Regional Sympathetic Blockade Attenuates Activation of Intestinal Macrophages and Reduces Gut Barrier Failure

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ABSTRACT

Background: Endotoxin-induced activation of monocytes may lead to extravasation of cells, excessive production of nitric oxide, and subsequent epithelial injury in the gut. Regional sympathetic blockade by means of thoracic epidural anesthesia has been implicated to protect the epithelial barrier. This study tested the hypothesis that thoracic epidural anesthesia decreases epithelial permeability by attenuating monocytic production of nitric oxide and nitrosative stress.

Methods: Rats were anesthetized, hemodynamically monitored, and mechanically ventilated. Endotoxemia was induced by an intravenous bolus injection of Escherichia coli lipopolysaccharide. Either lidocaine 2% or normal saline was injected as a bolus, followed by a continuous infusion via an epidural catheter. Three hundred minutes after injection of lipopolysaccharide or normal saline, gut epithelial permeability to fluorescein isothiocyanate-dextran (4 kDa), intestinal expression of inducible nitric oxide synthase by macrophages, and lipid peroxidation represented by 8-isoprostane tissue concentration were quantified.

Results: Thoracic epidural anesthesia significantly attenuated the endotoxin-induced increases in gut epithelial permeability (437 [293, 492] vs. 628 [532, 1,042] ng/ml, median [quartiles], P = 0.03), expression of nitric oxide synthase (2 [1,2] vs. 7 [5,8] cells per 384 µm², P = 0.003), macrophage infiltration, and lipid peroxidation (22,460 ± 11,476 vs. 37,840 ± 17,551 pg/ml, mean ± SD, P = 0.05).

Conclusions: Thoracic epidural anesthesia attenuates endotoxin-induced gut epithelial injury. This is likely due to a decrease in monocytic extravasation and intestinal nitrosative stress. As possible mechanisms, direct nerve-immune interplay, a reduction in plasma catecholamines, or a systemic lidocaine effect has to be considered.

What We Already Know about This Topic
- Increased intestinal epithelial permeability associated with bacterial translocation may occur during systemic inflammation, hemorrhagic shock, and other pathophysiologic conditions. Thoracic epidural anesthesia has been shown to increase intestinal perfusion and to attenuate leukocyte adhesion.

What This Article Tells Us That Is New
- This study in normotensive rats exposed to endotoxemia shows that thoracic epidural anesthesia decreases epithelial permeability, monocytic extravasation, and inducible form of nitric oxide synthase expression in the gut.

INCREASED intestinal epithelial permeability has been reported in various pathophysiologic conditions, such as ileus, hemorrhagic shock, and systemic inflammation. Animal models of systemic inflammation have shown the association of increased intestinal permeability and bacterial translocation, which may contribute to multiple organ failure. Intravenous administration of lipopolysaccharide (LPS) is a widely used model of systemic inflammation. It is associated with activation of monocytes, which recognize the bacterial antigen via toll-like receptor 4 and cluster of differentiation 14. Subsequent immune cell adhesion to the vascular endothelium, extravasation to intestinal tissue, and excessive production of nitric oxide by the inducible form of nitric oxide synthase (iNOS) in macrophages may affect epithelial cells in the gut and induce gut barrier dysfunction.

Regional sympathetic blockade by means of thoracic epidural anesthesia (TEA) has been shown to increase intestinal...
perfusion \(^7\)–\(^10\) attenuate leukocyte adhesion \(^11\)–\(^12\) and reduce patient morbidity \(^13\) in systemic inflammation. Furthermore, a protective effect on the epithelial barrier has been suggested in rabbits subjected to hypoxia \(^14\) and endotoxemia \(^15\). However, the mechanism involved in this latter phenomenon remains unclear.

The release of nitric oxide and inflammatory mediators by macrophages seems to be a crucial step in the dysregulation of tight junction proteins, which are responsible for the intestinal barrier function. \(^16\)–\(^17\) We, therefore, tested the hypothesis that TEA decreases epithelial permeability by reducing macrophage-derived nitrosative stress. In a rodent model of normotensive endotoxemia, effects of TEA on intestinal epithelial permeability, iNOS expression by macrophages, nitric oxide metabolites, and lipid peroxidation in intestinal tissue were quantified.

Materials and Methods

Animals

We used male Sprague-Dawley rats \((297 \pm 30 \text{g, mean } \pm \text{SD})\), which were purchased (Charles River Laboratories International, Inc., Sulzfeld, Germany) and housed in our animal facility under standardized conditions. All animals were handled according to the National Research Council’s guide on the care and use of laboratory animals \(^18\) and had free access to standard chow (Altromin Spezialfutter GmbH und Co.KG, Lage, Germany) and water \textit{ad libitum}. All experiments were approved by the local animal care committee (Landesamt für Gesundheit und Soziales, Berlin, Germany).

Experimental Protocol

General anesthesia was provided by a subcutaneous injection of urethane \((1.5 \text{g/kg, Sigma-Aldrich-Chemie GmbH, Deisenhofen, Germany})\) and ketamine \((50 \text{mg/kg, Pharmacia & Upjohn GmbH, Erlangen, Germany})\). Ketamine was supplemented approximately every 2 h by \(15–30 \text{mg/kg}\). Under general anesthesia, epidural catheterization, tracheotomy, as well as vascular cannulation, were performed. Recording of hemodynamic values was started, and animals received an intravenous bolus injection of \(5 \text{ml of NaCl 0.9% solution (Boehringer Ingelheim KG Delta-Pharma, Pfulling, Germany). Then, a continuous intravenous infusion of NaCl 0.9% (6 ml/h) and mechanical ventilation with an oxygen air mix (30/70%) were started. After a stabilization period of 15 min, blood was analyzed, and animals were randomly assigned to the following groups (table 1): group LPS+/TEA− \(\text{n = 16}\) received an intravenous bolus injection of \textit{Escherichia coli} LPS \((50 \text{mg/kg, serotype 026:B6, Sigma-Aldrich})\) and an epidural bolus injection \((30 \text{µl})\) of NaCl 0.9%, followed by a continuous infusion \((30 \text{µl/h, Genie Plus, Kent Scientific Corporation, Torrington, CT})\). In group LPS+/TEA+ \(\text{n = 12}\), endotoxin injection was paralleled by an epidural infusion of lidocaine 2% \((\text{B. Braun Melsungen AG, Melsungen, Germany})\) in analogous volumes to NaCl 0.9%. Group LPS−/TEA− \(\text{n = 9}\) received NaCl 0.9% instead of endotoxin and lidocaine. Three hundred minutes after injection of LPS or NaCl 0.9%, blood was analyzed and a loop of ileum was ligated for determination of epithelial permeability. Then blood and organs were harvested, shock frozen in liquid nitrogen, and stored at \(-80°C\) until quantification of nitric oxide metabolites and lipid peroxidation.

For immunohistochemical analysis of iNOS expression, five additional animals of each group were used. At the end of the experimental protocol, animals were transcardially perfused with 60 ml phosphate-buffered saline \((\text{PBS, pH = 7.4, Biochrom AG, Berlin, Germany})\) and 300 ml cold PBS containing paraformaldehyde 4% \((\text{fixative solution})\). Then three pieces of ileum \((\text{approximately 3 cm of length})\) were excised, postfixed at \(4°C\) for 90 min in fixative solution, and cryoprotected overnight at \(4°C\) in PBS containing sucrose 10%. The tissues were then embedded in \textit{Tissue-Tek® O.C.T.™} compound \((\text{Miles Laboratories Inc., Elkhart, IN})\) and frozen at \(-80°C\) until further processing. Then 8-micron-thick sections prepared on a cryostat were mounted onto gelatine-coated slides.

Instrumentation

Animals were tracheotomized and intubated using a polyethylene tube \((\text{PE 205, Portex Ltd., Hythe, United Kingdom})\). Epidural catheterization was performed as described before. \(^10\) Briefly, a polyethylene catheter of 200 mm length \((\text{PE 10, Portex})\) with a dead space of 5 µl was placed via a midline incision along the spinal processes of L2–S1. Subsequent suture and tape fixation prevented displacement of the catheter. After each experiment, the correct position of the catheter in the epidural space was confirmed by autopsy with a mean segmental level of T7. Former experiments had revealed a minimum spread of lidocaine to segments T4 to L1 associated with a plasma concentration of lidocaine of 0.33 µg/ml in endotoxemic rats. \(^10\) TEA in those experiments significantly reduced endotoxin-induced plasma concentration of adrenaline. \(^10\)

Table 1. Experimental Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Intravenous LPS Bolus</th>
<th>Epidural Infusion</th>
<th>n</th>
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<tbody>
<tr>
<td>LPS+/TEA−</td>
<td>50 mg/kg in 1 ml NaCl 0.9%</td>
<td>NaCl 0.9%, 30 µl bolus followed by 30 µl/h</td>
<td>16</td>
</tr>
<tr>
<td>LPS+/TEA+</td>
<td>50 mg/kg in 1 ml NaCl 0.9%</td>
<td>Lidocaine 2%, 30 µl bolus followed by 30 µl/h</td>
<td>12</td>
</tr>
<tr>
<td>LPS−/TEA−</td>
<td>1 ml NaCl 0.9%</td>
<td>NaCl 0.9%, 30 µl bolus followed by 30 µl/h</td>
<td>9</td>
</tr>
</tbody>
</table>

LPS = lipopolysaccharide; TEA = thoracic epidural anesthesia.
The right carotid artery and both external jugular veins were cannulated (PE 50, Portex) for monitoring of blood pressure and infusion of fluids or central venous pressure monitoring, respectively. Continuous hemodynamic monitoring was performed using pressure transducers (model p23 ID, Gould-Statham Instruments Inc., Hato Rey, Puerto Rico) connected to arterial and venous lines via three-way cocks and a recorder (Gould Pilot, Gould Instruments, Bal-lainvilliers, France). Mechanical ventilation with an oxygen/air mix (30/70%, Animal Respirator Advanced 4601-1, TSE Systems GmbH, Bad Homburg, Germany) was controlled by expired gas analysis (TSE Systems) to provide normoventilation. Arterial blood was analyzed for blood gases, acid base status, electrolytes (RapidlabTM 348, Chiron Diagnostics GmbH, Fernwald, Germany), and blood cell counts (Coulter® Ac-T™ Analyzer, Beckman Coulter, Inc., Miami, FL). Body temperature was kept at 36°C throughout the experiment using a warming pad.

**Quantification of Intestinal Epithelial Permeability**

Epithelial permeability was assessed using a ligated loop model as described elsewhere. Briefly, the ileum was incised and ligated at both ends of a 10-cm-long loop with the distal incision 5 cm proximal to the cecum. Before tying the ligature, feces were removed by cautiously flushing the segment with PBS, and a solution of 4-kDa fluorescein isothiocyanate–conjugated dextran (10 mg in 1 ml of PBS; Sigma-Aldrich) was injected into the intestinal segment via a flexible cannula. The gut loop was then wrapped in wet gauze and covered with a plastic film. After 30 min of incubation, blood was sampled from the portal vein and centrifuged to separate the plasma. The plasma was stored at –80°C until quantification of fluorescence with a spectrofluorometer (excitation at 480 nm, extinction at 520 nm, FluoroMax-2, TECAN Deutschland GmbH, Crailsheim, Germany). Absorption was measured at 405 nm.

**Immunohistochemistry**

For immunohistochemical detection of iNOS, tissue specimens were prepared as described previously. Endogenous peroxidase was inhibited by incubating with 10% (vol/vol) H2O2 for 30 min. To prevent nonspecific binding, tissue sections were incubated for 60 min in PBS containing 0.3% Triton X-100, 1% bovine serum albumin, and 10% goat serum. Then, the tissue sections were incubated overnight at room temperature with a polyclonal rabbit anti-rat iNOS (Santa Cruz Biotechnology, Inc., Heidelberg, Germany, dilution 1:1,000) antiserum supplemented with 3% normal goat serum and 0.3% Triton X-100. Thereafter, the slides were washed in PBS for 6 × 10 min and exposed to the appropriated biotinylated secondary antibody goat anti-rabbit immunoglobulin (Vector Laboratories, Inc., Burlingame, CA) for 1–2 h. After washing in PBS for 3 × 10 min, tissue sections were then incubated with avidin–biotin-conjugated peroxidase for 45 min. Finally, the sections were washed and stained with 3',3'-diaminobenzidine tetrahydrochloride (Sigma-Aldrich) containing 0.01% H2O2 in 0.05 M Tris-buffered saline (pH 7.6) for 3–5 min. After the enzyme reaction, the sections were washed in tap water, counterstained with thionin, then dehydrated in alcohol, cleared in xylene, and mounted in dibutyl-phthalate-polyisystrene-xylene (Merck KGA, Darmstadt, Germany). Quantification of iNOS-immunoreactive cells was performed by an examiner blinded to the protocol as described previously. The mean number of iNOS-immunoreactive cells were counted in four sections per animal and 10 squares (384 μm2 each) (objective ×20; eyepiece ×10). Immune cells were identified by the following morphologic criteria: (1) as polymorphonuclear leukocytes by large cell bodies and multisegmented nuclei; (2) as macrophages/monocytes by large cell bodies, vacuolated cytoplasm, and irregular-shaped nuclei; and (3) as lymphocytes by small cell bodies, large nuclei, and small amounts of cytoplasm.

**Quantification of Nitric Oxide Metabolites and Lipid Peroxidation**

Tissue concentrations of nitric oxide metabolites and 8-isoprostanate, a product of lipid peroxidation, were quantified in shock frozen tissue specimen stored at –80°C until further processing. For quantification of nitric oxide metabolites, tissue samples were homogenized in PBS and centrifuged at 10,000 g for 20 min. The supernatant was ultrafiltered with 10 kDa cut-off filter units (Millipore GmbH, Schwalbach, Germany). Samples then were assayed for nitrate and nitrite content using a colorimetric assay (Cayman Chemical Company, Ann Arbor, MI). Absorption was measured at 540 nm (Spectra Rainbow Thermo Reader and Magellan Software V 2.22, TECAN Deutschland GmbH, Crailsheim, Germany). For quantification of 8-isoprostanate, intestinal samples were homogenized in a buffer containing sodium hydrogen phosphate, EDTA, and indomethacin and centrifuged at 20,000 g for 15 min. The supernatant was analyzed for 8-isoprostanate concentration using a colorimetric assay (Cayman Chemical). Absorption was measured at 405 nm.

**Statistical Analysis**

All tests were performed using Sigma Stat 3.10 (Systat Software GmbH, Erkrath, Germany). A sample size analysis using SD values from the literature generated a sample size of six animals. For randomization of animals we used a closed envelope protocol. The LPS-/TEA– group was excluded from the randomization procedure when interim analysis of data revealed that variability of epithelial permeability in endotoxemic animals was higher than assumed. Due to a time delay between the animal experiments and the quantification of epithelial permeability and due to dropouts in the analysis of single samples, group sizes became inhomogeneous and differed between variables, respectively.
Retrospective sample size analysis yielded a sample size of 12 animals assuming an SD of 40%, a difference in mean values of 25%, a probability of error of 5%, and a power of 80%.

Normality of data was tested using the Kolmogorov–Smirnov analysis. Hemodynamic variables were analyzed using a Friedman repeated measures ANOVA on ranks with Tukey post hoc analysis. Hemodynamic data were depicted as mean and SEM for clear presentation. Other data were tested using a one-way ANOVA (with Student–Newman–Keuls as post hoc analysis) or a Kruskal–Wallis ANOVA on ranks (with Dunn test as post hoc analysis) as appropriate. Intragroup comparisons in laboratory data were performed using the Wilcoxon test, followed by the Bonferroni post hoc analysis. The comparison between arterial and portal venous values in epithelial permeability was performed using the Mann–Whitney test, followed by the Bonferroni post hoc analysis. To calculate exact P values, nonparametric data presented in the abstract were analyzed analogously. For all statistical tests, significance was assumed at P values less than 0.05.

Results
The amount of missing values was 1.59%. Missing values were not handled with a complete case analysis.

Hemodynamic Variables
Mean arterial pressure increased in all groups after bolus administration of NaCl 0.9% and transiently decreased with bolus injection of LPS in endotoxemic groups (fig. 1A). The combination of LPS administration and sympathetic blockade led to the most pronounced decrease in mean arterial pressure. Blood pressure was stable during the further course of the experiments in all groups.

Heart rate at baseline was higher in LPS−/TEA− animals without any changes during the experiment (fig. 1B). Heart rate increased similarly in both endotoxemic groups after the administration of LPS.

Central venous pressure remained stable throughout the experiments in all groups, without differences among groups (fig. 1C).

Laboratory Variables
All laboratory variables were comparable among groups at baseline (table 2). Arterial partial pressure of oxygen increased throughout the experiment and was higher in endotoxemic animals with sympathetic blockade compared to healthy controls after 300 min of endotoxemia (table 2). All animals were normoventilated at 300 min of endotoxemia, but pH decreased due to metabolic acidosis with lower base excess values in the endotoxemic groups (table 2). Sodium plasma concentration showed only small increases at 300 min of endotoxemia, whereas blood cell counts were lower in endotoxemic animals compared to healthy controls (table 2).

Epithelial Permeability
Plasma concentration of fluorescent dye after permeation from the gut lumen was determined in arterial and portal venous blood (fig. 2). Concentration of dye was significantly increased only in arterial and venous blood of endotoxemic animals without sympathetic blockade. TEA significantly attenuated the increase of dye concentration in portal venous blood (fig. 2).
Intestinal Expression of iNOS

Expression of iNOS in intestinal tissue was increased in all animals receiving endotoxin (fig. 3, A and B) compared to healthy controls (fig. 3C). However, the expression was significantly higher in endotoxemic animals without TEA (fig. 3A) compared to animals with TEA (fig. 3B).

Histomorphologic Identification of Macrophages in Intestinal Tissue

Cells expressing iNOS could be identified as macrophages according to the above described histomorphologic criteria (for macrophages: by large cell bodies, vacuolated cytoplasm, and irregular-shaped nuclei). The number of macrophages equaled the number of cells expressing iNOS. As a consequence, quantification of macrophage infiltration was analogous to quantification of iNOS expression (graph not displayed, fig. 3).

Blood and Tissue Concentrations of Nitric Oxide Metabolites

Concentrations of nitric oxide metabolites were significantly increased in blood and all investigated tissues of endotoxemic animals without sympathetic blockade. Values were lower in TEA animals without reaching statistical significance (table 3).

Intestinal Lipid Peroxidation

In endotoxemic animals without TEA, tissue concentration of intestinal 8-isoprostane was significantly higher compared to both other groups. TEA animals showed values comparable to healthy animals (fig. 4).

Discussion

This experimental animal study investigated the effects of regional sympathetic blockade on endotoxin-induced epithelial permeability and intestinal inflammation. We demonstrated in endotoxemic rats that TEA decreased gut...
epithelial permeability, monocytic iNOS expression, and nitrosative stress.

**Hemodynamic Variables**

Bolus injection of endotoxin led to a short transient decrease in mean arterial pressure and a lasting increase in heart rate (fig. 1, A and B). Hypotension associated with bolus injection of endotoxin has been discussed to be mediated by a sudden release of nitric oxide and prostacyclin from vascular endothelium. After this short period of hypotension, endotoxemic animals showed mean arterial pressure values comparable to healthy control animals, consistent with a model of normotensive endotoxemia. Heart rate baseline values were higher in healthy animals compared to both endotoxemic groups, although all animals did not differ in treatment at that time point. Heart rate did not change in healthy animals, whereas it increased in animals after endotoxin injection. Tachycardia is the heart’s response to systemic inflammation and has been demonstrated to go along with increases in plasma catecholamines in former experiments.

Epidural bolus infusion of lidocaine had a synergistic hypotensive effect, which can be well explained by blockade of the sympathetic innervation of resistance vessels. However, mean arterial pressure stabilized quickly also in TEA animals without intergroup differences in heart rate, implying that the heart was outside the sympathetic block. This is in line with the results of former experiments on the epidural spread of lidocaine in the same rat model of TEA. Furthermore, stable mean arterial pressure and comparable central venous pressure values (fig. 1C) between groups demonstrate adequate volume therapy in all animals.

**Laboratory Variables**

All animal groups presented with mild acidosis at the end of the experiment (table 2). This can be attributed to the infusion of normal saline, which is known to cause hyperchloremic acidosis with greater volumes infused. Normal saline is one of the standard fluids used for volume replacement in animal experiments; nevertheless, using

![Fig. 3. Immunohistochemical staining and quantification of inducible nitric oxide synthase in the gut (brown color) and identification of macrophages in intestinal tissue. iNOS = inducible nitric oxide synthase; LPS = lipopolysaccharide; TEA = thoracic epidural anesthesia. A, Group LPS+TEA-. B, Group LPS+/TEA+. C, Group LPS-/TEA-. Arrows show examples of macrophages expressing iNOS. Scale bar = 20 µm. Data in the graph are median, quartiles and range. *P < 0.05 versus LPS-/TEA−, # P < 0.05.](image)
Epidural Anesthesia Protects Gut Epithelial Barrier

A balanced fluid acidosis might have been prevented. Furthermore, the use of a colloidal resuscitation fluid instead of normal saline would have significantly decreased the amount of resuscitation fluid. Base excess values were lower in endotoxemic animals (table 2) consistent with a well-described mitochondrial effect of endotoxin, leading to an increase in serum lactate. Lower pH values were found in TEA animals (table 2), which also had highest values in partial pressure of carbon dioxide, albeit in the normal range.

The number of all blood cells decreased with the course of endotoxemia (table 2), which can be interpreted as adhesion of these cells to the vascular endothelium.

Intestinal Epithelial Permeability
Injection of endotoxin induced a significant increase in epithelial permeability (fig 2). Endotoxin-induced epithelial permeability to intraluminal fluorescent dye was significantly decreased by TEA (fig. 2). This is in line with the results of Kosugi et al., who found less epithelial permeability and also demonstrated less histologic signs of tissue injury with TEA in endotoxemic rabbits. We were able to differentiate between the portal and the systemic circulation and found less intergroup differences in the systemic circulation, which may be due to hepatic clearance of the dye. Although not investigated in our study, bacterial translocation to the systemic circulation may have been affected by TEA, postulating a strong correlation of epithelial permeability and bacterial translocation in rats.

As a possible cellular mechanism for the increase in epithelial permeability during endotoxemia, excessive production of nitric oxide in monocytic cells with a subsequent damage of epithelial tight junction proteins has been described. Intestinal Monocytic Cell Infiltration and Production of Nitric Oxide

Our experiments demonstrated that endotoxemia was associated with increased infiltration of iNOS-expressing macrophages in intestinal tissue (fig. 3). The inflammatory pathway after injection of E. coli LPS has been described in detail. It includes binding of LPS to a binding protein and initiating a mediator cascade via toll-like receptor 4 on monocytes. One consequence is the excessive production of the reactive nitrogen species nitric oxide by monocytic cells. The detection of an increased amount of nitric oxide metabolites in different tissues demonstrated the systemic inflammatory effect of LPS (table 3).

TEA was associated with less tissue infiltration of iNOS-expressing macrophages (fig. 3) and a smaller amount of nitric oxide metabolites in organs inside and outside the sympathetic block (table 3). The attenuation effect of TEA on nitric oxide production in different tissues, including the intestine, was less pronounced than the effect on intestinal iNOS expression and did not reach statistical significance. This could be due to other sources of nitric oxide production, such as endothelial cells, mast cells, and smooth muscle cells, which may not have been influenced by TEA. Nevertheless, an effect of TEA on nitric oxide production is visible in all organs.

Effects on macrophages in organs within the sympathetic block may be explained by a direct communication between

### Table 3. Blood and Tissue Concentration of Nitric Oxide Metabolites

<table>
<thead>
<tr>
<th>LPS+/TEA−</th>
<th>LPS+/TEA+</th>
<th>LPS−/TEA−</th>
</tr>
</thead>
<tbody>
<tr>
<td>Portal venous blood, µM/l</td>
<td>108 (91,134) *</td>
<td>71 (58,102) *</td>
</tr>
<tr>
<td>Intestine, nM/g tissue</td>
<td>133 (96,207) *</td>
<td>104 (93,116) *</td>
</tr>
<tr>
<td>Liver, nM/g tissue</td>
<td>66 (59,79) *</td>
<td>45 (27,67)</td>
</tr>
<tr>
<td>Lung, nM/g tissue</td>
<td>111 (103,139) *</td>
<td>91 (76,103) †</td>
</tr>
<tr>
<td>Heart, nM/g tissue</td>
<td>123 (97,130) *</td>
<td>81 (71,104) †</td>
</tr>
<tr>
<td>Kidney, nM/g tissue</td>
<td>107 (95,120) *</td>
<td>94 (85,99) *</td>
</tr>
</tbody>
</table>

Data are median and quartiles.
* P < 0.05 vs. LPS−/TEA−; † P < 0.05 vs. LPS+/TEA−.
LPS = lipopolysaccharide; TEA = thoracic epidural anesthesia.

Fig. 4. Intestinal concentration of lipid peroxidation product 8-isoprostane. LPS = lipopolysaccharide; TEA = thoracic epidural anesthesia; Data are mean and SD. * P < 0.05 versus LPS−/TEA−; † P < 0.05.
sympathetic nerves and immune cells (nerve-immune interplay), which has been previously demonstrated for the parasympathetic system.\textsuperscript{34,35}

Organs outside the blocked region (as demonstrated for nitric oxide metabolites in heart and lung), however, can only be influenced by a systemic effect. A possible explanation is that TEA reduces tissue infiltration of nitric oxide-producing monocyctic cells via a decrease in catecholamine plasma concentration. Catecholamines have been demonstrated to induce expression of adhesion molecules on endothelium and immune cells\textsuperscript{36} and to increase iNOS expression.\textsuperscript{37} A reduction of plasma concentration of adrenaline has been shown in this model of TEA,\textsuperscript{10} which makes this a likely mechanism. Furthermore, this would be a possible explanation for the beneficial effects of β-receptor blocking drugs in sepsis.\textsuperscript{38} Another explanation, however, is a systemic effect of epidurally applied local anesthetic. Albeit the systemic concentration of lipid peroxidation in this model was determined to be low (0.33 µg/ml),\textsuperscript{10} effects of lidocaine on leukocyte adhesion\textsuperscript{29} and cytokine release\textsuperscript{39} have been described by other authors.

Although not focused in our experiments, an effect of TEA on the endothelial surface layer, glycocalyx, may explain parts of our results. Intravenous LPS has been shown to deteriorate the glycocalyx\textsuperscript{40,41} and to cause the release of nitric oxide and prostacyclin from the vascular endothelium, leading to increased adhesion of leukocytes.\textsuperscript{42} Protective effects of TEA on the glycocalyx, therefore, may be a challenging subject for further investigations.

**Intestinal Lipid Peroxidation**

Lipid peroxidation in intestinal tissue was only found to be increased in endotoxemic animals without TEA (fig. 4). In contrast, endotoxemia in combination with TEA was associated with 8-isoprostane values comparable to healthy animals (fig. 4). This suggests a protective effect of TEA on intestinal tissue. Because lipid peroxidation is catalyzed by free radicals,\textsuperscript{43} which may result from nitrosative stress, this protective effect of TEA may be directly due to the attenuation of intestinal nitric oxide production. An additional mechanism could be a mitigating effect of TEA on activation and extravasation of granulocytes, with less release of peroxidases from these cells into intestinal tissue. Former experiments had demonstrated a decrease in hemorrhage-induced leukocyte adhesion with TEA.\textsuperscript{11} Differentiation of white blood cells, however, was not possible.

**Conclusion**

Regional sympathetic blockade by means of thoracic epidural anesthesia decreased endotoxin-induced epithelial permeability of the gut. This is likely due to an inhibition of monocyctic cell infiltration into intestinal tissue, with less production of nitric oxide and less nitrosative tissue injury as verified by reduced lipid peroxidation. As possible mechanisms, direct nerve-immune interplay, a reduction in plasma catecholamines, or a systemic lidocaine effect has to be considered.

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