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Endothelium-dependent modulation of the pressor activity of arginine vasopressin in the isolated superior mesenteric arterial bed of the rat

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1 Pressor responses to arginine vasopressin (AVP) were determined in the rat isolated superior mesenteric arterial bed perfused with Krebs-Henseleit solution and compared with those to noradrenaline.

2 In control preparations the maximum pressor response to the peptide was 34 ± 3 mmHg and the ED_{50} was 21 ± 4 mu ($n = 11$). The maximal pressor response to noradrenaline (30 μg) was 100 ± 6 mmHg ($n = 8$). After removal of the functional endothelium with the detergent CHAPS, the maximum pressor response to AVP increased to 64 ± 4 mmHg and the ED_{50} decreased to 7.7 ± 2.0 mu ($n = 11$) but the response to 10 μg noradrenaline was unaffected.

3 Nordihydroguaiaretic acid (2.5 μM) significantly increased the maximum pressor response to AVP from 41 ± 2 mmHg to 86 ± 8 mmHg ($n = 9$); the ED_{50} was unchanged. Methylene blue (50 μM) also increased the maximum response from 41 ± 3 mmHg to 87 ± 13 mmHg ($n = 8$) without affecting the ED_{50}. Neither treatment significantly affected the response to 10 μg noradrenaline.

4 Neither indomethacin (10 μM) nor BW755C (10 μM) had significant effects upon either the maximal response or ED_{50} for AVP nor did they affect the response to 10 μg noradrenaline.

5 In 6 preparations SKF-525A significantly increased both the ED_{50}, from 9.8 ± 2.1 to 22 ± 2 mu, and the maximum response, from 36 ± 2 to 70 ± 3 mmHg.

6 It is concluded that the pressor response to AVP in this vascular bed is modulated, in the presence of functional endothelium, by the simultaneous release of endothelium-derived relaxing factor.

Introduction

Arginine vasopressin (AVP) is a neurohypophyseal hormone which alters water permeability in the kidney (Dousa & Valtin, 1976) and, in addition, it is a potent constrictor of vascular smooth muscle (Altura, 1975). However, it has recently been found that AVP inhibits myogenic tone in the canine basilar artery and relaxes the precontracted artery. Both of these inhibitory effects are abolished by removal of the endothelium (Katusic *et al.*, 1984). The endothelium of vascular tissue produces a humoral factor (or factors) termed endothelium-dependent relaxing factor(s) (EDRF), which relaxes the underlying vascular smooth muscle (Furchgott & Zawadzki, 1980). Thus release of EDRF by AVP may account for the inhibitory properties observed by Katusic *et al.* (1984). A further demonstration of a vasorelaxant response to AVP has been made in the rabbit renal circulation where AVP produces vasoconstriction followed by significant vasodilatation (Seino *et al.*, 1985). However, the cyclo-oxygenase inhibitor indomethacin was found to potentiate the vasoconstriction and attenuate the vasodilatation. Thus it has been proposed that, in this preparation, AVP stimulates the production of vasodilator prostanoids, which modify the pressor effects of AVP.

The mesenteric circulation of the rat receives approximately one-fifth of the cardiac output (Nichols *et al.*, 1985) and, hence, regulation of this bed may make significant contributions towards systemic blood pressure and circulating blood volume. AVP causes vasoconstriction in this region of the vasculature (Altura, 1975) and specific binding sites for the peptide have been found in membranes prepared from the rat mesenteric arterial bed (Schiffrin & Genest, 1983). However, there have been no reports of a vasorelaxant action in this vascular

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region and so we describe here a study undertaken in order to examine whether or not the vasoconstrictor effects of AVP were modulated in the mesenteric circulation by the vascular endothelium in the rat.

Methods

Isolated perfused mesentery

The isolated, Krebs-Henseleit perfused, superior mesenteric vascular bed of the rat was prepared essentially as described by McGregor (1965). Briefly, heparinized (1000 u kg^{-1}, i.p.) male Wistar rats (280–400 g; Bantin and Kingman, Hull) were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}, i.p.). The superior mesenteric artery was cannulated with polyethylene tubing (PP50; Portex, Hythe, Kent), and the associated vascular bed was perfused, by means of a Harvard Type 1203A peristaltic perfusion pump, at 2 ml min^{-1} with Krebs-Henseleit solution containing (mM): NaCl 118, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, $CaCl_2$ 2.5, and glucose 5.5. The Krebs-Henseleit solution was gassed with 5% CO_2 in O_2 and maintained at 37 ± 1°C. The period between the stopping of the blood supply and start of perfusion with the Krebs-Henseleit solution did not exceed 1 min. The mesentery was carefully dissected away from the abdominal organs and placed on a Perspex block, again maintained at 37 ± 1°C. The tissue was overlaid with Nescofilm (Hoslab, London) to minimize surface damage. Changes in perfusion pressure were measured by means of a Bell & Howell pressure transducer (Type 4-442-001) placed close to the end of the outflow cannula and coupled to Grass model 79D polygraph. Zero pressure was calibrated to zero flow. Since the rate of perfusion was constant at 2 ml min^{-1}, changes in perfusion pressure could be directly related to changes in vascular resistance.

Following a 30 min equilibration period, the vasoconstrictor agents were administered into the perfusion circuit in bolus doses of 100 μl. AVP was administered every 12 min to minimize desensitization and dose-response curves for AVP were constructed in each tissue before and after various manipulations.

Removal of the endothelium was achieved by perfusion of the mesentery with the zwitterionic detergent CHAPS (3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulphonate) at 0.3% (w/v) in distilled water for 90 s followed by a 45 min re-equilibration period. This procedure has been shown by histology to remove >90% of the endothelial cells from the vascular bed (Hiley *et al.*, 1987b). In each experiment functional removal of the endothelium was confirmed by demonstration that 100 ng of acetylcholine was unable to cause a reduction of the pressor response to 10 μg of noradrenaline on co-administration (Hiley *et al.*, 1987b).

Drugs

Nordihydroguaiaretic acid (Sigma, Poole, Dorset) was added to the perfusion fluid to give a final concentration of 2.5 μM. An equilibration period of 60 min was found necessary. Methylene blue (Fisons, Loughborough, Leicestershire) was continuously inflused into the perfusion circuit to achieve a final concentration of 50 μM and a second dose-response curve to AVP was constructed 10 min after the start of infusion. Indomethacin (Sigma) was added to the perfusion fluid to achieve a final concentration of 10 μM; the tissue was perfused with indomethacin for 30 min before administration of the pressor agents. BW 755C (3-amino-1-(3-trifluoromethyl)-2-pyrazoline hydrochloride; the kind gift of Dr J. Salmon, Wellcome Research Laboratories, Beckenham, Kent) was added to the perfusion fluid to achieve a final concentration of 10 μM and a 30 min re-equilibration period was allowed to elapse before determination of a new dose-response curve. SKF-525A (proadifen, 2-dimethylaminoethyl diphenylpropyl acetate; Smith Kline & French Laboratories, Welwyn, Hertfordshire) was dissolved in the Krebs-Henseleit solution to give a concentration of 10 μM and 30 min was allowed for equilibration.

Stock solutions (1 mg ml^{-1}) of noradrenaline bitartrate (Koch Light, Haverhill, Suffolk) were prepared in saline containing ascorbic acid (1 mg ml^{-1}). Stock solutions of indomethacin (1 mg ml^{-1}; Sigma) and nordihydroguaiaretic acid (1 mg ml^{-1}) were prepared in absolute ethanol. BW 755C (1 mg ml^{-1}) and sodium nitroprusside (1 mg ml^{-1}; Koch-Light) were prepared in saline and protected from the light. Methylene blue (1 mg ml^{-1}) was prepared in saline. Vasopressin (20 u ml^{-1}; Pitressin, Parke-Davis, Pontypool, Gwent) was supplied in 0.5% chlorbutol. All dilutions were made in 0.9% (w/v) NaCl.

Data analysis and statistics

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

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

where R is the increase in perfusion pressure on AVP administration, A is the dose of AVP, R_{max} is the maximal increase in perfusion pressure and n_H is a slope function. A modified Marquardt procedure was used as implemented in the Harwell routine VB01A on the Cambridge University IBM 3081 mainframe computer (Hiley *et al.*, 1987a). All values are given as the mean ± 1 s.e.mean and *n* represents

the number of preparations. Results obtained were compared by Student's unpaired t test, and a P value of less than 0.05 was considered to be statistically significant.

Results

Figure 1 shows that AVP (0.003–0.3 u) produced dose-related increases in mesenteric perfusion pressure up to a calculated maximum increase of 34 ± 3 mmHg with an ED_{50} of 22 ± 4 mu ($n = 11$). The maximum noradrenaline pressor response obtained in this preparation occurred with 30 μg and was 100 ± 6 mmHg ($n = 8$). The responses to both noradrenaline and AVP in this preparation were stable for up to 5 h. The AVP pressor response was relatively slow to develop, reaching its peak in approximately 1 min, and waned over a period of 2–3 min; noradrenaline responses peaked within 10 s and declined over 1 min. It was observed that after a dose of AVP, responses to noradrenaline were potentiated for up to 10 min. The potentiation of the noradrenaline pressor response was maximal 5 min after a dose of 0.3 u AVP when the response to 10 μg of noradrenaline was 123 ± 9 mmHg compared to 91 ± 6 mmHg (control; $n = 10$). This represents a potentiation of $37 \pm 6\%$ and did not appear to be affected by any of the experimental interventions used in this study.

Perfusion of the mesentery with 0.3% CHAPS produced a leftward shift in the log dose-response curve to AVP (Figure 1) with a significant ($P < 0.05$) increase in the mid-point sensitivity; the ED_{50} was reduced to 7.7 ± 2.0 mu after CHAPS. Also, the calculated maximum pressor response was significantly ($P < 0.001$) increased to 64 ± 4 mmHg ($n = 11$). Perfusion of the mesentery with CHAPS had no significant effects upon the vascular reactivity to noradrenaline; the pressor responses to 10 μg of noradrenaline being 94 ± 6 mmHg for the control and 96 ± 8 mmHg following perfusion of the mesentery with CHAPS ($n = 10$). However, basal perfusion pressure was significantly ($P < 0.001$) increased from 8.6 ± 0.7 mmHg to 17.2 ± 1.6 mmHg by the treatment ($n = 11$). Treatment of the mesentery with CHAPS did not significantly alter the ability of sodium nitroprusside to reduce noradrenaline induced tone; 10 μg of nitroprusside produced reductions of $52 \pm 2\%$ ($n = 10$) and $54 \pm 3\%$ ($n = 10$) respectively in control and treated tissues.

Nordihydroguaiaretic acid (2.5 μM) significantly ($P < 0.05$) increased the vascular reactivity to AVP; in 9 preparations the maximum response was increased from a control value of 41 ± 2 mmHg to 86 ± 8 mmHg following addition of nordihydroguaiaretic acid (Figure 2). The ED_{50} values were not significantly altered by the treatment, being

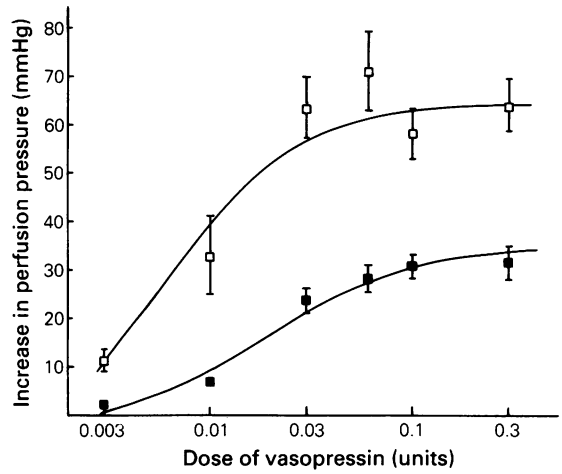

Figure 1 The effects of perfusion with 0.3% CHAPS on the pressor responses to arginine vasopressin (AVP) in the isolated, Krebs-Henseleit perfused, superior mesenteric arterial bed of the rat in 11 preparations: (■) show mean responses obtained before and (□) give the responses obtained after perfusion with the detergent. The lines are those calculated in the curve fitting procedure and are described by the values for ED_{50} and R_{max} given in the text and by n_H values of 1.6 ± 0.3 (control) and 1.7 ± 0.5 (after CHAPS). The bars show 1 s.e.mean.

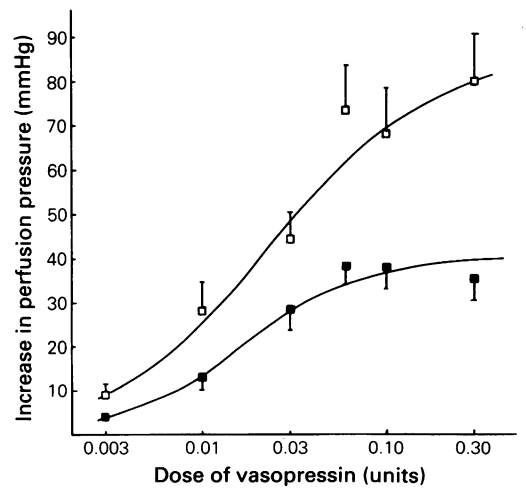

Figure 2 Effects of nordihydroguaiaretic acid (2.5 μM) on the pressor responses to arginine vasopressin (AVP) in the isolated superior mesenteric arterial bed of the rat: (■) show mean control increases in perfusion pressure in the absence of, and (□) responses obtained in the presence of, nordihydroguaiaretic acid. The curves shown are those obtained from the fitting procedure and the values of ED_{50} and R_{max} are given in the text; the slope functions were 1.7 ± 0.2 (control) and 1.5 ± 0.2 (experimental). The vertical bars show 1 s.e.mean and $n = 9$.

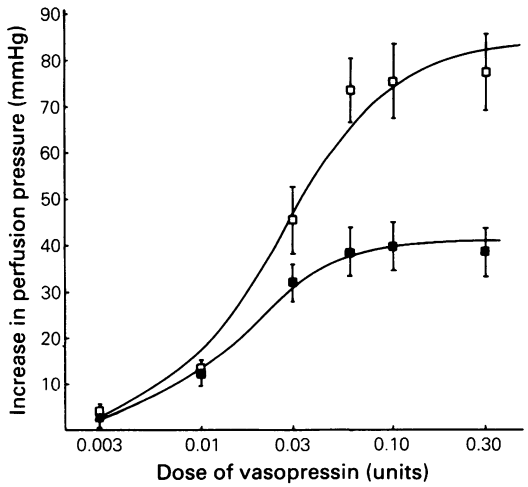

Figure 3 Pressor responses to arginine vasopressin (AVP) in the perfused superior mesenteric arterial bed of the rat in the presence and absence of methylene blue (50 μM) ($n = 8$). Results obtained before perfusion with methylene blue are given by (■) and those in its presence by (□). The curves are those obtained by the fitting procedure and have the values for ED_{50} and R_{max} given in the text; values for n_H were 1.4 ± 0.2 (control) and 1.1 ± 0.2 (methylene blue). The bars show 1 s.e.mean.

16 ± 2 mu and 28 ± 6 mu respectively for the control and after nordihydroguaiaretic acid perfusion. Perfusion with nordihydroguaiaretic acid had no significant effects upon the pressor responses to 10 μg of noradrenaline which were 92 ± 8 mmHg prior to treatment and 89 ± 8 mmHg following perfusion with nordihydroguaiaretic acid ($n = 9$). Basal perfusion pressure was also unaltered in these 9 tissues by perfusion with nordihydroguaiaretic acid; before treatment it was 10.1 ± 0.9 mmHg and during it was 9.7 ± 0.5 mmHg.

Methylene blue (50 μM) produced a similar significant ($P < 0.05$) enhancement of the maximum increase in perfusion pressure from 41 ± 3 mmHg to 87 ± 13 mmHg ($n = 8$), while the ED_{50} was not significantly altered being 16 ± 4 mu in the control and 24 ± 10 mu during the infusion (Figure 3). Methylene blue, at this concentration was without effect upon the reactivity to 10 μg of noradrenaline, (91 ± 10 mmHg control; 89 ± 7 mmHg during infusion) or basal perfusion pressure (16.6 ± 2.0 mmHg, control; 16.0 ± 2.3 mmHg, methylene blue; $n = 8$ for all values).

Indomethacin (10 μM) had no significant effects either upon the maximum pressor response or the mid-range sensitivity for AVP. Control values were 45 ± 5 mmHg and 29 ± 8 mu respectively for the maximum pressor response and the ED_{50}, while following addition of indomethacin they were 43 ± 5 mmHg for the maximum pressor response and 35 ± 8 mu for the ED_{50} ($n = 10$). The slope function was also unchanged with values of 1.4 ± 0.1 (control) and 1.8 ± 0.3 (indomethacin). Indomethacin did not significantly alter the noradrenaline (10 μg) pressor response which was 89 ± 6 mmHg prior to the addition of indomethacin and 93 ± 7 mmHg following the treatment of the 10 preparations with indomethacin. Basal perfusion pressure was unchanged by indomethacin, being 12.0 ± 1.4 mmHg during perfusion compared to a control value of 10.7 ± 0.8 mmHg ($n = 10$).

BW 755C (10 μM) had no significant effects upon either the ED_{50} or the maximum pressor response for AVP in 6 preparations, the ED_{50} values being 19 ± 4 mu for the control and 27 ± 12 mu following the perfusion. The maximum pressor responses were 39 ± 3 mmHg for the control and 43 ± 8 mmHg after the treatment. The slope functions were 1.6 ± 0.2 (control) and 1.8 ± 0.2 (BW 755C). BW 755C had no significant effects upon the vascular reactivity to noradrenaline; 10 μg of noradrenaline produced a pressor response of 97 ± 8 mmHg following BW 755C perfusion which compares with a control value of 92 ± 7 mmHg. Basal perfusion pressures were 16.3 ± 2.1 mmHg (control) and 13.1 ± 2.6 mmHg (BW 755C).

In 6 preparations, a dose-response relationship to AVP was determined in the presence of 10 μM SKF-525A. This resulted in significant increases in both ED_{50} (control, 9.8 ± 2.1 mu; SKF-525A, 22 ± 2 mu; $P < 0.01$) and the maximum response (control, 36 ± 2 mmHg; SKF-525A, 70 ± 3 mmHg; $P < 0.001$). The slope functions, n_H, were 1.8 ± 0.4 (control) and 1.6 ± 0.1 (in the presence of SKF-525A). The pressor responses to 10 μg noradrenaline were 90 ± 14 mmHg before and 93 ± 10 mmHg after the addition of SKF-525A to the perfusing fluid. Basal perfusion pressure was similarly unaltered by the treatment being 15.0 ± 1.8 mmHg before perfusion and 16.3 ± 2.2 mmHg following addition of the agent ($n = 6$).

Discussion

This study shows that the pressor activity of AVP in the superior mesenteric arterial bed is modulated by a process which is dependent on the presence of a functioning endothelium and which can therefore be regarded as an opposing indirect vasorelaxant action of the peptide. Attempts to show a direct vasorelaxant effect in preparations preconstricted with noradrenaline (infusion of 100 $\mu g\,min^{-1}$) were successful in so far as reduction of the induced tone did occur

but quantitation was not possible because of the potentiation of the noradrenaline response by the AVP. The potentiation was marked and was such that the response to 10 μg noradrenaline became approximately 20% larger than the maximum response observed in the absence of AVP effects. Fortunately the effect was short-lived and no potentiation was detectable 10 min after a dose of AVP. The apparent lack of effect of all the treatments upon the potentiation suggests that the potentiation was independent of an intact endothelium, EDRF or arachidonic acid metabolism. This contrasts the findings of Karmazyn *et al.* (1978) who found that this potentiation appeared to be dependent on some aspect of cyclo-oxygenase function since it was not present in mesenteric preparations pretreated with indomethacin and into which prostaglandin E_2 was reinfused.

Perfusion of blood vessels with the zwitterionic detergent CHAPS in distilled water has been shown by Tesfamariam *et al.* (1985) and in our laboratory (Hiley *et al.*, 1987b) to be efficient at removing the vascular endothelium without any significant effects upon the underlying vascular smooth muscle. This is shown functionally by unchanged vasoconstrictor responses to noradrenaline and abolition of the vasorelaxation to acetylcholine after successful removal of the endothelium. In this study the vasorelaxant properties of sodium nitroprusside were unaffected by the treatment, showing that CHAPS does not affect the guanylyl cyclase-dependent processes underlying the relaxation of vascular smooth muscle in response to nitrovasodilators (Schultz *et al.*, 1977). The increased vascular reactivity and sensitivity of the mesentery towards AVP following CHAPS perfusion suggests that the endothelium produces vasodilator metabolites, in response to AVP, which are capable of modulating the pressor activity of AVP. This process is lost upon CHAPS treatment.

Vascular tissue produces vasodilator prostanoids, principally prostaglandin I_2 (Moncada *et al.*, 1976), which have been localised to the vascular endothelium (Moncada *et al.*, 1977). The involvement of prostanoids in the modulation of the AVP pressor response was assessed by treatment of the mesentery with the cyclo-oxygenase inhibitor indomethacin. The lack of effect of indomethacin upon the responses to AVP shows that this peptide does not provoke the liberation of vasodilator prostanoids in the mesenteric circulation. This is in contrast to the renal circulation of the rabbit in which Seino *et al.* (1985) showed that indomethacin attenuated the renal vasodilator response to intrarenal infusion of AVP.

The ability of nordihydroguaiaretic acid, which is both a dual lipoxygenase and cyclo-oxygenase inhibitor and an antioxidant, to inhibit the modulation of the AVP pressor response may be independent of the action of this agent upon these arachidonic acid metabolising enzymes since BW 755C, also an inhibitor of both enzymes, was without effect. Indeed, nordihydroguaiaretic acid has been shown to interact with EDRF in transit through a cascade system (Griffith *et al.*, 1984) and, in the isolated mesentery, nordihydroguaiaretic acid has been shown to attenuate the endothelium-dependent relaxations to acetylcholine while BW 755C was without effect (Hiley *et al.*, 1987b). Thus the present results are consistent with the inhibition of the vasodilator component of the response to AVP being an interaction of nordihydroguaiaretic acid with EDRF produced in response to the peptide rather than inhibition of lipoxygenase and cyclo-oxygenase.

Endothelium-dependent relaxations are mediated by a rise in guanosine 3′:5′-cyclic monophosphate (cyclic GMP) in the vascular smooth muscle (Holzmann, 1982; Rapoport & Murad, 1983) following stimulation of the soluble guanylyl cyclase (Förstermann *et al.*, 1986; Martin *et al.*, 1988). Methylene blue inhibits this stimulation of guanylyl cyclase (Gruetter *et al.*, 1981). In this study the augmentation of the pressor activity of AVP by methylene blue suggests that the modulation is mediated by a rise in tissue cyclic GMP. It is unlikely that the enhancement was a result of decreasing basal cyclic GMP as methylene blue is without effect upon these levels of the nucleotide (Gruetter *et al.*, 1981). It is also interesting to note that the degree of potentiation given by methylene blue was very similar to those given by destruction of the endothelium with CHAPS or the probable destruction of released EDRF by nordihydroguaiaretic acid.

In this study removal of the endothelium and pharmacological inhibition of endothelium-dependent relaxations but not of the cyclo-oxygenase and lipoxygenase pathways augmented the vasoconstrictor activity of AVP. This suggests that, in addition to causing vasoconstriction, AVP acts upon the vascular endothelium causing the release of EDRF which limits the pressor activity of AVP. The release of EDRF by vasoconstrictor agents, leading to self-modulation has also been reported for noradrenaline (Eglème *et al.*, 1984) and for histamine, angiotensin II and the α_2-adrenoceptor agonist UK 14304 (Bullock *et al.*, 1986). None of the manipulations carried out in the present study altered the pressor activity of 10 μg noradrenaline. Hence, in the superior mesenteric arterial bed the noradrenaline pressor response is not appreciably modulated by the concomitant release of prostanoids or EDRF. The lack of modulation of the noradrenergic pressor response in the mesentery contrasts with the α_2-adrenoceptor mediated release of EDRF in the rat aorta suggested by

Eglème *et al.* (1984). A long-term reduction of the effectiveness of noradrenaline as a pressor agent after administration of some α_2-adrenoceptor agonists has been shown to occur in the rat superior mesenteric arterial bed both in the isolated, Krebs-Henseleit perfused and the *in situ*, blood perfused preparations, but this was found to be almost entirely independent of α_2-adrenoceptor activation (Hiley *et al.*, 1987a).

SKF-525A enhanced the pressor response to AVP to a similar extent to the increases seen with CHAPS and methylene blue. A primary action of this substance is to inhibit the activity of cytochrome P450-dependent mono-oxygenases (Mannering, 1971) although it does have other actions including binding to muscarinic cholinoceptors (Taylor *et al.*, 1980). An endothelial cytochrome P450 has been reported (Singer *et al.*, 1984; Abraham *et al.*, 1985) and some workers have found that inhibitors of this cytochrome, including SKF-525A, inhibit endothelium-dependent relaxation (Peach *et al.*, 1985). In view of the fact that cytochrome P450 can metabolise arachidonic acid to bioactive products, including a vasorelaxant 5,6-epoxide (Carroll *et al.*, 1987; Proctor *et al.*, 1987), it has been suggested that EDRF might be an arachidonic acid derivative. However, some doubt must be attached to this hypothesis as a result of the discovery that nitric oxide possesses many of the properties of EDRF and appears to be released from the endothelium (Palmer *et al.*, 1987). Also Förstermann *et al.* (1988) have reported that, in bioassay experiments in which EDRF was allowed to come into contact with inhibitors after its release but before its action on the assay tissue, SKF-525A interacts with the factor in solution rather than with its production or action on the recipient cells. This interaction in solution could explain the enhancement of the pressor response to AVP in the rat superior mesenteric bed reported here. Nevertheless, it should be noted that pretreatment of rats with phenobarbitone, a potent inducer of cytochrome P450, not only increased the concentration of the cytochrome in the superior mesenteric arterial bed but also increased the potency of acetylcholine as an endothelium-dependent vasodilator (by decreasing the ED_{50}) without affecting the endothelium-independent vasodilator response to sodium nitroprusside (Randall & Hiley, 1988).

Basal perfusion pressure was unaltered by both nordihydroguaiaretic acid and methylene blue which may suggest that basally released EDRF (Griffith *et al.*, 1984) has little part to play in the determination of vascular resistance in the superior mesenteric arterial bed under the conditions used here. The modest increase in basal perfusion pressure associated with CHAPS perfusion may reflect vessel obstruction as a consequence of endothelial cell destruction. The lack of effects of the cyclo-oxygenase inhibitor indomethacin and of the dual lipoxygenase/cyclo-oxygenase inhibitor BW 755C suggests that cyclo-oxygenase and lipoxygenase products do not influence basal tone in this preparation.

Endothelium-dependent modification of vascular reactivity to AVP in the mesentery may be physiologically and pathologically significant. Endothelial damage or disruption of endothelium dependent relaxations, for example in hypercholesterolemia (Chappell *et al.*, 1985; Verbeuren *et al.*, 1986), would increase vascular reactivity towards AVP which may have significant effects upon total peripheral resistance and may contribute towards the genesis of cardiovascular disease.

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