Role of prostaglandins in determining the increased cardiac sympathetic nerve activity in ovine sepsis

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Booth LC, Ramchandra R, Calzavacca P, May CN. Role of prostaglandins in determining the increased cardiac sympathetic nerve activity in ovine sepsis. Am J Physiol Regul Integr Comp Physiol 307: R75–R81, 2014. First published April 30, 2014; doi:10.1152/ajpregu.00450.2013.—Effective treatment of sepsis remains a significant challenge in intensive care units. During sepsis, there is widespread activation of the sympathetic nervous system, which is thought to have both beneficial and detrimental effects. The sympathoexcitation is thought to be partly due to the developing hypotension, but may also be a response to the inflammatory mediators released. Thus, we investigated whether intracarotid infusion of prostaglandin E₂ (PGE₂) induced similar cardiovascular changes to those caused by intravenous infusion of Escherichia coli in sheep and whether inhibition of prostaglandin synthesis, with the nonselective cyclooxygenase inhibitor indomethacin, administered at 2 and 8 h after the onset of sepsis, reduced sympathetic nerve activity (SNA), and heart rate (HR). Studies were performed in conscious sheep instrumented to measure mean arterial pressure (MAP), HR, cardiac SNA (CSNA), and renal SNA (RSNA). Intracarotid infusion of PGE₂ (50 ng·kg⁻¹·min⁻¹) increased temperature, CSNA, and HR, but not MAP or RSNA. Sepsis, induced by infusion of E. coli, increased CSNA, but caused an initial, transient inhibition of RSNA. At 2 h of sepsis, indomethacin (1.25 mg/kg bolus) increased MAP and caused reflex decreases in HR and CSNA. After 8 h of sepsis, indomethacin did not alter MAP, but reduced CSNA and HR, without altering baroreflex control. These findings indicate an important role for prostaglandins in mediating the increase in SNA in sepsis, as this may lead to development of more targeted treatments.

The extent to which sympathetic activation during sepsis results from reduced baroreceptor inhibition due to the hypotension or to actions of inflammatory mediators, such as prostaglandins, is unclear. Both intracarotid and central administration of prostaglandin E₂ (PGE₂) have been shown to produce similar changes to those seen during sepsis: fever, tachycardia, and an increase in RSNA (3, 4). Furthermore, inhibitors of the cyclooxygenase (COX) pathway, which inhibit prostaglandin production, prevent the tachycardia and the release of catecholamines and stress hormones in response to endotoxin in humans (25), as well as the increase in RSNA in response to LPS in anesthetized rats (30). In addition, the late increase in RSNA following transient, mild exposure to LPS in rabbits was reduced following acetylsalicylate administration (26).

In view of the evidence that PGE₂ increases heart rate (HR) and that COX inhibitors prevent endotoxin-induced tachycardia, we investigated whether intracarotid administration of PGE₂ increased cardiac sympathetic nerve activity (CSNA) and HR in normal conscious sheep. To determine the role of prostaglandins in driving the increased CSNA in sepsis, we examined the effect of treatment with indomethacin, a nonselective inhibitor of COX 1 and COX2, on the resting levels and baroreflex control of heart rate and CSNA in conscious sheep with early hyperdynamic sepsis.

METHODS

Experiments were conducted on 12 adult Merino ewes, housed in individual metabolic cages, with free access to food and water. The response to infusion of PGE₂ was examined in nine sheep (38 ± 2 kg). At least 24 h later, the effect of indomethacin in sepsis was determined in 10 sheep, seven of which were common to the previous group (39 ± 1 kg). Experiments were conducted once sheep were accustomed to laboratory conditions and human contact. The experimental procedures were approved by the Animal Ethics Committee of the Florey Institute of Neuroscience and Mental Health under guidelines laid down by the National Health and Medical Research Council of Australia.

Surgical procedures. All sheep underwent two surgeries separated by at least 2 wk of recovery. Anesthesia was induced by intravenous thiopental sodium (15 mg/kg) and, following intubation, was maintained with 1.5–2.0% isoflurane in an O₂ and air mixture. In the first surgery, sheep were prepared with carotid arterial loops. In the second operation, intrafascicular recording electrodes were implanted in the left cardiac and renal sympathetic nerves (24). In all operations, animals were treated with intramuscular antibiotics (900 mg, ilium Propen; procaine penicillin; Troy Laboratories, Smithfield, NSW, Australia.

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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) at the start of surgery, then 4 h and 24 h postsurgery.

On the day before implantation of recording electrodes, arterial and venous cannulas were inserted into the carotid artery and jugular vein. Temperature was measured using a thermocouple (Physitemp Instruments, Clifton, NJ) inserted 20 cm into a jugular venous catheter. Experiments were started on conscious sheep 4 days after implantation of the electrodes.

**Measurement of SNA.** CSNA and RSNA were recorded differentially between the pair of electrodes with the best signal-to-noise ratio (24). The signal was amplified ($\times 100,000$) and filtered (bandpass, 300–1,000 Hz), displayed on an oscilloscope, and passed through an audio amplifier and loudspeaker. 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). Data were analyzed on a beat-to-beat basis using custom-written routines in the Spike 2 program. Recordings were made for a 15-min resting period, and this level, minus background noise, was defined as the 100% baseline level. The techniques for spike counting per heartbeat and subtraction of background noise were as previously described (32).

**Generation of baroreceptor reflex curves.** Baroreceptor reflex curves were constructed from data collected during infusions of phenylephrine and sodium nitroprusside, infused in random order (32). Briefly, phenylephrine and sodium nitroprusside (67 $\mu$g/ml in sterile saline) were infused at 30, 60, 120, and 300 ml/min for $\sim$1 min at each dose, resulting in a slow, steady change in blood pressure. To construct baroreflex curves, SNA burst size, determined as the number of discriminated spikes between successive diastolic pressures minus background, was divided by heart period (32). These data were sorted by diastolic blood pressure and averaged in groups of 10. To allow data from individual animals to be grouped, spike counts were normalized by calculating the percent change in SNA from the mean activity recorded during the 15-min resting period, described above. Percent SNA was plotted against diastolic pressure and regression analysis performed (4-parameter sigmoid relationship; Sigmaplot, Systat Software, San Jose, CA). For generation of the HR baroreflex curve, data were sorted by systolic blood pressure (32).

**Experimental design.** Studies were performed in a total of nine sheep for the PGE2 infusions, seven of which had RSNA and six of which had CSNA recordings. Sepsis was performed in 10 sheep, eight of which had RSNA and seven of which had CSNA recordings.

**PGE2 infusion.** Mean arterial pressure (MAP), CSNA, RSNA, temperature, and HR (calculated from systolic peaks) were recorded for 30 min before and during a 30-min bilateral intracarotid infusion of PGE2 (50 ng·kg$^{-1}$·min$^{-1}$; Sigma-Aldrich, Castle Hill, NSW, Australia), dissolved in ethanol (stock solution of 1 mg/ml) and diluted in 60 ml of sterile saline. Arterial baroreflex curves were generated during the control period and 10 min after the start of the PGE2 infusion.

**Indomethacin treatment during sepsis.** During the experiment, 15-min measurements of MAP, CSNA, and RSNA were made at hourly intervals. During the first hour of the control period, baroreflex curves were generated. At the end of the control period, sepsis was induced by intravenous infusion of live *E. coli* [3 $\times$ 10$^9$ colony-forming units (cfu) in 15 ml of saline over 15 min] and then maintained with a continuous infusion of 1.5 $\times$ 10$^9$ cfu/h for 8 h. After 2 and 8 h of *E. coli* infusion, arterial baroreflex curves were generated before and after intravenous infusions of indomethacin (1.25 mg/kg; Sigma-Aldrich) dissolved in 2 ml Na$_2$CO$_3$ and 13 ml sterile saline, infused through a Millipore filter over 15 min. Data from the 15 min prior to indomethacin infusion were used as the preindomethacin control, which was compared with data from a 15-min period during indomethacin when cardiovascular changes had stabilized, before the postindomethacin baroreflex curves were generated.

At the end of the protocol, sheep were killed with an intravenous overdose of pentobarbital sodium (100 mg/kg). To establish that the 2-h dose of indomethacin did not influence the later responses, in a pilot study in two sheep, we established that the development of sepsis from 3 to 8 h and the response to indomethacin at 8 h did not appear to be altered by previous treatment with indomethacin. The finding that temperature increased back to preindomethacin levels within 45

![Fig. 1. Cardiovascular changes with bilateral intracarotid infusion of prostaglandin E2 (PGE2). Left: temperature (Temp; °C), heart rate (HR; bpm), mean arterial blood pressure (MAP; mmHg), cardiac sympathetic nerve activity (CSNA; %baseline), and renal SNA (RSNA; %baseline) are shown 5 min before and during the first 10 min of PGE2 infusion (started at vertical dotted line). Data points are 1-min averages ± SE. Right: variables during the 5-min control period (C) and in the last 2 min of PGE2 infusion before the start of baroreflex curve generation (PGE2). Data were analyzed using paired t-test. **P < 0.005 PGE2 vs. C.](http://ajpregu.physiology.org/)

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min of the end of infusion of indomethacin is further evidence that in sepsis, the ability of indomethacin to inhibit the synthesis of endogenous prostaglandins was of short duration. In initial studies, a third dose of indomethacin was administered after 24 h of sepsis, but this led to severe renal and cardiovascular failure; therefore, subsequent experiments were ended after the 8-h indomethacin treatment.

Statistics. Statistical analysis was performed in R and Rcmdr (11, 23). Data sets were assessed for normality and log-transformed or square root-transformed as appropriate. For negative data, differences from baseline were also considered. Changes in cardiovascular variables and baroreflex data in response to PGE2 were analyzed using paired sample t-tests. The cardiovascular variables and baroreflex data in the indomethacin and sepsis experiment were tested using repeated-measures ANOVA with Greenhouse-Geisser correction, and post hoc analysis was performed using paired sample t-tests with Bonferroni correction for multiple comparisons. P < 0.05 was considered statistically significant. Normally distributed data are presented as means ± SE, and log and square root-transformed data are presented as geometric mean (95% CI).

RESULTS

Response of normal sheep to bilateral intracarotid infusions of PGE2. Intracarotid infusion of PGE2 did not change MAP [92 (84.99) vs. 92 (85.98) mmHg], but significantly increased HR [78 (69.89) to 113 (93.146) bpm; P < 0.05; Fig. 1]. There was an increase in CSNA [102 (100.105) to 179 (152.209) % baseline; P < 0.005], whereas RSNA was unchanged [100 (97.104) to 97 (86.108) % baseline]. PGE2 infusion significantly increased temperature (39.2 ± 0.3°C, P < 0.05) followed by a decrease to control levels by 8 h (38.7 ± 0.2°C; P < 0.005).

With PGE2 infusion, there was a significant increase in the lower plateau of the HR baroreflex curve (P < 0.01 compared with baseline; Table 1, Fig. 2). There were no significant differences in any parameter of the CSNA or RSNA baroreflex curves.

Responses to intravenous infusion of E. coli. Sepsis was induced by intravenous infusion of live E. coli. Temperature was elevated at 2 and 8 h of sepsis (baseline: 38.8 ± 0.2°C; 2 h: 40.2 ± 0.1°C, P < 0.05; 8 h: 40.7 ± 0.4°C, P < 0.05). Infusion of E. coli caused an initial increase in MAP at 2 h [from 86 (82.90) to 99 (95.104) mmHg; P < 0.05] followed by a decrease to control levels by 8 h [87 (83.91) mmHg; Fig. 3]. There was no significant increase in HR at 2 h of sepsis [77 (70.85) vs. 79 (69.90) bpm; not significant (NS)]; but, by 8 h of sepsis, HR was significantly elevated above control values [127 (120.135) bpm; P < 0.05]. Similarly, after 2 h of sepsis, CSNA was not significantly increased [101 (99.103) vs. 102 (64.148) % baseline; NS]; however, by 8 h, CSNA had more than doubled [232 (175.297) % baseline P < 0.05; Fig. 3]. In contrast, RSNA was significantly lower after 2 h of sepsis [100 (99.102) to 23 (9.44) % baseline; P < 0.05] and then increased back to baseline values by 8 h of sepsis [129 (101.159) % baseline; Fig. 3].

Effect of treatment of septic sheep with indomethacin. The effectiveness of the dose of indomethacin used was demonstrated by the finding that it significantly reduced temperature at both 2 and 8 h of sepsis (to 38.4 ± 0.3°C and 39.2 ± 0.3°C, respectively; P < 0.05 vs. preindomethacin). Treatment with indomethacin at 2 h of sepsis significantly increased MAP [to 112 (104.120) mmHg; P < 0.05], decreased HR [to 54 (50.59) bpm; P < 0.05] and reduced CSNA [to 20 (8.37) % baseline; P < 0.05; Fig. 3].

At 8 h of sepsis, indomethacin had no effect on MAP [87 (83.91) vs. 91 (84.98) mmHg; NS], but reduced HR from 127 (120.135) to 103 (94.113) bpm (P < 0.05). CSNA decreased from 232 (175.297) to 114 (55.193) % baseline (P < 0.05). After 8 h of sepsis, RSNA was similar to control levels, and the level was not significantly altered by indomethacin.

Effect of indomethacin on the baroreflex control of HR, CSNA, and RSNA in sepsis. The baroreflex control of HR, CSNA, and RSNA was tested at 2 and 8 h of sepsis, before and after indomethacin infusion. After 2 and 8 h of sepsis, there were significant increases in the lower plateau of the HR baroreflex curve (55 ± 3 bpm to 69 ± 5 bpm at 2 h; P < 0.05, and to 85 ± 6 at 8 h; P < 0.05), which were not affected by indomethacin administration (Table 2, Fig. 4).

The baroreflex control of CSNA and RSNA was unchanged at 2 h and 8 h of sepsis, although there was a tendency for a change in the gain of the CSNA reflex (P = 0.088, Table 2, Fig. 4). Treatment with indomethacin had no effect on the baroreflex control of CSNA or RSNA at either 2 or 8 h of sepsis.

DISCUSSION

This study investigated the role of prostaglandins in mediating the cardiovascular changes in early hyperdynamic sepsis in conscious sheep. In the first study, in normal sheep, bilateral intracarotid infusion of PGE2 increased CSNA, HR, and temperature, with no changes in MAP or RSNA, or in the baroreflex control of sympathetic nerve activity. These responses are similar to changes seen in septic sheep (24), suggesting they may be mediated by PGE2. To examine the role of prostaglandins in sepsis, we studied the effect of the nonselective COX inhibitor, indomethacin, at 2 and 8 h following the start of E. coli infusion. The findings indicate that prostaglandins, possibly together with downstream inflammatory mediators, contribute to the increased CSNA and HR in sepsis.

Table 1. Baroreflex parameter variables before and during bilateral intracarotid infusions of PGE2: effect of PGE2 on the baroreflex control of HR, CSNA and RSNA

<table>
<thead>
<tr>
<th>Variable</th>
<th>Heart Rate</th>
<th>CSNA</th>
<th>RSNA</th>
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<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>PGE2</td>
<td>Baseline</td>
</tr>
<tr>
<td>Range, bpm/%baseline</td>
<td>108 ± 8</td>
<td>88 ± 11</td>
<td>317 (234,297)</td>
</tr>
<tr>
<td>Max gain</td>
<td>−4.1 ± 0.5</td>
<td>−4.4 ± 0.8</td>
<td>−10.3 ± 1.4</td>
</tr>
<tr>
<td>BPso2, mmHg</td>
<td>96 ± 3</td>
<td>105 ± 4*</td>
<td>69 ± 5</td>
</tr>
<tr>
<td>Lower plateau, bpm/%baseline</td>
<td>60 ± 5</td>
<td>87 ± 9*</td>
<td>2 ± 3</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE or geometric mean [95% confidence interval (CI)] and were analyzed using paired sample t-tests. BPso2, arterial pressure at midpoint of heart rate range. *P < 0.05 vs. baseline.
In normal sheep, bilateral intracarotid infusions of PGE2 increased temperature and heart rate, in agreement with previous studies (4, 5). In addition, we showed that PGE2 caused a rightward shift in the HR baroreflex curve and increased the lower plateau of the curve, changes that partly accounted for the increase in HR. Furthermore, we showed that PGE2 increased resting CSNA, but there were no significant changes in baroreflex control of CSNA. The increase in the bottom plateau of the HR baroreflex curve, with no similar change in the CSNA baroreflex curve, is, thus, most likely due to a reduction in vagal tone. This is supported by previous findings that propranolol alone did not prevent PGE2-induced tachycardia (4). In contrast, we found that PGE2 did not alter the resting level or baroreflex control of RSNA. Overall the responses to PGE2 were similar to changes following administration of live E. coli in conscious sheep (24), suggesting that PGE2 may be an important pathophysiological mediator of the response to hypodynamic sepsis.

In sepsis, there are large increases in the levels of proinflammatory cytokines, but these molecules do not have direct effects on the brain because they do not cross the blood-brain-barrier (BBB). There is, however, evidence that cytokines, including TNFα and IL-1β, act on perivascular cells to induce COX-2 activity, leading to production of PGE2 that crosses the BBB and activates E-prostanoid receptors on central neurons to modulate cardiovascular control and sympathetic nerve activity (15, 34). Indeed, intracarotid infusions of PGE2 have been shown to increase arterial pressure and heart rate (4), and intracerebroventricular PGE2 increased MAP, HR, and RSNA (35). The bilateral intracarotid infusion of PGE2 in the current study was designed to primarily target the central nervous system, but the lack of an increase in MAP may reflect some spillover of PGE2 into the periphery where it has vasodilatory effects (8, 9), which may have counteracted a central sympathetically mediated action to increase arterial pressure.

Although it is well established that inhibition of prostaglandin synthesis in sepsis reduces fever (10), only a few studies have investigated the effects of COX inhibitors on sympathetic activity in sepsis. In anesthetized rats with LPS-induced endotoxemia, indomethacin did not change the fall in MAP, but it prevented the increase in RSNA (30) and, in human volunteers administered E. coli endotoxin, treatment with ibuprofen attenuated the increases in heart rate and plasma catecholamines (25). The effects of COX inhibitors on the resting levels or baroreflex control of CSNA have not, however, been investigated. Therefore, the present study investigated the effects on CSNA of inhibiting prostaglandin synthesis early in sepsis (2 h) and in the tachycardic prehypotensive stage of hypodynamic sepsis (8 h). Two hours after the start of E. coli infusion, intravenous indomethacin increased MAP, similar to the transient increase in MAP seen with indomethacin in normal sheep (18) and reduced HR and CSNA. The decrease in these variables is most likely a reflex response to the indomethacin-induced increase in MAP. In support of this notion, indomethacin did not affect the baroreflex control of HR, CSNA, or RSNA after 2 h of sepsis. At both 2 and 8 h of sepsis, intravenous indomethacin reduced the elevated body temperature to normal, indicating the effectiveness of the dose administered.

After 8 h of sepsis, sheep were tachycardic, CSNA was elevated to ~200% of baseline levels, and RSNA had returned to control levels. Interestingly, in contrast to what was seen after 2 h of sepsis, indomethacin did not increase MAP, suggesting a shift from prostaglandin-dependent vasodilatation to other vasodilator mechanisms, possibly nitric oxide. Supporting this, rats administered endotoxin did not show widespread increases in nitric oxide until 3 h postendotoxin (31), and we have found that after 24 h of nonhypotensive, hypodynamic sepsis in sheep, intrarenal infusion of L-NAME increased MAP and total peripheral resistance (14).

Despite not increasing blood pressure, indomethacin at 8 h of sepsis decreased CSNA and HR toward normal levels but
had no effect on RSNA. These effects of indomethacin suggest that prostaglandins, possibly PGE2 or downstream inflammatory mediators, have direct central actions that increase CSNA and HR at this stage of sepsis. Although CSNA was close to normal values after indomethacin at 8 h, HR had not completely returned to control levels. This is not surprising as additional mechanisms, such as reduced baroreflex inhibition with developing hypotension and reduced vagal tone, are likely to contribute to the increased HR. Indeed, a reduction in vagal tone in sepsis has been shown using spectral analysis of HR in anesthetized endotoxic rabbits (29). In addition, vagal impairment would account for the increase in the bottom plateau of the HR baroreflex curve in sepsis, since this part of the CSNA baroreflex was unchanged at 2 and 8 h of sepsis. *P < 0.05 vs. baseline, **P < 0.05 vs. preindomethacin value, paired t-test with Bonferroni correction.

Fig. 3. Cardiovascular changes during sepsis induced by administration of Escherichia coli and responses to indomethacin at 2 and 8 h. Left: Temperature (Temp;°C), heart rate (HR; bpm), mean arterial blood pressure (MAP; mmHg), cardiac sympathetic nerve activity (CSNA; % baseline), and renal SNA (RSNA; % baseline) following E. coli infusion (started at vertical dotted line). The solid vertical arrows represent the period of indomethacin administration and baroreflex curve generation 2 and 8 h after the start of sepsis (I). Right: variables at baseline (C; solid bar), 15 min before indomethacin (B; open bars), and 15 min after stabilization during indomethacin infusion (A; shaded bars) at 2 and 8 h of sepsis. *P < 0.05 vs. baseline, **P < 0.05 vs. preindomethacin value, paired t-test with Bonferroni correction.
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reflex-mediated in response to the changes in MAP. Although 
contrast, the responses of RSNA to indomethacin were mainly 

their downstream inflammatory mediators may mediate the 
increased in CSNA during this stage of sepsis.

Perspectives and Significance

These studies demonstrate the differential control of sympa-
thetic outflow to the heart and kidneys by prostaglandins, 
firstly by the ability of PGE2 to increase cardiac, but not renal 
SNA, and secondly by the ability of indomethacin to reduce 
CSNA, but not RSNA, in sepsis. The similar patterns of 
response to PGE2 and sepsis suggest that prostaglandins or 
their downstream inflammatory mediators may mediate the 
increases in CSNA and HR in mild hyperdynamic sepsis, and 
this was confirmed by the responses to indomethacin. In 
contrast, the responses of RSNA to indomethacin were mainly 
reflex-mediated in response to the changes in MAP. Although 
this study investigated the differential effects of indomethacin 
on renal and cardiac SNA in sepsis, the effects on other 
sympathetic nerves, such as those innervating muscle, gut, and 
spleen, remain to be determined. It also remains to be deter-
mined whether selectively reducing the increase in CSNA early 
in sepsis is associated with improved outcome in hyperdy-

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Disclosures

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

Author Contributions

Author contributions: L.C.B., R.R., and P.C. performed experiments; L.C.B. analyzed data; L.C.B., R.R., and C.N.M. interpreted results of experiments; L.C.B. prepared figures; L.C.B. drafted manuscript; L.C.B., P.C., and C.N.M. edited and revised manuscript; L.C.B., R.R., P.C., and C.N.M. ap-
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