Septic shock induces distinct changes in sympathetic nerve activity to the heart and kidney in conscious sheep

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Septic shock induces distinct changes in sympathetic nerve activity to the heart and kidney in conscious sheep. Am J Physiol Regul Integr Comp Physiol 297: R1247–R1253, 2009. First published September 2, 2009; doi:10.1152/ajpregu.00437.2009.—Sepsis and septic shock are the chief cause of death in intensive care units, with mortality rates between 30 and 70%. In a large animal model of septic shock, we have demonstrated hypotension, increased cardiac output, and tachycardia, together with renal vasodilatation and renal failure. The changes in cardiac sympathetic nerve activity (CSNA) that may contribute to the tachycardia have not been investigated, and the changes in renal SNA (RSNA) that may mediate the changes in renal blood flow and function are unclear. We therefore recorded CSNA changes in renal SNA (RSNA) that may mediate the changes in renal output. There was little correlation between the changes in RSNA and renal blood flow, suggesting that the renal vasodilatation was mediated mainly by other mechanisms.

Acute renal failure; renal blood flow

Sepsis and septic shock remain a major challenge in medicine; they are the chief cause of death in intensive care units with mortality rates between 30 and 70% (15). A cardiovascular hallmark of septic shock is systemic vasodilatation and hypotension, which has been proposed to be mediated largely by increased production of nitric oxide (5, 16). The fall in arterial pressure is accompanied by increases in heart rate (HR) and renal sympathetic nerve activity (RSNA) (12, 18).

Although these are well-established responses to bacterial infection, the mechanisms have not been fully elucidated, and recordings of cardiac sympathetic nervous activity (CSNA) have not been made in septic shock. An important role for increased CSNA as a cause of the tachycardia is the finding that β-blockade returned the elevated HR to control levels in conscious rabbits treated with lipopolysaccharide (LPS) (10) and prevented the tachycardia in febrile humans (2). The factors leading to an increase in CSNA in septic shock have not been investigated but may include unloading of arterial baroreceptors in response to the hypotension and altered baroreflex control.

The majority of studies that have investigated the changes in SNA during sepsis have shown increases in RSNA in response to endotoxemia induced by administration of LPS (17, 18). The relevance of LPS models of sepsis in rodents to human sepsis is uncertain as LPS administration in rodents is usually associated with a hypodynamic circulation (systemic vasodilatation, hypotension, and a fall in cardiac output) (7). These findings are in contrast to the hyperdynamic circulation (systemic vasodilatation, hypotension, and an increase in cardiac output) commonly seen in human septic patients. We have developed a large animal model of septic shock that simulates the human condition of fever, tachycardia, tachypnea, hyperlactatemia, hypoxemia, oliguria, hypotension, and high cardiac output (3, 6, 7, 19). In this setting, despite renal vasodilatation and a large increase in renal blood flow, animals developed oliguria and a decrease in creatinine clearance. This is in contrast to the fall in renal blood flow seen in models of sepsis with a hypodynamic circulation (1, 4).

To establish the changes in SNA to the heart and kidney in hyperdynamic septic shock, we have recorded CSNA and RSNA following administration of live Escherichia coli to conscious sheep. In this well-characterized model of septic shock, the increases in HR and renal blood flow are initiated at different time points (3, 7, 19), so we have examined the temporal changes in activity in these sympathetic nerves during the development and maintenance phases of sepsis. To determine whether the arterial baroreflex control of SNA to the heart and kidney is altered at different phases of septic shock, we examined the relationships of CSNA and RSNA to arterial pressure at different times after administration of E. coli.

METHODS

Adult merino ewes (34–47 kg body wt) were housed in individual metabolic cages in association with other sheep. Experiments were started when sheep were accustomed to laboratory conditions and human contact. Sheep were fed a diet of oaten chaff (800 g/day), and water was offered ad libitum. All experiments were approved by the Animal Experimentation Ethics Committee of the Howard Florey Institute.

Surgical procedures. Experiments were conducted in two groups of sheep; one group was instrumented for cardiac and renal sympathetic nerve recording, and the second group was instrumented to record renal blood flow. Before the nerve recording studies, sheep underwent two aseptic surgical procedures, each separated by 2 wk of recovery. For all surgery, anesthesia was induced with intravenous thiopental sodium (15 mg/kg) and, following intubation, was maintained with 1.5–2.0% isoflurane/O2. In the first stage, sheep were prepared with a carotid arterial loop. Briefly, a carotid artery was isolated and exteriorized in a fold of skin to form a carotid arterial loop, allowing easy access for arterial cannulation. In a separate operation, infrasaccular electrodes...
were implanted in the left or right renal nerves (9) and, during the same surgery, in the left cardiac sympathetic nerves (20). Experiments were conducted on standing, conscious sheep, and to minimize any effect of surgical stress, experiments were not started until 4 days after implantation of the electrodes. In a second group of sheep with carotid arterial loops, flow probes (4 mm; Transonic Systems) were implanted on the left renal artery for the measurement of renal blood flow, as previously described (8). In all operations, animals were treated with intramuscular antibiotics (900 mg, Ilium Prophen, procaine penicillin; Troy Laboratories, Smithfield, New South Wales, Australia or Mavlab, Queensland, Australia) at the start of surgery and then for 2 days postoperatively. Postsurgical analgesia was maintained with intramuscular injection of flunixin meglumine (1 mg/kg; Troy Laboratories or Mavlab) at the start of surgery and then 4 and 16 h postsurgery.

On the day before implantation of recording electrodes, arterial and venous cannulas were inserted into the carotid artery and jugular vein, as described previously (20). In addition, a bladder catheter was inserted at least 8 h before the start of the protocol. Measurement of SNA. CSNA and RSNA were recorded differentially between the pair of electrodes with the best signal-to-noise ratio. The signal was amplified (×100,000) and filtered (band pass, 300–1,000 Hz), displayed on an oscilloscope, and passed through an audio amplifier and loud speaker. Sympathetic nerve activity (5,000 Hz) and arterial blood pressure (100 Hz) were recorded on computer using a CED Micro 1401 interface and Spike 2 software (Cambridge Electronic Design, Cambridge, UK). Studies were performed in a total of 10 conscious sheep. RSNA was recorded in seven animals; two of these animals had simultaneous CSNA recordings. CSNA was also recorded in an additional three sheep (n = 5 for CSNA).

Experimental protocols. During the experiment, 5-min measurements of resting CSNA, RSNA, central venous pressure, and arterial pressure were made at hourly intervals. Urine was collected in hourly lots from the bladder catheter using a fraction collector. During the first hour of the control period, baroreflex curves were generated by measuring CSNA, RSNA, and HR responses to increases and decreases in arterial pressure induced by intravenous administration of incremental doses of phenylephrine and sodium nitroprusside, as described previously (9). At the end of the control period, sepsis was induced by intravenous infusion of live E. coli (3 × 109 colony forming units in 20 ml of saline over 10 min). The arterial baroreflex curves were also generated at 8, 15, and 32 h after infusion of E. coli.

At the end of the control period and at 8, 15, and 32 h after infusion of E. coli, arterial blood samples were obtained for analysis of blood lactate (ABL System 625; Radiometer Medical, Copenhagen, Denmark) and plasma creatinine (Synchro LXS System; Beckmann Coulter, Fullerton, CA). At these times, urine samples were collected for measurement of creatinine (Synchro LXS System) and used to calculate creatinine clearance (creatine urine/creatinine plasma × urine volume/time).

In a separate group of eight sheep implanted with renal artery flow probes at least 2 wk earlier, the effect of administration of E. coli on renal blood flow was determined. On the day before the experiment, cannulas were inserted into the carotid artery and jugular vein for measurement of arterial pressure and for infusion, respectively. Arterial pressure and renal blood flow were measured every 10 min for 10 s at 50 Hz. After a control period of 5 h, E. coli (3 × 109 colony forming units in 20 ml of saline over 10 min) was infused intravenously and sheep were monitored for a further 16 h.

Data analysis. Data were analyzed on a beat-to-beat basis using custom-written routines in the Spike 2 program as previously described (20). Briefly, for each heartbeat, the program determined diastolic, systolic, and mean arterial pressure, heart period, and the number of discriminated spikes of SNA above threshold between the following diastolic pressures, a measure of burst size. The threshold was set just above background so that spikes from small bursts were counted. The background noise of SNA was taken as the spikes per second during the highest dose of phenylephrine when SNA was abolished in the control period. This background noise was subtracted from all the data collected. Baroreceptor reflex curves were constructed from data collected during infusion of phenylephrine and nitroprusside. Data are means ± SE and were analyzed using repeated-measures ANOVA (SigmaStat, Access Softtek, version 2.03). If a significant effect was found, post hoc comparisons were made at time points 1, 2, 4, 8, and 16 h vs. the control time points using contrast coefficients. P ≤ 0.05 was considered statistically significant.

RESULTS

Hemodynamic and renal function parameters. Consistent with our previous results, administration ofE. coliinduced hypertension with a delayed onset (3, 7, 19). About 5 h after the bolus of E. coli, blood pressure started to decrease, reached a minimum after 9 h, and remained depressed for the remainder of the protocol (Figs. 1 and 2). In contrast, the increase in HR in response to E. coli was not delayed, with significant increases after 2, 4, 8, and 16 h. The pattern of change in CSNA after E. coli infusion indicated a significant increase in CSNA over time, with significant increases after 8 and 16 h (Figs. 1 and 2). After administration of E. coli, the changes in CSNA correlated with those in HR (P < 0.01; mean R2 value = 0.54 ± 0.08). In contrast to the progressive increase in CSNA, administration of E. coli was associated with a large, transient decrease in RSNA (P < 0.05 at 2 h) (Figs. 1 and 2). After this, there was an increase in RSNA to ~180% of control levels, and it remained elevated for the remainder of the protocol.

Urine output increased briefly after induction of sepsis, reaching a peak at 4 h, and then by 8 h had decreased to below 30% of control levels (Fig. 1). Serum creatinine was significantly increased 8 h after E. coli infusion (from 72 ± 2 to 105 ± 5 μM, P < 0.05) before gradually recovering toward control levels (82 ± 5 μM after 32 h of E. coli). Glomerular filtration rate, as estimated by creatinine clearance, was significantly decreased at 8 h after induction of sepsis (from 90 ± 21 to 34 ± 7 ml/min, P < 0.05) before recovering toward control levels (98 ± 22 ml/min after 32 h). Arterial baroreflex control of HR, CSNA, and RSNA. After induction of sepsis, the range of baroreflex-mediated changes in HR was significantly decreased (Fig. 3 and Table 1). This was because in sepsis, increases in arterial pressure caused a much smaller bradycardia than in the normal state, resulting in a significant increase in the lower plateau of the curve. There was also a significant decrease in the maximum gain of the HR baroreflex curve during sepsis (Fig. 3 and Table 1). The increased HR after induction of sepsis meant that the resting point was shifted higher on the curve at 8 and 15 h after E. coli infusion before returning toward normal levels at 32 h.

In contrast to the reduced fall in HR during phenylephrine-induced increases in arterial pressure in sepsis, the baroreceptor-induced changes in CNSA were similar in the normal and septic states (Fig. 4). On the other hand, during decreases in arterial pressure, CSNA was increased to a greater extent during sepsis, resulting in a significant increase in the range of the baroreflex relationship between diastolic blood pressure and CSNA from 8 to 32 h after E. coli infusion (Fig. 4). There were no significant changes in any of the other baroreflex parameters during sepsis (Fig. 4 and Table 1). There was a significant increase in the resting level of CSNA after induction of sepsis, and this remained elevated for the remainder of the protocol.
The range of the baroreflex relationship between diastolic blood pressure and RSNA tended to increase during septic shock, but this did not reach statistical significance (Fig. 5). There were no significant changes in any of the other baroreflex parameters during sepsis. The resting level of RSNA was significantly increased 8 h after induction of sepsis.

Changes in renal blood flow in septic shock. Administration of E. coli in the second group of animals led to changes in hemodynamic parameters similar to those of the first group. There was a delayed decrease in mean arterial pressure over time, whereas there was an immediate biphasic increase in HR that lasted the for remainder of the recording period (Fig. 6). There was an immediate increase in renal blood flow that remained elevated throughout the recording protocol. Renal vascular conductance also increased in a biphasic manner (Fig. 6).

DISCUSSION

This study used a well-characterized model of hyperdynamic sepsis in conscious sheep in which administration of live E. coli caused hypotension, tachycardia, increased renal blood flow, and a decrease in renal function. There were differential changes in the measured variables suggesting that the responses to E. coli were evoked by separate mechanisms. The novel findings of this study are the organ-specific changes in SNA. There was an increase in CSNA that was correlated with increases in HR, whereas there was a transient inhibition of RSNA followed by a prolonged stimulation. In addition, there were different effects on the range of the arterial baroreflex-induced changes in HR and SNA; it was depressed for HR, increased for CSNA, and unchanged for RSNA.

Changes in CSNA and HR during sepsis. It is well established that bacterial infection increases HR, but the changes in
CSNA that may contribute to this have not been determined. In conscious sheep, administration of live *E. coli* caused increases in both CSNA and HR. The high correlation between the changes in CSNA and HR support the notion that increased sympathetic drive to the heart contributes to the tachycardia during septic shock. In support of this, previous studies have demonstrated that β-blockade returned the elevated HR to control levels in conscious rabbits treated with LPS (10). It is important to note that other mechanisms may cause the initial tachycardia that began to develop before CSNA increased (Fig. 1).

Information from the arterial baroreflex curves also suggests that other mechanisms in addition to the increased CSNA play a role in causing the tachycardia. At 8 and 15 h after *E. coli* infusion, the lower plateau of the HR baroreflex curve was significantly increased (Fig. 3), despite CSNA being abolished at the higher pressures (Fig. 4). The increase in the lower plateau of the HR curve, by 60 beats/min, must therefore have resulted from a reduction in cardiac vagal activity or a direct effect of cytokines on the heart. In addition, the top plateau of the CSNA arterial baroreflex curves was increased at 8 and 15 h after *E. coli* infusion (Fig. 4), suggesting an additional central drive to increase CSNA. These results are in agreement with the observation that administration of live *E. coli* to conscious rats caused an increase in HR that was greater than that observed during baroreceptor unloading (13). The factors leading to the increase in the top plateau of the CSNA curve are unknown but may be due to a central effect of inflammatory

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**Fig. 2.** Raw data in a conscious sheep showing arterial pressure (AP), CSNA, and RSNA during a control period (A) and 2 (B), 8 (C), and 33 h after infusion of *E. coli* (D).
cytokines released in response to E. coli. Administration of cytokines has been shown to induce tachycardia (11), but the effects on CSNA have not been examined.

Changes in RSNA, renal blood flow, and renal function during sepsis. In agreement with our previous findings in this sheep model of hyperdynamic sepsis, treatment with E. coli induced an initial diuresis, oliguria, and a decrease in glomerular filtration rate, which developed in the presence of renal vasodilatation and an increase in renal blood flow (6, 19). During the initial 3 h after E. coli infusion, arterial pressure was unchanged and there was a significant inhibition of RSNA (Fig. 1), which may have contributed to the initial increase in renal blood flow (Fig. 6). The reduction in RSNA also may have contributed to the diuresis, although the finding that RSNA had returned to control levels at 4 h after E. coli infusion, when the diuresis was greatest, indicates that additional factors are involved. The sustained increase in renal blood flow in the presence of increased RSNA, which would promote vasoconstriction, indicates that other mechanisms must mediate this effect. The finding of large increases in the release of nitric oxide in septic shock indicates that this could be one mechanism causing the renal vasodilatation (5, 16).

The mechanism behind the transient reduction in RSNA remains unclear. The decrease in RSNA cannot be due to baroreceptor inhibition because there was no change in arterial pressure when RSNA was inhibited. Previous studies in con-

Table 1. Baroreflex parameter values obtained before and during septic shock induced by a bolus of Escherichia coli

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Sepsis 8 h</th>
<th>Sepsis 15 h</th>
<th>Sepsis 32 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>87±2</td>
<td>69±3*</td>
<td>75±3*</td>
<td>76±3*</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>70±5</td>
<td>167±9*</td>
<td>157±10*</td>
<td>123±9*</td>
</tr>
<tr>
<td>Renal sympathetic nerve activity, %</td>
<td>100</td>
<td>166±26*</td>
<td>147±24*</td>
<td>109±19</td>
</tr>
<tr>
<td>Cardiac sympathetic nerve activity, %</td>
<td>100</td>
<td>371±52*</td>
<td>343±70*</td>
<td>210±25*</td>
</tr>
</tbody>
</table>

Heart rate parameters

| Lower plateau   | 50±2          | 113±7*         | 92±12*         | 70±6*         |
| Range           | 124±12        | 69±6*          | 79±14*         | 87±8*         |
| BP_{50}, mmHg   | 97±3          | 100±4          | 105±4          | 97±3          |
| Maximum gain    | −6.0±0.8      | −4.4±0.8       | −3.3±0.5*      | −3.9±0.6*     |

Cardiac sympathetic nerve activity parameters

| Lower plateau   | 15±2          | −6±14          | 7±10           | −2±10         |
| Range           | 233±21        | 481±50*        | 512±90*        | 298±29*       |
| BP_{50}, mmHg   | 78±5          | 84±5           | 91±5           | 80±3          |
| Maximum gain    | −14.0±4.6     | −11.4±2.7      | −13.7±4.5      | −7.9±0.9      |

Renal sympathetic nerve activity parameters

| Lower plateau   | 6±6           | −8±4           | 2±8            | 2±3           |
| Range           | 199±23        | 286±70         | 219±43         | 197±30        |
| BP_{50}, mmHg   | 79±4          | 73±3           | 80±5           | 74±5          |
| Maximum gain    | −9.0±1.8      | −6.5±1.2       | −7.9±1.2       | −4.5±0.9      |

Values are means ± SE. BP_{50}, arterial pressure at midpoint of heart rate range. *P < 0.05 vs. control.
scious rabbits demonstrated that LPS caused an initial decrease in RSNA that was attributed to compensatory renal vasodilation in response to peripheral vasoconstriction (14). In this context, it is possible that the renal sympathoinhibition is part of a central patterned response that is evoked to raise body temperature in response to the infection. Our results suggest that the subsequent sustained increase in RSNA after E. coli administration was mediated largely by withdrawal of baroreceptor-mediated inhibition in response to the decreased blood pressure, since there was little change in the arterial baroreflex curves at different times during septic shock (Fig. 5). Other studies using endotoxin have found that the increase in RSNA persists in baroreceptor denervated animals (18, 21, 22), suggesting additional stimulation of RSNA in this setting by a direct central action of inflammatory cytokines.

**Perspectives**

This study demonstrates that in a large animal model of septic shock that mimics the changes seen in humans, there were increases in CSNA and HR that followed a similar pattern. The high correlation between the changes in CSNA and HR suggests that the increased sympathetic drive to the heart is an important factor causing the tachycardia and increased cardiac output seen in this model of sepsis, although reduced vagal tone may also play a role. The persistent increase in renal blood flow occurred in the presence of increased RSNA, indicating that mechanisms other than the renal sympathetic nerves played the major role in determining the renal vasodilation. It is likely that in sepsis, the increase in CSNA is beneficial given that it promotes an increase in cardiac output that maintains arterial pressure in the face of peripheral vasodilatation. Although the vasoconstrictor actions of increased RSNA are overridden by other factors, its action to increase renin release, and thus circulating angiotensin II, should help support arterial pressure.

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