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Disassociation of static and dynamic cerebral autoregulatory performance in healthy volunteers after lipopolysaccharide infusion and in patients with sepsis

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Berg RM, Plovsing RR, Ronit A, Bailey DM, Holstein-Rathlou NH, Møller K. Disassociation of static and dynamic cerebral autoregulatory performance in healthy volunteers after lipopolysaccharide infusion and in patients with sepsis. Am J Physiol Regul Integr Comp Physiol 303: R1127–R1135, 2012. First published October 17, 2012; doi:10.1152/ajpregu.00242.2012.— Sepsis is frequently complicated by brain dysfunction, which may be associated with disturbances in cerebral autoregulation, rendering the brain susceptible to hypoperfusion and hyperperfusion. The purpose of the present study was to assess static and dynamic cerebral autoregulation in a human experimental model of the systemic inflammatory response during early sepsis and in patients with advanced sepsis. Cerebral autoregulation was tested using transcranial Doppler ultrasound in healthy volunteers (n = 9) before and after LPS infusion and in patients with sepsis (n = 16). Static autoregulation was tested by norepinephrine infusion and dynamic autoregulation by transfer function analysis (TFA) of spontaneous oscillations between mean arterial blood pressure and middle cerebral artery blood flow velocity in the low frequency range (0.07–0.20 Hz). Static autoregulatory performance after LPS infusion and in patients with sepsis was similar to values in healthy volunteers at baseline. In contrast, TFA showed decreased gain and an increased phase difference between blood pressure and middle cerebral artery blood flow velocity after LPS (both P < 0.01 vs. baseline); patients exhibited similar gain but lower phase difference values (P < 0.01 vs. baseline and LPS), indicating a slower dynamic autoregulatory response. Our findings imply that static and dynamic cerebral autoregulatory performance may disassociate in sepsis; thus static autoregulation was maintained both after LPS and in patients with sepsis, whereas dynamic autoregulation was enhanced after LPS and impaired with a prolonged response time in patients. Hence, acute surges in blood pressure may adversely affect cerebral perfusion in patients with sepsis.

cerebral autoregulation; endotoxin; human; lipopolysaccharide; sepsis-associated encephalopathy; sepsis. The systemic inflammatory response to infection, is frequently complicated by acute brain dysfunction, also termed sepsis-associated encephalopathy (SAE), which is characterized by confusion, agitation, and impaired consciousness (13, 16). It is often the first symptom of sepsis and may herald an unfavorable neurocognitive outcome (6). Although the underlying mechanisms remain unresolved, SAE may involve changes in cerebral hemodynamics; accordingly, patients with sepsis appear to be particularly susceptible to cerebral ischemia and breaching of the blood-brain barrier (32, 33), both of which may be facilitated by a functional impairment of cerebral autoregulation (6).

Cerebral autoregulation encompasses cerebral arteriolar vasodilation and vasoconstriction as cerebral perfusion pressure decreases and increases, respectively, and functions to dampen the impact of changes in mean arterial blood pressure (MAP) on cerebral blood flow (CBF) (15, 27). Cerebral autoregulation is, thus, a homeostatic mechanism that protects the brain tissue from the potentially damaging effects of hypoperfusion and hyperperfusion. It may be described both in terms of “static” performance, that is, the ability of CBF to respond to longer-lasting changes in MAP, and in terms of “dynamic” performance concerning the changes occurring in CBF within the first few seconds after an acute change in MAP (1, 25). Static autoregulation has been reported to be maintained in patients with SAE (22), whereas dynamic autoregulation may be impaired (28). It is, however, presently unknown whether static and dynamic cerebral autoregulatory performance reflect entirely the same phenomenon and thus change in parallel, or whether one may be preserved, while the other is impaired. As of now, static and dynamic autoregulation has not been evaluated concurrently in patients with sepsis, nor have they been compared during early and advanced stages of disease.

In the present study, we evaluated static and dynamic cerebral autoregulation in healthy volunteers following an intravenous infusion of LPS, an established human in vivo model of...
the systemic inflammatory response that occurs during early sepsis (3), as well as in patients with severe sepsis or septic shock. We hypothesized that a dissociation of static and dynamic cerebral autoregulation would occur, in the sense that dynamic but not static autoregulation would be impaired, both in healthy volunteers after LPS infusion and in patients with advanced sepsis.

METHODS

The study was approved by the Scientific Ethical Committee of Copenhagen and Frederiksberg Municipalities, Denmark (file number H-A-2009-020 with amendments) and was performed in accordance with the Declaration of Helsinki.

Healthy volunteers. Nine healthy male volunteers aged 23 (mean, SD, 2) yr were enrolled in the study after providing oral and written informed consent. All were nonasthmatic, had an unremarkable medical history, with no signs of infection within 4 wk ahead of the trial day, and none took any regular medication. Before inclusion, volunteers underwent a thorough physical examination; a 12-lead ECG was obtained, and standard laboratory tests were performed. All tests were normal.

Volunteers reported to the intensive care unit (ICU) at 7:30 AM following a 12-h overnight fast and were placed in bed. They were subsequently catheterized with an antecubital catheter (for LPS infusion), and an arterial line was inserted in the left radial artery following local anesthesia (lidocaine, 20 mg/ml). After catheter insertion, the volunteer rested in the supine position with slight head elevation (∼20°) for 45 min before measurements were started. Heart rate (via a three-lead ECG), invasive blood pressure, and arterial oxygen saturation (by pulse oximetry) were continuously monitored, and medically qualified personnel were present at all times. Volunteers were allowed to drink tap water ad libitum during the study day. They were discharged after 12 h upon complete alleviation of symptoms and normalization of all vital parameters, and following a light meal and removal of catheters.

LPS infusion. Volunteers underwent a 4-h continuous intravenous infusion of purified Escherichia coli LPS (infusion rate, 0.5 ng·kg⁻¹·h⁻¹; Batch G2 B274, U.S. Pharmacopeial Convention, Rockville, MD). In this model, symptom scores and plasma TNF-α peak at ∼1 h after cessation of the infusion (37). The combined assessment of static and dynamic cerebral autoregulation, with a total duration of 80 min, was carried out at baseline, and immediately after cessation of the infusion.

Rectal temperature and arterial blood samples were obtained at baseline, hourly during the LPS infusion, and 2 h after cessation of the infusion. Volunteer symptoms were evaluated at these time points by means of a modified visual analog scale; the volunteer rated six symptoms (malaise, shivering, nausea, headache, myalgia, and dizziness) from 1 to 10, where a score of 1 implied that the symptom was not present at all, and 10 that it was the worst imaginable. The six individual scores were added together to yield a total symptom score with a minimal value of 6 and a maximal value of 60. Symptoms of encephalopathy were not systematically assessed during LPS infusion.

Patients. The study was conducted in a tertiary 20-bed noncardiothoracic ICU in a university hospital. The inclusion criteria for participation were 1) the presence of severe sepsis or septic shock according to consensus criteria (18), 2) 18–75 years of age, 3) the presence of a radial artery catheter, and 4) a diagnosis of sepsis within the past 72 h. The exclusion criteria were 1) clinical evidence or laboratory evidence of neurotrauma, cerebrovascular disease, neuroinfection, or pregnancy, and 2) a history of arterial hypertension. A total of 16 patients (two females) were included (for patient characteristics, see Table 1). Inclusion was preceded by oral and written informed consent from the next of kin, as well as from the patient’s general practitioner or the national health inspector. The patients were 59 (mean; SD, 12) yr old, and the study was conducted within 24 h after sepsis had been diagnosed in 10 patients, between 24 and 48 h in three patients, and between 48 and 72 h in the remaining three patients. All patients were mechanically ventilated at the time of the study; 11 by a pressure-regulated volume control mode, 4 by a pressure support mode, and 1 by a pressure control mode. The presedation Glasgow Coma Score (GCS) at admission to the ICU was 15 in eight patients, 12–14 in six patients, and 9 in two patients, and symptoms of encephalopathy, ranging from confusion to delirium and unconsciousness, were present in 10 patients prior to sedation. Propofol, fentanyl, and/or remifentanil were used as sedatives-ana-legics in 14 patients, none of which have been found to affect cerebral autoregulation in previous studies of elective surgical patients (9, 35, 39). Two patients received all three, seven received two, and five received one of these drugs. The median Ramsay score for all 16 patients (Ref. 4) was 4 (interquartile range, 4–5). Eleven of the patients were in a state of septic shock at the time of the study, as defined by the need for continuous infusion of one or more vasopressor drugs, despite intravenous fluid administration. Eight of these patients received norepinephrine alone (median, 0.08; interquartile range, 0.02–0.20 μg·kg⁻¹·min⁻¹), two received both norepinephrine (0.06 and 0.13 μg·kg⁻¹·min⁻¹, respectively) and epinephrine (0.08 and 0.13 μg·kg⁻¹·min⁻¹), or a combination of norepinephrine and epinephrine (0.04 and 0.08 μg·kg⁻¹·min⁻¹, respectively).

Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Patient</th>
<th>Gender</th>
<th>Age, yr</th>
<th>CNS Status</th>
<th>APACHE II</th>
<th>SOFA</th>
<th>Infectious Focus</th>
<th>Pathogen</th>
<th>ICU Stay, days</th>
<th>30-Day Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 M</td>
<td>47</td>
<td>Awake and alert</td>
<td>17</td>
<td>9</td>
<td>Lung</td>
<td>E.coli</td>
<td>77</td>
<td>Survived</td>
<td></td>
</tr>
<tr>
<td>2 M</td>
<td>45</td>
<td>Awake and alert</td>
<td>24</td>
<td>10</td>
<td>Blood</td>
<td>Unknown</td>
<td>6</td>
<td>Died</td>
<td></td>
</tr>
<tr>
<td>3 M</td>
<td>59</td>
<td>Confused</td>
<td>31</td>
<td>17</td>
<td>Blood</td>
<td>C. canimorsus</td>
<td>15</td>
<td>Survived</td>
<td></td>
</tr>
<tr>
<td>4 M</td>
<td>70</td>
<td>Awake and alert</td>
<td>30</td>
<td>8</td>
<td>Abdomen</td>
<td>Unknown</td>
<td>3</td>
<td>Survived</td>
<td></td>
</tr>
<tr>
<td>5 M</td>
<td>73</td>
<td>Awake and alert</td>
<td>23</td>
<td>13</td>
<td>Soft tissue</td>
<td>Klebsiella spp</td>
<td>7</td>
<td>Survived</td>
<td></td>
</tr>
<tr>
<td>6 F</td>
<td>74</td>
<td>Confused</td>
<td>31</td>
<td>12</td>
<td>Soft tissue</td>
<td>Staphylococci</td>
<td>6</td>
<td>Died</td>
<td></td>
</tr>
<tr>
<td>7 M</td>
<td>69</td>
<td>Delirious</td>
<td>23</td>
<td>7</td>
<td>Lung</td>
<td>Unknown</td>
<td>4</td>
<td>Died</td>
<td></td>
</tr>
<tr>
<td>8 M</td>
<td>58</td>
<td>Awake and alert</td>
<td>5</td>
<td>3</td>
<td>Soft tissue</td>
<td>F. necrophorum</td>
<td>8</td>
<td>Survived</td>
<td></td>
</tr>
<tr>
<td>9 M</td>
<td>52</td>
<td>Delirious</td>
<td>13</td>
<td>7</td>
<td>Blood</td>
<td>Staphylococci, streptococci</td>
<td>5</td>
<td>Survived</td>
<td></td>
</tr>
<tr>
<td>10 M</td>
<td>43</td>
<td>Somnolent</td>
<td>19</td>
<td>14</td>
<td>Soft tissue</td>
<td>Streptococci</td>
<td>15</td>
<td>Survived</td>
<td></td>
</tr>
<tr>
<td>11 M</td>
<td>46</td>
<td>Somnolent</td>
<td>13</td>
<td>6</td>
<td>Soft tissue</td>
<td>E.coli, staphylococci</td>
<td>6</td>
<td>Survived</td>
<td></td>
</tr>
<tr>
<td>12 M</td>
<td>63</td>
<td>Unconscious</td>
<td>21</td>
<td>10</td>
<td>Lung</td>
<td>Enterococci, S. marcescens</td>
<td>3</td>
<td>Survived</td>
<td></td>
</tr>
<tr>
<td>13 F</td>
<td>40</td>
<td>Somnolent</td>
<td>20</td>
<td>3</td>
<td>Soft tissue</td>
<td>E.coli, streptococci</td>
<td>5</td>
<td>Survived</td>
<td></td>
</tr>
<tr>
<td>14 M</td>
<td>67</td>
<td>Unconscious</td>
<td>33</td>
<td>14</td>
<td>Unknown</td>
<td>14</td>
<td>Survived</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 M</td>
<td>69</td>
<td>Awake and alert</td>
<td>18</td>
<td>2</td>
<td>Soft tissue</td>
<td>Unknown</td>
<td>3</td>
<td>Survived</td>
<td></td>
</tr>
<tr>
<td>16 M</td>
<td>69</td>
<td>Awake and alert</td>
<td>31</td>
<td>12</td>
<td>Lung</td>
<td>E. coli</td>
<td>23</td>
<td>Died</td>
<td></td>
</tr>
</tbody>
</table>

APACHE II, Acute Physiology and Chronic Health Evaluation II; SOFA, sequential organ assessment score; CNS, central nervous system (clinical neurocognitive status prior to sedation as evaluated by the physician on duty); ICU stay, total duration of the stay in the intensive care unit; F, female; M, male; E. coli, Escherichia coli; C. canimorsus, Capnocytophaga canimorsus; F. necrophorum, Fusobacterium necrophorum; S. marcescens, Serratia marcescens.

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Transcranial Doppler ultrasound. Unilateral linear middle cerebral artery blood flow velocity (MCAv) was estimated by the continuous measurement of backscattered Doppler signals using a 2-MHz pulsed transcranial Doppler (TCD) ultrasound system (Ez-Dop and Doppler-Box, Compumedics DWL, Singen, Germany). Changes in MCAv were considered to reflect changes in CBF, assuming an unchanged caliber of the middle cerebral artery. TCD has been validated for this purpose, and previous studies have found no effects of changes in blood pressure or PaCO_2 on the caliber of the middle cerebral artery (14, 24).

Following a standardized search technique (41), the Doppler probe was secured over the transtemporal window with an insonation depth of 45–60 mm. The Doppler probe was fixed on the left transtemporal window in healthy volunteers and in 13 patients. Because of difficulties insonating the left middle cerebral artery, the probe was fixed on the right transtemporal window in the remaining three patients. The Doppler probe was secured with an adjustable silicone headband in healthy volunteers and with a fixation rack in three patients. The Doppler probe was secured with an adjustable silicone headband in healthy volunteers and with a fixation rack in three patients. The arterial line was attached to a pressure transducer (invasive blood pressure module OptiMed, Compumedics DWL) to maintain the optimal insonation angle during measurements. The arterial line was secured over the transtemporal window with an insonation depth of 45–60 mm.

Invasive arterial blood pressure, TCD waveforms, and respiratory rate were sampled continuously at 1 Hz using an analog-to-digital converter (PowerLab 16/30, ADInstruments, Milford, MA) interfaced with a personal computer.

Static autoregulation. Steady-state testing of autoregulation was achieved by increasing MAP 25–30 mmHg by means of an intravenous infusion of norepinephrine. MAP was increased in 5-mmHg increments over 4–7 steps of pressure change, remaining 4–5 min at each pressure step. Static autoregulation was subsequently evaluated by resampling MAP and MCAv data at 10 Hz for the calculation of the slope of the regression line between the increase in MAP and the absolute and relative change in MCAv, respectively (20). Since MAP may have been located below the lower limit of autoregulation prior to norepinephrine infusion in some subjects, a computer program was used to identify a possible lower limit of autoregulation by a dual linear regression method (17, 31), which was modified to take a possible slope of the autoregulatory plateau into account (20) and was adapted for MatLab version R2011a (MathWorks, Natick, MA). If a lower limit of autoregulation was identified, the slope was calculated for the regression line, fitting only those points with MAP values exceeding the lower limit.

Dynamic autoregulation. In both healthy volunteers and patients, dynamic autoregulation was examined using transfer function analysis (42), which assesses spontaneous oscillations in MAP and MCAv. Invasive arterial blood pressure and TCD waveforms were recorded over a 20-min steady-state period and resampled at 10 Hz for spectral analysis. A 1,200-point Fourier transformation, with a 60-s overlap between sequential segments for Welch spectral estimation and smoothing by the Hanning window, was performed in MatLab version R2011a (MathWorks). Data from the low-frequency range (0.07–0.20 Hz) are presented specifically to identify changes in dynamic autoregulation (42), since MAP fluctuations in this frequency range are independent of the respiratory frequency (12). Dynamic autoregulation was subsequently evaluated by transfer gain, MAP-to-MCAv phase difference, and coherence. Transfer gain quantifies the function of cerebral autoregulation as a high-pass filter that buffers the changes in MCAv induced by transient fluctuations in MAP. The MAP-to-MCAv phase difference refers to the displacement of the MCAv waveform relative to the MAP waveform and may, therefore, be interpreted to reflect the time delay between changes in MAP and MCAv (40). We defined a phase shift as positive when MCAv leads MAP. Coherence reflects the fraction of spectral power (“variability”) of MCAv that can be linearly explained by the spectral power of MAP (25, 26). To ensure robust gain and MAP-to-MCAv phase difference estimates, only those gain and MAP-to-MCAv phase difference values where the corresponding coherence was >0.4 were used for subsequent analysis (40). Accordingly, a decrease in gain and/or an increased MAP-to-MCAv phase difference would be interpreted as evidence for enhanced dynamic autoregulation, and an increase in gain and/or a reduction in the MAP to MCAv phase difference would be interpreted as evidence for a functional impairment of dynamic autoregulation (26, 42).

Laboratory analyses. White blood cell counts were determined by an automated analyzer (Sysmex XE-2100,Sysmex Europe, Hamburg, Germany). Plasma was obtained by centrifuging whole blood in EDTA-containing tubes at 3,500 rpm at 4°C for 15 min, and the samples were kept at −80°C until analysis. Plasma concentrations of TNF-α and IL-6 were measured in duplicate by means of electro-chemiluminescent detection on a multiplex immunoassay using a SECTOR Imager 2400 (Meso Scale Diagnostics, Gaithersburg, MD), and mean concentrations were calculated. The assay sensitivity was determined from using two internal controls (human plasma) and was below 12% at the low end and below 8% at the high end of the standard curve for both cytokines.

Arterial oxygen tension (PaO_2), oxygen saturation-fraction (SaO_2), PaCO_2, pH, and hemoglobin were determined on a blood gas analyzer (ABL 725; Radiometer, Brønshøj, Denmark). Arterial oxygen content (CaO_2) was calculated as CaO_2 (mmol/l) = SaO_2 × hemoglobin (mmol/l) + PaO_2 (Torr) × 0.0013 mmol·l⁻¹·Torr⁻¹.

Statistics. A linear mixed model with a covariance structure fitted for nonequidistant data points was used to analyze the effects of LPS infusion on immune and systemic hemodynamic changes over time. If an overall effect of time was identified, the estimated individual differences from baseline were evaluated using the Tukey-Kramer adjustment for multiple comparisons. Standard residual diagnostics were used for model control, and data were logistically transformed to attain variance homogeneity and normal distribution of residuals if needed.

Because the majority of cerebral hemodynamic data did not follow a normal distribution, as evaluated by normality plots and Shapiro-Wilk’s test for normality, Wilcoxon’s signed-rank test was used for paired comparisons between baseline and after LPS infusion in healthy volunteers. A Mann-Whitney U-test was applied to test for differences between patients and healthy subjects at baseline and after LPS infusion, respectively, and P values were adjusted according to Holm’s sequential Bonferroni correction. Spearman correlations were applied to test the relationships between PaCO_2 and autoregulation in healthy volunteers and in patients, respectively, as well as the relationship between autoregulation, Ramsay score, and preadmission GCS in patients. A Friedman test was used to assess the effects of LPS infusion on total symptom scores (ordinal data) in volunteers. All P values were adjusted according to Holm’s sequential Bonferroni correction.

Unless otherwise stated, data are presented as median (interquartile range), and P < 0.05 was considered statistically significant. All statistical analyses were performed by SAS statistical software version 9.2 (SAS Institute, Cary, NC).

RESULTS

Clinical, inflammatory, and cardiovascular variables. LPS infusion induced a systemic inflammatory response with fever, leukocytosis, and elevated cytokine levels (Fig. 1A–D), as well as flu-like symptoms, with increases in all individual symptoms and subsequent increases in total symptom scores (P < 0.001; data not presented). TNF-α levels were higher in

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healthy volunteers after LPS infusion than in patients with sepsis, whereas IL-6 levels were similar (Fig. 1, C and D).

The LPS-induced inflammatory response was associated with an increased heart rate and a temporary decrease in MAP, yielding similar values to those in patients (Fig. 2, A and B). Furthermore, a hyperventilatory response with a decrease in PaCO₂ and increased arterial pH was induced by LPS infusion (Fig. 2, C–E); there was, however, no subsequent effect on CaO₂ (Fig. 2F). In contrast, and despite inspired oxygen fractions of 0.48 (0.40–0.65), as well as normal PaO₂, 81 (76–101) Torr [10.8 (10.1–13.4) kPa], and SaO₂, 0.98 (0.96–0.98), all patients had low CaO₂ levels (Fig. 2F), mainly due to low hemoglobin concentrations [5.7 (5.4–6.2) mmol/l].

Symptoms of encephalopathy were not systematically assessed in healthy volunteers, but volunteers were awake and fully alert during the cerebral hemodynamic measurements and exhibited no overt symptoms of encephalopathy during the study.

Static cerebral autoregulation. Static autoregulation was tested in all healthy volunteers, both at baseline and after LPS infusion, and in 14 patients. The measured MCAv values were lower after LPS infusion compared with baseline [baseline: 67 (59–77) cm/s; LPS: 56 (53–60) cm/s; P < 0.05]; patients did not differ, however, from healthy volunteers in any of the two conditions [56 (30–69) cm/s; P = 0.12 vs. baseline; P = 0.34 vs. LPS].

Norepinephrine infusion at a rate of 0.19 (0.15–0.26) µg·kg⁻¹·min⁻¹ increased MAP from 91 (86–98) to 110 (106–116) mmHg in healthy volunteers at baseline (P < 0.01) and after LPS, a norepinephrine infusion rate of 0.16 (0.11–0.23) µg·kg⁻¹·min⁻¹ increased MAP from 74 (73–83) to 104 (101–106) mmHg (P < 0.01). No lower limit for autoregulation was identified in any of the healthy volunteers, either at baseline or after LPS, that is, all data points of volunteers were located above this limit.

In patients, the norepinephrine infusion rate was increased from 0.02 (0.0–0.10) µg·kg⁻¹·min⁻¹ to 0.17 (0.15–0.30) µg·kg⁻¹·min⁻¹, which increased MAP from 75 (69–81) to 95 (88–110) mmHg (P < 0.01). A lower limit of autoregulation was identified in two patients, at a MAP of 77 and 84 mmHg, respectively; in the rest of the patients, all data points were located above the lower limit.

The slopes of the absolute autoregulation regression lines were 0.19 (0.06–0.36) cm·s⁻¹·mmHg⁻¹ for healthy volunteers at baseline, 0.06 [(-0.14–0.23) cm·s⁻¹·mmHg⁻¹ after LPS (P = 0.13 vs. baseline), and 0.43 (0.09–0.79) cm·s⁻¹·mmHg⁻¹ in sepsis (P = 0.20 vs. volunteers at baseline; P < 0.05 vs. volunteers after LPS). Similarly, the relative autoregulation regression line slopes did not differ between conditions in healthy volunteers, but a significant difference between healthy volunteers after LPS and patients was present (Fig. 3).

The absolute static autoregulatory regression line slopes were unrelated to PaCO₂ both at baseline and after LPS (baseline: ρ = 0.07; P = 0.87; LPS: ρ = 0.40; P = 0.29); likewise, the relative static autoregulatory regression line slopes were unrelated to PaCO₂, (baseline: ρ = 0.07; P = 0.87; LPS: ρ = 0.37; P = 0.33). There were no significant correlations between the slopes of the absolute static autoregulatory regression lines and Ramsay scores (ρ = -0.05, P = 0.86), GCS (ρ = -0.14, P = 0.64) or PaCO₂ (ρ = -0.03, P = 0.91) in patients, nor did the relative static regression line slopes exhibit any significant correlations to these variables: Ramsay scores
(ρ = −0.07, P = 0.83), GCS (ρ = 0.02, P = 0.96), or PaCO₂ (ρ = −0.01, P = 0.97). Neither absolute nor relative slopes differed between those patients that exhibited symptoms of encephalopathy and those that were awake and alert prior to sedation (all NS, data not presented).

Dynamic cerebral autoregulation. Recordings of spontaneous variations in MAP and MCAv were completed in all nine healthy volunteers at baseline and after LPS, and in all 16 patients. In one volunteer, coherence decreased below 0.4 after LPS infusion, and this subject was, therefore, omitted from further transfer function analysis. The spectral power of MAP was very low for the whole group of patients [0.2 (0.0–0.5) mmHg²/Hz]; consequently, the corresponding coherence values were low [0.34 (0.31–0.52)] and were only ≥0.4 in six patients, who were enrolled for subsequent transfer function analysis.

In the eight healthy volunteers, LPS infusion caused a decrease in gain and an increase in the MAP-to-MCAv phase difference. Compared with the healthy volunteers, patients exhibited lower MAP-to-MCAv phase differences (Table 2). There were no significant correlations between the dynamic autoregulation parameters and PaCO₂ in healthy volunteers, either at baseline (gain: ρ = 0.60, P = 0.12; MAP-to-MCAv phase difference: ρ = 0.12, P = 0.78) or after LPS (gain: ρ = −0.07, P = 0.87; MAP-to-MCAv phase difference: ρ = −0.12, P = 0.78). In patients, gain was unrelated to PaCO₂ (ρ = 0.31, P = 0.54), whereas the MAP-to-MCAv phase difference was inversely and significantly correlated to PaCO₂ (ρ = −0.83, P < 0.05). The dynamic autoregulation parameters were unrelated to Ramsay scores (gain: ρ = 0.29, P = 0.57; MAP-to-MCAv phase difference: ρ = −0.74, P = 0.09) or presedation GCS in patients (gain:
In the present study, we demonstrate that static cerebral autoregulatory performance in healthy humans is unaffected by LPS infusion and that it is similar in healthy volunteers at baseline and patients with sepsis. Moreover, we found that dynamic cerebral autoregulation is enhanced in healthy volunteers after LPS infusion with decreased transfer gain and increased MAP-to-MCAv phase differences, whereas patients with sepsis exhibited a somewhat different picture with lower MAP-to-MCAv phase differences.

LPS infusion induced a marked systemic inflammatory response similar to that of the very early stages of sepsis (3, 23, 37, 38), with progressive fever, leukocytosis, and elevated TNF-α, and IL-6 levels. In this model TNF-α levels and symptoms have previously been found to peak ~1 h after cessation of the infusion (37). Although we did not obtain blood samples at this time point in the present study, because this would have impeded the cerebral autoregulatory assessment, the clinical signs in volunteers corresponded well with this notion. Patients exhibited a systemic inflammatory response characteristic of the more advanced stages of sepsis, in which TNF-α may decline, while IL-6 levels typically remain high (3, 21, 37). Accordingly, TNF-α levels were higher in healthy volunteers after LPS than in patients, whereas IL-6 levels were similar (Fig. 1, C and D). The healthy volunteers developed other clinical and laboratory findings characteristic of the systemic inflammatory response syndrome encountered in septic patients (18), including increased heart rate and hyperventilation; in contrast, the patients enrolled in the present study had been septic for up to 72 h and were mechanically normoventilated. Therefore, we speculate that the differences observed between patients and volunteers in the present study may be due both to the difference in duration of the systemic inflammatory response and to the different levels of ventilation.

Static autoregulation was not found to be impaired in either healthy volunteers after LPS infusion or in patients with sepsis (Fig. 3), and there were no significant correlations between static autoregulatory performance and PaCO2 levels. This is in agreement with a previous study of eight patients with SAE in which static autoregulation was found to be preserved (22) but differs from a more recent study of 21 patients with severe sepsis or septic shock, which reported that static autoregulation was attenuated, particularly in patients with high PaCO2 levels (36). Our study does, however, differ fundamentally from the earlier studies with regard to the assessment of static autoregulation. In the majority of previous autoregulation studies on various clinical conditions, static performance is considered dichotomously as either being intact or absent, in many instances according to an arbitrarily defined threshold, which has typically been set at a 0.5% change in MCAv/mmHg change in MAP or higher (25). This is based on the classical view of cerebral autoregulation, which presupposes that CBF is in effect constant across values of MAP ranging from a lower limit of ~60 mmHg to an upper limit of ~150 mmHg (27). This view has, however, been challenged on several occasions (15, 25), and in a recent human experimental study, it was reported that MCAv (as a surrogate for CBF) changes by as much as ~0.8% per mmHg change in MAP when autoregulation is intact (20). A similar relationship was found in the present study, both in healthy volunteers and in patients with sepsis (Fig. 3). Static autoregulatory performance did, nevertheless, vary somewhat in the patient group, and it is possible that significant differences between groups could have been detected if a larger cohort had been studied. Furthermore, cerebral autoregulation would arguably have been classified as being absent, both in some healthy volunteers and patients if the dichotomous approach had been pursued; thus, 4 out of the

Table 2. Dynamic cerebral autoregulation after LPS infusion in healthy volunteers (n = 8) and in patients with sepsis (n = 6)

<table>
<thead>
<tr>
<th></th>
<th>Healthy Volunteers</th>
<th>LPS</th>
<th>Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAPsp, mmHg/Hz</td>
<td>2.8 (2.3–3.5)</td>
<td>1.4 (0.9–3.6)</td>
<td>2.4 (0.6–4.3)</td>
</tr>
<tr>
<td>MCAVsp, cm²/s²</td>
<td>5.4 (3.2–6.2)</td>
<td>2.1 (1.8–3.9)*</td>
<td>1.8 (1.0–2.0)</td>
</tr>
<tr>
<td>Gain, cm·mmHg⁻¹·s⁻¹</td>
<td>1.16 (1.07–1.34)</td>
<td>0.87 (0.77–0.89)**</td>
<td>0.69 (0.55–1.21)</td>
</tr>
<tr>
<td>Phase, radians</td>
<td>0.74 (0.68–0.91)</td>
<td>1.12 (1.10–1.31)**</td>
<td>0.17 (0.01–0.28)**†</td>
</tr>
<tr>
<td>Coherence, units</td>
<td>0.83 (0.81–0.87)</td>
<td>0.63 (0.54–0.79)*</td>
<td>0.74 (0.52–0.91)</td>
</tr>
</tbody>
</table>

Data are presented as median (interquartile range). Values for the spectral power of mean arterial blood pressure (MAPsp) and middle cerebral artery blood flow velocity (MCAVsp), as well as transfer gain, the MAP-to-MCAv phase difference, and coherence are presented in the low frequency range (0.07–0.20 Hz). Only data for subjects with coherence ≥0.4 are presented. *P < 0.05 vs. baseline; **P < 0.01 vs. baseline; †P < 0.01 vs. LPS.
9 volunteers and 8 of the 14 patients had a regression line slope above 0.5% change in MCAv per mmHg change in MAP. Static autoregulatory performance was, nonetheless, neither related to GCS nor to the presence of symptoms of encephalopathy prior to sedation in patients.

The enhancement of dynamic autoregulation after LPS infusion was unexpected and contrary to our working hypothesis. Although PaCO2 is considered one of the main regulators of cerebral hemodynamic function (15, 27) and hyperventilation has been found to be associated with a reduction in CBF after intravenous LPS administration (7, 23), hyperventilation is not likely to explain the observed changes in dynamic autoregulation per se. Thus, we did not find any significant correlations between the enhanced dynamic autoregulation and the decrease in PaCO2, and although a previous study found that voluntary hyperventilation enhances dynamic performance as evaluated by step-induced changes in MAP by the thigh cuff deflation technique (1), this involved much more profound hypocapnia [PaCO2 ∼22 Torr (2.93 kPa)] than encountered in the present study. Moreover, voluntary hyperventilation to similarly low PaCO2 values was not found to affect transfer gain or the MAP-to-MCAv phase difference in a later study (2). On the other hand, Brassard et al. (7) recently reported a significant correlation between transfer function indices of dynamic autoregulation and the hyperventilatory response after administration of LPS. In contrast to the present study, LPS was administered as an intravenous bolus, which causes a much briefer systemic inflammatory response with a very short-lived increase in circulating cytokines than that induced by a 4-h continuous infusion (37). It is possible that the effect of hyperventilation on cerebral hemodynamics is more pronounced under these specific circumstances (23). During the more prolonged inflammatory response elicited by a 4-h infusion, which may reflect the early systemic inflammatory response in sepsis more adequately, the impact of other pathophysiological components of the systemic inflammatory response on the cerebrovasculature, such as the associated hyperthermia or even the direct effects of circulating cytokines, may be augmented. With regard to hyperthermia, it has previously been demonstrated that passive heating to core temperatures similar to those induced by LPS increased only the MAP-to-MCAv phase difference, whereas gain was unaffected (19). Thus, although neither hyperventilation nor hyperthermia can fully explain the enhanced dynamic autoregulation observed after LPS per se, at least on the basis of the data at hand, it cannot be ruled out that they may act in synergy to cause the observed cerebral hemodynamic changes.

Patients exhibited a somewhat different dynamic autoregulatory pattern compared with healthy volunteers, with maintained gain, albeit at lower MAP-to-MCAv phase differences. This indicates impaired dynamic autoregulation in the sense that the magnitude of the cerebrovascular response to a given physiological change in MAP is preserved, but with a prolonged response time. Impaired dynamic autoregulation has similarly been reported in previous studies of patients with sepsis (28, 34). Because aging is not associated with alterations in gain or the MAP-to-MCAv phase difference in the low-frequency range (8, 40), our findings are not likely confounded by the differences in age between healthy volunteers and patients. The small sample size does, however, warrant caution with regard to any definitive conclusions in relation to putative causative factors based on these findings. We did find an inverse relationship between the MAP-to-MCAv phase differences and PaCO2 levels in patients, indicating less efficient dynamic autoregulation with increasing PaCO2 levels. This finding is supported by previous studies that have reported high PaCO2 levels in septic patients with impaired dynamic autoregulation (28, 34); it may, therefore, be that CO2 contributes to the observed slowing of cerebral autoregulatory responses. An additional factor that may also have profound effects on cerebral function, both directly and by impairing dynamic autoregulation, is the markedly low CaO2 values that were present in all patients (Fig. 2F). Thus, cerebral hypoxia has previously been demonstrated to impair dynamic autoregulation during acute inspiratory hypoxia (5).

The present study suggests that dynamic performance of cerebral autoregulation may be affected without concomitant effects on static performance. Although static and dynamic autoregulation was initially thought to be reflective of the same physiological phenomenon and to change in parallel in various physiological and pathophysiological conditions (1, 39), a few earlier studies support the notion that static and dynamic autoregulation may disassociate under certain circumstances. Thus, low-dose isoflurane impairs dynamic performance, as evaluated by step-induced changes in MAP by thigh cuff deflation, whereas static autoregulation is unaffected (35). Similar findings have been made in patients with acute ischemic stroke, where dynamic but not static autoregulation was found to be attenuated (10), a pattern that was evident up to 2 wk after the ictus (11). Thus, dynamic autoregulation may be relatively sensitive, and static autoregulation may be more robust to various physiological and pathophysiological events, which could imply that the response time of the cerebral circulation may be affected before its actual ability to respond adequately to steady-state changes in MAP.

The underlying mechanisms of impaired dynamic cerebral autoregulatory performance in sepsis remain obscure. Since recent studies suggest that neurovascular coupling is likewise slower in sepsis (29, 30), impaired dynamic autoregulation may reflect a general cerebral microvascular dysfunction that involves “indo- lence” of cerebrovascular responses, thus exposing the brain to intermittent hypoxia both during decreases in blood pressure and upon neuronal activation. Both cerebral hypoxia due to low CaO2 levels (anemic hypoxia) and changes in PaCO2 may be of importance to these changes, and may, indeed, be further augmented by cerebral microvascular dysfunction. A number of complementary mechanisms that were not addressed in the present study, such as the direct cerebrovascular effects of circulating proinflammatory mediators, as well as neuro-oxidative-nitrosative stress with concomitant effects on the vascular nitric oxide bioavailability, may be involved in this vicious cycle (6). Further studies focusing on the relationship between cerebral CO2 reactivity, cerebral oxygen vasoreactivity, neurovascular coupling, and dynamic autoregulation in patients with sepsis are warranted to elucidate their potential relationship to neuro-oxidative-nitrosative stress and the occurrence of encephalopathy at various stages of disease.

Perspectives and Significance

The findings of the present study imply that the static and dynamic properties of cerebral autoregulation may disassociate in sepsis; hence, dynamic autoregulation was enhanced in a
human-animal model that mimics the systemic inflammatory response during the very early stages of sepsis, whereas the dynamic autoregulatory response was slower in patients with severe sepsis or septic shock. In contrast, static autoregulation was maintained throughout. This suggests that the response time of the cerebral circulation may be affected before its actual ability to respond adequately to steady-state changes in MAP. Notwithstanding that the brief systemic inflammatory response evoked by LPS infusion may not have been sufficient to adversely affect dynamic autoregulation, the enhanced dynamic autoregulation observed after LPS may suggest that dynamic autoregulation is preserved during the very early stages of sepsis, whereas the prolonged response time of the autoregulatory responses in patients may reflect a progressive cerebral microvascular “indolence” during the course of sepsis into more advanced stages. This could potentially expose the brain to intermittent hypoxia and/or blood-brain barrier breaching upon fluctuations in blood pressure and, thus, contribute to the widespread ischemic zones and hemorrhages that have been observed in neuropathological studies of patients dying from septic shock (6, 32, 33). Thus, our findings suggest that neuroprotective strategies instituted for the prevention of encephalopathy and permanent cognitive deficits in critically ill patients with sepsis should take the potentially deleterious effects of acute surges in blood pressure on CBF into account.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES