Lingual, splanchnic, and systemic hemodynamic and carbon dioxide tension changes during endotoxic shock and resuscitation

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Guzman, Jorge A., Mathew S. Dikin, and James A. Kruse. Lingual, splanchnic, and systemic hemodynamic and carbon dioxide tension changes during endotoxic shock and resuscitation. J Appl Physiol 98: 108–113, 2005. First published July 30, 2004; doi: 10.1152/japplphysiol.00243.2004.—Sublingual and intestinal mucosal blood flow and PCO2 were studied in a canine model of endotoxin-induced circulatory shock and resuscitation. Sublingual PCO2 (PsCO2) was measured by using a novel fluorescent optode-based technique and compared with lingual measurements obtained by using a Stowe-Severinghaus electrode [lingual PCO2 (PlCO2)]. Endotoxin caused parallel changes in cardiac output, and in portal, intestinal mucosal, and sublingual blood flow (Qs). Different blood flow patterns were observed during resuscitation: intestinal mucosal blood flow returned to near baseline levels postfluid resuscitation and decreased by 21% after vasopressor resuscitation, whereas Qs rose to twice that of the preshock level and was maintained throughout the resuscitation period. Electrochemical and fluorescent PCO2 measurements showed similar changes throughout the experiments. The shock-induced increases in PsCO2 and PlCO2 were nearly reversed after fluid resuscitation, despite persistent systemic arterial hypotension. Vasopressor administration induced a rebound of PsCO2 and PlCO2 to shock levels, despite higher cardiac output and Qs, possibly due to blood flow redistribution and shunting. Changes in PlCO2 and PsCO2 paralleled gastric and intestinal PCO2 changes during shock but not during resuscitation. We found that the lingual, splanchnic, and systemic circulations follow a similar pattern of blood flow variations in response to endotoxin shock, although discrepancies were observed during resuscitation. Restoration of systemic, splanchnic, and lingual perfusion can be accompanied by persistent tissue hypercarbia, mainly lingual and intestinal, more so when a vasopressor agent is used to normalize systemic hemodynamic variables.

sublingual circulation; endotoxic shock; vasopressors

ALTHOUGH ASSESSMENT OF THE adequacy of tissue perfusion and oxygenation has been a major focus in the clinical management of critically ill patients, conventional hemodynamic and oxygen-derived physiological variables have been shown to be insensitive and may even be normal in the early stages of circulatory shock (3, 8). Recognition that tissue hypoxia may still be present in certain regional tissues, such as the gut and kidneys, despite normal or even elevated cardiac output (Qs), and that inadequate organ perfusion due to hemorrhage or other causes results in tissue hypercarbia, has shifted attention to examining tissue PCO2 as a marker for the presence or severity of perfusion failure (10, 14, 16, 17, 21, 23).

The architecture of gut mucosal microvasculature, a higher critical oxygen delivery compared with other organs and the body as a whole, and disproportionately greater vasoconstriction than other vascular territories in response to decreased Qs, make the gastrointestinal (GI) tract a highly sensitive target organ for detecting early or occult tissue dysoxia (12, 28, 30).

Various anatomic sites for monitoring GI PCO2 have been examined, and, although gastric (PgCO2), intestinal (PiCO2), esophageal, and rectal PCO2 have been studied as indicators of hypoperfusion in the experimental setting, the stomach is currently the preferred site for clinical use (5, 11, 27). Recently, sublingual PCO2 (PsCO2) has been proposed as an indicator of systemic or gut hypoperfusion (17, 23, 32, 34). PsCO2 paralleled changes in PgCO2 during experimental hemorrhage and also clinically. Because sublingual capnometry is disarmingly facile, is noninvasive, does not require administration of H2-receptor-blocking drugs, and has the potential to be less costly than conventional tonometry, it may emerge as a valid clinical alternative for monitoring tissue perfusion in the intensive care setting (19, 23).

However, although correlation between PsCO2 and PgCO2 has been shown to be acceptable, marked heterogeneity of regional splanchnic blood flow appears to be the rule during endotoxin-induced circulatory shock (11, 24). Additionally, recent evidence suggests that the stomach may not represent the most accurate GI site for detecting splanchnic ischemia (33). Furthermore, a large discrepancy between PgCO2 and PsCO2 was reported in one patient with bowel ischemia, suggesting that sublingual blood flow (Qs) may not parallel gut perfusion in all clinical conditions (19). To our knowledge, comparisons between Qs and splanchnic blood flow have only been reported in hemorrhagic shock models but not in models of septic shock, where peripheral blood flow redistribution and shunting are even more striking. All of the above considerations question the validity of PsCO2 as a surrogate marker of mucosal PiCO2 during endotoxin or septic shock.

We hypothesize that the sublingual and the intestinal mucosal circulations do not always correlate during different phases of endotoxin-induced circulatory shock and resuscitation. Using a canine model of endotoxin shock, we performed the following investigation to test whether the sublingual and intestinal mucosal circulations follow similar patterns of change during endotoxin-induced circulatory shock and resuscitation, and whether measurements of lingual PCO2 (PlCO2) or PsCO2 can be used to infer PgCO2 and PiCO2. The investigation also evaluated a novel, fluorescent optode-based technique for assessing PsCO2.

MATERIALS AND METHODS

Surgical preparation. This protocol was approved by the Animal Investigation Committee of Wayne State University. Mongrel dogs

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(14–22 kg) were fasted overnight and then anesthetized with an intravenous injection of pentobarbital sodium (30 mg/kg), endotracheally intubated, and placed on mechanical ventilation (model MA-1; Puritan Bennett, Pleasanton, CA) using a constant tidal volume (15 ml/kg). Respiratory rate was adjusted to achieve a baseline arterial PCO2 (PaCO2) of ~40 Torr. A femoral vein and artery were exposed by surgical dissection and cannulated with vascular catheters for continuous intravenous infusions of pentobarbital sodium (0.06 mg·kg⁻¹·min⁻¹) and normal saline solution, as well as for continuous monitoring of mean arterial blood pressure (MABP) and intermittent blood sampling for blood-gas and hemoglobin assays. A balloon-tipped, multilumen, thermoludition pulmonary artery catheter (Opticath; Abbott Laboratories, Morgan Hill, CA) was advanced through the femoral vein and guided into the pulmonary artery by pressure waveform analysis for measurement of Q̇ and pulmonary artery occlusion pressure (Ppao). Following a midline laparotomy, the duodenum and small intestine were displaced to expose the portal vein. After careful dissection, an 8-mm ultrasonic transit-time flow probe (model 8RS; Transonic Systems, Ithaca, NY) was placed around the vessel and secured with sutures to the adjacent lymphatic tissue. A 7-Fr catheter was advanced through the splenic vein to the portal vein for blood sampling. Correct positioning was confirmed by palpating the tip of the catheter through the wall of the portal vein.

Through a small antimesenteric ileostomy, a fiber-optic laser-Doppler flow probe (type R; Transonic Systems) was affixed to the intestinal mucosa for measurements of intestinal mucosal blood flow (Q̇i), and the ileostomy was closed without compromising perfusion in the area of interest. This methodology does not provide measurements of microvascular perfusion in absolute terms, but it has been validated previously as a reliable means of estimating relative changes in mucosal perfusion (26).

Through a second antimesenteric ileostomy, a double-lumen, balloon-tipped tonometry catheter was introduced into the intestinal lumen for continuous measurement of Plco2, and the ileostomy was closed. A second balloon-tipped tonometry catheter (TONO-14F, Datex-Ohmeda, Andover, MA) was inserted into the stomach through the esophagus for measuring PteCO2. After hemostasis was ensured, the laparotomy was closed. A Stowe-Severinghaus PCO2 electrode (no. 6752–00; Novametrix Medical Systems, Wallingford, CT) was affixed to the ventral paramedian surface of the tongue by using cyanoacrylate adhesive for continuous monitoring of tissue PCO2 at the lingual surface (PlcO2). A fiber-optic laser-Doppler flow probe (type I; Transonic Systems) was affixed to the floor of the mouth approximately midway between the incisors and the root of the tongue for measurements of Q̇. The animals were then allowed to stabilize for 45 min, during which time minute ventilation was readjusted, if necessary, to maintain PaCO2 at ~40 Torr. Core temperature was monitored by using the thermistor of the pulmonary artery catheter and maintained at 38.0 ± 0.5°C by using heating pads and overhead infrared lamps as necessary.

Measurements and calculations. Systemic arterial, mixed-venous, and portal venous blood samples were analyzed for Po2, PCO2, and pH by using an automated blood-gas analyzer (model 860; Bayer Diagnostics, Tarrytown, NY). Q̇ was measured by thermoludition and reported as the average of at least triplicate measurements. Hemodynamic pressures were measured by electronic transduction (Transpac; Abbott Laboratories). Portal vein blood flow (Q̇p) was measured ultrasonically (model T206; Transonic Systems), Q̇ and Q̇p were measured by laser-Doppler flowmetry (BLF21; Transonic Systems), PlCO2 was measured by using an automated electrochemical method (models 860; Novametrix Medical Systems) following calibration with precision gas mixtures. PteCO2 was also measured intermittently at two additional oral sites by using a fluorescent optode method (N-80 CapnoProbe SL System; Nellcor, Pleasanton, CA). The N-80 instrument contains a precision optical component that emits light at two wavelengths in the violet and blue portions of the visible spectrum. The fluorescence intensity generated by the violet wavelength is strongly sensitive to pH. The light is launched into an optical fiber and delivered to the tip of the disposable sensor. The green fluorescent light generated in the optode is directed back to the N-80 instrument through an optical fiber. The light is then radiometrically quantitated and directly correlated to PteCO2. Immediately following calibration, the optode sensors (SLS-1; Nellcor) were manually positioned within the oral cavity with the optical windows held firmly in place against the ventral paramedian surface of the tongue near its root (fluorescence PlCO2, and 2) the floor of the mouth (fluorescence PteCO2) approximately midway between the incisors and the root of the tongue. Measurements were rejected if they could not be completed within 3 min of calibration or the event of a calibration error. PteCO2 was monitored continuously by way of the balloon-tipped ileal catheter by using capnometric recirculating gas tonometry (9, 10). PteCO2 was monitored intermittently by automated reciprocal aspiration (Tonocap; Datex-Ohmeda) from the balloon-tipped gastric catheter. Both tonometry measurement techniques employ infrared spectrophotometry calibrated to a precision CO2 source.

Experimental procedure. After two consecutive sets of baseline measurements (hemodynamic values; arterial, mixed-venous, and portal blood gas, acid-base, and lactate values; Q̇, Q̇p, and Q̇; and electrochemical PlCO2, fluorescence PlCO2, fluorescence PteCO2, Pco2, and PlCO2) were obtained, maintenance intravenous fluids were discontinued, and endotoxic shock was induced by intravenous injection of 3 mg/kg E. coli LPS (serotype 0111:B4; Sigma-Aldrich, St. Louis, MO) over 5 min. Resuscitation was started 20 min after commencement of LPS. This resuscitation lasted 45 min and was divided into two phases. During the first 15 min, an intravenous infusion of isotonic saline solution was administered to target and maintain a Ppao similar to baseline. During the subsequent 30 min of the resuscitation period, an intravenous infusion of norepinephrine was initiated and titrated to achieve the baseline MABP level, if that goal could not be reached by intravascular volume expansion alone. Measurements were obtained at 10-min intervals before, during, and immediately after the LPS injection was initiated, and at 15-min intervals thereafter. Timing for LPS infusion was chosen based on previous experience at our laboratory and others, aiming to induce a decrease in systemic hemodynamic parameters of a magnitude comparable to that observed in a similar clinical scenario (11, 35).

Similarly, the resuscitation pattern was intended to reflect a response pattern comparable to clinically. Animals were then euthanized by injection of a saturated solution of potassium chloride through the right heart catheter.

Statistical analysis. Summary values are expressed as means ± SE. One-factor repeated-measures ANOVA was used to compare sequential measurements for each tested variable obtained between baseline and subsequent experimental time points. Dunnett’s test was used to make further comparisons if ANOVA revealed significant differences. The control value for Dunnett’s test was designated as the last measurement obtained at the end of the baseline period. Two-way repeated-measures ANOVA was used for comparisons of changes between Q̇ and Q̇p, between Q̇ and Q̇p, between electrochemical PlCO2, and fluorescence PteCO2, and between electrochemical PlCO2, and fluorescence PteCO2 over the experimental time points. Correlations were examined by using the Pearson product-moment statistic. Agreement analysis was performed by the method of Bland and Altman by using electrochemical measurements as the criterion standard (1). Probability values (two-tailed) of <0.05 were considered statistically significant. Statistical calculations were performed by using Excel (version 7.0; Microsoft, Redmond, WA) and SigmaStat (version 2.0; Jandel, San Rafael, CA) software.

RESULTS

Seven animals were studied (18 ± 1 kg). Systemic hemodynamic variables, venous Po2, and venoarterial carbon dioxide differences are shown in Table 1. In agreement with previous reports, endotoxin infusion induced a decrease in
heart rate followed by a return to near baseline levels after fluid resuscitation and then a tachycardic response during vasopressor resuscitation (3, 8, 13). MABP dropped by almost 50% 10 min post-LPS infusion. Although MABP improved with intravascular volume expansion, it did not reach preendotoxin levels. By protocol design, MABP at the end of the resuscitation period was comparable to baseline levels. Changes in tissue PCO2 are shown in Fig. 2. Figure 2, top, shows changes in PECO2 and PICO2. Both variables had a similar pattern of change; however, in accordance with previous reports, the change in PECO2 was more striking at the intestinal ports, the change in PCO2 was more striking at the intestinal sites (1, 14). Thus only PICO2 reached statistical significance. Electrochemical PICO2 and fluorescent PSCO2 changed comparably throughout the experiments. Changes in fluorescent PSCO2 followed a pattern similar to both electrochemical PICO2 and fluorescent PSCO2, but they did not achieve statistical significance. The shock-induced increases in fluorescent PSCO2 and electrochemical PICO2 were nearly reversed after fluid resuscitation, despite a MABP that remained below the baseline level. However, after initiation of the vasopressor, fluorescent PSCO2 and electrochemical PICO2 rebounded and remained at shock levels, despite normalization of MABP and a higher Qc and Qg. The mean bias between PICO2 by the Severinghaus electrode criterion standard method and the fluorescent optrode method was 38.8 ± 3.7 Torr on the glossal surface and 44.0 ± 3.2 Torr at the sublingual position.

Table 2 shows PCO2 gaps (tissue minus arterial blood values) at various monitoring sites. Baseline PICO2 was 32.2 ± 1 Torr, 32.2 ± 1.5 Torr post-LPS, 35.2 ± 2.2 15 Torr post-fluid resuscitation, and 39.9 ± 2.5 Torr at the end of resuscitation (P = not significant), probably reflecting increased ventilation-perfusion mismatch induced by the hyperdynamic status

<table>
<thead>
<tr>
<th>Table 1. Systemic hemodynamic variables, venous oxygen tension, and venoarterial carbon dioxide tension gradients at major experimental time points</th>
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<tr>
<td><strong>Baseline</strong></td>
</tr>
<tr>
<td>Heart rate,* beats/min</td>
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<tr>
<td>Cardiac output, m1kg⁻¹min⁻¹</td>
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<tr>
<td>MABP,* mmHg</td>
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<tr>
<td>PpaO2, mmHg</td>
</tr>
<tr>
<td>PICO2,Torr</td>
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<tr>
<td>PICO2,Torr</td>
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<tr>
<td>PICO2−PaCO2,Torr</td>
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<tr>
<td>PSCO2−PICO2,Torr</td>
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</table>

Values are means ± SE. E. coli lipopolysaccharide; MABP, mean arterial blood pressure; PpaO2, pulmonary artery occlusion pressure; PICO2, mixed-venous PO2; PpaCO2, arterial PO2; PICO2−PaCO2, mixed-venous PCO2−arterial PCO2 (PaCO2) difference; PICO2−PICO2, portal venous PCO2−PaCO2 difference. *P < 0.001 by repeated-measures ANOVA, †P < 0.05 compared with baseline by Dunnett’s multiple-comparison statistic.

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achieved during resuscitation. Differences in baseline PCO2 and Pco2 gap at various levels of the GI tract were observed and have been described previously (11, 33). At the level of the tongue, electrochemical Plco2-Paco2 was considerably higher than the other CO2 gaps, and this may be explained by the differences in measurement methodology (Severinghaus electrode vs. fluorescent optrode). All CO2 gaps increased after LPS infusion. At the end of resuscitation, CO2 gaps (by either measurement technique) returned to near-baseline levels; however, in accordance with Qt, PiCO2-PaCO2 remained at near shock levels.

**DISCUSSION**

Although measurement of gut mucosal PCO2 has proven to be a useful marker for hypoperfusion and for assessing the adequacy of resuscitation, the optimal site for clinical monitoring remains unclear (4, 5, 11, 17, 27). Our data show that changes in the tongue and sublingual territory in response to shock are almost indistinguishable from splanchnic changes; however, notable differences are apparent during resuscitation.

Endotoxin administration induced a drop in MABP of >50% and comparable decreases in systemic blood flow, splanchnic blood flow, and Qs. These findings are in accordance with previously reported data from our laboratory and by others (13, 17, 27). However, a different pattern was observed during resuscitation, depending on the territory monitored and the phase of resuscitation. After 15 min of aggressive fluid resuscitation, rebound to a hyperdynamic status was observed at the level of the large-vessel circulation, Qa and Qp. This rebound occurred, despite a mean arterial pressure that was almost 25% lower than the baseline level. After norepinephrine administration was initiated during the second phase of resuscitation, MABP returned to baseline levels and Qa continued to increase; however, Qp showed a sharp change to a downward trend, even though it remained higher than baseline levels. Concordant with previously reported decreases in Qs and jejunal blood flow observed when norepinephrine was administered to increase MABP to 20 mmHg above shock levels (32), these findings suggest a more selective vasoconstrictive effect of norepinephrine at the splanchnic vasculature.

At the level of the small-vessel circulation under study, a different response to resuscitative efforts was noted between the lingual and the ileal mucosal territories. Qt returned to baseline levels after fluid resuscitation but decreased by 20% after norepinephrine was initiated. This reduction could be secondary to a relatively selective splanchnic vasoconstrictive effect of norepinephrine with redistribution of blood flow away from the gut (13, 16). On the other hand, Qs achieved and maintained values twice higher than baseline throughout resuscitation. Explanations for this observation can only be hypothetical, because few investigations have examined the lingual or sublingual circulation during endotoxic shock. However, a study using orthogonal polarization of spectral imaging to assess the number of perfused sublingual vessels by size demonstrated that the normal (1.5:1) ratio of small-to-large vessel perfusion is reversed in sepsis (6). Therefore, it is possible that resuscitation increased shunting by enhancing flow to larger sublingual vessels rather than increasing capillary perfusion. This hypothesis is supported by our measurements of lingual and PsCO2, which demonstrated rebound increases during resuscitation with norepinephrine.

**Table 2. Tissue-arterial Pco2 gradients at various monitoring sites during major experimental time points**

<table>
<thead>
<tr>
<th>Time (min Postresuscitation)</th>
<th>Fluids</th>
<th>Vasopressor</th>
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<tbody>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluorescence PsCO2-Paco2 * Torr</td>
<td>10.9±2.5</td>
<td>26.2±4.2†</td>
</tr>
<tr>
<td>Fluorescence Plco2-Paco2 * Torr</td>
<td>13.5±1.9</td>
<td>23.9±2.1†</td>
</tr>
<tr>
<td>Electrochemical Plco2-Paco2 * Torr</td>
<td>44.9±4.3</td>
<td>70.3±7.9†</td>
</tr>
<tr>
<td>PiCO2-Paco2 * Torr</td>
<td>24.9±4.6</td>
<td>34.8±6.2</td>
</tr>
<tr>
<td>PgCO2-Paco2 * Torr</td>
<td>6.9±3.1</td>
<td>12.6±2.7</td>
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</tbody>
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<tr>
<th>10 min Post-LPS</th>
<th>20 min Post-LPS</th>
<th>15 min Postresuscitation (Fluids)</th>
<th>45 min Postresuscitation (Fluids + Vasopressor)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluorescence PsCO2-Paco2 * Torr</td>
<td>10.9±2.5</td>
<td>26.2±4.2†</td>
<td>16.0±4.2</td>
</tr>
<tr>
<td>Fluorescence Plco2-Paco2 * Torr</td>
<td>13.5±1.9</td>
<td>23.9±2.1†</td>
<td>20.6±0.7</td>
</tr>
<tr>
<td>Electrochemical Plco2-Paco2 * Torr</td>
<td>44.9±4.3</td>
<td>70.3±7.9†</td>
<td>66.8±7.7†</td>
</tr>
<tr>
<td>PiCO2-Paco2 * Torr</td>
<td>24.9±4.6</td>
<td>34.8±6.2</td>
<td>39.8±7.3†</td>
</tr>
<tr>
<td>PgCO2-Paco2 * Torr</td>
<td>6.9±3.1</td>
<td>12.6±2.7</td>
<td>11.3±2.3</td>
</tr>
</tbody>
</table>

Values are means ± SE. PsCO2, Plco2, Pco2, and PgCO2: sublingual, lingual, intestinal, and gastric PCO2, respectively; fluorescence PsCO2-Paco2, PsCO2 (fluorescent method)-Paco2 gradient; fluorescence Plco2-Paco2, Plco2 (fluorescent method)-Paco2 gradient; electrochemical Plco2-Paco2, Plco2 (electrochemical method)-Paco2 gradient. *P < 0.001 by repeated-measures ANOVA; †P < 0.05 compared with baseline by Dunnett’s multiple-comparison statistic.
Both electrochemical $P_{ICO_2}$ and fluorescence $P_{SCO_2}$ followed a similar pattern throughout the experiments, and, despite the differences in methodology and anatomic positioning, the percent change was almost identical for both techniques. As noted above, lingual $PCO_2$ and $PS_{CO_2}$ decreased to near-baseline levels during the initial phase of resuscitation (isotonic saline infusion) but rebounded to shock levels once the vasopressor infusion was started to achieve normalization of MABP. Whether this rise in tissue $PCO_2$ was a consequence of increased oxygen delivery levels between the two mucosal circulations (luminal volume) and possibly to differences in critical hypoperfusion results in lingual and splanchnic tissue hypercarbia that parallels changes in tissue $PCO_2$ observed at the levels of the gastric and intestinal mucosa. However, the most striking finding of our study was that restoration of systemic, splanchic, and lingual perfusion could accompany persistent tissue hypercarbia, more so when norepinephrine is used to normalize systemic hemodynamic variables. Our data support the notion that monitoring efforts directed at restoration of systemic hemodynamic variables may be misleading and that concomitant measurements of tissue $PCO_2$ provide helpful additional information.

DISCLOSURES
This study was funded in part by a grant from Nellcor Puritan Bennett.

REFERENCES


