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Hyporeactivity of mesenteric vascular bed in endotoxin-treated rats

Delia Mitolo-Chieppa [a,*], Miralma Serio [a], M. Assunta Potenza [a], Monica Montagnani [a], Gianfranco Mansi [a], Salvatore Pece [b], Emilio Jirillo [b], Jean-Claude Stoclet [c]

[a] *Institute of Pharmacology, Medical School, University of Bari, Policlinico, P.zza G. Cesare, 70124 Bari, Italy*

[b] *Institute of Microbiology, Medical School, University of Bari, Policlinico, P.zza G. Cesare, 70124 Bari, Italy*

[c] *Laboratoire de Pharmacologie Cellulaire et Moléculair, CNRS URA 600, Université Louis Pasteur Strasbourg, BP 24, 67401 Illkirch, France*

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Abstract

Vascular reactivity and activation of the nitric oxide (NO) pathway were investigated in perfused mesenteric vascular bed removed from rats 5 h after i.p. injection of bacterial lipopolysaccharide (*E. coli* lipopolysaccharide, 30 mg kg^{-1}). Lipopolysaccharide treatment induced hyporesponsiveness to noradrenaline. Maximal noradrenaline-induced vasoconstriction was significantly reduced in lipopolysaccharide-treated vs. untreated preparations. Continuous infusion of L-arginine (L-Arg) (0.2 mM) enhanced noradrenaline hyporeactivity of lipopolysaccharide-treated rats. N^{ω}-Nitro-L-arginine methyl ester (L-NAME) (0.2 mM), a non-selective inhibitor of NO synthase, failed to completely restore the noradrenaline hyporeactivity of lipopolysaccharide-treated + L-Arg-infused mesenteric vascular bed. After L-NAME treatment, Methylene blue (10 μM), a guanylate cyclase inhibitor, produced no additional increase of noradrenaline vasoconstriction in lipopolysaccharide-treated + L-Arg-infused mesenteric vascular bed, suggesting that an NO-independent activation of guanylate cyclase may be excluded. In lipopolysaccharide-treated preparations, L-Arg (0.2 mM) elicited a significant increase in nitrite production, which was antagonized by L-NAME. In conclusion, lipopolysaccharide-induced noradrenaline hyporesponsiveness of rat resistance vessels can only be partially explained by NO overproduction. Other mechanisms, probably related to vasoconstriction, may be involved.

Keywords: L-Arginine; Nitric oxide (NO); Nitric oxide (NO) synthase; Lipopolysaccharide; Septic shock; Mesenteric vascular bed; (Rat)

1. Introduction

Nitric oxide (NO) production from L-arginine (L-Arg) is catalyzed by constitutive or inducible NO synthase isoenzymes (Palmer et al., 1987). NO derived from constitutive NO synthase activation is involved in the regulation of blood vessel tone (Rees et al., 1989) and mediates vascular smooth muscle relaxation (Ignarro et al., 1987) by activating soluble guanylate cyclase, leading to a rise in cyclic guanosine 3′,5′-monophosphate (cGMP) (Stoclet, 1991).

Stimuli, such as bacterial lipopolysaccharides (Fleming et al., 1990) and cytokines (Busse and Mülsch, 1990), elicit inducible NO synthase protein synthesis over 3–6 h (Fleming et al., 1991a) in a variety of cells and tissues, including vascular endothelial and smooth muscle cells (Moncada et al., 1991; Förstermann et al., 1991). Experimental and clinical studies have suggested that excessive NO production by inducible NO synthase may be responsible for hypotension and aortic hyporesponsiveness to noradrenaline associated with septic shock (Julou-Schaeffer et al., 1991; Fleming et al., 1991b). Furthermore, it has been reported that inhibitors of the L-Arg/NO pathway are able to reduce hypotension, restore responsiveness to noradrenaline and prevent the increase in cGMP content induced by lipopolysaccharide (Gray et al., 1991; Thiemermann and Vane, 1990).

The activation of the L-Arg/NO pathway has been studied mostly in conductance vessels, such as the aorta. However, the complex haemodynamic events occurring in resistance vessels in the course of septic shock syndrome have yet to be fully elucidated.

With the aim of contributing more information on the putative mechanisms underlying the failure of peripheral resistance in septic shock, we carried out an investigation on perfused mesenteric vascular bed, an accepted experimental model for the study of resistance vessels.

* Corresponding author. Tel.: +39 80 5478448 (extension 9); fax: +39 80 5478444.

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We studied the responsiveness to noradrenaline and the time course of noradrenaline-induced vasoconstriction (recorded as variations in perfusion pressure) in mesenteric vascular bed preparations from rats treated in vivo with *E. coli* lipopolysaccharide, without and with infusion of L-Arg, in comparison with those of untreated control rats. The effects of L-Arg as well as the activation of the L-Arg/NO pathway were also assessed, using the NO inhibitor N^{ω}-nitro-L-arginine methyl ester (L-NAME), in both endothelium-intact and endothelium-denuded preparations. In addition, a probable NO-independent pathway of guanylate cyclase activation was investigated by using Methylene blue.

The presence of NO in samples of perfusate from mesenteric vascular bed of untreated and lipopolysaccharide-treated rats was determined by measuring the accumulation of nitrites, the predominant oxidation product of NO. Biochemical data were compared with the functional responses.

2. Materials and methods

2.1. Mesenteric vascular bed preparation

Male Wistar rats (250–300 g) were injected i.p. with *E. coli* lipopolysaccharide (serotype 055: B5) (30 mg kg^{-1} diluted in 0.1 ml saline/100 g body weight). 5 h later, they were heparinized (200 IU/ rat i.p.), then decapitated and exsanguinated, under ether anaesthesia. Wistar rats injected with sterile saline were used as control. The mesenteric vascular bed was prepared and perfused as described by McGregor (1965) and Criscione et al. (1984). A midline incision was made in the abdominal cavity and the superior mesenteric artery was cannulated via a small incision in the abdominal aorta, just rostral to the left renal artery, using a stainless steel cannula (diameter 0.90 × 20 mm). After removal of the entire intestine and associated vascular bed, the mesenteric vascular bed was separated from the intestine by cutting close to the intestinal wall. Only the arterial branches running from the superior mesenteric trunk to the jejunum were perfused, while all the other branches, including the ileo-colic, right colic, middle colic and the majority of ileal arteries, were tied off. In order to eliminate the internal blood, the mesenteric vascular bed was flushed with a modified Krebs-Henseleit solution of the following composition (mM): NaCl 113; KCl 4.8; $MgSO_4$ 1.2; NaH_2PO_4 1.2; $CaCl_2$ 1.2; $NaHCO_3$ 25; glucose 5.5. The isolated mesenteric vascular bed, mounted on a perfusion system, was perfused with modified Krebs-Henseleit solution, maintained at 37°C, at a constant flow rate of 5 ml min^{-1}, by means of a peristaltic pump (101-ISMATEC SA). A mixture of 95% O_2 and 5% CO_2 was bubbled continuously into the modified Krebs-Henseleit solution. The preparation was also superfused with modified Krebs-Henseleit solution at the rate of 0.5 ml min^{-1}, to prevent drying. Drug solutions were infused directly into the perfusate, proximal to the arterial cannula, using another peristaltic pump. All experiments were carried out in the presence of indomethacin (10 μM) to prevent the interference of cyclo-oxygenase products in lipopolysaccharide-stimulated NO synthesis (Gray et al., 1991). Changes in perfusion pressure were measured using a pressure transducer (ISOTEC) and recorded continuously on a polygraph (GRAPHTEC-WATANABE type 3310).

2.2. Experimental protocols

After an equilibration period of 30–40 min, the perfusion pressure, which is directly correlated to vascular resistance, was nearly 20 mm Hg (resting tone).

2.2.1. Protocol 1: concentration-response curves for noradrenaline

Concentration-response curves for noradrenaline were obtained in mesenteric vascular bed from untreated and lipopolysaccharide-treated rats by measuring the response to each concentration 15 min after addition of each dose, when steady state had been reached.

2.2.2. Protocol 2: NO synthase inhibition in endothelium-intact and endothelium-denuded preparations

The effect of L-NAME (0.2–0.5 mM), an inhibitor of both inducible and constitutive NO synthase isoforms, was evaluated on the contractile response elicited by a submaximal concentration of noradrenaline (basal noradrenaline response) obtained from concentration-response curves for noradrenaline constructed as in Protocol 1. The effect of L-NAME was measured 20 min after addition of the drug in endothelium-intact or endothelium-denuded preparations from untreated and lipopolysaccharide-treated rats.

Similar experiments were performed in preparations infused with 0.2 mM L-Arg, a concentration producing 70% of the maximum effect determined in L-Arg concentration-response curves and able to supply about a two-fold physiological concentration of L-Arg.

Removal of the endothelium (tested by almost complete lack of the vasodilatation elicited by 0.3 μM acetylcholine) was obtained by means of intermittent air flushing for 7 min directly into the mesenteric vascular bed (Burdet and Criscione, 1989).

2.2.3. Protocol 3: concentration-response curves for L-Arg and D-Arg

In noradrenaline-precontracted preparations from untreated and lipopolysaccharide-treated rats, L-Arg or D-Arg was infused in order to obtain concentration-response curves. The effect of each concentration was measured 15 min after addition of each dose.

2.2.4. Protocol 4: time course of noradrenaline perfusion pressure

The mesenteric vascular bed was continuously perfused with modified Krebs-Henseleit solution containing noradrenaline in order to induce vasoconstriction. Within a period of 30 min, the variations of noradrenaline-induced perfusion pressure were recorded in untreated and lipopolysaccharide-treated preparations, without or with L-Arg infusion (0.2 mM).

2.2.5. Protocol 5: effect of guanylate cyclase inhibitor

NO-independent activation of guanylate cyclase was evaluated with Methylene blue (10–20 μM), administered after L-NAME (0.2 mM) treatment in lipopolysaccharide-treated + L-Arg-infused preparations.The effect was measured 20 min after addition of the drug.

2.2.6. Protocol 6: assay for nitrite determination

The presence of NO in the perfusate was determined by measuring the amount of nitrites, the predominant stable oxidation product of NO. Nitrite determination was performed in untreated and lipopolysaccharide-treated preparations, routinely perfused with saline modified Krebs-Henseleit saline containing noradrenaline. In order to obtain detectable nitrite accumulation, samples of perfusate (1 ml) were collected during a period of 45 min at a flow rate of 0.03 ml min^{-1}, under continuous infusion of L-Arg (0.2 mM) alone or both L-Arg (0.2 mM) and L-NAME (0.2–0.5 mM). The results were compared with basal nitrite concentrations in the saline control samples.

Nitrite concentrations in the perfusate were determined using Griess' reagent (1% sulfanilamide, 0.1% napthylethylenediamine, 2.5% ortophosphoric acid) (Green et al., 1982). Briefly, Griess' reagent (1 ml) was mixed with an equal volume of perfusate, and optical density was determined at 550 nm, after 20 min. Nitrite concentrations were calculated on a standard curve constructed by using known concentrations of sodium nitrite from 1 to 50 μM.

2.3. Drugs

Norepinephrine hydrochloride, acetylcholine chloride (Aldrich, Milwaukee, WI, USA); L-arginine, D-arginine, N^{ω}-nitro-L-arginine methyl ester, Methylene blue trihydrate (Sigma, St. Louis, MO, USA) were dissolved in distilled water and then diluted in modified Krebs-Henseleit solution. Indomethacin (Sigma) was dissolved in 4% $NaHCO_3$ solution and lipopolysaccharide (*E. coli* serotype 055: B5, Sigma) was dissolved in saline (0.9 % NaCl).

2.4. Statistical analysis

Results are expressed as the mean ± S.E. for (n) rats per experimental group. Statistical comparisons were made using analysis of variance (ANOVA) and Student's t-test for paired and unpaired data, as appropriate. A P value of < 0.05 was considered statistically significant.

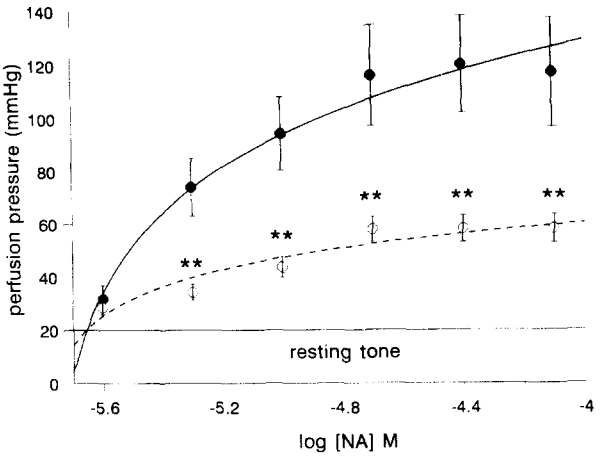

Fig. 1. Concentration-response curves elicited by noradrenaline (NA) in mesenteric vascular bed from untreated (●) and lipopolysaccharide (LPS)-treated rats (○). The effect of each concentration was measured in the steady state, 15 min after addition of each dose. Values are the mean ± S.E. of 7 (untreated rats) and 12 (LPS-treated rats) experiments. ** $P < 0.01$ vs. respective untreated values.

3. Results

3.1. Effects of lipopolysaccharide pretreatment on vascular reactivity to noradrenaline

Concentration-response curves for noradrenaline in mesenteric vascular bed are illustrated in Fig. 1. In vivo treatment with *E. coli* lipopolysaccharide produced a modification in the concentration-response curve for noradrenaline. The maximal vasocontriction was significantly reduced (by 60%) in comparison with that of untreated rats, while no significant change was observed in the potency.

3.2. Effect of L-NAME on vascular noradrenaline reactivity of endothelium-intact and endothelium-denuded preparations

The use of L-NAME, an inhibitor of both inducible and constitutive NO synthase isoforms, gave rise to the results shown in Fig. 2. In endothelium-intact preparations from untreated and lipopolysaccharide-treated groups, a basal noradrenaline response was obtained with submaximal concentrations of noradrenaline (5–10 μM in untreated and 10–20 μM in lipopolysaccharide-treated rats), determined from concentration-response curves constructed as above. Administration of L-NAME (0.2 mM) produced a significant enhancement (+50 mm Hg) of the basal response, which was equivalent in both groups. Endothelium denudation, assessed by almost complete lack of vasodilatation induced by acetylcholine, was performed in order to rule out constitutive NO synthase. This procedure caused a

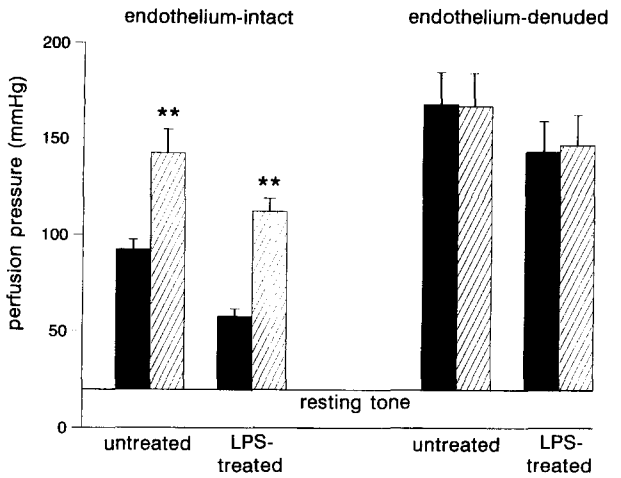

Fig. 2. Effect of addition of 0.2 mM L-NAME to noradrenaline (NA)-precontracted mesenteric vascular bed from untreated (5–10 μM NA) and lipopolysaccharide (LPS)-treated (10–20 μM NA) rats in endothelium-intact and endothelium-denuded preparations. The effect was measured 20 min after addition of L-NAME. Solid columns, control; hatched columns, L-NAME. Values are the mean ± S.E. of 5 experiments. ** $P < 0.01$ vs. respective control.

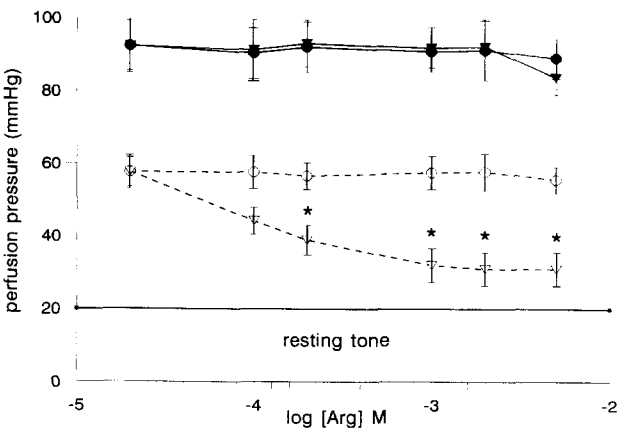

Fig. 3. Concentration-response curves elicited by L-Arg (triangles) and D-Arg (circles) in noradrenaline (NA)-precontracted mesenteric vascular bed from untreated (5–10 μM NA) (closed symbols) or lipopolysaccharide (LPS)-treated rats (10–20 μM NA) (open symbols). The effect of each concentration was measured 15 min after addition of each dose. Values are the mean ± S.E. of 10 (untreated rats) and 9 (LPS-treated rats) experiments. * $P < 0.05$ vs. D-Arg values of LPS-treated rats.

significant increase in the basal response to noradrenaline in untreated and lipopolysaccharide-treated rats; subsequent administration of L-NAME was ineffective in both groups. The same results were obtained using a lower basal perfusion pressure (evoked by 2.5 μM noradrenaline), or a higher concentration (0.5 mM) and more prolonged administration (over 40 min) of L-NAME (data not shown).

3.3. Effects of L-Arg and D-Arg additions

To investigate a possible lipopolysaccharide-induced activation of the L-Arg/NO pathway, the substrate of NO synthase, L-Arg, was infused in mesenteric vascular bed. In lipopolysaccharide-treated rats, L-Arg but not D-Arg produced a dose-dependent relaxation. The minimum significant effect was obtained with 0.2 mM L-Arg and the maximum effect (reduction by 65% of noradrenaline perfusion pressure) was reached with 1 mM. Neither L-Arg nor D-Arg was effective in the preparations obtained from untreated rats (Fig. 3).

3.4. Time course of noradrenaline-induced effects on perfusion pressure

Fig. 4 shows the time course of the noradrenaline-induced vasoconstriction of mesenteric vascular bed from untreated and lipopolysaccharide-treated rats without or with continuous infusion of L-Arg (0.2 mM). A rapid and transient increase in perfusion pressure (peak), reached within 1-2 min, did not significantly differ in all considered groups. In the steady state, reached from 10 min onward, noradrenaline (5–10 μM) produced a submaximal vasoconstriction (70–80% of the maximal response) that was not modified by infusion of L-Arg (0.2 mM). In lipopolysaccharide-treated rats, despite the use of higher noradrenaline concentrations (10–20 μM), the maximal vasoconstriction obtained was, at the steady state, constantly lower than that observed in untreated rats. After noradrenaline, the perfusion pressure of mesenteric vascular bed from lipopolysaccharide-treated rats (57.78 ± 5.1 mm Hg) was significantly depressed (by 48%) compared to that of untreated rats (92.4 ± 6.5 mm Hg). Moreover, lipopolysaccharide-induced noradrenaline hyporeactivity was enhanced by continuous and prolonged infusion of 0.2 mM L-Arg, which produced a further significant decrease

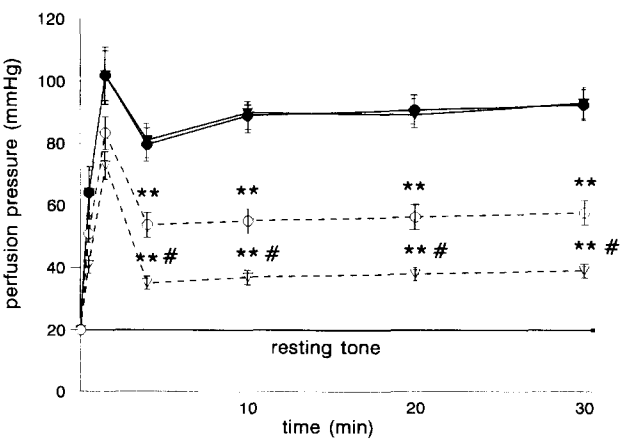

Fig. 4. Time course of noradrenaline (NA)-induced vasoconstriction of mesenteric vascular bed from untreated (5–10 μM NA) (closed symbols) or lipopolysaccharide (LPS)-treated rats (10–20 μM NA) (open symbols), without (circles) or with (triangles) continous infusion of 0.2 mM L-Arg. Values are the mean ± S.E. of 10 (untreated rats), 10 (untreated + L-Arg-infused rats), 17 (LPS-treated rats) and 9 (LPS-treated + L-Arg-infused rats) experiments. ** $P < 0.01$ vs. respective untreated values; # $P < 0.05$ vs. respective LPS-treated values.

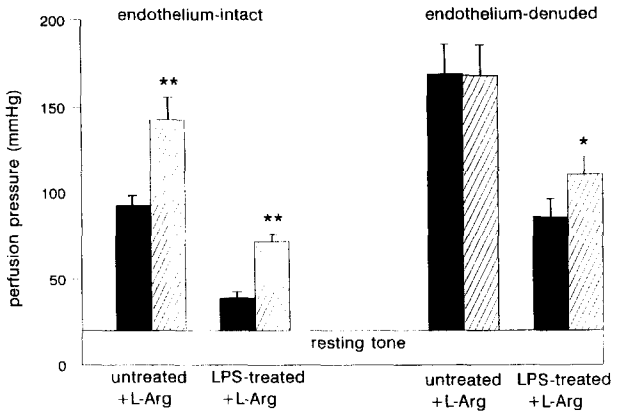

Fig. 5. Effect of addition of 0.2 mM L-NAME to noradrenaline (NA)-precontracted mesenteric vascular bed from untreated + L-Arg-infused (5–10 μM NA) and lipopolysaccharide (LPS)-treated + L-Arg-infused (10–20 μM NA) rats in endothelium-intact and endothelium-denuded preparations. The effect was measured 20 min after addition of L-NAME. Solid columns, control; hatched columns, L-NAME. Values are the mean ± S.E. of 5 (untreated + L-Arg-infused rats), 6 (LPS-treated + L-Arg-infused rats) experiments. * $P < 0.05$, ** $P < 0.01$ vs. respective control.

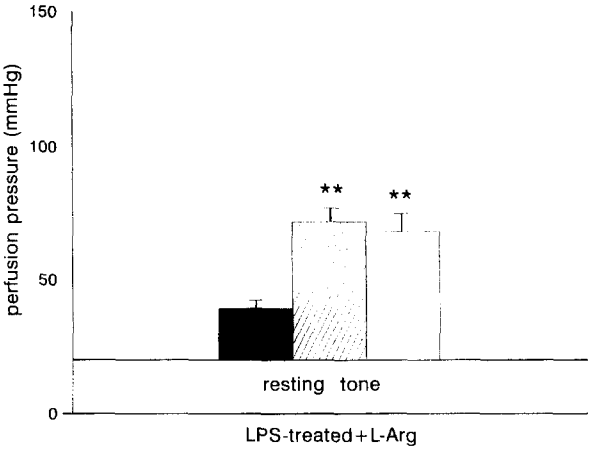

Fig. 6. Effect of addition of 10 μM Methylene blue after 0.2 mM L-NAME treatment in noradrenaline (NA)-precontracted (10–20 μM) mesenteric vascular bed from lipopolysaccharide (LPS)-treated + L-Arg-infused rats. The effect was measured 20 min after addition of each drug. Solid column, control; hatched column, L-NAME; open column, Methylene blue. Values are the mean ± S.E. of 4 experiments. ** $P < 0.01$ vs. control.

(from 57.78 ± 5.1 mm Hg to 39 ± 2.7 mm Hg) in the noradrenaline-induced perfusion pressure of lipopolysaccharide-treated rats.

3.5. Effect of L-NAME on vascular noradrenaline reactivity of endothelium-intact and endothelium-denuded preparations, infused with L-Arg

As shown in Fig. 5, in endothelium-intact preparations from lipopolysaccharide-treated rats infused with L-Arg (0.2 mM), 0.2 mM L-NAME produced a less marked increase in basal noradrenaline response (from 39 ± 2.7 mm Hg to 71.71 ± 4.7 mm Hg) than in untreated preparations (from 92.4 ± 6.6 mm Hg to 122.7 ± 15.2 mm Hg). It was unable to completely restore the response to the basal noradrenaline level of mesenteric vascular bed from untreated rats (92.4 ± 6.5 mm Hg). A higher concentration (0.5 mM) or prolonged administration (over 40 min) of L-NAME did not produce further effects (data not shown).

In endothelium-denuded preparations infused with 0.2 mM L-Arg, subsequent administration of L-NAME was able to further enhance ($P < 0.05$) the perfusion pressure of lipopolysaccharide-treated but not of untreated rats.

In another set of experiments, we tested aminoguanidine (0.2 mM), a potent inhibitor of inducible NO synthase. This drug, administered after 0.2 mM L-NAME, did not produce any additional increase in lipopolysaccharide-treated + L-Arg-infused preparations with or without endothelium (data not shown).

3.6. Effect of Methylene blue after L-NAME treatment

Fig. 6 shows that, after 0.2 mM L-NAME treatment, subsequent administration of 10 μM Methylene blue did not produce any additional increase in perfusion pressure in mesenteric vascular bed from lipopolysaccharide-treated + L-Arg-infused rats. A higher concentration (20 μM) and more prolonged administration (over 40 min) of Methylene blue were ineffective, too (data not shown).

3.7. Effect of L-Arg and L-NAME on nitrite production after lipopolysaccharide treatment

Nitrite, an oxidation product of the interaction of NO with molecular oxygen and water, is a useful parameter in monitoring NO synthase function. Nitrite accumulation was detected in the perfusate under (1) basal conditions, (2) continuous infusion of 0.2 mM L-Arg and (3) continuous infusion of both L-Arg (0.2 mM) and L-NAME (0.2–0.5 mM). The results are reported in Table 1. In untreated rats, addition of L-Arg did not modify the basal production of nitrites. In lipopolysaccharide-treated rats, L-Arg induced a significant increase in nitrite accumulation, which reached

Table 1
Nitrite concentration (μM) in samples of perfusate from mesenteric vascular bed

Perfusion	Untreated rats	Lipopolysaccharide-treated rats
Saline	1.70 ± 0.48	1.05 ± 0.15
Saline + L-Arg	1.51 ± 0.18	5.38 ± 0.17 [a,b]
Saline + L-Arg + L-NAME (0.2 mM)	1.63 ± 0.27	1.71 ± 0.75
Saline + L-Arg + L-NAME (0.5 mM)	1.67 ± 0.22	1.68 ± 0.69

Results are expressed as mean ± S.E. values of 5 experiments. [a] $P < 0.01$ vs. saline + L-Arg sample from untreated rats (grouped t-test). [b] $P < 0.01$ vs. saline and saline + L-Arg + L-NAME samples from LPS-treated rats (ANOVA and Newman-Keuls test). L-Arg: 0.2 mM.

approximately 5 times basal levels. In order to demonstrate that nitrite production was specifically due to L-Arg/NO pathway activation, perfusate nitrite levels were assessed after simultaneous infusion of both L-Arg and L-NAME. The results demonstrate that 0.2 mM L-NAME completely counteracted the L-Arg-stimulated increased nitrite production, restoring the basal nitrite levels. No significantly different results were obtained using 0.5 mM L-NAME.

Similar experiments, performed in the absence of L-NAME treatment, confirmed that the reduction in L-Arg-stimulated nitrite accumulation was specifically produced by L-NAME, and was not due to a spontaneous decrease in nitrite production. In fact, the increased nitrite concentration was not modified in the course of time under continuous infusion of L-Arg (data not shown).

4. Discussion

Several lines of evidence suggest that endotoxin, an initiator of septic shock syndrome, leads to induction of NO synthase with over-production of the vasodilator NO in conductance vessels (Radomski et al., 1990; Rees et al., 1990; Fleming et al., 1991c). This phenomenon is thought to underlie the impaired responsiveness to vasoconstrictors demonstrated in isolated aorta from rats treated with endotoxin (Wakabayashi et al., 1987; Julou-Schaeffer et al., 1990).

However, very few investigations have been carried out in resistance vessels in the course of endotoxin shock, in spite of the fact that resistance vascular districts are known to play a major role in the physiopathology of septic shock syndrome.

Our findings provide the first evidence that in vivo administration of *E. coli* lipopolysaccharide produces hyporesponsiveness to noradrenaline in rat perfused resistance vessels. In previous investigations, Schneider et al. (1992) and Mitchell et al. (1993) failed to find hyporesponsiveness to vasoconstrictor agents in mesenteric vessels from lipopolysaccharide-treated rats. It is possible that these different findings depend on different experimental conditions. In fact, Schneider et al. (1992) investigated noradrenaline-developed tension in third-generation mesenteric artery rings, while our experimental model is a perfused system of resistance vessels, reproducing in vivo conditions, in which stretch or shear stress produced by flow may enhance the release of the vasodilator NO from the endothelium (Ohno et al., 1990). The apparent discrepancy with Mitchell et al. (1993) results may be explained by the observation that these authors simply analyzed the earlier noradrenaline-induced peak contraction, which in our experiments, too, was not modified by lipopolysaccharide-treatment; indeed, solely in the steady state (15 min after noradrenaline infusion), did we observe a reduction in perfusion pressure, indicating a failure of peripheral resistance in lipopolysaccharide-treated rats.

In order to investigate NO pathway activation during endotoxaemia, we tested the effect of L-NAME, an inhibitor of both constitutive and inducible NO synthase.

L-NAME was able to produce the same increase in basal noradrenaline-induced vasoconstriction in mesenteric vascular bed from both lipopolysaccharide-treated and untreated rats. In this connection, it may be suggested that in rat mesenteric vascular bed, NO production by constitutive NO synthase plays an important role in regulating vascular tone. The observation of significantly higher levels of constitutive NO synthase activity in mesenteric vessels in comparison with aorta (Mitchell et al., 1993) seems to be consistent with this hypothesis. Furthermore, it would appear that the lipopolysaccharide-induced noradrenaline hyporeactivity was abolished by the removal of the endothelium, suggesting that it is an endothelium-dependent phenomenon. In these conditions, L-NAME was unable to further increase the noradrenaline-induced vasoconstriction, leading us to hypothesize that endothelium-independent NO overproduction may be excluded in endotoxaemic rats. Nevertheless, this assertion is questionable, because this set of experiments was carried out in the absence of L-Arg, the substrate necessary for demonstrating NO overproduction via an inducible NO synthase localized in vascular smooth muscle.

Biochemical data are in agreement with these findings, since there was no difference in nitrite concentration between untreated and lipopolysaccharide-treated preparations.

However, unlike endothelial cells, in which NO synthase production does not require addition of exogenous L-Arg (Hecker et al., 1990), smooth muscle cells, after NO synthase induction, may have a higher dependence on exogenous L-Arg for NO production (Julou-Schaeffer et al., 1990, 1991). On these grounds, separate sets of experiments were performed under continuous infusion of L-Arg. It was observed that the lipopolysaccharide-induced noradrenaline hyporeactivity was intensified by 0.2 mM L-Arg, which caused a further reduction in the noradrenaline-induced vasoconstriction. The constant presence of L-Arg during the perfusion was necessary to supply an about two-fold circulating Arg concentration (0.08 mM) (Cerra et al., 1979), which has been reported to be sufficient to induce maximal vascular NO-dependent hyporeactivity in vivo in lipopolysaccharide-treated rats (Gray et al., 1991).

Thus, only under these experimental conditions, which reproduce the humoral environment of in vivo septic shock, can the effect of NO inhibitors really be evaluated.

In this regard, the results showed that NO synthase inhibition produced different effects in the presence of L-Arg and in its absence. In fact, L-NAME enhanced noradrenaline-induced vasoconstriction, not only in endothelium-intact but also in endothelium-denuded preparations from lipopolysaccharide-treated + L-Arg-infused rats; no additional increasing effect was obtained after aminoguanidine treatment.

It seems that this enhancing effect in denuded preparations is due to an antagonism of NO overproduction via inducible NO synthase localized in vascular smooth muscle cells. This suggests that NO overproduction is implicated in vascular hyporeactivity and substrate availability becomes a rate-limiting factor for demonstrating inducible NO synthase activity in ex vivo experiments during endotoxaemia. Biochemical assay confirmed these results, in that enhancement of nitrite concentration, antagonizable by L-NAME, occurred only with exogenous L-Arg in lipopolysaccharide-treated preparations.

However, although increased activation of the L-Arg/NO pathway by lipopolysaccharide treatment is certainly involved, it does not entirely account for the noradrenaline hyporesponsiveness of mesenteric vascular bed, since L-NAME was unable to completely restore noradrenaline-induced vasoconstriction in lipopolysaccharide-treated + L-Arg-infused rats to the level in untreated rats.

To verify whether an NO-independent activation of guanylate cyclase by lipopolysaccharide may be responsible for the L-NAME-resistant component of vascular hyporesponsiveness, we tested Methylene blue, since it is able to completely restore lipopolysaccharide-induced noradrenaline hyporeactivity in conductance vessels (Wu et al., 1994; Beasley et al., 1994). In the rat mesenteric district, unlike the aorta, the contribution to endotoxin-induced hyporeactivity of lipopolysaccharide-triggered mediators, like guanylate cyclase activators, may be excluded since Methylene blue did not restore NO-independent hyporesponsiveness. However, an irreversible, guanylate cyclase-independent action of NO cannot be ruled out. Besides NO, other free radicals might contribute to lipopolysaccharide-induced vascular hyporeactivity, since it has been reported that superoxide anion may cause hyperpolarization and hydroxyl radical may activate guanylate cyclase interacting with another site of this enzyme (Marín and Rodríguez-Martínez, 1995).

In conclusion, L-Arg/NO pathway hyperactivation plays a key role in regulating the extent of mesenteric vascular bed hyporeactivity produced by lipopolysaccharide, but it only explains one component of this hyporeactivity. The remaining component, which can be evidenced without the substrate activating NO pathway, could be due to other mechanisms, probably strictly related to the vasoconstriction event, such as modified receptor sensitivity, or alteration of the signal transduction pathway. However, desensitization of α_1-adrenoceptors could also be excluded, since our data showed that no change in noradrenaline potency was observed after lipopolysaccharide treatment. Indeed, as previously reported in aortic rings (Bigaud et al., 1990), it may be hypothesized that in the mesenteric district, the NO-independent impairment of noradrenaline-induced vasoconstriction might be due to alterations in post-receptor mechanisms, thereby implicating Ca^{2+} handling within smooth muscle cells.

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