Modulation by nitric oxide of platelet-activating factor-induced albumin extravasation in the conscious rat

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1 The objective of this study was to assess whether or not endogenous nitric oxide (NO) could mediate the hypotensive response to platelet-activating factor (PAF) and modulate PAF-induced microvascular albumin leakage in the conscious rat.

2 PAF (0.19 and 1.9 nmol kg⁻¹, i.v.) evoked dose-dependent hypotension and significantly enhanced albumin extravasation in the large airways, pancreas, stomach and duodenum 15 min after its administration. Inhibition of NO synthesis by N^G-nitro-L-arginine methyl ester (L-NAME, 0.125–2 mg kg⁻¹, i.v.) produced marked dose-dependent increases in albumin accumulation (up to 290%) in large airways, liver, spleen, pancreas, kidney, stomach and duodenum as measured by the extravasation of Evans blue dye. L-NAME (2 mg kg⁻¹) treatment markedly potentiated PAF (1.9 nmol kg⁻¹)-induced albumin extravasation in these tissues, whereas it did not modify the hypotensive response to PAF.

3 Maintenance of mean arterial blood pressure at the level observed following 2 mg kg⁻¹ L-NAME by infusion of noradrenaline (620–790 ng kg⁻¹ min⁻¹) neither affected significantly albumin extravasation nor potentiated the permeability effect of PAF in the vascular beds studied with the exception of large airways, where noradrenaline mimicked the effects of L-NAME.

4 These results indicate that inhibition of endogenous NO formation leads to an increase in albumin extravasation and to potentiation of the vascular permeability effect of PAF, whereas the hypotensive action of PAF seems to be independent of NO formation in the conscious rat. These data suggest an important role for NO in the regulation of albumin extravasation.

Keywords: Nitric oxide, N^G-nitro-L-arginine methyl ester; N^G-monomethyl-L-arginine; albumin extravasation; hypotension; PAF; airways; gastrointestinal tract; kidney

Introduction

The non-thrombogenic and high solute and water permeability properties of the vascular endothelium have long been recognized as essential components of normal microvascular integrity. Endothelial cells are also an active site for the production of a large number of potent vasoactive substances essential in the regulation of vascular tone and reactivity. One of these substances, endothelium-derived relaxing factor (EDRF) has been identified as nitric oxide (NO) (Palmer *et al.*, 1987; Ignarro *et al.*, 1987) or a closely related nitroso compound (Myers *et al.*, 1989). NO acts as an endogenous nitrovasodilator via activation of vascular smooth muscle cytosolic guanylate cyclase (Murad, 1986; Furchgott & Vanhoutte, 1989). NO may also modulate the interactions between inflammatory cells and vascular endothelium. This notion is based on the fact that analogues of L-arginine, including N^G-monomethyl-L-arginine (L-NMMA) and N^G-nitro-L-arginine methyl ester (L-NAME), which inhibit NO synthesis (Rees *et al.*, 1990) also promote platelet aggregation (Radomski *et al.*, 1987a) as well as adhesion of platelets and neutrophil granulocytes to vascular endothelium (Radomski *et al.*, 1987b; Kubes *et al.*, 1991). These latter events would lead to endothelial dysfunction (Lefer *et al.*, 1991).

Platelet-activating factor (PAF) has been implicated as an important regulator of the cardiovascular system in various pathological conditions, including ischaemia-reperfusion (Filep *et al.*, 1989; Montruccio *et al.*, 1989) and endotoxin shock (Doebber *et al.*, 1985; Chang *et al.*, 1987). Impaired vascular relaxation (Lefer *et al.*, 1991) and enhanced vascular permeability (cf. Filep *et al.*, 1991a) are characteristic features of these conditions. Since endothelial dysfunction associating ischaemia-reperfusion can be attenuated by organic NO donors (Siegfried *et al.*, 1992), it is conceivable that

reduced release or action of NO may, in part, account for endothelial dysfunction (Lefer *et al.*, 1991). The purposes of the present experiments were to examine the role of endogenous NO in mediating the hypotensive action of PAF and to study whether or not inhibition of NO biosynthesis compromises PAF-induced increases in microvascular albumin extravasation in selected vascular beds of the rat.

Methods

The experiments were performed on conscious, chronically catheterized male Wistar rats weighing 230–290 g (Charles River Canada Inc., St. Constant, Québec, Canada). The animals were housed in individual metabolic cages and were prepared as described previously (Filep *et al.*, 1987). Briefly, under anaesthesia (ketamine, 75 mg kg⁻¹ and sodium pentobarbitone, 15 mg kg⁻¹) catheters were implanted into the abdominal aorta and vena cava through the central tail artery and left femoral vein, respectively. The venous catheter was led subcutaneously to the root of the tail. The catheters emerging from the tail were protected by an acrylic cuff glued to the tail. The cuff was connected to a stainless steel spiral and was fed through the loop of the metabolic cage. The animals were allowed to recover completely for at least 4 days following the surgical procedures. On the day of the experiment, following an equilibration period of 2 h, basal cardiovascular parameters were measured for 20 min before drug administration. During the experiments the animals could move freely and had free access to food and water. Mean arterial blood pressure was monitored continuously with an electromanometer using a COBE CDX III pressure transducer (Digi-Med, Louisville, KY, U.S.A.). Protein extravasation was estimated by measuring tissue accumulation of Evans blue dye, which binds to plasma albumin (Rawson,

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1943). Previous studies have demonstrated close correlation for the exudation of Evans blue dye and radioiodinated serum albumin (Udaka *et al.*, 1970; Rogers *et al.*, 1989). All procedures followed were in accordance with the recommendations of the Canadian Council of Animal Care.

Experimental protocols

Initial experiments showed that L-NMMA and L-NAME at a dose of 25 and 2 mg kg⁻¹, respectively, evoked near maximal (93–97%) increases in mean arterial blood pressure. Therefore, these doses of L-arginine analogues, unless otherwise stated, were used in subsequent experiments. In the second series of experiments, the ability of L-NMMA and L-NAME to inhibit acetylcholine-induced decrease in mean arterial blood pressure was examined. The hypotensive responses to graded doses of acetylcholine (1–8 nmol kg⁻¹, injected i.v. at 3 to 5 min intervals) were compared before and after treatment with L-arginine analogues. In some animals, mean arterial blood pressure was maintained at the level observed following L-arginine analogues by the continuous infusion of noradrenaline (620–790 ng kg⁻¹ min⁻¹, 6.9 µl min⁻¹) for 10 min before generation of a dose-response curve. Each animal served as its own control. The effects of L-NAME on protein extravasation were studied in additional experiments. The animals were treated with 0.125, 0.5 or 2 mg kg⁻¹ L-NAME or its vehicle (0.9% NaCl) for 10 min, then Evans blue dye (20 mg kg⁻¹, 25 mg ml⁻¹ in 0.9% NaCl) was injected i.v. In the last series of experiments, Evans blue dye was injected together with PAF (0.9 or 1.9 nmol kg⁻¹) or its vehicle in the absence and presence of L-NAME (2 mg kg⁻¹, 10 min before injection of PAF). In order to compare directly the responses to PAF before and after NO blockade, the effects of PAF were studied in animals receiving noradrenaline infusion. Ten minutes after injection of PAF or its vehicle, the animals were anaesthetized with sodium pentobarbitone (50 mg kg⁻¹, i.v.) and were perfused with 40 ml 0.9% NaCl through a catheter inserted into the abdominal aorta. The perfusion was completed within 5 min (i.e. 15 min after injection of PAF). Portions of pulmonary tissues, liver, spleen, pancreas, kidney, stomach, duodenum and dorsal skin were excised and weighed. Tissue Evans blue

dye content was measured by spectrophotometry following extraction with formamide as described previously (Filep *et al.*, 1991b). Evans blue content of each sample was expressed as µg per g dry tissue weight to avoid underestimation of changes due to plasma fluid extravasation.

Drugs

Platelet-activating factor (1-O-hexadecyl-2-O-acetyl-sn-glycero-3-phosphocholine) was purchased from Bachem (Bubendorf, Switzerland), L-NMMA was from Research Biochemicals Inc. (Natick, MA, U.S.A.), noradrenaline hydrochloride, L-NAME and Evans blue dye were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). All drugs were dissolved in 0.9% NaCl immediately before use.

Statistics

Results are expressed as means ± s.e.mean. Statistical evaluation of the data was performed by Dunn's multiple contrast hypothesis test (Dunn, 1964) when various treatments were compared to the same control or by Wilcoxon signed rank test and Mann-Whitney U test for paired and unpaired observations, respectively. A level of $P < 0.05$ was considered significant for all tests.

Results

Effects of L-NAME and L-NMMA on the depressor response to PAF

Intravenous bolus administration of L-NMMA (25 mg kg⁻¹) or L-NAME (2 mg kg⁻¹) increased mean arterial blood pressure from 108 ± 3 to 157 ± 5 mmHg ($n = 8$) and from 106 ± 3 to 158 ± 4 mmHg ($n = 8$), respectively. The pressor effect of L-NMMA was a transient response, returning to baseline by 45–50 min. L-NAME produced a sustained pressor effect, mean arterial blood pressure remained elevated by 50 mmHg for up to 90 min. Acetylcholine evoked similar depressor responses in control and L-NMMA or L-NAME-treated animals (Figure 1) in spite of differences in the prein-

Figure 1 Hypotensive effects of acetylcholine in conscious rats before and after treatment with N^G-nitro-L-arginine methyl ester (L-NAME, 2 mg kg⁻¹), N^G-monomethyl-L-arginine (L-NMMA, 25 mg kg⁻¹) or noradrenaline (NA, 620–790 ng kg⁻¹ min⁻¹). Mean arterial blood pressure was 107 ± 2 mmHg ($n = 9$), 158 ± 4 mmHg ($n = 8$), 151 ± 8 mmHg ($n = 6$) and 156 ± 5 mmHg ($n = 10$) before injection of acetylcholine in control (a), L-NAME (b), L-NMMA (c) and noradrenaline-treated (d) groups, respectively. Values are means ± s.e.mean. * $P < 0.05$; ** $P < 0.01$ (compared to noradrenaline-treated group).

Figure 2 Hypotensive response to PAF in conscious rats following administration of 0.9% NaCl (control, a), N^G-nitro-L-arginine (L-NAME, 2 mg kg⁻¹, b), N^G-monomethyl-L-arginine (L-NMMA, 25 mg kg⁻¹, c) or noradrenaline (NA, 620–790 ng kg⁻¹ min⁻¹, d). Mean arterial blood pressure was 108 ± 3 mmHg ($n = 6$), 151 ± 4 mmHg ($n = 6$), 150 ± 3 mmHg ($n = 6$) and 151 ± 2 mmHg ($n = 6$) before injection of PAF in control, L-NAME, L-NMMA and noradrenaline-treated animals, respectively. Values are means ± s.e. mean.

jection mean arterial blood pressure levels. To circumvent these differences, mean arterial blood pressure was elevated by infusion of noradrenaline to comparable levels (156 ± 5 mmHg, $n = 10$) as seen after L-arginine analogues. In this paradigm, the hypotensive effects of acetylcholine were significantly reduced by both L-NMMA and L-NAME (Figure 1). The inhibition ranged from 30 to 41%. In contrast, the maximum depressor responses to PAF were identical in L-NMMA and L-NAME-treated animals and during noradrenaline infusion (Figure 2).

Effects of L-NAME on protein extravasation

In agreement with previous studies, intravenous bolus injection of L-NAME (0.125 – 2 mg kg⁻¹) elicited a dose-dependent pressor effect (Table 1). This was accompanied by a dose-dependent increase in tissue Evans blue content in all vascular beds studied with the exception of the pulmonary parenchyma (Table 1). However, the sensitivity of various vascular beds to L-NAME treatment showed considerable variation. For example, significantly enhanced protein extravasation was detected in the stomach following L-NAME at a dose as low as 0.125 mg kg⁻¹, whereas a 16 fold higher dose was required to evoke significant increases in Evans blue content in the spleen (Table 1).

Effects of L-NAME on PAF-induced albumin extravasation

Injection of L-NAME (2 mg kg⁻¹) evoked increases of 120 to 190% in tissue Evans blue dye content in the bronchus, pancreas, kidney, stomach and duodenum. L-NAME increased protein extravasation by 74, 108 and 66% in the trachea, liver and spleen, respectively (Table 2). No significant changes in Evans blue content were detected in the pulmonary parenchyma and skin (Table 2). Maintenance of mean arterial blood pressure at the level observed following L-NAME administration by infusion of noradrenaline did not enhance albumin extravasation, except in the pulmonary circulation. Evans blue content of pulmonary tissues did not differ significantly following L-NAME and noradrenaline treatments (Table 2).

As expected, PAF produced a dose-dependent increase in albumin extravasation in most vascular beds studied (Table 2). When PAF (1.9 nmol kg⁻¹) was injected following L-NAME, protein extravasation was markedly enhanced in the large airways, stomach and duodenum (Table 2). A significant increase in Evans blue content was observed even in the pulmonary parenchyma, where neither PAF nor L-NAME by itself enhanced albumin extravasation (Table 2). In the pancreas, combined administration of L-NAME and PAF produced smaller, but still significant increases in tissue Evans

Table 1 Effects of N^G-nitro-L-arginine methyl ester (L-NAME) on mean arterial blood pressure (MABP) and protein extravasation in conscious rats

	Con ($n = 5$)	L-NAME (mg kg ⁻¹)		
		0.125 ($n = 4$)	0.5 ($n = 4$)	2 ($n = 5$)
Basal MABP (mmHg)	105 ± 2	104 ± 3	108 ± 3	105 ± 3
Maximum increase in MABP (mmHg)	1 ± 1	$16 \pm 1^*$	$24 \pm 4^*$	$45 \pm 3^{**}$
Protein extravasation (μ g Evans blue dye per g dry tissue weight)				
Trachea	52 ± 2	66 ± 4	$73 \pm 4^*$	$95 \pm 8^{**}$
Bronchus	44 ± 4	75 ± 12	$78 \pm 14^{**}$	$93 \pm 17^{**}$
Pulmonary parenchyma	73 ± 7	92 ± 13	101 ± 17	106 ± 12
Liver	47 ± 5	54 ± 7	$74 \pm 10^*$	$102 \pm 9^{**}$
Spleen	90 ± 11	86 ± 16	113 ± 10	$148 \pm 23^*$
Pancreas	32 ± 5	38 ± 6	$93 \pm 12^*$	$91 \pm 10^{**}$
Kidney	51 ± 4	55 ± 6	$84 \pm 16^*$	$123 \pm 32^{**}$
Stomach	64 ± 6	$95 \pm 7^*$	$112 \pm 10^*$	$158 \pm 24^{**}$
Duodenum	74 ± 10	88 ± 11	$172 \pm 15^*$	$216 \pm 18^{**}$

Values are means $\pm$ s.e.mean. The animals were treated with L-NAME (0.125 – 2 mg kg⁻¹ i.v. in a volume of 10μ l 0.9% NaCl per 100 g body weight) or its vehicle (Con, control) for 10 min, then Evans blue dye (20 mg kg⁻¹) was injected i.v. The rats were anaesthetized and perfused with 0.9% NaCl 10 min after injection of the dye. $^*P < 0.05$; $^{**}P < 0.01$ compared to control by Dunn's multiple contrast hypothesis test.

Table 2 Effects of platelet-activating factor, N^G-nitro-L-arginine methyl ester (L-NAME) and noradrenaline (NA) on albumin extravasation in selected organs in conscious rats

	Con	PAF (0.19 nmol kg ⁻¹)	PAF (1.9 nmol kg ⁻¹)	L-NAME	L-NAME plus PAF (1.9 nmol kg ⁻¹)	NA	NA plus PAF (1.9 nmol kg ⁻¹)
Trachea	62 ± 6	99 ± 4	$116 \pm 10^*$	$108 \pm 12^*$	$152 \pm 13^{**}$	$118 \pm 21^*$	$161 \pm 17^{**}$
Bronchus	47 ± 6	63 ± 6	$112 \pm 10^*$	$104 \pm 9^*$	$227 \pm 38^{**}$	$162 \pm 28^*$	$215 \pm 15^{**}$
Pulmonary parenchyma	81 ± 7	74 ± 5	92 ± 12	110 ± 8	$171 \pm 27^*$	$135 \pm 13^*$	$145 \pm 27^*$
Liver	53 ± 5	52 ± 2	53 ± 4	$110 \pm 10^{*†}$	$111 \pm 20^{*†}$	58 ± 4	61 ± 6
Spleen	118 ± 8	139 ± 8	131 ± 8	$195 \pm 23^{**††}$	$212 \pm 24^{**††}$	113 ± 8	138 ± 10
Pancreas	38 ± 5	61 ± 6	$186 \pm 19^{**}$	$100 \pm 12^{*†}$	$89 \pm 9^*$	43 ± 5	$103 \pm 20^*$
Kidney	55 ± 8	64 ± 6	71 ± 6	$136 \pm 20^{**†}$	$121 \pm 14^{**††}$	70 ± 6	74 ± 7
Stomach	71 ± 5	68 ± 7	$140 \pm 5^*$	$155 \pm 9^{*††}$	$316 \pm 48^{**††}$	77 ± 6	$163 \pm 19^*$
Duodenum	90 ± 9	123 ± 4	$196 \pm 7^*$	$252 \pm 21^{**††}$	$360 \pm 46^{**††}$	102 ± 16	$212 \pm 17^*$
Skin	32 ± 5	34 ± 8	35 ± 9	43 ± 4	39 ± 8	41 ± 3	38 ± 7

Values are expressed as Evans blue dye, μ g g⁻¹ of tissue (dry weight) mean $\pm$ s.e.mean for 6 experiments. The animals were given L-NAME (2 mg kg⁻¹), noradrenaline (620 – 790 ng kg⁻¹ min⁻¹) or 0.9% NaCl (Con, control) for 10 min before i.v. bolus injection of platelet-activating factor (PAF, 0.19 or 1.9 nmol kg⁻¹) plus Evans blue dye (20 mg kg⁻¹). The rats were anaesthetized and perfused with 0.9% NaCl 10 min after the injection of PAF. The permeability measurement was made 15 min after injection of PAF. Organ distribution of Evans blue dye was determined as described in Methods. $^*P < 0.05$; $^{**}P < 0.01$ (compared to control by Dunn's multiple contrast hypothesis test); $^†P < 0.05$; $^††P < 0.01$ (compared to noradrenaline); $^‡P < 0.05$; $^‡‡P < 0.01$ (compared to noradrenaline plus PAF).

blue content than either L-NAME or PAF by itself (Table 2). Injection of PAF during noradrenaline infusion evoked similar changes in tissue Evans blue dye content in the lung to those observed when PAF was administered to L-NAME-treated animals (Table 2). On the other hand, noradrenaline infusion did not potentiate albumin extravasation elicited by PAF in the other vascular beds studied, whereas a decreased response to PAF was detected in the pancreas in these animals (Table 2).

Discussion

The present results show that inhibition of endogenous NO synthesis does not affect the hypotensive response to PAF, whereas it leads to potentiation of albumin extravasation elicited by PAF in selected vascular beds of conscious rats.

In confirmation of earlier observations (Whittle *et al.*, 1989; Furchgott & Vanhoutte, 1989; Rees *et al.*, 1990; Vargas *et al.*, 1991), the present study also demonstrates that L-arginine analogues induce large increases in mean arterial blood pressure in the normotensive rat. At a dose of 25 and 2 mg kg⁻¹ respectively, both L-NMMA and L-NAME yielded near maximal pressor effect. This is widely regarded as an indicator of inhibition of endogenous NO production. However, depressor responses to acetylcholine, which exerts its *in vitro* vasorelaxant action through liberation of EDRF/NO (cf. Furchgott & Vanhoutte, 1989) were similar in control rats and in animals treated with L-arginine analogues. To circumvent the influence of the level of inherent vascular tone (i.e. normal or elevated blood pressure) on responses to acetylcholine, mean arterial blood pressure was adjusted by noradrenaline infusion to levels similar to those observed following L-NMMA or L-NAME treatment. In this paradigm, significant attenuation, but not complete inhibition of the acetylcholine-induced depressor response could be observed. Previous studies investigating the effect of L-arginine analogues on the peak hypotensive effect of acetylcholine in the rat resulted in controversial results as inhibition (Whittle *et al.*, 1989), significant attenuation (Vargas *et al.*, 1991) and lack of effect (Gardiner *et al.*, 1990; Van Gelderen *et al.*, 1991) have been reported. Although the reasons for these conflicting results are not clear, the present results indicate that endogenous NO partially mediates the hypotensive action of acetylcholine in conscious rats. These observations also suggest that i.v. injection of acetylcholine does not provide a simple means of quantifying the degree of NO blockade. Unlike acetylcholine, under no conditions did we find that the absolute falls in mean arterial blood pressure elicited by PAF were attenuated in the presence of NO synthesis inhibitors. These observations are consistent with those of Boughton-Smith *et al.* (1992) who reported that L-NMMA does not attenuate PAF (100 nmol kg⁻¹ min⁻¹)-induced fall in mean arterial blood pressure in anaesthetized rats. Therefore, it is feasible that the depressor action of PAF is independent of NO.

Administration of L-NAME resulted in a dose-dependent increase in albumin extravasation in the large airways, liver, spleen, pancreas, kidney, stomach and duodenum, but not in the pulmonary parenchyma and skin. Recently, it has been reported that L-NAME (10 mg kg⁻¹) or L-NMMA (50 mg kg⁻¹) have no significant effect on the vascular leakage in the guinea-pig trachea and main bronchi (Kuo *et al.*, 1992) and rat gastrointestinal tract (Boughton-Smith *et al.*, 1992), respectively. Although the reasons for the different results are not known at present, it might be related to differences in the action of L-NAME on the airways in different species and to differences in the metabolism of L-NMMA and L-NAME in the rat. Indeed, L-NMMA, but not L-NAME is rapidly metabolized by endothelial cells to L-citrulline, and subsequently to L-arginine (Hecker *et al.*, 1990), the precursor of NO. The enhanced albumin accumulation cannot be attributed to an elevation in mean arterial blood pressure and

consequently to an increase in perfusion pressure, since no significant changes in tissue Evans blue dye content were observed following noradrenaline infusion in all vascular beds studied except the pulmonary circulation. Furthermore, L-NAME has been reported to increase transcapillary protein fluxes without elevating capillary hydrostatic pressure in the cat ileum (Kubes & Granger, 1992). These findings are consistent with the notion that capillary hydrostatic pressure is not the primary determinant of microvascular protein extravasation (Grega *et al.*, 1986). An increase in the capillary surface area by L-NAME is also unlikely as vasoconstrictors generally cause derecruitment of capillaries (Grega *et al.*, 1986; Kubes & Granger, 1992). Therefore, it is plausible to assume that L-NAME treatment leads to interendothelial cell gap formation and consequently to opening of the large-pore system, the dominant macromolecular pathway operant in inflammation (Grega *et al.*, 1986). In the pulmonary circulation, a rapid increase in perfusion pressure (over 40 cmH₂O) would lead to widening and disruption of endothelial junctions (Tsukimoto *et al.*, 1990). Thus, it is likely that enhanced pulmonary protein extravasation is a consequence of endothelial damage due to either L-NAME or noradrenaline.

Inhibition of NO production by L-NAME resulted in enhanced tissue Evans blue dye content 15 min after administration of PAF in the large airways, spleen, stomach and duodenum. In contrast, no further elevation in plasma leakage by L-NMMA was reported in the gastrointestinal tract following infusion of PAF in anaesthetized rats (Boughton-Smith *et al.*, 1992). However, interpretation of results obtained with L-NMMA is complicated by the fact that L-NMMA is rapidly metabolized to L-arginine (see above), which, in turn, could reverse its inhibitory action on NO synthase. Furthermore, differences in the experimental protocols used by Boughton-Smith *et al.* (1992) and in the present study (i.e. anaesthetized versus conscious rats, infusion versus bolus injection of PAF) might also be responsible for the conflicting results. Interestingly, combined administration of L-NAME and PAF evoked a significant increase in albumin extravasation in the pulmonary parenchyma, where neither PAF nor L-NAME by itself produced significant changes. Noradrenaline infusion did not mimic the effect of L-NAME on albumin extravasation elicited by PAF in all but the pulmonary vascular beds studied. These observations suggest that with the exception of the pulmonary circulation, the potentiating effect of L-NAME on PAF-induced albumin accumulation cannot be attributed to an increase in perfusion pressure. In the lung, endothelial damage caused by high perfusion pressure may potentiate albumin accumulation evoked by PAF. On the other hand, the vasoconstrictor effect of L-NAME could mask any oedema-inducing effect of PAF, as was observed in the pancreas. Similarly, the potent vasoconstrictor action of PAF in the renal circulation (Sherf *et al.*, 1986) may be responsible for the slightly decreased albumin extravasation seen following L-NAME plus PAF administration compared to the effect of L-NAME.

Although high perfusion pressure-induced structural changes in the capillaries may explain the permeability enhancing effect of L-NAME in the pulmonary circulation, the mechanism by which inhibition of NO production increases protein extravasation in other vascular beds remains unclear. Inhibition of NO formation promotes adhesion of platelets and neutrophil granulocytes to the vascular endothelium (Radomski *et al.*, 1987b; Kubes *et al.*, 1991), an event that is known to induce endothelial dysfunction (Lefer *et al.*, 1991). The findings that a monoclonal antibody directed against the common β -subunit (CD18) of the leukocyte integrins significantly attenuated L-NAME-induced protein fluxes in the cat ileum (Kubes & Granger, 1992) give further support to this hypothesis. Another possibility is that local accumulation of superoxide and/or hydrogen peroxide released by endothelial cells (Zweier *et al.*, 1988) or by neutrophil granulocytes activated by PAF (O'Flaherty *et al.*,

1981; Shaw *et al.*, 1981) may account for the increased permeability (Del Maestro *et al.*, 1981). Since NO may scavenge small amounts of superoxide (Rubanyi & Vanhoutte, 1986; Gryglewski *et al.*, 1986), inhibition of its production would result in accumulation of free radicals.

In conclusion, the present findings show that L-NMMA and L-NAME do not affect the hypotensive response to PAF, whereas they markedly augment PAF-induced albumin extravasation in selected vascular beds of conscious rats. These

findings suggest an important role for NO in the regulation of albumin extravasation.

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References

- BOUGHTON-SMITH, N.K., DEAKIN, A.M. & WHITTLE, B.J.R. (1992). Actions of nitric oxide on the acute gastrointestinal damage induced by PAF in the rat. *Agents Actions, Special Conference Issue*, C3-C9.
- CHANG, S.W.V., FEDDERSEN, C.O., HENSON, P.M. & VOELKEL, N.F. (1987). Platelet-activating factor mediates hemodynamic changes and lung injury in endotoxin treated rats. *J. Clin. Invest.*, **79**, 1498-1509.
- DEL MAESTRO, R.F., BJORK, J. & ARFORS, K.E. (1981). Increase in microvascular permeability induced by enzymatically generated free radicals. I. In vivo study. *Microvasc. Res.*, **22**, 239-254.
- DOEBBER, T.W., WU, M.S., ROBBINS, J.C., CHOY, B.M., CHANG, M.N. & SHEN, T.Y. (1985). Platelet-activating factor (PAF) involvement in endotoxin-induced hypotension in rats. Studies with PAF receptor antagonist kadsurenone. *Biochem. Biophys. Res. Commun.*, **129**, 799-808.
- DUNN, O.J. (1964). Multiple comparisons using rank sums. *Technometrics*, **6**, 241-252.
- FILEP, J., BRAQUET, P. & MOZES, T. (1991a). Significance of platelet-activating factor in mesenteric ischemia-reperfusion. *Lipids*, **26**, 1336-1339.
- FILEP, J., FÖLDES-FILEP, E. & FRÖLICH, J.C. (1987). Vascular responses to leukotrienes B₄, C₄ and D₄ following FPL 55712, indomethacin, saralasin, phentolamine and verapamil in the conscious rat. *Br. J. Pharmacol.*, **90**, 431-439.
- FILEP, J., HERMÁN, F., BRAQUET, P. & MOZES, T. (1989). Enhanced levels of platelet-activating factor in blood following intestinal ischemia in the dog. *Biochem. Biophys. Res. Commun.*, **158**, 353-359.
- FILEP, J.G., SIROIS, M.G., ROUSSEAU, A., FOURNIER, A. & SIROIS, P. (1991b). Effect of endothelin-1 on vascular permeability in the conscious rat: interactions with platelet-activating factor. *Br. J. Pharmacol.*, **104**, 797-804.
- FURCHGOTT, R.F. & VANHOUTTE, P.M. (1989). Endothelium-derived relaxing and contracting factors. *FASEB J.*, **3**, 2007-2018.
- GARDINER, S.M., COMPTON, A.M., KEMP, P.A. & BENNETT, T. (1990). Regional and cardiac haemodynamic responses to glyceryl trinitrate, acetylcholine, bradykinin and endothelin-1 in conscious rats: effect of N^G-nitro-L-arginine methyl ester. *Br. J. Pharmacol.*, **101**, 632-639.
- GREGA, G.J., ADAMSKI, S.W. & DOBBINS, D.E. (1986). Physiological and pharmacological evidence for the regulation of permeability. *Fed. Proc.*, **45**, 96-100.
- GRYGLEWSKI, R.J., PALMER, R.M.J. & MONCADA, S. (1986). Superoxide anion is involved in the breakdown of endothelium-derived vascular relaxing factor. *Nature*, **320**, 454-456.
- HECKER, M., MITCHELL, J.A., HARRIS, H.J., KATSURA, M., THIEMERMANN, C. & VANE, J.R. (1990). Endothelial cells metabolize N^G-monomethyl-L-arginine to L-citrulline and subsequently to L-arginine. *Biochem. Biophys. Res. Commun.*, **167**, 1037-1040.
- IGNARRO, L.J., BUGA, G.M., WOOD, K.S., BYRNS, R.E. & CHAUDHURI, G. (1987). Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. *Proc. Natl. Acad. Sci. U.S.A.*, **84**, 9265-9269.
- KUBES, P. & GRANGER, D.N. (1992). Nitric oxide modulates microvascular permeability. *Am. J. Physiol.*, **262**, H611-H615.
- KUBES, P., SUZUKI, N. & GRANGER, D.N. (1991). Nitric oxide: an endogenous modulator of leukocyte adhesion. *Proc. Natl. Acad. Sci. U.S.A.*, **88**, 4651-4655.
- KUO, H.P., LIU, S. & BARNES, P.J. (1992). The effect of endogenous nitric oxide on neurogenic plasma exudation in guinea-pig airways. *Eur. J. Pharmacol.*, **221**, 385-388.
- LEFER, A.M., TSAO, P.S., LEFER, D.J. & MA, X.L. (1991). Role of endothelial dysfunction in the pathogenesis of reperfusion injury after myocardial ischemia. *FASEB J.*, **5**, 2029-2034.
- MONTRUOCCHIO, G., ALLOATTI, G., TETTA, C., DE LUCA, R., SAUNDERS, R.N., EMANUELLI, G. & CAMUSSI, G. (1989). Release of platelet-activating factor from ischemic-reperfused rabbit heart. *Am. J. Physiol.*, **256**, H1236-H1246.
- MURAD, F. (1986). Cyclic guanosine monophosphate as a mediator of vasodilation. *J. Clin. Invest.*, **78**, 1-5.
- MYERS, P.R., GUERRA, R. Jr. & HARRISON, D.G. (1989). Release of NO and EDRF from cultured bovine aortic endothelial cells. *Am. J. Physiol.*, **256**, H1030-H1037.
- O'FLAHERTY, J.T., MILLER, C.H., LEWIS, J.C., WYNKLE, R.L., BASS, D.A., MCCALL, C.E., WAITE, M. & DE CHATELET, L.R. (1981). Neutrophil responses to platelet-activating factor. *Inflammation*, **5**, 193-201.
- PALMER, R.M.J., FERRIGE, A.G. & MONCADA, S. (1987). Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature*, **327**, 524-526.
- RADOMSKI, M.W., PALMER, R.M.J. & MONCADA, S. (1987a). Comparative pharmacology of endothelium-derived relaxing factor, nitric oxide and prostacyclin in platelets. *Br. J. Pharmacol.*, **92**, 181-187.
- RADOMSKI, M.W., PALMER, R.M.J. & MONCADA, S. (1987b). Endogenous nitric oxide inhibits human platelet adhesion to vascular endothelium. *Lancet*, **ii**, 1057-1058.
- RAWSON, R.A. (1943). The binding of T-1824 and structurally related diazo dyes by the plasma proteins. *Am. J. Physiol.*, **138**, 708-717.
- REES, D.D., PALMER, R.M.J., SCHULZ, R., HODSON, H.F. & MONCADA, S. (1990). Characterization of three inhibitors of endothelial nitric oxide synthase *in vitro* and *in vivo*. *Br. J. Pharmacol.*, **101**, 746-752.
- ROGERS, D.F., BOSCHETTO, P. & BARNES, P.J. (1989). Plasma exudation. Correlation between Evans blue dye and radiolabeled albumin in guinea pig airways *in vivo*. *J. Pharmacol. Met.*, **21**, 309-315.
- RUBANYI, G.M. & VANHOUTTE, P.M. (1986). Superoxide anions and hyperoxia inactivate endothelium-derived relaxing factor. *Am. J. Physiol.*, **250**, H822-H827.
- SHAW, J.O., PINCKARD, R.N., FERRIGNI, K.S., MCMANUS, L.M. & HANAHAN, D.J. (1981). Activation of human neutrophils with 1-O-hexadecyl/octadecyl-2-acetyl-sn-glycerol-3-phosphorylcholine (platelet-activating factor). *J. Immunol.*, **127**, 1250-1255.
- SHERF, H., NIES, A., SCHWERTSCHLAG, U., HUGHES, M. & GERBER, J.G. (1986). Hemodynamic effect of platelet-activating factor in the kidney *in vivo*. *Hypertension*, **8**, 737-741.
- SIEGFRIED, M.R., ERHARDT, J., RIDER, T., MA, X.L. & LEFER, A.M. (1992). Cardioprotection and attenuation of endothelial dysfunction by organic nitric oxide donors in myocardial ischemia-reperfusion. *J. Pharmacol. Exp. Ther.*, **260**, 668-675.
- TSUKIMOTO, K., MATHIEU-CASTELLO, D., PREDILETTO, R. & WERF, J.B. (1990). Structural basis of increased permeability of pulmonary capillaries with high transmural pressures. *Am. Rev. Respir. Dis.*, **141**, A297.
- UDAKA, K., TAKEUCHI, Y. & MOVAT, H.Z. (1970). Simple method for quantitation of enhanced vascular permeability. *Proc. Soc. Exp. Biol. Med.*, **133**, 1384-1387.
- VAN GELDEREN, E.M., HEILIGERS, J.P.C. & SAXENA, P.R. (1991). Haemodynamic changes and acetylcholine-induced hypotensive responses after N^G-nitro-L-arginine methyl ester in rats and cats. *Br. J. Pharmacol.*, **103**, 1899-1904.

- VARGAS, H.M., CUEVAS, J.M., IGNARRO, L.J. & CHAUDHURI, G. (1991). Comparison of the inhibitory potencies of N^G-methyl, N^G-nitro- and N^G-amino-L-arginine on EDRF function in the rat: evidence for continuous basal EDRF release. *J. Pharmacol. Exp. Ther.*, **257**, 1208–1215.
- WHITTLE, B.J.R., LOPEZ-BELMONTE, J. & REES, D. (1989). Modulation of the vasodepressor actions of acetylcholine, bradykinin, substance P and endothelin in the rat by a specific inhibitor of nitric oxide formation. *Br. J. Pharmacol.*, **98**, 646–652.
- ZWEIER, J.L., KUPPUSAMY, P. & LUTTY, G.A. (1988). Measurement of endothelial cell free radical generation: Evidence for a central mechanism of free radical injury in postischemic tissues. *Proc. Natl. Acad. Sci. U.S.A.*, **85**, 4046–4050.

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