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Role of nitric oxide in maintaining vascular integrity in endotoxin-induced acute intestinal damage in the rat

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1 The role of endogenous nitric oxide (NO) in maintaining intestinal vascular integrity following acute endotoxin (*E. coli.* lipopolysaccharide) challenge was investigated in the anaesthetized rat by use of N^G-monomethyl-L-arginine (L-NMMA), a selective inhibitor of NO synthesis.

2 L-NMMA (10–50 mg kg^{-1}, i.v.) pretreatment enhanced both the macroscopic and histological intestinal damage and the increases in vascular permeability, measured as the leakage of [^{125}I]-labelled human serum albumen, induced after 15 min by endotoxin (50 mg kg^{-1}, i.v.).

3 The effects of L-NMMA (50 mg kg^{-1}, i.v.) were enantiomer specific, as D-NMMA had no effect. Furthermore, these effects were reversed by L-arginine (300 mg kg^{-1}, i.v.), the precursor of NO synthesis but not by D-arginine (300 mg kg^{-1}, i.v.).

4 L-NMMA (10–50 mg kg^{-1}, i.v.) increased mean systemic arterial blood pressure but this does not appear to be the mechanism by which endotoxin-induced intestinal damage was enhanced, since similar systemic pressor responses induced by phenylephrine (10 μg kg^{-1} min^{-1}, i.v.), had no such effect.

5 The results suggest that synthesis of NO from L-arginine has a role in maintaining the microvascular integrity of the intestinal mucosa following acute endotoxin challenge.

Introduction

Endotoxic shock is characterized by hypotension, intravascular coagulation, increases in vascular permeability, haemoconcentration and gastro-intestinal damage. These effects of endotoxin may result from a direct action of this lipopolysaccharide component of bacterial cell walls on the vascular endothelium (Harlan *et al.*, 1983; Meyrick *et al.*, 1986) or as a consequence of the release of secondary mediators (Parker & Parillo, 1983). In recent studies the vasoactive mediators, platelet activating factor (PAF) and thromboxane A_2 (TXA_2), were found to play key roles in the gastro-intestinal haemorrhagic damage induced by endotoxin in the rat (Wallace *et al.*, 1987; Whittle *et al.*, 1987; Boughton-Smith *et al.*, 1989). In other studies prostacyclin, which is a potent vasodilator and inhibitor of platelet aggregation, attenuated the pathological effects of endotoxin (Lefer *et al.*, 1989; Krausz *et al.*, 1981; Smith *et al.*, 1985; Ditter *et al.*, 1988).

Other endogenous mediators that affect the vasculature may also have a protective role and therefore be involved as a defence mechanism in endotoxic shock. Interest has recently focused on nitric oxide (NO), the labile vasodilator formed from L-arginine by endothelial cells (Palmer *et al.*, 1987; 1988), and which was originally characterized as endothelium-derived relaxing factor or EDRF (Furchgott & Zawadzki, 1980; Furchgott, 1983). In addition to endothelial cells, NO can also be formed by other cells, including macrophages and neutrophils (Hibbs *et al.*, 1988; Marletta *et al.*, 1988; McCall *et al.*, 1989), which may be involved in endotoxic shock. The synthesis of NO by these cells can be selectively inhibited by the L-arginine analogue, N^G-monomethyl-L-arginine (L-NMMA) (Hibbs *et al.*, 1987; Palmer *et al.*, 1988; McCall *et al.*, 1989). Inhibition of endothelial-derived NO by L-NMMA in anaesthetized animals produces an increase in systemic arterial blood pressure and inhibits the hypotensive action of acetylcholine and other endothelium-dependent vasodilators (Rees *et al.*, 1989; Whittle *et al.*, 1989), suggesting that NO may have an important regulatory role on the vasculature *in vivo*. In the present study, therefore, the role of endogenous NO in maintaining vascular integrity in a model of endotoxin-induced acute intestinal damage in the rat was investigated using L-NMMA.

A preliminary account of some of this work has been presented to the British Pharmacological Society (Hutcheson *et al.*, 1990).

Methods

Endotoxin-induced jejunal damage

Male Wistar rats (225–275 g) which had been deprived of food but not water overnight (18–24 h) were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}, i.p.) and a 25 gauge needle (Butterfly-25, Venisystems) inserted into a tail vein. Lipopolysaccharide from *E. coli* (LPS) was administered as a bolus intravenous injection (4 ml kg^{-1}) at a dose of 50 mg kg^{-1}, which in preliminary dose-range studies gave a moderate degree of intestinal damage. Control animals received isotonic saline (4 ml kg^{-1}) by the same route. After 15 min, a segment of jejunum (6 cm) taken from a region 10–15 cm distal to the pylorus was opened longitudinally and gross macroscopic damage assessed by use of a scoring system of 0 (normal) to 3 (severe damage) based on the degree of hyperaemia and vasocongestion by an observer unaware of the treatment (Boughton-Smith *et al.*, 1989).

Histological damage, in wax-embedded sections (4 μm) of jejunum stained with haemotoxylin and eosin, was assessed under light microscopy in a randomised manner and a scoring system was used where; 0 = normal; 1 = focal regions of vasocongestion; 2 = extensive vasocongestion of the sub-epithelial vessels and congestion to the deeper mucosa; 3 = extensive vasocongestion of the entire depth of the mucosa and submucosal haemorrhage.

Plasma leakage

The vascular permeability and haemorrhage produced by LPS was determined as the gastrointestinal leakage of ^{125}I-labelled human serum albumen ([^{125}I]-HSA, 10 μCi, 37 GBq) administered 20–30 min before LPS or saline as a bolus intravenous injection (150 μl). After a further 15 min, a segment of jejunum (6 cm) was taken as described above, blotted dry, weighed (wet weight) and [^{125}I]-HSA measured with a gamma spectrom-

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eter (Nuclear Enterprises, NE1600). [^{125}I]-HSA was determined in plasma (100 μl) prepared (2 min; 900 g) from blood, drawn from the abdominal aorta and plasma leakage expressed as $\mu l\,g^{-1}$ of tissue.

Blood pressure and haematological analysis

A carotid artery of the anaesthetized rat was cannulated for measurement of systemic arterial blood pressure (Elcomatic EM 750A pressure transducer). In addition, blood samples (100 μl) were collected from the carotid arterial cannula, immediately prior to and 15 min after LPS, for the determination of leukocytes (WBC), erythrocytes (RBC) and haematocrit (HCT) on a Clay Adams Haematology Analyser 5.

Drug treatment

Groups of rats were pretreated with L-NMMA (10, 25 or 50 mg kg^{-1}), its D-enantiometer, D-NMMA (50 mg kg^{-1}) or saline, 15 min prior to LPS injection. The doses of L-NMMA were chosen from previous studies in which they were shown to inhibit endothelium-dependent vasodilatation *in vivo* and the *ex vivo* generation of NO by vascular tissue (Rees *et al.*, 1989; Whittle *et al.*, 1989). In experiments to determine the specificity of L-NMMA, L-arginine (150–300 mg kg^{-1}) or D-arginine (300 mg kg^{-1}) were injected intravenously 5 min after L-NMMA. To determine the importance of the systemic hypertension induced by L-NMMA, control studies were undertaken in which 5 min prior to LPS, the α-adrenoceptor agonist phenylephrine (10 μg kg^{-1} min^{-1}) was administered into a cannulated femoral vein, at a dose that provided a similar increase in blood pressure to that produced by L-NMMA (50 mg kg^{-1}) and continued for 20 min.

Drugs and materials

N^{G}-monomethyl-L-arginine (L-NMMA) and its enantiomer, D-NMMA, were synthesized in the Department of Medicinal Chemistry, WRL by Dr H. Hodson. *E. coli* lipopolysaccharide (0111:B4), L- and D-arginine and phenylephrine were from Sigma Chemical Company and [^{125}I]-labelled human serum albumen was from Amersham International.

Statistical analysis

The data are expressed as the mean ± s.e.mean of (n) rats per experimental group. Statistical comparisons for parametric data were made by Student's t test for unpaired data, except for haematological studies in which a Student's t test for paired data was used. The Mann-Whitney U-test was used for statistical comparisons of non-parametric data. A probability of $P < 0.05$ was considered as statistically significant.

Results

Jejunum damage

As shown in Figure 1, LPS (50 mg kg^{-1} i.v.) produced a significant level of damage in the rat jejunum after 15 min, ($P < 0.05$ compared to saline control) which was characterized macroscopically as a diffuse hyperaemia (damage score, 1.3 ± 0.2, $n = 15$). L-NMMA (10–50 mg kg^{-1}, i.v.) administered prior to LPS, dose-dependently enhanced the jejunal damage. The higher dose of L-NMMA produced extensive damage, with haemorrhage into the jejunal lumen. The enantiomer, D-NMMA (50 mg kg^{-1}) had no significant effect on LPS-induced jejunal damage (Figure 1). In control experiments, treatment with L-NMMA (50 mg kg^{-1}) alone did not produce jejunal damage ($n = 4$). Administration of L-arginine (150 and 300 mg kg^{-1}, i.v.) after L-NMMA, dose-dependently

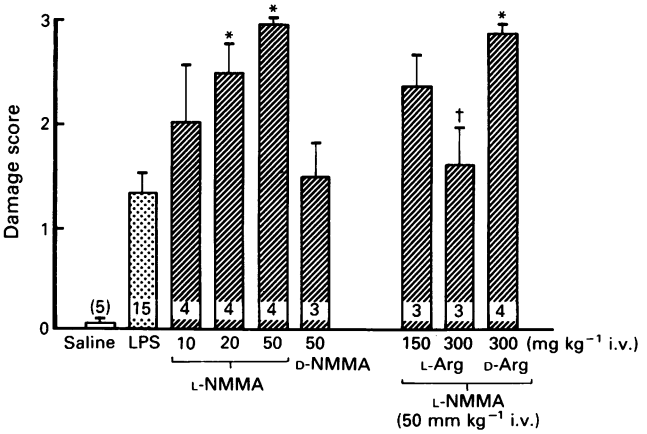

Figure 1 Enhancement of *E. coli* lipopolysaccharide (LPS; 50 mg kg^{-1}, i.v.)-induced macroscopic jejunal damage in the rat by pretreatment with N^{G}-monomethyl-L-arginine. (L-NMMA, 10–50 mg kg^{-1}, i.v.) and the effect of L-arginine (150 and 300 mg kg^{-1}, i.v.) or D-arginine (300 mg kg^{-1}, i.v.). Jejunal damage was scored macroscopically (0–3 scale) in a randomized manner in segments of tissue taken 15 min after LPS. Results are the mean and vertical bars indicate s.e.mean of n (number in column) rats per experimental group. Statistically significant difference from LPS control, is shown as * $P < 0.05$ and the effect of L-arginine as † $P < 0.05$.

inhibited the enhancement of LPS-induced jejunal damage. Thus, L-arginine (300 mg kg^{-1}) completely reversed the damage to the level produced by LPS alone, whereas D-arginine (300 mg kg^{-1}) had no significant effect (Figure 1). L-Arginine (300 mg kg^{-1}) alone had no significant effect on the jejunal damage produced by LPS alone ($n = 4$).

Histological damage

LPS produced moderate vasocongestion in the jejunal villi (score 0.9 ± 0.1, $n = 4$) as shown in Figure 2. However, as described for the macroscopic damage, pretreatment with L-NMMA (50 mg kg^{-1}) enhanced the jejunal vasocongestion produced by LPS and also induced distinct haemorrhage (Figure 2). Furthermore, the enhancement of histological damage was reduced (80 ± 24% inhibition, $n = 6$, $P < 0.05$) by L-arginine (300 mg kg^{-1}, i.v.) but not by D-arginine (Table 1).

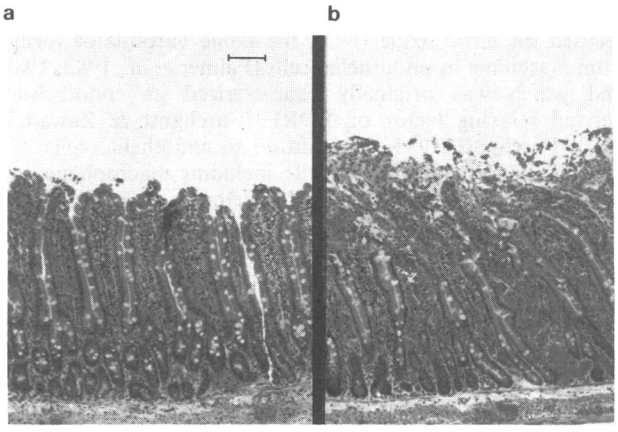

Figure 2 Section of jejunum from rat treated with *E. coli* lipopolysaccharide (LPS: 50 mg kg^{-1}, i.v.) (a) alone or (b) following pretreatment with N^{G}-monomethyl-L-arginine (L-NMMA, 50 mg kg^{-1}, i.v.). The vasocongestion following LPS is limited to the upper portion of the jejunal villi. In animals pretreated with L-NMMA, the vasocongestion and engorgement extent throughout the entire depth of the mucosa. In addition there is haemorrhage and loss of cells from the tips of the villi. (Haematoxylin and eosin).

Table 1 Enhancement of *E. coli* lipopolysaccharide (LPS)-induced jejunal damage by N^G-monomethyl-L-arginine (L-NMMA) and prevention by L-arginine

Treatment ($mg\,kg^{-1}$ i.v.)	(*n*)	*Histological damage*	*% LPS control*
LPS (50)	(4)	0.9 ± 0.1	100 ± 11
LPS + L-NMMA (50)	(6)	2.7 ± 0.2**	300 ± 22**
LPS + L-NMMA + L-arginine (300)	(6)	1.3 ± 0.4	144 ± 44
LPS + L-NMMA + D-arginine (300)	(7)	2.6 ± 0.3**	289 ± 33**

Histological jejunal damage after 15 min was assessed in sections (4 μm) stained with haematoxylin and eosin in a randomised blinded manner by a scoring system of 0 = normal; 1 = focal regions of vasocongestion; 2 = extensive vasocongestion of subepithelial vessels and congestion of deeper mucosa; 3 = extensive vasocongestion of the entire depth of the mucosa and mucosal haemorrhage. Results are given as mean ± s.e.mean of (*n*) experiments, where statistical significant difference from control using Mann-Whitney U-test is shown as ** $P < 0.01$.

Plasma leakage

LPS alone produced a significant plasma leakage into the jejunum (net leakage, $286 \pm 44\,\mu l\,g^{-1}$ of tissue, $n = 10$, $P < 0.001$) that was enhanced dose-dependently by pretreatment with L-NMMA (10–50 $mg\,kg^{-1}$) as shown in Figure 3. At the highest dose, L-NMMA (50 $mg\,kg^{-1}$) enhanced net plasma leakage by $70 \pm 4\%$ ($n = 13$, $P < 0.01$), whilst D-NMMA (50 $mg\,kg^{-1}$) had no effect. L-Arginine (300 $mg\,kg^{-1}$), inhibited the enhanced plasma leakage ($85 \pm 15\%$ inhibition, $n = 5$, $P < 0.05$) produced by L-NMMA (50 $mg\,kg^{-1}$, i.v.), whereas D-arginine (300 $mg\,kg^{-1}$, i.v.) was without effect (Figure 3).

LPS also induced plasma leakage in the stomach, duodenum and ileum (Figure 4) and this was also significantly ($P < 0.05$) enhanced by pretreatment with L-NMMA (50 $mg\,kg^{-1}$). There was, however, no significant plasma leakage in the colon after LPS alone, or following pretreatment with L-NMMA (Figure 4).

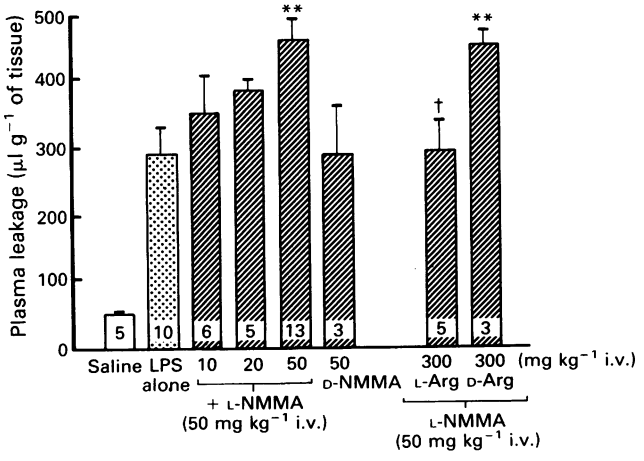

Figure 3 Enhancement of *E. coli* lipopolysaccharide (LPS; 50 $mg\,kg^{-1}$, i.v.) induced increases in intestinal plasma leakage by pretreatment with N^G-monomethyl-L-arginine (L-NMMA, 10–50 $mg\,kg^{-1}$, i.v.) and the effect of L-arginine (L-Arg, 300 $mg\,kg^{-1}$, i.v.) or D-arginine (D-Arg, 300 $mg\,kg^{-1}$ i.v.). The leakage of [^{125}I]-labelled human serum albumen after 15 min administered i.v. 30–40 min prior to LPS was measured in segments of rat jejunum. Results are the mean, and vertical bars indicate s.e.mean of *n* (number in column) rats per group. Statistically significant difference from LPS control is shown as ** $P < 0.01$ and the effect of L-arginine as † $P < 0.05$.

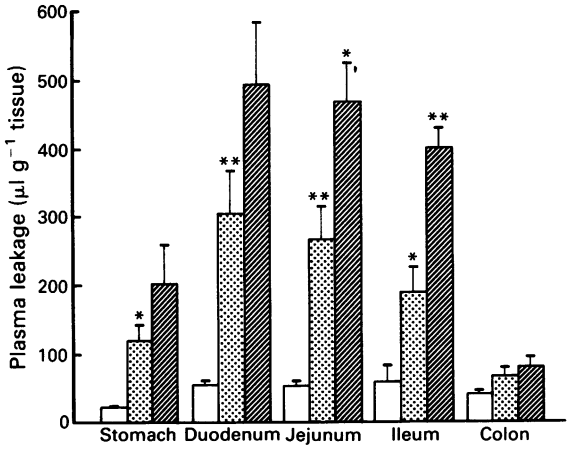

Figure 4 Increases in plasma leakage induced by *E. coli* lipopolysaccharide (LPS, 50 $mg\,kg^{-1}$, i.v.) in different regions of the gastrointestinal tract and enhancement by N^G-monomethyl-L-arginine (L-NMMA, 50 $mg\,kg^{-1}$, i.v.). The plasma leakage of [^{125}I]-labelled human serum albumen, administered 30–40 min before either saline (open columns) LPS (stippled columns) or LPS and L-NMMA (hatched columns) was measured in segments of gastro-intestinal tissue after 15 min. Results are the mean and vertical bars indicate s.e.mean of 4 rats per group. Statistically significant differences between either saline control vs LPS or between LPS vs LPS and L-NMMA are shown as * $P < 0.05$, ** $P < 0.01$.

Systemic arterial blood pressure

The initial resting mean systemic arterial blood pressure (BP) of 127 ± 4 mmHg was reduced to 90 ± 8 mmHg ($n = 11$, $P < 0.05$), 15 min after LPS. L-NMMA (10–50 mg kg^{-1}, i.v.) produced a dose-dependent increase in BP (of 22 ± 1 mmHg; 28 ± 3 mmHg and 31 ± 2 mmHg, at 10, 20 and 50 $mg\,kg^{-1}$ respectively, $P < 0.001$, $n = 4$), that persisted for the duration of the experiment (30 min). The systemic vasopressor effect of L-NMMA (50 $mg\,kg^{-1}$) did not, however, prevent the decrease in BP induced by LPS, which fell to a level (95 ± 5 mmHg, $n = 15$), not significantly different from that induced by LPS alone. When L-arginine (300 $mg\,kg^{-1}$) was administered 5 min after L-NMMA, BP returned to resting levels within 5 min, while D-arginine (300 $mg\,kg^{-1}$) did not reverse the pressor effects of L-NMMA ($n = 4$). D-NMMA (50 $mg\,kg^{-1}$) had no significant effect on BP ($n = 4$).

Haematological analysis

The mean haematological parameters for control blood samples were HCT, $48 \pm 1\%$; RBC, $6.8 \pm 2 \times 10^6\,mm^{-3}$ and WBC, $5.8 \pm 0.4 \times 10^3\,mm^{-3}$. LPS induced a small increase in HCT ($7 \pm 1\%$ increase above control, $n = 6$, $P < 0.05$) and RBC count ($12 \pm 1\%$ increase, $n = 6$; $P < 0.01$) after 15 min (Figure 5). Pretreatment with L-NMMA (50 $mg\,kg^{-1}$) markedly potentiated the increase in HCT and RBC produced by LPS (to $30 \pm 3\%$ and $21 \pm 5\%$ increase above control HCT and RBC respectively, ($n = 8$, $P < 0.01$). Despite the haemoconcentration induced by LPS alone and following pretreatment with L-NMMA, there was no significant change in WBC count. There was, however, a reduction in the WBC/RBC ratio of $11 \pm 1\%$ ($n = 6$) after LPS and of $18 \pm 2\%$ ($n = 8$) after L-NMMA and LPS, suggesting a relative loss of leucocytes from the circulation. The haematological parameters were not affected by L-NMMA (50 $mg\,kg^{-1}$) or L-arginine (300 $mg\,kg^{-1}$) alone ($n = 4$).

Effect of phenylephrine

Intravenous infusion of phenylephrine ($10\,\mu g\,kg^{-1}\,min^{-1}$) produced a sustained increase in BP of 32 ± 7 mmHg ($n = 3$), comparable to that induced by L-NMMA (50 $mg\,kg^{-1}$).

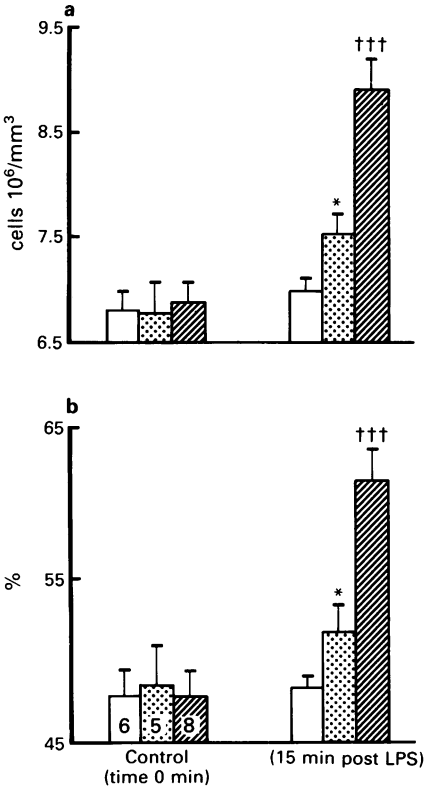

Figure 5 Enhancement of *E. coli* lipopolysaccharide (50 mg kg^{-1}, i.v.)-induced increases in (a) red blood cell count (RBC) and (b) haematocrit (HCT) by pretreatment (15 min) with N^G-monomethyl-L-arginine (L-NMMA, 50 mg kg^{-1}, i.v.). RBC and HCT were measured in blood samples collected from the carotid artery before (time 0) and 15 min after saline (open columns), LPS (stippled columns) or L-NMMA and LPS (hatched columns). Results are the mean, vertical bars indicate s.e.mean of 5–8 rats per group. Statistically significant difference from saline control is shown as * $P < 0.05$ and enhancement above LPS control as ††† $P < 0.001$.

However, concurrent administration of phenylephrine did not significantly affect the jejunal damage (score, 0.8 ± 0.6, $n = 3$) or net plasma leakage (215 ± 70 μl g^{-1} tissue, $n = 3$) induced by LPS.

Discussion

The present study demonstrates that the arginine analogue, L-NMMA, a specific inhibitor of NO synthesis (Palmer *et al.*, 1988), enhances LPS-induced intestinal damage that occurs after 15 min and that the enhancement is prevented by L-arginine, the physiological precursor of NO synthesis. These findings, therefore, indicate that the synthesis of NO from L-arginine has an important role in maintaining the integrity of the intestinal mucosa following acute challenge with LPS.

The enhancement by L-NMMA of the acute macroscopic and histological jejunal damage and the plasma leakage induced by LPS was enantiomer specific, since D-NMMA, which does not inhibit NO biosynthesis (Palmer *et al.*, 1988), had no effect, while the effects of L-NMMA were not reversed by D-arginine. The nature of the jejunal damage, which involved vasocongestion and distinct haemorrhage, implicates vascular injury as an important primary event. In addition to these local intestinal effects, L-NMMA also enhanced the increase in systemic erythrocyte count and haematocrit produced by LPS, suggesting changes in vascular permeability.

Although in the present study, L-NMMA did not attenuate the acute fall in blood pressure induced by this dose of LPS, its systemic vasopressor effects may have confounded any distinct inhibitory actions. Thus, the contribution of endogenous NO release in the complex cardiovascular changes associated with endotoxin shock is not clear. These systemic vasopressor actions of L-NMMA do not seem to be the mechanism of enhanced intestinal damage, since phenylephrine, infused at a dose sufficient to produce a similar vasopressor response as L-NMMA, did not enhance LPS-induced intestinal damage. It is possible however, that L-NMMA and phenylephrine may exert differential local responses in vascular beds, and the effect of L-NMMA on the jejunum may thus be a consequence of changes in regional microvascular blood flow. The inhibition of NO synthesis by L-NMMA may, by removing this endogenous vasodilator, indirectly produce local vasoconstriction and decrease intestinal vascular perfusion. Indeed, L-NMMA substantially decreases vascular conductance in the mesenteric vascular bed of conscious rats (Compton *et al.*, 1989; Gardiner, 1990). In addition, L-NMMA in comparable doses to those used in the present study, has been shown to decrease rat gastric mucosal blood flow (Pique *et al.*, 1989) and also to lead to mucosal damage when the release of other local vasodilator mediators was concurrently inhibited (Whittle *et al.*, 1990).

In previous studies, inhibitors of the potent vasoconstrictor TXA_2 attenuated the intestinal damage produced by LPS (Boughton-Smith *et al.*, 1989). Therefore, the extent of intestinal damage may depend on the local balance within the intestinal microcirculation of damaging vasoconstrictors and protective vasodilators. Indeed, in animal models of endotoxic shock, infusion of the potent vasodilator, prostacyclin, exerted protective effects (Lefer *et al.*, 1989; Krausz *et al.*, 1981; Smith *et al.*, 1985; Ditter *et al.*, 1988), which were attributed to vasodilatation, as well as to inhibition of platelet aggregation and suppression of thromboxane formation. Since NO is also a potent vasodilator that inhibits platelet aggregation, and in addition can inhibit platelet adhesion to the vascular endothelium (Radomski *et al.*, 1987a,b) it may, like prostacyclin, have a local protective role under these conditions. The simultaneous activation of adenylate cyclase by prostacyclin and of guanylate cyclase by NO, as has been demonstrated in platelets (Radomski *et al.*, 1986c), may produce a potentiating interaction on different cells affecting the vasculature, and thereby act synergistically to maintain vascular integrity. The effects of NO-releasing nitrovasodilators, alone or in combination with prostacyclin, in models of vascular damage such as that following endotoxic shock therefore warrant investigation.

The enhancement of acute intestinal damage by L-NMMA resulted in haemorrhage, and therefore the observed increases in intestinal plasma leakage ([^{125}I]-HSA) represent, at least in part, overt vascular damage. The marked enhancement by L-NMMA of LPS-induced haemoconcentration, suggests that a significant part of the plasma leakage is also due to a profound increase in vascular permeability. The mechanism by which the removal of NO by L-NMMA may reduce blood flow to the jejunum but paradoxically increase plasma leakage is not clear, but may reflect the substantial extent to which vascular integrity is compromised by LPS.

Several studies have shown that LPS can stimulate the synthesis of NO. Treatment *in vivo* with *E. coli* LPS increases the amount of NO_3^-, an oxidative product of NO, in the urine of rats (Wagner *et al.*, 1983) and in the blood of mice (Stuehr & Marletta, 1985). Furthermore, in a study *in vitro*, LPS stimulates the formation of NO-like activity by vascular endothelium (Salvemini *et al.*, 1989) and increases NO, NO_2^- and NO_3^- production by mouse macrophages, (Stuehr & Marletta, 1985; Marletta *et al.*, 1988). In addition, the synthesis of NO by rat neutrophils can be stimulated by the bacterial peptide fMet-Leu-Phe (FMLP) and leukotriene B_4 (McCall *et al.*, 1989). In the present study, therefore, the enhancement of acute intestinal damage by L-NMMA could be due to both inhibition of the basal synthesis of NO and also prevention of increases in NO synthesis by leukocytes or endothelial cells in response to LPS stimulation. The failure of L-arginine to reverse the intestinal damage produced by LPS alone suggests that under these conditions, there is sufficient endogenous substrate for NO synthesis to be maximally stimulated.

The mechanisms by which LPS increases vascular permeability, vascular damage and haemorrhage in the jejunum may also involve the generation of reactive oxygen molecules. Endotoxin can prime phagocytic leukocytes to release oxygen radicals (Pabst & Johnston, 1980; Weiss & LoBuglio, 1982) and can stimulate the formation of oxygen radicals indirectly by releasing secondary mediators, such as PAF, or by activation of the complement system which additionally stimulate neutrophils (Sacks *et al.*, 1978). There is considerable evidence that oxygen radicals derived from activated neutrophils can damage endothelial cells and produce increases in vascular permeability (Fantone & Ward, 1982, for review). In addition, the small intestine is also particularly sensitive to ischaemia-reperfusion injury, which has been shown in a cat model to be neutrophil- and oxygen radical-dependent (Granger *et al.*, 1981; Hernandez *et al.*, 1987). This may explain why in the present study, the plasma leakage induced by LPS and its enhancement by L-NMMA was greatest in the small intestine, with no effect on the colon. Therefore, the mechanism of intestinal injury by LPS may involve reactive radicals formed by either activated leukocytes, or possibly, by endothelial cells. Studies *in vitro* have shown that NO can interact with the superoxide radical (O_2^-) to produce a loss in activity of both moieties (Palmer *et al.*, 1987; McCall *et al.*, 1989). Furthermore, similar biological effects of stimulated neutrophils on platelet aggregation can be achieved by either scavenging O_2^- with superoxide dismutase or by the addition of L-arginine, which increases the level of NO formation (McCall *et al.*, 1989). Thus endogenous NO released from endothelial cells or activated phagocytic leukocytes may serve to reduce the acute microvascular damage produced by LPS by scavenging the O_2^- moiety.

The apparent loss of leukocytes from peripheral blood observed following LPS and its enhancement by L-NMMA may be due to neutrophil aggregates becoming lodged in the intestinal microcirculation. The formation of such aggregates has been previously described following PAF-induced gastrointestinal damage (Wallace & Whittle 1986) and may be an important underlying mechanism in the vascular damage produced by PAF and LPS. Neutrophil aggregation can be inhibited by NO (McCall *et al.*, 1988) and it is feasible that NO may also prevent neutrophil adhesion to the endothelium, as has previously been shown for the adhesion of platelets (Radomski *et al.*, 1987b). Such effects may therefore be additional mechanisms by which NO could limit neutrophil-dependent damage to the vascular endothelial cells.

The present study with L-NMMA suggests that endogenous NO has an important acute protective role in the intestinal microvasculature against blood-borne toxins and tissue-destructive mediators. The role of NO in other inflammatory conditions in which there are increases in vascular permeability involving either neutrophils, oxygen radicals or vasoactive mediators, thus requires investigation.

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