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Upregulation of Rho-kinase (ROCK-2) expression and enhanced contraction to endothelin-1 in the mesenteric artery from lipopolysaccharide-treated rats

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Abstract

Effects of bacterial lipopolysaccharide (*Escherichia coli* serotype, 055:B5, 20 mg kg^{-1}, i.p., for 6 h) and a Rho-kinase inhibitor, (+)-(*R*)-*trans*-4-(1-aminoethyl)-*N*-(4-pyridyl) cyclohexanecarboxamide dihydrochloride monohydrate, Y-27632 (10^{-9}–10^{-5} M) were investigated on the contractile responses of the rat mesenteric artery to phenylephrine (10^{-9}–$3x10^{-5}$ M), angiotensin-2 (10^{-10}–10^{-6} M) and endothelin-1 (10^{-10}–10^{-7} M). Moreover, alteration in the level of Rho-kinase (ROCK-2) expression was examined in the superior mesenteric artery obtained from saline- and lipopolysaccharide-treated rats by Western blotting. Endotoxemic rat mesenteric rings exhibited no different contractions to phenylephrine and angiotensin-2 but augmented contractile activity to endothelin-1. In the mesenteric artery obtained from the endotoxemic rats, acetylcholine-induced vasorelaxation did not differ; pD_2 value for acetylcholine was 7.85±0.12 in the endotoxemic rings; however, it was 7.81±0.15 in the control rings ($P>0.05$). Y-27632 induced relaxation, which was the same in the control arteries as in endotoxemic ones when contracting agent was phenylephrine. However, when endothelin-1 was used to precontract the rings, Y-27632 produced enhanced relaxation in endotoxemic vessels. pD_2 values for Y-27632 were, respectively, 7.69±0.12 and 8.20±0.10 in control and endotoxemic rings precontracted by endothelin-1 (10^{-8} M) ($P<0.01$). Moreover, Y-27632 (10^{-5} M) suppressed the contraction induced by angiotensin-2 (10^{-10}–10^{-6} M). Western blot analysis revealed that Rho-kinase was upregulated significantly in the mesenteric artery obtained from the rats treated with LPS for 6 h. In addition, serum NO_2^-/NO_3^- level, which was detected by Griess method, was 10.0±1.4 μM in endotoxemic rats; however, it was 6.6±0.5 μM in control ($P<0.05$). Taken together, these results show that the expression of the contractile protein Rho-kinase could be upregulated in endotoxemic mesenteric artery and this upregulation may be coincided with an enhanced contraction to endothelin-1 but not phenylephrine and angiotensin-2.

Keywords: Endothelin-1; Lipopolysaccharide; Mesenteric artery; Nitric oxide; Rho-kinase; Y-27632

1. Introduction

Bacterial toxins, particularly endotoxins such as lipopolysaccharide, which constitutes the outer membrane of gram-negative bacteria, are known to be the main mediators of the high morbidity and mortality rates in gram-negative septic shock (Titheradge, 1999). A reduction in peripheral vascular resistance is suggested to be one of the major determinant factors leading to death (Parratt, 1989). Inducible nitric oxide synthase (iNOS)-derived NO is generally held responsible for the attenuation of vascular responses to vasoconstrictors (Wakabayashi et al., 1991; Thiemermann, 1997), despite the possible involvement of some other mediators such as arachidonic acid metabolites and superoxide anions (Briones et al., 2002; Hori et al., 2001; Wakabayashi et al., 1991). In addition, lipopolysaccharide may cause a defect in the signal transduction induced by various vasoconstrictor receptors stimulation. Accordingly, it has been reported that altered or impaired adrenoceptor signal transduction is responsible for systemic

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vasodilatation and hyporesponsiveness in an endotoxemic model (Pleiner et al., 2002). Two main cellular mechanisms are acknowledged for the vascular hyporeactivity: (1) a decrease in intracellular Ca^{2+} concentration and (2) a decrease in Ca^{2+} sensitivity through enhanced myosin phosphatase activity, which causes dephosphorylation of myosin light chain, and thus counteracts smooth muscle contraction (Cohen et al., 1999; Wu et al., 1996). It has been known that NO could sequester intracellular Ca^{2+} through cGMP-dependent pathways (Yao and Huang, 2003; Schlossmann et al., 2003). Interestingly, NO has recently been reported to involve in the Ca^{2+} sensitisation phenomenon (Begum et al., 2000; Sauzeau et al., 2003), which is defined that contractile apparatus can be independent of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) (Somlyo and Somlyo, 1994). Accordingly, it has been reported that NO-induced relaxation is not associated with a decrease in $[Ca^{2+}]_i$ but may inhibit Ca^{2+} sensitisation, which is driven by Rho/Rho-kinase pathway (Bolz et al., 2003). The cross talk between NO and Rho signalling is controversial, in that on one hand, NO induces vasodilatation by inhibiting Rho/Rho-kinase pathway (Chitaley and Webb, 2002), and on the other hand, endothelium-dependent NO can upregulate RhoA expression (Etter et al., 2001).

In vascular smooth muscle, various agonists (i.e., phenylephrine, angiotensin-2, endothelin-1, etc.) stimulate G proteins to transduce the signal into cellular effects. Activation of G_q or G_{11} proteins generally results in phosphoinositidase activation followed by hydrolysis of phosphatidylinositol 4,5-bis-phosphate to diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP_3). However, when $G_{12/13}$ protein is activated, the signal is transmitted to the Rho/Rho-kinase pathway (Fukata et al., 2001). This novel pathway provides an alternative vasoconstrictor mechanism, which is independent of intracellular Ca^{2+} elevation, i.e., at a constant $[Ca^{2+}]_i$, the contractile apparatus may work (Somlyo and Somlyo, 1994). It has been reported that Rho/Rho-kinase pathway could be involved in the contractile activity of various tissues such as corpus cavernosum (Büyükafşar and Ün, 2003), gastric fundus (Büyükafşar and Levent, 2003), vas deferens (Büyükafşar et al., 2003), uterus (Tahara et al., 2002), urinary bladder (Wibberley et al., 2003) and rat mesenteric vascular bed (Büyükafşar et al., 2004). Furthermore, this signalling could be involved in some pathological states (see the reviews Wettschureck and Offermanns, 2002; Sandu et al., 2000). However, it has yet to be investigated whether altered Rho/Rho-kinase signalling may be involved in contractile responses of endotoxemic vascular preparations to various agonists.

Therefore, in the present study, first we examined effects of different agonists, namely, endothelin-1, phenylephrine and angiotensin-2, in the mesenteric arteries obtained from lipopolysaccharide-treated rats and compared the contractions induced by these agonists to those obtained from control (saline-treated) arteries. Moreover, relaxant effect of the Rho-kinase inhibitor, Y-27632, was evaluated in endotoxemic mesenteric arteries precontracted by the vasoconstrictors. In addition, possible alteration in the expression level of Rho-kinase protein in endotoxemic rat mesenteric artery was also investigated by Western blotting.

2. Materials and methods

2.1. Animals and tissue preparation

This study was performed in accordance with the Guide for the Care and Use of Laboratory Animals of Mersin University Centre for Experimental Medicine. Female Wistar rats were used in the study. The rats were randomly separated into two groups, one of which was injected 20 mg kg^{-1} *Escherichia coli* lipopolysaccharide (i.p. for 6 h) and the other group received saline (i.p. for 6 h). Six hours later, the rats were killed by a cervical dislocation following stunning by a blow to head. Thereafter, the chest was opened and the blood was immediately collected by cardiac puncture for NO_2^-/NO_3^- analysis. The abdomen was then opened and superior mesenteric artery of 15–20 mm long was isolated, cleared of connective and fat tissues and finally dissected out into a Petri dish containing Krebs solution. Approximately 2.5–3 mm rings in width were prepared and suspended between two hooks in an organ bath filled with Krebs's solution (composition in mM: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$, 25, KH_2PO_4 1.2, glucose 11, Na_2EDTA 0.3) under an initial tension of 0.3 g, which was chosen as optimum resting tension after preliminary experiments. The bath temperature was maintained at 37 °C.

2.2. Organ bath experiments

Following the equilibration of 1 h during which bath medium was replaced with fresh Krebs for every 15 min, all rings obtained from saline- (control) and lipopolysaccharide-treated rats were contracted by 80 mM KCl for 5 min. Thereafter, they were rinsed with Krebs solution and incubated for further 45 min. After this period, the rings were cumulatively contracted by endothelin-1 (10^{-10}–10^{-7} M), phenylephrine (10^{-9}–3×10^{-5} M) or angiotensin-2 (10^{-10}–10^{-6} M). Some rings were precontracted with either endothelin-1 (10^{-8} M) or phenylephrine (10^{-6} M) to induce a steady-state submaximal contraction and then a Rho-kinase inhibitor, Y-27632 (10^{-9}–10^{-5} M), was added increasingly. As angiotensin-2 did not induce a steady-state contraction but the active tone was gradually decreasing, Y-27632 (10^{-5} M) was incubated for 30 min to test its effect on this agonist-elicited contractile activity. In another series of experiments, acetylcholine was tested cumulatively (10^{-9}–10^{-5} M) in the rings obtained from saline- and LPS-treated rats to test endothelial integrity after LPS challenge.

2.3. Western blot analysis for Rho-kinase (ROCK-2)

Mesenteric arteries from saline- and lipopolysaccharide (20 mg kg^{-1}, i.p., for 6 h)-treated rats (n=4–5) were immediately isolated after sacrification, and connective tissue was dissected out. The artery segment of about 3–4 cm was then transferred to a Petri dish containing ice-cold Krebs solution. After removing the blood within the lumen by gently squeezing, it was homogenized with the lyses buffer solution [compositions: Tris–HCl (pH=7.4) 50 mM, NaCl 400 mM, EGTA 2 mM, EDTA 1 mM, dithiothreitol (DTT) 1 mM, phenylmethylsulphonyl fluoride (PMSF) 10 μM, leupeptine 10 μg ml^{-1}, pepstatin 1 μg ml^{-1}, benzamidine 1 mM]. The homogenate was centrifuged at 2000×g for 10 min at 4 °C to remove nuclei and unlysed cells, and the supernatant was removed. It was then used for protein analysis (with Lowry method) and Western blot analysis. Equal amounts of proteins (150 μg) were loaded in wells, electrophoresed on 8% polyacrylamide-sodium dodecyl sulphate (SDS) gels and then transferred to a nitrocellulose membrane overnight. The membrane was blocked with enhanced chemiluminescence (ECL) blocking agent (Amersham Biosciences, Freiburg, Germany) in Tris-buffered solution containing 0.05% tween-20 (TBS-T) for 1 h. It was then probed with a primary antibody raised against ROCK-2 (ROKα, Polyclonal IgG, Santa Cruz Biotechnology, CA, USA) at 1:200 dilution followed by horseradish peroxidase-conjugated secondary antibody (donkey anti-goat, 1:1000, Santa Cruz Biotechnology). The blots were then detected with an advanced chemiluminescence detection kit (Amersham Biosciences). After imaging, the membrane was stained with Coommasie Brilliant Blue and scanned. Thereafter, relative densities of the bands of ROCK-2 and total proteins were compared with the help of a computer program (Scion Image, USA).

2.4. Chemicals used

Angiotensin-2, ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA), ethylenediamine tetraacetic acid (EDTA) disodium, dithiothreitol, phenylmethylsulphonyl fluoride (PMSF), leupeptine, benzamidine, Tris–HCl, *E. coli* lipopolysaccharide, acetylcholine chloride and phenylephrine hydrochloride were obtained from Sigma (St. Louis, USA). Potassium chloride (KCl), glycine and dodecylsulphate sodium salt were purchased from Merck (Darmstadt, Germany). (+)-(*R*)-*trans*-4-(1-aminoethyl)-*N*-(4-pyridyl) cyclohexanecarboxamide dihydrochloride monohydrate (Y-27632) and endothelin-1 were obtained from Tocris Cookson (Bristol, UK). Primary antibody for ROCK-2 and HRP-conjugated secondary antibody were obtained from Santa Cruz Biotechnology. ECL advance kit was purchased from Amersham Biosciences. The kit was used according to the manufacturer's guide. Potassium chloride, phenylephrine hydrochloride, endothelin-1, angiotensin-2 and Y-27632 were dissolved in distilled water.

2.5. Statistical evaluations

All data represent means±standard error of the mean (S.E.M.) of n observations. Contractions to the agonists were expressed as percent of 80 mM KCl-induced tone. Relaxations to Y-27632 were evaluated as percent reductions of active tone induced by the agonists. For statistical comparison, one-way analysis of variance (ANOVA) followed by the Bonferroni post hoc test or Student t-test, if appropriate, was used. A P-value less than 0.05 was considered significant. Graphs were drawn by use of GraphPad Prism 3.0 program (GraphPad software, San Diego, CA, USA).

3. Results

3.1. Effects of potassium chloride, endothelin-1, phenylephrine and angiotensin-2 on the mesenteric artery obtained from saline- and lipopolysaccharide-treated rats

KCl (80 mM), endothelin-1 (10^{-9}–10^{-7} M), phenylephrine (10^{-9}–3×10^{-5} M) and angiotensin-2 (10^{-10}–10^{-6} M) all produced contraction in the rat mesenteric artery. pD_2 values (−log EC_{50} values) were 7.69±0.12, 7.53±0.11 and

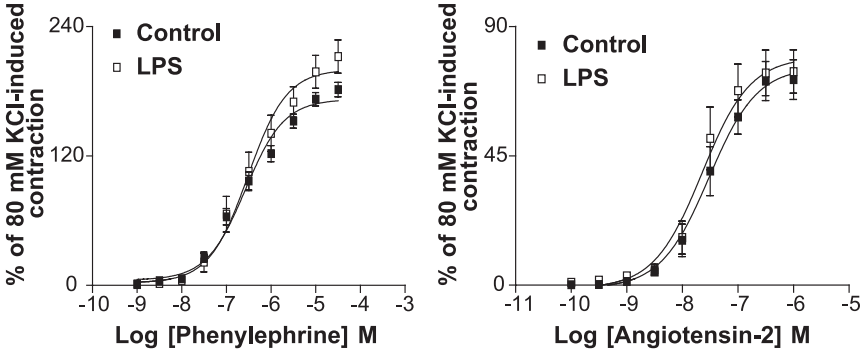

Fig. 1. Unchanged contractions to phenylephrine (n=8–9, left panel) and angiotensin-2 (n=8–10, right panel) in the mesenteric artery obtained from the lipopolysaccharide-treated rats (20 mg kg^{-1}, i.p., for 6 h). The contractions were expressed as percent of 80 mM KCl-elicited contraction. Data represent means±S.E.M.

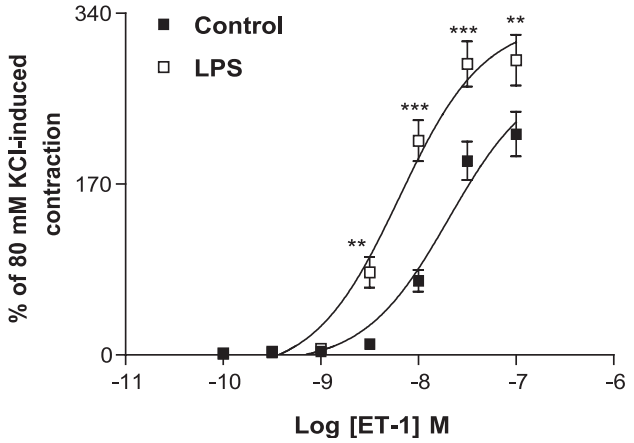

Fig. 2. Enhanced contraction to endothelin-1 (n=6–9) in the mesenteric artery obtained from the lipopolysaccharide-treated rats (20 mg kg^{-1}, i.p., for 6 h). The contractions were expressed as percent of 80 mM KCl-elicited contraction. Data represent means±S.E.M. **: P<0.01, ***: P<0.001. Comparison was made by one-way analysis of variance (ANOVA) followed by Bonferroni post hoc test.

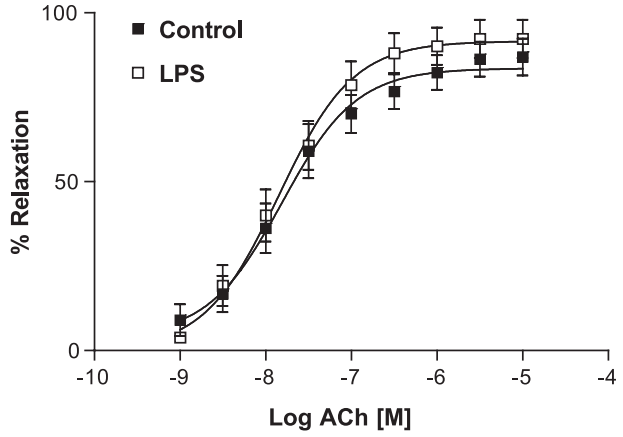

Fig. 4. Effect of LPS treatment (20 mg kg^{-1}, i.p., for 6 h) on acetylcholine-induced relaxation in the rat mesenteric rings, which were submaximally contracted by phenylephrine. Results were expressed as means±S.E.M. for 4–7 observations. Note that LPS treatment did not change endothelium-dependent vasodilatation in the mesenteric artery.

6.57±0.06 for endothelin-1, angiotensin-2 and phenylephrine, respectively. Lipopolysaccharide treatment had no effects on phenylephrine and angiotensin-2-induced contraction (Fig. 1), whereas it significantly enhanced endothelin-1-induced contractions (Fig. 2). The contraction elicited by KCl (80 mM) was not attenuated by lipopolysaccharide challenge in that the contractions were 322.5±23.5 mg (n=29) and 279.4±17.8 mg (n=42) in control and endotoxemic rings (P>0.05).

3.2. Effect of Rho-kinase inhibitor, Y-27632, on the mesenteric arteries obtained from saline- and lipopolysaccharide-treated rats

Y-27632 relaxed mesenteric arteries in a concentration-dependent manner. However, it produced less relaxation in the rings precontracted by endothelin-1 relative to phenylephrine. pD_2 values for Y-27632 were 6.25±0.08 and 5.68±0.09 when the vessels were precontracted by phenylephrine and endothelin-1, respectively (P<0.001). LPS treatment shifted the concentration–response curve to the left when the mesenteric artery was precontracted by endothelin-1 but not phenylephrine (Fig. 3). Since angiotensin-2-induced contractile activity did not reach steady state, i.e., tended to decrease, Y-27632 (10^{-5} M) was incubated for 30 min and then concentration–response curve to angiotensin-2 was obtained. The Rho-kinase inhibitor conspicuously suppressed the contraction. pD_2 values for angiotensin-2 were 7.52±0.11 and 7.85±0.18 (P>0.05) in the absence and presence of Y-27632. However, E_{max} values were 75.89±2.79 and 12.37±0.79 in the absence and the presence of Y-27632, respectively (P<0.001).

3.3. Effects of LPS challenge on acetylcholine-induced relaxation

Acetylcholine evoked concentration-dependent vasodilatations in the mesenteric artery obtained from saline-treated rats, which were not different from those in the artery from

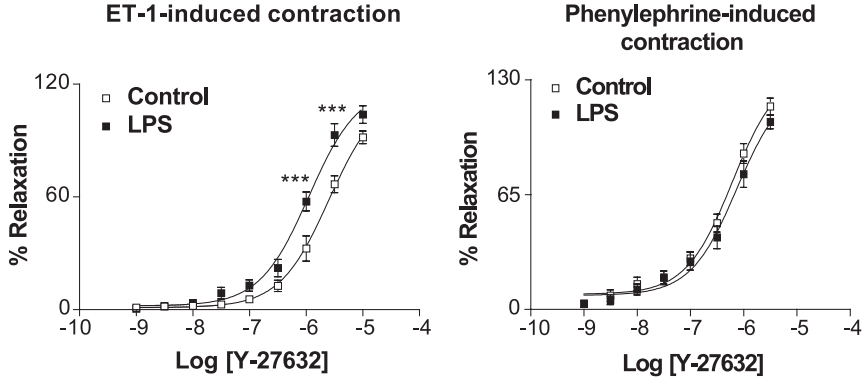

Fig. 3. The Rho-kinase inhibitor, Y-27632-induced relaxation in the mesenteric arteries obtained from saline and lipopolysaccharide (20 mg kg^{-1}, i.p., for 6 h), which were precontracted, by endothelin-1 (left panel) and phenylephrine (right panel). Results were expressed as means±S.E.M. for 9–11 observations. ***: P<0.001. Comparison was made by one-way analysis of variance (ANOVA) followed by Bonferroni post hoc test.

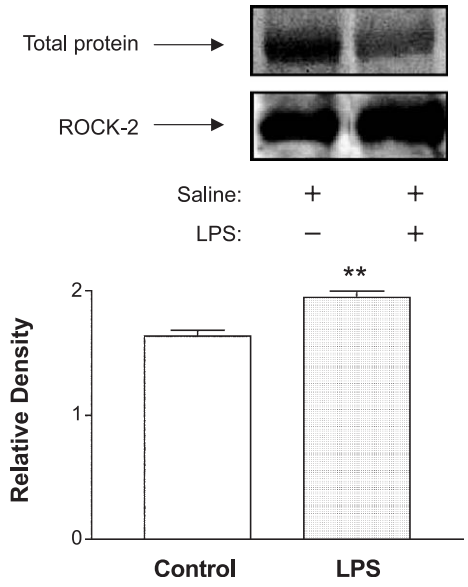

Fig. 5. Western blot for Rho-kinase (ROCK-2, ROKα) in the rat mesenteric arteries from saline- and LPS (20 mg kg^{-1}, i.p., for 6 h)-injected rats. Homogenates of the mesenteric arteries (n=3 from each) was submitted to sodium dodecyl sulphate (SDS)-PAGE with 8% polyacrylamide and then transferred to a nitrocellulose membrane. The membrane was blocked with the enhanced chemiluminescence-blocking agent (ECL advance) in Tris-buffered solution containing 0.05% tween-20 (TBS-T) for 1 h. It was then probed with a primary antibody raised against ROCK-2 (ROKα, Polyclonal IgG) at 1:200 dilution followed by horseradish peroxidase-conjugated secondary antibody (donkey antigoat, 1:1000). The density of protein bands was quantitatively analysed by a computer-based program, Scion Image. **: P<0.01, Student t-test for unpaired observation was used for the comparison.

saline-treated rats. pD_2 values for acetylcholine were, respectively, 7.81±0.15 and 7.85±0.12 in the control and endotoxemic vessels (P>0.05) (Fig. 4).

3.4. NO_2^-/NO_3^- level in the serum obtained from saline- and lipopolysaccharide-treated rats

Stable NO metabolites, NO_2^- and NO_3^-, were found to be significantly increased in the blood of LPS-treated rats. The NO_2^-/NO_3^- levels were 6.6±0.5 μM (n=8) and 10.0±1.4 μM (n=6) in the serum of control and endotoxemic rats, respectively (P<0.05).

3.5. Expression of Rho-kinase in the mesenteric arteries obtained from saline- and lipopolysaccharide-treated rats

Western blot analysis revealed that treatment of rats with lipopolysaccharide for 6 h upregulated Rho-kinase expression in their mesenteric arteries (Fig. 5).

4. Discussion

The Rho-kinase inhibitor, Y-27632, produced relaxation in the control mesenteric artery rings precontracted by phenylephrine and endothelin-1. Moreover, it significantly suppressed angiotensin-2-elicited contraction. However, in endothelin-1-precontracted vessels, Y-27632 caused less relaxation, compared to phenylephrine-precontracted vessels, probably due to the different association of endothelin receptors to Rho-signalling. Alternatively, involvement of Rho-kinase pathway in the endothelin-1-induced contraction might be less than that in phenylephrine-elicited contractions. In other words, excitatory endothelin receptors may be coupled to some other cellular pathways, i.e., protein kinase C (Lan et al., 2004) in addition to the Rho/Rho-kinase signalling. On the other hand, it has been recently reported that endothelin-1 and its receptor complex can be internalised and active for a long time as much as several hours, leading to long-lasting contraction (Bkaily et al., 2003). This might also render endothelin-1-induced contraction more resistant to vasodilators including Y-27632.

With respect to contraction, in the endotoxemic rings, there was no attenuation in the contractile activity to KCl, phenylephrine and angiotensin-2. However, the endothelin-1-induced contraction was substantially enhanced in the vessels obtained from the endotoxemic rats. This seems not to be due to the possible damage of vascular endothelium by LPS challenge since acetylcholine-elicited vasorelaxation has not changed in the endotoxemic vessels. It has been generally reported that lipopolysaccharide treatment has not modified α-adrenoceptor-mediated contraction in mesenteric arteries relative to other arteries such as aorta (Briones et al., 2002; Martinez et al., 1996; Mitchell et al., 1993; Schneider et al., 1992; Thiemermann, 1997). Furthermore, neurogenic (sympathetic) contraction of the mesenteric arteries obtained from lipopolysaccharide-treated rats was not modified (Ohlmann et al., 2000). This might show that either overproduced nitric oxide (NO) was not able to inhibit the signal transduction triggered by α-adrenoceptors and angiotensin receptors or upregulated Rho-kinase might compensate any decrease in the contractile responses to the vasoconstrictors in the mesenteric artery.

On the other hand, in endotoxemic conditions, there is a reduction in peripheral vascular resistance to vasoconstrictors (Thiemermann, 1997), which is suggested to be one of the major determinant factors leading to death (Parratt, 1989). Attenuation of vascular responses to vasoconstrictors in endotoxemia is generally attributed to NOS-derived NO (Wakabayashi et al., 1991). In this study, lipopolysaccharide treatment also increased serum NO_2^-/NO_3^- level. The mechanism by which NO could impair vascular contraction may involve a reversible deficiency of $[Ca^{2+}]_i$ (Piepot et al., 2002). In addition, it has been reported that there could be a decrease in Ca^{2+} sensitivity through enhanced myosin phosphatase activity, which causes dephosphorylation of myosin light chain, and thus counteracts smooth muscle contraction (Cohen et al., 1999; Wu et al., 1996). Lately, it has been suggested that endogenous NO may also affect Ca^{2+} sensitisation phenomenon, which is mainly governed

by Rho/Rho-kinase signalling by regulating RhoA activation (Carter et al., 2002; Sauzeau et al., 2003). In this study, there was no hyporeactivity to phenylephrine and angiotensin-2 in the endotoxemic mesenteric artery where the main vasodilatory regulator of vascular tone is generally endothelium-derived hyperpolarizing factor rather than NO itself. We did not measure tissue nitrite/nitrate content in the mesenteric artery obtained from the endotoxemic rats. However, blood level of these NO metabolites was increased, but probably, this may not be an enough inhibitory stimulus that was able to cause vascular hyporesponsiveness to vasoconstrictors in the mesenteric artery. On the contrary, there was a substantial augmentation in endothelin-1-induced contraction after lipopolysaccharide challenge. This enhancement coincided with the upregulation of Rho-kinase expression. However, although significant, the expression rate of the enzyme was small, and thus this may be explored at shorter and longer periods of lipopolysaccharide challenge. Furthermore, activation of the enzyme should also be investigated. Nevertheless, based on these data, perhaps endothelin-1 receptors-induced signalling might be diverted to Rho-kinase pathway in endotoxemic state. In other words, lipopolysaccharide may induce an increase in the coupling of endothelin receptors to RhoA, which then activates Rho-kinase. In association with this, Y-27632-induced vasodilatation was significantly enhanced in the mesenteric rings obtained from LPS-treated rats, which was precontracted by endothelin-1. This may imply that not only the expression but also the activation of Rho-kinase enzyme could be increased in the endotoxemic state. In addition, endothelin-1-induced contraction was also augmented in the endotoxemic guinea-pig mesenteric artery (Jones et al., 1999), confirming our data. It has been proposed that endothelin-1 antagonists may be therapeutic advantages for the impaired tissue perfusion, which is key step in the multiorgan failure seen in sepsis since these agents surprisingly attenuated sepsis-induced intestinal vasoconstriction in arteries and venulae despite diminished hyporeactivity to vasoconstrictors (Wilson et al., 1993; Miura et al., 1996). Moreover, the importance of endothelin system in human septic shock was demonstrated by a clear correlation between plasma level of endothelin and morbidity and mortality in septic patients (Pittet et al., 1991; Weitzberg et al., 1991).

It has been reported that exposure of endothelial cells to lipopolysaccharide increased several mitogen-activated protein kinases (MAPK) (Arditi et al., 1995; Schumann et al., 1996). An interaction between nuclear factor κB (NF-κB) and the Rho family as small GTPases was suggested recently (Perona et al., 1997). Furthermore, it has been shown that Rho proteins are essential for lipopolysaccharide-induced NF-κB activation (Hippenstial et al., 2000).

Consequently, lipopolysaccharide treatment induces a complex set of events, which may compensate the inhibitory effect of iNOS-derived NO or some other inhibitory compounds in the rat mesenteric resistance artery. For instance, upregulation of Rho-kinase expression may be one of these events. Recently, it has been reported that there could be a cross talk between NO and Rho/Rho-kinase signalling in that NO is necessary for the activation of RhoA protein (Sauzeau et al., 2003). In contrast, this signalling may negatively regulate and inhibit the expression of endothelial NOS (eNOS) (Ming et al., 2002). Moreover, inhibition of NOS strengthened RhoA and Rho-kinase-mediated contraction in rat aorta (Carter et al., 2002).

In conclusion, the results of the present study provide mechanistic evidence for the enhanced vasoconstriction to endothelin-1 in the endotoxemic rat mesenteric artery. Moreover, as with endothelin antagonist, Rho-kinase inhibitors might be potential therapeutic agents for the prevention of the intestinal vasoconstriction, which is the main event that triggers tissue hypoperfusion and consequently multiorgan failure in sepsis, although selectivity of these inhibitors is the major apparent problem for their use because Rho-kinase seems to be ubiquitously expressed in the body.

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