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Detergent and methylene blue affect endothelium-dependent vasorelaxation and pressure/flow relations in rat blood perfused mesenteric arterial bed

Michael D. Randall & [1]C. Robin Hiley

Department of Pharmacology, University of Cambridge, Hills Road, Cambridge CB2 2QD

1 The autoperfused superior mesenteric arterial bed of the rat was used to study the opposition by carbachol of pressor responses to noradrenaline and the effects of methylene blue, the detergent CHAPS and indomethacin on pressure/flow relations.

2 Carbachol (1 ng–3 μg) reduced the pressor response to 1 μg noradrenaline in a dose-dependent manner with an $ED_{50} = 25.5 \pm 6.0$ ng and a maximum inhibition of $51.8 \pm 1.9\%$. Perfusion of the mesenteric bed with 0.3% CHAPS in saline for 150 s abolished the reduction by carbachol of the noradrenaline pressor response. The effect of carbachol was also abolished by infusion of 1% methylene blue into the mesenteric vacular bed. Indomethacin (5 mg kg^{-1}) was without significant effect on the carbachol opposition of noradrenaline pressor responses. None of the 3 treatments had any effect on the response to 1 μg noradrenaline.

3 Perfusion with CHAPS before determination of pressure/flow relations, or infusion of methylene blue during their determination, steepened the regression of pressure upon flow to the same extent; at all the flow rates used (0.4–3.54 ml min^{-1}) pressure was greater as a result of the treatment than in control animals. Pretreatment with indomethacin had no effect on pressure/flow relations.

4 It is concluded that carbachol opposition to noradrenaline pressor responses in the blood perfused superior mesenteric arterial bed of the rat shows the characteristics of being mediated by endothelium-dependent relaxing factor (EDRF). Since vascular resistance increases more rapidly than in controls when the endothelium is functionally inhibited by treatment with CHAPS or perfusion with methylene blue, it appears that EDRF has a role *in vivo* in the modulation of myogenic vascular tone.

Introduction

The vascular endothelium is known to release a humoral factor or factors, termed endothelium-derived relaxant factor (EDRF), when it is stimulated by a variety of agents (Furchgott & Zawadzki, 1980; Furchgott, 1983). The EDRF acts on the underlying smooth muscle to stimulate soluble guanylyl cyclase thus causing an increase in cyclic GMP (Holzmann, 1982; Rapoport *et al.*, 1983; Martin & White, 1987) which brings about protein phosphorylation (Rapoport *et al.*, 1983) leading in turn to a decrease in the smooth muscle tone. The liberation of EDRF from unstimulated preparations has also been reported (Griffith *et al.*, 1984), while removal of the endothelium has been shown to lead to a reduction in levels of cyclic GMP in the vascular smooth muscle (Rapoport & Murad, 1983; Miller *et al.*, 1984). It is thus probable that EDRF is released tonically and not just in response to agonists. This hypothesis is supported by the observation that increases in flow rates through perfused canine femoral arteries lead to greater amounts of a non-prostanoid substance emerging in the effluent which relaxes recipient assay tissues (Rubanyi *et al.*, 1986). It would appear therefore that basal release of EDRF may be regulated by fluid flow and a possible stimulus mechanism is found in cultured endothelial cells which contain channels for Ca^{2+} (Lansmann *et al.*, 1987) and K^+ (Olesen *et al.*, 1988) activated by increases in shear stress. It is therefore possible that the endothelium may act as a sensor of blood flow

[1] Author for correspondence.

and liberate EDRF to modulate the myogenic vasoconstriction which is a response to increased blood flow (Bayliss, 1902).

Most of the previous studies on the role of endothelium in vascular control have been carried out in isolated blood vessels, cultured cells or vascular beds perfused with physiological salt solutions. EDRF is known to interact with blood components such as haemoglobin (Martin *et al.* 1985) and platelets (Azuma *et al.*, 1986) and so it is important to obtain evidence for a physiological role in blood perfused systems. In this respect, Angus *et al.* (1983) showed that endothelium-dependent relaxation to acetylcholine and substance P occurred in the blood-perfused femoral artery of the dog, but this is not a resistance vessel and it is in these vessels that important modulation of vascular smooth muscle tone may occur (Griffith *et al.*, 1987). Thus one aim of this study was to show that relaxations to carbachol in the *in situ* blood-perfused superior mesenteric artery preparation, in which perfusion pressure is monitored in order to determine changes in resistance vessel tone, had characteristics of endothelium-dependence. It was then intended to examine the effects of both chemical destruction of endothelial integrity and pharmacological inhibition of endothelium-dependent relaxations on vascular performance by determining pressure/flow relations.

Methods

Male Wistar rats (250–330 g; Bantin and Kingman, Hull) were anaesthetized with 120 mg kg^{-1} sodium thiobutabarbitone (Inactin; BYK Gulden, Konstanz, F.R.G.) given i.p. A tracheal cannula was inserted to allow them to breath air spontaneously and the left jugular vein was cannulated with PP25 tubing for the administration of drugs and 0.9% saline at 6 ml h^{-1} (to prevent volume depletion). The right common carotid artery was cannulated (with PP50 tubing) and connected to a Bell & Howell type 4–422–0001 pressure transducer coupled to a Grass Model 79D polygraph in order to record central arterial pressure. Heart rate was derived from the pressure wave by means of a Grass 7P44 tachograph preamplifier. Rectal temperature was maintained at 37°C by means of a homeothermic blanket system (BioScience, Sheerness, Kent).

The preparation used for *in situ* blood perfusion of the superior mesenteric bed was similar to that described by Jackson & Campbell (1980) and has been described in detail by Hiley *et al.* (1985). Briefly, a mid-line incision was made in the abdomen and the intestines were displaced to allow the positioning of ligatures around the abdominal aorta distal to the origin of the renal arteries and around the superior mesenteric artery shortly after its origin. Surgery was halted for 20 min to allow haemostasis before the i.v. administration of 1000 u kg^{-1} of heparin. The abdominal aorta was cannulated with PP60 tubing which led in turn to a Harvard type 2903 servo-controlled pump, a bubble trap (which also eliminates the pulses in the flow) and a heat exchanger to reheat the blood to 37°C. The circuit, the dead space of which was less than 1.5 ml, was initially filled with 0.9% saline to maintain the circulating volume in the rat; this procedure had no significant effects upon the haematocrit. The blood was returned to the animal through the superior mesenteric artery by means of a PP50 inflow cannula. The period of ischaemia experienced by the bed during cannulation did not exceed 1 min. The input pressure was measured by means of a second Bell & Howell transducer placed a fixed distance from the end of the inflow cannula.

The pressure recorded by the transducer is determined by the sum of vascular resistance in the perfused bed and the resistance of the inflow cannula through which the blood flows between the transducer and the artery. Correction for the resistance of this cannula was made by determining the pressure drop across the cannula; this was done by recording the actual pressure resulting from passing the blood of the animal through the cannula alone at each flow rate at the end of the experiment and subtracting these values from the recorded pressures. This procedure has been shown to give values in agreement with the actual pressure in the vascular bed (Hiley *et al.*, 1985). At the end of each experiment 75 μl arterial blood was taken from the carotid artery for haematocrit determination.

Drugs were administered into the extracorporeal circuit proximal to the heat exchanger in volumes up to 200 μl. Noradrenaline and carbachol were co-administered (Hiley *et al.*, 1987) and the vasodilator effects of the carbachol were assessed as a reduction in the pressor response to a standard submaximal dose of 1 μg noradrenaline. The vasorelaxant effects of sodium nitroprusside were assessed in a similar manner. Pressure/flow relations were established over a range of 8 flow rates, from 0.4 to 3.54 ml min^{-1}. When drugs were continuously infused into the extracorporeal circuit, the rate of infusion was added to the flow rate delivered by the peristaltic pump. Each flow rate was used at least 4 times and was changed every 2 min according to a Latin square design.

Functional destruction of the endothelium was achieved by perfusing the mesenteric vascular bed with a 0.3% (w/v) solution of the detergent CHAPS (3 - [(3 - cholamido - propyl) - dimethylammonio] - 1 - propane sulphonate) in 0.9% saline at 2 ml min^{-1} for 150 s in place of blood. The animal was allowed to

stabilize for 20 min before the start of an experimental determination. In each case functional destruction of the endothelium was confirmed by the inability of 1 μg of carbachol to reduce the pressor response to the standard 1 μg dose of noradrenaline.

For administration of methylene blue, a 1% (w/v) solution of the dye in saline was continuously infused into the extracorporeal circuit at a rate of 0.1 ml min^{-1}. It was found that, 5 min after the start of the infusion, inhibition of the vasorelaxant properties of carbachol was complete and experiments were started. In control experiments, 0.9% saline was infused in place of the methylene blue and, in both cases, the infusion was in place of the intravenous saline infusion.

Pretreatment with indomethacin was achieved by a bolus injection of indomethacin (5 mg kg^{-1}) into the jugular vein prior to the setting up of the extracorporeal circuit. The injection volume was 2 ml kg^{-1}.

Statistical and data analysis

All the values are given as the mean $\pm$ s.e.mean and the number of animals in each group is represented by *n*. Comparison between means was carried out by Student's unpaired *t* test or, if specifically stated in the text, one-way analysis of variance.

Comparison of the regression of perfusion pressure on flow rate was made by analysis of covariance with correction being made for the curvature of the lines (Snedecor & Cochran, 1980). $\bar{y}$ refers to the mean elevation of the regression line at the point ($\bar{x}$, $\bar{y}$); values of $\bar{x}$ are not given as they are the same for each investigation as the flow rates used were the same for both control and experimental animals in a given comparison.

Dose-response curves were analysed by fitting the logistic equation:

$$R = \frac{R_{max} \cdot A^{n_H}}{ED_{50}^{n_H} + A^{n_H}}$$

where R is the reduction of the pressor response to the standard dose of noradrenaline, A is the dose of carbachol, R_{max} the maximal response, n_H is the slope function and ED_{50} the dose of carbachol producing half the maximal reduction in the pressor response to noradrenaline. A modified Marquardt procedure was used as implemented in the Harwell routine VB01A on the Cambridge University IBM 3081 (Aceves *et al.*, 1985)

Drugs

All were made up on the day of the experiment. Carbachol (Sigma, Poole, Dorset), methylene blue (Fisons, Loughborough, Leicestershire), CHAPS (Sigma) and sodium nitroprusside (Koch-Light, Haverhill, Suffolk), were all dissolved in saline. A 1 mg ml^{-1} solution of noradrenaline as the bitartrate (Koch-Light) was made up in a 1 mg ml^{-1} solution of ascorbate and diluted in saline. Indomethacin (Sigma) was initially dissolved in a 5% solution of sodium bicarbonate and then diluted in saline. The solutions of both noradrenaline and sodium nitroprusside were protected from the light.

Results

Effect of CHAPS perfusion on carbachol opposition of the noradrenaline pressor response

Noradrenaline produced dose-dependent increases in the perfusion pressure of the superior mesenteric arterial bed without (except at doses over 10 μg) affecting systemic blood pressure. Throughout the study a submaximal dose of noradrenaline (1 μg) was used to establish tone against which the vasorelaxant properties of carbachol and sodium nitroprusside could be assessed; this dose of noradrenaline increased perfusion pressure by 135 $\pm$ 9 mmHg (n = 14). Carbachol (1 ng–3 μg) reduced the noradrenaline pressor response in a dose-dependent manner (Figure 1); the curve fitting procedure gave n_H = 0.62 $\pm$ 0.05, ED_{50} = 25.5 $\pm$ 6.0 ng and a calculated maximal reduction of 51.8 $\pm$ 1.9% in the noradrenaline pressor response. The higher doses of carbachol (1 μg and 3 μg) were found to have systemic effects consisting of transient hypotension and bradycardia. Perfusion of the mesentery with CHAPS abolished the vasorelaxant properties of carbachol (Figure 1). However, this treatment was without significant effects upon the noradrenaline pressor response which was 163 $\pm$ 16 mmHg (n = 6) following CHAPS treatment. The effectiveness of sodium nitroprusside in opposing the pressor effects of noradrenaline was similarly unaffected, the calculated maximum reductions being 47.3 $\pm$ 3.3% in the control and 45.5 $\pm$ 2.4% following perfusion (n = 6 in both cases). Perfusion with the detergent did not alter central cardiovascular parameters; the control heart rate was 396 $\pm$ 8 min^{-1} and mean arterial pressure was 117 $\pm$ 5 mmHg (n = 11), while following detergent perfusion of the mesentery the heart rate was 383 $\pm$ 14 min^{-1} and the mean arterial pressure was 95 $\pm$ 7 mmHg (n = 6). The haematocrit was well-maintained following the treatment, being 37.8 $\pm$ 1.1% in the controls and 36.3 $\pm$ 2.2% after CHAPS perfusion (n = 6 in both groups).

Effect of methylene blue on carbachol opposition of the noradrenaline pressor response

Perfusion of the mesentery with methylene blue abolished the ability of carbachol to oppose the

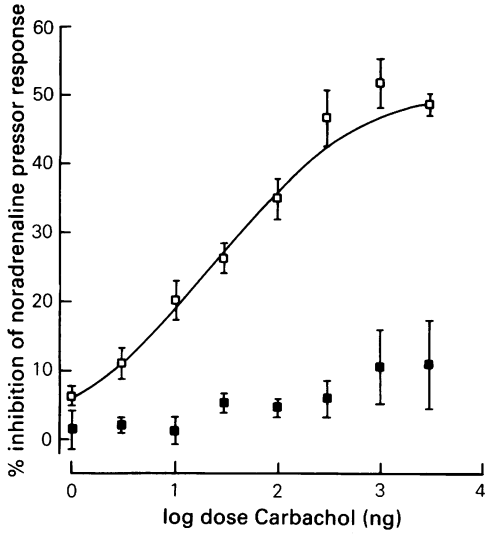

Figure 1 Reduction of the pressor response to 1 μg noradrenaline by co-administered carbachol in the *in situ* blood-perfused superior mesenteric arterial bed of the rat: (□; $n = 8$–11) show data obtained before, and (■; $n = 7$–11) show data obtained after, perfusion of the mesentery with 0.3% CHAPS in saline for 150 s. The line drawn through the control points is that obtained in the non-linear least squares fitting procedure and the values are given as the mean with s.e. mean shown by vertical bars.

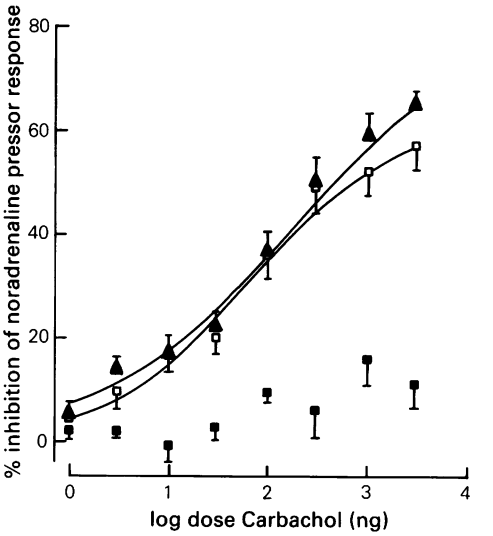

Figure 2 Opposition of the pressor response to 1 μg noradrenaline by co-administered carbachol in the *in situ* blood-perfused superior mesenteric arterial bed of the rat: (□; $n = 6$–9) give control data; (▲; $n = 6$) are results obtained after administration of 5 mg kg^{-1} indomethacin; and (■; $n = 6$–8) show data obtained during the infusion of 1% methylene blue. The lines drawn through the data points for the control and indomethacin curves are those calculated by the non-linear least squares fitting procedure. The values are given as mean with the bars representing s.e.mean.

pressor effect of noradrenaline (Figure 2) but in order to achieve full blockade it was found necessary to infuse the agent continuously. Methylene blue treatment did not alter the systemic effects of the highest doses of carbachol used (1 μg and 3 μg); after 1 μg carbachol, blood pressure and heart rate fell by 10.0 ± 1.8 mmHg and 6.3 ± 3.2 min^{-1} ($n = 6$; control) and 13.3 ± 8.4 mmHg and 15.0 ± 6.1 min^{-1} ($n = 6$; methylene blue). For 3 μg the control values were falls of 40.4 ± 4.1 mmHg and 27.5 ± 1.9 min^{-1} whilst, after methylene blue, the falls were 40.0 ± 7.5 mmHg and 21.7 ± 3.8 min^{-1} ($n = 6$ for all values). Methylene blue did not alter central arterial pressure, heart rate or the mesenteric vascular response to noradrenaline (Table 1).

Effect of pretreatment with indomethacin on carbachol opposition of the noradrenaline pressor response

Pretreatment with indomethacin did not alter the vasorelaxation to carbachol (Figure 2), with control values of $n_H = 0.59 \pm 0.08$, $ED_{50} = 74.4 \pm 29.4$ ng,

Table 1 Central arterial pressure, heart rate and mesenteric pressor response to 1 μg noradrenaline in the presence of indomethacin or methylene blue

	Control ($n = 9$)	*Methylene blue* ($n = 8$)	*Indomethacin* ($n = 6$)
Mean arterial pressure (mmHg)	107 ± 3	112 ± 7	102 ± 5
Heart rate (min^{-1})	394 ± 13	386 ± 10	400 ± 17
Pressor response to noradrenaline (mmHg)	110 ± 1	111 ± 14	107 ± 5

Indomethacin was given i.v. (5 mg kg^{-1}). Methylene blue was infused into the superior mesenteric arterial bed as a 1% solution at a rate of 0.1 ml min^{-1}. Noradrenaline was administered close arterially into the superior mesenteric artery in a volume of 0.1 ml.
There were no significant differences between any of the three values for each parameter (assessed by one way analysis of variance).

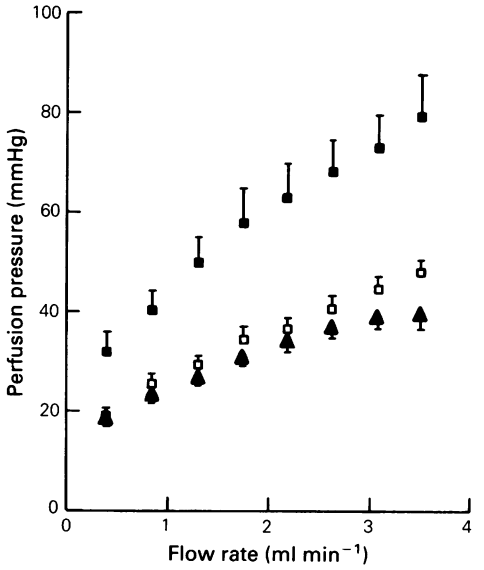

Figure 3 Pressure/flow relations of the *in situ* blood-perfused superior mesenteric arterial bed of the rat after treatment with indomethacin or perfusion with 0.3% CHAPS: (□) show results obtained in control animals; (▲) give the data from animals treated with 5 mg kg^{-1} indomethacin; and (□) show the values obtained in rats in which CHAPS had been perfused through the mesenteric bed for 150 s. The values are given as mean with the bars representing s.e.mean; $n = 6$ for each group.

and a calculated maximum inhibition of 64.5 ± 5.5% ($n = 9$) compared to values obtained after indomethacin of $n_H = 0.43 \pm 0.08$, $ED_{50} = 82.9 \pm 37.3$ ng and a calculated maximum inhibition of 71.5 ± 3.9% ($n = 6$). Indomethacin did not change mean arterial pressure or heart rate nor was the mesenteric vascular response to noradrenaline altered (Table 1).

Effect of CHAPS upon pressure/flow relations

Treatment with CHAPS was found to alter significantly the pressure/flow relationship (Figure 3) with the slope of the regression being steeper in the group pretreated with CHAPS; in the control rats ($n = 6$) regression of pressure on flow rate gave a mean slope of 9.00 ± 0.79 mmHg min ml^{-1} and a mean elevation ($\bar{y}$) of 34.7 ± 1.6 mmHg whereas in the CHAPS-perfused preparations the mean slope was 15.0 ± 2.2 mmHg min ml^{-1} and mean elevation ($\bar{y}$) was 57.9 ± 3.2 mmHg ($n = 6$). Analysis of convariance showed both variables to be significantly different between the control and the CHAPS preparations with $P < 0.01$. The intercepts of the regression lines on the y axis, representing calculated pressure at zero flow rate were 28.5 ± 1.8 mmHg in the CHAPS-treated and 21.1 ± 4.4 mmHg in the control group; these values were not significantly different.

Effect of indomethacin upon pressure/flow relations

For indomethacin (Figure 3) the slope of the regression of pressure on flow rate was 7.06 ± 0.65 mmHg min ml^{-1} which was not significantly different from that in the control group. However, $\bar{y}$ for indomethacin was 30.9 ± 1.2 mmHg which was significantly lower than in the controls ($P < 0.01$). Pressure at zero flow for indomethacin was determined as 17.1 ± 1.1 mmHg, and this did not differ significantly from the control.

Effect of methylene blue upon pressure/flow relations

Pressure/flow relations were established for methylene blue perfused preparations ($n = 7$) against another set of controls ($n = 5$) in which saline was infused into the extracorporeal circuit in place of methylene blue (Figure 4). The mean slope of the regression line for methylene blue was significantly greater ($P < 0.01$) than that for the control group, being 15.2 ± 1.3 mmHg min ml^{-1} compared to 9.22 ± 0.85 mmHg for the control, and the mean elevation ($\bar{y}$) was also significantly greater ($P < 0.01$) for

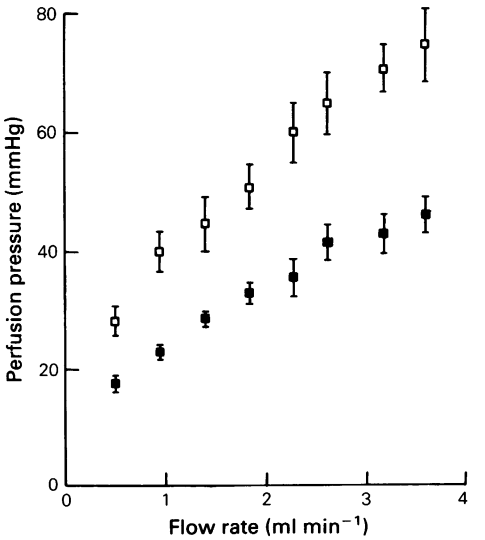

Figure 4 Effect of infusion of 1% methylene blue through the *in situ* blood-perfused superior mesenteric arterial bed of the rat on pressure/flow relations: (■; $n = 5$) are the control values and (□; $n = 7$) are those obtained in the presence of methylene blue. The values are given as mean with the bars representing s.e.mean.

the methylene blue ($\bar{y} = 54.1 \pm 2.4$ mmHg) compared to the control rats ($\bar{y} = 33.5 \pm 0.2$ mmHg). Calculated pressure at zero flow was 24.6 ± 4.5 mmHg in the methylene blue and 14.6 ± 1.6 mmHg in the control groups; these values were not significantly different by analysis of covariance. Treatment with methylene blue did not lead to significant changes in heart rate ($376 \pm 8\,min^{-1}$, control; $403 \pm 8\,min^{-1}$, experimental), mean arterial pressure (106 ± 10 mmHg, control; 104 ± 7 mmHg, experimental) or haematocrit ($41.4 \pm 3.4\%$, control; $42.7 \pm 0.9\%$, experimental).

Discussion

This study clearly demonstrates that *in vivo*, carbachol is able to oppose noradrenaline-induced tone in the rat superior mesenteric arterial bed. Further, the vasorelaxant properties of carbachol were abolished by perfusion of the mesentery with the detergent CHAPS. Perfusion of the rat mesenteric arterial bed *in vitro* with detergents has also been shown to lead to inhibition of the relaxant properties of muscarinic agents and has been shown by histology to be accompanied by endothelial destruction (Burdet *et al.*, 1986; Byfield *et al.*, 1986; Hiley *et al.*, 1987). The perfusion time for the detergent in the present study was greater than that used *in vitro* but this may reflect the greater complexity of the bed *in vivo* which contains all the capillary beds, especially those in the intestinal wall itself, which are absent in the commonly used *in vitro* preparations. Perfusion of the mesentery with CHAPS did not result in significant central cardiovascular effects or haemolysis but was able to abolish the opposition by carbachol of noradrenaline pressor responses without altering the opposition by the endothelium-independent vasorelaxing agent, sodium introprusside or, indeed, the ability of the vascular smooth muscle to contract in response to noradrenaline. Thus this treatment represents a selective tool for the study of endothelium-dependent relaxations in individual vascular beds *in vivo*.

The endothelium-dependent nature of the effect of carbachol was confirmed by the ability of methylene blue to abolish its opposition of the noradrenaline pressor response. Methylene blue is thought to inhibit endothelium-dependent relaxations at the level of guanylyl cyclase (Gruetter *et al.*, 1981; Martin *et al.*, 1985). It is unlikely that the effects of methylene blue in this study were due to the anti-muscarinic properties of the agent (Cook, 1926) as the systemic muscarinic effects of carbachol were well-maintained throughout the infusion of methylene blue. It is also unlikely that the effects of methylene blue were attributable to effects upon cyclo-oxygenases (Martin & Drazan, 1987) since we found that the effects of methylene blue were not the same as those produced by pretreatment with indomethacin. The relatively high doses of methylene blue used both in this study and that of Hogan *et al.* (1988), compared to those required *in vitro* (typically 10–50 μM; e.g. Martin *et al.* (1985), probably reflects the appreciable plasma-protein binding of the agent.

Pretreatment with indomethacin, at the dose used by Jackson & Campbell (1980) and Kitagawa *et al.* (1987) to inhibit peripheral cyclo-oxygenases in the intact rat, had no effect upon the vasorelaxant properties of carbachol which supports the conclusion that the carbachol opposition of the noradrenaline pressor response was not in any way mediated by vasodilator prostanoids.

Both perfusion with CHAPS and infusion of methylene blue, at levels which this study show attenuate endothelium-dependent relaxations, significantly altered pressure/flow relations in the mesentery to the same extent. Both treatments increased the gradient of the pressure/flow plot which may be equated with an increase in mean vascular resistance across the bed. The lack of effect of the cyclo-oxygenase inhibitor indomethacin upon the pressure/flow relations effectively rules out the involvement of prostanoids in the regulation of local haemodynamics. This accords with the findings of Rubanyi *et al.* (1986) who showed that, although increased flow rates increased the amount of the prostacyclin metabolite (6-keto $PGF_{1\alpha}$) emerging in the effluent from canine femoral arteries, indomethacin was able to decrease the levels of 6-keto $PGF_{1\alpha}$ without altering the flow-dependent vasodilator properties of the effluent. It is possible that the alterations of the pressure/flow relations obtained in the present study reflect an inhibition of the flow-induced release of EDRF while the lack of effect of the treatments upon pressure at zero flow suggests that vascular compliance was not significantly altered by the treatments. Thus the alteration in pressure/flow relations cannot be attributed to vessel obstruction as a consequence of the treatments.

Release of EDRF has been shown to occur not only in response to stimulation by agonists but also tonically (Miller *et al.*, 1984) and in response to changes in fluid flow rate (Smiesko *et al.*, 1985; Pohl *et al.*, 1986). Smiesko *et al.* (1985) and Pohl *et al.* (1986) independently reported that flow-induced vasodilatation in canine femoral arteries was dependent upon an intact endothelium. Also, in canine femoral arteries, Kaiser *et al.* (1986) have shown that the flow-induced vasodilatation was sensitive to pharmacological inhibition of endothelium-dependent relaxations. Recent evidence from single channel recording suggests that endothelial cells contain cation channels that are sensitive to changes

in shear stress at levels close to those encountered in blood vessels. Lansman *et al.* (1987) reported the presence of stretch-activated calcium channels in endothelial cells, and suggested that these channels might be involved in the release of humoral factors in a flow-dependent manner. More recent studies (Olesen *et al.*, 1988) have reported that the endothelium contains potassium channels which are regulated by changes in fluid motion at the endothelial surface and it has been suggested that potassium-induced hyperpolarization of the endothelial cells leads to the liberation of EDRF.

Whatever the cause of release of EDRF in the resistance vessels of the blood-perfused superior mesenteric arterial bed under the conditions used in the present study (i.e. tonic or shear stress-induced), we have shown that treatments which inhibit endothelium-dependent relaxations to agonists have appreciable effects upon pressure/flow relations and thus vascular resistance across the bed. Thus, not only does the release of EDRF in the absence of agonists modulate the increase in perfusion pressure with increases in fluid flow in vascular beds perfused with physiological salt solution (Griffith *et al.*, 1987), but we have shown that EDRF has the same role in a blood-perfused system. The myogenic increase in perfusion pressure, which was first described by Bayliss (1902), if unmodulated would lead to instability in the vascular system. Opposition by EDRF thus confers stability upon the vascular system and would limit the work required for the circulation of blood, this latter effect would be most important in resistance beds. This has been supported by the work of Griffith *et al.* (1987) who, using microangiographic techniques, were able to show that, in the rabbit ear artery perfused with Holman's solution, the vascular endothelium maintains a fourth power relationship between diameter and flow and that at high flow rates the pressure gradient becomes independent of flow, which reduces the work required for tissue perfusion. This is lost upon inhibition of endothelium-dependent relaxations.

The present study clearly shows that the effects of carbachol in a blood perfused system *in situ* exhibit the characteristics of endothelium-dependence. Further, in conditions in which carbachol is no longer effective as a vasorelaxant, but in which the endothelium-independent vasodilator nitroprusside maintains its effect, perfusion pressure increases to a much greater extent as blood flow is increased. This shows that EDRF liberation fulfils an important physiological role in acute vascular regulation as a modulator of the myogenic response to increased flow and thus limits the work of tissue perfusion. Pathological inhibition of endothelial functioning, for example in hypercholesterolemia (Verbeuren *et al.*, 1986; Freiman *et al.*, 1986) may chronically alter EDRF release thus altering vascular control and vascular resistance which may contribute towards the elevation of arterial pressure.

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