Induction of endotoxin tolerance improves lung function after warm ischemia in dogs

IVAR FRIEDRICH,1 JAN SPILLNER,1 ER-XIONG LU,2 MARKUS BARNSCHEIDT,2 OLIVER KUSS,3 ARMIN SABL OTZKI,1 F. ULRICH SCHADE,2 AND JOCHEN BÖRGERMANN1

Thoracic and Cardiovascular Surgery,3 Institute of Epidemiology, Biometrics and Informatics, Martin-Luther-University, 06097 Halle; and 2Clinical Research Group Shock and Multiorgan Failure, Essen University, 45128 Essen, Germany

Submitted 6 May 2002; accepted in final form 29 August 2002


PULMONARY ISCHEMIA-REPERFUSION injury (I/R injury) represents a common clinical phenomenon after lung transplantation, pulmonary embolism, and cardiac surgery with extracorporeal circulation (19, 20, 23). It is commonly believed that reperfusion activates NADPH oxidase and the subsequent release of reactive oxygen species (ROS). ROS release results in lipid and protein oxidation, causing cellular damage. Proinflammatory cytokines have been suspected to amplify the local effects of I/R and to promote systemic injury after I/R. Much evidence suggests that the release of tumor necrosis factor (TNF)-α from activated macrophages plays a pivotal role (18). Several studies showed protective effects of strategies based on the inhibition of proinflammatory mediators such as TNF-α antibodies and TNF-α binding protein (7, 28).

Despite the experimental efficacy of inhibiting single mediators, various clinical trials did not yield satisfying results (2). Although benefits resulting from the removal of single mediators may be limited to certain experimental models, the balance of pro- and anti-inflammatory factors appears to be the primary determinant in critical situations, including I/R. How this balance is controlled remains poorly understood. But there is some evidence that various strategies of proinflammatory priming, such as heat stress (15), ischemic preconditioning (12), or administration of bacterial cell wall components like endotoxin (8), alter the inflammatory equilibrium so that subsequent injuries are attenuated considerably. The present study addresses the issue of a possible protective effect of endotoxin pretreatment (endotoxin tolerance [ET]) on pulmonary reperfusion injury. Injection of endotoxin induces a strong inflammatory response in animals and humans because of the associated release of proinflammatory cytokines. If repeated endotoxin doses are administered over time, only a minor inflammatory response (such as fever or leukopenia/leukocytosis) is evoked, and the production of factors such as TNF-α remains low. This phenomenon is called ET. ET is not limited to endotoxin or bacterial infections but can also be protective in hemorrhagic shock (1), myocardial infarction (3), or kidney failure (14) resulting from I/R injury.

The following two phases of ET can be distinguished: early and late tolerance. The early phase begins several hours after the initial endotoxin treatment. This early phase is obviously related to cellular mechanisms because macrophages isolated in this phase show an altered response to endotoxin in vitro (21) and exert highly protective activities when transferred to normal mice (10). The changes caused by the early phase protect against reperfusion injury.

In contrast, late-phase tolerance occurs ~24–48 h after endotoxin exposition as a result of antibody formation against the O-antigens of endotoxin. Although several experimental models of I/R injury have addressed the effects of ET, no studies exist on...
the complications of pulmonary reperfusion. This study investigates the potential protective effects of ET on pulmonary I/R injury.

MATERIALS AND METHODS

The study and protocol were approved by the local authorities and the institutional animal care committee. Foxhounds (27–33 kg) of either sex were alternatively assigned to the control group (C; n = 6) and ET group (n = 6). Experimental animals were kept under 12:12-h day-night cycles and had ad libitum access to food and water until 12 h before surgery. Research was conducted in accordance with federal statutes and regulations relating to animals and experiments involving animals. All experiments were performed under sterile conditions and by the same cardiothoracic surgeon. All animals were hemodynamically stable and did not require catecholamine support during surgery or the postoperative course.

Surgical Procedure

After intravenous anesthesia with thiopentone (30 mg/kg body wt) and oropharyngeal intubation, the animals were ventilated with a fractional O2 inspiration rate of 0.3 in body wt) and oropharyngeal intubation, the animals were kept under 12:12-h day-night cycles and had ad libitum access to food and water until 12 h before surgery. After intravenous anesthesia with thiopentone (30 mg/kg body wt) and oropharyngeal intubation, the animals were kept under 12:12-h day-night cycles and had ad libitum access to food and water until 12 h before surgery. After intravenous anesthesia with thiopentone (30 mg/kg body wt) and oropharyngeal intubation, the animals were kept under 12:12-h day-night cycles and had ad libitum access to food and water until 12 h before surgery.

After chest tube insertion and wound closure, the animals were handled. One femoral arterial line and a pulmonary-arterial catheter (Swan Ganz; Baxter Healthcare, Irvine, CA) were placed to obtain blood samples. Pressure transducers (Millar Micro Tip; Millar Instruments, Houston, TX) were positioned at the midheart level and referenced to atmospheric pressure. Mean arterial pressure (MAP), mean pulmonary arterial pressure (PAP), central venous pressure (CVP), and pulmonary capillary wedge pressure (PCWP) were recorded. With the use of a Sirecust 9000 Monitor (Siemens Medical Products, Erlangen, Germany), cardiac output (CO) was assessed by thermodilution. Cardiac index (CI = CO/m2 body surface area), pulmonary vascular resistance indexes [PVRI = (PAP − PCWP)/80/CI], and systemic vascular resistance index [SVRI = (MAP − CVP) × 80/CI] were calculated. Pulmonary vascular resistance indexes of the LLepr and the RLaat lung were derived from left and right pulmonary artery flows and main pulmonary artery and left atrial pressures. The body surface area (BSA) was calculated as follows: BSA (m2) = 0.1 × [body wt (kg)]^{2/3}.

Bronchoalveolar Lavage

Before reperfusion, a bronchoalveolar lavage (BAL) of only upper lobes was performed via a flexible bronchoscope (Olympus BF-P10; Olympus, Hamburg, Germany). For bronchoscopic lavage, the scope was wedged in the upper lobe bronchus, and 20-ml aliquots of sterile saline were injected and recovered by gentle aspiration (recruitment ~60%). After 8 h of reperfusion, the right and the left lungs were lavaged, excluding the upper lobes. For whole lung lavage, the bronchus of the lower lobe was cannulated, the cannula was secured with a transbronchial suture, and lavage was performed with 500 ml sterile saline (recruitment ~70%), passively instilled at a pressure of 40 cmH2O. The BAL fluids (BALF) were filtered through sterile gauze, collected on ice, and immediately centrifuged at 200 g (4°C, 10 min) to sediment cellular material. The cell pellet was separated into aliquots in 0.05 M guanidinium thiocyanate buffer and snap-frozen in liquid nitrogen. Alveolar protein content (APC) of this cell-free BALF was measured using a commercially available kit based on the bicinchoninic acid assay (13).

Induction of ET

ET was induced by daily intravenous injection of endotoxin isolated from Escherichia coli serotype (for in vivo use) purchased from Sigma Chemical (St. Louis, MO). Before and after injection, blood samples were withdrawn. The endotoxin dose was increased from 5 μg/kg on day 1, 10 μg/kg on day 2, 20 μg/kg on day 3, 40 μg/kg on day 4, to 60 μg/kg on days 5 and 6. The animals were under close veterinarian observation during the entire period of tolerance induction. On days 1 and 2, febrile temperatures and “common cold”-like signs were apparent. Later doses were tolerated well in six animals that remained free of any signs of illness from day 3 on. One animal died from pulmonary embolism on day 2, and another dog had to be killed on day 2 because of severe abdominal pain. The autopsy revealed bowel obstruction from intestinal volvulus.

Pulmonary Function

Central venous, arterial, and left and right pulmonary venous blood samples were obtained on an hourly basis. Blood gases, Hb, and potassium concentrations were measured with a blood gas analyzer (ABL 4; Radiometer, Copenhagen, Denmark). One time per hour, dynamic (“respirator”) compliance, mean and peak ventilation pressures, and minute volume were read from the respirator and recorded.

Hemodynamic Monitoring

A femoral arterial line and a pulmonary-arterial catheter (Swan Ganz; Baxter Healthcare, Irvine, CA) were placed to measure pulmonary and systemic hemodynamics and to obtain blood samples. Pressure transducers (Millar Micro Tip; Millar Instruments, Houston, TX) were positioned at the midheart level and referenced to atmospheric pressure. Mean arterial pressure (MAP), mean pulmonary arterial pressure (PAP), central venous pressure (CVP), and pulmonary capillary wedge pressure (PCWP) were recorded. With the use of a Sirecust 9000 Monitor (Siemens Medical Products, Erlangen, Germany), cardiac output (CO) was assessed by thermodilution. Cardiac index (CI = CO/m2 body surface area), pulmonary vascular resistance indexes [PVRI = (PAP − PCWP)/80/CI], and systemic vascular resistance index [SVRI = (MAP − CVP) × 80/CI] were calculated. Pulmonary vascular resistance indexes of the LLepr and the RLaat lung were derived from left and right pulmonary artery flows and main pulmonary artery and left atrial pressures. The body surface area (BSA) was calculated as follows: BSA (m2) = 0.1 × [body wt (kg)]^{2/3}.

Bioassay of TNF

TNF bioactivity was determined in a cytolytic cell assay in the mouse fibrosarcoma cell line WEHI 164, clone 13 (gift of
In brief, for TNF quantification, the WEHI cells (2 × 10⁴ cells/well) were incubated with serial dilutions of sera in microtiter plates. After 18 h, 10% 3-(4,5-dimethylthiazol-2-yli)-2,5 diphenyltetrazolium bromide (MTT, 100 μg/well; Sigma Chemical, Munich, Germany) was added. The reaction was stopped after 4 h by adding 5% formic acid in isopropanol, and the content of reduced MTT was read in a micro-ELISA autoreader (MR5000; Dynatech Laboratories, Alexandria, VA). The TNF titer is expressed in units per milliliter of plasma with recombinant murine TNF-α kindly donated by Dr. T. Hartung, University of Konstanz). It was found that this antiserum neutralized all cytolytic activity measured with the WEHI cell assay. To test interference of plasma with recombinant murine TNF-α, TNF (80 units; Genzyme, Munich, Germany) was incubated in the presence of the sera to be tested, and the WEHI assay was carried out as described above.

Statistics

Data are presented as means ± SE, unless otherwise stated. For statistical analysis, we selected nonparametric ANOVA models to account for possible nonnormality of data and small sample sizes (6). All analyses allowed for the fact that measurements were repeatedly (over time and from the two different lungs) taken from the same dogs. Pairwise comparisons were Bonferroni adjusted to avoid spurious significances. Significance levels are expressed as P < 0.05, P < 0.01, or P < 0.001 for comparisons between the control and ET groups or when comparing time points and baseline values. Graphs were generated with Sigma Plot 4 (Jandel Scientific, Ratingen, Germany), and statistical calculations were performed with SAS Version 8e (SAS Institute, Cary, NC).

RESULTS

Induction of ET

During induction of ET, body temperature, white blood counts (WBC), and TNF serum levels were monitored after endotoxin challenge.

Body temperature was assessed daily 2 h after endotoxin injection (Fig. 1). After the first endotoxin injection, a febrile response with core temperatures of up to 40°C developed. In spite of higher amounts of endotoxin injected on the following days, the body temperature increase was considerably attenuated. This reaction was paralleled by a strong increase in TNF serum levels after the first injection of endotoxin, but TNF serum levels did not peak with later endotoxin doses (Fig. 2).

Pulmonary Reperfusion Injury

Gas exchange. In the control group, a 3-h warm ischemic period with consecutive reperfusion for 8 h resulted in progressive deterioration of gas exchange in both the I/R-injured left lung (LL_rep, Fig. 3A) and, to a minor extent, the noninjured RL_nat (Fig. 3B). After the onset of reperfusion (60 min), RL_nat nearly recovered to baseline PO₂ values (144.3 ± 6.5 mmHg). A significant difference between ET and control was not detectable in the RLNat. The reperfused lungs in either group showed a highly significant decrease in oxygenation. In the ET group, gas exchange recovered within 60 min after initial deterioration. After 480 min, the decrease in oxygenation after reperfusion in the control group remained significantly below that of the ET group (ET: 172.7 ± 12.9 vs. control 66.1 ± 7.2 mmHg). ET resulted in marked improvement of gas exchange in the LL_rep. In both LL_rep (in the ET group) and RL_nat, the range of pulmonary venous PO₂ values was not significantly different from baseline values.

![Fig. 1. Body core temperature 2 h after endotoxin injection. Values are given as means ± SE. *P < 0.05 for the comparison of individual time points with baseline data.](http://ajplung.physiology.org/)

![Fig. 2. Tumor necrosis factor (TNF) serum levels before and after endotoxin injection. Venous blood samples were withdrawn directly before endotoxin injection and 2 h afterward. Values are given as means ± SE. ***P < 0.001 for the comparison of individual time points with baseline data. EXP, experiment.](http://ajplung.physiology.org/)
Compliance. Independent ventilation of the left and right lung permitted separate assessment of dynamic compliances. At baseline, compliance was found to be 35 ml/0.1 kPa. After I/R (baseline vs. 5-min reperfusion), dynamic compliance dropped significantly in both the reperfused and the native organ and stayed significantly below baseline for the remaining observation time (P < 0.001). This held true for both the control and the ET group. The effect was more pronounced in the LLrep (Fig. 4A) than in the RLnat (Fig. 4B). Although RLnat compliance values were not different between the various groups, LLrep compliance was markedly higher in the ET group throughout the experiment.

Pulmonary hemodynamics. After reperfusion, the control group PAP were higher than the respective starting pressures in the ET group (Table 1). In the control group, transpulmonary gradient showed an increase directly after reperfusion (Fig. 5).

Fluid accumulation. Control data were taken from a previous lung transplant study in dogs where the recipient left lung was excised. In this study group, a “normal value” was determined for foxhound lungs. The wet-to-dry ratio in this group was 5.6 ± 0.23, which is comparable to the data given in the literature (12). The LLrep wet-to-dry ratio increased approximately threefold above baseline (16.7 ± 1.2; Fig. 6). Although not as pronounced, pulmonary fluid accumulated in the RLnat of the control group (the wet-to-dry ratio was found to be increased: 9.9 ± 0.6). No significant change was detected in the ET group, however (RLnat: 6.2 ± 0.5). Reperfused lungs in the ET group also showed an increase in water accumulation, but the...
Reperfusion. Data are from ET animals and animals without endo-
toxin pretreatment (control). Values are given as means ± SE for 6
lungs each. Significance levels are as indicated.

### Table 1. Systemic hemodynamics

<table>
<thead>
<tr>
<th>Reperfusion, min</th>
<th>Baseline</th>
<th>5</th>
<th>30</th>
<th>60</th>
<th>120</th>
<th>240</th>
<th>360</th>
<th>480</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3.7 ± 0.4</td>
<td>7.2 ± 1.2*</td>
<td>5.7 ± 1.1</td>
<td>4.5 ± 0.6</td>
<td>4.7 ± 0.7</td>
<td>4.7 ± 0.5</td>
<td>4.5 ± 0.6</td>
<td>5.1 ± 0.4</td>
</tr>
<tr>
<td>ET</td>
<td>3.3 ± 0.5</td>
<td>3.5 ± 0.21†</td>
<td>3.9 ± 0.3</td>
<td>4.8 ± 0.4</td>
<td>4.5 ± 0.3</td>
<td>4.8 ± 1.3</td>
<td>5.1 ± 0.8</td>
<td>4.6 ± 0.4</td>
</tr>
<tr>
<td><strong>MAP</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>79.3 ± 2.3</td>
<td>81.9 ± 4.1</td>
<td>76 ± 4.8</td>
<td>86.7 ± 7.2</td>
<td>87 ± 2.9</td>
<td>80.4 ± 4.7</td>
<td>77.4 ± 5</td>
<td>77.9 ± 4</td>
</tr>
<tr>
<td>ET</td>
<td>83.6 ± 6.3</td>
<td>75.4 ± 3.6</td>
<td>74.8 ± 3.4</td>
<td>81.4 ± 5.1</td>
<td>81.4 ± 7.7</td>
<td>75.2 ± 3.4</td>
<td>75.8 ± 3.2</td>
<td>72.2 ± 3.7</td>
</tr>
<tr>
<td><strong>SVRI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1,713 ± 202.8</td>
<td>1,007 ± 168.5*</td>
<td>977 ± 103.5*</td>
<td>1,014 ± 102.7*</td>
<td>1,224 ± 133.7</td>
<td>1,378 ± 167.6</td>
<td>1,350 ± 135</td>
<td>1,335 ± 137.7</td>
</tr>
<tr>
<td>ET</td>
<td>1,858 ± 211.3</td>
<td>1,653 ± 50.2‡</td>
<td>1,431 ± 75.1‡</td>
<td>1,525 ± 108.4‡</td>
<td>1,261 ± 146.9</td>
<td>1,519 ± 97.8</td>
<td>1,470 ± 134.1</td>
<td>1,355 ± 114.7</td>
</tr>
<tr>
<td><strong>CVP</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3.3 ± 0.7</td>
<td>4.5 ± 0.9</td>
<td>4.5 ± 0.6</td>
<td>4.2 ± 0.9</td>
<td>4.2 ± 1.2</td>
<td>3.3 ± 0.6</td>
<td>3.5 ± 0.7</td>
<td>3.5 ± 0.6</td>
</tr>
<tr>
<td>ET</td>
<td>3 ± 0.9</td>
<td>3.6 ± 1.1</td>
<td>3.6 ± 0.8</td>
<td>3.4 ± 1</td>
<td>3.6 ± 0.9</td>
<td>2.8 ± 0.8</td>
<td>3 ± 0.8</td>
<td>3 ± 0.8</td>
</tr>
<tr>
<td><strong>PAP</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>11.7 ± 1.1</td>
<td>15.8 ± 1.3*</td>
<td>18.4 ± 2.3*</td>
<td>18.2 ± 2.1*</td>
<td>16 ± 0.9*</td>
<td>15.6 ± 1*</td>
<td>15.6 ± 1.2*</td>
<td>17 ± 1.9*</td>
</tr>
<tr>
<td>ET</td>
<td>11.5 ± 1</td>
<td>11.7 ± 0.3†</td>
<td>11.5 ± 1†</td>
<td>11.5 ± 1†</td>
<td>12 ± 0.5†</td>
<td>12.7 ± 0.8†</td>
<td>13.6 ± 0.7</td>
<td>13.6 ± 1.1</td>
</tr>
<tr>
<td><strong>PCWP</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6.3 ± 0.7</td>
<td>7.2 ± 0.8</td>
<td>8.2 ± 1.1</td>
<td>7.8 ± 1</td>
<td>8.8 ± 0.9</td>
<td>8.2 ± 1</td>
<td>8.8 ± 1.1</td>
<td>8.3 ± 1.2</td>
</tr>
<tr>
<td>ET</td>
<td>5 ± 0.3</td>
<td>6 ± 0.9</td>
<td>5.4 ± 0.7</td>
<td>5 ± 0.7</td>
<td>6 ± 0.8</td>
<td>5.8 ± 1.2</td>
<td>7.6 ± 0.9</td>
<td>7.8 ± 0.7</td>
</tr>
<tr>
<td><strong>PVRI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>131.1 ± 43.6</td>
<td>114.8 ± 28.6</td>
<td>151.3 ± 48.8</td>
<td>173 ± 26</td>
<td>158.6 ± 46.6</td>
<td>145.3 ± 36.2</td>
<td>148.6 ± 17</td>
<td>168.9 ± 21.8</td>
</tr>
<tr>
<td>ET</td>
<td>130.9 ± 25.7</td>
<td>124.4 ± 20.2</td>
<td>107.8 ± 10.5</td>
<td>97 ± 19†</td>
<td>102.9 ± 19.1</td>
<td>136.8 ± 22.5</td>
<td>105.1 ± 21.2</td>
<td>87.6 ± 26.3†</td>
</tr>
</tbody>
</table>

Values are means ± SE. CI, cardiac index; MAP, mean arterial pressure; CVP, central venous pressure; PAP, pulmonary arterial pressure; PCWP, pulmonary capillary wedge pressure; PVRI, pulmonary vascular resistance index; ET, endotoxin. Baseline data were obtained after intubation and anesthesia induction. Data from ET-pretreated animals are compared with those from animals without pretreatment (control) and between baseline values and the time points of 5, 30, 60, 120, 240, 360, and 480 min of reperfusion within each group. Derived data (SVRI and PVRI) were obtained from each experiment. *P < 0.05 for the comparison of individual time points with baseline data; †P < 0.05 and ‡P < 0.01 for the comparison between ET and the control group.

Edema formation was significantly higher in the control than in the ET group.

**APC.** After 8 h of reperfusion, L.L.L.L. (2.273 ± 419.6 µg/ml) and R.L.L.L. (270.8 ± 88.8 µg/ml) APC were significantly higher than baseline (66.7 ± 7.0 µg/ml) in controls and in the ET group (Fig. 7A). Alveolar protein load, however, was much higher in the L.L.L.L. than in the R.L.L.L. group (~9-fold higher). Interestingly, alveolar protein levels were markedly lower in the ET group, especially in L.L.L.L. (869.5 ± 185 µg/ml).

**Phospholipid protein quotient.** After 8 h of reperfusion, the phospholipid protein quotient (PPQ) of control group BALF was significantly different from that of the ET group (Fig. 7B). Compared with baseline values (0.47 ± 0.01), PPQ was significant lower in the L.L.L.L. (0.05 ± 0.01). PPQ decreased much more in the control group, however. The PPQ in the ET group was lower.

---

![Fig. 5. Time course of transpulmonary pressure gradient. Data were obtained before warm ischemia of the left lung and during 8 h of reperfusion. Data are from ET animals and animals without endotoxin pretreatment (control). Values are given as means ± SE for 6 lungs each. Significance levels are as indicated.](image)

![Fig. 6. Wet-to-dry ratio before and after 8 h of reperfusion. The wet-to-dry ratio is significantly different between the control and ET groups. Compared with baseline values, wet-to-dry ratios increase significantly in the R.L.L.L. and L.L.L.L. of the control group. Data are from ET animals and animals without endotoxin pretreatment (control). Values are given as means ± SE for 6 lungs each.](image)
One of the characteristics of ET is its suppressive effect on the synthesis of TNF and other cytokines such as interleukin (IL)-1. Therefore, it was proposed that more IL-10, a very potent inhibitor of cytokine synthesis in macrophages, might be produced in ET (24). Such results from in vitro studies in which macrophages and/or monocytes were “hyposensitized” could not be reproduced in in vivo models. Mice with a deletion of the IL-10 gene developed ET under endotoxin treatment (4), and macrophages isolated from endotoxin-tolerant mice showed a significantly decreased capacity to produce both IL-10 and TNF (10).

Some recent studies have addressed the question of how in vitro endotoxin exposure changes intracellular signal transduction in macrophages. Phosphorylation of MAP kinases and translocation of nuclear factor-κB to the nucleus were found to be downregulated in cells pretreated with endotoxin, which impaired the synthesis of cytokines such as TNF (22, 29). Furthermore, in vitro pretreatment of monocytes with endotoxin resulted in accumulation of the p50 monomer of nuclear factor-κB, which led to the formation of p50p50 homodimers instead of p50p65 heterodimers (31). Although the heterodimer transactivates genes with the appropriate binding sites in their promoters, p50p50 homodimers are not able to do so; therefore, these cells had lost their ability to respond to endotoxin stimulation with cytokine synthesis. If these mechanisms are also responsible for in vivo ET has not been determined. There is some evidence, however, that inhibitory activities to cytokine and particularly to TNF synthesis circulate in endotoxin-tolerant mice (27). Such activities are not present in normal animals. Macrophages from endotoxin-tolerant mice produce this inhibitor when activated by endotoxin in vitro, whereas macrophages from normal mice are not endowed with this capacity (10). The nature of this inhibitor has not yet been identified.

In the present study, the effects of ET were investigated in a dog model of 3 h of warm lung ischemia followed by 8 h of reperfusion. This model differed in several aspects from customary setups. Both lungs were ventilated separately but synchronized. Catheters in the pulmonary veins of the lower lobes permitted gas exchange measurements of the reperfused, and the native, contralateral lung. This experimental setup avoids snaring procedures, which potentially influence blood pressure and blood flow in the pulmonary vascular system. The posture of the animals was prone during the observation time to reduce the effects of positioning on gas exchange and ventilation/perfusion matching (5). Several beneficial effects were anticipated as follows: 1) there was no contralateral lung overexpansion that would have resulted from ventilating the reperfused and the native lung (which have different compliances) through one tube. Overexpansion may lead to ventilator-induced lung injury (VILI). VILI effects would have been superimposed on I/R effects; 2) there was no “underventilation” resulting from the poor compliance of the reperfused lung with subsequent atelectasis and ventilation/perfusion mis-

**DISCUSSION**

For a long time, ET was thought to be specific for endotoxin (17, 26). Although this may hold true for tolerance against pyrogens, a number of experimental studies demonstrated that beneficial effects also extend to hemorrhagic shock (32), myocardial infarction (9), hepatic reperfusion injury (8), and acute renal failure (14). ET seems to protect against assaults that are not primarily induced by endotoxin, including assaults seen in clinically relevant syndromes. Understanding of the underlying mechanisms may be of significant clinical importance.
match. With separate ventilation, the pathophysiological changes caused by I/R can be observed, without being obscured by muddling effects from the experimental setup.

ET was assessed by TNF measurements before and 2 h after injection of endotoxin. The first injection was consistently followed by fever. In addition, WBC (mainly neutrophils) decreased significantly 2 h postinjection, and TNF serum levels increased considerably. A rebound of leucocytes with WBC of up to 40,000/μl was detectable, primarily resulting from polymorphonuclear neutrophil recruitment 24 h after endotoxin injection (data not shown). On the following injections, ET became apparent: TNF levels were low, and no fever developed. Whole blood could not be stimulated to produce TNF. During the subsequent days, the endotoxin dose was increased, but inflammatory signs like fever and WBC changes disappeared. After 6 days of endotoxin injections, no sign of inflammatory response was detectable. When the dogs underwent the surgical procedure after 2 days of recovery, no signs of inflammation were left. After reperfusion, oxygenation was diminished markedly in both the reperfused and the native lung. The impairment in the contralateral (right) organ may be because of the right lateral position during the surgical procedure. This is supported by the fact that gas exchange normalized within 1 h. Inflammation of the contralateral organ by blood-borne mediators liberated from the reperfused organ (remote injury) did not worsen gas exchange in either the control or the ET group. Immediately after reperfusion, oxygenation of the reperfused lung was highly significantly impaired in the control group and did not improve during the observation time. Induction of ET before I/R preserved normal oxygenation parameters during the experimental course. Reperfusion after 3 h of ischemia resulted in a significant decrease of contralateral pulmonary compliance. The compliance of the reperfused organ dropped even more. No differences in pulmonary compliance between control and the ET groups were detectable in the native organ. In contrast in the reperfused lung, the control group displayed significantly lower pulmonary compliance at all times. This finding may relate to the profound increase in protein leakage in the reperfused lung, especially in the control group. Plasma proteins are strong inhibitors of the surface tension reduction properties of pulmonary surfactant. The inhibition of surfactant function leads to an increase in pulmonary compliance (16).

PPQ assessment shows the relationship between plasma leakage and reduced pulmonary compliance. In both the ET group and, more pronounced, control group, PPQ was reduced in the reperfused organ. Previous studies show that plasma proteins impair surfactant function. Diminished surfactant function increases the surface tension of the intraalveolar lining fluid, leading to dropping pulmonary compliance and finally in atelectasis, ventilation perfusion mismatch, and impaired gas exchange (13). The increased plasma protein permeability of the vascular/alveolar barrier is paralleled by edema forming in the reperfused lungs. Interestingly, water content of the native organ, indicated by wet-to-dry ratios, did not increase compared with baseline after ET. On the other hand, a significant increase in water accumulation was found in the control group (even more than in the reperfused lung after ET induction). Wet-to-dry ratios were not corrected for total blood volume because the filling pressures were not increased and fluid substitution was guided by hemodynamic parameters. Therefore, pulmonary edema after reperfusion is best explained by an increase of permeability for water.

ET does not suppress all signs of reperfusion injury. A variety of pathogenic mechanisms (e.g., radical formation) is operative after warm I/R, and it is not likely that any type of injury can be attenuated by induction of ET. Our results indicate that functional parameters are still impaired: pulmonary edema is more pronounced and intraalveolar protein content is increased, even in endotoxin-tolerant animals. However, all observed parameters were improved compared with the control group. Interestingly, in the ET group, the native lung had a lower wet-to-dry ratio, less intra-APC, and a higher PPQ, all indicators of diminished injury. This may be because of reduced liberation of blood-borne inflammatory mediators from the reperfused organ acting on the nonreperfused lung. The hypothesis that the inflammatory response is reduced needs to be validated in an animal model that permits comprehensive investigation of immunological mechanisms. Currently, such methods are only available for small animals, not for canines. This study is the first to address ET and pulmonary I/R injury. Comparative data are therefore not available. However, several studies have addressed the effects of ET on I/R injury in other organs. With a murine model of 45-min warm ischemia of the left kidney and excision of the right kidney, Heemann et al. (14) demonstrated 100% survival at 48 h in the ET compared with 25% in the control group. Histologically, a lower number of leucocytes were found to infiltrate the kidney, and apoptotic tubular cells were less frequent in the ET group. Comparison of creatinine levels showed superior organ function in the ET group and renal failure in the controls. In a brain ischemia model in rats, Toyoda et al. (30) found smaller infarcted areas after pretreatment with diphosphoryl lipid A, a nontoxic species of lipid A (30). Superoxide dismutase activity was increased at the infarction site, and myeloperoxidase activity was reduced. Furthermore, in a rabbit model of myocardial infarction, the infarct size was reduced after ET induction. Coronary artery occlusion and reperfusion led to an increase in TNF serum levels in the control but not in the ET group. Belosjorow et al. (3) explained this phenomenon with increased TNF inhibitory activity after endotoxin pretreatment. Flohé et al. (11) found in a rat model that endotoxin pretreatment and subsequent endotoxin shock reduced TNF-α mRNA in lung and liver tissue in the ET group. A detailed study by Salkowski et al. (25) addressed pulmonary and hepatic gene expression after cecal ligation and puncture in mice. mRNA expression of proinflammatory cytokines like
TNF-α, IL-6, and granulocyte-macrophage colony-stimulating factor was profoundly reduced. Not only proinflammatory but also anti-inflammatory mediators like IL-10 and IL-1 receptor antagonist were suppressed after tolerance induction with monophosphoryl lipid A (25).

This study demonstrates that ET provides significant protection against I/R injury of the lung. The underlying protective mechanisms are still unclear. Elucidating these mechanisms may open the gates for clinical strategies to control the I/R syndrome.

This work was supported by grants from the Hans und Gerti Fischer Stiftung fuer Herz und Kreislaufforschung, Essen Germany and Deutsche Forschungsgemeinschaft (Schm 74/13–2).

REFERENCES


