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Endotoxin elicits nitric oxide release in rat but prostacyclin synthesis in human and bovine vascular smooth muscle cells

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Abstract

Lipopolysaccharide (LPS) exposure to cells and tissues can mimic the biochemical events leading to septic shock. Previous data demonstrated a massive upregulation of prostaglandin endoperoxide H_2 synthase (PGHS-2), but not NO synthase-2 (NOS-2) in bovine smooth muscle cells (SMC) between 2 and 12 h of LPS exposure. This caused an abundant release of prostacyclin (PGI_2) by constitutive PGI_2-synthase as a counterregulation to a dysfunctional endothelium. We here report that human as well as bovine SMC mainly respond by the induction of PGHS-2 and the subsequent release of PGI_2, whereas rat SMC exhibited a distinct induction of NOS-2 and released significantly higher amounts of ·NO compared with cattle and human. The induction of either PGHS-2 or NOS-2 in the three different species investigated seems to be mutually exclusive in the time window of 2–24 h. This finding should be considered in the setup of experimental models for the investigation of septic shock.

Keywords: Vascular smooth muscle cells; Endotoxin; Prostaglandin endoperoxide H_2 synthase; Nitric oxide synthase; Prostacyclin

Septic shock is associated with an imbalance of the finely tuned homeostatic network in the vasculature [1,2]. Understanding of the biochemical events occurring in sepsis is essential for a rational pharmacological treatment, and hence animal and cellular models are being investigated to study the sequence of events from the first inflammatory responses to endothelial dysfunction, and finally death which still occurs in a high percentage of patients [3–5]. The rat model has been intensively used for many studies although the higher resistance of rats and their lower mortality upon endotoxin (lipopolysaccharide, LPS) exposure are well known [6].

Bovine vascular endothelial cells under resting conditions release basal amounts of prostacyclin (PGI_2) and nitric oxide (·NO) which both exert anti-aggregatory, anti-adhesive, and vasodilatory properties [7–9]. Exposure to LPS results in increased endothelial superoxide ($^{\bullet}O_2^-$) formation which interacts with ·NO in a nearly diffusion-limited reaction to form peroxynitrite [10,11]. This reactive intermediate was found capable to inhibit endothelial PGI_2-synthase by nitration of a tyrosine-residue near the active site [12,13]. An upregulation of $^{\bullet}O_2^-$ therefore inhibits the release of both vasodilators PGI_2 and ·NO. Since endothelial prostaglandin endoperoxide synthase-1 (PGHS-1) which supplies PGI_2-synthase with substrate remains active under such conditions, 15-hydroxy-prostaglandin 9,11 endoperoxide (PGH_2) is still produced and can activate the thromboxane A_2 (TxA_2)/PGH_2 receptor on vascular smooth muscle cells resulting in additional vasoconstriction [14,15]. These

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events which require no de novo protein synthesis can be observed within the first hour of stimulation and prepare the stage for leukocyte adhesion and emigration [16]. A second phase in the activation of the vasculature is initiated after roughly 2 h and involves the induction of early immediate genes in vascular smooth muscle cells. Under such conditions, PGHS-2 was observed upregulated in bovine aortic smooth muscle cells (SMC) to supply the so far dormant constitutive PGI_2 synthase with substrate [17]. It was further deduced that this PGHS-2-dependent PGI_2 generation vitally contributes to the highly elevated 6-keto-$PGF_{1\alpha}$ levels and the extreme hypotension observed in patients with septic shock syndromes [18].

Notably, no significant nitration of PGI_2 synthase was observed in these bovine SMC which was explained by the lack of NOS-2 induction in this tissue. This appeared to be at variance with reports of intense NOS-2 induction in the rat aorta [19,20] and led us to investigate potential differences in the response of rat and bovine aortic SMC towards LPS. In order to compare with the situation in humans we included SMC from human aortic segments. As an essential result it was verified that rat aortic SMC responded to LPS by an elevated output of ˙NO as a consequence of NOS-2 induction whereas human and bovine SMC responded mainly by a PGHS-2-dependent PGI_2 release and only marginal formation of ˙NO.

Materials and methods

Cell culture. In pre-studies whole endothelium-denuded aortic segments were incubated with LPS, however such incubations were lacking sufficient oxygen supply when incubated for several hours. We therefore prepared primary cultures of human, bovine, and rat aortic smooth muscle cells (SMC) which were obtained by collagenase digestion (Type CLS; Biochrom, Berlin, Germany) of endothelium-denuded aortic vessel segments. Cells were grown in M199-medium (Biochrom, Berlin, Germany) supplemented with 10% FCS and 1% penicillin/streptomycin. SMC were allowed to rest in serum-free medium 24 h prior to experiments which were performed with cells of passage 1 (bovine) and 3 (human + rat), maintained in a 37 °C humidified incubator in an atmosphere of 95% air and 5% CO_2. During passage, cells were pooled and seeded out to achieve identical cell numbers since shape and growth pattern of the cells made it difficult to obtain reliable counts for standardization. Cells were utilized for experiments when confluence was reached. This method allowed comparison between the different species. LPS (*Escherichia coli*; Serotype 026:B26, 10 μg/ml for all experiments) was obtained from Sigma, St. Louis, USA.

PGHS activity assay. Cells were washed twice, collected in cold PBS, and centrifuged at 1000*g* for 3 min. The pellet was dissolved in lysis buffer (20 mM Hepes, 1% Triton X-100, 1% aprotinin, and 10% glycerol, pH 7.5) for 30 min. Following centrifugation at 12,000*g* for 1 min, the supernatant was incubated with reaction buffer (80 mM Tris–HCl, 0.8 mM phenol, 10 μg/ml hematin, and 17.2 μM [^{14}C]AA) for 2 min. The reaction was terminated by the addition of ethyl acetate/2 M citric acid (30:1). The organic phase was spotted by glass capillaries onto silica TLC plates (Silica 60, Merck, Darmstadt, Germany) and subjected to chromatography. The solvent consisted of ethyl acetate:2,2,4-trimethylpentane:acetic acid:water (110:50:20:100). Plates were exposed to a phosphorImager™ screen overnight. For reading the screen, a phosphorImager™ system from Molecular Dynamics, USA, was used. Quantification was performed by the detection of total PGHS metabolites utilizing ImageQuant™ software.

NOS activity assay. Cells were homogenized by sonication in PBS. NOS activity was measured by the conversion of [^{14}C]L-arginine to [^{14}C]L-citrulline. Forty microliters of supernatant was incubated for 30 min at 37 °C with 40 μl assay buffer containing 32.3 mM Hepes with 0.8% glycerol, 6 μM FAD (Sigma), 6 μM FMN (Sigma), 100 μg/ml CaM (BioMol), 3 mM DTT (Lancaster), 5 μM [^{12}C]L-arginine (Sigma), 5 μM [^{14}C]L-arginine (Amersham), and 250 μM NADPH (BioMol). After 30 min, the reaction was stopped by the addition of 120 μl methanol. One hundred microliters of the mixture was spotted by glass capillaries onto a silica TLC plate (Silica 60, Merck) and subjected to chromatography. The solvent consisted of ammonium hydroxide (30%), chloroform, methanol, and water in a 2:0.5:4.5:1 mixture. Plates were exposed to a phosphorImager™ screen overnight. For reading the screen, a phosphorImager™ system from Molecular Dynamics, USA, was used. Quantification was performed by using ImageQuant™ software.

Quantitative PCR analysis. Total RNA isolation was performed according to the guanidine isothiocyanate/phenol method described in the manufacturer's instructions (Peqlab, Erlangen, Germany). For reverse transcription, murine leukemia virus reverse transcriptase (Superscript, Invitrogen, California, USA) and oligo(dT)15 primers (Promega, Mannheim, Germany) were used. The reaction was performed at 42 °C for 60 min. PCR amplification was carried out in a LightCycler Instrument (Roche Diagnostics, Mannheim, Germany) using the PGHS-2 specific sense primer: 5′-ATC TTT GGG GAG ACC ATG GTA GA-3′ and anti-sense primer: 5′-ACT GAA TTG AGG CAG TGT TGA TG-3′; for bovine/human NOS-2 sense primer: 5′-GGC TGG CGG GCG AGG CGT TTC-3′ and anti-sense primer: 5′-GCT GCT TCA GGG TGG GGG CCA-3′; for rat NOS-2 sense primer: 5′-AGT GTC AGT GGC TTC CAG CTC-3′ and anti-sense primer: 5′-AGT GTC AGT GGC TTC CAG CTC-3′; for human/bovine GAPDH sense primer: 5′-ACC CTC AAG ATT GTC AGC AAT GC-3′ and anti-sense primer 5′-GTC GTC ATA AGT CCC TCC ACG AT-3′; and for rat GAPDH sense primer: 5′-TCC ATG ACC GTT GTC AGC AAT GC-3′ and anti-sense primer: 5′-GTG GTC ATT AGC CCT TCC ACG AT-3′. Settings were used as follows: denaturation at 95 °C for 15 s, annealing at 65 °C for 5 s, and amplification at 72 °C for 19 s. Fifty cycles were run before the reaction was stopped. Amplification was followed in real time and its crossing points were used for evaluation. After PCR, a melting curve analysis for checking primer specificity was carried out. GAPDH served for standardization.

Western-blot analysis. Proteins were separated electrophoretically by 8% SDS–PAGE and then transferred to nitrocellulose membranes (Hybond-C extra, Amersham, GB) by semidry blotting. The membrane was blocked for 2 h in 5% milk powder. It was then incubated with the primary antibodies for 2 h at room temperature or at 4 °C overnight and for 45 min with a peroxidase conjugated secondary antibody at room temperature. Bands were visualized using the ECL Western blot system (Interchim, Montlucon, France). α-PGHS-2, α-NOS-2, and α-SMC-actin monoclonal antibodies were purchased from Transduction Laboratories, Lexington, USA. α-PGI_2-synthase polyclonal antibody was a kind gift from Prof. Tanabe, Osaka, Japan.

Measurements of prostanoids and nitrite in cell culture supernatants. 6-keto-$PGF_{1\alpha}$, was determined by using commercially available EIA-kits (Assay Designs, Ann Arbor, USA) according to the manufacturer's instructions. During passage, cells for a single experiment were pooled and seeded out to achieve identical cell numbers since shape and growth pattern of the cells made it difficult to obtain reliable counts for standardization. This method allowed better comparison

between different experiments. Nitrite (NO_2^-) as the stable end product of ·NO formation was measured by the Griess assay. Briefly, 30 μl of 12.5 μM sulfanilamide and 30 μl of 6 M HCl were mixed with a 300 μl sample at room temperature and incubated for 5 min. Absorbance was measured before and after the addition of 25 μl *N*-(1-naphthyl)ethylenediamide (12.5 μM) at 560 nm using a microtiter plate reader. Nitrite concentrations were calculated from a $NaNO_2$ standard curve in the range of 0.5–50 μM. Nitrate was quantified after conversion to nitrite by nitrate reductase (Roche, Mannheim, Germany) prior to the Griess reaction.

Results

Smooth muscle cells from rat, bovine, and human aortae were cultivated and used from passage 1 to 3. As described earlier, LPS addition (10 μg/ml) to bovine SMC caused a rapid increase of prostaglandin endoperoxide synthase-2 (PGHS-2) mRNA after 3 h followed by a steady decline until 24 h after LPS exposure (Fig. 1A). Primary SMC from human aortic segments showed the same time course, whereas cells from rat aortae were lacking significant PGHS-2 induction in the time frame investigated. For PGHS-2 protein the Western blots indicated qualitatively the same trend (Fig. 1B) with a slightly earlier peak at 6 h for human cells and at about 12 h for bovine cells. For rat SMC only a small band appeared after 6 h which remained low at 12 and 24 h.

For NOS-2 mRNA and protein mass the induction pattern between the three species was just reverse: a rapid NOS-2 mRNA increase after 6 h (Fig. 2A) with a parallel expression of NOS-2 protein (Fig. 2B) in rat SMC and no detectable staining in Western blots for human and bovine NOS-2 although the sensitivity of the antibody was in the same range (data not

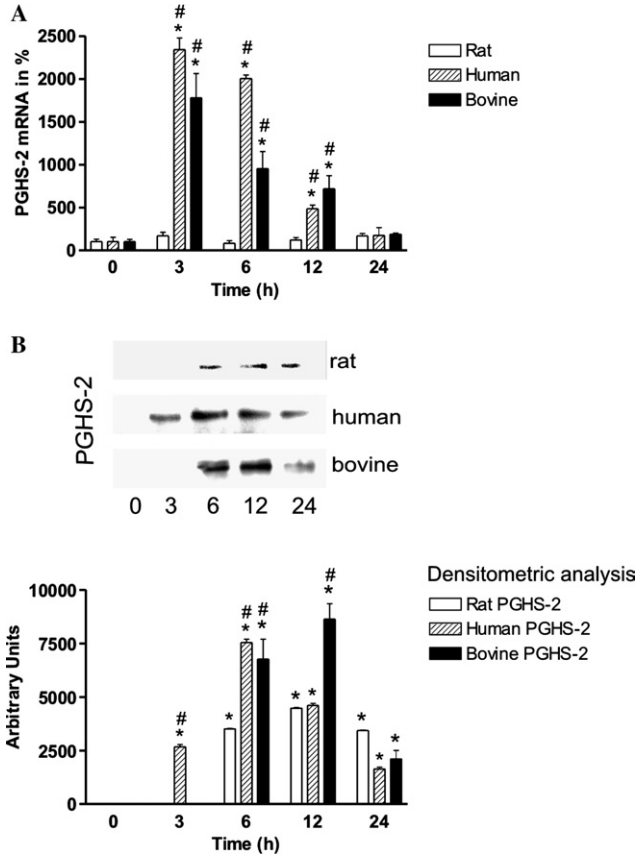

Fig. 1. PGHS-2 mRNA induction in aortic SMC (A). LPS (10 μg/ml) was added at $t = 0$, cells were incubated for the time periods indicated. Human and bovine SMC exhibited a distinct induction of PGHS-2 whereas induction in rat SMC was nearly negligible. PGHS-2 protein expression followed its corresponding mRNA in human and bovine SMC whereas expression in rat was detected to be much weaker (B). Note that incubation time of X-ray detection film was three times longer in case of rat PGHS-2 blot. Western blots are representative of three separate experiments which were evaluated by densitometry as demonstrated. Values are means ± SD [$n = 4$ in (A); $n = 3$ in (B)]. *Inner-group differences $P < 0.05$ vs. values at $t = 0$. #Inter-group differences $P < 0.05$ of human and bovine vs. rat values.

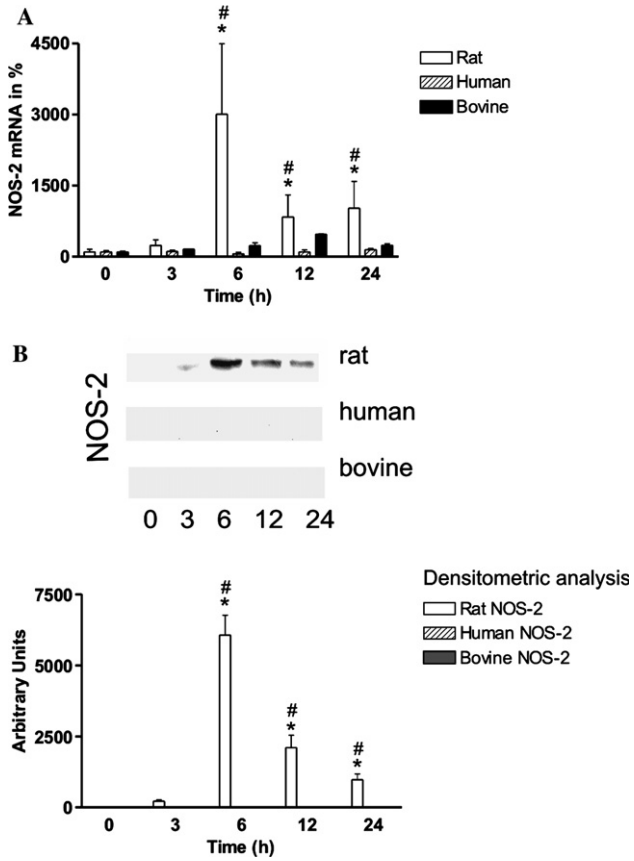

Fig. 2. NOS-2 induction in aortic SMC. Cells were treated as in Fig. 1. Rat SMC demonstrated a clear induction of NOS-2 mRNA (A) as well as NOS-2 protein (B) while induction in human and bovine cells was only minimal. Densitometric evaluation of NOS-2 protein expression (B) underlined a maximal induction after 6 h of LPS exposure followed by a gradual decline in rat SMC. Values are means ± SD [$n = 4$ in (A); $n = 3$ in (B)], *inner-group differences $P < 0.05$ vs. values at $t = 0$. #Inter-group differences $P < 0.05$ of rat vs. human values.

shown). When measuring enzymatic activities of PGHS, the conversion of [^{14}C]arachidonate did not result in any significant values in the first 3 h after LPS treatment in line with the absence of PGHS-2 (Fig. 3A). Human and bovine SMC greatly increased this conversion of [^{14}C]arachidonate to prostanoids after 6 h which decreased at 12 and 24 h (Fig. 3A). As an apparent discrepancy, the activity of 6-keto-$PGF_{1\alpha}$ formation increased from 3 to 24 h continuously (Fig. 3B) at variance with the declining total PGHS activity from 6 to 24 h. This interesting observation seems to be connected with a different time-dependent coupling of PGHS-2 to PGI_2 synthase but was not further investigated in the context of the present study. In agreement with the data in Fig. 1, rat SMC did not display significant upregulation of cyclooxygenase activities but elevated NOS-activity after 3 h and maintained an about fourfold increase between 6 and 24 h (Fig. 4A). As a consequence, nitrite accumulated about sixfold in rat SMC over 24 h whereas human and bovine SMC showed a basal activity which led to only modest nitrite levels after 24 h (Fig. 4B). It should be taken into account that such levels arise from a constant basic activity, and hence the steady increase with time results from an unchanged basal NOS activity.

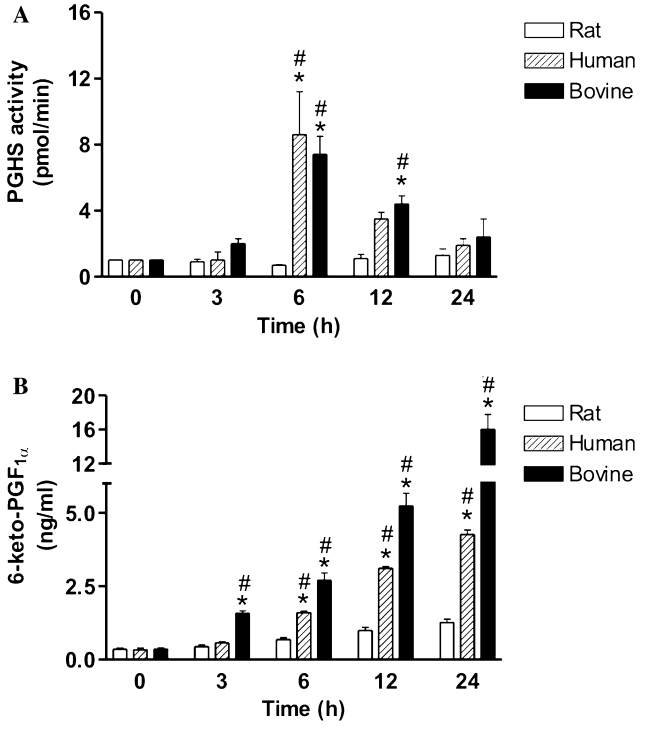

Fig. 3. Total PGHS activity of SMC homogenates (A) revealed an upregulation only in human and bovine cells. 6-Keto-$PGF_{1\alpha}$ as the stable hydrolysis product of PGI_2 was profoundly upregulated in human and bovine SMC while rat SMC exhibited a constant but moderate release (B). 6-Keto-$PGF_{1\alpha}$ release by bovine SMC between 12 and 24 h was definitely higher than in human cells. Values are means ± SD ($n = 4$). *Inner-group difference $P < 0.05$ vs. values at $t = 0$. #Inter-group difference $P < 0.05$ of human and bovine vs. rat values.

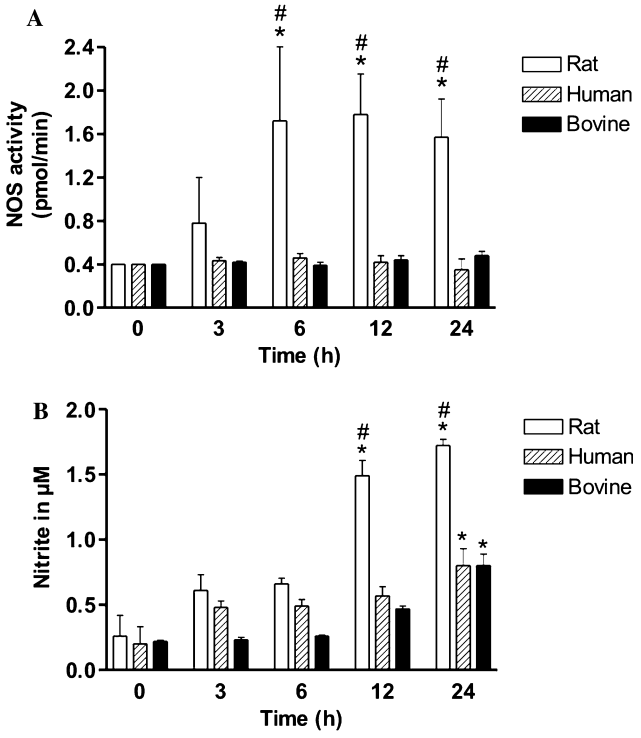

Fig. 4. Total NOS activity of SMC homogenates (A) indicates an upregulation only in rat SMC while NOS activity in human and bovine cells remained constant over time. Nitrite detection (B) as indicator of ·NO formation illustrates distinct higher rates released by rat SMC. Human and bovine SMC demonstrated a far weaker but constant basal formation of ·NO. Values are means ± SD ($n = 4$). *Inner-group difference $P < 0.05$ vs. values at $t = 0$. #Inter-group difference $P < 0.05$ of rat vs. human values.

Discussion

Knowledge of the biochemical events in developing sepsis and septic shock is crucial for a therapeutic approach of this still highly lethal pathophysiological situation. Sepsis and its associated syndromes are accompanied by severe hypotension which mainly arises from a PGI_2 or ·NO-mediated elevation of intracellular cAMP or cGMP in vascular smooth muscle cells and the subsequent downregulation of free Ca^{2+} [2,21]. Since in progressive stages of endotoxin exposure the vascular endothelium becomes dysfunctional and fails to serve as a regulator of vessel tone [2], autocrine stimulation of the smooth muscle mainly accounts for the extreme hypotension observed under these conditions. Several studies described in the literature were performed with rats as the model of choice, however our results revealed dramatic species-specific differences in the response of vascular smooth muscle cells to LPS. In contrast to human and bovine SMC, cells from the rat responded by

the distinct induction of NOS-2 and only moderate elevation of PGHS-2. Although ·NO in principal can replace PGI_2 and vice versa, there was obviously no combined action of these two mediators in the smooth muscle within a species in contrast to the situation in the endothelium which was demonstrated to release ·NO and PGI_2 synchronously [22,23]. At first sight it appears rather unusual that the early immediate genes PGHS-2 and NOS-2 are not induced in parallel since both should depend on NF-κB signalling [24,25]. However, studies on the promoter region of PGHS-2 revealed that induction of PGHS-2 is far more complicated and depends on other transcription factors like cAMP responsive element binding protein [26] or methylation of the promoter region [27]. With respect to the biochemical events leading to endothelial dysfunction, the species-dependent exclusive release however could be regarded as an effective mechanism to allow a sustained autocrine dilation of the smooth muscle in response to prolonged endotoxin exposure. In endothelial cells, proinflammatory stimuli like TNFα or LPS cause an elevation of $\cdot O_2^-$ formation which interacts with ·NO to form peroxynitrite [10]. In the following step, peroxynitrite nitrates and inhibits endothelial PGI_2-synthase and therefore the release of both ·NO and PGI_2 becomes inhibited. Interestingly in bovine SMC exposed to LPS up to 24 h, no nitration and inhibition of PGI_2-synthase was observed [17]. This can be explained partially by the higher antioxidative capacity of SMC compared with endothelial cells, but the most intriguing explanation for this lack of inhibition may arise from the observed lack of significant ·NO formation since $\cdot O_2^-$ generation is also upregulated in SMC in response to LPS [28]. In rat mesangial cells for example, induction of both NOS-2 and PGHS-2 was detected and about half of PGI_2-synthase became nitrated and inhibited under such conditions [29]. It has to be noted that the mutual exclusion in smooth muscle cells is limited to the time window between 2 and 24 h. Stimulation of rat aortic vessel for more than 24 h revealed induction of PGHS-2 [30] whereas bovine and human SMC in later stages demonstrated an upregulation of NOS-2 (unpublished). It could be speculated that in humans the late induction of NOS-2 may provide a mechanism to guarantee anti-thrombotic and dilatory properties up to roughly 24 h of vascular activation. A rise of ·NO could then facilitate nitration of PGI_2-synthase and yield the pro-aggregatory and vasoconstrictive state observed in the final stages of septic shock patients. In contrast, rat SMC responded mainly by the induction of NOS-2. The resistance of NOS-2 against oxidative stress, e.g., by peroxynitrite is much higher (≈10 μM) [31] compared with that of PGI_2-synthase (≈50 nM) [13]. PGI_2-synthase is also more vulnerable to certain fatty acid hydroperoxides which may arise during oxidative stress conditions [32]. This difference may contribute to the known higher resistance of rats against bacterial infections compared with other species and should be considered when data obtained in the rat model are transferred to the situation in humans.

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References

[1] R.C. Bone, The pathogenesis of sepsis, Ann. Intern. Med. 115 (1991) 457–469.
[2] S.J. Wort, T.W. Evans, The role of the endothelium in modulating vascular control in sepsis and related conditions, Br. Med. Bull. 55 (1999) 30–48.
[3] J.E. Parrillo, Pathogenic mechanisms of septic shock, N. Engl. J. Med. 328 (1993) 1471–1477.
[4] P. Vallance, J. Collier, K. Bhagat, Infection, inflammation, and infarction: does acute endothelial dysfunction provide a link? Lancet 349 (1997) 1391–1392.
[5] M.A. Martin, Epidemiology and clinical impact of gram-negative sepsis, Infect. Dis. Clin. North Am. 5 (1991) 739–752.
[6] M.J. Schultz, T. van der Poll, Animal and human models for sepsis, Ann. Med. 34 (2002) 573–581.
[7] G. Noll, T.F. Luscher, The endothelium in acute coronary syndromes, Eur. Heart J. 19 (1998) C30–C38.
[8] R.F. Furchgott, J.V. Zawadzki, The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine, Nature 288 (1980) 373–376.
[9] S. Moncada, J.R. Vane, Arachidonic acid metabolites and the interactions between platelets and blood-vessel walls, N. Engl. J. Med. 300 (1979) 1142–1147.
[10] M. Bachschmid, S. Thurau, M.H. Zou, V. Ullrich, Endothelial cell activation by endotoxin involves superoxide/NO-mediated nitration of prostaglandin synthase and thromboxane receptor stimulation, FASEB J. 17 (2003) 914–916.
[11] R.E. Huie, S. Padmaja, The reaction of NO with superoxide, Free Radic Res. Commun. 18 (1993) 195–199.
[12] M. Zou, C. Martin, V. Ullrich, Tyrosine nitration as a mechanism of selective inactivation of prostacyclin synthase by peroxynitrite, Biol. Chem. 378 (1997) 707–713.
[13] P. Schmidt, N. Youhnovski, A. Daiber, A. Balan, M. Arsic, M. Bachschmid, M. Przybylski, V. Ullrich, Specific nitration at tyrosine 430 revealed by high resolution mass spectrometry as basis for redox regulation of bovine prostacyclin synthase, J. Biol. Chem. 278 (2003) 12813–12819.
[14] D.E. Mais, D.L. Saussy, A. Chaikhouni, P.J. Kochel, D.R. Knapp, N. Hamanaka, P.V. Halushka, Pharmacologic characterization of human and canine thromboxane A2/prostaglandin H2 receptors in platelets and blood vessels: evidence for different receptors, J. Pharmacol. Exp. Ther. 233 (1985) 418–424.
[15] M.L. Ogletree, D.N. Harris, R. Greenberg, M.F. Haslanger, M. Nakane, J. Pharmacological actions of SQ 29,548, a novel selective thromboxane antagonist, Pharmacol. Exp. Ther. 234 (1985) 435–441.
[16] R.J. Gumina, P.J. Newman, D. Kenny, D.C. Warltier, G.J. Gross, The leukocyte cell adhesion cascade and its role in myocardial ischemia–reperfusion injury, Basic Res. Cardiol. 92 (1997) 201–213.

[17] S. Schildknecht, M. Bachschmid, A. Baumann, V. Ullrich, COX-2 inhibitors selectively block prostacyclin synthesis in endotoxin-exposed vascular smooth muscle cells, FASEB J. 18 (2004) 757–759.
[18] P.V. Halushka, H.D. Reines, S.E. Barrow, I.A. Blair, C.F. Dollery, W. Rambo, J.A. Cook, W.C. Wise, Elevated plasma 6-keto-prostaglandin F1 alpha in patients in septic shock, Crit. Care Med. 13 (1985) 451–453.
[19] D.M. Fries, E. Paxinou, M. Themistocleous, E. Swanberg, K.K. Griendling, D. Salvemini, J.W. Slot, H.F. Heijnen, S.L. Hazen, H. Ischiropoulos, Expression of inducible nitric-oxide synthase and intracellular protein tyrosine nitration in vascular smooth muscle cells: role of reactive oxygen species, J. Biol. Chem. 278 (2003) 22901–22907.
[20] D.D. Rees, S. Cellek, R.M. Palmer, S. Moncada, Dexamethasone prevents the induction by endotoxin of a nitric oxide synthase and the associated effects on vascular tone: an insight into endotoxin shock, Biochem. Biophys. Res. Commun. 173 (1990) 541–547.
[21] R.F. Furchgott, Role of endothelium in responses of vascular smooth muscle, Circ. Res. 53 (1983) 557–573.
[22] P. Akarasereenont, J.A. Mitchell, Y.S. Bakhle, C. Thiermann, J.R. Vane, Comparison of the induction of cyclooxygenase and nitric oxide synthase by endotoxin in endothelial cells and macrophages, Eur. J. Pharmacol. 273 (1995) 121–128.
[23] P. Akarasereenont, Y.S. Bakhle, C. Thiemermann, J.R. Vane, Cytokine-mediated induction of cyclo-oxygenase-2 by activation of tyrosine kinase in bovine endothelial cells stimulated by bacterial lipopolysaccharide, Br. J. Pharmacol. 115 (1995) 401–408.
[24] Q. Xie, Y. Kashiwabara, C. Nathan, Role of transcription factor NF-kappaB/Rel in induction of nitric oxide synthase, J. Biol. Chem. 269 (1994) 4705–4708.
[25] C.C. Lin, L.D. Hsiao, C.S. Chien, C.W. Lee, J.T. Hsieh, C.M. Yang, Tumor necrosis factor-alpha-induced cyclooxygenase-2 expression in human tracheal smooth muscle cells: involvement of p42/p44 and p38 mitogen-activated protein kinases and nuclear factor-kappaB, Cell Signal. 16 (2004) 597–607.
[26] X. Ma, Q. Yang, K.T. Wilson, N. Kundu, S.J. Meltzer, A.M. Fulton, Promoter methylation regulates cyclooxygenase expression in breast cancer, Breast Cancer Res. 6 (2004) R316–R321.
[27] K. Kirtikara, R. Raghow, S.J. Laulederkind, S. Goorha, T. Kanekura, L.R. Ballou, Transcriptional regulation of cyclooxygenase-2 in the human microvascular endothelial cell line, HMEC-1: control by the combinatorial actions of AP2, NF-IL-6 and CRE elements, Mol. Cell. Biochem. 203 (2000) 41–51.
[28] C. Patterson, J. Ruef, N.R. Madamanchi, P. Barry-Lane, Z. Hu, C. Horaist, C.A. Ballinger, A.R. Brasier, C. Bode, M.S. Runge, Stimulation of a vascular smooth muscle cell NAD(P)H oxidase by thrombin: evidence that p47(phox) may participate in forming this oxidase in vitro and in vivo, J. Biol. Chem. 274 (1999) 19814–19822.
[29] M.H. Zou, T. Klein, J.P. Pasquet, V. Ullrich, Interleukin 1β decreases prostacyclin synthase activity in rat mesangial cells via endogenous peroxynitrite formation, Biochem. J. 336 (1998) 507–512.
[30] D. Bishop-Bailey, S.W. Larkin, T.D. Warner, G. Chen, J.A. Mitchell, Characterization of the induction of nitric oxide synthase and cyclo-oxygenase in rat aorta in organ culture, Br. J. Pharmacol. 121 (1997) 125–133.
[31] J.P. Pasquet, M.H. Zou, V. Ullrich, Peroxynitrite inhibition of nitric oxide synthases, Biochimie 78 (1996) 785–791.
[32] J.A. Weaver, J.F. Maddox, Y.Z. Cao, I.K. Mullarky, L.M. Sordillo, Increased 15-HPETE production decreases prostacyclin synthase activity during oxidant stress in aortic endothelial cells, Free Radic Biol. Med. 30 (2001) 299–308.