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ORIGINAL ARTICLE

Comparison of the effects of vasopressin and norepinephrine on organ perfusion during septic shock in streptozotocin-induced diabetic rats

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Abstract
Purpose The purpose of this study was to compare the effects of norepinephrine (NE) and vasopressin on systemic hemodynamics, renal and mesenteric artery blood flow, inflammatory response and inducible nitric oxide synthase (iNOS) activity during endotoxin shock in streptozotocin-induced diabetic rats.
Methods The study was designed to include three sets of experiments: (1) measurement of changes in systemic hemodynamics and mesenteric and renal artery blood flow; (2) measurement of biochemical variables; and (3) measurement of iNOS activity in the mesenteric artery. Systemic hemodynamics, regional artery blood flow changes and biochemical variables were assessed before treatment and 1, 2 and 3 h after treatment.
Results Vasopressin, but not NE, prevented the decreases in aortic blood flow, but did not restore mesenteric artery blood flow. In addition, vasopressin partially restored renal artery blood flow in diabetic rats. Plasma nitrite levels and iNOS activity in the mesenteric artery were elevated after intravenous LPS in diabetic rats. Endotoxin-induced decreases in mesenteric arterial blood flow were partially restored by vasopressin with nonselective NOS inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME), in diabetic rats. Moreover, L-NAME prevented increases in plasma nitrite levels and iNOS activity in the mesenteric artery. In contrast, endotoxin-induced decreases in renal arterial blood flow were partially restored by vasopressin with L-NAME, but not by NE in diabetic rats.
Conclusions Nitric oxide may be one possible contributor to reduced sensitivity of the mesenteric and renal arteries to vasopressin during septic shock in streptozotocin-induced diabetic rats.

Keywords Septic shock · Mesenteric artery blood flow · Nitric oxide · Vasopressin · Norepinephrine

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Introduction

Septic shock induced by Gram-negative bacteria represents a major complication in critical care medicine that can lead to multiple organ failure, although the molecular and cellular events resulting in the development and progression of this condition have yet to be clarified [1].

One therapeutic approach to the treatment of hyperdynamic sepsis and hypotension is to administer catecholamines to maintain sufficient mean arterial pressure (MAP) and cardiac output [2–4]. However, no clear consensus has been reached regarding which specific vasopressors should be used [4]. Although dopamine has been recommended by an American consensus conference [4], norepinephrine (NE) was considered equivalent by a European consensus conference [3]. Some researchers, however, have shown that administration of NE leads to strong vasoconstriction in the splanchnic area, which might induce ischemia of the gastrointestinal tract [5]. Other research has demonstrated the efficacy of using vasopressin to control hemodynamics

during septic shock. Tsuneyoshi et al. [6] examined the effects of vasopressin in 16 patients who remained persistently hypotensive despite infusions of pharmacological doses of catecholamines, and found that MAP was increased after the administration of vasopressin. Another report by Guzman et al. [7] showed the efficacy of using vasopressin instead of catecholamines for the stabilization of hemodynamics during septic shock. In contrast, Klinzing et al. [8] questioned the superiority of vasopressin over NE during septic shock.

Some reports have shown that nitric oxide (NO) is a key mediator in the effects of catecholamines or vasopressin. Tsuneyoshi et al. [9] examined NE-evoked isometric tension in the mesenteric arterial ring isolated from omentum in patients with septic shock, and found that N^G-nitro-L-arginine methyl ester (L-NAME) restored the reduced sensitivity to vasopressor agents. Patel et al. [10] suggested the role of inducible NO synthase (iNOS) regarding the attenuation of vasopressin-dependent signal transduction. These reports strongly indicate that NO induced by iNOS during sepsis might be one of the determining factors associated with the sensitivity of hemodynamics or organ blood flow to vasoactive agents.

Many reports have described functional abnormalities in the cardiovascular systems of patients with diabetes mellitus [11, 12]. Although the primary cause of altered cardiovascular function in the acute phase of diabetes remains unclear, some reports suggest that iNOS is activated in rats with streptozotocin-induced diabetes [11, 12]. Cheng et al. [12] showed that depressed cardiac function in rats with streptozotocin-induced diabetes is associated with the activation of iNOS, and that administration of selective iNOS inhibitors could restore cardiovascular responses to NE.

Therefore, the present study compared the effects of NE and vasopressin on systemic hemodynamic variables, renal and mesenteric artery blood flow, inflammatory response and iNOS activity during endotoxin shock in diabetic rats.

Materials and methods

Study protocols were in accordance with the ethical principles provided by the Experimental Animal Laboratory of Gunma University School of Medicine.

Male seven-week-old Wistar rats (body weight 250–350 g) were maintained in wire-mesh cages with ad libitum access to standard laboratory feed and water, under a 12-h light/dark cycle, at 22°C.

Experimental protocol

The study was designed to include three sets of experiments: (1) measurement of changes in systemic hemodynamics and mesenteric and renal artery blood flow (each group $n = 7$, total number of rats = 28); (2) measurement of biochemical variables (each group $n = 7$, total number of rats = 28); and (3) measurement of iNOS activity in the mesenteric artery (each group $n = 10$, total number of rats = 40). A total of 96 animals were used in these experiments.

Diabetic model

The diabetic model was induced in accordance with our previous studies [13, 14], using a bolus injection of streptozotocin (60 mg/kg body weight; Sigma Chemicals, St Louis, MO, USA) into the tail vein of the rats under anesthesia induced using 1–2% sevoflurane. Three days later, hyperglycemia comprising >300 mg/dl in tail vein blood was confirmed in all rats in the fasting state, using a glucose analyzer (Antesense II; Daikin, Osaka, Japan). Rats were considered to be diabetic and were used for this study if hyperglycemia was identified (>300 mg/dl).

Group assignments

Rats in the first set of experiments were randomly divided into four groups: Group 1 ($n = 7$), control; Group 2 ($n = 7$), receiving lipopolysaccharide (LPS) (*Escherichia coli* endotoxin, 10.0 mg/kg intravenous bolus); Group 3 ($n = 7$), receiving intravenous LPS and NE (continuous infusion at 0.2 μg/kg/min); and Group 4 ($n = 7$), receiving LPS and vasopressin (0.04 IU/min). A 10.0-mg/kg dose of endotoxin is capable of causing 50% lethality within 6 h [15–17]. Infusion of NE or vasopressin was started 30 min after LPS administration.

The most common septic shock model involves single bolus injection of endotoxin [18, 19], as this model is reproducible and simple to prepare. In addition, the model offers a useful tool for examining the effects of therapeutic drugs on hemodynamic changes induced by sepsis [19].

To identify the appropriate dosage of continuous infusion of vasopressin on this endotoxin shock model, we tried to examine the effects of three dosages of vasopressin (0.01, 0.04 and 0.08 IU/min) on systemic hemodynamics, and found that 0.04 IU/min was the most effective dosage to improve systemic hemodynamics induced by sepsis in nondiabetic rats (data not shown).

In a previous study, we found that the NE dosage used in this study (0.2 μg/kg/min) was the most effective dosage for improving systemic hemodynamics induced by sepsis in nondiabetic rats [20].

Rats were anesthetized using intraperitoneal injection of pentobarbital (50 mg/kg). After tracheotomy, the rats were connected to an SN-480-7 volume-cycled ventilator (Shinano Manufacturing, Tokyo, Japan) with 30% O_2, 70%

N_2 and 1% isoflurane. Rectal temperature was monitored using a temperature controller (CMA/150^R). A 2-ml bolus of saline solution was injected subcutaneously to maintain fluid balance. For simultaneous measurement of MAP and heart rate (HR), the right femoral artery was cannulated using a 2-Fr high-fidelity micromanometer catheter. Drugs were administered intravenously through a polyethylene catheter (PE50) placed in the dorsal vein. The catheter in the right femoral artery was connected to a PowerLab hemodynamic monitoring system (BioRes, Nagoya, Japan). Changes in HR, MAP, and ascending aortic, renal and mesenteric artery blood flows were measured using ultrasonic flow probes (Transonic Systems, NY, USA) before (baseline) and 1, 2 and 3 h after intravenous LPS injection. The ascending aortic, renal and mesenteric arteries were prepared and visualized. At 3 h after LPS administration, rats were killed by injection of an overdose of pentobarbital via the dorsal vein.

We added experiments on nondiabetic rats ($n = 4$ in each group) for the same four groups to compare the effects of NE and vasopressin on hemodynamic variables between nondiabetic and diabetic rats, and assessed changes in plasma nitrite levels over time for both groups ($n = 4$ in each group).

Biochemical measurements

To exclude the influence of blood sampling on hemodynamic variables and flow, hemodynamic and biochemical parameters were also measured in another 28 animals divided into the same four groups. To extrapolate data from these to the experimental set of animals in which hemodynamic variables were evaluated, rats were exposed to identical experimental conditions for systemic measurements. Plasma concentrations of lactate, glucose, tumor necrosis factor (TNF)α and interleukin (IL)-1β and partial pressures of arterial blood gases were measured before (baseline) and 1 and 3 h after LPS injection, using 2.0 ml of blood collected from the femoral artery. Partial pressures of arterial blood gases were analyzed using an ABL3 acid–base laboratory machine (Radiometer, Copenhagen, Denmark). Plasma TNFα activity was quantified by measuring cytotoxicity against L929 cells in rabbit serum. IL-1β levels were measured using ELISA kits (IL-1; R&D Systems, Tokyo, Japan). After measuring hemodynamic and biochemical parameters, rats were killed by pentobarbital overdose.

Measurement of nitrate/nitrite

Plasma levels of nitrate and nitrite, indicating the biosynthesis of NO by iNOS activity, were measured at baseline and 1, 2 and 3 h after intravenous injection, as we have previously described [14]. Samples collected in tubes containing ethylene diamine tetraacetic acid (EDTA) were centrifuged at 4,000$\times g$ for 20 min. After reducing plasma nitrate to nitrite using nitrate reductase (670 mU/ml) and NADPH (160 μM) at room temperature for 16–17 h, the supernatant was decanted. Triplicate samples (100 μL/tube) were diluted to 1 mL with double-distilled water, and then 100 μL of fresh Griess reagent (1% sulfanilamide in 5% concentrated H_3PO_4 acid and 0.1% naphthylethylenediamine dihydrochloride in H_2O) was added. The nitrite concentration was measured at 550 nm against a standard curve of sodium nitrite.

Measurement of iNOS activity

The calcium-independent conversion of L-arginine to L-citrulline in endothelium of mesenteric artery homogenates indicates iNOS activity [14]. Endothelium sections ($n = 10$ per group, with $n = 4$ at 1 h, $n = 3$ at 2 h, $n = 3$ at 3 h) at baseline and 1, 2 and 3 h after intravenous injection were scraped into 50 mM Tris–HCl containing 0.1 mM EDTA and 1 mM phenylmethylsulfonyl fluoride (pH 7.4), then homogenized in the same buffer on ice. Homogenates (50 μL) were added to 10 mL tubes (warmed to 37°C) that contained 100 μL of reaction buffer (10 μL of [^{3}H]-L-arginine; 150–200 cpm/pM), NADPH (1 mM), calmodulin (30 μM), tetrahydrobiopterin (5 μM) and 2 μM EGTA). Samples were incubated for 30 min at 37°C. The reaction was terminated by adding cold (4°C) stop buffer (pH 5.5), containing 100 mM HEPES, and 12 mM EDTA. Reaction mixtures were applied to columns containing Dowex 50 W (8% crosslinked, Na^+ form) and eluted. A liquid scintillation counter (Aloka 650; Aloka, Tokyo, Japan) was used to measure [^{3}H]-L-citrulline activity. Enzyme activity is expressed as femtomoles of L-citrulline produced per milligram of total protein per minute. Protein was measured by the Bradford method using bovine serum albumin as the standard (BioRad, Richmond, CA, USA).

To study the possibility that reduced iNOS activity in the endothelium of diabetic rats may facilitate the beneficial effects of NE or vasopressin on mesenteric or renal artery blood flow, we performed an additional experiment in which rats were co-administered nonselective NOS inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME), at 5 mg/kg intraperitoneally in 0.5 mL of saline immediately after intravenous LPS injection for possible prevention of increased iNOS activity in endothelium among diabetic rats treated with NE and vasopressin.

In this additional experiment, we focused on the effects of the time courses of hemodynamic variables, plasma NO levels and iNOS activity in endothelium of the mesenteric artery only at 3 h in the LPS i.v. and L-NAME groups of diabetic rats treated with NE and vasopressin.

Statistical analysis

All data are presented as arithmetic means ± SD. After confirming equal variance among groups using the Bartlett test, multiple comparisons were performed using analysis of variance. Scheffe's method was used to compare means. Values of $P < 0.05$ were considered statistically significant. All statistical analyses were performed using StatView^{R} 5.0 software (Abacus Concepts, Berkeley, CA, USA).

Results

Figures 1, 2, and 3 and Table 1 show the time courses of changes in blood flow of the ascending aorta and mesenteric and renal arteries in the four groups among both nondiabetic and diabetic rats, as measured by ultrasonic flow probes. LPS administration significantly decreased these blood flows in the four groups in both nondiabetic and diabetic rats.

More profound decreases in mesenteric and renal blood flows were seen in diabetic rats as compared with those in nondiabetic rats (maximum decrease in mesenteric artery among diabetic rats: 29% of baseline value; maximum decrease in mesenteric artery among nondiabetic rats: 40% of baseline value). Infusion of NE or vasopressin returned ascending aorta blood flows to baseline values in both nondiabetic and diabetic rats (Fig. 1a, b). However, infusions of NE and vasopressin had no effect on mesenteric artery blood flow in diabetic rats, and only partially restored mesenteric artery blood flow in nondiabetic rats (Fig. 2a, b). Infusion of NE or vasopressin partially restored renal artery blood flow toward baseline in nondiabetic rats, and produced a more effective return of renal artery blood flow in nondiabetic rats (Fig. 3a). Diabetic rats showed no effect of NE on renal artery blood flow, and only a partial effect of vasopressin on renal artery blood flow (Fig. 3b).

Table 2 shows plasma nitrite levels in the four groups for both nondiabetic and diabetic rats. Plasma nitrite levels significantly increased from 1 to 3 h after LPS in Groups 2–4 compared with baseline. In addition, larger increases in plasma nitrite levels were observed in each of the four groups for diabetic rats compared with nondiabetic rats.

To confirm the role of NO in the diabetic condition during sepsis, the next experiments were performed only in diabetic rats. Table 3 shows the time courses of variables in the four groups for diabetic rats. In Group 2, MAP was decreased at 1 and 3 h after LPS administration, while the decrease in MAP induced by LPS was ameliorated in Groups 3 and 4. No significant changes in PaO_2, $PaCO_2$ or hematocrit were noted in Group 1 during the study. PaO_2

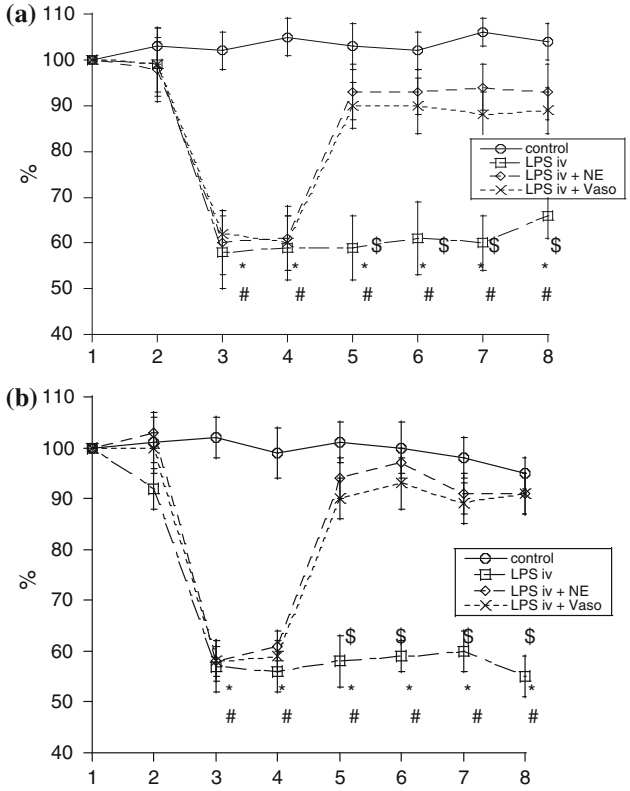

Fig. 1 Time courses of changes in ascending aortic blood flow in the four groups for nondiabetic (**a**) and diabetic (**b**) rats. *1* Before LPS administration. *2* At the time of LPS administration. *3* 15 min after LPS administration. *4* 30 min after LPS administration (start of continuous infusion of NE or vasopressin). *5* 30 min after start of continuous infusion of NE or vasopressin. *6* 1 h after start of continuous infusion of NE or vasopressin. *7* 2 h after start of continuous infusion of NE or vasopressin. *8* 3 h after start of continuous infusion of NE or vasopressin. $^{*}p < 0.05$ compared with before LPS administration within each group. $^{\#}p < 0.05$ compared with control group at each time point. $^{\$}p < 0.05$ compared with the other three groups at each time point. Group 1, control; Group 2, LPS i.v.; Group 3, LPS i.v. + NE; Group 4, LPS i.v. + vasopressin

was significantly decreased at 3 h after LPS in Groups 2, 3 and 4 compared with baseline, and the decrease in PaO_2 was greater in Group 2 than in the other three groups at 3 h. Plasma glucose levels were significantly increased at 3 h after LPS in Groups 2, 3 and 4 compared with baseline, and the increase in plasma glucose level was greater in Group 2 than in the other three groups at 3 h. Plasma lactate levels were significantly increased at 1 and 3 h after LPS. Values of TNFα and IL-1β in Group 1 did not appear to change throughout the study, but were significantly increased in Groups 2–4 at 1 and 3 h after LPS.

Table 4 shows the iNOS activity of endothelium in the mesenteric artery for the four groups in diabetic rats. The iNOS activity of endothelium in the mesenteric artery was significantly increased from 1 to 3 h after LPS in Groups 2, 3 and 4 compared with baseline, and the increase in iNOS

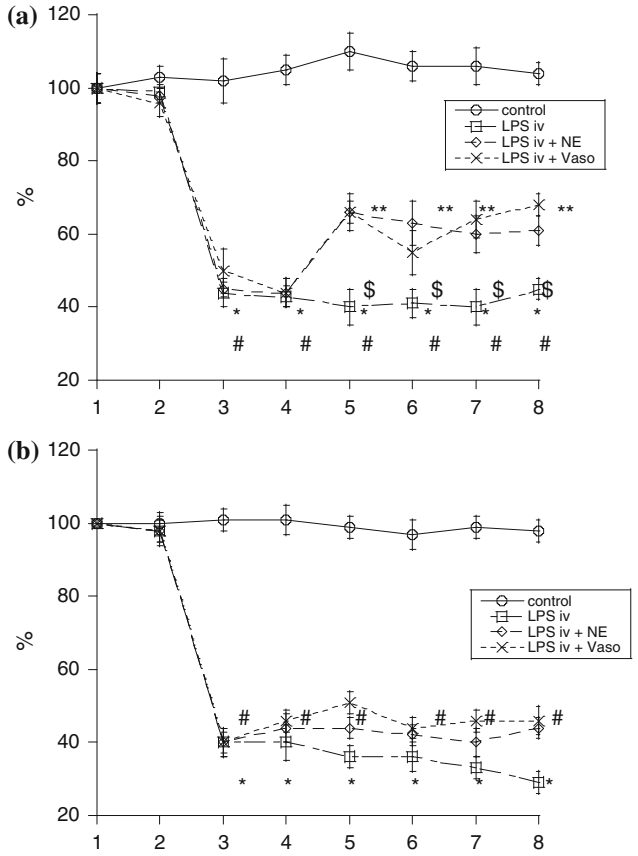

Fig. 2 Time courses of changes in mesenteric artery blood flow in the four groups for nondiabetic (**a**) and diabetic (**b**) rats. *1* Before LPS administration. *2* At the time of LPS administration. *3* 15 min after LPS administration. *4* 30 min after LPS administration (start of continuous infusion of NE or vasopressin). *5* 30 min after start of continuous infusion of NE or vasopressin. *6* 1 h after start of continuous infusion of NE or vasopressin. *7* 2 h after start of continuous infusion of NE or vasopressin. *8* 3 h after start of continuous infusion of NE or vasopressin. $^{*}p < 0.05$ compared with before LPS administration within each group. $^{\#}p < 0.05$ compared with control group at each time point. $^{\$}p < 0.05$ compared with the other three groups at each time point. Group 1, control; Group 2, LPS i.v.; Group 3, LPS i.v. + NE; Group 4, LPS i.v. + vasopressin

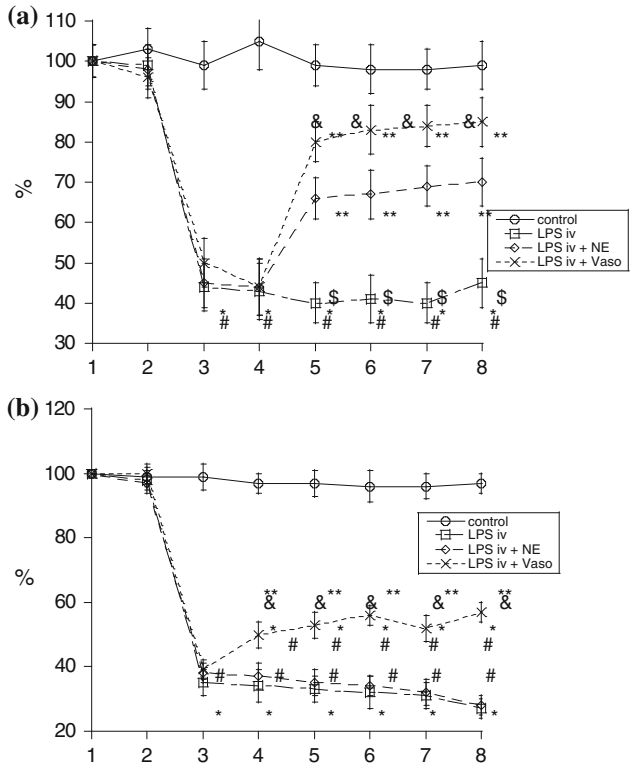

Fig. 3 Time courses of changes in renal artery blood flow in the four groups for both nondiabetic (**a**) and diabetic (**b**) rats. *1* Before LPS administration. *2* At the time of LPS administration. *3* 15 min after LPS administration. *4* 30 min after LPS administration (start of continuous infusion of NE or vasopressin). *5* 30 min after start of continuous infusion of NE or vasopressin. *6* 1 h after start of continuous infusion of NE or vasopressin. *7* 2 h after start of continuous infusion of NE or vasopressin. *8* 3 h after start of continuous infusion of NE or vasopressin. $^{*}p < 0.05$ compared with before LPS administration within each group. $^{**}p < 0.05$ compared with the LPS i.v. group at each time point. $^{\#}p < 0.05$ compared with control group at each time point. $^{\$}p < 0.05$ compared with the other three groups at each time point. $^{\&}p < 0.05$ compared with the LPS i.v. + NE group at each time point. Group 1, control; Group 2, LPS i.v.; Group 3, LPS i.v. + NE; Group 4, LPS i.v. + vasopressin

activity was higher in Group 2 than in the other three groups at 3 h.

Figures 4, 5 and 6 show the time courses of changes in systemic, mesenteric and renal artery blood flows in the four groups with co-administration of L-NAME. Co-administration of L-NAME partially restored ascending aortic blood flows, and completely restored ascending aortic blood flows with vasopressin and NE (Fig. 4). Furthermore, co-administration of L-NAME with vasopressin allowed partial restoration of mesenteric artery blood flows, and completely restored renal blood flow (Figs. 5, 6). In contrast, co-administration of L-NAME with NE achieved partial restoration of mesenteric and renal blood flows (Figs. 5, 6).

Plasma nitrite levels and iNOS activities in the four groups returned to control values with L-NAME treatment (plasma nitrite level at 3 h: control, 0.4 ± 0.2 μM; LPS i.v., 0.7 ± 0.3 μM; LPS i.v. + NE, 0.8 ± 0.3 μM; LPS i.v. + vasopressin, 0.8 ± 0.4 μM; iNOS activity of endothelium in mesenteric artery at 3 h: control, 0.4 ± 0.1 fmol/mg/min; LPS i.v. 0.9 ± 0.4 fmol/mg/min; LPS i.v. + NE, 0.7 ± 0.3 fmol/mg/min; LPS i.v. + vasopressin, 0.8 ± 0.4 fmol/mg/min).

Discussion

Continuous infusion of NE or vasopressin could prevent endotoxin-induced deleterious changes in systemic hemodynamics, but differential effects on mesenteric and renal

Table 1 Changes in ascending aortic, mesenteric and renal blood flows over time for the four groups

	Baseline				1 h				3 h			
	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
(a) Nondiabetic rats												
Ascending aortic (ml/min)	11.8 ± 1.2	11.0 ± 1.3	11.5 ± 1.3	11.6 ± 1.4	11.5 ± 1.1	6.4 ± 1.2*,#	10.3 ± 1.3	10.2 ± 1.2	11.4 ± 1.3	6.7 ± 1.0*,#	10.9 ± 1.1	10.6 ± 0.08
Mesenteric (ml/min)	1.50 ± 0.08	1.52 ± 0.07	1.54 ± 0.06	1.50 ± 0.07	1.46 ± 0.06	0.60 ± 0.06*,#	0.99 ± 0.07*	1.01 ± 0.05*	1.48 ± 0.05	0.66 ± 0.07*,#	1.0 ± 0.07*	1.02 ± 0.08*
Renal (ml/min)	1.42 ± 0.05	1.44 ± 0.04	1.40 ± 0.04	1.45 ± 0.04	1.45 ± 0.03	0.54 ± 0.03*,#	0.90 ± 0.05*,$	1.10 ± 0.05*	1.43 ± 0.04	0.57 ± 0.04*,#	0.93 ± 0.05*,$	1.17 ± 0.07*
(b) Diabetic rats												
Ascending aortic (ml/min)	11.8 ± 1.2	11.8 ± 1.2	11.2 ± 1.0	11.0 ± 1.1	11.6 ± 0.8	7.0 ± 0.8*,#	10.9 ± 0.8	10.3 ± 0.8	11.2 ± 1.0	6.6 ± 1.0*,#	10.4 ± 1.1	10.1 ± 1.0
Mesenteric (ml/min)	1.50 ± 0.08	1.47 ± 0.06	1.51 ± 0.08	1.47 ± 0.06	1.47 ± 0.06	0.54 ± 0.06*,#	0.64 ± 0.06*	0.66 ± 0.05*	1.48 ± 0.06	0.44 ± 0.06#	0.67 ± 0.05*	0.69 ± 0.07*
Renal (ml/min)	1.44 ± 0.08	1.40 ± 0.07	1.42 ± 0.08	1.39 ± 0.07	1.39 ± 0.05	0.45 ± 0.05*,$	0.49 ± 0.07*,$	0.79 ± 0.05*	1.40 ± 0.05	0.39 ± 0.07*,$	0.40 ± 0.05*,$	0.80 ± 0.06*

All data are expressed as mean ± SD

Group 1: control, Group 2: receiving lipopolysaccharide (LPS: *Escherichia coli* endotoxin, 10.0 mg/kg intravenous bolus), Group 3: receiving intravenous LPS and NE (continuous infusion at 0.2 μg/kg/min), Group 4: receiving LPS and vasopressin (0.04 IU/min)

* $p < 0.05$ compared with baseline

\# $p < 0.05$ compared with the other three groups

$ $p < 0.05$ compared with Group 4

blood flows were seen during endotoxin shock in diabetic rats. Moreover, co-administration of NOS inhibitor could partially restore renal blood flow with vasopressin, but not with NE in diabetic rats.

Hyperglycemia or the diabetic condition have been reported to influence the production of proinflammatory cytokines. Esposito et al. [21] showed that patients with impaired glucose tolerance (IGT) display higher baseline levels of TNFα and IL-6 compared with normal controls. Moreover, people with IGT show a higher and more sustained cytokine release in response to acute pulses of hyperglycemia. Endotoxins are known to induce proinflammatory cytokines [1–3], so endotoxic effects may be aggravated under diabetic conditions. Furthermore, abnormal NOS and NO production in cardiac and vascular smooth muscle are reportedly found in the diabetic condition [22]. An increase in the production of NO induced by iNOS plays a pivotal role in the pathogenesis of diabetic adverse effects [23].

Numerous reports have noted that administration of NE could improve systemic hemodynamics, but whether administration of NE can improve intestinal perfusion during septic shock remains controversial [24–27]. Agents such as phenylephrine and NE that display alpha-adrenergic effects have been considered to cause intestinal ischemia by decreasing intestinal perfusion during septic shock [24]. Our findings are consistent with previous reports that NE induces global mesenteric vasoconstriction [5]. Guzman et al. [7] also reported that continuous infusion of NE at 0.2 μg/kg/min was unable to improve portal or mucosal blood flows as measured by ultrasonographic probes during septic shock. In contrast, Di Giantomasso et al. [28] found no effects on mesenteric blood flow after NE infusion at 0.4 μg/kg/min. These effects were also observed by Revelly et al. [29] and De Backer et al. [25]. Such discrepancies might be attributable to differences in species, fluid resuscitation and anesthesia.

In contrast to NE, the results of using vasopressin for septic shock have been controversial. Tsuneyoshi et al. [9] demonstrated that low-dose vasopressin infusion (0.04 units/min) increased MAP, systemic vascular resistance, and urine output in patients with vasodilatory septic shock and hyporesponsiveness to catecholamines. With regard to the effects of vasopressin on the splanchnic region, other controversial studies have examined the efficacy of using vasopressin for septic shock. Klinzing et al. [8] demonstrated that vasopressin infusion in doses sufficient to replace the vasopressor NE tended to increase splanchnic and blood flow despite substantial reductions in cardiac output for patients with septic shock. Martikainen et al. [30] examined the effects of vasopressin on systemic and splanchnic hemodynamics and metabolism during endotoxin shock in pigs, and found that vasopressin

Table 2 Time courses of changes in plasma nitrite levels (μM) in the four groups

Group	Pretreatment	After treatment		
		1 h	2 h	3 h
(a) Nondiabetic rats				
1. Control	1.1 ± 0.2	1.2 ± 0.2	1.2 ± 0.2	1.2 ± 0.2
2. LPS iv	1.2 ± 0.2	3.3 ± 1.1*,#	5.5 ± 1.9*,#,$	9.5 ± 2.0*,#,$
3. LPS iv + NE	1.4 ± 0.2	1.9 ± 1.0$	3.4 ± 1.4*,$	4.4 ± 1.9*,$
4. LPS iv + Vaso	1.3 ± 0.2	1.7 ± 0.6	2.9 ± 1.1*4	4.1 ± 1.3*,$
(b) Diabetic rats				
1. Control	2.2 ± 0.2	2.3 ± 0.2	2.3 ± 0.3	2.1 ± 0.2
2. LPS iv	2.3 ± 0.2	5.3 ± 1.3*,$,#	9.9 ± 3.5*,$,#	15.2 ± 3.9*,$,#
3. LPS iv + NE	2.0 ± 0.2	3.3 ± 1.1*,$	5.4 ± 2.3*,$	7.4 ± 2.5*,$
4. LPS iv + Vaso	2.0 ± 0.2	3.0 ± 0.7*,$	4.9 ± 2.1*,$	7.1 ± 1.0*,$

All data are expressed as means ± SD

* $p < 0.05$ compared with pretreatment values

# $p < 0.05$ compared with other groups at each time point

$ $p < 0.05$ compared with Group 1 at each time point

decreased cardiac output and blood flow in the superior mesenteric artery and portal vein, whereas hepatic arterial blood increased. Malay et al. [31] found dose-related changes in the vasoconstrictive effect of vasopressin in septic shock. These results showed that differential infusion doses of vasopressin may result in dose-dependent and organ-specific vasoconstriction or relaxation. Until now, no reports have examined the comparative effects of vasopressin or NE on organ blood flow during septic shock in diabetic rats. Increased plasma NO levels were observed in diabetic rats at the pretreatment point compared with those in nondiabetic rats, possibly due to iNOS activation. Moreover, some differences were observed in the effects of NA and vasopressin on the mesenteric and renal artery blood flow between nondiabetic rats and diabetic rats. This finding strongly suggests that inhibition of LPS-induced NO production may help to ameliorate the hemodynamic instability and cytokine overproduction associated with endotoxic shock in diabetics. To clarify the role of NO in the effects of vasopressin or NE on hemodynamics during septic shock in diabetic rats, we examined the effects of co-administration of L-NAME with vasopressin or NE on hemodynamics, and found that co-administration of L-NAME with vasopressin almost completely restored mesenteric and renal blood flows, whereas NE did not. Our study confirmed that the difference in the effects of vasopressin or NA between nondiabetic rats and diabetic rats may be in part attributable to the overproduction of NO.

Many studies have indicated that there is considerable regional heterogeneity in the reactivity of blood vessels to vasopressin, with vasopressin producing marked constriction in cutaneous, splanchnic and muscle vessels, dilatation or weak constriction in the renal vasculature, no effect or dilatation in the pulmonary vasculature, and dilatation in coronary arteries [31–33]. These differences are thought to be related to the specific animal species and experimental procedures used, and also to the relative distribution and importance of V1 and V2 vasopressin receptors and the role of the endothelium in vascular reactivity to vasopressin [31, 32]. Garcia-Villalon et al. [32] showed that effects of vasopressin on endothelium are mediated by the release of NO, and that these effects are inhibited by the administration of L-NAME. Activity of iNOS was more strongly provoked by the hyperglycemic condition observed in the present study, so prevention of provoked iNOS activity seems likely to improve organ blood flow in diabetic rats. We speculated that the blocking of iNOS, eNOS or nNOS by L-NAME may be attributable to endothelial contraction or relaxation in the mesenteric and renal arteries, and the induction of differential effects of organ perfusion during sepsis.

Various studies have implicated iNOS in the pathogenesis of endotoxin-induced hemodynamic instability and organ dysfunction [18, 34]. However, L-NAME is a non-selective NOS blocker, so another NOS, such as eNOS or nNOS, may also be blocked by L-NAME. Indeed, Ichinose et al. [34] showed that selective iNOS inhibitors prevented systemic, cardiac and pulmonary hemodynamic dysfunction, and showed that although L-NAME prevented systemic hypotension, administration impaired cardiac function. Other studies [35–38] have shown the deleterious effects of L-NAME on mortality rate compared with the use of nonselective iNOS inhibitors. In contrast, another study showed that L-NAME administration improves ventricular performance in streptozotocin-induced diabetic rats [39]. Moreover, Connelly et al. [40] reported that the pathogenesis of sepsis was characterized by an initial activation

Table 3 Changes in variables over time for the four groups (diabetic rats)

	Baseline				1 h				3 h			
	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
Heart rate (beats/min)	288 ± 24	279 ± 22	290 ± 24	289 ± 19	280 ± 23	358 ± 24*	323 ± 29	320 ± 20*	301 ± 28	377 ± 36*	335 ± 30*	345 ± 30*
MAP (mmHg)	88 ± 4	87 ± 4	82 ± 5	83 ± 3	86 ± 5	59 ± 7$^{*,\#}$	88 ± 10	89 ± 11	86 ± 5	49 ± 12$^{\#}$	79 ± 12	80 ± 11
PaO_2 (mmHg)	171 ± 15	168 ± 15	170 ± 11	177 ± 10	170 ± 10	161 ± 17	155 ± 19	167 ± 20	170 ± 13	98 ± 19$^{*,\#}$	120 ± 20*	127 ± 16*
$PaCO_2$ (mmHg	36 ± 4	35 ± 3	35 ± 4	36 ± 5	37 ± 6	38 ± 7	39 ± 6	40 ± 7	39 ± 5	41 ± 8	37 ± 5	38 ± 5
Ht (%)	35 ± 3	35 ± 3	35 ± 5	35 ± 4	33 ± 5	35 ± 4	35 ± 4	36 ± 5	35 ± 5	35 ± 4	34 ± 7	35 ± 5
Glucose (mg/dL)	339 ± 16	345 ± 20	350 ± 26	333 ± 22	345 ± 21	399 ± 35*	387 ± 23*	362 ± 29*	339 ± 18	429 ± 44$^{*,\#}$	390 ± 31*	388 ± 29*
Lactate (mM)	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.4 ± 0.2	3.1 ± 1.2$^{*,\#}$	1.6 ± 1.1*	1.5 ± 1.1*	0.3 ± 0.2	9.9 ± 2.3$^{*,\#}$	3.5 ± 2.2$^{*,\$}$	2.6 ± 0.7*
RT (°C)	37.5 ± 0.2	37.5 ± 0.2	37.4 ± 0.3	37.3 ± 0.4	37.4 ± 0.2	37.9 ± 0.5	37.9 ± 0.4	37.8 ± 0.3	37.1 ± 0.2	38.8 ± 0.6*	38.4 ± 0.6*	38.47 ± 0.6*
TNFα (ng/ml)	n.d.	n.d.	n.d.	n.d.	n.d.	4.9 ± 1.2$^{*,\#}$	3.0 ± 1.3*	2.9 ± 1.0*	n.d.	4.1 ± 2.0*	3.0 ± 1.9*	2.7 ± 1.7*
IL-1β (ng/ml)	n.d.	n.d.	n.d.	n.d.	n.d.	1.0 ± 0.2$^{*,\#}$	0.9 ± 0.4*	0.7 ± 0.3*	n.d.	3.9 ± 1.1$^{*,\#}$	1.7 ± 0.5*	1.8 ± 0.6*

All data are expressed as mean ± SD

Ht, hematocrit; *RT*, rectal temperature; *IL*, interleukin; *TNF*, tumor necrosis factor; *n.d.*, not detectable

Group 1 ($n = 7$), control; Group 2 ($n = 7$), receiving lipopolysaccharide (LPS: *Escherichia coli* endotoxin, 10.0 mg/kg intravenous bolus); Group 3 ($n = 7$), receiving intravenous LPS and NE (continuous infusion at 0.2 μg/kg/min); and Group 4 ($n = 7$), receiving LPS and vasopressin (0.04 IU/min)

* $p < 0.05$ compared with baseline

# $p < 0.05$ compared with the other three groups

$ $p < 0.05$ compared with Group 4

Table 4 Time courses of changes in inducible nitric oxide synthase (iNOS) activity in rat mesenteric artery in the four groups (fmol/mg/min) among diabetic rats

Group	Pretreatment	After treatment		
		1 h	2 h	3 h
1. Control	0.3 ± 0.1	0.3 ± 0.2	0.4 ± 0.1	0.4 ± 0.1
2. LPS iv	0.3 ± 0.1	1.1 ± 0.2*	2.9 ± 0.5*,$,#	3.6 ± 0.4*,$,#
3. LPS iv + NE	0.4 ± 0.1	0.9 ± 0.3*	1.5 ± 0.4*,$	2.1 ± 0.3*,$
4. LPs iv + Vaso	0.3 ± 0.2	1.0 ± 0.2*	1.6 ± 0.5*,$	2.2 ± 0.5*,$

All data are expressed as means ± SD

Group 1 ($n = 7$), control; Group 2 ($n = 7$), receiving lipopolysaccharide (LPS: *Escherichia coli* endotoxin, 10.0 mg/kg intravenous bolus); Group 3 ($n = 7$), receiving intravenous LPS and NE (continuous infusion at 0.2 μg/kg/min); and Group 4 ($n = 7$), receiving LPS and vasopressin (0.04 IU/min)

* $p < 0.05$ compared with pretreatment values

# $p < 0.05$ compared with other groups at each time point

$ $p < 0.05$ compared with Group 1 at each time point

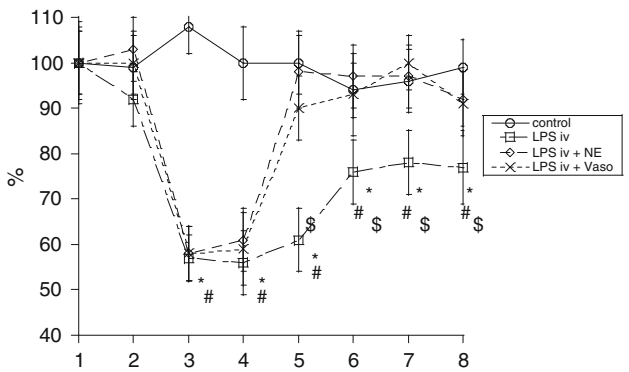

Fig. 4 Time courses of changes in ascending aortic blood flow in the four groups with L-NAME in diabetic rats. *1* Before LPS administration. *2* At the time of LPS administration. *3* 15 min after LPS administration. *4* 30 min after LPS administration (start of continuous infusion of NE or vasopressin). *5* 30 min after start of continuous infusion of NE or vasopressin. *6* 1 h after start of continuous infusion of NE or vasopressin. *7* 2 h after start of continuous infusion of NE or vasopressin. *8* 3 h after start of continuous infusion of NE or vasopressin. *$p < 0.05$ compared with before LPS administration within each group. #$p < 0.05$ compared with the control group at each time point. $$p < 0.05$ compared with the other three groups at each time point. Group 1, control; Group 2, LPS i.v.; Group 3, LPS i.v. + NE; Group 4, LPS i.v. + vasopressin

of eNOS, so that eNOS could be deleterious during endotoxemia. Further extensive studies are thus needed to clarify these findings.

Study limitations

The diabetic condition used in this study is an acute-phase diabetic model, whereas most clinically observed diabetic patients suffer from a long-standing hyperglycemic condition. Therefore, the present findings may not resemble the clinical situation. However, acute hyperglycemia

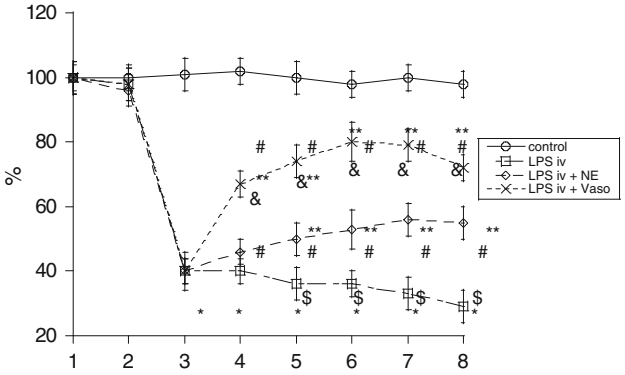

Fig. 5 Time courses of changes in mesenteric artery blood flow in the four groups with L-NAME in diabetic rats. *1* Before LPS administration. *2* At the time of LPS administration. *3* 15 min after LPS administration. *4* 30 min after LPS administration (start of continuous infusion of NE or vasopressin). *5* 30 min after start of continuous infusion of NE or vasopressin. *6* 1 h after start of continuous infusion of NE or vasopressin. *7* 2 h after start of continuous infusion of NE or vasopressin. *8* 3 h after start of continuous infusion of NE or vasopressin. *$p < 0.05$ compared with before LPS administration within each group. **$p < 0.05$ compared with the LPS i.v. group at each time point. #$p < 0.05$ compared with the control group at each time point. $$p < 0.05$ compared with the other three groups at each time point. &$p < 0.05$ compared with the LPS i.v. + NE group at each time point. Group 1, control; Group 2, LPS i.v.; Group 3, LPS i.v. + NE; Group 4, LPS i.v. + vasopressin

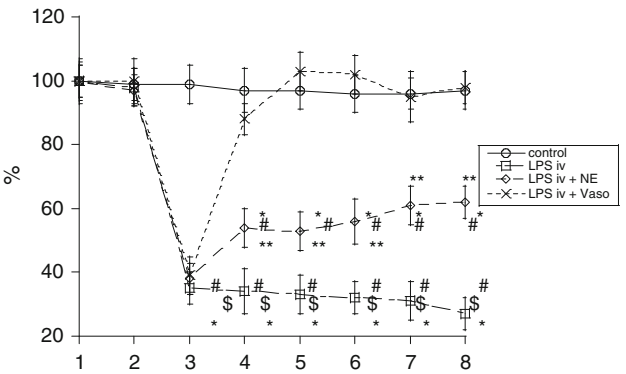

Fig. 6 Time courses of changes in renal artery blood flow in the four groups with L-NAME in diabetic rats. *1* Before LPS administration. *2* At the time of LPS administration. *3* 15 min after LPS administration. *4* 30 min after LPS administration (start of continuous infusion of NE or vasopressin). *5* 30 min after start of continuous infusion of NE or vasopressin. *6* 1 h after start of continuous infusion of NE or vasopressin. *7* 2 h after start of continuous infusion of NE or vasopressin. *8* 3 h after start of continuous infusion of NE or vasopressin. *$p < 0.05$ compared with before LPS administration within each group. **$p < 0.05$ compared with the LPS i.v. group at each time point. #$p < 0.05$ compared with control group at each time point. $$p < 0.05$ compared with the other three groups at each time point. Group 1, control; Group 2, LPS i.v.; Group 3, LPS i.v. + NE; Group 4, LPS i.v. + vasopressin

sometimes occurs during sepsis, so the present results may at least be useful for clarifying hemodynamic instability under hyperglycemic conditions.

The present study focused on hemodynamic variables, and did not examine the effects of vasopressin and NE on microcirculation, so we cannot rule out the possibility that ischemic changes associated with impaired microcirculation may occur during septic shock. In addition, the absolute blood flow measured by ultrasonic flow probes was low compared to physiological values in this study. Many factors such as anesthetic agents and dosage or surgical intervention may modulate hemodynamic variables and cardiac output. Thus, it is possible that this low blood flow may impact on our results.

In this study, a single dose of L-NAME was infused during septic shock in rats, as our previous study showed that this dose was sufficient to completely prevent iNOS activity during septic shock [18]. Other doses of L-NAME may potentially result in different hemodynamic changes during sepsis in diabetic rats. In addition, although we did not measure left ventricular (LV) function, other studies have shown that LV function is impaired while systemic hemodynamics are improved by L-NAME [37, 38].

We have shown that administration of vasopressin or NE attenuates the endotoxin-induced production of proinflammatory cytokines in diabetic rats. Although our study did not show the underlying mechanisms, one possibility is that improved organ perfusion, particularly mesenteric perfusion, may decrease the production of proinflammatory cytokines in peripheral organs.

In conclusion, NO is one contributor to the reduced sensitivity of the mesenteric and renal arteries to vasopressin during septic shock in streptozotocin-induced diabetic rats.

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