Autoregulative function in the brain in an endotoxic rat shock model

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Abstract. Objective and design: Autoregulative function in the brain gets relevant in hypodynamic conditions of a sepsis syndrome. We investigated the temporal pattern and dose dependent effects of LPS-induced shock on autoregulative function in rats.

Material and subjects: Chloralose-anesthetized and mechanically ventilated male CD-rats (n = 30).

Treatment: Animals were subjected to vehicle, 1 or 5 mg/kg b.w. lipopolysaccharide (LPS) from E. coli given intravenously.

Methods: Autoregulative function was tested repeatedly with a carotid compression technique assessing the transient hyperemic response ratio (THRR) in the cortex with laser Doppler flowmetry up to 270 min. THRR data from exsanguination experiments served as controls.

Results: Despite lower blood pressure levels in the high dose group (control: 114 ± 7 mmHg; 1 mg/kg LPS group: 82 ± 16 mmHg; 5 mg/kg LPS group: 62 ± 16 mmHg; p < 0.05) progressive cerebral hyperemia occurred similarly in both groups. Compared to exsanguination experiments autoregulative compensation for lower blood pressure levels was lacking in the high LPS dose group at the end of experiments.

Conclusions: Cerebral autoregulation was affected by LPS-induced shock supporting the notion of vasoregulative failure in endotoxic shock

Key words: Cerebral autoregulation – Lipopolysaccharide – Sepsis – Shock – Cerebral blood flow – Rat

Introduction

Sepsis and systemic inflammatory response syndrome (SIRS) are the leading causes of mortality in intensive care units [1, 2]. Abnormalities in microcirculatory organ perfusion with subsequent organ dysfunction characterizes early stages of severe sepsis and septic shock [1, 3, 4]. Previously, we found indication of early microcirculatory failure in the brain investigating the neurovascular coupling mechanism in a rat model of endotoxic shock [5].

Another important vasoregulative mechanism of the brain is the cerebral autoregulation, which maintains constant cerebral perfusion despite cerebral perfusion pressure changes [6, 7]. In hypodynamic states of septic shock when blood pressure levels decline, integrity of the cerebral autoregulation might gain clinical relevance for the patient [8, 9]. Severe hypotension was associated with the occurrence of septic encephalopathy [10]. Failure of the cerebral autoregulation has been documented in many disease processes but reports are conflicting in sepsis syndromes [11–13].

To repeatedly investigate autoregulative function in rats during LPS-induced shock we referred to a carotid compression technique and obtained the transient hyperemic response ratio (THRR). Because of a progressive decline in blood pressure data from exsanguination experiments with similar blood pressure levels served as controls [7].

Materials and methods

General preparation

All procedures performed on the animals were in strict accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and approved by the local Animal Care and Use Committee.

Adult male CD-rats (280–310 g) were initially anesthetized with 1.5% halothane in a 1:1 N₂O/O₂ mixture of gases, tracheotomized, paralyzed with pancuronium bromide (0.2 mg·kg⁻¹·h⁻¹), and artificially ventilated (Harvard Rodent Ventilator; Harvard, South Natick, MA, U.S.A.). Arterial blood gas analyses and pH were measured repeatedly as needed and at least every 30 min (Blood gas analyzer model Rapidlab 348, Bayer Vital GmbH, Fernwald, Germany). In addition, glucose and lactate levels were measured repeatedly (Glukometer Elite XL, Bayer Vital GmbH, Fernwald, Germany; Lactate pro, Arkray Inc. European
Office, Duesseldorf, Germany). The right femoral artery and vein were cannulated for mean arterial blood pressure recording, blood sampling, and drug administration. Rectal body temperature was maintained at 37°C using a feedback-controlled heating pad. Since pH changes have inverse effects on cerebral blood flow permissive hyperventilation was allowed. At the end of experiments blood was collected to determine the cell destruction markers for neurons (neuron specific enolase,NSE) and astrocytes (S-100B) from the LPS/vehicle groups for reasons of comparison to previous data [5].

The head of each animal was fixed in a stereotaxic frame, the apex of the skull was exposed, and the bone over the left parietal cortex was thinned with a saline-cooled drill to allow transcranial laser-Doppler flowmetry (LDF) [14]. The laser probe (BRL-100, Harvard Apparatus, Massachusetts, USA) was placed 3.5 mm lateral and 1 mm rostral to the bregma in accordance with the coordinates of the forepaw lying in the center of the vascular territory of the middle cerebral artery [15]. The LDF velocity signal and the systemic mean arterial blood pressure were recorded continuously and processed on a personal computer running data acquisition software (Neurodyn, HSE, March-Hugstetten, Germany).

Vascular studies

Compression of the common carotid artery was undertaken ipsilaterally to the recording site with a clamping device (HSE, March-Hugstetten, Germany) using a vascular clip with a closing pressure of 0.25 N (Vessel clip, Aesculap, Trossingen, Germany). Therefore, clamping is non-traumatic for the vessel, and guarantees reversibility of the occlusion. Special care was undertaken not to come into contact with the vagus nerve. Clamping for 10s was alternated with 10s periods of clip release. The laser-Doppler recordings enabled induced blood-flow velocity responses to be obtained [5, 16].

As a measure of autoregulative function the transient hyperemic response ratio (THRR) was used. It indicates the peak increase in LDF signal after release of compression in relation to resting flow velocities [17,18]. The parameter was calculated according to the following formula:

$$\text{THRR} = \left( \frac{F_{\text{MAX}}}{F_{\text{BASE}}} - 1 \right) \times 100$$

where $F_{\text{MAX}}$ indicates the maximal blood-flow velocity increase after opening the clip. The Doppler measures flow velocity rather than flow and data are usually given as arbitrary units. However, according to the calculation of the THRR values indicate signal changes which have been demonstrated to correlate quite well with flow changes [14].

To exclude a possible nitric oxide related interference of halothan narcosis was replaced by intravenous application of $\alpha$-chloralose (80 mg/kg) (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) approximately 60 min before stimulation experiments. Supplementary doses of chloralose (30 mg/kg) were given every hour.

### Table 1. Group averaged data for lactate, glucose, pH, pCO$_2$, hemoglobin, resting cerebral blood flow velocity (CBFV) change, and blood pressure for all groups. Data are given as mean ± SD. Low lactate levels indicate values below the lower range of measure. Statistical results are given from Sheffé post hoc test as: *<0.05; **<0.01; ***<0.001 (control vs. sepsis groups) or +<0.05; +++ < 0.001 between sepsis groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Glucose [mg/dL]</th>
<th>Lactate [mmol/L]</th>
<th>pH</th>
<th>Blood pressure [mmHg]</th>
<th>pCO$_2$ [mmHg]</th>
<th>Hemoglobin [mg/l]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Base</td>
<td>End</td>
<td>Base</td>
<td>End</td>
<td>Base</td>
<td>End</td>
</tr>
<tr>
<td>Vehicle</td>
<td>±10 ±8</td>
<td>-</td>
<td>±0.06</td>
<td>±0.06</td>
<td>±8</td>
<td>±3.5</td>
</tr>
<tr>
<td>1 mg/kg b.w.</td>
<td>±8 ±8*</td>
<td>-</td>
<td>±0.6</td>
<td>±0.07***</td>
<td>±14</td>
<td>±12**</td>
</tr>
<tr>
<td>5 mg/kg b.w.</td>
<td>±11 ±7*</td>
<td>-</td>
<td>±0.6</td>
<td>±0.05***</td>
<td>±6</td>
<td>±13***</td>
</tr>
</tbody>
</table>

### Laboratory assays

At the end of the experiments the blood samples were drawn into tubes containing aprotonin (Trasyloil, Bayer AG, Leverkusen, Germany), immediately centrifuged and separated, after which plasma was stored at −80°C until analysis. The GAD levels were determined using an enzyme-linked immunosorbent assay (NEL EIA kit; Hoffmann-La Roche, Basel, Switzerland). The S-100B protein was determined with an immunoluminometric assay (Sangtec 100 LIA; Sangtec Medical, Bromma, Sweden) using monoclonal antibodies specific for the beta subunit of the S-100 protein.

### Study design

To demonstrate the effects of different LPS-doses over time each 10 rats were subjected to either 1 mg/kg or 5 mg/kg per body weight LPS i.v. (Lipopolysaccharid E. coli, O111:B4, Sigma-Aldrich Chemie GmbH, Germany) or vehicle alone (0.5 ml 0.9% NaCl). From previous studies it was known that 1 mg/kg LPS resulted in a moderate blood pressure decrease with a pressure stabilization in the range of 70 to 80 mmHg, whereas 5 mg/kg led to a progressive decline close to the lower limit of cerebral autoregulation (in rats: 50 to 60 mmHg [6,7,16]) within 5h [5]. Experiments were performed in random order. The injections were given slowly within 2-3 minutes. A moderate volume therapy of 3-6ml/h 0.9% NaCl was allowed for blood pressure stabilization.

To assess autoregulative integrity under different blood pressure conditions THRR values were compared to similar blood pressure levels using the exsanguinations technique [7]. Prior to and after LPS administration cerebral autoregulation was measured in distinct time intervals up to 270 minutes. Exsanguinations experiments were performed in 10 rats. By withdrawing blood via an arterial line into a heparinized syringe blood pressure levels can be manipulated reversible and accurately [7]. THRR data were obtained under mean arterial blood pressure levels in the ranges from 60–70, 70–80, 80–90, 90–100, 100–120 mmHg.

### Statistics

THRR data from the low and high LPS dose groups were compared to blood pressure matched THRR data from the exsanguinations experiments. If appropriate, a repeated ANOVA was performed to assess differences between groups. In case of significance a Scheffé post hoc test was applied. If assumptions of normal distribution and equality of variances could not be assured in statistic tests, a nonparametric Paired-Sign or Kolmogorov-Smirnov test was undertaken instead (Statview, SAS, USA). The significance level $p$ was set to 0.05.