Intensive Care Med (2003) 29:292–300
DOI 10.1007/s00134-002-1611-0

EXPERIMENTAL

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Effects of epinephrine and norepinephrine on hemodynamics, oxidative metabolism, and organ energetics in endotoxemic rats

Received: 27 March 2002
Accepted: 13 November 2002
Published online: 14 January 2003
Springer-Verlag

An editorial regarding this article can be found in the same issue (http://dx.doi.org/10.1007/s00134-002-1596-8).

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Abstract *Objective:* To determine whether epinephrine increases lactate concentration in sepsis through hypoxia or through a particular thermogenic or metabolic pathway.
Design: Prospective, controlled experimental study in rats.
Setting: Experimental laboratory in a university teaching hospital.
Interventions: Three groups of anesthetized, mechanically ventilated male Wistar rats received an intravenous infusion of 15 mg/kg *Escherichia coli* O127:B8 endotoxin. Rats were treated after 90 min by epinephrine (n=14), norepinephrine (n=14), or hydroxyethyl starch (n=14). Three groups of six rats served as time-matched control groups and received saline, epinephrine, or norepinephrine from 90 to 180°min. Mean arterial pressure, aortic, renal, mesenteric and femoral blood flow, arterial blood gases, lactate, pyruvate, and nitrate were measured at baseline and 90 and 180 min after endotoxin challenge. At the end of experiments biopsy samples were taken from the liver, heart, muscle, kidney, and small intestine for tissue adenine nucleotide and lactate/pyruvate measurements.
Measurements and results: Endotoxin induced a decrease in mean arterial pressure and in aortic, mesenteric, and renal blood flow. Plasmatic and tissue lactate increased with a high lactate/pyruvate (L/P) ratio. ATP decreased in liver, kidney, and heart. The ATP/ADP ratio did not change, and phosphocreatinine decreased in all organs. Epinephrine and norepinephrine increased mean arterial pressure to baseline values. Epinephrine increased aortic blood flow while renal blood low decreased with both drugs. Plasmatic lactate increased with a stable L/P ratio with epinephrine and did not change with norepinephrine compared to endotoxin values. Nevertheless epinephrine and norepinephrine when compared to endotoxin values did not change tissue L/P ratios or ATP concentration in muscle, heart, gut, or liver. In kidney both drugs decreased ATP concentration.
Conclusions: Our data demonstrate in a rat model of endotoxemia that epinephrine-induced hyperlactatemia is not related to cellular hypoxia.

Keywords Tissue lactate concentration · Tissue adenosine 5′-triphosphate concentration · Epinephrine · Septic shock · Lactate/pyruvate ratio

Introduction

The circulatory, metabolic, and energy impairments observed in septic shock patients are generally the result of a bacterial challenge. The human response to this challenge usually includes increased cardiac output, decreased peripheral resistance, and low arterial pressure. In such situations vasopressor catecholamines are currently recommended after volume loading [1]. Indeed catecholamines exhibit major effects on the cardiovascular system, flow distribution, cellular metabolism, and immunity [2]. Among these, epinephrine and norepinephrine are widely used in septic shock treatment. The receptor profile of epinephrine is complex, and its pharmacological effects are dose-dependent. At low doses epinephrine acts predominantly on the peripheral β_1- and β_2-adrenergic receptors. However, as the dose of epinephrine is increased, the α_1-adrenergic receptor-mediated vasoconstrictor effects predominate. Moreover, epinephrine exhibits major metabolic effects and increases glucose and lactate concentration through an increase in hepatic glucose output. Epinephrine also stimulates the release of lactate from skeletal muscle in a dose-dependent manner for oxidation or for gluconeogenesis (Cori cycle). Conversely, norepinephrine has a small effect on carbohydrate metabolism and acts predominantly on the peripheral α_1-adrenergic receptors. Indeed, while demonstrating similar effects on systemic hemodynamics, epinephrine and norepinephrine-dobutamine do not induce identical effects on lactate metabolism and regional flow [3]. In contrast to norepinephrine-dobutamine, when used in septic shock, epinephrine increases lactate level with a slightly enhanced lactate/pyruvate (L/P) ratio, decreases global splanchnic flow [4] and elevates tonometric mucosal PCO_2 gap (tonometer PCO_2, $PaCO_2$), a surrogate marker of gastric mucosal metabolism and/or perfusion [3, 4].

The Task Force of the American College of Critical Care Medicine and the Society of Critical Care Medicine used these observations to recommend the use of epinephrine only in patients who fail to respond to traditional therapies [1]. Nevertheless, invasive arterial and pulmonary artery monitoring, although essential to the rational management of septic shock, may take time to establish. Fluid therapy and vasoactive therapy may be immediately required to maintain an acceptable blood pressure. In this setting there is good reason to choose a broad spectrum catecholamine such as epinephrine or dopamine rather than a pure α-adrenergic agonist which could cause substantial reductions in cardiac output or in place of a pure β-agonist such as dobutamine which can exacerbate vasodilation and hypotension through its β_2-adrenergic action. Moreover, epinephrine is commonly used worldwide, and its reported mortality does not differ from that other vasopressors [5]. In dopamine-resistant septic shock Duranteau et al. [6] demonstrated that epinephrine increases gastric blood flow more than norepinephrine using gastric laser Doppler and gastric tonometry, suggesting that the epinephrine-induced increase in PCO_2 gap is due to the calorigenic effects of epinephrine [7]. In cardiosurgical patients Totaro et al. [8] demonstrated that epinephrine increases lactate levels without any change in L/P ratio. Thus it is unclear whether epinephrine increases lactate concentration through hypoxia or through a metabolic pathway.

The aim of this experimental study was to compare the effects of epinephrine and norepinephrine using an endotoxic shock model on (a) global and regional hemodynamics, (b) systemic and tissue lactate and pyruvate levels, and (c) tissue high energy phosphates. This approach may provide new insights into the question of whether changes in lactate concentration in epinephrine-treated rats are more likely related to direct effects on glycolysis or to cellular hypoxia.

Methods

The experiments were conducted in adherence to the Guiding Principles on the Care and Use of Animals prescribed by the American Physiological Society.

Animal preparation and monitoring

Wistar rats (300–320 g) fasted overnight were anesthetized with thiopental sodium (30 mg/kg intraperitoneally). Additional doses were given when necessary (indicated by presence of interdigital reflexes). Tracheostomy was performed, and the animals were ventilated. Right jugular vein and carotid artery were cannulated. Rectal temperature was monitored continuously and maintained at 37°C. Abdominal aortic blood flow as a reflection of cardiac output and renal, mesenteric, and right femoral blood flows were measured with a perivascular probe and a small animal flowmeter (T206, Transonic Systems, Ithaca, N.Y., USA). Arterial blood pressure was monitored continuously using a disposable pressure transducer (5265014 Viggo-Spectramed, Bilthoven, The Netherlands), allowing us to use a short arterial line (6 cm), and an amplifier-recorder system (Sirecust 302A, Siemens, Berlin, Germany). Arterial blood samples were used to determine pH, aO_2, and $PaCO_2$.

Experimental protocols

The model used was previously validated in our laboratory as a hypotensive and hypodynamic model inducing lactic acidosis and decreased muscular blood flow and muscle pH [9, 10].

Three groups of six rats served as time-matched control groups and received, respectively, saline, epinephrine (Epi), or norepinephrine (Nor) from 90 to 180 min. Previous reports [11, 12] have shown epinephrine at both 0.2 and 1 $\mu g\ kg^{-1}\ min^{-1}$ to increase lactate levels. We investigated in four rats the effects of epinephrine and norepinephrine at 0.2 and 1 $\mu g\ kg^{-1}\ min^{-1}$ on mean arterial pressure (MAP), heart rate, and lactate level (data not shown). Although 1 $\mu g\ kg^{-1}\ min^{-1}$ epinephrine increased lactate levels to a higher extent, it also increased the heart rate by more than 30%, leading us to choose 0.2 $\mu g\ kg^{-1}\ min^{-1}$. No difference was found for norepinephrine between 0.2 and 1 $\mu g\ kg^{-1}\ min^{-1}$.

Endotoxic shock was induced by 15 mg/kg endotoxin (*Escherichia coli* O127:B8, Sigma, St. Quentin Fallavier, France) intravenously infused from 0 to 20 min. All the rats received hydroxyethyl starch (5 ml/h). From 90 to 180 min the endotoxin-treated rats randomly received hydroxyethyl starch (5 ml/h; Endo, n=14), epinephrine (Endo-Epi, n=14), or norepinephrine (Endo-Nor, n=14) titrated to increase MAP to baseline values (±10%). The same amount of fluid was infused in all rats, i.e., 5 ml/h. The assignment of treatment was randomized and the investigator was blinded to the treatment [13]. Glucose, lactate, pyruvate, acetoacetate, β-hydroxybutyrate, nitrates and arterial gas were measured at baseline 90 and 180 min after endotoxin infusion.

Analytic sampling procedures

Analysis of pyruvate, β-hydroxybutyrate, and acetoacetate

Pyruvate, β-hydroxybutyrate, and acetoacetate: arterial blood samples were immediately deproteinized by addition of iced perchloric acid (1 mol/l) and immediately analyzed. Pyruvate was measured by enzymatic UV method. β-Hydroxybutyrate and acetoacetate were measured by enymatic method using β-hydroxybutyrate-dehydrogenase reaction involving NADH for acetoacetate and NAD for β-hydroxybutyrate. The range of normal values is 40–68 µmol/l for pyruvate, 20–80 µmol/l for acetoacetate, and 60–170 µmol/l for β-hydroxybutyrate. The arterial ketone body ratio was defined as the ratio between acetoacetate and β-hydroxybutyrate [14].

The analytical range is 0–10,000 µmol/l for lactate, 0–300 µmol/l for pyruvate, 10–500 µmol/l for acetoacetate, and 10–2000 µmol/l for 3-hydroxybutyrate. Run-to-run precision expressed as coefficient of variation is 1.5% for lactate, 5.9% for pyruvate, 7.1% for acetoacetate, and 6.6% for 3-hydroxybutyrate.

Serum nitrates were measured according to the method of Green et al. [15]. Serum samples were deproteinized before analysis by using sulfosalicylic acid, centrifuged, and added to a buffer containing 5% NH_4Cl and 5%NaOH. Samples were injected onto a column filled with copper-plated cadmium filings to reduce nitrates to nitrites. The column effluent was mixed with Griess reagent. Nitrite concentration was determined by measurement of the absorbance at 546 nm and compared with a standard solution of sodium nitrate. Normal values were less than 40 µmol/l. Equivalent volumes of isotonic saline replaced the withdrawn blood.

Tissue adenine nucleotide and lactate/pyruvate measurements

At the end of the experiments, with the animal still alive, tissue samples were rapidly taken with stainless steel tongs precooled in liquid nitrogen from the liver, right kidney, jejunum, leg muscle, and heart, immediately frozen in liquid nitrogen, and stored at 80°C. Two separate experimenters took part in the sampling procedure in order to keep its total duration below 2 s. ATP, ADP, AMP, and 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 mm and 210 nm. Tissue pyruvate and lactate were assayed enzymatically as previously described. 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 adenine nucleotide pool (ATP plus ADP plus AMP), the ATP/ADP ratio, and the adenylate energy charge [ATP+0.5 ADP)/(ATP+ADP+AMP] [16].

Data presentation and statistics

Results are expressed as mean ±SD. A repeated-measures one-way analysis of variance was used to evaluate within-group differences. Difference between groups was tested using a two-way analysis of variance (repeated time measurements and treatment as independent variables). When the F values were significant at the 5% level, further pairwise comparisons were made using Dunnett's test for the effect of time and Bonferroni's correction for the effects of treatment at specific times. Linear regression analysis was used to assess the relationship between blood flow and ATP value. Differences were considered statistically significant at $p<0.05$.

Results

Systemic and regional hemodynamics

Saline

MAP, heart rate, and aortic, mesenteric, renal, and femoral blood flow did not change during the experimentation period (Figs. 1, 2, 3).

Epinephrine and norepinephrine

Both drugs induced a slight increase in MAP (from 118±11 and 120±9 to 129±11 and 130±8 mmHg, respectively, $p<0.05$). Aortic, renal, mesenteric, and femoral blood flow slightly increased ($p<0.05$; Figs. 1, 2, 3).

Endotoxin

Heart rate increased from 369±9 to 430±14 bpm, $p<0.05$). MAP decreased dramatically during endotoxin infusion from 115±11 to 76±8 mmHg (vs. control values, $p<0.01$; Fig. 4). Aortic blood flow decreased ($p<0.01$; Fig. 4). Mesenteric and renal blood flow decreased without variation in the regional to systemic ratio ($p<0.01$; Figs. 5, 6). Femoral blood flow and the femoral/aortic blood flow ratio fell (approx. 40% and 50% vs. control values, respectively, $p<0.05$).

Endo-Epi and Endo-Nor

The mean efficient dose of epinephrine and norepinephrine was 2.8±0.4 and 3.1±0.4 µg kg^{-1} min^{-1}, respectively. Both drugs increased heart rate (from 370±8 and 360±9 to 431±10 and 420±12 bpm, respectively, $p<0.05$). Both drugs increased MAP to baseline values ($p<0.01$; Fig. 4). Epinephrine slightly increased aortic blood flow compared to norepinephrine ($p<0.05$; Fig. 4). Epinephrine and norepinephrine did not change mesenteric blood flow vs. endotoxin (Fig. 5). Nevertheless, the

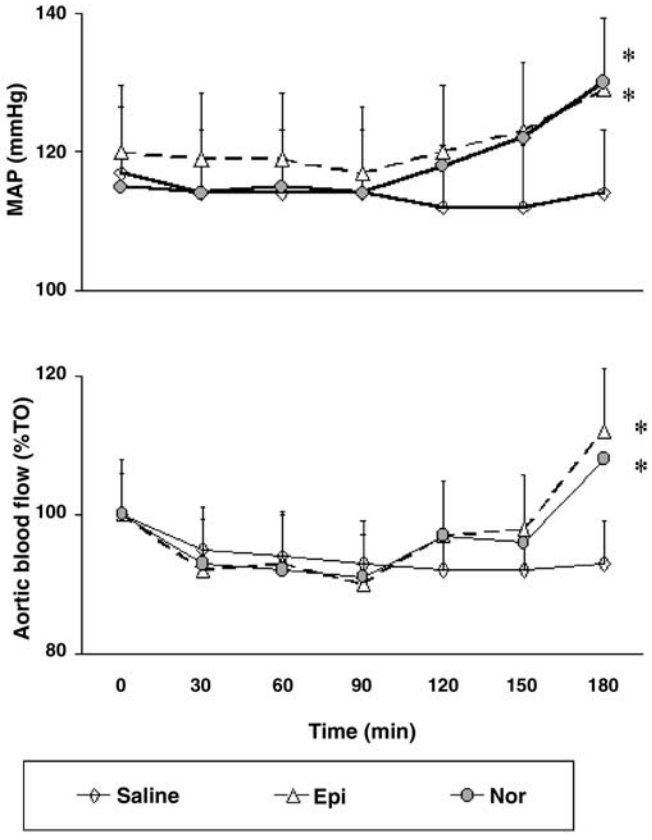

Fig. 1 Time course of mean arterial pressure and aortic blood flow in control rats without treatment (saline) or treated from 90 to 180 min with epinephrine or norepinephrine. Values are mean ±SD. *p<0.05 vs. control group

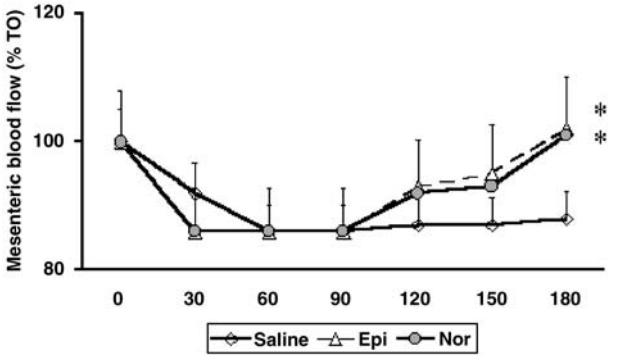

Fig. 2 Time course of mesenteric blood flow in control rats without treatment (saline) or treated from 90 to 180 min with epinephrine or norepinephrine. Values are mean ±SD. *p<0.05 vs. control group

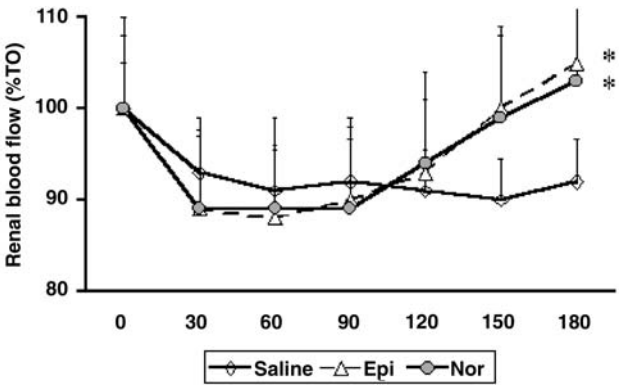

Fig. 3 Time course of renal blood flow in control rats without treatment (saline) or treated from 90 to 180 min with epinephrine or norepinephrine. Values are mean ±SD. *p<0.05 vs. control group

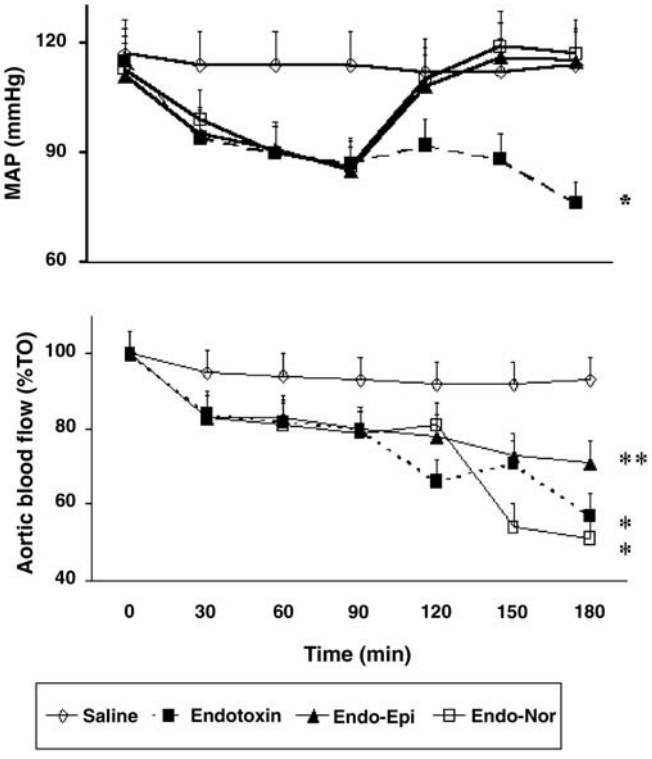

Fig. 4 Time course of mean arterial pressure and aortic blood flow in control rats (saline) and endotoxemic rats without treatment (endotoxin) or treated from 90 to 180 min with epinephrine (*Endo-Epi*) or norepinephrine (*Endo-Nor*). Values are mean ±SD. *p<0.05 vs. control group, **p<0.05 vs. control, endotoxin, and Endo-Nor group

mesenteric/aortic blood flow ratio slightly decreased in the epinephrine group (p<0.05) but did not change in the norepinephrine group. Renal blood flow (Fig. 6) and the renal/aortic blood flow ratio decreased with epinephrine and norepinephrine (p<0.01). Femoral blood flow decreased in the norepinephrine group (approx. 10%, vs. endotoxin values, p<0.05) and slightly increased in the epinephrine group (approx. 10%, vs. endotoxin values, p<0.05).

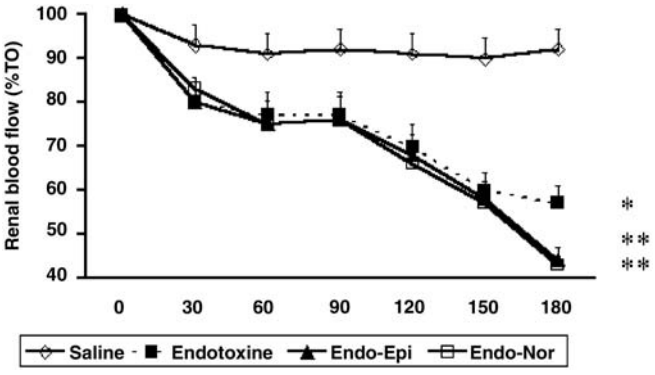

Fig. 5 Time course of mesenteric blood flow in control rats (saline) and endotoxemic rats without treatment (endotoxin) or treated from 90 to 180 min with epinephrine (*Endo-Epi*) or norepinephrine (*Endo-Nor*). Values are mean ±SD, *p<0.05 vs. control group

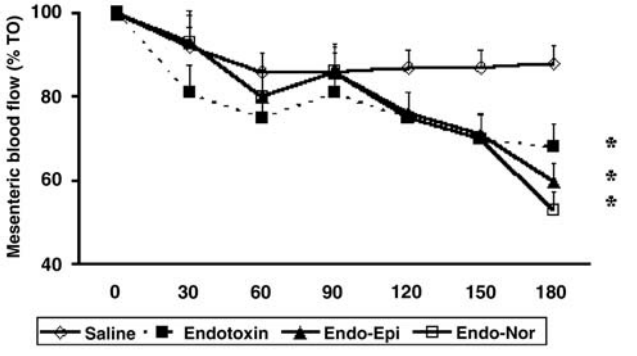

Fig. 6 Time course of renal blood flow in control rats (saline) and endotoxemic rats without treatment (endotoxin) or treated from 90 to 180 min with epinephrine (*Endo-Epi*) or norepinephrine (*Endo-Nor*). Values are mean ±SD. *p<0.05 vs. control group, **p<0.05 vs. endotoxin group.

Acid-base equilibrium and lactate metabolism

Arterial bicarbonate remained stable in control group (24±2 to 25±3 mmol/l) and decreased in all endotoxin groups (24±2 to 16±34 mmol/l in endotoxin group, 26±2 to 15±4 mmol/l in epinephrine group and 25±2 to 16±3 mmol/l in norepinephrine group, p<0.05). Arterial pH was stable in control group (7.35±0.01 to 7.35±0.01), decreased in endotoxin, epinephrine and norepinephrine groups, respectively (7.35±0.02 to 7.26±0.06, 7.35±0.02 to 7.22±0.06, and 7.36±0.02 to 7.22±0.08, p<0.05). Lactate levels did not increase in the saline group and slightly increased (p<0.05) with a normal L/P ratio in the epinephrine-alone treated group. Lactate concentration and L/P ratio increased in endotoxin-treated rats (p<0.05). With respect to endotoxin values lactate increased during epinephrine infusion (p<0.05) without change in the L/P ratio. Lactate and the L/P ratio did not change during norepinephrine infusion. The arterial ketone body ratio did not change in controls and decreased in all endotoxin-treated rats (p<0.05; Table 1).

Tissue lactate and lactate/pyruvate ratio

In the epinephrine-alone group epinephrine increased muscular lactate without change in the L/P ratio. In endotoxin-treated rats lactate and the L/P ratio increased in all organs (p<0.05; Table 2). With respect to endotoxin values no changes occurred in catecholamine-treated rats.

Adenine nucleotide, phosphocreatinine, and energy charge

No change occurred in adenine nucleotide levels in saline, epinephrine, and norepinephrine groups. In the endotoxin groups no change occurred in muscle. ATP concentration and energy charge of liver decreased in all endotoxin treated groups (p<0.05) without any change in the ATP/ADP ratio or in the adenine nucleotide pool. In

Table 1 Time course of arterial metabolic parameters. Values at baseline and 180 min in controls (*saline*) in controls treated with epinephrine (*Epi*) or norepinephrine (*Nor*) and endotoxemic rats without treatment (*Endo*) or treated from 90 to 180 min with epinephrine (*Endo-Epi*) or norepinephrine (*Endo-Nor*)

	Glucose (mmol/l)		Lactate (mmol/l)		Lactate/pyruvate ratio		Arterial ketone body ratio	
	Baseline	180 min	Baseline	180 min	Baseline	180 min	Baseline	180 min
Saline	2.34±0.30	2.56±0.50	1.6±0.7	1.9±1.0	16±5	19±7	0.92±0.3	1.1±0.5
Epi	2.40±0.32	3.50±0.70*	1.5±0.6	2.1±0.5*	15±5	16±4	1.2±0.4	1.4±0.5
Nor	2.40±0.40	2.60±0.50	1.5±0.5	1.6±0.4	16±4	17±5	1.1±0.4	0.9±0.5
Endo	2.20±0.50	2.45±0.60	1.5±0.7	4.5±2.1*	16±4	31±12*	1.39±0.4	0.60±0.4*
Endo-Epi	2.30±0.40	4.60±1.30*	2.0±0.8	5.1±2.0**	15±3	28±9*	1.1±0.5	0.5±0.2*
Endo-Nor	2.50±0.50	3.70±0.50*	1.3±0.4	4.4±2.1*	16±4	32±12*	0.95±0.5	0.50±0.3*

*p<0.05 vs. baseline in the same group, **p<0.05 vs. baseline in the same group and compared with other groups

Table 2 Course of tissue lactate (nmol/mg wet/weight) and lactate/pyruvate (*L/R*) ratio. At baseline and 180 min in control (*saline*) and endotoxemic rats without treatment (*Endo*) or treated from 90 to 180 min with epinephrine (*Endo-Epi*) or norepinephrine (*Endo-Nor*)

	Muscle	Liver	Gut	Kidney	Heart
Lactate					
Saline	2.2±1.2	1.2±0.7	2.0±0.9	0.75±0.4	1.2±0.3
Epi	4.2±1.3*	1.4±0.6	1.8±0.7	0.80±0.5	1.1±0.2
Nor	2.7±1.0	1.3±0.5	1.9±0.8	0.60±0.2	1.5±0.3
Endo	7.1±2.0*	4.2±1.2*	4.3±0.9*	3.6±1.5*	7.3±2.2*
Endo-Epi	6.1±2.0*	4.8±1.3*	6.6±1.5*	4.2±1.1*	5.0±1.5*
Endo-Nor	6.3±2.0*	4.4±1.2*	5.2±1.1*	4.1±1.2*	6.0±1.2*
L/P ratio					
Saline	22±8	24±6	14±5	19±10	17±8
Epi	19±6	22±5	14±5	17±8	18±9
Nor	20±5	22±7	15±4	18±9	20±9
Endo	82±12*	60±14*	29±9*	42±12*	35±9*
Endo-Epi	76±15*	70±14*	33±7*	80±18*	42±10*
Endo-Nor	80±14*	75±19*	32±9*	85±12*	40±9*

*$p<0.05$ vs. saline

kidney endotoxin, norepinephrine and epinephrine decreased ATP concentrations, but the decrease was higher in catecholamine-treated rats ($p<0.05$). ATP in heart decreased in all endotoxin-treated rats ($p<0.05$; Table 3). Phosphocreatinine (PCr) did not change in control rats but decreased in all organs during endotoxemia ($p<0.05$). Renal energy charge decreased in norepinephrine-treated rats. No change occurred in muscle, small intestine, or heart energy charge during endotoxin challenge and treatments. The ATP/ADP ratio and adenine nucleotide pool did not change in any of the groups (Table 3). Aorta blood flow was correlated with liver ($r=0.65$, $p<0.001$), kidney ($r=0.55$, $p<0.01$), and heart ($r=0.407$, $p<0.05$) ATP. Renal blood flow was correlated with kidney ATP level ($r=0.75$, $p<0.01$). Mesenteric blood flow was not correlated with gut ATP level.

Course of nitrate concentration

After 3 h of endotoxin administration plasmatic nitrate increased from 8±5 to 113±20 μmol/l ($p<0.01$). No change occurred during catecholamine administration. Nitrate levels did not change in the control group throughout the time-course of the experiment.

Table 3 High-energy phosphate concentration in control and endotoxemic rats: ATP (nmol/mg wet weight), ATP/ADP ratio, adenine nucleotide pool (nmol/mg wet weight), and energy charge. At baseline and 180 min in control (*saline*) and endotoxemic rats without treatment (*Endo*) or treated from 90 to 180 min with epinephrine (*Endo-Epi*) or norepinephrine (*Endo-Nor*)

	Muscle	Liver	Gut	Kidney	Heart
ATP					
Saline	4.8±1	2.6±0.15	1.13±0.1	1.10±0.10	3.7±0.4
Epi	5.0±1	2.8±0.16	1.02±0.1	1.05±0.10	3.5±0.4
Nor	5.0±0.8	2.7±0.13	1.09±0.1	1.00±0.10	3.8±0.4
Endo	5.0±0.5	1.5±0.10*	1.15±0.1	0.80±0.20*	3.0±0.4*
Endo-Epi	5.1±1	1.3±0.12*	0.90±0.1	0.70±0.10**	2.7±0.3*
Endo-Nor	5.0±0.8	1.3±0.14*	1.13±0.1	0.45±0.05**	2.5±0.4*
ATP/ADP					
Saline	4.5±0.6	2.4±0.3	4.1±0.4	2.5±0.1	1.8±0.1
Epi	4.3±0.6	2.3±0.3	4.3±0.4	2.3±0.1	1.9±0.1
Nor	4.2±0.6	2.2±0.3	4.1±0.3	2.4±0.1	2.0±0.1
Endo	4.1±0.4	2.3±0.2	4.2±0.3	2.7±0.1	1.6±0.1
Endo-Epi	4.5±0.5	2.0±0.3	4.1±0.4	2.0±0.1	1.3±0.1
Endo-Nor	4.7±0.5	2.2±0.2	4.2±0.3	2.3±0.1	1.7±0.1
Adenine nucleotide pool					
Saline	6.0±0.5	3.5±0.2	2.7±0.4	2.2±0.4	6.5±0.1
Epi	6.2±0.5	3.6±0.2	2.9±0.5	2.0±0.3	6.8±0.1
Nor	6.1±0.5	3.6±0.2	2.8±0.4	2.0±0.4	6.6±0.1
Endo	5.2±0.4	3.1±0.4	2.6±0.5	2.2±0.3	5.7±0.2
Endo-Epi	5.7±0.5	3.0±0.5	2.3±0.4	2.0±0.4	5.2±0.3
Endo-Nor	5.4±0.6	3.0±0.4	2.1±0.3*	1.7±0.3	5.2±0.4
Energy charge					
Saline	0.9±0.01	0.85±0.01	0.82±0.03	0.84±0.02	0.82±0.02
Epi	0.9±0.01	0.88±0.01	0.84±0.03	0.85±0.02	0.84±0.02
Nor	0.9±0.01	0.86±0.01	0.82±0.03	0.86±0.02	0.83±0.02
Endo	0.9±0.01	0.48±0.02*	0.80±0.02	0.71±0.01	0.78±0.01
Endo-Epi	0.9±0.01	0.45±0.02*	0.74±0.02	0.71±0.02	0.72±0.02
Endo-Nor	0.9±0.01	0.50±0.01*	0.80±0.02	0.65±0.02*	0.80±0.02

*$p<0.05$ vs. saline,
**$p<0.05$ vs. Endo

Discussion

The major aim of this investigation was to determine whether exogenous catecholamines have a detrimental effect on lactate metabolism and high energy phosphate in a rat model of endotoxemia. Our results establish that endotoxin have major effects on lactate metabolism and decreased high energy phosphate in liver, heart, and kidney. Conversely, epinephrine and norepinephrine have minor effects on metabolism and only impaired high energy phosphate in kidney.

Characterization of the septic model

The model used was previously characterized as a hypotensive and hypodynamic model. Despite volume loading endotoxin decreased both MAP and aortic blood flow. This may be due to a combination of decreased afterload and decreased myocardial contractility. The decrease in mesenteric and renal blood flows was entirely related to the decrease in aortic blood flow since the regional to systemic ratio did not change. Conversely, muscular blood flow decreased with a decrease in the systemic to regional ratio arguing for blood flow redistribution.

Effect of endotoxin on lactate and ketones metabolism

In the present study blood lactate levels during endotoxin challenge dramatically increased with elevated L/P ratio. This increase was paralleled in tissue since lactate and the L/P ratio also increased in all organs. In this respect our results confirm previously published results [17, 18]. This increase in the tissue L/P ratio argues for an hypoxic or a cytopathic mechanism. The L/P ratio is of particular interest in evaluating the NADH/NAD ratio. Since it cannot be directly measured, the NADH/NAD ratio can be estimated in cytosol from the L/P ratio and in mitochondria from the acetoacetate/β-hydroxybutyrate ratio. We recently demonstrated that the L/P and arterial ketone body ratios are, respectively, elevated and decreased in the early phase of catecholamine treated septic shock [19], suggesting that lactic acidosis is associated with a decrease in cytoplasmic and mitochondrial redox state. In the present model, the decrease in redox potential might be related to an hypoxic mechanism through a decrease in mean perfusion pressure and cardiac output or through a direct cytopathic effect of endotoxin [20].

We also demonstrated a decrease in the arterial ketone body ratio. This ratio depends mainly on hepatic blood flow and hepatic mitochondrial function. These observations, i.e., an increase in the L/P ratio, decrease in the arterial ketone body ratio associated with a decrease in liver ATP, and unchanged liver ATP/ADP ratio, suggest an ischemic or hypoxic mechanism.

Effect of endotoxin on ATP and PCr levels

Data relating to the effects of sepsis on tissue levels of adenine nucleotides are plentiful but conflicting and thus difficult to interpret [21]. One reason for the variance in results may be the septic models that were employed, i.e., the species, doses of lipopolysaccharide, intensity of resuscitation, early or late sepsis, and degree of hypotension. Therefore our results should be cautiously interpreted with regards to our model.

Endotoxin decreased ATP levels in heart, kidney, and liver and had no effects on muscle ATP. These findings are in accordance with those of previous studies on energy metabolism in endotoxic or bacteremic shock [17, 22, 23]. In these organs the ATP content in our control and endotoxemic rats agrees perfectly with that reported in these studies. By contrast, we did not find any variations in gut ATP content in our experimental model. Gut ATP in the sepsis model has not been extensively studied. Liaudet et al. [24] and Schmidt et al. [25] in a less severe model of rodent endotoxemia found a decrease in ATP content in the jejunum. This discrepancy with our results might be explained by the difference in biopsy timing, a higher resuscitated model (5 vs. 2 ml/h), or a selective decrease in mucosal ATP [26]. In the present study ATP/ADP ratios remained unchanged, indicating an intact phosphorylation in the respiratory chain [27, 28].

Effects of epinephrine and norepinephrine on hemodynamics

Both drugs increased MAP to near baseline values. Nevertheless, aortic blood flow was better maintained with epinephrine. On the other hand, norepinephrine had no effect on mesenteric blood flow partition while epinephrine decreased the mesenteric/aortic blood flow ratio, confirming experimental [29] and human data. In norepinephrine-treated rats aortic blood flow decreased at the expense of femoral blood flow. Conversely, in epinephrine groups the slight increase in aortic blood flow was beneficial to femoral blood flow. Both drugs decreased the renal/aortic blood flow ratio when compared to endotoxin alone, suggesting a vasoconstrictor effect on kidney vascular bed. It is clear that in the present model the effects of agents with strong β- adrenergic effects as epinephrine may have more beneficial effects in an hypodynamic model of shock than agents with less potent β effect than norepinephrine. Such an effect may limit the translation of these results to patients in hyperdynamic septic shock.

Effects of epinephrine and norepinephrine on lactate metabolism

As previously demonstrated [11, 12], epinephrine but not norepinephrine increased lactate levels in healthy and endotoxin treated rats. Epinephrine significantly increased both lactate and pyruvate levels leading to stable a L/P ratio. Conversely, muscular lactate in the control-Epi group was increased, confirming that the observed lactate increase in epinephrine-treated rats was due mainly to muscle production. Chasiotis et al. [30] demonstrated that glycogenolysis in muscle is the likely cause of the increased lactate release after epinephrine infusion. Similar results were found in cardiosurgical patients [8]. In human septic shock epinephrine increases lactate levels without any increase in the L/P ratio when the latter is normalized to pH [3]. Physiologically, epinephrine enhances glycogenolysis with a net increase in pyruvate production. This mechanism is mediated via a β_2-adrenergic-mediated stimulation of muscle and hepatic phosphorylase, and inhibition of glycogen synthetase. Moreover, epinephrine stimulates Na^+-K^+-ATPase activity in isolated skeletal muscles [31]. This effect is mimicked by other β-adrenergic agonists and by analogues of cyclic AMP and is blocked by β-receptor blockade or by inhibition of the Na^+-K^+-ATPase with ouabain. These observations suggest that epinephrine increases ATP consumption by the Na^+-K^+ pump, thereby stimulating muscle glycolysis and lactate production in a glycolytic metabolic compartment [32]. Stimulation of Na^+-K^+-ATPase would generate ADP, thereby raising phosphofructokinase activity and accelerating aerobic glycolysis and thus increasing lactate level [33]. Additionally, epinephrine inhibits the pyruvate dehydrogenase complex. This action limits pyruvate oxidation rate. Epinephrine stimulates the release of lactate from skeletal muscle in a dose-dependent manner for oxidation or for gluconeogenesis (Cori cycle). Additionally, the epinephrine-induced increase in lactate concentration was identical in control and endotoxin-treated rats (±0.6 mmol/l) despite a tenfold higher dose of epinephrine. Our data confirm that endotoxin-treated rats are less responsive to an epinephrine-induced increase in lactate concentration [12]. This might be due to the difference between high-dose endotoxin and human sepsis since endotoxin overwhelmed the capability of the animal to mobilize its normal physiological adaptation and defense mechanisms. Nevertheless, similar data were obtained with dobutamine in metabolic-induced stress in human volunteers [34]. Similar effects on the L/P ratio were found on tissue levels: neither drug in endotoxin-treated rats increased the L/P ratio compared to endotoxin alone except for kidney, where the L/P ratio increased significantly in drug-treated rats. The increase in L/P level in the kidney may be related to an hypoxic state since it was associated with a major decrease in renal perfusion.

Effects of epinephrine and norepinephrine on ATP, energy charge, and PCr levels

In muscle, heart, liver, and gut neither drug had an effect on ATP levels. Nevertheless, during catecholamine administration, cell necrosis may have occurred especially in the gut and interfered with tissue lactate or ATP since dead cells do not produce lactate or consume ATP. On the other hand, in kidney the increase in lactate and L/P levels was associated with a decrease in ATP in the norepinephrine and epinephrine groups. Thus it can be concluded that in this hypotensive and hypokinetic model of endotoxinic shock epinephrine and norepinephrine have detrimental effects on renal perfusion and renal energetic state. In similar circumstances dopexamine [35] has also proven its effectiveness in improving renal blood flow and oxygenation.

In summary, our study demonstrates that epinephrine and norepinephrine have favorable effects on MAP and aortic blood flow. The distribution of blood flow differed depending on the use of epinephrine or norepinephrine. The use of epineprine was associated with an increase in lactate level with a stable L/P ratio. Epinephrine or norepinephrine use was not associated with deleterious effects on tissue lactate [36] or high-energy phosphate except in kidney where both drugs had some adverse effects through a selective decrease in renal perfusion. Hence our data demonstrate that epinephrine-induced hyperlactatemia is probably related to direct effects on carbohydrate metabolism and not to cellular hypoxia.

Acknowledgements The authors thanks Pr. O. Lesur (Sherbrooke, Québec, Canada) and Pr. R. Nevière (Lille, France) for their helpful comments. This work was carried out in the Laboratoire d'Exploration Fonctionnelle Rénale, Faculté de Médecine, Vandoeuvre-les-Nancy, France. The study was supported in part by a grant from Nestle-Perrier France.

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