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Interactions of constitutive nitric oxide with PAF and thromboxane on rat intestinal vascular integrity in acute endotoxaemia

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1 The involvement of endogenous platelet activating factor (PAF) and thromboxane A_2 in the acute microvascular damage in the ileum and colon induced by the nitric oxide (NO) synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME) following endotoxin administration was investigated in the rat over a 1 h period.

2 Administration of L-NAME (1–10 mg kg^{-1}, s.c.) concurrently with *E. coli* lipopolysaccharide (LPS; 3 mg kg^{-1}, i.v.) dose-dependently increased vascular permeability in the ileum and colon, as determined by the leakage of radiolabelled albumin, and caused macroscopic mucosal damage in the ileum determined 1 h later. Neither LPS administration nor L-NAME (5 mg kg^{-1}) alone affected resting vascular permeability.

3 Infusion of phenylephrine (10 μg kg^{-1} min^{-1}, i.v. for 1 h) caused an elevation in blood pressure similar to that found following L-NAME administration (5 mg kg^{-1}, i.v. or s.c.), but did not increase intestinal vascular permeability, when administered with LPS (3 mg kg^{-1}, i.v.).

4 The increased vascular permeability in the ileum and colon and macroscopic damage in the ileum, induced by L-NAME (5 mg kg^{-1}, s.c.) and LPS (3 mg kg^{-1}, i.v.) was dose-dependently inhibited following s.c. pretreatment (15 min before challenge) with the thromboxane synthase inhibitors, OKY 1581 (5–25 mg kg^{-1}) or 1-benzyl-imidazole (1–50 mg kg^{-1}), or with the thromboxane receptor antagonist, BM 13177 (0.2–2 mg kg^{-1}).

5 Pretreatment with the cyclo-oxygenase inhibitor, indomethacin (2–5 mg kg^{-1}, s.c., 15 min before challenge) reduced the microvascular injury in the ileum and colon and macroscopic lesions in the ileum, observed after the concurrent administration of L-NAME and LPS.

6 Pretreatment (15 min) with the PAF-receptor antagonists, WEB 2086 (0.5–1 mg kg^{-1}, s.c.) or BN 52021 (2.5–10 mg kg^{-1}, s.c.) likewise attenuated this intestinal vascular injury.

7 Combined administration of low doses of 1-benzyl-imidazole (1 mg kg^{-1}) with WEB 2086 (0.5 mg kg^{-1}) 15 min before L-NAME and LPS challenge, abolished this vascular damage and macroscopic injury.

8 These results suggest that PAF and thromboxane A_2 are released acutely following challenge with a low dose of endotoxin. However, these mediators do not appear to injure the intestinal microvascular bed unless NO synthase is concurrently inhibited. Such findings support the protective role of constitutively-formed NO, counteracting the injurious vascular actions of cytotoxic mediators released under pathological conditions.

Keywords: Nitric oxide; PAF; thromboxanes; intestinal mucosal injury; intestinal vascular permeability; N^G-nitro-L-arginine methyl ester

Introduction

Nitric oxide (NO), formed from L-arginine, plays an important role in the maintenance of gastrointestinal microvascular and mucosal integrity (Whittle *et al.*, 1990; Kubes & Granger, 1992; Whittle, 1993). The beneficial action of endogenous NO, synthesized by the Ca^{2+}-dependent constitutive enzyme in the early phase of endotoxaemia, has been suggested from the findings that administration of the NO synthase inhibitor, N^G-monomethyl-L-arginine (L-NMMA) augmented the acute mucosal damage in the gastrointestinal tract provoked by high doses of endotoxin (Hutcheson *et al.*, 1990). Moreover, L-NMMA or the more potent NO synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME) induces an acute substantial increase of vascular permeability in the rat small and large intestine following the intravenous injection of low doses of endotoxin (László *et al.*, 1994a).

The mechanism of the acute effects of endotoxin on the gastrointestinal integrity are not fully understood and may result from a direct injurious action on the vascular endothelium (Harlan *et al.*, 1983; Meyrick *et al.*, 1986). However, following exposure to high doses of lipopolysaccharide (LPS), such intestinal injury appears to cause the early release of tissue damaging secondary mediators, including platelet-activating factor (PAF) or thromboxane A_2 (Wallace *et al.*, 1987; Boughton-Smith *et al.*, 1989). An injurious role or PAF as a mediator of gastrointestinal mucosal damage is supported by the observation that PAF induces gastric haemorrhagic necrosis (Rosam *et al.*, 1986) and causes significant microvascular injury in the gastrointestinal tract (Wallace *et al.*, 1987; Boughton-Smith *et al.*, 1992; Filep & Földes-Filep, 1993). Furthermore, the immediate hypotension following the administration of high doses of endotoxin can be prevented by the PAF-receptor antagonist, WEB 2086 (Casals-Stenzel, 1987; Szabó *et al.*, 1993). In other studies, the increased survival rate after the treatment with thromboxane synthase inhibitors and thromboxane receptor antagonists indicates a pathological involvement of thromboxane A_2 in endotoxaemic states (Wise *et al.*, 1980; Halushka *et al.*, 1983; Olanoff *et al.*, 1985).

The aim of the present study was to evaluate the possible

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role of PAF and thromboxanes in the acute intestinal mucosal damage and microvascular injury provoked by inhibition of NO synthesis with L-NAME following challenge by a low dose of endotoxin in the rat. Thus, the effects of two structurally unrelated PAF-receptor antagonists, BN 52021 and WEB 2086 (Casals-Stanzel, 1987; Koltai et al., 1991a,b) have been investigated. Likewise, the actions of the thromboxane synthase inhibitor, 1-benzyl-imidazole and the non-imidazole compound OKY 1581, along with the thromboxane receptor antagonist BM 13177 (Hamid-Bloomfield & Whittle, 1989; Boughton-Smith et al., 1989) and the cyclooxygenase inhibitor, indomethacin, have been evaluated.

Part of this work has been presented to the British Pharmacological Society (László et al., 1994b).

Methods

Experimental protocol

Male Wistar rats (225–275 g) were fasted overnight, but received water *ad libitum*. Under halothane anaesthesia *E. coli* lipopolysaccharide (3 mg kg^{-1}, i.v.) ^{125}I-labelled human serum albumin ([^{125}]-HSA; 2 μCi kg^{-1}, i.v.) and L-NAME (1–10 mg kg^{-1}, s.c.) were injected. Indomethacin (2 and 5 mg kg^{-1}), OKY 1581 (5–25 mg kg^{-1}), 1-benzyl-imidazole (BZI; 1–50 mg kg^{-1}), BM 13177 (0.2–2 mg kg^{-1}), BN 52021 (2.5–10 mg kg^{-1}) or WEB 2086 (0.1–1 mg kg^{-1}) were administered s.c. 15 min prior to LPS and L-NAME (5 mg kg^{-1}, s.c.) treatment. The doses of these agents were selected on the basis of previous studies in the rat (Casals-Stanzel, 1987; Wallace et al., 1987; Boughton-Smith et al., 1989; Hamid-Bloomfield & Whittle, 1989; Martinez-Cuesta et al., 1992).

In further studies, BZI (1 mg kg^{-1}, s.c.) and WEB 2086 (0.5 mg kg^{-1}, s.c.) were injected concurrently, 15 min before challenge with LPS (3 mg kg^{-1}, i.v.) and L-NAME (5 mg kg^{-1}, s.c.). Plasma leakage in the ileum and colon, and mucosal damage in the ileum was determined after 1 h.

For the evaluation of intestinal macroscopic injury and vascular permeability changes, tissue was removed 1 h after endotoxin injection.

Plasma leakage

As a measure of vascular endothelial damage, plasma leakage of [^{125}I]-HSA was determined in the ileum and colon. Under halothane anaesthesia, blood was collected from the abdominal aorta into syringes containing trisodium citrate (final concentration 0.318%) and centrifuged (10,000 *g*, 10 min, 4°C). The [^{125}I]-HSA content of the plasma and segments of the ileum and colon was determined in a gamma-spectrometer (Nuclear Enterprises NE 1600) and the albumin content in intestinal tissue was calculated as described previously (Boughton-Smith et al., 1993). Values from control tissue were subtracted from the values of treated tissue and the data were expressed as change in (Δ) plasma leakage, μl plasma g^{-1} tissue.

Evaluation of macroscopic lesions

Segments of the ileum were photographed and the gross macroscopic damage to the ileum was assessed by an observer unaware of the experiment, using a scoring system 0–IV, where 0 was the normal tissue; I = mild hyperaemia; II = more severe hyperaemia with one or two vasocongested streaks; III = vasocongested streaks and IV = confluent vasocongestion. It was not, however, possible to grade macroscopic mucosal injury in the colon, since although the colonic tissue appeared oedematous, well-demarcated damage was not clearly apparent.

Comparison of the effect of phenylephrine infusion and L-NAME on blood pressure and plasma leakage

Systemic arterial blood pressure was measured over a 1 h period from the right carotid artery of rats under pentobarbitone anaesthesia (60 mg kg^{-1}, i.p.) using a blood pressure transducer (Elcomatic) connected to a Grass Polygraph. L-NAME (5 mg kg^{-1}) was administered s.c. or intravenously as a bolus injection and phenylephrine was infused into the tail vein (10 μg kg^{-1} min^{-1}).

In a separate study, the phenylephrine infusion (10 μg kg^{-1} min^{-1}, i.v.) was initiated concurrently with LPS administration (3 mg kg^{-1}, i.v.) in pentobarbitone-anaesthetized rats. Plasma leakage in the ileum and colon were determined 1 h later. Likewise, the action of L-NAME (5 mg kg^{-1}, s.c.) administered concurrently with LPS on intestinal plasma leakage in pentobarbitone-anaesthetized rats was determined after 1 h.

Materials

E. coli lipopolysaccharide (0111:B4), indomethacin (dissolved in 1.25% sodium bicarbonate), phenylephrine and L-NAME (dissolved in isotonic saline) were obtained from Sigma Chemical Co. (Poole, Dorset). ^{125}I-labelled human serum albumin was from Amersham International (U.K.). OKY 1581 (sodium (E)-3[4-(3-pyridylmethyl)phenyl]-2-methylacrylate) was a gift from ONO Pharmaceuticals, 1-benzylimidazole and BM 13177 (4-[2-benzyenesulphonamide)-ethyl]-phenoxyacetic acid) were synthesized in the Wellcome Research Laboratories, BN 52021 (ginkgolide B, 9H-1,7a-(epoxymethanol)-1H,6aH, cyclopenta[c][2-3-b]furo-[3,2′:3,4] cyclopenta-[1,2-d]furan 5,9,12-[4H]trione, 3 *tert*-butylhexahydro 4,7b,11,hydroxy-8-methyl) and WEB 2086 ((3-[4-(2-chlorophenyl)-9-methyl-6H-thieno[3,2-f][1,2,4]triazolo-[4,3-a] [1,4]diazepine-2-yl]-1-(4-morpholinyl)-1-propanone) were supplied by IHB-IPSEN Research Laboratories and by Boehringer Ingelheim K.G., respectively. The compounds were dissolved in isotonic saline or in the case of BN 52021, in the supplied vehicle, which was used in control studies.

Statistics

Data are expressed as mean ± s.e.mean of (*n*) observations. For statistical comparisons, analysis of variance with the Bonferroni test was utilised. $P < 0.05$ was taken as significant.

Results

Effect of L-NAME on endotoxin-induced intestinal plasma leakage and mucosal damage

Administration of LPS (3 mg kg^{-1}, i.v.) did not cause plasma leakage in the ileum or colon (Figure 1), or macroscopic injury in the ileum (Table 1) when determined 1 h after challenge. Concurrent administration of L-NAME (1–10 mg kg^{-1}, s.c.) with LPS, however, dose-dependently induced plasma leakage in the intestine with a maximum increase of Δ430 ± 59 and Δ315 ± 40 μl g^{-1} tissue in the ileum and in the colon, respectively ($P < 0.001$), as shown in Figure 1. The combination of LPS and L-NAME (5 mg kg^{-1}, s.c.) also provoked macroscopic injury in the ileum over a 1 h period (Table 1). Administration of L-NAME (5 mg kg^{-1}, s.c.) alone did not cause plasma leakage in the ileum and colon ($n = 8$ and 11).

Effect of thromboxane synthase inhibitors and receptor antagonist on intestinal plasma leakage and macroscopic damage

The administration of the thromboxane synthase inhibitors, OKY 1581 (5–25 mg kg^{-1}, s.c.) and BZI (1–50 mg kg^{-1}, s.c.)

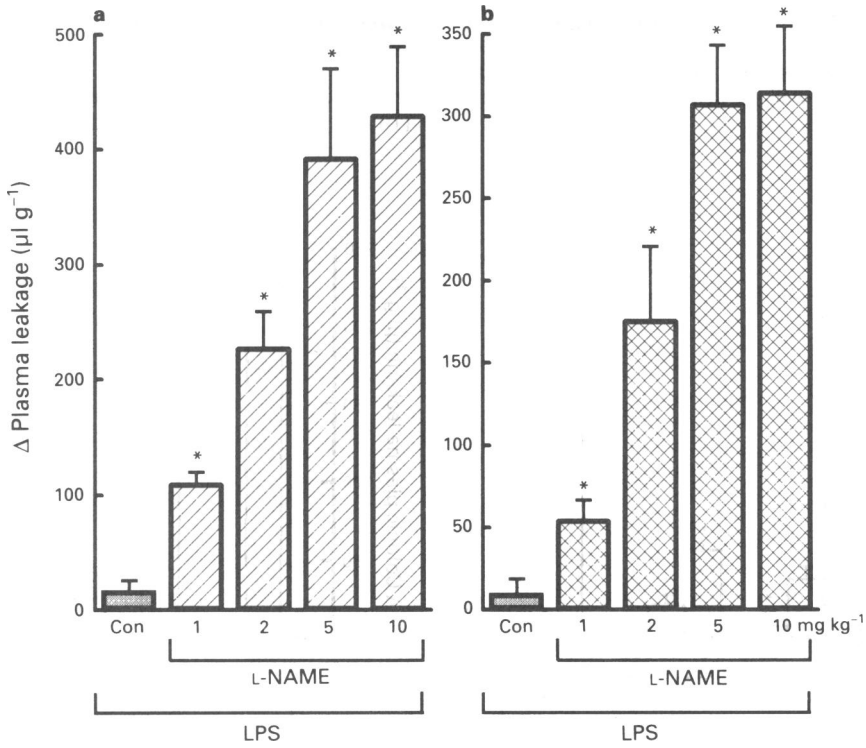

Figure 1 Potentiation of lipopolysaccharide (LPS, 3 mg kg^{-1}, i.v.)-induced vascular leakage of plasma, determined using radiolabelled albumin, in (a) rat ileum and (b) colon by concurrent administration of N^G-nitro-L-arginine methyl ester (L-NAME, 1–10 mg kg^{-1}, s.c.) after 1 h. The columns show the leakage of plasma (Δ μl g^{-1} tissue) 1 h after injection of LPS alone (stippled column) or LPS with L-NAME (hatched columns). Data are given as the mean ± s.e.mean of 12 (control) and 8, 8, 10, 8 rats per group, respectively; statistical significance is shown as $*P < 0.05$ compared to the LPS-alone group (Con).

Table 1 Macroscopic injury induced by concurrent administration of lipopolysaccharide (LPS, 3 mg kg^{-1}, i.v.) and N^G-nitro-L-arginine methyl ester (L-NAME, 5 mg kg^{-1}, s.c.) in the mucosa of the rat ileum: inhibition by pretreatment (15 min, s.c.) with the thromboxane synthase inhibitors, OKY 1581 and 1-benzyl-imidazole (BZI), the thromboxane receptor antagonist, BM 13177 and the platelet-activating factor receptor antagonists, WEB 2086 and BN 52021

Treatment	*Dose* (mg kg^{-1})	*Damage index*
Saline		0.13 ± 0.13
LPS		0.54 ± 0.22
LPS + L-NAME		2.57 ± 0.30*
+ OKY 1581	5.0	1.75 ± 0.31
	10.0	1.50 ± 0.19*
	25.0	0.88 ± 0.13*
+ BZI	5.0	1.08 ± 0.18*
	50.0	0.50 ± 0.19*
+ BM 13177	0.2	2.60 ± 0.24
	2.0	0.60 ± 0.24*
+ WEB 2086	0.1	3.13 ± 0.23
	1.0	1.22 ± 0.22*
+ BN 52021	2.5	2.80 ± 0.29
	10.0	1.05 ± 0.25*

Data are expressed as a damage index (on a 0–4 scale) and calculated as mean ± s.e.mean of at least 4 rats per group, where statistical significance is shown as $*P < 0.05$ compared with the LPS group, $*P < 0.05$ vs. LPS + L-NAME group.

dose-dependently attenuated plasma leakage in the ileum and colon (Figures 2 and 3), and prevented the macroscopic injury in the ileum induced by LPS and L-NAME (Table 1).

The thomboxane receptor antagonist, BM 13177 (0.2–2 mg kg^{-1}, s.c.) also dose-dependently inhibited ileal and colonic plasma leakage (Figures 2 and 3), and protected the ileum against macroscopic injury induced by LPS and L-NAME (Table 1).

Effects of PAF antagonists on intestinal plasma leakage and macroscopic damage

The plasma leakage in the ileum and colon, caused by LPS and L-NAME was dose-dependently diminished by pretreatment with the PAF-receptor antagonists, WEB 2086 (0.1–1 mg kg^{-1}, s.c.) or BN 5202 (2.5–10 mg kg^{-1}, s.c.) as shown in Figure 4. These agents also significantly reduced the macroscopic injury in the ileum (Table 1).

Effect of indomethacin on intestinal plasma leakage and mucosal injury

Indomethacin (2 and 5 mg kg^{-1}, s.c.) reduced the intestinal plasma leakage induced by the concurrent administration of LPS and L-NAME (by 93 ± 3% and 100% in the ileum, and 88 ± 6% and 100% in the colon, respectively, $P < 0.001$; $n = 4–8$), and also protected the ileum against macroscopic damage (by 68 ± 16% and 93 ± 5%, respectively; $P < 0.001$).

Interaction between BZI and WEB 2086 on intestinal plasma leakage and mucosal injury

Administration of a low dose of BZI (1 mg kg^{-1}, s.c.) had no effect on plasma leakage induced by the concurrent administration of LPS and L-NAME in the ileum and colon, and did not protect the ileal mucosa against macroscopic lesions (Figure 5). Administration of WEB 2086 (0.5 mg kg^{-1}, s.c.) reduced L-NAME and LPS-induced plasma leakage in the ileum and colon (by 59 ± 6% and 62 ± 5%, respectively; $P < 0.01$; $n = 8$ and 10), but did not reduce significantly ileal

macroscopic injury. By contrast, when BZI (1 mg kg^{-1}, s.c.) and WEB 2086 (0.5 mg kg^{-1}, s.c.) were injected together prior to the administration of LPS and L-NAME, ileal and colonic plasma leakage as well as ileal macroscopic damage was abolished (Figure 5).

Effect of phenylephrine infusion on blood pressure and intestinal plasma leakage

In pentobarbitone-anaesthetized rats, phenylephrine infusion (10 μg kg^{-1} min^{-1}, i.v.) caused a similar elevation in blood

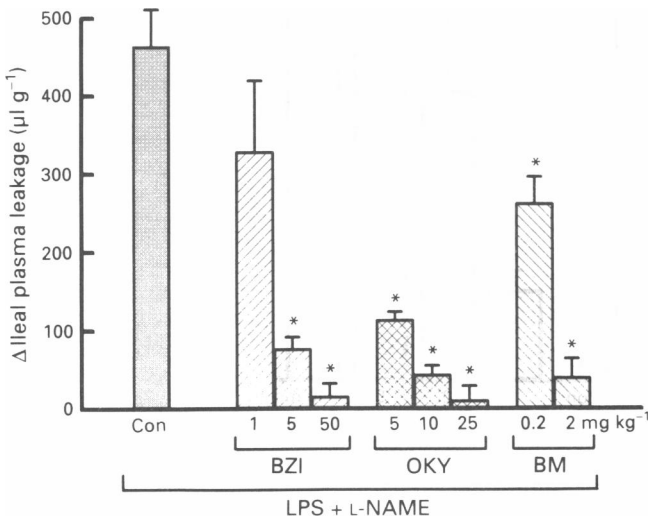

Figure 2 Inhibition by OKY 1581 (OKY, 5–25 mg kg^{-1}, s.c.), 1-benzyl-imidazole (BZI, 1–50 mg kg^{-1}, s.c.) and BM 13177 (BM, 0.2–2 mg kg^{-1}, s.c.) pretreatment (15 min before endotoxin) of vascular leakage of radiolabelled albumin induced by concurrent administration of lipopolysaccharide (LPS, 3 mg kg^{-1}, i.v.) and N^{G}-nitro-L-arginine methyl ester (L-NAME, 5 mg kg^{-1}, s.c.) in the ileum. Albumin leakage (expressed as plasma leakage, Δ μl g^{-1} tissue) was determined 1 h after challenge. Data are shown as the mean ± s.e.mean of 12 (control) and 6, 10, 8, 6, 7, 7, 4, and 4 rats per group, respectively; statistical significance is shown as $*P < 0.05$ compared to the LPS + L-NAME group (Con).

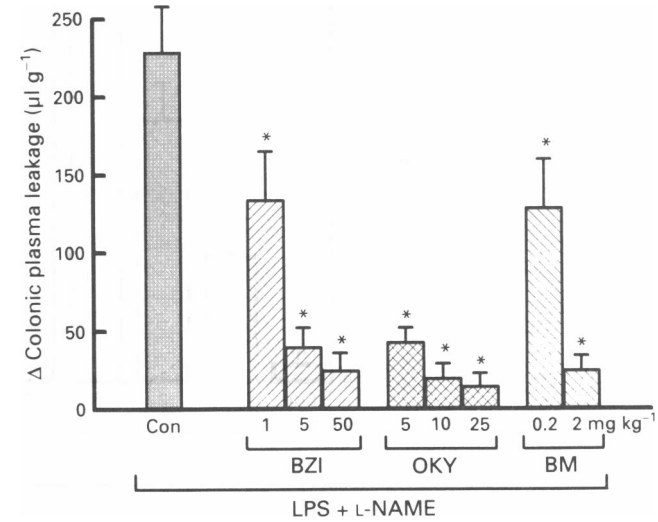

Figure 3 Inhibition by OKY 1581 (OKY, 5–25 mg kg^{-1}, s.c.), 1-benzyl-imidazole (BZI, 1–50 mg kg^{-1}, s.c.) and BM 14177 (BM, 0.2–2 mg kg^{-1}, s.c.) pretreatment (15 min before endotoxin) of vascular leakage of radiolabelled albumin induced by concurrent administration of lipopolysaccharide (LPS, 3 mg kg^{-1}, i.v.) and N^{G}-nitro-L-arginine methyl ester (L-NAME, 5 mg kg^{-1}, s.c.) in the colon. Albumin leakage (expressed as plasma leakage, Δ μl g^{-1} tissue) was determined 1 h after challenge. Data are shown as the mean ± s.e.mean of 12 (control) and 6, 10, 8, 6, 7, 7, 4, and 4 rats per group, respectively; statistical significance is shown as $*P < 0.05$ compared to the LPS + L-NAME group (Con).

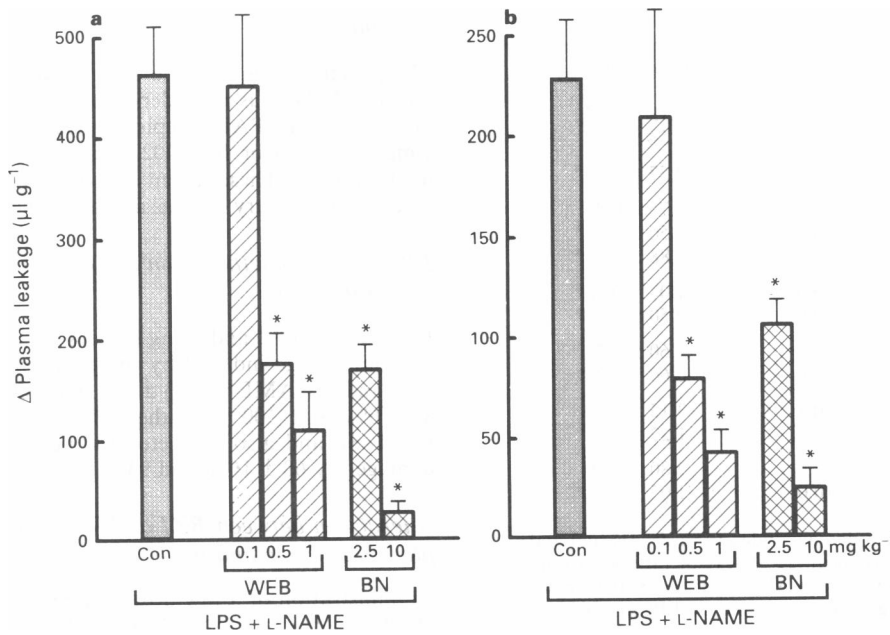

Figure 4 Inhibition by WEB 2086 (WEB, 0.1–1.0 mg kg^{-1}, s.c.) and BN 52021 (BN, 2.5–10 mg kg^{-1}, s.c.) pretreatment (15 min before endotoxin) of vascular leakage of radiolabelled albumin induced by concurrent administration of lipopolysaccharide (LPS, 3 mg kg^{-1}, i.v.) and N^{G}-nitro-L-arginine methyl ester (L-NAME, 5 mg kg^{-1}, s.c.) in the ileum (a) and colon (b). Albumin leakage (expressed as plasma leakage, Δ μl g^{-1} tissue) was determined 1 h after challenge. Data are shown as the mean ± s.e.mean of 12 (control) and 7, 12, 9, 6 and 8 rats per group, respectively; statistical significance is shown as $*P < 0.05$ compared to the LPS + L-NAME group (Con).

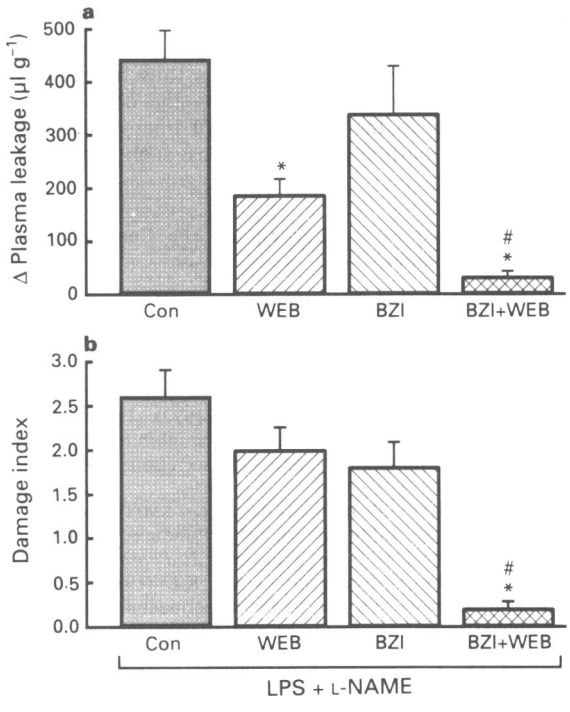

Figure 5 Effect of WEB 2086 (WEB, 0.5 mg kg^{-1}, s.c.) and 1-benzyl-imidazole (BZI, 1 mg kg^{-1}, s.c.) pretreatment (15 min before endotoxin) on vascular leakage (a) and macroscopic damage (b) induced by concurrent administration of lipopolysaccharide (LPS, 3 mg kg^{-1}, i.v.) and N^G-nitro-L-arginine methyl ester (L-NAME, 5 mg kg^{-1}, s.c.) in the ileum. Albumin leakage (expressed as plasma leakage, μl g^{-1} tissue) and macroscopic damage (damage index: 0–4) were determined 1 h after challenge. Data are shown as the mean ± s.e.mean of 10 (control) and 8, 8 and 10 rats per group, respectively; statistical significance is shown as $^*P < 0.05$ compared to the LPS + L-NAME group (Con), $^\#P < 0.05$ vs. WEB or BZI groups.

pressure (BP) to that observed following the bolus administration of L-NAME (5 mg kg^{-1}, i.v.) over a 1 h period. Thus, the BP changes were Δ 46 ± 11 and Δ 41 ± 6 mmHg after 15 and 60 min of phenylephrine infusion, compared with Δ 48 ± 11 and Δ 43 ± 5 mmHg at similar times after L-NAME administration ($P < 0.001$ for each compared with values from control, untreated rats; $n = 4$ and 5). Administration of LPS (3 mg kg^{-1}) had no significant effect on BP over the experimental period (Δ − 7 ± 11 mmHg after 1 h). In LPS challenged rats, the increase in BP, determined 15 min following intravenous administration of L-NAME (5 mg kg^{-1}) or infusion of phenylephrine (10 μg kg^{-1} min^{-1}), was Δ 37 ± 9 and Δ 52 ± 6 mmHg, respectively ($n = 4$; $P < 0.01$ from control, untreated rats), while after 1 h, the BP values were Δ 24 ± 8 and Δ 22 ± 2 mmHg, respectively ($n = 4$). Likewise, the increase in BP following subcutaneous administration of L-NAME (5 mg kg^{-1}) in LPS-treated rats was Δ 36 ± 7 mmHg after 15 min and Δ 23 ± 6 mmHg after 1 h ($n = 5$). Thus, following LPS administration, the changes in BP caused by these doses of L-NAME (either by the i.v. or s.c. route) were not significantly different from those of the intravenous infusion of phenylephrine at either time-point during the 1 h observation period.

Phenylephrine infusion (10 μg kg^{-1} min^{-1}, i.v.) had no effect on plasma leakage in the ileum and colon of LPS-treated pentobarbitone-anaesthetized rats during the 1 h investigation period (Δ 0 ± 5 and Δ 0 ± 4 μl g^{-1} in ileal and colonic tissue in the LPS alone group, and Δ 0 ± 19 and Δ 0 ± 3 μl g^{-1} tissue in the LPS and phenylephrine group, respectively; $n = 4$ and 6). By contrast, administration of L-NAME (5 mg kg^{-1}, s.c.) and LPS in pentobarbitone-anaesthetized rats increased plasma leakage in the ileum and colon by Δ 473 ± 16 and Δ 329 ± 45 μl g^{-1} tissue, respectively ($n = 5$, $P < 0.001$).

Discussion

This study confirms our recent observations that the administration of a low dose of LPS does not cause any acute vascular or mucosal damage of the intestine (Boughton-Smith *et al.*, 1993), whereas extensive acute injury can be provoked by concurrent administration of the NO synthase inhibitor, L-NAME (László *et al.*, 1994a). The increased plasma leakage in the ileum and colon following concurrent administration of L-NAME and endotoxin over a 1 h period appears unrelated to elevation of systemic arterial blood pressure and a consequent increase in microvascular hydrostatic pressure, since phenylephrine infusion, in a dose that increased blood pressure to a similar extent to L-NAME, did not affect intestinal vascular permeability following endotoxin administration. It is possible however, that there are differences in the local changes in intestinal blood flow with these two agents, but interpretation of comparative studies on the mucosal microcirculation would be complex. These acute actions of L-NAME have previously been shown to be abolished by L-arginine (László *et al.*, 1994a) and thus appear to reflect inhibition of constitutive NO synthase, expression of the inducible NO synthase in rat intestinal tissue not being detected until 3 h after endotoxin challenge (Salter *et al.*, 1991; Boughton-Smith *et al.*, 1993).

Administration of high doses of endotoxin acutely elevates the tissue concentration of both PAF and thromboxane A_2 in the rat intestine, and these mediators may be involved the associated damage in the intestinal vasculature and mucosa (Whittle *et al.*, 1987; Boughton-Smith *et al.*, 1989). In the present study, low doses of endotoxin in combination with L-NAME were used to induce intestinal injury and unlike a previous study over 15 min using high doses of endotoxin and L-NMMA (Hutcheson *et al.*, 1990), plasma leakage was observed in the colon as well as in the ileum after 1 h. Under these conditions the PAF-receptor antagonists, WEB 2086 and BN 52021 reduced plasma leakage and mucosal damage in both intestinal tissues during this early phase of endotoxaemia. Furthermore, the thromboxane synthase inhibitors, 1-benzyl-imidazole and OKY 1581, as well as the thromboxane receptor antagonist, BM 13177 inhibited such plasma leakage and mucosal damage. The reduction by indomethacin of the plasma leakage and mucosal injury is likely to reflect inhibition of thromboxane formation, as seen previously when endotoxin alone was used (Boughton-Smith *et al.*, 1989), and suggests that cyclo-oxygenase products do not play a key role in the maintenance of microvascular integrity in the early phase of endotoxaemia.

The potent vasodilator actions of endogenous NO may protect the intestinal microvascular bed and the consequent mucosal injury by effectively opposing the vasoconstrictor actions of thromboxane A_2 (Whittle, 1993). Furthermore, NO may prevent microthrombotic occlusion of the microvasculature provoked by thromboxane, as it can inhibit platelet aggregation and the adhesion of platelets to the vascular endothelium (Radomski *et al.*, 1987a,b). Since PAF-induced microvascular stasis and mucosal damage appears to involve the aggregation of neutrophils and other inflammatory cells in the microcirculation (Wallace & Whittle, 1986; Whittle *et al.*, 1986) and neutrophil adhesion is implicated in vascular injury (Kubes *et al.*, 1990), NO could also maintain intestinal microcirculatory perfusion by preventing such neutrophil aggregation and adhesion (McCall *et al.*, 1988; Kubes *et al.*, 1991). Indeed, S-nitroso-*N*-acetyl-penicillamine, a spontaneous generator of NO, inhibits gastrointestinal plasma leakage induced by endotoxin and by PAF (Boughton-Smith *et al.*, 1990; 1992) and PAF-induced increase of vascular permeability in the gut can be potentiated by L-NAME (Filep & Földes-Filep, 1993).

The present study suggests that PAF and thromboxanes are released following the administration of low doses of endotoxin, but do not cause acute injury to the intestinal microvasculature unless there is concurrent inhibition of NO synthase. The pronounced inhibitory effect of the combination of PAF-receptor antagonist and thromboxane synthase inhibitor could suggest interactions between the damaging effects of these endogenous mediators. It therefore appears that the maintenance of intestinal microvascular and mucosal integrity during the acute phase of endotoxaemia depends on complex regulatory mechanisms, involving the balance between the release of aggressive mediators and the beneficial actions of NO. These present findings thus further support the protective role of NO, formed by the constitutive NO synthase enzyme, in the modulation of microvascular integrity under acute pathological conditions, such as in the early phase of sepsis.

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