Contractions to Histamine in Pulmonary and Mesenteric Arteries from Endotoxemic Rabbits: Modulation by Vascular Expressions of Inducible Nitric-Oxide Synthase and Histamine H$_1$-Receptors

NAOYUKI MATSUDA, YUICHI HATTORI, XIAO-HONG ZHANG, HIROYUKI FUKUI, OSAMU KEMMOTSU, and SATOSHI GANDO

Departments of Pharmacology (Y.H.) and Anesthesiology & Critical Care Medicine (N.M., X.H.Z., O.K., S.G.), Hokkaido University School of Medicine, Sapporo, Japan; and Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Tokushima, Tokushima, Japan (H.F.)

Received May 8, 2003; accepted July 2, 2003

ABSTRACT

The inducible isoform of nitric-oxide synthase (iNOS) is highly expressed after induction of endotoxemia and contributes to vascular hypocontractility in endotoxemia. Circulating levels of histamine are elevated in animal models of sepsis and in patients with septic shock. This study assessed whether the vascular effects of histamine play a significant role in the pathophysiology of endotoxemic shock despite the hyporesponsiveness to vasoconstrictors associated with iNOS up-regulation. Rabbits were rendered endotoxemic by lipopolysaccharide (LPS; 100 μg/kg, i.v.). In mesenteric arteries taken from animals at 6 h of LPS administration, the contractile response to histamine was significantly impaired but histamine-evoked contractions in pulmonary arteries were unchanged. Western blot revealed a drastic increase in iNOS expression in mesenteric vessels after LPS, but endotoxin-induced iNOS increase was not so marked in pulmonary vessels. On the other hand, expression of endothelial nitric-oxide synthase was suppressed under LPS challenge in both types of vessels. In the presence of N$\text{G}$-nitro-L-arginine or (S)-ethylisothiourea used for iNOS inhibition, histamine-evoked contractions of endotoxemic pulmonary and mesenteric vessels were significantly enhanced. This was possibly associated with a dramatic increase in H$_1$-receptor expression at the gene and protein levels, as determined by Northern blot and immunoblot analyses. Furthermore, we found that LPS-induced endotoxemia caused prominent increases in production of histamine through induction of histidine decarboxylase in tissues, including blood vessels. From these results, we propose that histamine may contribute to the development of endotoxin-induced pulmonary hypertension.

Sepsis is a serious systemic disorder characterized by generalized vasodilation, hyporesponsiveness of the vascular smooth muscle of the systemic circulation to pressor agents, including catecholamines, and hypotension (Suffredini et al., 1989; Parrillo et al., 1990). Peripheral tissue oxygen uptake and utilization are eventually compromised by the low blood pressure, and such abnormalities contribute to end organ damage and, ultimately, to death (Ziegler et al., 1991). ARDS is a serious complication of sepsis. The characteristic pathophysiology involves nonhydrostatic permeability pulmonary edema and refractory hypoxemia (Danzer et al., 1979). Pulmonary hypertension, together with elevated pulmonary vascular resistance, is frequently detected in patients with severe ARDS and is associated with an increased mortality (Zapol and Snider, 1977). It has been suggested that a number of potentially important chemical mediators are involved in the pathogenesis of septic ARDS, including histamine (Tranbaugh and Lewis, 1984).

Histamine levels in the plasma are elevated in animal models of sepsis (Hinshaw et al., 1961; Spink et al., 1964; Schumer, 1971) as well as in patients with septic shock (Nagy, 1990). In our recent work using endotoxemic rabbits, the sustained elevation of plasma histamine has been shown to be associated with the time-dependent increases in protein expression of HDC in tissues (Matsuda et al., 2002). A significant increase in the plasma histamine level has been also reported in a rat model of ARDS produced by phospholipase

ABBREVIATIONS: ARDS, acute respiratory distress syndrome; HDC, histidine decarboxylase; NO, nitric oxide; NOS, nitric-oxide synthase; iNOS, inducible nitric-oxide synthase; eNOS, endothelial nitric-oxide synthase; LPS, lipopolysaccharide; L-NNA, N$\text{G}$-nitro-L-arginine; (S)-ethyl-ITU, (S)-ethylisothiourea; PSS, physiological salt solution; kb, kilobase(s).
A2 injection (Stömer and Steinmann, 1989). Histamine can affect both pulmonary and systemic vascular responses (Ahmed et al., 1982), lung mechanics (Hutchinson et al., 1982), and pulmonary microvascular permeability (Brigham et al., 1980). Experimental studies using the porcine model of septic ARDS have shown that treatment with histamine receptor blockers in combination with blockade of a number of inflammatory mediators ameliorates abnormal parameters and improves animal survival time (Sielaff et al., 1987; Byrne et al., 1990). These facts strongly suggest an important role for histamine in the etiology of sepsis-induced pulmonary derangements.

To date, little is known about the role of histamine in the vascular responses to sepsis in vitro. It has been shown that H1- and H2-receptors mediate pulmonary vasoconstriction and vasodilatation, respectively, but stimulation of either receptor produces systemic vasodilatation (Ahmed et al., 1982). Thus, histamine may contribute to the pulmonary hypertension (by H1-receptor-mediated vasoconstriction) that occurs in septic ARDS. However, expressions of the inducible form of nitric-oxide synthase (iNOS) can be stimulated in blood vessels harvested from endotoxemic animals after treatment with LPS (Knowles et al., 1990; Griffiths et al., 1995; Hom et al., 1995). Because induction of iNOS in blood vessels results in impaired responses to vasoconstrictor stimuli (Fleming et al., 1991; Griffiths et al., 1995; Hom et al., 1995), presumably from the generation of large amounts of NO, histamine-evoked contraction may be attenuated in pulmonary arteries from LPS-treated animals. The first aim of this study, therefore, was to assess the pulmonary and mesenteric arterial responses to histamine in vitro in the absence and presence of L-NNA, an inhibitor of NOS, or (S)-ethyl-ITU, a selective iNOS inhibitor, when rabbits were rendered endotoxemic by LPS and to determine whether the effects of L-NNA are associated with the expression levels of iNOS in each vascular tissue. Second, we wanted to assess the effect of in vivo pretreatment of LPS on gene and protein expressions of histamine H1-receptors in vascular tissues and, in particular, to demonstrate whether the expressions can modify histamine-evoked contractile responses. We also examined whether the expression level of HDC protein is increased in vascular tissues of this endotoxemic model.

Materials and Methods

Animal Model. Male New Zealand White rabbits weighing 2 to 2.5 kg were injected intravenously with LPS (100 µg/kg; Escherichia coli 055; List Biological Laboratories Inc., Campbell, CA) or an equivalent volume of sterile saline (sham; 2 ml/body) 6 h before being killed by overdose of pentobarbital (60 mg/kg, i.v.). Our previous studies with this endotoxemic model demonstrated that animals show little or no histamine in the vascular tissues at 6 h after treatment with LPS (Knowles et al., 1990; Griffiths et al., 1995; Hom et al., 1995). Because induction of iNOS in blood vessels results in impaired responses to vasoconstrictor stimuli (Fleming et al., 1991; Griffiths et al., 1995; Hom et al., 1995), presumably from the generation of large amounts of NO, histamine-evoked contraction may be attenuated in pulmonary arteries from LPS-treated animals. The first aim of this study, therefore, was to assess the pulmonary and mesenteric arterial responses to histamine in vitro in the absence and presence of L-NNA, an inhibitor of NOS, or (S)-ethyl-ITU, a selective iNOS inhibitor, when rabbits were rendered endotoxemic by LPS and to determine whether the effects of L-NNA are associated with the expression levels of iNOS in each vascular tissue. Second, we wanted to assess the effect of in vivo pretreatment of LPS on gene and protein expressions of histamine H1-receptors in vascular tissues and, in particular, to demonstrate whether the expressions can modify histamine-evoked contractile responses. We also examined whether the expression level of HDC protein is increased in vascular tissues of this endotoxemic model.

Organ Bath Experiments. At 6 h after injection of LPS or vehicle, the main pulmonary artery and the main branch of the superior mesenteric artery were carefully removed after induction of terminal anesthesia with pentobarbital. The vessels were then trimmed of the surrounding tissues under a dissecting microscope and cut into rings of 4-mm length. Care was taken to ensure that the endothelium was not damaged during the processing of the tissue preparation. The ring was suspended by a pair of stainless steel hooks in a water-jacketed bath filled with 25 ml of PSS. The composition of PSS was 118 mM NaCl, 4.7 mM KCl, 1.2 mM MgCl2, 2.5 mM CaCl2, 1.2 mM KH2PO4, 25.0 mM NaHCO3, and 10.0 mM glucose. The solution in the bath was gassed with 95% O2 and 5% CO2, and its temperature was maintained at 37°C. The ring was stretched until an optimal tension of 2 g was loaded and then allowed to equilibrate for ≥60 min before the start of the recordings. Isometric tension was monitored with a transducer and recorded by a pen recorder. The rings were repeatedly challenged with 40 mM K+ until the high-K+-induced contractions reached a constant value. High-K+ PSS was prepared by substitution of KCl for NaCl on an equimolar basis. Concentration-response curves for histamine-induced contractions were constructed in a cumulative manner by increasing the concentration of the agonist in steps of 0.5 log units. The vessel was exposed to each concentration of histamine until the contractile response reached a peak, which usually occurred within 5 min. When L-NNA or (S)-ethyl-ITU was used, they were added to the bath 30 or 45 min before administration of histamine.

At the completion of each experiment, the rings were carefully blotted dry and weighed. Contractile responses were expressed as milligrams of developed tension per milligram of tissue wet weight to normalize the differences in the cross-sectional area of the ring preparation.

Western Blot Analysis. Immunoblotting was performed as demonstrated in our previous report (Matsuda et al., 2000b). Samples of tissue homogenate were subjected to electrophoresis on polyacrylamide gels, and proteins were transferred to polyvinylidine difluoride filter membrane. After blocking with 1% bovine serum albumin in phosphate-buffered saline, the membranes were incubated with specific antibody recognizing HDC, H1-receptor, eNOS, or iNOS. For HDC recognition, we used a mouse monoclonal antibody that was kindly provided by Prof. A. Ichikawa and Dr. S. Tanaka (Department of Physiological Chemistry, Graduate School of Pharmaceutical Sciences, Kyoto University, Kyoto, Japan; Tanaka et al., 1998). For recognizing H1-receptor, eNOS, and iNOS, we used the following commercially available antibodies: anti-human H1-receptor rabbit polyclonal antibody (Chemicon International, Temecula, CA), anti-human eNOS rabbit polyclonal antibody (Affinity BioReagents, Golden, CT) and anti-rabbit iNOS mouse monoclonal antibody (Affinity BioReagents). After extensive washing with phosphate-buffered saline containing 0.05% Tween 20 to remove any nonspecifically bound primary antibodies, the membranes were incubated with horseradish peroxidase-conjugated anti-mouse antibody or anti-rabbit antibody. Finally, the blots were visualized with the enhanced chemiluminescence detection system (Amersham Biosciences UK Ltd., Little Chalfont, Buckinghamshire, UK), exposed to X-ray film, and analyzed by NIH Image Software.

Total RNA Extraction and Northern Blot Analysis. Total RNA was extracted from tissues by the guanidine thiocyanate-phenol-chloroform method with Isogen (Nippon Gene Co., Ltd., Toyama, Japan) used routinely in our laboratory (Matsuda et al., 1999). RNA purity was determined by the ratio of optical density measured at 260 and 280 nm (OD260/OD280), and RNA quantity was estimated at OD260.

RNA (20 µg per lane) was subjected to electrophoresis on agarose/formaldehyde gels and then transferred to a Hybond-N+ nylon membrane (Amersham Biosciences UK Ltd., Little Chalfont, Buckinghamshire, UK). The membrane was prehybridized in prewarmed rate-enhanced hybridization buffer (Rapidhyb buffer; Amersham Biosciences UK Ltd.) at 65°C for 60 min. The guinea pig histamine H1-receptor cDNA (Horio et al., 1993) was kindly provided by Prof. E. Senba (Department of Anatomy, Wakayama Medical College, Wakayama, Japan). The cDNA probe was radiolabeled using a random primer labeling system (Rediprime; Amersham Biosciences UK Ltd.) in the presence of [32P]dCTP (6000 Ci/mmole; PerkinElmer Life Sciences, Boston, MA). The blot was washed at high stringency in 2 × standard saline citrate/0.1% SDS at room temperature for 10 min and in 1 × standard saline citrate/
pretreatment with 100 nM chlorpheniramine, a H₁-receptor was quantitated by counting the radioactivity using a bioimaging analyzer system (Fuji BAS 2000; Fuji Photo Film, Tokyo, Japan), as described previously (Matsuda et al., 1999). Ethenium bromide staining was used as a control to verify gel loading, and expression of H₁-receptor mRNA was normalized as the ratio of H₁-receptor mRNA over 28 S ribosomal RNA.

Measurement of Plasma Histamine. An arterial blood sample was rapidly collected into chilled tubes containing EDTA-2Na and centrifuged (4°C) to separate the plasma. The plasma was stored at −80°C until the day of assay. The plasma histamine concentration was determined by use of a sensitive radioimmunoassay (Eiken Chemical Co., Ltd., Tokyo, Japan).

Statistical Analysis. Values are means ± S.E. Statistical assessment of the data were made by Student’s t test or a repeated-measures one-way analysis of variance followed by Bonferroni’s multiple comparison test when appropriate. P < 0.05 was taken as significant.

Results
Changes in Plasma Concentrations of Histamine. The basal concentration of histamine in the plasma was 258 ± 91 nM (n = 5) in the rabbit. As demonstrated in our recent report (Matsuda et al., 2002), administration of a moderate dose of LPS (100 μg/kg) resulted in immediate and sustained increases in plasma concentrations of histamine. Thus, plasma histamine concentrations were significantly elevated from baseline early after injection of LPS, with a peak concentration at 30 min (14.5 ± 1.3 μM). Although declining thereafter (8.8 ± 0.6 μM at 1 h), plasma concentrations of histamine remained much higher than the baseline value throughout the 10 h of the study (10.9 ± 1.0 μM at 6 h and 9.9 ± 0.9 μM at 10 h).

Histamine Responses of Isolated Blood Vessels. Histamine produced concentration-dependent contractions of pulmonary and mesenteric segments taken from both rabbits at 6 h after sham and LPS treatment. Pretreatment of these vessels from sham-treated animals with 10 μM cimetidine, a H₂-receptor antagonist, had no significant effect on histamine-induced contractions (EC₅₀ = 27 ± 8 μM for pulmonary and 22 ± 8 μM for mesenteric artery, n = 6), but pretreatment with 100 nM chlorpheniramine, a H₁-receptor antagonist, resulted in a marked shift of the concentration-response curve for histamine to the right so that the EC₅₀ values with chlorpheniramine pretreatment was significantly greater than those without any pretreatment (pulmonary, 460 ± 47 μM versus 56 ± 18 μM, P < 0.001; mesenteric, 445 ± 39 versus 12 ± 7 μM, P < 0.001). The same results were obtained in pulmonary and mesenteric arterial rings from LPS-treated animals. Thus, it was indicated that histamine-induced contractions of pulmonary and mesenteric arteries are entirely mediated through activation of H₁-receptors irrespective of sham or LPS treatment.

As shown in Fig. 1A, the contractile responses to histamine were similar in pulmonary arteries from sham- and LPS-treated rabbits. However, incubation with 100 μM L-NNa resulted in greater contractions to histamine in pulmonary arteries from LPS-treated than in those from sham-treated animals (Fig. 1B). In the presence of L-NNa, the maximal contractions was 41 ± 10 and 98 ± 17 mg/mg wet weight in sham and endotoxemic pulmonary arterial rings, respectively (n = 5, P < 0.05). Similarly, in the presence of 10 μM (S)-ethyl-ITU, a selective iNOS inhibitor, the contractile responses to higher concentrations of histamine were significantly greater in pulmonary arteries from LPS-treated than in those from sham-treated animals (Fig. 1C).

In mesenteric arteries, histamine-induced contractions were significantly depressed by 6-h endotoxemia (Fig. 2A). Thus, the EC₅₀ value was significantly greater in endotoxemic compared with sham-treated vessels (80 ± 19 versus 22 ± 8 μM, n = 6, P < 0.05), although the decrease in the maximal contraction was statistically insignificant (216 ± 15 versus 248 ± 34 mg/mg wet weight). When mesenteric arteries were incubated with L-NNa, histamine-induced contractions were evidently enhanced in the endotoxemic group (Fig. 2B). The maximum force of contraction elicited by histamine was 252 ± 31 versus 539 ± 70 mg/mg wet weight, respectively, in vessels from vehicle- and LPS-treated animals (n = 6, P < 0.01). Incubation with (S)-ethyl-ITU also resulted in a significant increase in contractions to higher concentrations of histamine in endotoxemic mesenteric arteries (Fig. 2C).

In neither pulmonary nor mesenteric arteries from septic animals was the contractile response to phenylephrine altered by inhibition of NOS with L-NNa. The maximal contractions to phenylephrine in endotoxemic pulmonary and mesenteric arterial rings (189 ± 23 and 557 ± 78 mg/mg wet weight) were comparable with the corresponding sham-treated vessels (215 ± 27 and 446 ± 89 mg/mg wet weight, n = 5–7).

Nor was any relaxation to histamine seen when pulmonary and mesenteric arteries from either sham- or LPS-treated rabbits were incubated with cimetidine (10 μM), a selective H₂-receptor antagonist. When mesenteric arterial rings were pretreated with 100 μM L-NNa, the relaxations to phenylephrine induced by cimetidine were significantly impaired in endotoxemic mesenteric arteries (Fig. 2D). A similar impairment of relaxation to phenylephrine was observed when mesenteric arterial rings were incubated with 100 μM (S)-ethyl-ITU (Fig. 2E). However, the relaxations to phenylephrine induced by cimetidine and (S)-ethyl-ITU were markedly enhanced in the presence of 10 μM L-NNa (Fig. 2F).
animals were precontracted with phenylephrine \( (n = 4 \text{ for each}) \). The intactness of functional endothelium in vessels employed was confirmed by the ability of acetylcholine to elicit full relaxation.

**Protein Expression of HDC in Vascular Tissues.** Immunoblot analysis using the mouse monoclonal antibody against HDC indicated the presence of two forms of HDC with different molecular sizes, 53 and 74 kDa, in rabbit vascular tissues. It is assumed that the 74-kDa form of HDC is the cytosolic enzyme, the 53-kDa form is the particulate enzyme, and the two forms are interconverted in the luminal area of the endoplasmic reticulum (Tanaka et al., 1998). As presented in Fig. 3A, only the 53-kDa band was faintly detected in pulmonary arteries, whereas both the two bands with 53 and 74 kDa were strongly found in mesenteric arteries. In pulmonary arteries, however, the 74-kDa band was evidently induced at 6 h after LPS administration. Furthermore, we found a strikingly higher level of the two forms of HDC in endotoxemic mesenteric arteries. However, because the 53-kDa form of HDC is recognized as an active form (Tanaka et al., 1998), the relative levels of the 53-kDa band were quantitatively analyzed and the result showed an about 3-fold increase in the active form of HDC in the two types of vessels from 6-h endotoxemic animals compared with control vessels (Fig. 3B).

**Gene and Protein Expressions of Vascular Histamine H1-Receptors.** Histamine H1-receptor immunological detection was performed using anti-human H1-receptor polyclonal antibody, which recognized a 57-kDa band in rabbit vascular tissues (Fig. 4A). The band was evidently lighter in mesenteric than in pulmonary arteries from control animals. Induction of sepsis by LPS injection resulted in a marked increase in H1-receptor expression, and no difference was found in its expression level between the two vessels. Densitometric quantification of the signal showed that the expression level was 1.7- and 3.5-fold higher in 6-h endotoxemic than in control pulmonary and mesenteric arteries, respectively (Fig. 4B).

Northern blot analysis of rabbit vascular tissues indicated that the increase in H1-receptor protein expression in endotoxemia occurred at the level of gene expression. As reported in the cerebrum and heart of this species (Matsuda et al., 2002), vascular tissues expressed 3.3- and 3.9-kb transcripts corresponding to H1-receptor mRNA (Fig. 5A). The transcript levels of H1-receptors were increased 1.6- to 2.1-fold and 1.7- to 2.5-fold in pulmonary and mesenteric arteries, respectively.
controls. The locations of ribosomal RNAs (28 S and 18 S) are indicated. Ethidium bromide staining was used as a control for gel loading (lower panel). B, summary of quantification of densitometric measurement of H1-receptor mRNA normalized as the ratio of its mRNA level over 18 S level. Means ± S.E. (n = 5) are shown. *** P < 0.001 compared with the respective controls. ### P < 0.001 compared with the corresponding values obtained with control pulmonary artery.

Fig. 5. Northern blot analysis of histamine H1-receptor mRNA in pulmonary and mesenteric arteries from control (C) and 6-h endotoxemic (LPS) rabbits. A, representative autograph of Northern blot analysis of H1-receptor mRNA expression. H1-receptor mRNA was detected as major two bands of 3.3 kb and 3.9 kb by using a 32P-labeled cDNA fragment (0.50-kb) of guinea pig H1-receptor as a probe. The locations of ribosomal RNAs (28 S and 18 S) are indicated. Ethidium bromide staining was used as a control for gel loading (lower panel). B, summary of quantification of densitometric measurement of H1-receptor mRNA normalized as the ratio of its mRNA level over 18 S level. Means ± S.E. (n = 5) are shown. *** P < 0.001 compared with the respective controls. ### P < 0.001 compared with the corresponding values obtained with control pulmonary artery.

Fig. 5. Northern blot analysis of histamine H1-receptor mRNA in pulmonary and mesenteric arteries from control (C) and 6-h endotoxemic (LPS) rabbits. A, representative autograph of Northern blot analysis of H1-receptor mRNA expression. H1-receptor mRNA was detected as major two bands of 3.3 kb and 3.9 kb by using a 32P-labeled cDNA fragment (0.50-kb) of guinea pig H1-receptor as a probe. The locations of ribosomal RNAs (28 S and 18 S) are indicated. Ethidium bromide staining was used as a control for gel loading (lower panel). B, summary of quantification of densitometric measurement of H1-receptor mRNA normalized as the ratio of its mRNA level over 18 S level. Means ± S.E. (n = 5) are shown. *** P < 0.001 compared with the respective controls. ### P < 0.001 compared with the corresponding values obtained with control pulmonary artery.

Discussion

Sepsis is characterized by hypotension, peripheral vasodilatation, and hyporesponsiveness of vascular smooth muscle to pressor agents such as catecholamine. It is suggested that iNOS, which generates large amounts of NO, is a critical mediator of such diminished vascular contractility in sepsis (Fleming et al., 1991; Griffiths et al., 1995; Hom et al., 1995). The present study demonstrates that histamine-evoked contraction in rabbit mesenteric arteries is subject to sepsis-induced hyporesponsiveness. Thus, the contractile response to histamine of mesenteric arteries taken from endotoxemic rabbits was profoundly attenuated. However, LPS treatment caused no impairment of the contractile response to histamine in rabbit pulmonary arteries. This difference seems to be related to different expression levels of iNOS between the two types of vessels. The endotoxin-induced increase in relative protein contents for iNOS was more marked in mesenteric (9.4-fold) than pulmonary (2.1-fold) arteries. Our results suggest that in vivo treatment with LPS leads to iNOS up-regulation and vascular hypocontractility in mesenteric but less in pulmonary vessels. This finding may provide a basis for the results of past investigations that iNOS induction-associated vascular hypocontractility was not observed in the pulmonary circulation (Nelson et al., 1991; Suba et al., 1992; Spath et al., 1994; Fullerton et al., 1995) despite the presence of iNOS mRNA in pulmonary vessels after in vivo LPS (Griffiths et al., 1995).

Although the gene that encodes iNOS is not thought to be expressed in normal vessels (Kibbe et al., 1999), iNOS was found to be constitutively expressed in rabbit mesenteric and pulmonary arteries under baseline conditions in the present study. However, this is no surprise. In recent reports, iNOS gene expression has been noted in human umbilical vein endothelial cells obtained from normal pregnancies (Wang et al., 2003) and in normal human saphenous vein (Dattilo et al., 1997). Furthermore, immunohistochemical iNOS staining has shown that its expression is localized to vascular smooth muscle cells as well as bronchial epithelial and smooth muscle cells in normal rat lungs (Steudel et al., 1999). Such evident iNOS expression in normal vascular tissues would reflect that vessels may be naturally exposed to proinflammatory cytokines or microbial products even in the absence of bacterial infection.

It is well documented that pharmacological inhibitors of NOS can improve the hyporesponsiveness to the contractile agents in vessels taken from LPS-treated animals (Fleming et al., 1991). In the presence of l-NNA, histamine-evoked contraction was modified in both pulmonary and mesenteric
arteries from endotoxemic rabbits such that there was greater contraction in septic vessels at higher histamine concentrations. The same results were also obtained in the presence of the selective iNOS inhibitor (S)-ethyl-ITU. Thus, vessels from endotoxemic animals had a significantly greater maximal contraction to histamine compared with controls in the experiments using (S)-ethyl-ITU. This substantial enhancement of contractions in septic vessels after incubation with L-NNA or (S)-ethyl-ITU seemed to be specific to histamine, because we were unable to demonstrate any significant difference in phenylephrine-induced maximal contraction in the presence of L-NNA between control and septic vessels.

The results of our experiments using histamine H1- and H2-receptor antagonists, chlorpheniramine and cimetidine, imply that activation of H2-receptors results in histamine-evoked contractions in both pulmonary and mesenteric arteries. As recently demonstrated in myocardial tissues (Matsuda et al., 2002), our Northern blotting showed that gene expression of H1-receptors was markedly increased in both pulmonary and mesenteric arteries after induction of endotoxemia by LPS. We also found that H2-receptors were more abundantly expressed in those vessels from endotoxemic rabbits compared with controls, as determined by immunoblot analysis. The parallel behavior of protein and mRNA expressions indicates that endotoxin causes an increase in vascular H2-receptor synthesis at the level of transcriptional regulation. On the basis of the observation that the contractile responses of pulmonary and mesenteric arteries to histamine were mediated entirely through H2-receptors, we suggest that up-regulation of vascular H2-receptor expression after endotoxin administration may be centrally involved in the increase in histamine-induced contractions in endotoxemic vessels in the presence of L-NNA. When the NOS inhibitor was absent, however, the generation of NO by iNOS induction after endotoxemia could apparently override the H2-receptor up-regulation, resulting in the lack of potentiation of the histamine vascular response.

No relaxation was seen when histamine was added to pulmonary and mesenteric vessels either from control or endotoxemic rabbits precontracted with phenylephrine. Thus, histamine does not seem to be capable of producing eNOS-dependent relaxation in the rabbit vessels employed herein. Our result with mesenteric arteries is in contrast to the in vivo study where histamine causes systemic vasodilation (Ahmed et al., 1982). The effects of histamine on vascular tone may vary depending on the type of vessel (conductance or resistance) being studied. Alternatively, histamine may exert eNOS-dependent vasodilatation in mesenteric vascular bed.

Interestingly, we found that eNOS expression was prominently down-regulated in endotoxemic vessels. In previous studies using cultured cells, down-regulation of eNOS expression, triggered by increased NO formation via LPS-induced iNOS expression, has been reported (Buga et al., 1993; MacNaul and Hutchinson, 1993). This study represents the first report that eNOS expression is markedly down-regulated in blood vessels taken from LPS-treated animals. In agreement with this eNOS down-regulation, impaired endothelium-dependent relaxations have been shown in blood vessels from endotoxemic animals (Myers et al., 1995; Wiel et al., 2000). However, definitive evidence for a negative feedback loop between iNOS and eNOS obviously awaits further elucidation.

Circulating levels of histamine were markedly elevated during endotoxin shock, as fully demonstrated in our recent work (Matsuda et al., 2002). We found that expression of the active form of HDC, by which histamine is synthesized from L-histidine in mammalian tissues, were drastically increased in pulmonary and mesenteric arteries after endotoxemia. This could result in locally elevated levels of histamine concentrations in the blood vessel wall. Furthermore, we provided evidence of up-regulation of H1-receptors in endotoxemic vessels. The influence of these changes on vascular tone could be more marked in the pulmonary circulation, because the regional difference in induction of iNOS by endotoxin was achieved between pulmonary and systemic vessels. Thus, histamine may play a role in the development of pulmonary hypertension during sepsis. The present finding that histamine-evoked contractions of endotoxemic pulmonary and mesenteric vessels were significantly enhanced in the presence of L-NNA or (S)-ethyl-ITU suggests that the harmful effect of histamine on not only the pulmonary circulation but also the intestinal circulation may be exaggerated when NOS inhibitors and even selective iNOS inhibitors are given after endotoxemia induction. This may partly explain why the use of NOS inhibitors was detrimental during endotoxemia (Wright et al., 1992; Parker and Adams, 1993; Pastor et al., 1994).

In summary, we examined the effects of LPS-induced sepsis on vascular reactivity to histamine, mRNA and protein expressions of H1-receptors, protein expression of HDC, the only enzyme that forms histamine in mammals, and iNOS and eNOS protein expressions in pulmonary and systemic conductance vessels. As expected, LPS treatment markedly increased iNOS expression and impaired histamine-evoked contractions in mesenteric arteries. However, induction of iNOS by LPS was not so pronounced in pulmonary arteries, and inhibition of NOS significantly enhanced the histamine response in both types of vessels from LPS-treated rabbits. The hyper-responsiveness to histamine of endotoxemic vessels in the presence of the NOS and selective iNOS inhibitors is in agreement with this eNOS down-regulation, impaired endothelium-dependent relaxations have been shown in blood vessels from endotoxemic animals. Thus, histamine may exert eNOS-dependent vasodilatation in mesenteric vascular bed.
seemed to be associated with a dramatic increase in vascular gene and protein levels of H2-receptors. Moreover, there was sustained overproduction of histamine after LPS treatment caused by increased HDC expression in tissues, including blood vessels. From these data, we suggest that histamine may play a contributory role in the genesis of pulmonary hypertension in vivo during endotoxemia. However, additional work will be required to document the presence of pulmonary hypertension in our endotoxemic model and the therapeutic effect of H2-receptor antagonists on the abnormal pulmonary circulation of this model.

Acknowledgments

We thank Yoshika Takahashi, Mika Takenaka, and Kazuaki Yagasaki for excellent technical assistance.

References


Address correspondence to: Dr. Yuichi Hattori, Department of Pharmacology, Hokkaido University School of Medicine, Sapporo 060-8638, Japan. E-mail: yhattori@med.hokudai.ac.jp

Vascular Histamine during Endotoxemia 181