Epinephrine induces tissue perfusion deficit in porcine endotoxin shock: evaluation by regional CO$_2$ content gradients and lactate-to-pyruvate ratios

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MATERIALS AND METHODS

The institutional animal care and use committee of the University of Kuopio approved the study protocol. Anesthesia. Twelve domestic female pigs (27 ± 1 kg) were used in the study. Animals were fasted for 24 h before the experiment with free access to water. The gastrointestinal tract was emptied with osmotic laxative (Colonsteril; Orion, Espoo, Finland). For the experiment, the animals were premedicated with an intramuscular injection of atropine 0.05 mg/kg. Sedation was induced with ketamine 10 mg/kg and xylazine 2 mg/kg. The ear vein was cannulated, and anesthesia was induced with sodium thiopentone 5–15 mg/kg. Anesthesia and analgesia were maintained with continuous infusions of sodium thiopentone 5 mg·kg$^{-1}$·h$^{-1}$ and fentanyl 30 μg·kg$^{-1}$·h$^{-1}$ during the surgical manipulation, after which the fentanyl infusion rate was reduced to 5 μg·kg$^{-1}$·h$^{-1}$. Tracheotomy was performed, and the lungs were ventilated with a tidal volume of 10–15 ml/kg to maintain normocapnia (arterial CO$_2$ 33.8–41.3 mmHg) and FiO$_2$ was adjusted to keep arterial partial pressure of O$_2$ above 13.3 kPa. Positive end expiratory pressure of 5–8 cmH$_2$O was applied during the experiment. Cefuroxime (Zinacef; 750 mg) was given intravenously at the beginning of the surgical procedures. Neuromuscular blockade was established with continuous infusion of pancuronium (1–4 mg/h). To limit the number of animals to a minimum, we did not randomize control groups (endotoxin without drug intervention and operated animals without endotoxin or drug intervention) for this experiment. Instead, we referred to the control animals from earlier experiments, which were completed during the same time period (18).

Animal preparation. The left femoral artery was cannulated for arterial blood sampling and for monitoring of systemic arterial blood pressure. The right jugular vein was dissected free and cannulated for pulmonary artery catheter (Arrow; Arrow International, Reading, PA) and hepatic vein catheter (model MPA2 Angiographic catheter; Boston Scientific, Maple Grove, MN) to measure pulmonary artery occlusion pressure (PAOP) and to collect blood samples. Probes for ECG and O$_2$ saturation were inserted. A full midline laparotomy was

performed, and the urinary bladder was catheterized and drained. The stomach was emptied with orogastric drain. The position of the hepatic catheter was confirmed manually.

The portal vein and hepatic arteries were dissected free. Precalibrated ultrasonic flow probes were inserted around the vessels. Mesenteric, colonic, portal, and gastric veins were cannulated (single lumen catheter; Arrow) for regional blood sampling. Microdialysis capillaries were inserted on the surface of the jejunum for intraperitoneal lactate measurement. The laparotomy was closed in two layers.

**Hemodynamic monitoring.** Systemic and pulmonary arterial pressures were recorded with quartz pressure transducers, and displayed continuously on a multimodular recorder and monitor (model CS3; Datex-Ohmeda Instrumentarium, Helsinki, Finland). Automated data filtering (2-min median) was used when the continuous parameters were collected (Deio Instrumentarium). Heart rate was measured from the ECG. PAOP was measured hourly. Cardiac output was measured by the thermodilution technique (mean value of 3 measurements) with room temperature saline injections of 5 ml.

**Blood flow measurements.** Regional blood flows were measured with ultrasonic transit-time flow probes (Transonic Systems, Ithaca, NY) from the hepatic artery and portal vein. Signals were recorded by flow meters (Flowmeters T108 and T208; Transonic Systems, Ithaca, NY). The flow data were stored with computer software (Windaq; DATAQ instruments, Akron, OH). In vivo zero flow signals were recorded at the end of each experiment.

**Fluid management.** For maintenance fluid therapy, 5 ml·kg⁻¹·h⁻¹ saline 0.9% was infused throughout the experiment. Fluid resuscitation with Ringer’s acetate and hydroxyethyl starch 1:1 (Hemohes; Braun, Melsungen, Germany) was performed to achieve PAOP > 5 mmHg with or without allowing central venous pressure to exceed 12 mmHg. Blood glucose was maintained between 4.5 and 7.0 mM with a 30% glucose infusion throughout the experiment.

**Microdialysis.** Microdialysis capillaries were manufactured in our laboratory (25). Dialysate (2 μl/min) was collected for half an hour before endotoxin infusion, before vasopressor infusion, and at the end of the experiment in 30-min fractions. Microdialysate lactate concentrations were analyzed within 15 min (model 2300 Stat Plus; Yellow Springs Instruments, Yellow Springs, OH). Microdialysate glycerol concentrations were determined (model CMA600; CMA, Stockholm, Sweden) after the samples had been stored at −20°C.

**Experimental protocol.** After the surgical procedures, the animals were stabilized for 6 h and randomized into epinephrine (n = 6) and norepinephrine (norepinephrine, n = 6) groups. Escherichia coli endotoxin (lipopolysaccharide 0111:B4; Difco Laboratories, Detroit, MI) infusion was started at a rate of 0.25 μg·kg⁻¹·h⁻¹, and after 1–3 h, the infusion rate was doubled repeatedly every 30–45 min to achieve hypotensive shock [mean systemic arterial pressure (SAPm) < 60 mmHg]. When hypotensive shock was achieved, either epinephrine or norepinephrine infusion was started (randomization using sealed opaque envelopes). We adjusted the infusion rate for 4 h aiming to maintain SAPm at >70 mmHg. Two animals in both groups received additional epinephrine (0.02 ± 0.01 mg) to prevent hemodynamic collapse during the first hour of endotoxin infusion. Duration of endotoxin infusion before hypotensive shock was 17 ± 2 h in the epinephrine group and 20 ± 1 h in the norepinephrine group (not significant). Cardiac output (CO) was measured by the bolus thermodilution method and displayed on a thermistor catheter cinoid Doppler (Transonic Systems, Ithaca, NY). Cardiac output was calculated as

\[ \text{CO} = \frac{Q_{\text{flow}}}{C_{\text{Doppler}}} \]

where \( Q_{\text{flow}} \) is the measured flow rate and \( C_{\text{Doppler}} \) is the cardiac output derived from the Doppler signal. The total splanchnic blood flow (sum of portal venous and hepatic arterial blood flows) increased in the norepinephrine group. The total amounts of epinephrine and norepinephrine infused were 6.7 ± 2.7 and 4.1 ± 0.6 mg.

**RESULTS**

All animals survived to the end of the experiment. The initial infusion rate of epinephrine and norepinephrine was 0.01 μg·kg⁻¹·min⁻¹ and the infusion rates of vasopressors necessary to reverse hypotension were 0.93 ± 0.4 μg·kg⁻¹·min⁻¹ in the epinephrine and 0.66 ± 0.1 μg·kg⁻¹·min⁻¹ in the norepinephrine group. The total amounts of epinephrine and norepinephrine infused were 6.7 ± 2.7 and 4.1 ± 0.6 mg.

**Systemic and regional hemodynamics and oxygen transport.** Both epinephrine and norepinephrine effectively reversed hypotension. Norepinephrine, but not epinephrine infusion increased cardiac output. The total splanchnic blood flow (sum of portal venous and hepatic arterial blood flows) increased in response to norepinephrine infusion (P = 0.016, Table 1). Epinephrine decreased portal venous blood flow in five of six animals over the 4-h study period, regardless of the direction of change in cardiac output (Fig. 1). Concomitantly, epinephrine maintained the total splanchnic blood flow. Epinephrine was associated with modestly higher splanchnic oxygen extraction (P = 0.078, Mann-Whitney U-test) (Table 2). In addition, regional gastric RQ increased from 0.95 ± 0.01 at baseline to 1.59 ± 0.20 after 4 h of infusion in the epinephrine group, whereas norepinephrine was associated with a lower RQ after shock and a 4-h norepinephrine infusion of 0.81 ± 0.02 at baseline and 1.07 ± 0.07 (P = 0.016 between the groups) after infusion.

**Plasma (actual) bicarbonate was calculated by the analyzer according to the formula given by the supplier.** Lactate measurements were performed immediately after collecting the samples. The pyruvate samples were analyzed within 48 h of collection. Before analysis, the samples were frozen and kept at −20°C. Hemoglobin concentration and hemoglobin oxygen saturation were determined by using an analyzer (Hemoxymeter OSM3, Radiometer) in porcine mode.

**Calculations and statistical analysis.** All data are presented as medians with interquartile ranges. Data are truncated to baseline, hypotensive endotoxin shock before drug intervention (0 h), and after 4 h from the beginning of drug infusion (4 h). The Mann-Whitney U-test was done to evaluate differences between groups at the baseline, before vasopressor infusions, and at 4 h when appropriate. Within-group analysis (changes from shock 0 h to shock 4 h) was done with nonparametric analysis of variance for repeated measurements (Friedman test). A P value of <0.05 was used to indicate statistical significance. Blood carbon dioxide contents and regional CO₂ content gradients were calculated as first devised by Giovannini et al. (8) and applied by Jakob et al. (12). First, we calculated the arterial CO₂ content from the bound form of carbon dioxide in plasma (CaCO₂p) and red blood cells (CaCO₂r), dissolved carbon dioxide, and hematocrit. We used the Henderson-Hasselbalch equation to determine CaCO₂p from the partial tension of carbon dioxide in arterial blood and arterial pH. CaCO₂p was then determined, because it is known that the ratio between CaCO₂r and CaCO₂p is a function of pH and oxygen saturation. Venous-to-arterial CO₂ content gradient was then calculated from its two components by using the mathematical model described by Giovannini et al. (8): 1) the increase related to change in partial tension of CO₂ from arterial to venous blood, and 2) the increase in CO₂ content related to change in hemoglobin oxygen saturation from arterial to venous blood (Haldane effect). Regional respiratory quotient (RQ) was calculated as the ratio of regional venous-to-arterial CO₂ content gradient to regional-to-arteriovenous O₂ content gradient. Blood O₂ content was calculated with the standard formula of CO₂ = [Hb (g/dl)·SO₂·1.34 (ml O₂/g Hb)] + [0.0031 (ml O₂·mmHg⁻¹·dl⁻¹)·PO₂].
Table 1. Systemic blood pressure, cardiac output (CO), and regional blood flows in epinephrine-treated (Epi) and norepinephrine-treated (NE) animals

<table>
<thead>
<tr>
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<th>Baseline</th>
<th>Shock 0 h</th>
<th>Shock 4 h</th>
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<tbody>
<tr>
<td>SAPm, mmHg</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Epi</td>
<td>103±8</td>
<td>57±1</td>
<td>73±1*</td>
</tr>
<tr>
<td>NE</td>
<td>109±10</td>
<td>58±2</td>
<td>74±2*</td>
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<tr>
<td>CO 1/min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epi</td>
<td>3.6±0.3</td>
<td>3.6±0.2†</td>
<td>3.9±0.7</td>
</tr>
<tr>
<td>NE</td>
<td>2.8±0.3</td>
<td>2.6±0.3</td>
<td>5.2±0.7*</td>
</tr>
<tr>
<td>Qsplanch, ml/min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epi</td>
<td>998±90§</td>
<td>985±65§</td>
<td>950±110</td>
</tr>
<tr>
<td>NE</td>
<td>668±59</td>
<td>688±103</td>
<td>1010±91*</td>
</tr>
<tr>
<td>Qporta, ml/min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epi</td>
<td>896±85</td>
<td>888±64§</td>
<td>825±98</td>
</tr>
<tr>
<td>NE</td>
<td>601±59</td>
<td>521±57</td>
<td>834±73*</td>
</tr>
<tr>
<td>Qha, ml/min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epi</td>
<td>102±13</td>
<td>97±11</td>
<td>124±16</td>
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<tr>
<td>NE</td>
<td>67±10</td>
<td>167±47</td>
<td>176±49</td>
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Values are means ± SE; n = 6 animals per group. SAPm, mean systemic arterial pressure; Qsplanch, splanchnic blood flow; Qporta, portal vein blood flow; Qha, hepatic arterial blood flow. *P < 0.05, within the group (shock 4 h–0 h, Friedman test); †P < 0.05, between the groups (Mann-Whitney U-test).

Table 2. Systemic and regional oxygen extraction ratios (OER) in Epi-treated (n = 6) and NE-treated (n = 6) animals

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Shock 0 h</th>
<th>Shock 4 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systemic OER</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epi</td>
<td>36±7</td>
<td>37±6</td>
<td>52±5</td>
</tr>
<tr>
<td>NE</td>
<td>44±6</td>
<td>48±2</td>
<td>45±3*</td>
</tr>
<tr>
<td>Splanchnic OER</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epi</td>
<td>58±4</td>
<td>68±6</td>
<td>78±8</td>
</tr>
<tr>
<td>NE</td>
<td>60±6</td>
<td>64±6</td>
<td>66±4</td>
</tr>
</tbody>
</table>

Values are means ± SE in percent; n = 6 animals per group. *P < 0.05, within the group (shock 4 h–0 h, Friedman test).

Systemic and regional CO2 exchange. Arterial PCO2 increased gradually and comparably in the two groups. Contrary to partial tension of carbon dioxide, arterial CO2 content decreased markedly in the epinephrine group compared with norepinephrine-treated animals (Fig. 2). Concomitantly, arterial plasma bicarbonate decreased by 30 ± 3% in the epinephrine group as opposed to stable bicarbonate in norepinephrine group (Fig. 2). Arterial CO2 content was highly correlated with the bicarbonate concentration in the epinephrine (r² = 0.94) but not in the norepinephrine group (r² = 0.55) (Fig. 2).

Epinephrine infusion was associated with more than twofold increase of a portal venous-to-arterial PCO2 gradient (from 6.9 ± 0.7 at baseline to 15.6 ± 2.5 mmHg at the end of the experiment, P < 0.05), whereas during the infusion of norepinephrine, the portal venous-to-arterial PCO2 gap remained low and stable (8.3 ± 0.9 at baseline vs. 9.2 ± 0.6 mmHg the end of the experiment). There was, however, only a minor (25%) increase in portal venous-to-arterial CO2 content gradient from 3.9 ± 0.3 to 4.9 ± 0.7 ml/dl in the epinephrine, compared with stable CO2 content gradient over the prehepatic tissues in the norepinephrine group (from 4.4 ± 0.4 to 3.8 ± 0.4 ml/dl). In the epinephrine group, at the end of the experiment, 20 ± 1% and in the norepinephrine group, 23 ± 2% of the CO2 content gradient over prehepatic tissues could be explained by the Haldane effect only (changing hemoglobin oxygen saturation over the tissue).

A more detailed evaluation of three regions within the visceral tissues revealed that epinephrine induced a marked increase in venous-to-arterial PCO2 gradient in the stomach and a true increase in CO2 content gradient with only 16 ± 3% accounted for by the Haldane effect compared with norepinephrine-treated animals in which both PCO2 gradient and CO2 content gradient remained low with 25 ± 1% accounted for by the Haldane effect (Fig. 3).

Mesenteric venous-to-arterial PCO2 gradient had a tendency to increase (P = 0.075) twofold after 4 h of epinephrine, whereas norepinephrine was associated with a low mesenteric PCO2 gradient. CO2 content gradient was constant in both epinephrine and norepinephrine groups (Fig. 3). The Haldane effect explained 19 ± 1 and 25 ± 1% of CO2 content gradient at the end of the experiment in the epinephrine and norepinephrine groups, respectively.

We detected a 75 ± 19% increase in portal venous-to-arterial PCO2 gradient in the colon after 4 h of epinephrine infusion during endotoxin shock. Norepinephrine was associated with stable and low colonic venous-to-arterial PCO2 gradient. In the epinephrine group venous-to-arterial CO2 content gradient remained constant, whereas in the norepinephrine group CO2 content gradient decreased by 15 with 20 ± 2% explained by the Haldane effect.

Systemic and regional lactate and pyruvate metabolism. During the 4-h period of epinephrine infusion, arterial lactate increased fivefold (P < 0.001), whereas norepinephrine did not change arterial lactate concentration. The gastric venous-to-arterial lactate gradient had a tendency to increase in animals treated with epinephrine (P = 0.075). Regional venous-to-arterial lactate gradients did not increase in any of the regions during infusion of norepinephrine (Table 3). Intraperitoneal lactate release occurred on the surface of the jejunum in the epinephrine group. Infusion of norepinephrine was not associated with intraperitoneal lactate release. (Fig. 4). Furthermore, epinephrine increased intraperitoneal glyceral concentration to...
Fig. 2. Arterial partial tension of CO2 (PCO2), arterial CO2 content, arterial (plasma) bicarbonate (HCO3⁻), and CO2 content-HCO3⁻ concentration relation in epinephrine (black, n = 6)- or norepinephrine (gray, n = 6)-treated animals at the baseline, after hypotensive endotoxin shock was established (0 h) and after 4 h of vasopressor infusion (4 h). *P < 0.05, within the group (shock 4–0 h, Friedman test). §P < 0.05, between the groups (Mann-Whitney U-test).

Fig. 3. Regional gastric (top row), jejunal (middle row), and colonic (bottom row) venoarterial (v-a) CO2 partial tension gradients (left panels), venoarterial CO2 content gradients (middle panels), and proportional magnitude of Haldane effect (right panels) in epinephrine (black, n = 6)- and norepinephrine (gray, n = 6)-treated animals at the baseline, after hypotensive endotoxin shock was established (0 h) and after 4 h of vasopressor infusion (4 h). *P < 0.05, within the group (shock 4 h–0 h, Friedman test). §P < 0.05, between the groups (Mann-Whitney U-test).
518 ± 107 μM, whereas after norepinephrine infusion, glycero remained low at 27 ± 8 μM (P < 0.05 between the groups).

The arterial lactate-to-pyruvate ratio as well as regional venous lactate-to-pyruvate ratios increased in all monitored areas in the epinephrine group, whereas there were no changes in lactate-to-pyruvate ratios in the norepinephrine group (Table 4).

**DISCUSSION**

The main findings of the present study may be summarized as follows. As expected, both epinephrine and norepinephrine reversed systemic hypotension induced by prolonged endotoxin challenge, and epinephrine infusion was associated with systemic hyperlactatemia and acidosis. Moreover, we assume that high systemic lactate concentration, apparently released from tissues other than the viscera, masked the effect of epinephrine on lactate metabolism in visceral tissues, and therefore, evaluation of tissue perfusion and metabolism by means of lactate only is limited. However, both systemic and regional venous lactate-to-pyruvate ratios (a marker of cytotoxic redox status) do suggest that epinephrine may, indeed, have untoward effects on tissue perfusion and/or metabolism. In support of this concept is the increase of, not only regional venous-to-arterial PCO2 gradients, but also CO2 content gradients with a low magnitude of the Haldane effect. In addition, intraperitoneal lactate and glycerol release may indicate anaerobic visceral metabolism and degrading cellular membranes, respectively. We suggest that epinephrine does have “superselective” regional adverse effects on gastric perfusion and metabolism within an otherwise preserved splanchnic circulation.

The limitations of this experiment must be considered: small sample size, endotoxin shock as a limited model of sepsis, and possibly species differences. Furthermore, our protocol focused strictly on gradually developing hypotensive shock (mean arterial pressure > 60 mmHg, regardless of the other hemodynamic parameters) as a trigger for vasoactive drug intervention. Apparently by chance, the systemic and regional blood flow patterns varied between the groups at the time when it became necessary to start the vasopressor infusion. However, because cardiac output and splanchnic blood flow were higher in the epinephrine group before drug intervention, it is likely that the effect, if any, of the different blood flow patterns would be favoring the epinephrine group. Secondly, all the metabolic markers indicate that the groups were comparable or favoring the epinephrine group at this time point. Finally, we cannot rule out the possibility that the increase in intraperitoneal lactate merely reflects systemic hyperlactatemia. Also, increasing glycerol may signal lipolysis rather than cellular membrane disintegration (7). More specifically, epinephrine may have stimulated glycerol transport from omental adipocytes through one of the aquaporins (15).

Both epinephrine and norepinephrine are widely used in septic shock to reverse hypotension and preserve perfusion pressure. Even recently, several investigators have addressed the question of whether epinephrine, as opposed to other vasoactive drugs, exerts harmful effects on tissue perfusion. This concern is on the basis of clinically common arterial hyperlactatemia and acidosis in conjunction with epinephrine infusion. Methods by which the assumed adverse effects have
been evaluated are various, including measurements of lactate, lactate-to-pyruvate ratio, ketone-to-body ratio, tonometry, regional blood flow, oxygen transport, and tissue perfusion (16, 17, 21, 27). We wanted to add to the knowledge of the effects of both norepinephrine and epinephrine by rigorous regional venous sampling, intraperitoneal microdialysis, and above all, regional venous-to-arterial CO2 content gradients with an estimation of the Haldane effect in a controlled randomized model of endotoxin shock.

Systemic and regional blood flow and oxygen transport. Systemic and splanchnic blood flows increased during norepinephrine infusion as opposed to epinephrine, which had no major effect on either. On the basis of these findings, one could argue that systemic and splanchnic perfusions were by and large maintained in both the epinephrine and norepinephrine groups. On the other hand, portal venous blood flow decreased in five of six animals, regardless of the direction of change in cardiac output during epinephrine infusion. Splanchnic and systemic oxygen consumption remained at the same level as before vasopressor infusions. However, systemic oxygen extraction increased up to 52% and splanchnic oxygen extraction up to 78% during epinephrine infusion. Therefore, we suggest that oxygen delivery was nearly marginal, especially in parts of the splanchnic vascular region when epinephrine was used for increasing perfusion pressure. Additionally, the calculated regional gastric RQ remained markedly higher in the epinephrine and norepinephrine groups. On the other hand, portal venous lactate-to-pyruvate ratios together with high venous lactate-to-pyruvate ratio, we interpret these findings as an indication of inadequate perfusion in gastric tissue when epinephrine is used to treat endotoxin-related hypotension. Finally, the marked increase in gastric RQ is consistent with the presence of regional hypoperfusion and hypoxia during epinephrine administration. Indeed, in these conditions, the RQ is better defined as an “exchange ratio,” because its value does not reflect a true RQ, but a series of gas exchange interactions, which commonly occur in low blood flow and tissue hypoxia (9). Another aspect and interpretation of the results is that the decreasing proportional magnitude of the Haldane effect during endotoxin shock represents low-CO2 buffering capacity of hemoglobin and other proteins as suggested by Giovannini et al. (10).

In conclusion, epinephrine induced a marked increase in lactate-to-pyruvate ratio in various visceral tissues. More importantly, increasing venous-to-arterial PCO2 gradients indicated that visceral perfusion defect might be present. High CO2 content gradients with decreasing Haldane effect and high regional venous lactate-to-pyruvate ratios together with high regional RQ pinpointed the most pronounced perfusion deficiency in the gastric wall when epinephrine, as opposed to norepinephrine, was used in endotoxin shock. In addition, intraperitoneal glycerol, a possible marker of cell membrane disintegration, increased when epinephrine was used as the vasopressor. As a clinical implication, we suggest that epinephrine may induce selective visceral tissue hypoperfusion and therefore should not be considered as a first line vasopressor.

GRANTS

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REFERENCES


