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Nitric oxide modulates vascular permeability in the rat coronary circulation

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1 The objective of the present study was to assess whether inhibition of nitric oxide (NO) production could modulate vascular permeability in the coronary circulation in conscious rats.

2 Intravenous injection of N^G-nitro-L-arginine methyl ester (L-NAME, 2 mg kg^{-1}) resulted in a slowly developing hypertension and evoked twofold increases in vascular permeability in the left ventricle and right atrium as measured by the extravasation of Evans blue dye. Maintenance of mean arterial blood pressure at the level observed following L-NAME injection by infusion of noradrenaline (620–820 ng kg^{-1} min^{-1}) did not induce significant protein extravasation in the coronary circulation.

3 L-NAME treatment markedly enhanced (up to 490%) protein extravasation both in the left ventricle and right atrium in response to platelet-activating factor (PAF, 1.9 nmol kg^{-1}, i.v.) and endothelin-1 (1 nmol kg^{-1}, i.v.). Noradrenaline infusion potentiated (up to 69%) endothelin-1-induced protein extravasation. The permeability effect of PAF was only slightly enhanced by noradrenaline.

4 The present findings indicate that inhibition of endogenous NO synthesis leads to an increase in protein extravasation and to potentiation of the permeability effects of PAF and endothelin-1 in the coronary circulation. These results also suggest that NO may be an important regulator of vascular permeability under physiological and pathological conditions.

Keywords: Nitric oxide; platelet-activating factor; endothelin-1; N^G-nitro-L-arginine methyl ester; protein extravasation; ischaemia-reperfusion; coronary circulation; inflammation

Introduction

Early myocardial oedema is a characteristic feature of the inflammatory response associated with acute myocardial ischaemia, especially with reperfusion of ischaemic tissues (Steenbergen *et al.*, 1985; Tranum-Jensen *et al.*, 1987). Recent studies have shown that ischaemia-reperfusion results in elevated plasma concentrations of platelet-activating factor (PAF) (Filep *et al.*, 1989; Montrucchio *et al.*, 1989) and endothelin-1 (Yasuda *et al.*, 1990; Watanabe *et al.*, 1991). Among other mediators, both PAF and endothelin-1 have been implicated as contributors to oedema formation during ischaemia-reperfusion (Braquet *et al.*, 1989; Filep *et al.*, 1991).

Nitric oxide (NO) synthesized by vascular endothelial cells (Ignarro *et al.*, 1987; Palmer *et al.*, 1988) or certain blood-borne cells (Ignarro, 1989) is a potent vasodilator substance. Endothelium-derived NO appears to play a role in the regulation of vascular tone and blood pressure (Ignarro, 1989; Rees *et al.*, 1989) and in the control of regional blood flow (Gardiner *et al.*, 1990). However, the actions of NO extend beyond its vasodilator potential, as inhibition of platelet aggregation (Radomski *et al.*, 1987a) and adhesion of platelets and neutrophil granulocytes to endothelial cells have also been reported (Radomski *et al.*, 1987b; Kubes & Granger, 1992). The objectives of the present experiments were to study whether or not inhibition of NO synthesis by N^G-nitro-L-arginine methyl ester (L-NAME) (Rees *et al.*, 1990) could affect microvascular protein extravasation and to examine whether or not L-NAME could modify the permeability effect of PAF and endothelin-1 in the coronary circulation in conscious rats.

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Methods

The experiments were performed on conscious, chronically catheterized male Wistar rats (235–315 g). The animals were kept 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}, i.p.) 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 connected to a stainless steel spiral and were fed through the top of the metabolic cage. The cuff was glued to the tail. The animals were allowed to recover completely for at least 4 days following the surgical procedure. 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 Statham P23 dB pressure transducer. To measure protein extravasation Evans blue dye was used as a marker of vascular permeability.

Experimental protocols

On the day of the experiments, following an equilibration period of 2 h, PAF (1.9 nmol kg^{-1}), endothelin-1 (1 nmol kg^{-1}) or their vehicle was injected i.v. together with Evans blue dye (20 mg kg^{-1}, 25 mg ml^{-1} in 0.9% NaCl). These doses of PAF and endothelin-1 have previously been shown to enhance protein extravasation in selected vascular beds in rats (Filep *et al.*, 1991). Some animals were pretreated with L-NAME (2 mg kg^{-1}), an inhibitor of NO synthesis (Rees *et al.*, 1990), for 10 min before injection of PAF or endothelin-1. Preliminary experiments showed that increasing the dose of L-NAME did not cause further increase in arterial blood pressure. In some rats, mean arterial blood pressure was maintained at the level observed following L-NAME injection by the continuous i.v. infusion of noradrenaline (620–820

ng kg^{-1} min^{-1}, 6.9 μl min^{-1}) for 10 min before injection of PAF or endothelin-1. Ten min after the injection of PAF or endothelin-1, the animals were anaesthetized with sodium pentobarbitone (50 mg kg^{-1}, i.v.) and the heart was perfused with 40 ml of 0.9% NaCl through a catheter inserted into the aorta. Portions of the anterior wall of the left ventricle and the right atrium were excised and weighed. Approximately half of each tissue was put in formamide (4 ml per g wet weight tissue at 20°C for 24 h), while the other half was dried at 60°C for 24 h. The amount of Evans blue extracted in formamide was determined by spectrophotometry. Evans blue content of each sample was expressed as μg dye per g dry weight of tissue to avoid underestimation of changes due to plasma fluid extravasation.

Materials

Endothelin-1 was a gift from Dr A. Fournier (INRS-Santé, Montreal, Canada). PAF (1-O-hexadecyl-2-O-acetyl-sn-glycero-3-phosphorylcholine) was purchased from Bachem, Bubendorf, Switzerland; L-NAME, noradrenaline hydrochloride and Evans blue dye were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. All chemicals were dissolved in 0.9% NaCl immediately before the experiments.

Statistics

Results are expressed as means ± s.e.mean. Statistical analysis of the data were performed by Dunn's multiple contrast hypothesis test (Dunn, 1964) and by Mann-Whitney U test. A $P<0.05$ level was considered significant for all tests.

Results

Bolus i.v. injection of endothelin-1 (1 nmol kg^{-1}) increased myocardial Evans blue content on average by 84 and 123% in the left ventricle and right atrium, respectively, whereas PAF (1.9 nmol kg^{-1}) caused slight, statistically non-significant increases in both tissues (Figures 1, 2). Intravenous administration of L-NAME (2 mg kg^{-1}) resulted in a slowly developing sustained hypertension, with the maximal change occurring between 6 and 8 min after injection (Figure 3). Thereafter mean arterial blood pressure remained stable for the next 20 min. L-NAME produced an increase of 108 and 89% in Evans blue content in the left ventricle (Figure 1) and right atrium (Figure 2), respectively. Maintenance of blood pressure at the level observed following L-NAME injection by infusion of noradrenaline (620–820 ng kg^{-1} min^{-1}) did not affect protein extravasation significantly (Figures 1, 2). The time course of changes in mean arterial blood pressure in response to PAF and endothelin-1 was similar in animals receiving L-NAME or noradrenaline, except that L-NAME slightly attenuated the magnitude and duration of the depressor effect of endothelin-1 (36 ± 2 mmHg vs. 48 ± 4 mmHg decrease in L-NAME and noradrenaline-treated animals, respectively, $n=6$, $P<0.05$). The maximum depressor responses to PAF were 80 ± 3 mmHg and 78 ± 3 mmHg in L-NAME and noradrenaline-treated animals, respectively ($n=6$, $P>0.1$).

L-NAME treatment markedly potentiated the permeability effect of both PAF and endothelin-1, resulting in 198 and 490% increases in tissue Evans blue content in the left ventricle, respectively (Figure 1). Similarily, tissue Evans blue content in the right atrium increased on average by 160 and 373% in L-NAME-treated animals in response to PAF and endothelin-1, respectively (Figure 2). Noradrenaline infusion enhanced PAF-induced protein extravasation by 30 and 11% in the left ventricle and right atrium, respectively (Figures 1, 2). These changes were, however, statistically not significant. On the other hand, noradrenaline infusion significantly potentiated the permeability effects of endothelin-1 both in the ventricle and atrium, as evidenced by the 69 and 24%

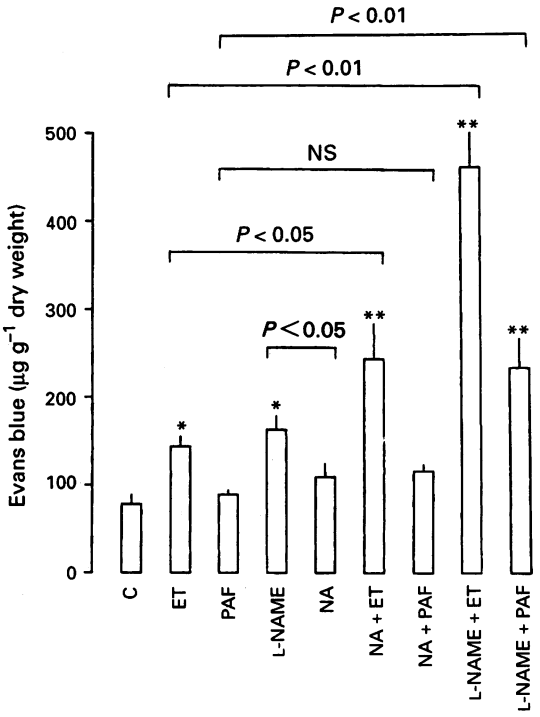

Figure 1 Effects of N^G-nitro-L-arginine methyl ester (L-NAME) on protein extravasation in the left ventricle of conscious rats. The animals were pretreated with L-NAME (2 mg kg^{-1}), noradrenaline (NA, 620–820 ng kg^{-1} min^{-1}) or 0.9% NaCl (control, C) for 10 min before i.v. bolus injection of endothelin-1 (ET, 1 nmol kg^{-1}) or PAF (1.9 nmol kg^{-1}) plus Evans blue dye (20 mg kg^{-1}). The rats were killed 10 min after injection of endothelin-1 or PAF. Values are means of 6 experiments with s.e.mean shown by vertical lines. *$P<0.05$; **$P<0.01$ (compared to control by Dunn's multiple contrast hypothesis test).

increases in tissue Evans blue content, respectively (Figures 1, 2). Combined administration of L-NAME and PAF evoked 2 and 2.3 fold higher increases in protein extravasation in the left ventricle and right atrium, respectively, than those detected following PAF injection during noradrenaline infusion ($n=6$, $P<0.01$). Similarly, tissue Evans blue content in the left ventricle and right atrium were 1.9 and 1.7 times higher, respectively, in response to endothelin-1 in L-NAME-treated animals than those evoked by endothelin-1 in animals receiving noradrenaline infusion ($n=6$, $P<0.01$).

Discussion

The present results indicate that inhibition of NO synthesis increases microvascular protein extravasation and potentiates the permeability effect of PAF and endothelin-1 in the rat coronary circulation. Furthermore, L-NAME did not modify the depressor effect of PAF, whereas it slightly attenuated both the magnitude and duration of the depressor response to endothelin-1. Although we have not measured *in vivo* NO formation, it might be expected that with the supramaximal dose of L-NAME used, NO synthase would be completely blocked. Thus, it seems likely that the depressor effect of PAF and a major part of the depressor action of endothelin-1 may occur independently of NO formation.

Inhibition of NO formation with L-NAME resulted in about twofold increases in protein extravasation both in the left ventricle and right atrium. It seems unlikely that an increase in perfusion pressure (Humphries *et al.*, 1991), and consequently in capillary hydrostatic pressure could account for the increased protein extravasation, since elevation of mean arterial blood pressure by infusion of noradrenaline did

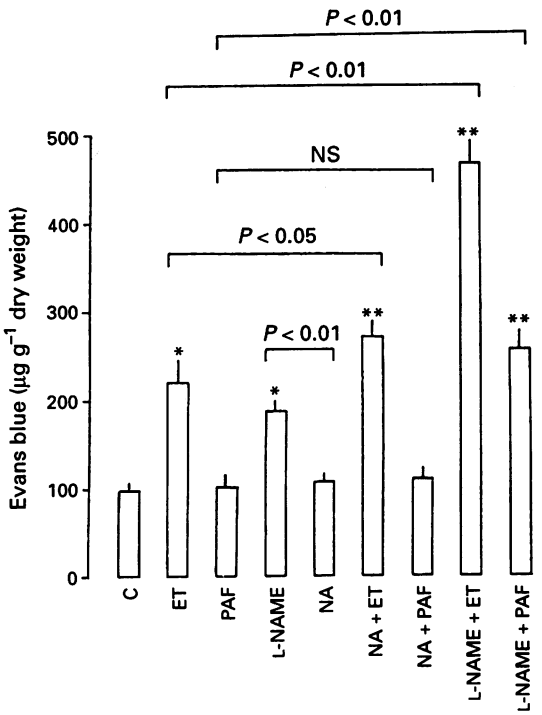

Figure 2 Effects of N^G-nitro-L-arginine methyl ester (L-NAME) on protein extravasation in the right atrium of conscious rats. The animals were pretreated with L-NAME (2 mg kg^{-1}), noradrenaline (NA, 620–820 ng kg^{-1} min^{-1}) or 0.9% NaCl (control, C) for 10 min before i.v. bolus injection of endothelin-1 (ET, 1 nmol kg^{-1}) or PAF (1.9 nmol kg^{-1}) plus Evans blue dye (20 mg kg^{-1}). The rats were killed 10 min after injection of endothelin-1 or PAF. Values are means of 6 experiments with s.e.mean shown by vertical lines. $*P<0.05$; $**P<0.01$ (compared to control by Dunn's multiple contrast hypothesis test).

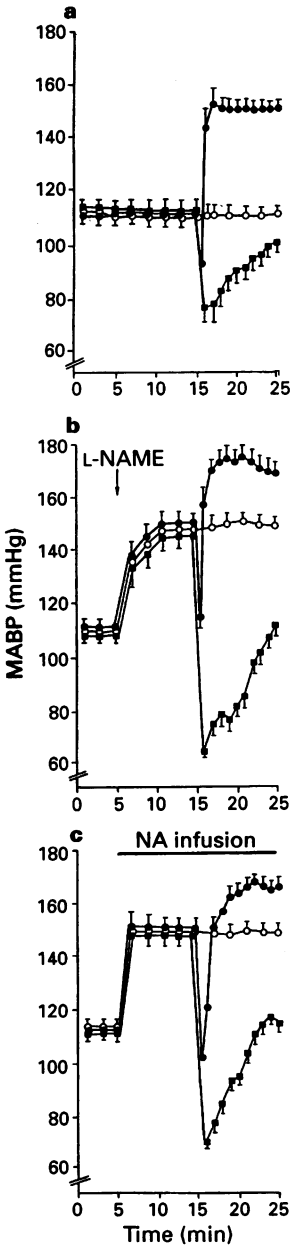

Figure 3 Blood pressure responses to endothelin-1 and PAF in untreated conscious rats (a), following N^G-nitro-L-arginine methyl ester (L-NAME) administration (b) and during infusion of noradrenaline (c). L-NAME (2 mg kg^{-1}) was injected i.v. or infusion of noradrenaline (NA, 620–820 ng kg^{-1} min^{-1}) was started at 5 min. Endothelin-1 (1 nmol kg^{-1}) (●), PAF (1.9 nmol kg^{-1}) (■) or 0.9% NaCl (○) were injected i.v. at 15 min in a volume of 4 μl per 100 g body weight. Values are means of 6 experiments with s.e.mean shown by vertical lines.

not mimic the effects of L-NAME on vascular permeability. Furthermore, L-NAME has recently been reported to decrease capillary hydrostatic pressure in the cat mesenteric circulation (Kubes & Granger, 1992). The enhanced protein extravasation by L-NAME cannot probably be attributed to an increase in capillary surface area for protein filtration since vasoconstrictors like L-NAME generally cause derecruitment of capillaries (Granger *et al.*, 1989). Another possibility might be that NO can preserve endothelial function. Indeed, inhibition of NO synthesis promotes adhesion of platelets and neutrophil granulocytes to the endothelium (Radomski *et al.*, 1987a,b; Kubes & Granger, 1992), an event that is known to induce endothelial dysfunction (Lefer *et al.*, 1991). Since the continuous release of NO may serve to scavange small amounts of superoxide released by endothelial cells, inhibition of NO synthesis might result in accumulation of superoxide radicals (Kubes & Granger, 1992), which, in turn, can enhance microvascular permeability (Del Maestro *et al.*, 1981).

Mediator-stimulated increase in protein extravasation is thought to be attributable primarily to induction of inter-endothelial cell gap formation exclusively in the venules (Bjork & Smedegard, 1983; Grega *et al.*, 1986). This leads to opening of the variable large-pore system, the dominant macromolecular transport pathway operant in inflammation (Grega *et al.*, 1986). Although activation of this transport pathway appears to be independent of haemodynamic changes, elevation of microvascular hydrostatic pressure can promote protein filtration if large pores are open (Grega *et al.*, 1986). The present findings with noradrenaline infusion are consistent with this hypothesis. By inducing capillary venoconstriction, noradrenaline is capable of increasing capillary hydrostatic pressure. However, this alone would not lead to enhanced protein filtration, as was confirmed in the present study. On the other hand, the increased capillary hydrostatic pressure might have potentiated endothelin-1-induced protein extravasation. A similar tendency was observed when PAF and noradrenaline administration was combined. However, these changes were not statistically significant probably due to the weak effect of PAF on protein extravasation in the coronary circulation and the relatively low number of observations.

The present study also showed that L-NAME markedly potentiated protein extravasation evoked by PAF and endothelin-1. Furthermore, Evans blue dye contents in the left ventricle and right atrium were approximately twofold higher when these mediators were injected in the presence of L-

NAME than in the presence of noradrenaline despite the fact that L-NAME and noradrenaline evoked similar changes in mean arterial blood pressure. Thus, it seems unlikely that the potentiating effect of L-NAME could be attributed to an increase in perfusion pressure. Since unlike noradrenaline, L-NAME has been reported to decrease rather than increase capillary hydrostatic pressure (Kubes & Granger, 1992), one may assume that L-NAME and noradrenaline do not enhance the permeability response to PAF and endothelin-1 through common mechanisms. Indeed, the potentiating effect of L-NAME could be explained by endothelial dysfunction secondary to inhibition of NO formation (see above). The findings that NO donors can decrease myocardial necrosis and attenuate endothelial dysfunction in the early phase of myocardial ischaemia-reperfusion (Siegfried *et al.*, 1992) lend further support to this hypothesis.

In conclusion, the present data demonstrate that inhibition of endogenous NO synthesis results in an increase in protein extravasation and potentiation of the permeability effects of PAF and endothelin-1 and suggest an important role for NO in regulating vascular permeability in the coronary circulation under physiological and pathological conditions.

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