Downregulation of Angiotensin II Type 1 Receptors During Sepsis

Michael Bucher, Karl-Peter Ittner, Jonny Hobbhahn, Kai Taeger, Armin Kurtz

Abstract—Our study aimed to characterize the mechanisms underlying the attenuated cardiovascular responsiveness toward the renin-angiotensin system during sepsis. For this purpose, we determined the effects of experimental Gram-negative and Gram-positive sepsis in rats. We found that sepsis led to a ubiquitous upregulation of NO synthase isoform II expression and to pronounced hypotension. Despite increased plasma renin activity and plasma angiotensin (Ang) II levels, plasma aldosterone concentrations were normal, and the blood pressure response to exogenous Ang II was markedly diminished in septic rats. Mimicking the fall of blood pressure during sepsis by short-term infusion of the NO donor sodium nitroprusside in normal rats did not alter their blood pressure response to exogenous Ang II. Therefore, we considered the possibility of an altered expression of Ang II receptors during sepsis. It turned out that Ang II type 1 receptor expression was markedly downregulated in all organs of septic rats. Further in vitro studies with rat renal mesangial cells showed that NO and a combination of proinflammatory cytokines (interleukin-1β, tumor necrosis factor-α, and interferon-γ) downregulated Ang II type 1 receptor expression in a synergistic fashion. In summary, our data suggest that sepsis causes a systemic downregulation of Ang II type 1 receptors that is likely mediated by proinflammatory cytokines and NO. We suggest that this downregulation of Ang II type 1 receptors is the main reason for the attenuated responsiveness of blood pressure and of aldosterone formation to Ang II and, therefore, contributes to the characteristic septic shock. (Hypertension. 2001;38:177-182.)

Key Words: shock ■ renin-angiotensin system ■ blood pressure ■ mesangium ■ cytokines ■ nitric oxide

Sepsis and septic shock are characterized by systemic vasodilation, arterial hypotension, and a high mortality rate. The underlying pathogenetic mechanisms are complex and still not clearly understood. There is some evidence that NO plays an important role in mediating sepsis-induced circulatory failure. It is well known that lipopolysaccharide (LPS), a component of the outer membrane of Gram-negative organisms, and lipoteichoic acid (LTA), a cell wall component of Gram-positive organisms, induce widespread tissue formation of NO, leading to characteristic septic shock. However, findings that sepsis-induced vascular hyporeactivity to vasoconstrictors is not completely reversed by NO synthase (NOS) inhibition suggest that there may be other or additional pathways involved in septic circulatory failure. The renin-angiotensin system is reportedly activated during sepsis, whereas a diminished blood pressure response to angiotensin (Ang) II has been shown. The hemodynamic effects of Ang II are thought to be mediated by Ang II type 1 (AT1) receptors, of which there have been identified 2 subtypes, AT1a and AT1b, in the rat. Although increasing evidence from in vitro studies indirectly suggests that the function of AT1 receptors might be altered during septic conditions, the available data are unclear and contradictory. Increased Ang II binding in cultured vascular smooth muscle cells has been reported after incubation with endotoxin. Differential effects of proinflammatory cytokines on Ang II binding and AT1 mRNA in vascular smooth muscle cells have been shown. In this context, it has been reported that interleukin-1α (IL-1α) but not tumor necrosis factor-α (TNF-α) or interferon-γ (IFN-γ) increases Ang II binding and AT1 mRNA levels, whereas the combination of IL-1α, TNF-α, and IFN-γ decreases Ang II binding. Recently, it has been shown that IFN-γ alone inhibits AT1 receptor expression. In cardiac fibroblasts, it has been reported that TNF-α upregulates AT1 receptor expression. In view of these first, albeit contradictory, in vitro findings, we considered the possibility that AT1 receptor function might be diminished during sepsis and thus could account for the attenuated biological efficacy of Ang II during sepsis. To address this issue, we determined AT1 receptor expression in different organs of rats during experimental Gram-negative or Gram-positive sepsis. In addition, the effect of cytokines and of NO on AT1 receptor expression was investigated in primary cultures of rat renal mesangial cells.

Methods

Animal Experiments

All animal experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (NIH publication, No. 93-23, revised 1985) and were approved by the local ethics
committee. Rats received (1) Ringer’s solution intravenously (control rats), (2) 10 mg/kg IV LPS (Escherichia coli), or (3) 10 mg/kg IV LTA (Staphylococcus aureus), and were euthanized 12 or 24 hours (n=6 per group) after injection.

For Ang II dose-response studies, the rats were anesthetized with sevoflurane under controlled ventilation. The carotid artery was cannulated for measurement of systemic blood pressure. Doses of Ang II (0.0125 to 0.5 μg/kg) were administered intravenously 12 hours after injection of LPS or vehicle, and blood pressure response was determined. To imitate LPS-induced arterial hypotension, the NO donor sodium nitroprusside (SNP) was acutely administered, and responsiveness to Ang II was determined.

Cell Culture
Primary cultures of mesangial cells were obtained as previously described.20 The cells were incubated for 12 hours with (1) serum-free medium (control), (2) a mixture of IL-1β (50 ng/mL), TNF-α (100 ng/mL), and IFN-γ (500 U/mL), (3) a mixture of these cytokines and Nω-nitro- l-arginine methyl ester (L-NAME, 1 mmol/L or 5 mmol/L) in arginine-free medium, (4) the NO donor SNP (500 μmol/L), or (5) the NO donor S-nitroso-N-acetylpenicillamine (SNAP, 500 μmol/L).

Determination of AT1a and AT1b Receptor, NOS II, and β-Actin mRNA
Total RNA from adrenal gland, kidney, liver, lung, heart, and mesangial cells was extracted as previously described.20 AT1a and AT1b mRNAs as well as NOS II and β-actin mRNAs were measured by RNase protection assay as previously described.20,21

Radioiodinated Binding Assays
Cells (3×105 cells per well seeded in 24-well culture plates and pretreated as indicated above) were washed with PBS and incubated at 37°C for 15 minutes with 10 or 100 pmol/L [125I]-Ang II (2000 Ci/mmol) in 100 mmol/L NaCl, 25 mmol/L Tris, 4.5 mmol/L MgCl2, (Tris-buffered saline), and 0.25% BSA (pH 7.4) in the absence or presence of unlabeled Ang II (1 μmol/L for nonspecific binding). Then the cells were washed with ice-cold PBS and solubilized with 1N NaOH. Radioactivity was measured by γ counting. All binding data were related to total cellular protein content.

Hepatic plasma membranes (100 μg total protein) were incubated at 37°C for 20 minutes with 50 or 500 pmol/L [125I]-Ang II in Tris-buffered saline with 0.1% BSA and protease inhibitors (pH 7.4) in the absence or presence of unlabeled Ang II (1 μmol/L for nonspecific binding). The incubation was terminated by the addition of 4 mL ice-cold incubation buffer, followed by vacuum filtration over glass fiber filters presoaked in incubation buffer. The filters were rapidly washed with incubation buffer, and filter-bound radioactivity was measured.

Determination of Plasma Renin Activity, Plasma Ang II Concentration, and Plasma Aldosterone Concentration
Plasma renin activity, plasma Ang II, and plasma aldosterone concentrations were determined by using commercially available radioimmunoassay kits.

Statistical Analysis
Data were analyzed by ANOVA, followed by Student’s t test. A value of P<0.05 was considered significant.

An expanded Methods section can be found in an online data supplement available at http://www.hypertensionaha.org.

Results
Effect of LPS and LTA on the Activity of the Renin-Angiotensin-Aldosterone System
Plasma renin activity was, on average, 6.5 μg Ang I · L⁻¹ · h⁻¹ in normal rats and increased ~13-fold and ~5.5-fold at 12 hours and ~4.5-fold and ~2-fold at 24 hours after injection of LPS or LTA, respectively. Plasma Ang II levels were ~6.2 ng/L and increased in parallel with plasma renin activity after injection of LPS or LTA. In contrast, plasma aldosterone concentrations (~67 ng/L in normal rats) were not altered during sepsis (Figure 1).

Effect of LPS on Blood Pressure Response to Ang II
Mean arterial blood pressure (MAP) of anesthetized rats was ~112 mm Hg (Figure 2). Graded bolus injections of Ang II (0.0125 to 0.5 μg/kg) caused a dose-related increase in MAP of ~60 mm Hg after injection of 0.5 μg/kg Ang II. Injection of LPS caused a decrease of MAP to ~60 mm Hg 12 hours after application, whereas vehicle-injected rats remained normotensive. The pressure response to Ang II was clearly diminished in LPS-injected rats compared with vehicle-injected rats. Thus, MAP increased by only ~14 mm Hg after injection of 0.5 μg/kg Ang II in septic rats compared with 57 mm Hg in nonseptic rats. Because sepsis led to the systemic upregulation of NOS II (Figures 3 to 5) and consequently to an enhanced formation of NO, we considered the possibility that the vasodilator activity of NO may be responsible for the reduced pressor activity during sepsis. Therefore, we aimed to mimic the LPS-induced arterial hypotension by acute infusion of the NO donor SNP into nonseptic anesthetized rats, which lowered MAP to levels similar to those found with LPS. In these animals, however, the pressor activity of Ang II remained normal, suggesting...
that it is not the vasodilator activity of NO that causes the attenuated responsiveness to Ang II during sepsis.

Therefore, we considered the possibility that the reduced action of Ang II might be due to alterations at the level of AT$_1$ receptors, which confer the vasopressor activity of Ang II. For this purpose, we semiquantified AT$_1$ mRNA abundance in different organs of septic rats.

Effect of LPS and LTA on AT$_1$ Receptor and NOS II Gene Expression in the Adrenal Gland

We found that AT$_{1a}$ mRNA was downregulated to 16% and 30% of the control level 12 hours after LPS or LTA injection and increased thereafter to $\approx 36\%$ and $\approx 54\%$ of the control level 24 hours after application of LPS or LTA, respectively (Figure 3). Similarly, AT$_{1b}$ mRNA was time-dependently downregulated 12 and 24 hours after injection of LPS or LTA. At the same time, NOS II mRNA was induced 12 hours and 24 hours after LPS or LTA injection, and the increase was stronger with LPS than with LTA.

Effect of LPS and LTA on AT$_1$ Receptor Gene Expression, 125I-Ang II Binding, and NOS II Gene Expression in the Liver

In the liver, LPS also resulted in decreases of AT$_{1a}$ and AT$_{1b}$ mRNA (13% and 7% after 12 hours and 64% and 22% after 24 hours of the control level, respectively; Figure 4, data not shown for LTA). This downregulation of AT$_1$ mRNA was paralleled by a time-dependent decrease of 125I-Ang II binding. Twelve hours after injection of LTA, both AT$_{1a}$ and AT$_{1b}$ receptors were also downregulated (43% and 47%, respectively). The decrease was smaller than after LPS injection. By 24 hours after LTA injection, AT$_1$ receptor expression tended to be lower than control, but the difference did not reach significance. NOS II mRNA was induced 12 hours and 24 hours after LPS or LTA injection, and the increase was stronger with LPS than with LTA.
Effect of LPS and LTA on AT1 Receptor and NOS II Gene Expression in the Lung, Kidney, and Heart

Injection of LPS also downregulated AT1 receptor mRNA in the lung, heart, and kidney (Figure 5, data not shown for LTA). In the lung and the heart, AT1 expression returned to baseline 24 hours after injection, although in the kidney, AT1 expression was still depressed 24 hours after LPS injection. As was the case with LPS, AT1 receptor expression was downregulated after LTA injection in the lung, heart, and kidney but to a lesser extent than after LPS injection. LPS also caused a stimulation of NOS II gene expression in the lung, heart, and kidney 12 hours after injection. In the lung and the heart, control levels of NOS II expression were reached again 24 hours after LPS injection. In the kidney, NOS II mRNA was still elevated compared with control. LTA caused a similar time-dependent change in NOS II gene expression in the lung, heart, and kidney, although the induction was smaller than after LPS injection.

To characterize the mechanisms by which sepsis could lead to downregulation of AT1 receptors at the cellular level, we used primary cultures of rat renal mesangial cells, which express both AT1a and AT1b receptors. We considered proinflammatory cytokines, such as IL-1β, TNF-α, and IFN-γ, as well as NO (which all are strongly produced during sepsis), as possible molecular mediators of sepsis.

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Effect of Cytokines and NO on AT1 Receptor Gene Expression, 125I-Ang II Binding, and NOS II Gene Expression in Mesangial Cells

AT1 receptor mRNA in mesangial cells was downregulated to <1% of control after 12 hours of incubation with the mixture of cytokines (IL-1β, TNF-α, and IFN-γ) (Figure 6). This downregulation of AT1 mRNA was paralleled by a decrease of 125I-Ang II binding to 10% of the control value. At the same time, NOS II mRNA was strongly induced by the cytokines. To address a possible mediator role of NO in the downregulation of AT1 receptor expression, we examined the effect of NO donors on AT1 receptor expression as well as the influence of inhibition of NO formation on the effects of cytokines. Incubation of mesangial cells with the NO donors SNAP or SNP also caused a downregulation of AT1 receptor mRNA to ~30% and ~55% of control, respectively, and a decrease of 125I-Ang II binding to 50% (SNAP) of control. Inhibition of endogenous NO formation by L-NAME in arginine-free medium moderately attenuated the downregulation of AT1 mRNA by cytokines to 6% of the control value and the decrease of 125I-Ang II binding to 23% of the control value.

Discussion

The aim of the present study was to investigate the mechanisms underlying the reduced biological efficacy of Ang II during sepsis. To induce sepsis, we used 2 established...
maneuvers, namely, intravenous injection of LPS for the induction of Gram-negative sepsis and intravenous injection of LTA for the induction of Gram-positive sepsis. In accordance with previous observations, these 2 maneuvers caused a strong stimulation of NOS II gene expression in various organs, indicating the efficacy of our model. Also in accordance with previous reports, we observed an activation of the renin-angiotensin system during sepsis and a pronounced hypotension, associated with a markedly reduced pressor effect of Ang II. Notably, plasma aldosterone concentrations remained normal in spite of the increased plasma concentrations of Ang II. In view of the strong induction of NOS II during sepsis, it was conceivable for us that the attenuated pressor activity of Ang II in septic beings might be related to massive overproduction of NO. However, our further findings render this possibility less likely, inasmuch as mimicking LPS-induced arterial hypotension by acute infusion of the NO donor SNP into nonseptic animals did not alter the vascular reactivity to Ang II. These findings suggest that the vascular hyporeactivity to Ang II during sepsis is not mainly related to NO-mediated vasodilation. Therefore, considering the attenuated response of blood vessels and of aldosterone secretion to Ang II during sepsis led to the hypothesis that Ang II receptor function might be altered. In fact, we found that the expressions of the AT1 receptor mRNAs were strongly downregulated in the adrenal gland, kidney, liver, lung, and heart during septic conditions. Because Ang II receptor binding was also strongly reduced in vivo during sepsis, we infer that sepsis leads to a systemic downregulation of AT1 receptors by decreasing AT1 mRNA and that this downregulation accounts for the attenuated effect of Ang II on vascular resistance and on aldosterone secretion. This conclusion is in good accordance with a previous report indirectly suggesting a possible involvement of Ang II receptors in the pathophysiology of septic circulatory failure. It was found in this context that the initial vasodilation in response to LPS infusion in rats was opposed by Ang II, whereas 2 hours after the onset of LPS infusion, the vasopressor effect of Ang II faded.

To obtain more information about the pathways along which septic conditions lead to downregulation of AT1 receptors, we studied the regulation of AT1 receptor expression in vitro with regard to mediator substances considered to be relevant for septic reactions. In particular, we considered the influence of proinflammatory cytokines and of NO on AT1 receptor expression in primary cultures of rat renal mesangial cells. The sum of the findings obtained suggests that both NO and cytokines are capable of suppressing AT1 receptor expression at the mRNA and protein levels. These findings are in accordance with previous observations that NO donors decrease AT1 mRNA as well as the number of Ang II receptors in cultured vascular smooth muscle cells. They also fit with the reports that IL-1α, TNF-α, and IFN-γ decrease Ang II binding to vascular smooth muscle cells in an NO-dependent fashion. Therefore, all of these in vitro data obtained by us and others not only support our in vivo observations but also indicate that the expression of the AT1 receptor genes are regulated both by NO and by cytokines. Which signaling pathways downregulate AT1 receptor gene expression is yet unknown and requires further investigation. Further experiments will also be required to determine whether the receptor expressions for other vasoconstrictors, such as norepinephrine or vasopressin, are also decreased during sepsis, because not only the pressor action of Ang II but also the pressor actions of norepinephrine and vasopressin are attenuated during sepsis.

All together, our data suggest that cytokines and NO act in concert to downregulate AT1 receptors during sepsis, causing an attenuation of AT1 receptor–mediated effects, such as vasoconstriction and aldosterone secretion. In consequence, the activated renin-angiotensin system is not sufficient to counteract the vasodilatory action of NO that causes the cardiovascular collapse during sepsis.

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References


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