine in the enalapril group may have interfered with mitochondrial respiration remains to be elucidated (29). Systemic and renal oxygen consumption were similar in groups with and without enalapril. Theoretically, increased fractional excretion of sodium in enalapril-treated animals should have resulted in lower renal oxygen demands because sodium reabsorption is a major determinant of kidney oxygen consumption (30). However, mismatch between renal oxygen consumption and oxygen used for active sodium reabsorption, which accounts for 80% of renal oxygen consumption, has been reported (31). It is also possible that inflammation may have increased renal basal oxygen consumption.

Unexpectedly, animals treated with enalapril received a significantly larger average hourly amount of fentanyl. This group was studied after the randomized norepinephrine and angiotensin II groups; slight genetic differences in opioid pharmacokinetics between the groups can therefore not be excluded. Furthermore, enalapril has been shown to alter cytochrome P450 activity (32). Alternatively or additionally, the low

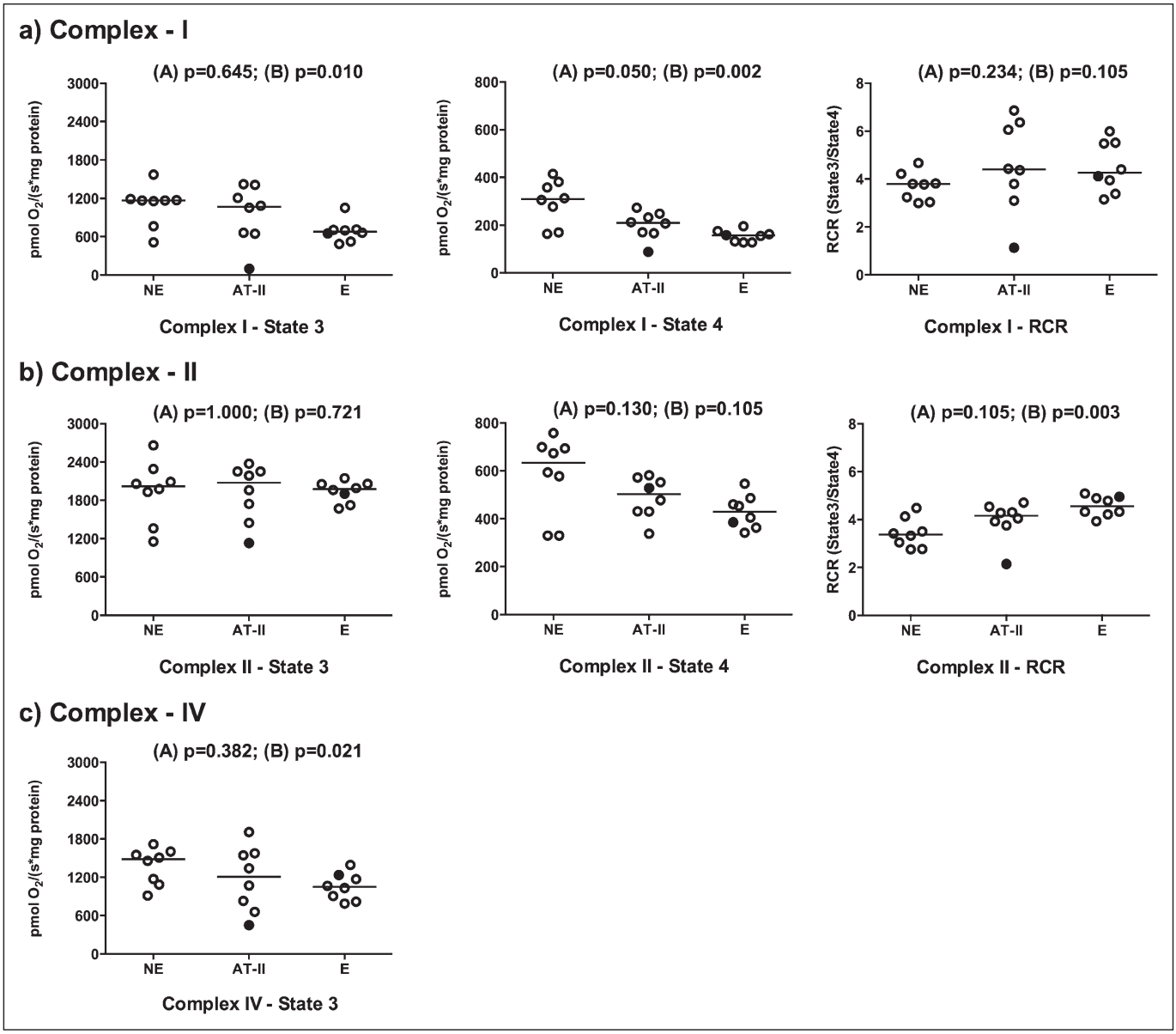

Figure 4. Isolated kidney mitochondrial respiration of complex I (**a**), complex II (**b**), and complex IV (**c**), respectively. NE = norepinephrine group, AT-II = angiotensin II group, E = enalapril + norepinephrine group. State 3 and 4 oxygen consumption is expressed as pmol/s/mg protein. State 3: active respiration after addition of adenosine diphosphate (ADP). State 4: respiration after consumption of ADP. RCR: respiratory control ratio (oxygen consumption of state 3/state 4). *Horizontal lines* represent median values. *Filled circles* represent animals that died early. *p* values: Mann-Whitney *U* test comparing (A) AT-II versus NE and (B) E versus NE.

cerebral perfusion pressure in the enalapril group and/or the high norepinephrine doses these animals received may have interfered with their reaction to painful stimuli.

All septic animals in our study needed glucose administration to maintain normal blood glucose concentrations, a feature characteristic to this porcine sepsis model. LPS infusion has been associated with reduced circulating glucose, insulin, and insulin-like growth factor-1 in pigs (33). This may be related to physiologically reduced leptin concentrations, a potent regulator of food intake and metabolism. Animals pretreated with enalapril required a significantly smaller amount of glucose infusion compared with the norepinephrine group. We can only speculate that this was related to an increased endogenous glucose production rate as a consequence of the administration of more than three times higher norepinephrine doses in this group (34, 35).

As always, the fluid management in the present study can be questioned. We used an approach adopted from the sepsis guidelines (2), with regular clinical assessment of the animals including hemodynamics, tissue perfusion variables and diuresis, and added fluid bolus only as long as cardiac output increased. We believe that increased filling pressures and cardiac output, maintained diuresis, and normal serum lactate concentrations at study end indicate that fluid management was adequate. Finally, the results from the enalapril group should be interpreted with caution since this group was not

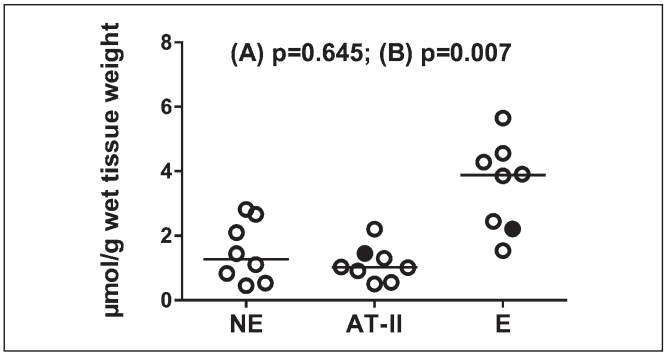

Figure 5. Adenosine triphosphate content in skeletal muscle samples at the end of the experiment ($n = 8$ per group). NE = norepinephrine group, AT-II = angiotensin II group, E = enalapril + norepinephrine group. *Horizontal lines* represent median values. *Filled circles* represent animals that died early. *p* values: Mann-Whitney *U* test comparing (A) AT-II versus NE and (B) E versus NE.

randomized. Further limitations are the relatively small group sizes and multiple testing with the risk of spurious false-positive findings.

CONCLUSIONS

In conclusion, we demonstrate that exogenous angiotensin II infusion was as efficient as norepinephrine to maintain arterial blood pressure and cardiac output. Exogenous angiotensin II did not increase the prevalence of AKI or deteriorate mitochondrial respiration. In the context of our resuscitation protocol, arterial blood pressure goals could not be achieved when angiotensin-converting enzyme was inhibited before and during sepsis. Creatinine concentrations increased despite maintained renal blood flow and urinary output, and renal mitochondrial respiration efficiency was increased with enalapril treatment. The robustness of these findings and the long-term effects of interference with the renin-angiotensin system on renal function and mitochondrial respiration in sepsis should be further evaluated.

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