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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 exsanguinations 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 characterises 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 exsanguinations 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 N_2O/O_2 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

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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.

Group	Glucose [mg/dL]		Lactate [mmol/L]		pH		Blood pressure [mmHg]		pCO_2 [mmHg]		Hemoglobin [mg/l]	
	Base	End	Base	End +++	Base	End	Base	End +	Base	End	Base	End
Vehicle	90 ±10	85 ±8	-	-	7.44 ±0.06	7.44 ±0.06	115 ±8	120 ±10	38.2 ±3.5	38..9 ±4.8	136 ±6	139 ±8
1 mg/kg b.w.	89 ±8	65 ±8*	-	1.7 ±0.6	7.45 ±0.06	7.38 ±0.07***	120 ±14	80 ±12**	38.3 ±2.5	33.6 ±2.7*	132 ±5	128 ±11
5 mg/kg b.w.	92 ±11	61 ±7*	-	3.5 ±0.6	7.45 ±0.06	7.35 ±0.05***	117 ±6	65 ±13***	37.8 ±3.9	33.1 ±2.8*	135 ±9	126 ±17

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 10 s was alternated with 10 s 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:

$$THRR = [(F_{MAX}/F_{BASE})-1]*100$$

where F_{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 with 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 α-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.

Laboratory assays

At the end of the experiments the blood samples were drawn into tubes containing aprotinin (Trasylol, Bayer AG, Leverkusen, Germany), immediately centrifugated and separated, after which plasma was stored at –80 °C until analysis. The NSE levels were determined using an enzyme-linked immunosorbent assay (NSE 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 5 h [5]. Experiments were performed in random order. The injections were given slowly within 2–3 minutes. A moderate volume therapy of 3–6 ml/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 exsanguination 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 Schefé 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.

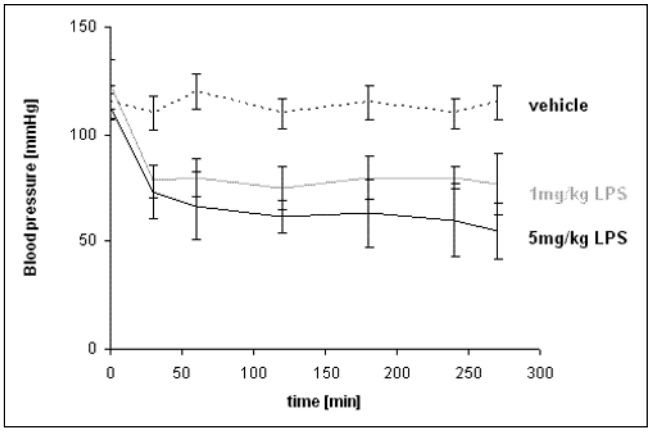

Fig. 1. Blood pressure (BP) data for the LPS/vehicle groups during the time course of experiments given as mean ± SD. The data from the vehicle group are given as serrated curve. The gray curve indicates data from the 1 mg/kg LPS group, the black curve that from the 5 mg/kg group.

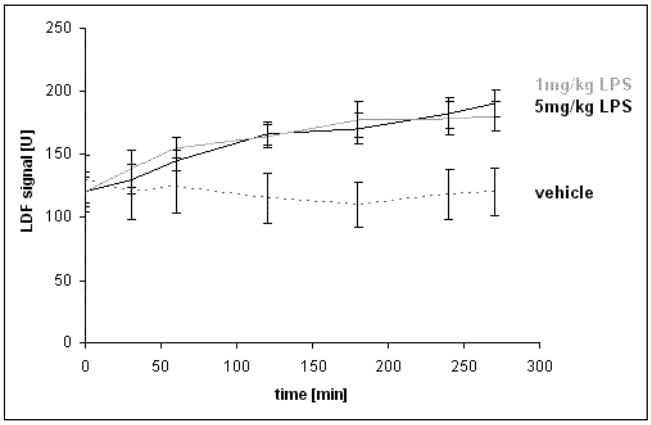

Fig. 2. Laser Doppler flow data during the time course of experiments for the LPS/vehicle groups given as mean ± SD. The data from the vehicle group are given as serrated curve. The gray curve indicates data from the 1 mg/kg LPS group, the black curve that from the 5 mg/kg group. Progressive cerebral hyperemia (+40 %) occurred in both sepsis groups.

Results

No rat died from LPS injection. Table 1 indicates the data for glucose, lactate, pH, blood pressure, pCO_2, and hemoglobin for the three experimental groups. Parameters remained stable in the vehicle group. In the 5 mg/kg LPS group changes were more pronounced than in the 1 mg/kg LPS group. Cell destruction parameters were similar to previously published data with the same endotoxic shock model. In the vehicle group NSE was 0.3 ± 0.1 ng/ml and S-100B was 0.6 ± 0.2 ng/ml. NSE increased to 2.0 ± 0.5 ng/ml in the 1 mg/kg LPS group and to 2.5 ± 0.3 ng/ml in the 5 mg/kg group. S-100B increased to 5 ± 2.2 ng/ml and 14 ± 1.9 ng/ml respectively. Glucose was substituted if needed maintaining levels above 60 mg/dl. Changes in blood pressure and cerebral resting blood flow velocities are given graphically in the figures 1 and 2. Figure 3 indicates the THRR data for the 1 mg/kg LPS and in figure 4 for the 5 mg/kg LPS group together with the blood pressure matched THRR values from the exsanguinations experiments. A compensative increase in the THRR value was seen under lower blood pressure conditions in the exsanguinations group which was lacking in the 5 mg/kg LPS group. The difference was statistically significant indicating impaired autoregulative compensation under low pressure conditions in the severe sepsis group. Reproducibility of the THRR-test can be seen in figure 5 for the vehicle group.

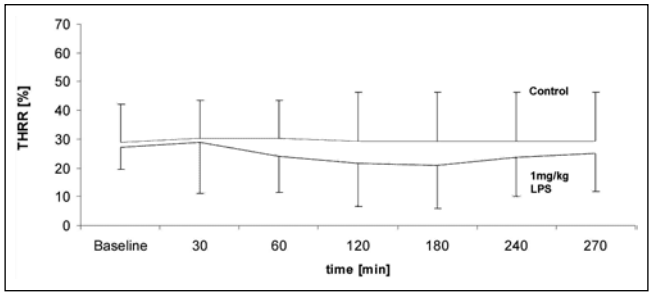

Fig. 3. Time course of the THRR after a 1 mg/kg LPS challenging as compared to responses from exsanguinations experiments with corresponding blood pressure levels. Although lower, THRR values did not reach significance as compared to exsanguinations data.

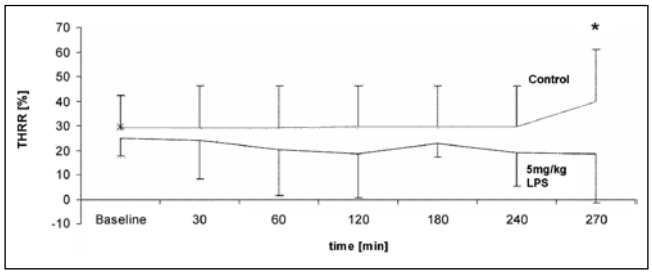

Fig. 4. Time course of the THRR after a 5 mg/kg LPS challenge. The lacking increase in THRR at the end of experiments under lower blood pressure levels indicates impaired autoregulative function. From the data of the exsanguinations experiments increase in the THRR would have been expected. Significance levels are given as following: * $p < 0.05$.

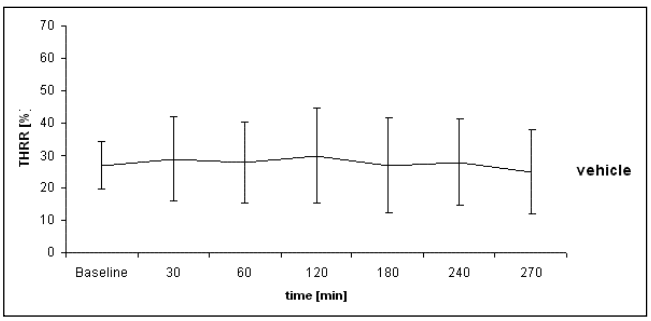

Fig. 5. THRR values for the vehicle group given as mean ± SD indicating the reproducibility of the technique.

Discussion

From the present study it appeared that autoregulative function was more robust against an endotoxin challenge as compared to the neurovascular coupling mechanism. Only

in the 5 mg/kg LPS group and at the end of experiments lacking increase of the THRR compensating for the lower blood pressure levels resulted in significance. These findings contrast to an early vasoregulative failure of the neurovascular coupling mechanism in the same rat model of endotoxic shock [5]: Performing electric forepaw stimulation the evoked flow velocity responses over the somatosensory cortex declined 60 min after sepsis induction and preceded changes in somatosensory evoked potentials by 120 min. However, sepsis related impairment of cerebral vasoregulative mechanisms might in part explain the higher cerebrovascular risk and more frequent occurrence of brain ischemia in more severe sepsis patients. Accordingly, our data support the concept of intensive monitoring and early therapy initiation.

Different techniques were used to assess cerebral autoregulative function [6]. In the present study, the carotid compression technique regarded as most appropriate, because exsanguination experiments or medical regimens are not applicable without interference with the sepsis syndrome. A leg cuff test, which is most widely used in clinical settings, is not applicable in small animal models. The carotid compression technique modulates cerebral perfusion pressure and has been established as an alternative technique to classical tests relying on systemic blood pressure variation [6, 7, 17, 18]. Due to a common trunk of the anterior cerebral artery in rats cross flow between vascular territories is less variable than in humans [19]. The laser Doppler technique has the time resolution needed to assess fast aspects of the cerebral autoregulation [5, 14, 16]. Relative signal changes correlate well with flow changes [14, 16]. In contrast to autoradiographic techniques vasoregulative function and repetitive testing can be performed easily with the LDF-technique [14].

We choose the present model in favour of a cecal ligature and puncture (CLP) model since time course as well as severity of the shock syndrome is more standardized. The invasiveness of the present approach might not be a relevant disadvantage since intensive monitoring and optimized treatment of rats a sepsis syndrome from the beginning is recommended. An early initiation of therapy and glucose control resulted in a better outcome in clinical studies [20, 21].

In vivo determination of NO-levels in the brain or brain vessels cannot be performed easily in the present model. However, generally it is well accepted that sepsis related cerebral hyperemia and catecholamine resistent vasoparalysis are related to overt NO production [22]. The time course of cerebral hyperemia which started 2 h after sepsis induction nicely matches to reported data of changes in NO-levels in the brain [22]. It was shown that the occurrence of cerebral hyperemia could be blocked by iNOS inhibitors [22]. However, the decrease in pH levels would have only accounted for a 10 % increase in cerebral perfusion [23].

Controversies in literature concerning autoregulative function can be explained by the different stimulation techniques, varying time points of investigation and different types and severity of sepsis syndromes [11, 24–27]. Some groups using classical autoregulative tests did not find relevant changes in cerebral blood flow regulation and concluded unaffected cerebral autoregulation [11] whereas others found significant affection of autoregulative function [12]. Some insignificant studies were performed at an early phase being in accordance with our initial data [11]. At later stages and also consistent with the results from late stages in the high dose LPS-group a considerable reduction in autoregulative function was reported [12, 24]. Administration of small doses of endotoxin (4 ng/kg) in healthy volunteers did not change cerebral metabolic rate, cerebral vascular resistance, and resting cerebral blood flow and was not associated with cerebral dysfunction [25]. However, in more severe cases in which higher toxin doses are assumable (septic patients) [26] or actually have been given (animal models) [22] significant hyperemia and autoregulative failure was present. A recent study in a CLP rat model did not find changes in resting cerebral blood flow 24 h after sepsis induction using quantitative autoradiography [27]. Unfortunately the authors did not perform serial measurements to exclude a transient cerebral hyperemia and also did not investigate autoregulative function. However, since blood pressure levels remained above 100 mmHg [27] a direct comparability to the present study of septic shock might also not be given.

Summary

We suggest a dose-dependent and timely cascaded change in microcirculatory integrity of the brain vasculature. It appears from previous data that the cerebral autoregulation is more robust against sepsis related changes than the neurovascular coupling underlining the complexity of sepsis related microcirculatory dysfunction in the brain. Although monitoring of autoregulative function in patients with endotoxic shock is still recommended we hypothesize that the breakdown of the neurovascular coupling might be more relevant for the occurrence of early septic encephalopathy. Furthermore, comparison of autoregulative data from septic patients to normal values might lead to false negative interpretation. Further investigations have to follow to investigate also the effects of treatment regimes such as catecholamine administration or selective iNOS inhibition on the vasoregulative mechanisms of the brain.

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