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The effect of endotoxin on sympathetic responses in the rat isolated perfused mesenteric bed; involvement of nitric oxide and cyclo-oxygenase products

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1 The effects of endotoxin on the vasoconstrictor responses to sympathetic nerve stimulation (SNS) were investigated in the rat isolated perfused mesenteric bed.

2 Rats received either saline (0.1 ml h^{-1}) or endotoxin (2.5 mg kg^{-1} h^{-1}) intravenously for 4 h; the mesenteric beds were then isolated, perfused with Krebs and prepared for SNS (50 V, 3 ms, 7-40 Hz).

3 SNS caused a frequency-dependent vasoconstrictor response which was abolished by either tetrodotoxin (10^{-7} M), prazosin (2.4×10^{-7}M) or guanethidine (2.4×10^{-7} M).

4 In mesenteric vascular beds removed from rats infused with endotoxin, there were markedly impaired vasoconstrictor responses to SNS, although responses to noradrenaline were not modified.

5 Removal of the endothelium with distilled water prevented endotoxin-induced impairment of vasoconstrictor responses to SNS, without modifying these responses in preparations from control rats.

6 Pretreatment with dexamethasone (3 mg kg^{-1} i.p. 1 h before commencing endotoxin or saline infusions) did not modify responses to SNS in control rats but prevented the effects of endotoxin.

7 Both L-NAME (10^{-3} M) and indomethacin (10^{-5} M) restored responses to SNS in preparations from endotoxin-treated rats without modifying these responses in control preparations. However, co-administration of L-NAME and indomethacin markedly augmented responses in both control and endotoxin-treated preparations.

8 The effects of L-NAME were reversed by addition of L-arginine (10^{-3} M).

9 The data suggest that endotoxin impairs the release of noradrenaline and that this effect is secondary to increased production of nitric oxide and prostanoids, possibly by the endothelium.

Keywords: Endotoxin; mesenteric vascular bed; endothelium; nitric oxide; cyclo-oxygenase; dexamethasone; sympathetic stimulation

Introduction

One of the consequences of the administration of bacterial lipopolysaccharide (endotoxin) to experimental animals, or its release from Gram negative organisms in patients with sepsis, is impaired responsiveness to vasoconstrictor agents such as noradrenaline (Parratt 1973; Fink *et al.*, 1985; Auclair *et al.*, 1986; Evequoz *et al.*, 1987) and to sympathetic nerve stimulation (Gray *et al.*, 1990a; Guc *et al.*, 1991; Tomikawa & Okabe, 1992). This reduced responsiveness, which also occurs in pithed animals (Guc *et al.*, 1990; 1992) and in blood vessels isolated from animals given endotoxin (Pomerantz *et al.*, 1982; Wakabayashi *et al.*, 1987; Bigaud *et al.*, 1990) has been shown to be due mainly to the induction of nitric oxide synthase (NOS) and to a subsequent elevation of guanosine 3':5'-cyclic monophosphate (cyclic GMP) (Julou-Schaeffer *et al.*, 1990; Fleming *et al.*, 1990; 1991; Gray *et al.*, 1991). This enzyme induction is inhibited by prior administration of dexamethasone (Knowles *et al.*, 1990; Rees *et al.*, 1990).

Although most of the effects of nitric oxide in impairing vascular responsiveness are at the post-junctional level, because it occurs not only with noradrenaline but with almost all other vasoconstrictor agents including calcium (Bigaud *et al.*, 1990; Gray *et al.*, 1990b; Guc *et al.*, 1990), there is some evidence that endotoxin may also impair the release of noradrenaline from sympathetic neurones (Tomikawa & Okabe, 1992) and that endothelium-derived substances such as nitric oxide might themselves reduce transmitter release (Tesfamariam *et al.*, 1987; Cohen & Weisbrod 1988; Sakuma *et al.*, 1992). The difficulty in examining this possibility in many experimental situations is that endotoxin and nitric oxide markedly influence post-junctional receptor responses to the neural transmitter noradrenaline. We have shown previously (Gray *et al.*, 1988; Bouvier *et al.*, 1994) that responses to exogenous noradrenaline are unimpaired in the mesenteric vascular bed following endotoxin administration, both *in vitro* and *in vivo*, an observation that has since been confirmed for other vasoconstrictor substances such as thromboxane, endothelin, phenylephrine and 5-hydroxytryptamine (5-HT) (Mitchell *et al.*, 1993). We now show that in this particular vascular bed endotoxin results in impaired responsiveness to sympathetic nerve stimulation, and that this is in contrast to responsiveness to exogenous noradrenaline. Further, we show that this is endothelium-dependent, and may involve both nitric oxide and cyclo-oxygenase products.

Methods

Male Sprague-Dawley rats, weighing between 250 and 300 g, were anaesthetized with sodium pentobarbitone (60 mg kg^{-1} by i.p. injection). The right common carotid artery was catheterized for the measurement of blood pressure, and both right and left jugular veins were cannulated for the continuous administration, throughout the experiment, of anaesthetic (sodium pentobarbitone, 36 mg kg^{-1} h^{-1}) and of lipopolysaccharide derived from *Escherichia coli* (055:B5, Boivin preparation, Difco Laboratories, 2.5 mg kg^{-1} h^{-1} in

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0.1 ml h^{-1} saline for 4 h). Control animals received an i.v. infusion of saline at the same rate and volume. In some experiments dexamethasone (3 mg kg^{-1}) was given by i.p. injection 1 h before commencing the endotoxin or saline infusions.

The trachea was cannulated and the animals were allowed to breathe spontaneously. If required artificial ventilation was provided with room air by a Harvard respirator (rate 48 strokes min^{-1}, stroke volume 1.5 ml per 100 g body weight). Body temperature was recorded using a rectal thermistor probe and was maintained at 37 ± 0.5°C using an incandescent lamp placed over the abdomen. Animals were allowed to stabilise for 20–30 min before the administration of endotoxin. At the end of the experiment (i.e. after 4 h of endotoxin or saline administration) the mesenteric beds of the rats were removed as described below.

Isolated perfused mesenteric bed

The abdominal cavity was opened by a mid-line incision through the linea alba and the mesenteric bed excised using the procedure described by McGregor (1965). Animals were given heparin (1000 u kg^{-1} i.v.) just before cannulation of the mesenteric artery and removal of the preparation from the rats. The mesenteric bed was perfused through a cannula inserted into the superior mesenteric artery with Krebs-Henseleit solution of the following composition (mM): NaCl 118.4, KCl 4.7, $MgSO_4$ H_2O 1.2, KH_2PO_4, $2H_2O$ 1.2, $NaHCO_3$ 25, $CaCl_2$ 2.5, and glucose 11.1 in distilled water. This solution was maintained at 37°C, bubbled with 5% CO_2 and 95% O_2 and perfused at a constant rate (2 ml min^{-1}; Gilson Minipuls 2, Anachem). The isolated, perfused preparations were placed on a Petri dish, which was supported in a heated water bath (37°C). The perfusate, which flowed from the cut ends of the vessels at the intestinal margin of the mesentery, was removed at a rate of 2 ml min^{-1} to prevent accumulation in the bath. The tissue was allowed to equilibrate for 30 min before the experiments were started.

The preparations were subjected to sympathetic nerve stimulation (SNS) through a ring electrode around the superior mesenteric artery by a Grass stimulator with supramaximal rectangular pulses (50 V) and a pulse duration of 3 ms. Tissues were allowed to return back to consistent basal prestimulation conditions by only stimulating the nerves every 15–20 min. To determine the possible roles of nitric oxide and prostanoids in the responses to SNS, the preparations were perfused with Krebs-Henseleit solution containing either N^G-nitro-L-arginine methyl ester (L-NAME; 1.3×10^{-3} M), or indomethacin (10^{-5} M) or a combination of both, 20 min before the first stimulation.

In some preparations, changes in perfusion pressure were assessed following the administration of noradrenaline (10^{-8}–10^{-4} M) before, and after, endothelial removal with distilled water (see below) and, following this treatment, in the presence of a combination of indomethacin (10^{-5} M) and L-NAME (1.3×10^{-3} M). The concentration of L-NAME was determined in preliminary experiments and was the lowest concentration producing maximal (39 ± 7%) inhibition of the vasodilator response to acetylcholine in this preparation.

Endothelial removal

To remove endothelial cells the preparations were perfused with distilled water (bubbled with 5% CO_2, 95% O_2, at 37°C) for 5 min (Adeagbo *et al.*, 1994). Endothelium removal was confirmed by the absence of relaxation when acetylcholine (10^{-6} M) was administered to preparations preconstricted with noradrenaline (10^{-5} M).

Drugs

The following drugs were used; (±)-noradrenaline hydrochloride, heparin sodium, N^G-nitro-L-arginine methyl ester hydrochloride, tetrodotoxin, prazosin hydrochloride, guanethidine monosulphate, acetycholine hydrochloride, indomethacin and dexamethasone (all obtained from Sigma Laboratories) and lipopolysaccharide (*Escherichia coli*; Difco). Sodium chloride, potassium chloride, magnesium sulphate, sodium hydrogen carbonate, potassium dihydrogen orthophosphate, D-glucose and calcium chloride were obtained from BDH, Poole, Dorset. Sodium pentobarbitone (Sagatal) was obtained from May & Baker, Dagenham, Essex. Noradrenaline solutions were freshly prepared in distilled water containing ascorbic acid (5 mM) and lyophylised endotoxin was dissolved in 0.9 w/v sodium chloride solution. Indomethacin was dissolved in absolute alcohol and diluted in Krebs-Henseleit solution to give a final concentration of 0.01% alcohol. This concentration did not modify responses to SNS. All other drugs were dissolved in distilled water and then diluted with Krebs-Henseleit solution.

Statistical analysis of data

Results are expressed throughout as means ± s.e.mean and were analysed by an unpaired Student's *t* test, one way ANOVA followed by a Tukey-Kramer multiple comparison test, or a two way ANOVA for single or repeated measurements as appropriate. A *P* value of less than 0.05 was considered to be significant.

Where preparations were subjected to three stimulating frequencies (10, 20, 30 Hz) (or several concentrations of noradrenaline) a two factor repeated measure ANOVA was undertaken to investigate whether or not there was a significant difference between groups or if there was a significant interaction between group and frequencies (or concentrations). Differences were investigated further by applying a one way ANOVA at each frequency (concentration) followed by a Tukey-Kramer multiple comparison test. Statistical analysis was performed by use of Minitab for Windows.

Results

The infusion of saline did not modify mean arterial blood pressure or heart rate (Table 1). Table 1 also shows that endotoxin had no significant effect on mean arterial blood pres-

Table 1 Mean systemic arterial blood pressure (mmHg) and heart rate (beats min^{-1}) in anaesthetized rats infused with either saline or *E. coli* endotoxin (2.5 mg kg^{-1} h^{-1}) for 4 h

	Time (h)					
	0 h		2 h		4 h	
	Pressure	*Heart rate*	*Pressure*	*Heart rate*	*Pressure*	*Heart rate*
Saline (control)	125 ± 2	390 ± 10	120 ± 2	385 ± 10	114 ± 2	368 ± 15
Endotoxin	124 ± 2	353 ± 15	79 ± 4*	307 ± 15*	68 ± 3**	299 ± 28*
Saline after Dex	130 ± 3	391 ± 30	128 ± 2	409 ± 7	119 ± 3	406 ± 12
Endotoxin after Dex	135 ± 2	373 ± 19	96 ± 1**	386 ± 13†	85 ± 5**‡	377 ± 17*†

*$P<0.05$; **$P<0.001$ compared with corresponding values at 0 h, †$P<0.05$; ‡$P<0.01$ compared with endotoxin alone, $n=7$ for each treatment. The reduction in blood pressure following endotoxin administration was less marked in those rats pretreated with dexamethasone (Dex, 3 mg kg^{-1} 1 h previously).

sure after 1 h of infusion but significantly reduced pressure by 2 h and at the end of the experiments (4 h). This reduction in arterial pressure was less marked in rats pretreated with dexamethasone (Table 1).

Vasoconstrictor responses to sympathetic nerve stimulation and to noradrenaline

Sympathetic nerve stimulation (SNS) produced a frequency dependent increase in perfusion pressure which was abolished by either 10^{-7} M tetrodotoxin ($n=4$), by prazosin (2.4×10^{-7} M; $n=4$) or by guanethidine (2.4×10^{-7} M; $n=4$). These studies indicate that the increase in perfusion pressure was mediated by the release of noradrenaline from noradrenergic nerves. Preparations removed from rats given endotoxin also showed a frequency-dependent increase in perfusion pressure to SNS (at 10 to 40 Hz) but these responses were significantly reduced compared with preparations taken from saline-treated rats (Figure 1). This increase in perfusion pressure to SNS in endotoxin preparations was also blocked by either tetrodotoxin, prazosin or guanethidine.

Noradrenaline increased perfusion pressure to the same extent in preparations removed from saline or endotoxin treated rats (Figure 2).

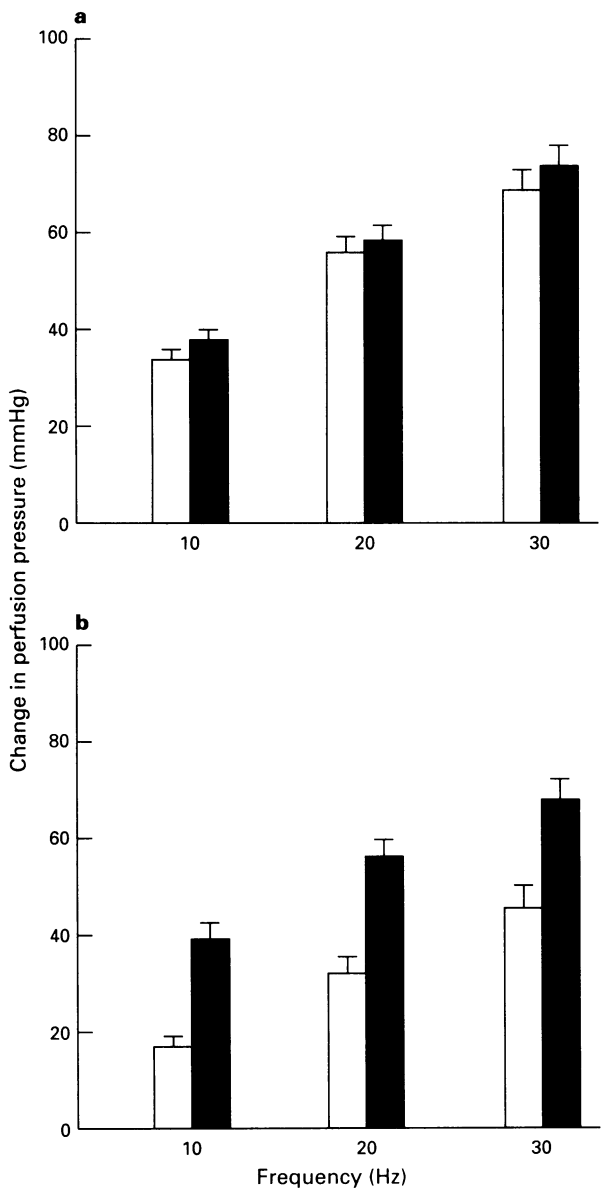

Figure 1 Changes in perfusion pressure in mesenteric vascular beds removed from rats infused for 4 h with saline as control (a) and from rats infused with endotoxin ($2.5\,mg\,kg^{-1}\,h^{-1}$ b) for 4 h and subjected to sympathetic nerve stimulation. The open columns are responses in preparations with an intact endothelium and the solid columns are from preparations denuded of endothelium by perfusing the preparation with distilled water. Endotoxin markedly suppressed the responses to sympathetic nerve stimulation and these were restored towards control by denuding the preparation of endothelium. Removal of the endothelium had no effect on responses to SNS in preparations removed from control, saline-infused rats. (Two way ANOVA repeated measures: Group, $P<0.01$; frequency, $P<0.01$, Group × frequency, NS. A Tukey-Kramer test following ANOVA at each frequency shows $P<0.01$ at each frequency for endothelial removal in preparations from endotoxin-treated rats; $n=10$ for each group).

The effects of removal of the endothelium on the changes in perfusion pressure in response to sympathetic nerve stimulation

The resting perfusion pressure, at a flow rate of 2 ml min^{-1}, was 27 ± 1 mmHg ($n=15$) in preparations taken from saline infused rats and 27 ± 1.1 mmHg in those taken from endotoxin-infused rats. During the 5 min infusion with distilled water there was a transient increase in basal perfusion pressure (of 90 ± 2.7 mmHg) but after 30 min the perfusion pressure had decreased to 29 ± 1 mmHg ($n=15$). Removal of the endothelium in preparations from saline-treated rats did not significantly alter the pressor responses to sympathetic nerve stimulation (Figure 1), although they were slightly greater at each of the three stimulation frequencies; in preparations removed from endotoxin-treated rats there was a marked and significant increase in the pressor responses to SNS (Figure 1). In the mesenteric vascular bed the pressor

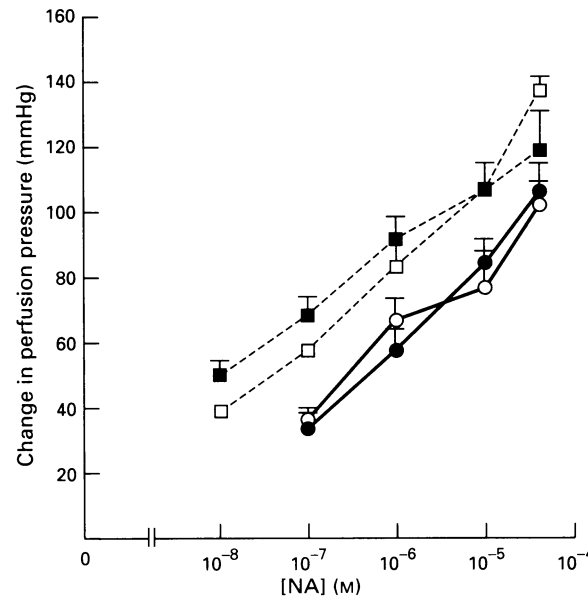

Figure 2 Noradrenaline (NA) dose-response curves for changes in perfusion pressure in isolated perfused mesenteric vascular beds removed from rats infused with saline for 4 h (○) or with endotoxin ($2.5\,mg\,kg^{-1}\,h^{-1}$; ●) for 4 h. Removal of the endothelium in these preparations potentiated the noradrenaline response in both control preparations (□) and in those removed from endotoxin-treated rats (■). (Two way ANOVA for repeated measures comparing noradrenaline responses in endothelium denuded and intact control preparations; effect of dose, $P<0.01$; effect of denuding endothelium, $P<0.01$; interaction NS. Comparing noradrenaline responses in endothelium intact and denuded preparations from endotoxin treated rats; effect of dose, $P<0.01$; effect of denuding, $P<0.01$; interaction NS; $n=6$ for each group).

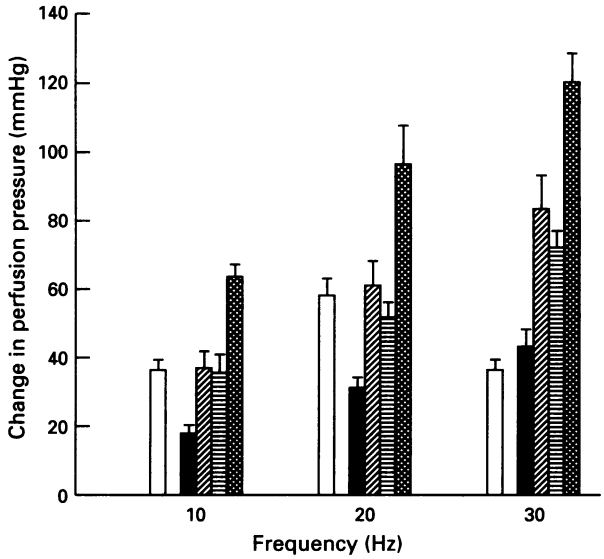

Figure 3 The effect of sympathetic nerve stimulation (SNS) on perfusion pressure in isolated mesenteric vascular beds removed from rats infused with endotoxin ($2.5\,mg\,kg^{-1}\,h^{-1}$; solid columns) and in the presence of L-NAME (diagonal hatched columns), indomethacin (horizontal hatched columns) or a combination of both (cross-hatched columns). For comparison, open columns are the responses from preparations removed from saline infused rats (see also Figure 5). The presence of either L-NAME or indomethacin restores the responsiveness to SNS in endotoxin-treated preparations, whereas the effect is greatly augmented when both inhibitors are present. (Two-way ANOVA repeated measures: Groups, $P<0.01$; frequencies, $P<0.01$; Groups x frequency, NS. One way ANOVA at each frequency followed by Tukey-Kramer multiple comparison test; indomethacin vs endotoxin, $P<0.05$; L-NAME vs endotoxin, $P<0.01$; L-NAME + indomethacin vs endotoxin $P<0.001$; indomethacin or L-NAME vs indomethacin + L-NAME, $P<0.01$; $n=8$ for each group).

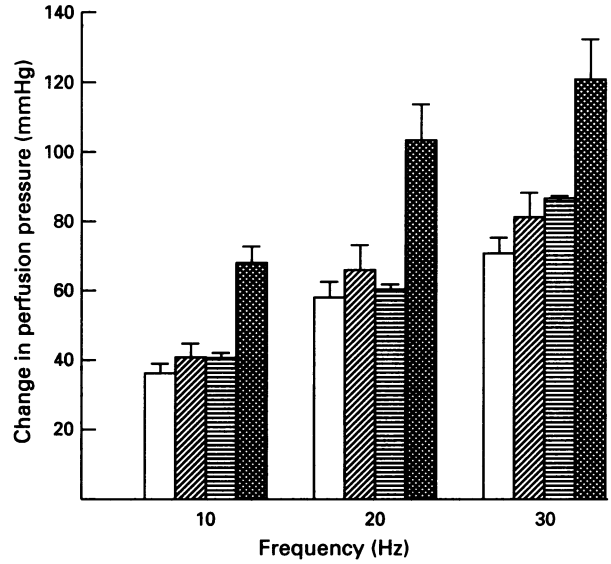

Figure 4 The effect of sympathetic nerve stimulation (SNS) on changes in perfusion pressure in isolated mesenteric vascular beds removed from rats infused with saline for 4 h. The control responses (open columns) are unaffected by either L-NAME (diagonal hatched columns) or indomethacin (horizontal hatched columns) but are greatly augmented when both inhibitors are present in the perfusing fluid (cross-hatched columns). (Two way ANOVA repeated measures: Groups, $P<0.01$; frequency, $P<0.01$; Groups x frequency, NS. Tukey-Kramer test following one way ANOVA at each frequency; L-NAME + indomethacin vs control, $P<0.01$; $n=7$ for each group).

responses to exogenous noradrenaline, which were unaffected by endotoxin (Figure 3), were potentiated by endothelial removal irrespective of whether the preparations had been taken from saline (Urabe *et al.*, 1991, and Figure 2) or endotoxin infused animals (Figure 2).

The impaired pressor responses to SNS in mesenteric preparations removed from endotoxin-treated rats were restored to those seen in the control preparations by either L-NAME (1.3×10^{-3} M), added to the Krebs Henseleit solution 20 min before stimulation, or by indomethacin (10^{-5} M; Figure 3). Addition of L-arginine (10^{-3} M) to the Krebs had no effect in control preparations but reversed the effect of L-NAME (change in perfusion pressure (mmHg) at 20 Hz; control 58.6 ± 3.8; endotoxin 32.9 ± 2.9 ($P<0.01$ vs control); endotoxin + L-NAME 57.0 ± 4.9; endotoxin + L-NAME + L-arginine 34.0 ± 5.0 ($P<0.01$ vs endotoxin + L-NAME), $n=5$). Neither L-NAME nor indomethacin had any effect on the pressor responses to SNS in mesenteric preparations removed from rats which had been infused with saline rather than endotoxin (Figure 4). However, in the presence of a combination of both L-NAME and indomethacin the pressor responses to SNS were greatly exaggerated in preparations taken both from saline (Figure 4) and from endotoxin treated rats (Figure 3). The L-NAME/indomethacin combination had no effect on pressor responses to exogenous noradrenaline in preparations denuded of endothelium (compare Figures 2 and 5). Similarly, the combination of L-NAME and indomethacin failed to augment the response to SNS in preparations removed from saline or endotoxin treated rats when the endothelium had been removed; change in perfusion pressure (mmHg) at

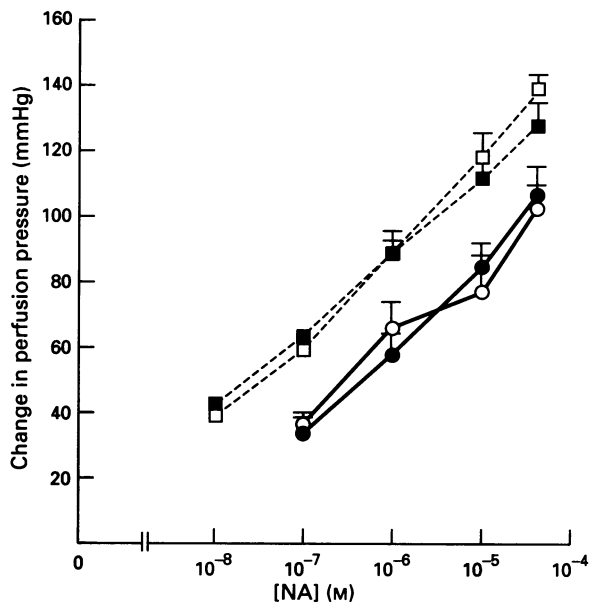

Figure 5 The effect of noradrenaline (NA) on changes in perfusion pressure in isolated perfused mesenteric vascular beds removed from rats infused with either saline (○) or endotoxin ($2.5\,mg\,kg^{-1}\,h^{-1}$; ●) for 4 h and the effect of denuding the endothelium and perfusing with a combination of L-NAME and indomethacin (□ and ■, respectively). There is no hyporeactivity to noradrenaline in this preparation and denuding the preparations of endothelium and infusing with a combination of a nitric oxide synthase inhibitor and cyclo-oxygenase inhibitor did not further augment responses in these preparations (compare Figure 2). (In control preparations; two way ANOVA for repeated measures; effect of denuding endothelium, $P<0.01$; effect of dose, $P<0.01$; interaction, NS. In preparations from endotoxin-treated rats; effect of denuding together with L-NAME and indomethacin, $P<0.01$; effect of dose, $P<0.01$; interaction, NS; $n=6$ for each group).

20 Hz; control, 58.6±3.8; control, denuded, L-NAME/indomethacin 60±1.6 (NS); endotoxin 31.8±2.7 ($P<0.01$ vs control) endotoxin, denuded L-NAME/indomethacin; 50.0±1.2 ($P<0.01$ vs endotoxin), $n=7$.

The effects of dexamethasone pretreatment on the responses of the mesenteric vascular bed to sympathetic nerve stimulation

Dexamethasone (3 mg kg^{-1}) was given by i.p. injection 1 h before the commencement of the saline or endotoxin infusions. It had no effect on either heart rate or blood pressure in saline infused animals but attenuated the bradycardia seen in rats infused with endotoxin (Table 1); blood pressures in rats pretreated with dexamethasone were slightly higher at 2 and 4 h into the endotoxin infusion (Table 1). At the end of the 4 h infusion period the preparations were removed and subjected to SNS. Dexamethasone pretreatment had no effect on the responses of mesenteric preparations taken from saline-treated rats but restored completely the impaired responsiveness to SNS of preparations removed from endotoxin-treated rats. This is illustrated in Figure 6.

Discussion

To distinguish between the possibility of postjunctional impairment (with nitric oxide acting as a functional antagonist to oppose constriction by elevating cyclic GMP; Vo *et al.*, 1992) and a prejunctional effect on sympathetic neurotransmission, requires a vascular bed where, unusually, responses to noradrenaline itself are unimpaired by endotoxin administration. Unlike other vascular beds (for example the tail artery preparation used by Vo *et al.*, 1992, and by Thorin & Atkinson, 1994) the responses of the mesenteric vascular bed to exogenous noradrenaline are unimpaired by endotoxin; in contrast, as we have shown, the responses to sympathetic nerve stimulation are indeed impaired. There is some recent evidence

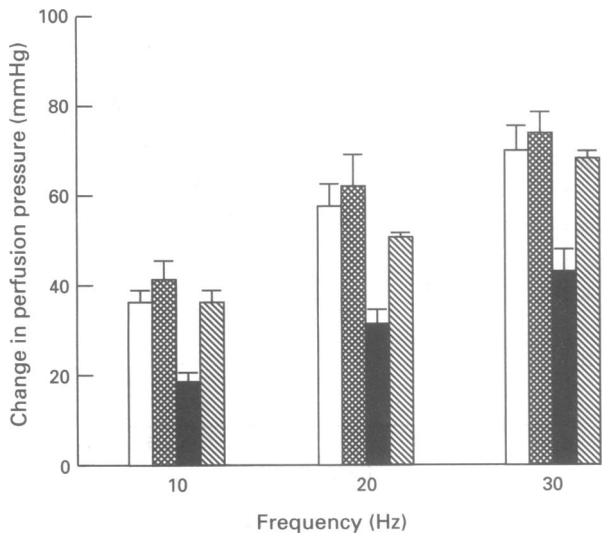

Figure 6 Changes in perfusion pressure in isolated mesenteric vascular beds during sympathetic nerve stimulation (SNS) and the effect of prior treatment with dexamethasone. Dexamethasone had no effect on responses to SNS in preparations removed from rats infused with saline for 4 h (open columns - control; cross-hatched columns - control following dexamethasone treatment) whereas it completely restored the depressed responses in preparations removed from endotoxin-treated rats (solid columns (control) and diagonal-hatched columns (dexamethasone-pretreated)). (Two way ANOVA for repeated measures; groups, $P<0.01$; frequency, $P<0.01$. Groups × frequency interaction, NS. Tukey-Kramer test following one way ANOVA at each frequency dexamethasone + endotoxin vs endotoxin; $P<0.01$; $n=8$ for each group).

that noradrenaline release is inhibited by nitric oxide (or some other endothelium-derived inhibitory factor) in the rat tail artery following transmural electrical stimulation (Thorin & Atkinson, 1994). Since, under conditions of endotoxaemia nitric oxide formation by endothelial, as well as vascular smooth muscle cells, is enhanced (Fleming *et al.*, 1991), one might expect inhibition by endothelium-derived products such as nitric oxide to be more pronounced under conditions of endotoxaemia. This indeed, seems to be the case.

In this particular preparation removal of the endothelium did not greatly alter the responses to sympathetic nerve stimulation (Figure 2), an observation that is in contrast to that observed in other vascular beds e.g. the rat tail artery (Bucher *et al.*, 1992) and by Li & Duckles (1992) in the rat mesenteric bed. The reason for the difference between our results and those of Li and Duckles (1992) must be speculative but these authors used a higher perfusion rate (5 ml min^{-1}) and different methods of both endothelium removal (saponin) and sympathetic nerve stimulation. However, in the present study, removal of the endothelium from preparations obtained from endotoxin-treated rats reversed the endotoxin-induced depression of responsiveness to sympathetic nerve stimulation (Figure 2). This might suggest a role for the endothelium in mediating endotoxin-induced impairment of responsiveness to nerve stimulation in the mesenteric vascular bed, although under normal conditions, the endothelium does not appear to modify greatly the activity of noradrenergic nerves in this preparation. Where endothelial removal augments responses to nerve stimulation (Hynes *et al.*, 1988; Bucher *et al.*, 1992; Li & Duckles, 1992), neuronally released noradrenaline may stimulate endothelial cells, presumably via α_2-adrenoceptors, to release relaxing factors. Stimulated release of endothelium-derived relaxing factor (EDRF) by exogenous agonists acting on endothelial α_2-adrenoceptors has been demonstrated previously in dog arteries (Angus *et al.*, 1986). Our data indicate that this does not happen in mesenteric preparations under normal conditions and are in agreement with other studies in the rabbit carotid artery (Tesfamariam *et al.*, 1987; Cohen & Weisbrod, 1988). However, we suggest that following administration of endotoxin there is induction of nitric oxide synthase, and probably also cyclo-oxygenase, in endothelial cells as well as in smooth muscle cells. A general induction of a calcium-independent nitric oxide synthase (NOS) in the mesentery following endotoxin administration has been demonstrated by Mitchell *et al.* (1993). This was maximal at 6 h but was also apparent at 3 h. A resultant increase in the ability of endothelial cells to release substances, such as NO and prostacyclin, on nerve stimulation or an enhancement of a continuous, basal, release of these mediators from endothelial cells, might then lead to inhibition of noradrenaline release by a prejunctional negative feedback action on sympathetic neurones. The fact that responses to noradrenaline itself are unaltered in preparations taken from endotoxin-treated rats (Figures 3 and 6), whether or not the endothelium is present, suggests that postjunctional responses are unimpaired.

An alternative interpretation to impaired noradrenaline release could be the enhanced release of some, as yet unidentified, vasodilator neurotransmitter in response to SNS in endotoxin-treated preparations. This could be nitric oxide itself, as in certain non-vascular smooth muscle preparations, like the bovine retractor penis muscle (Sheng *et al.*, 1992) or the guinea-pig trachea (Li & Rand, 1991), or calcitonin gene-related peptide (CGRP), which is released in the rat mesenteric vascular bed during field stimulation (Kawasaki *et al.*, 1988; 1990). This latter possibility seems unlikely in view of the facts that (a) the impaired responses are endothelium-dependent (CGRP-induced vasodilatation appears to be endothelium-independent in the rat perfused mesenteric bed; Li & Duckles, 1992) and (b) that this impairment is modified by either indomethacin or an inhibitor of the L-arginine NO pathway, or both.

The distinction between the effects of sympathetic nerve stimulation and of exogenous noradrenaline in mesenteric

preparations following endotoxin administration is also clear from the studies of Tomikawa & Okabe (1992). They showed that mesenteric artery ring preparations taken from rabbits injected with endotoxin 20 h (but not 5 h) previously had markedly depressed responses to field stimulation but not to exogenous noradrenaline (compare Figures 3 and 4 of their paper). They concluded that, at least in rabbits, endotoxin inhibits transmitter release from noradrenergic nerve endings because of an increased sensitivity of prejunctional inhibitory receptors. They also provided evidence that muscarinic and α_2-adrenoceptors were involved. Although this effect of endotoxin on noradrenergic transmission was, in their hands, totally independent of endothelial cells, it was linked with protein synthesis since it was not seen after the administration of actinomycin D or cycloheximide, indicating that some step sensitive to these inhibitors led to the activation or sensitization of inhibitory prejunctional receptors. We suggest that these steps include the induction of both iNOS and cyclo-oxygenase 2, which are dexamethasone sensitive.

We investigated the nature of the inhibitory substances presumed to be released from endothelial cells by preventing the synthesis of nitric oxide (with L-NAME) and/or the synthesis of prostanoids (with indomethacin). The results show that inhibition of either NO synthesis or of prostanoids restored responsiveness to SNS in mesenteric preparations removed from endotoxin-treated rats, without having any significant effect in control preparations. L-Arginine reversed the effects of L-NAME, supporting the idea that the effect of L-NAME was mediated via NOS inhibition. However, when both inhibitors were present, responses to SNS were markedly augmented not only in preparations removed from endotoxin-treated animals (Figure 4) but also in control preparations (Figure 5), whereas their presence had no effect on responses to exogenous noradrenaline, at least in the absence of endothelium (Figure 6). Removal of the endothelium abolished the augmenting effect of the L-NAME/indomethacin combination on SNS in endotoxin-treated preparations, suggesting the endothelium as the source of nitric oxide/cyclo-oxygenase products. It is not easy to interpret these data but we suggest that under conditions of endotoxaemia either nitric oxide or a cyclo-oxygenase product, the synthesis of which is markedly increased by nitric oxide (Sautebin *et al.*, 1995), acts as a brake on noradrenergic transmission. In normal preparations the release of *either* of these inhibitory substances is insufficient in itself to modify neurotransmission. However, if both inhibitory mediators are prevented from being released, sympathetic transmission and noradrenaline release are greatly enhanced, especially under conditions of endotoxaemia.

In summary, we think it unlikely that the changes we have observed which result from SNS are due to a modification of postjunctional α_2-adrenoceptors, because impaired responsiveness to exogenous noradrenaline has not been seen in this preparation either by ourselves (Gray *et al.*, 1988) or by others (Mitchell *et al.*, 1993). Further, the enhanced responsiveness to noradrenaline which occurs in this preparation after endothelial denudation (e.g. Figure 3) is the same whether the preparations are removed from endotoxin or saline-treated animals, and presumably indicates an α-adrenoceptor mediated release, from endothelial cells of nitric oxide (see also Fleming *et al.*, 1990). The finding that responses to sympathetic nerve stimulation are impaired in endotoxaemia, without a modification of postsynaptic responses to noradrenaline itself, and that this impairment is endothelium-dependent, suggests that there is an enhanced release of endothelium-derived factors (which include nitric oxide and a prostanoid) under these conditions which feeds back on sympathetic nerves to inhibit noradrenaline release by an, as yet, undefined prejunctional mechanism. This is even more pronounced following endotoxin administration than it is under normal conditions, as originally suggested by Tesfamarian *et al.* (1987) and by Thorin & Atkinson (1994). We suggest that this impairment of neurogenically invoked vasoconstriction contributes to the vasoplegia and unrelenting systemic hypotension which is characteristic of animals administered endotoxin and of patients with sepsis (reviewed by Parratt, 1995).

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