Cytokine-mediated downregulation of vasopressin V\textsubscript{1\textalpha} receptors during acute endotoxemia in rats

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Received 30 August 2001; accepted in final form 24 November 2001

Bucher, Michael, Jonny Hobbhahn, Kai Taeger, and Armin Kurtz. Cytokine-mediated downregulation of vasopressin V\textsubscript{1\textalpha} receptors during acute endotoxemia in rats. Am J Physiol Regulatory Integrative Comp Physiol 282: R979–R984, 2002. First published November 29, 2001; 10.1152/ajpregu.00520.2001.—The reduced pressure response to vasopressin during acute sepsis has directed our interest to the regulation of vasopressin V\textsubscript{1\textalpha} receptors. Rats were injected with lipopolysaccharide for induction of experimental gram-negative sepsis. V\textsubscript{1\textalpha} receptor gene expression was downregulated in the liver, lung, kidney, and heart during endotoxemia. Inasmuch as the concentrations of proinflammatory cytokines such as interleukin-1β, tumor necrosis factor-α, and interferon-γ were highly increased during sepsis, the influence of these cytokines on V\textsubscript{1\textalpha} receptor expression was investigated in primary cultures of hepatocytes and in the aortic vascular smooth muscle cell line A7r5. V\textsubscript{1\textalpha} receptor expression was downregulated by the cytokines in a nitric oxide-independent manner. Blood pressure dose-response studies after injection of endotoxin showed a diminished responsiveness to the selective V\textsubscript{1} receptor agonist Phe\textsuperscript{3},Ile\textsuperscript{6},Orn\textsuperscript{8}-vasopressin. Our data show that sepsis diminishes responsiveness to the selective V\textsubscript{1} receptor agonist studied dose-response studies after injection of endotoxin showed a diminished responsiveness to the selective V\textsubscript{1} receptor agonist Phe\textsuperscript{3},Ile\textsuperscript{6},Orn\textsuperscript{8}-vasopressin. Our data show that sepsis diminishes responsiveness to marginally V\textsubscript{1\textalpha} receptors and suggest that this effect is likely mediated by proinflammatory cytokines. We propose that this downregulation of V\textsubscript{1\textalpha} receptors contributes to the attenuated responsiveness of blood pressure in response to vasopressin and, therefore, contributes to the circulatory failure in septic shock.

SHOCK DUE TO SEPSIS HAS INCREASED in incidence during the past 50 years and is now one of the most common causes of death in intensive care units. Despite considerable therapeutic advances, mortality from septic shock remains high. Pathogenetically, sepsis and septic shock are characterized by systemic vasodilation, leading to arterial hypotension, multiple organ dysfunction, and death (21). The pathogenetic mechanism of this vasodilation is multifactorial and not clearly understood. Nitric oxide (NO) production due to induction of NO synthase isoform II (NOS II) has been assumed to mediate sepsis-induced vasodilation (16, 21). However, findings that arterial hypotension, as well as vascular hyporeactivity, is only slightly alleviated by NOS II inhibition suggest that other or additional pathways may be involved in septic circulatory failure (5, 22, 27, 31). Alterations in vasoconstrictor mechanisms have been reported. Inappropriate low plasma vasopressin levels have been suggested to contribute to the hypotension in advanced vasodilatory septic shock (14). In models of acute sepsis, vasopressin is markedly increased (26, 32, 33); in addition, a reduced pressor response to the exogenous hormone has been shown (8, 26, 30). The pathogenetic mechanisms of this phenomenon, called vasoplegia, are not clearly understood. One could imagine that the receptors for vasoconstrictors could be altered during sepsis, leading to diminished pressor effects in response to the respective agonist. We previously demonstrated that sepsis leads to a systemic downregulation of ANG II type 1 receptors (4). Therefore, we were interested also in the regulation of the receptor mediating the vasoconstrictive response to vasopressin, which is thought to be the vasopressin subtype 1A (V\textsubscript{1\textalpha}) receptor (17), during experimental endotoxemia. For this purpose, we determined V\textsubscript{1\textalpha} receptor gene expression in different organs of rats with acute gram-negative sepsis. In addition, the regulation of V\textsubscript{1\textalpha} receptors was investigated at a cellular level in response to typical endogenous mediators of sepsis. It is well known that the proinflammatory cytokines interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ), as well as NO, are predominantly active during inflammation (21). Therefore, the effect of these mediators on the expression of V\textsubscript{1\textalpha} receptors was determined in vitro in rat hepatocytes and aortic vascular smooth muscle cells (VSMCs).

METHODS

Animal experiments. All animal experiments were conducted in accordance with the National Institutes of Health guidelines and German laws relating to the protection of animals and were approved by the local ethics committee. Male Sprague-Dawley rats (200–250 g) were injected intravenously with Ringer solution (control rats) or lipopolysaccharide (LPS; Escherichia coli, 10 mg/kg iv; Sigma) and killed by decapitation 3, 6, 12, or 24 h (n = 6 per group) after injection.
V1A receptor gene expression in vivo. V1A receptor gene expression was determined in major rat organs before and after injection of LPS. V1A mRNA was abundantly expressed in the liver. In the lung, kidney, and heart, V1A mRNA was expressed to a much lower extent (Fig. 1, inset). In the liver, V1A mRNA was downregulated to ~2% of the control level 6 h after LPS injection and increased thereafter to 5 and 16% of the control level 12 and 24 h after injection, respectively (Fig. 1). Also, in the lung, kidney, and heart, V1A mRNA was downregulated to 31, 75, and 84% of the control level after 6 h of endotoxemia, respectively. In the lung and heart, V1A mRNA was also depressed after 12 and 24 h, whereas in the kidney, V1A mRNA had returned to control values at this time point. To exclude the possibility that the weak V1A mRNA signal in the lung and heart is due to trapped blood cells in these organs, we also assayed the total RNA yield from 2 ml of whole blood for V1A mRNA and found no specific V1A mRNA hybridization signal in whole blood (data not shown).

![Fig. 1. Effect of lipopolysaccharide (LPS; 10 mg/kg) on vasopressin type 1A (V1A) receptor mRNA in rat liver (A), lung (B), kidney (C), and heart (D) 6, 12, and 24 h after intravenous injection. Values are related to signals obtained for β-actin mRNA. Inset: absolute values (counts per minute (cpm)) for V1A mRNA relative to total RNA. Values are means ± SE of 6 animals per group. *P < 0.05 vs. control. Error bars are partially within the size of the symbols.](http://ajpregu.physiology.org/10.1152/ajpregu.00303.2002)
Cytokine concentrations. We further aimed to characterize the mechanisms along which V₁A receptors could be downregulated at a cellular level. As possible molecular mediators of sepsis, we considered proinflammatory cytokines, such as IL-1β, TNF-α, and IFN-γ, which are known to mediate a variety of effects during sepsis. As shown in Fig. 2, these three cytokines were strongly induced in the liver in the chosen model of sepsis.

V₁A receptor expression in hepatocytes. To investigate the possible role of proinflammatory cytokines in the regulation of V₁A receptor expression, we stimulated primary cultures of rat hepatocytes with the cytokines known to be abundantly generated in the liver during endotoxemia and determined hepatocellular V₁A mRNA as well as specific ¹²⁵I-vasopressin binding to hepatocytes. Incubation of hepatocytes with IL-1β (50 ng/ml), TNF-α (100 ng/ml), or IFN-γ (500 U/ml) for 8 h decreased V₁A mRNA to 35, 20, and 34% of control, respectively (Fig. 3). The combination of the cytokines additively downregulated V₁A receptor gene expression to 13% of control (Fig. 3A). In parallel, ¹²⁵I-vasopressin binding to the hepatocytes was reduced after incubation with the cytokines to 46, 43, 55, and 20% of control, respectively (Fig. 3B).

It is well known that cytokines lead to significant tissue formation of NO through the induction of NOS II activity. Therefore, we considered the possibility that increased NO production mediates the effects of the cytokines on the downregulation of V₁A receptors. Blocking of NO synthesis by coincubation of the cytokines with the NOS inhibitor L-NAME (1 mmol/l), however, did not change cytokine-induced downregulation of V₁A receptor mRNA (Fig. 4A) and ¹²⁵I-vasopressin binding (Fig. 4B). Also, incubation of hepatocytes with the NO donor SNAP (500 μmol/l) did not change V₁A mRNA (Fig. 4A) and specific ¹²⁵I-vasopressin binding (Fig. 4B) compared with control.

V₁A receptor expression in VSMCs. To investigate whether the cytokine-induced downregulation of V₁A receptors was restricted to hepatocytes or was a more general effect, we also incubated the aortic VSMCs (A7r5 cell line) with the cytokines. Incubation of VSMCs with IL-1β (50 ng/ml), TNF-α (100 ng/ml), or IFN-γ (500 U/ml) decreased V₁A mRNA, with TNF-α having the strongest effect (Fig. 5). The combination of the cytokines produced an even stronger downregulation of V₁A receptor gene expression in aortic VSMCs (Fig. 5A) than the single cytokines. In parallel, ¹²⁵I-vasopressin binding to A7r5 cells was reduced after incubation with the cytokines (Fig. 5B).

The contribution of NO to the cytokine-induced downregulation of V₁A receptors was also investigated in aortic VSMCs. As demonstrated for hepatocytes, blocking of NO synthesis by coincubation with the cytokines and L-NAME did not influence cytokine-induced downregulation of V₁A receptor mRNA. Incubation of aortic VSMCs with the NO donor SNAP (500 μmol/l) did not change V₁A mRNA compared with control (Fig. 6A). The characteristic changes in V₁A mRNA were paralleled by changes in specific ¹²⁵I-vasopressin binding (Fig. 6B).
Blood pressure response. Because our data suggested that V1A receptors are downregulated in several rat organs during endotoxemia, we aimed to determine the pathophysiological significance of this receptor downregulation for the vascular hyporeactivity in response to vasopressin. We therefore performed blood pressure-response studies using the selective V1 receptor agonist Phe2,Ile3,Orn8-vasopressin. Inasmuch as V1A receptor gene expression was mostly downregulated 6 h after LPS injection, we performed dose-response studies after 6–8 h. In vehicle-injected anesthetized rats, mean arterial blood pressure (MAP) was 102 mmHg, and graded bolus injections of Phe2,Ile3,Orn8-vasopressin (10–300 pmol/kg) caused a dose-related increase in MAP of 70 mmHg after injection of 300 pmol/kg Phe2,Ile3,Orn8-vasopressin (Fig. 7). At 6 h after injection of LPS, MAP was decreased to 58 mmHg, and the pressure response to Phe2,Ile3,Orn8-vasopressin was diminished compared with nonseptic rats. Thus, at that time, MAP increased by only 47 mmHg after injection of 300 pmol/kg Phe2,Ile3,Orn8-vasopressin.

DISCUSSION

Several in vivo and in vitro studies demonstrate a diminished pressor response to vasopressin during acute experimental sepsis (8, 26, 30). Despite its potential pathophysiological significance with regard to circulatory failure during sepsis, the factors responsible for the diminished vascular reactivity in response to vasopressin remain undefined. The vasoconstrictive response to vasopressin is mediated through the V1A receptor (17). We speculated that an altered expression of this receptor subtype could be involved in the diminished pressor response to vasopressin during acute sepsis. Therefore, we investigated V1A receptor expression in LPS-injected rats as one of the most common models of gram-negative sepsis. This maneuver caused an arterial hypotension and an increase in cytokine concentrations in various organs, indicating the efficacy of our model. We found a pronounced downregulation of V1A receptor mRNA in the liver as well as in the lung, kidney, and heart during experimental sepsis. These results are in good agreement with a study reporting diminished hepatic [3H]vasopressin binding in the rat after endotoxin infusion (23).

Proinflammatory cytokines and NO are known to be abundantly generated mediators during sepsis (21). Therefore, we were interested in the influence of these mediators on the expression of V1A receptors. Because of the multiplicity of factors that can influence the regulation of receptor expression, it has been impossible to examine the specific and independent effect of one mediator in vivo. For this purpose, we used two in vitro cell culture systems to exclude other variables.

![Fig. 4. Effect of a mixture of IL-1β, TNF-α, and IFN-γ on V1A receptor mRNA (A) and 125I-vasopressin binding (B) in hepatocytes after 8 h of incubation. mRNA values are related to signals obtained for β-actin mRNA; binding data are given for incubation with 125I-vasopressin (500 pmol/l) and related to total cellular protein content. Values are means ± SE of 4 experiments, each performed in duplicate culture dishes. *P < 0.05 vs. control.](http://ajpregu.physiology.org/doi/10.220.33.1)

![Fig. 5. Effect of IL-1β, TNF-α, IFN-γ, or a mixture of these cytokines (cytokines) on V1A receptor mRNA (A) and 125I-vasopressin binding (B) in aortic vascular smooth muscle (A7r5) cells after 8 h of incubation. mRNA values are related to signals obtained for β-actin mRNA; binding data are given for incubation with 125I-vasopressin (1 nmol/l) and related to total cellular protein content. Values are means ± SE of 4 experiments, each performed in duplicate culture dishes. *P < 0.05 vs. control.](http://ajpregu.physiology.org/doi/10.220.33.1)
The downregulation of V1a receptors in the liver may also be of relevance for some metabolic aberrations during sepsis. Alterations in glucose metabolism occur frequently during sepsis and include hyper- and hypoglycemia. It has been shown that vasopressin-stimulated glycogen phosphorylase α activation is less responsive in isolated rat hepatocytes after continuous infusion of endotoxin (23). The diminished expression of hepatic V1a receptors during sepsis could account for this observation.

Characteristics similar to those we suggest for the regulation of V1a receptor expression in this study have been observed for the ANG II type 1 receptor, which mediates the vasoconstrictor action of ANG II. So we have shown that a combination of IL-1β, TNF-α, and IFN-γ downregulates not only V1a receptor expression but also ANG II type 1 receptor expression in vitro (4). Therefore, proinflammatory cytokines seem to play a key role in the pathogenesis of septic shock. On one hand, these cytokines mediate a significant tissue production of the vasodilatory molecule NO, which causes the cardiovascular collapse during sepsis. On the other hand, these cytokines lead to a pronounced downregulation of pressor receptors for ANG II and vasopressin, resulting in a decreased vasoconstrictor responsiveness to these agonists. Consequently, ANG II and vasopressin are not able to counteract the overshooting vasodilatory action of NO during acute sepsis. It is not known along which signaling pathways the V1a receptor gene expression is downregulated by proinflammatory cytokines at the cellular level. The expression of functional receptors for IL-1, TNF-α, and IFN-γ has not been investigated in this study, but data from the literature indicate that these cytokine receptors should be expressed in hepatocytes and VSMCs, with the exception of IFN-γ receptors, which are only weakly or not expressed in normal hepatocytes (1, 6, 11-13, 18, 24, 28). Which downstream signaling pathways are

and uniquely demonstrated an NO-independent effect of proinflammatory cytokines to downregulate V1a receptors in hepatic and vascular tissue.

To investigate whether the downregulation of V1a receptors is of relevance for the vascular hyporeactivity to vasopressin during experimental sepsis, we performed blood pressure-response studies with a selective V1 receptor agonist 6 h after injection of vehicle or LPS, when V1a receptor gene expression was mostly depressed in all organs investigated. The diminished pressure responsiveness to the V1 receptor agonist suggests the relevance of the V1a receptor for the vascular hyporeactivity to vasopressin during acute endotoxemia. These results fit with previous reports showing decreased blood pressure response (26, 30) as well as decreased microvascular vasoconstrictive responses (8) to vasopressin during acute experimental sepsis. An increased pressor sensitivity to vasopressin has been reported in advanced vasodilatory septic shock. That study demonstrated a blood pressure-elevating effect of vasopressin in five septic patients, but the effect has not been compared with a nonseptic control group (15). These results are not in contrast to our findings, because we also found a blood pressure responsiveness to a V1 receptor-selective vasopressin analog during endotoxemia, which, however, was attenuated compared with normal rats.

Fig. 6. Effect of a mixture of IL-1β, TNF-α, and IFN-γ, without (cytokines) and with l-NAME (cytokines + l-NAME) or the NO donor SNAP on V1a receptor mRNA (A) and 125I-vasopressin binding (B) in aortic vascular smooth muscle (A7r5) cells after 8 h of incubation. mRNA values are related to signals obtained for β-actin mRNA; binding data are given for incubation with 125I-vasopressin (1 nmol/l) and related to total cellular protein content. Values are means ± SE of 4 experiments, each performed in duplicate culture dishes. *P < 0.05 vs. control.

Fig. 7. Effect of LPS (10 mg/kg) on mean arterial blood pressure (MAP) response to bolus injections of the selective V1 receptor agonist Phe³,Ile⁴,Orn³-vasopressin 6 h after intravenous injection of LPS or vehicle. Values are means ± SE expressed as absolute values of MAP (inset) and the change in MAP (ΔMAP) from the same experiments; n = 6 animals per group. *P < 0.05 vs. vehicle.
involved in the downregulation of vasopressin receptors requires further investigation.

Together, our data suggest that proinflammatory cytokines downregulate V1A receptor expression during sepsis, causing an attenuation of the V1A receptor-mediated vasoconstriction. This downregulation of V1A receptor gene expression likely contributes to the development of cardiovascular failure during sepsis.

The expert technical assistance of A. Seefeld and G. Wilberg is gratefully acknowledged.

REFERENCES