Activation of the PGI₂/IP System Contributes to the Development of Circulatory Failure in a Rat Model of Endotoxic Shock

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Abstract—Prostacyclin levels are increased in septic patients and several animal models of septic shock, and selective inhibition of cyclooxygenase-2 improved cardiovascular dysfunction in rats treated with lipopolysaccharide (LPS). Here, we examine the specific role of prostacyclin and of the receptor for prostacyclin (IP) in the development of LPS-induced circulatory failure. Intravenous injection of LPS (10 mg/kg) into male Sprague-Dawley rats caused a strong increase in plasma prostacyclin levels, which was paralleled by a decrease in blood pressure and an increase in heart rate. Moreover, LPS injection increased the mRNA expression of the IP receptor in the heart, aorta, lung, liver, adrenal glands, and kidneys. Cotreatment with the IP antagonist CAY-10441 (1, 10, 30, and 100 mg/kg) dose-dependently moderated the LPS-induced changes in mean arterial blood pressure, heart rate, cardiac output, and systemic vascular resistance. The development of cardiovascular failure was ameliorated by CAY-10441 in spite of the typical LPS-induced increases in plasma levels of cytokines and NO. In vitro, cytokines dose- and time-dependently induced IP expression in rat vascular smooth muscle cells. Incubation of cells with the stable IP agonist iloprost in the presence of the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine resulted in higher cAMP levels in cytokine-treated cells compared with untreated cells. Taken together, our data demonstrate a prominent role of the prostacyclin/IP system in the development of LPS-induced cardiovascular failure. (Hypertension. 2008;52:330-335.)

Key Words: prostacyclin ▪ LPS ▪ CAY-10441 ▪ blood pressure ▪ sepsis

Prostacyclin (PGI₂) is a major product of the arachidonic acid metabolism formed in the vascular endothelium by the action of the enzymes cyclooxygenase (COX) and prostacyclin synthase (PGIS). PGI₂ mediates its effects through a G protein–coupled receptor (IP), which is located on vascular smooth muscle cells (VSMCs).² Stimulation of IP by PGI₂ leads to an increase in cAMP, which likely mediates relaxation of VSMCs.²

Vasodilation is a major symptom of infection and inflammation, occurring systematically during sepsis. In the past years, it became obvious that an increased expression of the inducible isoform of NO synthase contributes fundamentally to septic shock. However, in contrast to the effect of the inducible isoform of NO synthase inhibitory drugs on hypotension in shock,³,⁴ administration of lipopolysaccharide (LPS) still causes hypotension in the inducible isoform of NO synthase–deficient mice,⁵,⁶ suggesting that NO is not the only mediator of LPS-induced hypotension.

Increased levels of PGI₂ have been reported in patients under septic shock and in animals treated with LPS or proinflammatory cytokines.⁷–⁹ Because PGI₂ is a potent vasodilator, one may assume that PGI₂ could be involved in the development of septic shock. Recent evidence suggests that the inducible isoform of COX, COX-2, is the main responsible enzyme for the increased production of PGI₂ in VSMCs.¹⁰,¹¹ In line with this, it has been shown that inhibition of COX-2 attenuates the fall in blood pressure or improves vascular endothelial dysfunction in endotoxemic animals.¹²,¹³ Therefore, we hypothesized that the PGI/IP system could especially contribute to the development of LPS-induced septic shock.

Materials and Methods

Animal Experiments

All of the animal experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and with the local ethics committee. Male Sprague-Dawley rats (200 to 250 g; n=8 per group) received 1 mL of Ringer’s solution (i.v., control), Escherichia coli–derived LPS (10 mg/kg i.v.), vehicle (10% polyethylene glycol–200 in isotonic NaCl, i.p.) or the IP antagonist CAY-10441 (i.p., Cayman Chemical, also known as RO1138452)⁴.

Measurement of Hemodynamic Parameters

Five hours after injection of LPS, mean arterial blood pressure (MAP) was measured as described.¹² Heart rate (HR), cardiac output (CO), systemic vascular resistance (SVR), and pressure were measured by a 2.0-Fr Millar tip catheter (Model SPR-838, Millar...
Instruments). A recovery period of 30 minutes was allowed to establish steady-state conditions.

Cell Culture
Rat VSMCs (Dominion Pharmakine; passage 4 to 10) were incubated for 24 hours with medium (control) or increasing concentrations of a combination of cytokines (the 100% mix consisted of tumor necrosis factor [TNF]-α [100 ng/mL], interleukin [IL]-1β [50 ng/mL], and interferon-γ [100 ng/mL]; Tebu-Bio). cAMP accumulation was determined 20 hours after incubation of VSMCs with cytokines and with 10 μmol/L of indomethacin or in cells incubated only with indomethacin. Cells were then incubated with 3-isobutyl-1-methylxanthine (500 μmol/L) for 15 minutes. Thereafter, cells were stimulated with iloprost (100 nmol/L) or iloprost in combination with CAY-10441 (1 μmol/L) for 30 minutes. The supernatant was removed, cells were lysed, and cAMP concentration was directly measured in the samples using an ELISA (direct cAMP kit, Assay Designs).

Determination of IP and β-Actin mRNA Expression
Total RNA from tissues and cells was extracted, reverse transcribed, and real-time PCR or RNase protection assay for IP and β-actin was carried out as described previously.15,16

Western Blot Analysis for IP and β-Actin
Membrane fractions of total protein were prepared as described previously.17 Western blot analysis was performed using a primary rabbit polyclonal antibody against IP (Cayman Chemical; 1:1000) and a horseradish peroxidase–conjugated secondary anti-rabbit polyclonal antibody (Santa Cruz Biotechnology; 1:1000). For β-actin, a monoclonal antibody (1:5000; Sigma) and goat antimouse secondary antibody (1:5000; Santa Cruz Biotechnology) were used. Quantitative assessment of band densities was performed densitometrically.

Determination of Plasma Levels of Cytokines, Nitrate/Nitrite, and 6-Keto Prostaglandin F1α
Plasma concentrations of cytokines, nitrate/nitrite, and 6-keto prostaglandin (PG) F1α were determined by using ELISA kits (R&D Systems and Cayman Chemical).

Statistics
Data were analyzed by ANOVA with multiple comparisons followed by the t test with Bonferroni’s adjustment. P<0.05 was considered significant.

Results
Effect of LPS on Mean Arterial Pressure, HR, and Plasma Levels of 6-keto-PGF1α
MAP and HR remained stable in the control group. LPS injection resulted in a decrease in MAP and in an increase in HR 3, 6, and 12 hours after administration. Plasma levels of 6-keto-PGF1α, the stable metabolite of PGI2, were not altered in the control animals but clearly increased 3, 6, and 12 hours after LPS treatment (Table).

Effect of LPS on IP Expression
We further investigated the influence of LPS on the expression of IPs in cardiovascular tissues. We found that IP mRNA expression was increased 1.7-, 2.9-, and 3.2-fold in thoracic aortas 3, 6, and 12 hours after LPS injection, respectively. Similarly, LPS increased IP mRNA expression in lungs, hearts, livers, adrenal glands, and kidneys (Figure 1A). In addition, LPS injection for 6 hours increased IP mRNA expression 2.6-, 2.0-, 1.6-, and 2.7-fold in the right atria, left atria, right ventricle, and left ventricle, respectively (Figure 1B). We further examined the protein expression of IP receptors in lungs and found that the expression was increased 1.7-, 2.1-, and 2.0-fold 3, 6, or 12 hours after LPS injection, respectively (Figure 1C).

Effect of CAY-10441 on Cardiovascular Parameters
Because we found an activation of PGI2/IP system by LPS treatment, we further investigated the impact of this activation on the development of hypotension in LPS-treated rats. We, therefore, used the IP receptor antagonist CAY-10441 and investigated the effect 6 hour after LPS injection. MAP in control animals was 78±4 mm Hg. Sole treatment with CAY-10441 did not alter MAP. LPS treatment decreased MAP to 51±4 mm Hg, and cotreatment with CAY-10441 dose-dependently attenuated the fall in blood pressure to 66±4 mm Hg at the highest dose level (Figure 2A). HR in control animals was 363±7 bpm. CAY-10441 did not alter HR. HR increased in animals treated with LPS to 518±14 bpm, and cotreatment with CAY-10441 dose-dependently attenuated the rise in HR to 425±13 mm Hg at the highest dose level (Figure 2B). CO in control animals was 63.2±4.2 mL/min and was not altered by sole treatment with CAY-10441. LPS injection increased CO to 92.8±7.4 mL/min, and cotreatment with CAY-10441 dose-dependently attenuated the rise in CO to 73.7±6.4 mL/min at the highest dose level (Figure 2C). SVR in control animals was 1.10±0.07 mm Hg·mL−1·min and was not affected by treatment with CAY-10441. SVR decreased to 0.47±0.03 mm Hg·mL−1·min in animals treated with LPS, and cotreatment with CAY-10441 dose-dependently attenuated the fall in SVR to 0.77±0.07 mm Hg·mL−1·min at the highest dose level (Figure 2D).

Effect of CAY-10441 on Plasma Levels of Cytokines and NO
Plasma values for TNF-α, IL-1β, and interferon-γ and the sum of nitrate and nitrate were strongly increased by LPS challenge. Treatment with CAY-10441 did not alter basal or LPS-induced plasma values (Figure 3).

Effect of Cytokines on IP mRNA Expression and cAMP Formation in VSMCs
To gain insight into the regulation and functional importance of our in vivo findings, we finally investigated the effect of cytokines in VSMCs. Incubation of VSMCs with a mixture of...
cells, iloprost increased cAMP levels \( \approx 38\)-fold. This increase was also inhibited by CAY-10441 (Figure 4C).

**Discussion**

In line with previous studies, we observed a strong increase in plasma levels of 6-keto-PGF\(_{1\alpha}\), which was accompanied by a time-dependent fall in systolic blood pressure and an increase in HR after injection of LPS.\(^7\)\(^8\) Under physiological conditions, it has been suggested that the endothelium regulates vascular tone by a constitutive formation of endothelial-isoform of NO synthase–derived NO and COX-1–derived PGI\(_2\).\(^18\)\(^19\) However, during inflammation, endothelial cells are capable of producing PGI\(_2\) in a COX-2–dependent manner.\(^20\) Notably, during severe sepsis, it has been suggested that high levels of endotoxin result in a complete loss of the endothelium and of course also of its function.\(^21\) In this context, it has been shown that VSMCs are also able to produce PGI\(_2\) in a COX-2–dependent manner in response to LPS.\(^10\) Thus, it seems likely that the strong rise in 6-keto-PGF\(_{1\alpha}\) plasma levels observed in our in vivo study may be the result of an induction of COX-2 leading to increased levels of PGI\(_2\). In line with this, it has been found that inhibition of COX-2 improves vascular endothelial dysfunction induced by LPS.\(^13\) In addition, renal-specific PGIS transgenic mice, which have an increased renal PGI\(_2\) formation, respond to LPS injection already with a low-dose of LPS, which did not alter renal function in wild-type mice.\(^32\)

It is well known that PGI\(_2\) exerts its effects on VSMCs via activation of IP receptors.\(^23\) Recently, we have shown that the expression of several G-protein–coupled receptors is decreased on LPS challenge.\(^24\)\(^25\) Thus, a decrease in the expression of IPs may counteract the effect of an increase in plasma PGI\(_2\) levels. We now found that the expression of the IP is increased in vivo after an LPS challenge. This new in vivo finding fits very well with in vitro findings, demonstrating that LPS, IL-1\(\beta\), and TNF-\(\alpha\) can increase the expression of IP mRNA.\(^26\)\(^28\) Moreover, these data may provide an explanation for the effectiveness of PGI\(_2\) or PGI\(_2\) agonists to increase splanchic blood flow, especially in septic animals,\(^29\) and for the important role of PGI\(_2\) as an inflammatory mediator.\(^30\) The functional relevance of our observation is highlighted by the enhanced iloprost-induced cAMP synthesis after treatment of VSMCs with cytokines.

To define the functional relevance of an activation of the PGI\(_2\)/IP system in the development of LPS-induced cardiovascular failure, we investigated the effect of the IP receptor antagonist CAY-10441.\(^14\) Confirming a previous report, sole treatment with CAY-10441 did not influence MAP and HR.\(^31\) We now found that CAY-10441 attenuated the fall in blood pressure induced by LPS. This finding fits very well with the attenuation of LPS-induced hypotension by COX-2 inhibition.\(^12\) In line with this, the positive effect of cPTIO on LPS-induced cardiovascular failure and mortality has been linked in part to an inhibition of PGI\(_2\) synthesis via inactivation of PGIS.\(^32\) Furthermore, it has been suggested that the induction of COX-2 in the heart is involved in myocardial dysfunction\(^33\)\(^34\) and that increased PGI\(_2\) synthesis via COX-2 could in part mediate LPS-induced cardiac failure.\(^35\)\(^36\) Therefore, our data provide further evidence for the involvement of
COX-2–derived PGI₂ in endotoxin-induced cardiovascular dysfunction.

Because our findings suggest that the attenuation of hypotension by CAY-10441 does not result from a decreased production of NO, our data are in agreement with the concept that NO is a major substance leading to hypotension in septic rodent models. However, in larger mammalians and humans, NO overproduction does not occur to the same extent. Therefore, attenuation of hypotension by IP receptor antagonism might be more pronounced in humans.

Perspectives

Taken together, our data demonstrate an LPS-induced activation of the PGI₂/IP system, which is probably mediated by proinflammatory cytokines. We infer from our findings that PGI₂ is of particular relevance for the development of
A

![Image of IP protein VSMC](image)

**Figure 4.** (A) Time-dependent and (B) dose-dependent effects of cytokines on IP mRNA expression in VSMCs. Inserts show representative blots for IP protein expression in cultured VSMCs. C, Effect of iloprost (100 nmol/L) or the combination of cytokines on IP protein expression in VSMCs. Inserts show representative blots for IP protein expression in cultured VSMCs.

LPS-induced cardiovascular failure. Additional animal and human studies are necessary to clarify whether inhibition of the IP receptor is beneficial in the treatment of septic circulatory failure. Possible effects of IP antagonism on renal, hepatic, and pulmonary function and on blood homeostasis would be areas of interest in this context, as well as the question of whether therapeutic strategies taken from experimental sepsis are useful under clinical conditions.

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**Disclosures**

None.

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