Disruption of COX-2 and eNOS does not confer protection from cardiovascular failure in lipopolysaccharide-treated conscious mice and isolated vascular rings

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Address for reprint requests and other correspondence: B. L. Jensen, Dept. of Cardiovascular and Renal Research, Institute of Molecular Medicine, Univ. of Southern Denmark, J. B. Winslow’svej 21, 3, DK-5000 Odense C, Denmark (e-mail: bljensen@health.sdu.dk).

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Stehr M, Madsen K, Vanhoutte PM, Hansen PB, Jensen BL. Disruption of COX-2 and eNOS does not confer protection from cardiovascular failure in lipopolysaccharide-treated conscious mice and isolated vascular rings. Am J Physiol Regul Integr Comp Physiol 301: R412–R420, 2011. First published May 4, 2011; doi:10.1152/ajpregu.00823.2010. —It was hypothesized that a serial stimulation of vascular cyclooxygenase-2 (COX-2) with subsequent activation of endothelial nitric oxide synthase (eNOS) is responsible for decrease in blood pressure, cardiac performance, and vascular reactivity in endotoxemia caused by LPS. The hypothesis was tested in catheterized, conscious, freely moving, wild-type mice and mice (C57BL/6J background) with targeted deletion of COX-2 and eNOS that were given an intravenous LPS bolus (2 mg/kg, 055:B5). In vitro studies were performed on murine aorta rings. LPS caused a concomitant decrease in mean arterial blood pressure (MAP) and heart rate (HR) that was significant after 3 h and was sustained throughout the experiment (8 h). The LPS-induced changes in MAP and HR were not different from control in COX-2−/− and eNOS−/− mice. A prostacyclin receptor antagonist (BR5064) blocked the hypotensive effect of a prostacyclin agonist (beraprost), but did not attenuate the LPS-induced decrease in MAP and HR. LPS decreased eNOS and neuronal NOS mRNA abundances in several organs, while inducible NOS mRNA was enhanced. In aortic rings, LPS suppressed α1-adrenoceptor-mediated vascular tone. Inhibition of COX-2 activity (NS 398), disruption of COX-2, endothelium removal, or eNOS deletion (eNOS−/−) did not improve vascular reactivity after LPS, while the NO synthase blockers L-NMMA and L-NMMA prevented loss of tone. COX-2 and eNOS activities are not necessary for LPS-induced decreases in blood pressure, heart rate, and vascular reactivity. Inducible NOS activity appears crucial. COX-2 and eNOS are not obvious therapeutic targets for cardiovascular rescue during gram-negative endotoxic shock.

iNOS; prostacyclin; nitric oxide; endothelium

GRAM-NEGATIVE BACTERIAL ENDOTOXEMIA with sepsis is a systemic response to infection that occurs with an increasing incidence. The systemic inflammatory response may be complicated by cardiovascular collapse with fatal blood pressure decline and multiorgan failure (8). It remains a significant challenge to define pharmacologic targets for intervention that protect and improve cardiovascular performance. Cyclooxygenase-2 (COX-2) expression and activity is stimulated in macrophages and endothelial cells by gram-negative bacterial wall-derived LPS (1). The levels of the potent vasodilators prostaglandin E2 (PGE2) and prostacyclin (PGI2) are increased in patients with septic shock (6). The effect of COX inhibitors on survival and cardiovascular parameters in septic shock is, however, contradictory. In septic patients, ibuprofen reduced urinary levels of metabolites of prostacyclin and thromboxane A2 but not the incidence or duration of shock or the rate of survival (7, 24). Ibuprofen rapidly reversed hypotension in dogs exposed to endotoxin (2) and improved several vascular parameters in pigs (21). Indomethacin improved survival but had no significant effect on mean arterial blood pressure (MAP) in dogs exposed to endotoxin (22), whereas in rats, indomethacin did not reduce the mortality rate (3). In anesthetized rats, the LPS-induced acute decrease in arterial blood pressure was attenuated significantly by a selective COX-2 inhibitor (rofecoxib) and a nonselective COX inhibitor (indomethacin) (25). Mice with targeted deletion of COX-2 (19) and mice treated with COX-2 inhibitors (34) exhibit improved survival when challenged with LPS, but the cardiovascular effects of LPS have not previously been examined in COX-2−/− mice. Nitric oxide (NO) produced by inducible NO synthase (iNOS) is of major importance for changes in blood pressure during sepsis (23). Apparently, the activity of endothelial NOS (eNOS) is required for the induction of iNOS, and arterial blood pressure is protected in LPS-treated anesthetized eNOS−/− mice (17, 38), although lethality is not affected (35). PGE2 stimulates eNOS activity and PGE2-mediated vasodilatation depends on the presence of endothelium and eNOS (27). A similar endothelium-dependency is seen also for PGI2-induced vasodilatation (36). These findings prompted the hypothesis that serial activation of COX-2 and eNOS by LPS may account for the involvement of both enzymes in the circulatory response to LPS. This hypothesis predicts that functional deletion of COX-2 or eNOS protects cardiovascular status equally well against LPS-induced cardiovascular depression through attenuated vasodilatation of resistance vessels. The hypothesis was tested in vivo in mice with targeted disruption of COX-2 and eNOS that were challenged with Escherichia coli-derived LPS (055:B5). Arterial blood pressure and heart rate were measured continuously in freely moving mice with chronic indwelling catheters mounted in a swivel system. To further address the involvement of the vasculature, in vitro myograph experiments were performed with isolated, LPS-treated, aortic rings prepared from wild-type, COX-2−/−, or eNOS−/− mice.

MATERIALS AND METHODS

Mice

The investigation conformed with the Guide for the Care and Use of Laboratory Animal published by the US National Institutes of
Health (NIH publication no. 85-23, revised 1996), and the experimental protocol was approved by the Danish Animal Experiments Inspectorate (no. 2009/561-1749) under the Danish Ministry of Justice. All experiments in mice were performed in accordance with the Danish national guidelines for care and handling of animals. C57BL/6J wild-type mice and C57BL/6J mice with targeted deletion of COX-2 and eNOS were studied. COX-2⁻/⁻ mice and their wild-type littermates (COX-2⁺/⁺) from heterozygous breeding were obtained from Jackson Laboratories on a predominant C57BL/6 background and further backcrossed for seven generations (C57BL/6) before experiments. Genotyping was done with DNA from tail biopsies using the REDExtract-NAssay tissue PCR kit (Sigma). PCR used the following primers: (COX-2: 5'-GCC-CTG-AAT-GAA-CTG-CAG-GAC-G-3', 5'-CAC-GGG-TAG-CCA-AGC-CTA-TGT-C-3'; (COX-2⁺/⁺) 5'-CAC-ATG-AGA-ATC-AGG-TCC-GG-3' and 5'-ACC-TCT-GGC-ATG-CTC-TTC-C-3'. In studies with eNOS⁻/⁻ mice (Jackson Laboratories), C57BL/6J wild-type mice were used as controls. Mice were 8–10 wk of age and were kept on a 12:12-h light-dark cycle with free access to standard mice chow and tap water.

**Blood Pressure Measurement**

Mice were anesthetized (50 mg/kg ketamine and 10 mg/kg xylazine ip) and catheters were placed in the femoral artery and vein for blood pressure measurement and drug infusion, respectively (27). Mice were then placed in individual cages, and catheters were attached to a swivel (Instech Laboratories) so that the mice were able to move freely. Mice recovered for 4 days after the operation, and in that period the arterial catheters were kept open with infusion of 10 μl/h heparin solution (100 units in glucose). Before the experiment, the arterial line was connected to a pressure transducer, and data were collected at 200 Hz. Systolic, diastolic, and mean blood pressures were normalized at resting tension, and the viability of the vascular smooth muscle and endothelial cells were tested by adding phenylephrine (at increasing concentrations: EC₅₀, EC₉₀, and EC₁₀₀) followed by acetylcholine (10⁻⁶ M). After normalization, the rings were contracted with phenylephrine (EC₅₀) for 1 h, and the experiment was ended by adding acetylcholine (10⁻⁶ M).

**Experimental series. Series 1**: rings from wild-type mice (C57BL/6J) were incubated with LPS (50 μg/ml) or vehicle; **series 2**: rings from COX-2⁻/⁻ mice were incubated with LPS or vehicle and rings from C57BL/6J were incubated with LPS and the selective COX-2 inhibitor eNOS, endothelial nitric oxide synthase; iNOS, inducible NOS; nNOS, neuronal NOS, COX-2, cyclooxygenase-2; GAPDH, liver and aorta; TBP, kidney; Tbcc, heart.

**Measurements of eNOS, iNOS, Neuronal NOS, and COX-2, α₁a- and β₁-Adrenoceptor mRNA and eNOS Protein**

**Quantitative-PCR.** Total RNA from kidney, liver, aorta, and heart from control and LPS-treated animals was isolated by Trizol reagent extraction according to the manufacturer’s instructions (Invitrogen) and used as template for quantitative-PCR analysis as described by Stubbe et al. (37). cDNA amplification was made using iQ SYBR Green Supermix (Bio-Rad, Hercules, CA), and primer sequences are shown in Table 1. A melting curve analysis confirmed specificity of each product for each plate setup, and a standard curve was constructed by plotting threshold cycle values against serial dilutions. Purified PCR product was used to generate a standard curve.

**Western blot analysis.** Liver tissue was homogenized using a sucrose/imidazole buffer modified from Jensen et al. (29). Next, 30 μg of protein was separated on a 4–15% Tris-HCl gel (Bio-Rad) and transferred to a Immobilin-P polyvinylidene difluoride membrane (Millipore). Membranes were blocked with 5% nonfat dry milk in Tris-buffered saline Tween-20 (TBST) for 1 h followed by incubation overnight at 4°C with primary antibody diluted 1:1,000 in 5% nonfat dry milk in TBST. The primary antibody was anti-eNOS (ab5589; Abcam, Cambridge, UK). The antigen-antibody complex was visualized by horseradish peroxidase-conjugated secondary antibody (cat. no. P0448, diluted 1:2,000; Dako) using the ECL plus Western Blotting Detection System (GE Healthcare, H illerød, Denmark). Antibody against β-actin (1:2,000, Abcam) was used for loading control.

**Isometric Force Measurements in Aorta Rings**

Isometric force measurements in aorta rings were obtained in a Halpern-Mulvany myograph (model 610 M; Danish Myo Technology, Aarhus, Denmark). The mice were killed by a blow to the head followed by decapitation. The thoracic aorta was rapidly excised and placed in ice-cold physiological salt solution (PSS) as described (27). This was followed by dissection and cutting the aorta into rings (1.5- to 2-mm length) before incubation at 37°C with LPS (50 μg/ml, E. coli, 055:B5; Sigma) or vehicle for 18 h. Incubation media contained DMEM (t-glutamine and HEPES; GIBCO) with 0.5% BSA (7.5%, GIBCO), penicillin (100 μ/ml, Sigma), and streptomycin (100 μg/ml, Sigma). The rings were then suspended in the myograph at 37°C in PSS with LPS, equilibrated with 5% CO₂ in air (pH of 7.4). The rings were normalized at resting tension, and the viability of the vascular smooth muscle and endothelial cells were tested by adding phenylephrine (at increasing concentrations: EC₅₀, EC₉₀, and EC₁₀₀) followed by acetylcholine (10⁻⁶ M). After normalization, the rings were contracted with phenylephrine (EC₅₀) for 1 h, and the experiment was ended by adding acetylcholine (10⁻⁶ M).

**Experimental series. Series 1**: rings from wild-type mice (C57BL/6J) incubated with LPS (50 μg/ml) or vehicle; **series 2**: rings from COX-2⁻/⁻ mice were incubated with LPS or vehicle and rings from C57BL/6J were incubated with LPS and the selective COX-2 inhibitor

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Table 1. Primer sequences

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<th>Product</th>
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<th>Antisense, 5'-/3'</th>
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<td>GCC-ATA-GAT-GAG-CTG-GCT-CTG</td>
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NS398 (10 μmol/l) or LPS alone; series 3: rings from eNOS−/− mice incubated with LPS or vehicle, and rings from C57BL/6J were incubated with LPS and the NOS inhibitor Nω-nitro-L-arginine methyl ester (l-NAME; 10−4 mol/l) and LPS or l-NAME alone; series 4: The endothelium was removed in rings from C57BL/6J by 1% Triton X-100 followed by incubation with LPS and L-NAME, LPS alone, or vehicle; series 5: rings from wild-type mice (C57BL/6J) incubated with LPS (50 μg/ml) and the iNOS inhibitor 1400W (1 μmol/l; Cayman Chemicals) or LPS alone. LPS, l-NAME and 1400W were dissolved in PSS.

**Statistical Evaluation**

Blood pressure data were integrated over 0.5 h before LPS and thereafter for each hour. Changes in mean ± SE of blood pressure with time were compared by one-way ANOVA. If ANOVA was significant, blood pressure values after LPS were compared with blood pressure before LPS (control) by unpaired Student’s t-test with Bonferroni correction. P < 0.05 was considered significant. Between groups (e.g., COX-2 genotypes) blood pressure decrease after LPS at 8 h (∆blood pressure) was compared by unpaired Student’s t-test.
Data testing vascular reactivity and expression levels of specific mRNA and protein molecules were evaluated by unpaired Student’s t-test. For each series, separate controls were always run in parallel. P < 0.05 was considered significant.

RESULTS

Effect of LPS on Arterial Blood Pressure and Heart Rate in Wild-Type Mice

Blood pressure was followed for 8 h after LPS or vehicle injection. Baseline MAP in catheterized, freely moving C57BL/6 mice was 106.5 ± 2.2 mmHg and heart rate was 613.1 ± 11.7 beats/min. Injection of a vehicle bolus (isotonic NaCl) had no acute effect on MAP or heart rate. After LPS injection (2 mg/kg iv), blood pressure decreased significantly after 2.5 h and stabilized at a level ~75 mmHg after 6 h, which was an average decline of 32.2 ± 3.8 mmHg (Fig. 1, A and B; n = 11). There was an almost synchronous decrease in heart rate after LPS at 3 h, which stabilized at a rate averaging 50% of control (~300 beats/min) at 6 h (Fig. 1, C and D).

In separate mice, circulatory parameters were followed for 20 h after LPS injection (n = 6). These mice survived, and blood pressure did not decrease further between 6 and 20 h. None of the tested mice died during the experiments (Fig. 1, B and D, inserts, right). Analysis of tracks with higher time resolution (average each 15 min) confirmed the above; blood pressure declined significantly at 150 min with no significant change in heart rate until 180 min, which then also declined.

Effect of LPS on Arterial Blood Pressure and Heart Rate in COX-2−/− Mice and COX-2+/+

The resting MAP in C57BL/6J mice with targeted deletion of COX-2 (COX-2−/−) averaged 112.3 ± 3.3 mmHg and in C57BL/6J wild-type littermates (COX-2+/+) 106.0 ± 3.3 mmHg was not significantly different (Fig. 2A). There was no blood pressure difference between genders in COX-2−/− mice. Bolus injection of LPS resulted in a blood pressure decrease that was significant after 2 h in COX-2−/− and then stabilized ~80 mmHg (Fig. 2, A and B). The change in blood pressure value after LPS was 26.2 ± 3.6 mmHg for COX-2−/− and 31.7 ± 4.9 mmHg for COX-2+/− and not significantly different between the two strains. Heart rate was 622.2 ± 20.6 beats/min in resting COX-2−/− mice, and an LPS bolus decreased it to 421.5 ± 45.9 beats/min (Fig. 2, C and D). The decrease in heart rate caused by LPS...
was not significantly different between wild-type littersmates and COX-2−/− (ΔHR 244.1 ± 55 and 202.7 ± 56 beats/min, respectively).

**Effect of a Prostacyclin Receptor Antagonist on the LPS-Induced Cardiovascular Functional Decline**

Efficient blockade of prostacyclin was controlled by bolus injection of the stable prostacyclin receptor agonist beraprost (20 μg/kg). In wild-type mice infused with vehicle, beraprost injection yielded an acute, reversible decrease in blood pressure (Fig. 3A). BR5064 (0.1 mg/kg bolus followed by 0.005 mg·kg−1·h−1 continuous infusion) abolished the effect of prostacyclin agonist infusion (Fig. 3B). Administration of BR5064 had no significant effect on resting blood pressure or heart rate (Fig. 3, C and D). BR5064 infusion to mice did not prevent a significant decrease in blood pressure in response to an LPS bolus (Fig. 3, C and D). Thus, after 4 h, blood pressure had decreased significantly in LPS-treated, BR5064-infused mice, and then stabilized at this level for the duration of the experiment. The change in blood pressure (19.7 ± 3.8 mmHg) was not significantly different from that observed in vehicle-infused, LPS-treated mice. Heart rate decreased significantly in response to LPS in BR5064-infused mice (Fig. 3, C and D). Administration of BR5064 to mice did not prevent a significant decrease in heart rate after LPS treatment (Fig. 3, C and D). Heart rate decreased significantly in response to LPS in BR5064-infused mice in a way not significantly different from that in vehicle-infused mice (Fig. 3, E and F).

**Effect of LPS on Arterial Blood Pressure and Heart Rate in eNOS−−/− Mice**

C57BL/6J mice with targeted deletion of eNOS displayed a significantly elevated resting blood pressure compared with wild-type mice: MAP was 130.5 ± 3.1 mmHg compared with 106.5 ± 2.2 mmHg in control mice (Fig. 4A). Heart rate was 657.4 ± 13 beats/min in eNOS−−/− mice (n = 6) compared with 613.1 ± 12 beats/min in wild-type mice, which was not significantly different. In eNOS−−/− mice, LPS treatment yielded a significant decrease in MAP that was apparent 3 h after the bolus injection (Fig. 4, A and B). Blood pressure then stabilized at a level ~90 mmHg. The ΔMAP decrease at 6 h was 43.2 ± 5 mmHg with no significant difference between wild-type control mice and eNOS−−/− mice. In response to LPS, heart rate displayed a significant decrease after 3 h and stabilized ~300 beats/min (Fig. 4, C and D). The decrease in heart rate after 6 h was 350.3 ± 19 beats/min in wild-type mice and was not significantly different between wild-type and eNOS−−/− mice.

**Effect of LPS on Contraction of Aortic Rings In Vitro**

Segments of thoracic aorta from C57BL/6J mice were preincubated with LPS and were subsequently suspended in a myograph. The α1-adrenoceptor agonist phenylephrine was used at EC50 (2 × 10−7 mol/l) for 60 min to examine the ability of the rings to maintain tone. After incubation with LPS (50 μg/ml), the preparations exhibited a reproducible and significant reduction of contractility in response to phenylephrine (n = 6) compared with control rings (n = 5) (Fig. 5A). At the end of each experiment, acetylcholine was added to test endothelial function. Acetylcholine relaxed the control rings, whereas acetylcholine induced a significant contraction in LPS-treated rings (Fig. 5C). LPS-treated rings from COX-2−−/− mice (n = 5) displayed a suppression of phenylephrine-induced tone that was indistinguishable from that observed in aorta harvested from wild-type mice (Fig. 5B). The COX-2 antagonist NS-398 had no significant effect on the vascular reactivity of LPS-exposed rings (Fig. 5B, n = 6). Aortic segments from eNOS−−/− mice incubated with LPS also showed a reduced contractility (n = 5) to phenylephrine stimulation not significantly different from that of arteries from wild-type mice (Fig. 5B, n = 6). Inhibition of NOS activity by
the addition of L-NAME or the iNOS inhibitor 1400W improved phenylephrine-induced contractility in LPS-exposed rings (each series \( n = 6 \)) to a level not significantly different from control rings or from preparations incubated with L-NAME only (Fig. 5B). Removal of the endothelium abolished acetylcholine-mediated relaxations but did not affect contractility determined as the response to EC\(_{50}\) of phenylephrine (16.1 \( \pm \) 2.2 mN, \( n = 5 \), Fig. 5B). Incubation of rings without endothelium with LPS reduced contractility (4.1 \( \pm \) 1.0 mN, \( n = 6 \)) in response to phenylephrine to a similar extent as that observed in preparations with endothelium (Fig. 5B). Incubation of LPS-treated rings without endothelium with L-NAME resulted in a significantly improved contraction to phenylephrine (12.43 \( \pm \) 1.6 mN, \( n = 6 \)) compared with LPS-treated aorta without endothelium not exposed to the NOS inhibitor; there was no significant difference in contractility compared with control rings without LPS and endothelium.

**Effect of LPS on eNOS, iNOS, Neuronal NOS, COX-2, and Adrenoceptor Expression in Mouse Organs**

Total RNA from control and LPS-treated wild-type and COX-2\(^{-1/-}\) mouse liver, aorta, kidney, and heart was subjected to quantitative RT-PCR analysis. In liver and kidney, systemic LPS treatment yielded a significantly elevated level of iNOS mRNA in wild-type and COX-2\(^{-1/-}\) (Fig. 6A). COX-2 mRNA was increased in liver (Fig. 6D). In aorta and left cardiac ventricle there were no significant change in iNOS and COX-2 mRNA level (Fig. 6, A–D). The level of eNOS mRNA in liver, aorta, and heart was significantly reduced in response to LPS infusion in wild-type mice but not in COX-2\(^{-1/-}\), whereas no significant change was observed in the kidney (Fig. 6B). Neuronal NOS (nNOS) mRNA was significantly downregulated in kidney and liver in LPS-treated wild-type and COX-2\(^{-1/-}\) mice, with the same tendency in aorta and heart (Fig. 6C). Total eNOS protein abundance in liver tissue was not changed by LPS treatment in wild-type and in COX-2\(^{-1/-}\) mice as determined by Western blot analysis (Fig. 6F). In contrast, iNOS protein level was significantly increased by LPS in wild-type and COX-2\(^{-1/-}\) mice compared with control (Fig. 6E). The abundance of \( \beta_1 \)-adrenoceptor mRNA in left ventricle was significantly decreased in wild-type and COX-2\(^{-1/-}\) in response to LPS (Fig. 6G). In aorta, there was no significant change in mRNA abundance of the \( \alpha_{1a} \)-adrenoceptor (Fig. 6H).

**DISCUSSION**

The present study shows that LPS-induced suppression of heart rate and blood pressure in conscious, freely moving mice is not attenuated by targeted deletion of COX-2 or eNOS, or by pharmacologic inhibition of prostacyclin receptor. Results from isolated aortic rings exposed to LPS corroborated the interpretation that COX-2 and the endothelium/eNOS has no major role in the depression of vascular reactivity caused by LPS. By contrast, selective iNOS inhibition rescued vascular contractility. LPS induced marked and opposite changes in the expression of iNOS (up), nNOS (down), and eNOS (down) mRNAs in vivo, which supports a crucial role of iNOS activity for the decline in cardiovascular function, in line with previous findings. Taken in conjunction, the present data show that activity of COX-2 or eNOS and therefore a serial coupling between them is less likely as cause for and therapeutic target of cardiovascular collapse in LPS-mediated endotoxemia.

The present report provides cardiovascular data obtained at the systemic level in conscious freely moving COX-2\(^{-1/-}\) mice, which constitute an attractive model to study the role of the enzyme independently of nonselective actions of COX inhibitors and the variable pharmacokinetic conditions that prevail.
during sepsis (18). The present model of LPS-induced endotoxemic shock resulted in hypotension and reduced heart rate with onset after 2 to 3 h that lasted for at least 20 h while mice survived. The “mild” LPS dosing regimen (2 mg/kg iv) is different from a study designed to examine survival, where COX-2−/− mice were significantly protected from death compared with COX-2+/+ in response to LPS (3 daily injections of 40 mg/kg ip or 100 mg/kg ip once) (19). The present observations suggest that the better survival in COX-2−/− mice and in mice given COX-2-selective blockers (4, 34) is less likely to be caused by an improved cardiovascular function. Of note, two selective COX-2 inhibitors and the glucocorticoid-receptor agonist dexamethasone showed equal ability to suppress the excretion of prostacyclin metabolite 6-keto-PGF1α in rats after LPS, but only dexamethasone improved arterial pressure and attenuated the rise in plasma levels of nitrite and nitrate caused by LPS (30). This finding supports a modest role of COX-2 activity for blood pressure decline compared with NOS activity. Efficient in vivo blockade of prostacyclin receptors was obtained, but the prostacyclin antagonist did not attenuate the LPS-mediated suppression of MAP and heart rate. COX-2 being the dominant source for systemic prostacyclin (31), the present finding is in accord with the lack of effect of COX-2 on systemic hemodynamics (13). In rats subjected to endotoxemic shock, both a COX-2 inhibitor, rofecoxib, and a prostacyclin antagonist, CAY-10441, attenuated the LPS-mediated decrease in blood pressure (25, 26). These experiments were performed during isoflurane anesthesia and used 5 times the present dose of...
of LPS (10 mg/kg) given intravenously. Baseline blood pressure was 80–90 mmHg and during LPS at or below 50 mmHg. These different experimental conditions may explain the discrepancies with the present findings obtained in conscious mice, although species differences may also be involved. Rats display a hyperdynamic circulatory response to LPS with increased heart rate and cardiac output (almost doubled), while blood pressure drops and systemic vascular resistance is markedly lowered (26). This suggests a sequence where the primary event is either vasodilatation with a compensatory increase in heart rate or a directly mediated inflammatory tachycardia inducing a reflex decrease in peripheral resistance. In mice, however, the basal heart rate approximated 600 beats/min, and in no case was an increase in response to LPS recorded. A decline in blood pressure was noted first, and within 30 min this was followed by a decline in heart rate with no compensatory tachycardia. The almost synchronous drop in blood pressure and heart rate suggest inadequate responsiveness in or effect of the sympathetic nervous system and/or suppressed inotropic state of the myocardium. Cytokines downregulate α-adrenoceptors in various organs during endotoxemia in rats (11), whereas the β3-adrenoceptor is upregulated in human myocardium from septic patients (32). Our results show a decrease of β1-adrenoceptor expression to 10% of control level in left ventricular myocardium, while aortic α1-adrenoceptor expression showed no alteration. If present at the level of functional receptors, this myocardial downregulation could cause a lower sensitivity towards sympathetic stimulation.

The lack of contribution of COX-2-derived prostanooids in the blood pressure response to LPS is in accord with modest and restricted upregulation of COX-2 mRNA in the liver. The data agree with previous data from mice where COX-2 changes transiently in vivo (20). In accord with earlier reports (28, 35), MAP was increased by ~30 mmHg in eNOS+/− mice. A significant role has been attributed to eNOS in endotoxemic (17, 38) and anaphylactic (14) blood pressure decrease, and the COX products PGE2 and prostacyclin can activate eNOS (22, 27, 33). The present data indicate that both in conscious mice and in isolated vascular rings, eNOS is downregulated and does not contribute to cardiovascular suppression. eNOS protein abundance is also decreased in endothelial cells in a model of cecal ligation and puncture in rats (39). Thus LPS modulates the expression of the three NOS isoforms differently since both eNOS and nNOS were suppressed and iNOS stimulated. nNOS is expressed in vascular smooth muscle cells (10) and endothelium (5). It contributes to endothelium-dependent relaxation in mouse aorta (12), and in accordance with our findings, nNOS is also downregulated in cardiac myocytes from rats in a model of endotoxic shock (16). The suppression of vascular eNOS abundance is in accord with disappearance of the relaxation to acetylcholine. Actually, and surprisingly, in isolated preparations incubated with LPS, acetylcholine caused an increase in tension rather than a relaxation. This probably reflects the induction of endothelium-dependent contractions, as has been observed with another cytokine in the aorta of the rat (9).

The present data rather support that iNOS is the major source of NO in response to LPS, since iNOS mRNA was enhanced several times in liver and kidney, while in vitro, L-NAME, as well as selective iNOS inhibition, normalized LPS-suppressed vessel reactivity. This is in accord with observations in LPS-challenged awake mice, where systemic NO production depended solely on iNOS and where eNOS-derived NO was reduced (23). This finding has been reproduced also in vitro, where iNOS activity suppressed eNOS expression and abolished acetylcholine-mediated dilatation in aortic rings of the mouse (15). The present in vitro observations suggest that LPS-stimulated iNOS activity in the arterial media layer is sufficient to suppress vascular reactivity. Although endothelial cells also may respond to LPS by upregulation of COX-2 (1) and iNOS, this appears not to be necessary for suppression of vascular reactivity. The role of the endothelium in sepsis is, although, not completely clear.

Perspectives and Significance

In summary, the present study shows that a nonlethal, acute, inflammatory challenge by infusion of gram-negative bacterial wall-derived LPS to conscious unrestrained mice reduces simultaneously heart rate and MAP in vivo and vascular reactivity in vitro, independently of COX-2, prostacyclin receptors, and endothelium/eNOS activity. Thus, at least in the mouse, a serial coupling between COX-2 and eNOS activities is unlikely to account for acute cardiovascular depression during endotoxemia, while iNOS appears to be of major significance.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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