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Endothelium-derived relaxing factor and the effects of acetylcholine and histamine on resistance blood vessels

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1 The role of endothelium-derived relaxing factor (EDRF) in the action of vasodilator (acetylcholine, histamine, nitroprusside) and vasoconstrictor (noradrenaline, vasopressin) drugs on vascular resistance in the isolated perfused kidney and mesentery of the rat was studied.

2 Acetylcholine (EC_{50} = 0.18 ± 0.05 nmol and 3.1 ± 0.06 nmol, n = 8) and histamine (EC_{50} = 31.2 ± 4.9 nmol and 46.2 ± 3.9 nmol, n = 8) produced dose-related vasodilatation in noradrenaline-preconstricted (i.e. 'high tone') rat renal and mesenteric blood vessels. The response to both vasodilators (but not nitroprusside) was abolished by infusion of CHAPS (4.7 mg ml^{-1}, 30 s). By use of an immunocytochemical staining procedure CHAPS was demonstrated to remove vascular endothelial cells lining intrarenal blood vessels.

3 Gossypol (3 μM), metyrapone (10 μM) and nordihydroguaiaretic acid, (NDGA, 30 μM), presumed inhibitors of EDRF biosynthesis, reduced or abolished the response to acetylcholine and histamine in perfused kidney and mesentery of the rat without affecting vasodilatation due to nitroprusside. Mepacrine (10 μM) similarly abolished the response to acetylcholine and histamine but in addition, reduced the response to nitroprusside in both preparations.

4 Methylene blue (100 μM), a presumed antagonist of the effect of EDRF, abolished vasodilatation due to acetylcholine and histamine and reduced the response to nitroprusside in perfused rat kidney and mesentery. Superoxide dismutase, SOD (15 u ml^{-1}), was without effect.

5 While CHAPS treatment significantly augmented the vasoconstrictor response to both noradrenaline and vasopressin in perfused renal and mesenteric vessels this effect was not mimicked by metyrapone or gossypol suggesting that the enhanced effect of vasopressor agents in CHAPS-perfused rat organs is due to the removal of a permeability barrier rather than impaired EDRF formation.

6 Responses to vasoconstrictor and vasodilator drugs in the perfused kidney and mesentery were obtained in the presence of indomethacin (8 μM) which produced in excess of 90% inhibition of prostacyclin (PGI_2) release as measured by radioimmunoassay of 6-oxo-prostaglandin $F_{1\alpha}$ (6-oxo-$PGF_{1\alpha}$) in the Krebs effluent.

7 We provide evidence that EDRF mediates the vasodilator response to acetylcholine and histamine in resistance blood vessels in perfused rat kidney and mesentery. The possibility that EDRF has a physiological role to play in regulating the calibre of resistance blood vessels is discussed.

Introduction

Endothelium-derived relaxing factor (EDRF) is a humoral substance released from vascular endothelial cells challenged with a number of vasoactive drugs including acetylcholine (Furchgott & Zawadzki, 1980), histamine (Van de Voorde & Leusen, 1983) and bradykinin (Gryglewski *et al.*, 1986). EDRF is chemically unstable with a reported half-life ranging from 6 s (Griffiths *et al.*, 1984) to 50 s (Forstermann *et al.*, 1986) and is probably broken down by superoxide anion (SO^-) released simultaneously in order to elicit autocatalytic destruction of the mediator (Gryglewski *et al.*, 1986; Rubanyi & Vanhoutte, 1986). Recently, Moncada and his colleagues have provided evidence that EDRF released by bradykinin or A23187 challenge of cultured pig aortic endothelial cells is indistinguishable from nitric oxide (NO) (Palmer *et al.*, 1987). EDRF is believed to relax vascular smooth muscle by activation of guanylate cyclase causing accumulation of intracellular cyclic GMP (Holzmann, 1982;

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Rapoport & Murad, 1983; Ignarro *et al.*, 1984). The possibility of a second, as yet unidentified, EDRF that causes vascular smooth muscle relaxation by an effect on the electrogenic Na-K pump has also been suggested (Vanhoutte, 1987).

The pharmacological activity of EDRF has been thoroughly investigated. It is well established that release of EDRF mediates the relaxant effect of drugs such as acetylcholine on preconstricted rings or bands of arteries and veins *in vitro* (for reviews see Furchgott, 1983, Vanhoutte *et al.*, 1986). In addition, EDRF released either from fresh vascular tissue or from cultured endothelial cells inhibits the aggregation of platelets suspended in physiological buffer (Furlong *et al.*, 1987), plasma (Azuma *et al.*, 1986) or whole blood (Bhardwaj & Moore, 1987) in response to standard aggregating agents, such as ADP or U46619. Similarly, NO is a potent inhibitor of platelet aggregation both in plasma and physiological salt solution (Radomski *et al.*, 1987).

The effect of EDRF on resistance blood vessels has received comparatively little attention. This is surprising since the calibre of such blood vessels determines the distribution of blood flow within organs and ultimately regulates blood pressure. We provide evidence in this paper that the vasodilator responses to acetylcholine and histamine (but not nitroprusside) in perfused rat renal and mesenteric resistance vessels are mediated by EDRF.

Methods

Perfused kidney and mesentery of rat

Rats (male, Sprague-Dawley, 250–350 g) were stunned by a blow to the head and exsanguinated. The mesentery and left kidney were removed and perfused (8 ml min^{-1}) with warmed (37°C), oxygenated (95% O_2: 5% CO_2) Krebs solution (composition, mM: NaCl 118, $NaHCO_3$ 25, $CaCl_2$ 1.9, $MgSO_4$ 1.19, KCl 4.75, KH_2PO_3 1.19, glucose 11.1, pH 7.2) as described by McGregor (1965) and Armstrong *et al.* (1976) respectively. Perfusion pressure was constantly monitored by means of a Bell & Howell pressure transducer connected to a Devices pen recorder. Drugs were injected in volumes less than 20 μl to prevent vascular effects due to an injection artifact. After an equilibration period of 30 min, noradrenaline and vasopressin were injected using dose cycle times of 3 min (kidney) and 5 min (mesentery). Vasodilator responses to acetylcholine, histamine and nitroprusside were assessed in so-called 'high tone' preparations, which were partially vasoconstricted by inclusion of a concentration of noradrenaline in the perfusing Krebs solution which produced approximately 60–80% of the maximal response. A dose cycle time of 5 min was employed for vasodilator drugs in both preparations. Kidneys and mesenteries were weighed before and at the end of the experiment.

Removal of endothelium by CHAPS

Vascular endothelial cells in the kidney and mesentery were removed by perfusion (30 s) with Krebs solution containing CHAPS (4.7 mg ml^{-1}). Removal of endothelial cells in the perfused rat kidney was verified immunocytochemically using a five stage anti-alkaline phosphatase alkaline phosphatase (APAAP) procedure essentially as described by Mason & Sammons (1978). Briefly, kidney sections (5 μm) were incubated (45 min) with the mouse monoclonal antibody, MRC OX-45. Binding of the monoclonal antibody was detected by incubation (30 min) of sections with rabbit anti-mouse immunoglobulin (Dako Ltd, 2109, diluted 1:50 in rat serum) followed thereafter by incubation (30 min) with a mouse anti-alkaline phosphatase alkaline phosphatase complex (Dako Ltd, D651). Endogenous tissue alkaline phosphatase activity was blocked with levamisole (1 M). Sections were reacted with napthol-AS-MX phosphate and Fast Red TR salt to reveal added alkaline phosphatase activity and thereafter lightly counterstained with Harris' haematoxylin prior to mounting in glycerin jelly. Sections were viewed by light microscopy.

Measurement of 6-oxo-prostaglandin $F_{1\alpha}$ release

Extracts of Krebs perfusate were assayed for 6-oxo-$PGF_{1\alpha}$ by means of a selective radioimmunoassay procedure. Briefly, 18 ml Krebs effluent were collected, acidified to pH 3.4 by dropwise addition of concentrated formic acid and extracted with Waters C-18 reverse phase Sep-Pak cartridges as described elsewhere (Berry *et al.*, 1986). Prostanoids were eluted with 10 ml ethyl acetate which was evaporated to dryness under a stream of air. Extraction efficicacy of radiolabelled 6-oxo-$PGF_{1\alpha}$ was $95 \pm 5\%$, ($n = 7$). Dried residues were resuspended in 0.5 ml distilled water and radioimmunoassayed for 6-oxo-$PGF_{1\alpha}$ with a commercially available kit. Antibody cross reactivity against PGE_2, $PGF_{2\alpha}$ and thromboxane B_2 (TxB_2) was less than 3%.

Statistics

Results show mean $\pm$ s.e.mean with the number of observations in parentheses. Statistically significant differences between groups were determined by Student's unpaired t test.

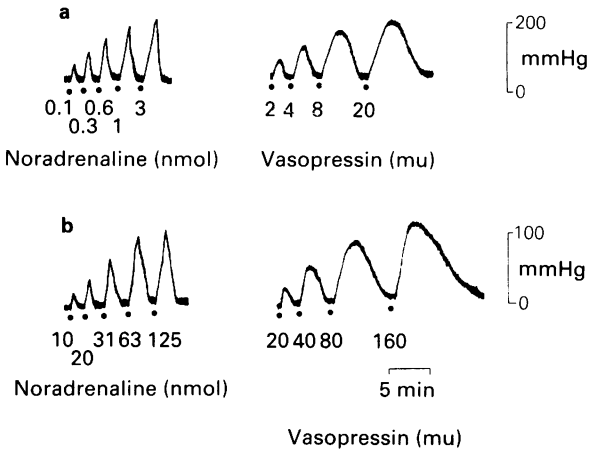

Figure 1 Representative traces showing the effect of bolus injection of noradrenaline and vasopressin in the isolated perfused rat kidney (a) and rat mesentery (b). Horizontal bar represents time scale. Vertical bar denotes scale for perfusion pressure in mmHg.

Materials

Acetylcholine chloride, histamine hydrochloride, indomethacin, mepacrine, methylene blue, metyrapone, sodium nitroprusside, noradrenaline bitartrate, nordihydroguaiaretic acid (NDGA) and superoxide dismutase (SOD) were obtained from Sigma and stock solutions (1 mg ml^{-1}) prepared fresh each day in saline. NDGA was used within 1 h of preparation. CHAPS (3-3 cholamidopropyl dimethylammonio 1-propanesulphonate) (Sigma) was dissolved (10 mg ml^{-1}) in Krebs solution. Napthol-AS-MX phosphate and Fast Red TR salt were obtained from Sigma. Harris' haematoxylin and glycerin jelly were purchased from BDH Ltd. 6-oxo-PGF$_{1\alpha}$ radioimmunoassay kit was purchased from Amersham International. Gossypol was the generous gift of Dr J.R.S. Hoult of this Department and the monoclonal antibody, MRC OX-45, was kindly provided by Dr A.F. Williams, MRC Cellular Immunology Unit, Sir William Dunn School of Pathology, University of Oxford.

Results

Perfused kidney and mesentery of rat

The perfused kidney and mesentery of the rat exhibited spontaneous vascular resistance of 164.4 ± 7.4 mmHg ($n = 26$) and 68.0 ± 4.7 mmHg ($n = 24$), respectively. Kidneys and mesenteries weighed at the end of the experiment following perfusion for 4 h gained approximately 1–4% of their starting weight.

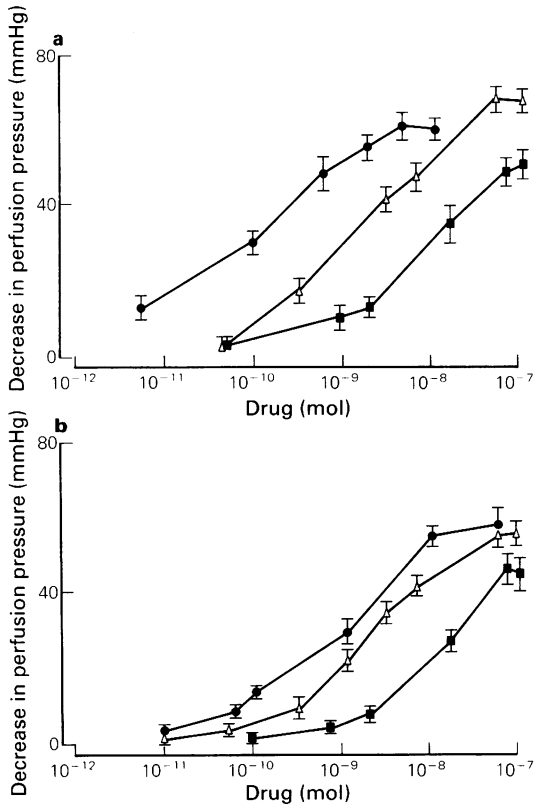

Figure 2 Vasodilator effect of acetylcholine (●), histamine (■) and nitroprusside (△) in noradrenaline-preconstricted isolated perfused rat kidney (a) and mesentery (b). Results show decrease in perfusion pressure in mmHg and are mean of $n = 10$; vertical bars show s.e.mean.

Noradrenaline and vasopressin elicited short-lived and dose-related increases in perfusion pressure indicating vasoconstriction of resistance blood vessels in both the perfused rat kidney and mesentery. Representative traces are shown in Figure 1. The maximum response which could be achieved with noradrenaline in the kidney was approximately 200 mmHg, almost double that in the mesentery (approximately 125 mmHg). The doses of noradrenaline (vasopressin in parentheses) required for half-maximal vasoconstriction (EC_{50}) were 0.6 ± 0.1 nmol (4.0 ± 0.7 mu) and 28.0 ± 2.5 nmol (62.0 ± 6.2 mu) in kidney and mesentery, respectively (all $n = 8$).

Noradrenaline added to the perfusing Krebs solution at a concentration of 0.1–0.5 μM in kidney and 100–150 μM in mesentery produced approximately 60–80% of the maximum response. No significant loss of vasoconstrictor tone was observed in such

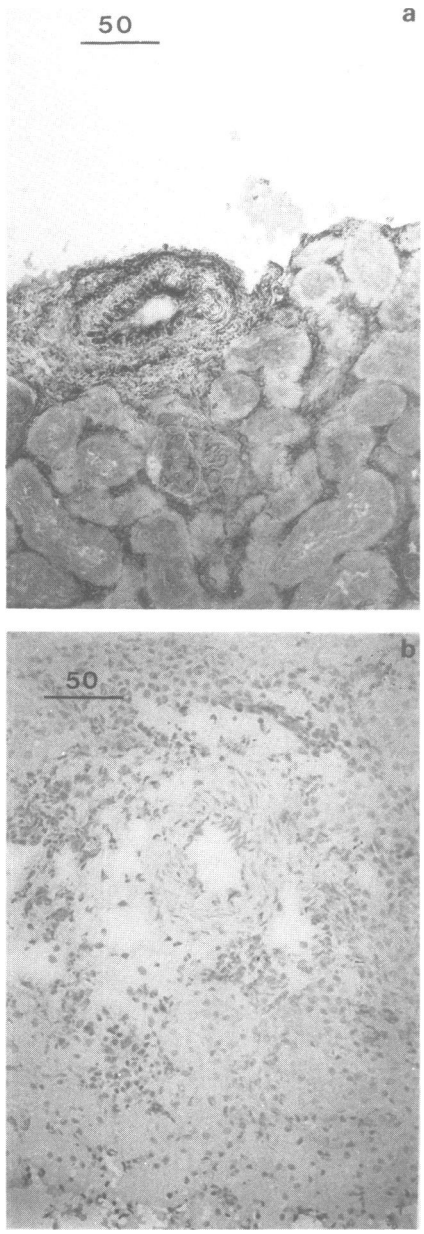

Figure 3 Representative photomicrographs showing intra-renal arteries approximately 80 μm in diameter from Krebs-perfused, control (a) and CHAPS-treated (b) rat kidneys. Notice the accumulation of black staining associated with the intimal surface of the blood vessel in (a) (centre of photograph) which is absent from the vessel shown in the centre of (b). Horizontal bar indicates size in μm.

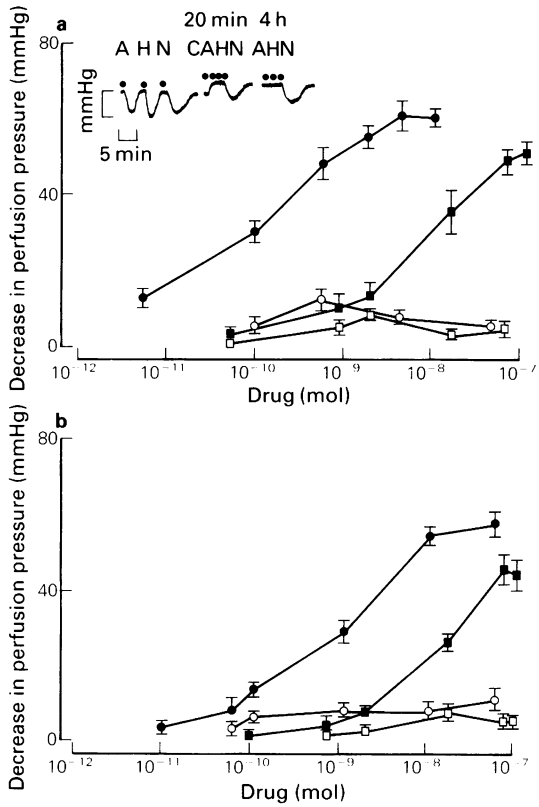

Figure 4 Vasodilator effect of acetylcholine (● ○) and histamine (■ □) in the noradrenaline-preconstricted isolated perfused rat kidney (a) and mesentery (b) before (closed symbols) and after (open symbols) removal of endothelium by CHAPS. Results show decrease in perfusion pressure measured in mmHg and are mean with s.e.mean shown by vertical bars; $n = 6$–10. A representative trace showing the effect of bolus injection of acetylcholine (A, 0.2 nmol), histamine (H, 25 nmol) and nitroprusside (N, 2 nmol) on perfusion pressure of noradrenaline-preconstricted rat kidney before, 20 min and 4 h after treatment with CHAPS (C) is shown in inset. Doses employed represent the EC_{50} values for each agonist in this preparation. Horizontal bar indicates time scale. Vertical bar denotes fall in perfusion pressure in mmHg.

preparations for periods of up to 4 h. Injection of acetylcholine, histamine and nitroprusside caused dose-related falls in perfusion pressure indicating vasodilatation of resistance blood vessels in both organs studied (Figure 2). Nitroprusside produced a more prolonged vasodilator effect than either acetylcholine or histamine. The EC_{50} values (all $n = 8$) for acetylcholine, histamine and nitroprusside in the perfused rat kidney (mesentery in brackets) were 0.18 ± 0.05 nmol (3.1 ± 0.06 nmol), 31.2 ± 4.9 nmol

(46.2 ± 3.9 nmol) and 2.3 ± 0.3 nmol (7.1 ± 0.9 nmol), respectively.

Effect of CHAPS on the response of perfused organs to vasoactive drugs

CHAPS administration resulted in removal of endothelial cells from intrarenal blood vessels in the perfused rat kidney. Although it is impossible to quantify accurately the extent of the removal of the renal vascular endothelium in these experiments, substantial removal of endothelial cells from intrarenal vessels (diameter 70–200 μm) as well as from glomeruli was observed in CHAPS-treated kidneys. In contrast, the vascular endothelium appeared intact in such vessels in slices prepared from control kidneys not exposed to CHAPS. Representative photomicrographs showing intra-renal arteries from control and CHAPS-treated kidneys are shown in Figure 3. In control experiments no endothelium-associated staining was detected in control kidney sections incubated with saline instead of MRC OX-45.

An increase in perfusion pressure of approximately 10–40 mmHg was observed immediately following CHAPS administration in 'high tone' perfused rat kidney and mesentery. Within 30 min of CHAPS administration the vasodilator response to acetylcholine and histamine was greatly reduced both in rat kidney and mesentery (Figure 4). This effect was not reversible for up to 4 h, after which the experiments were routinely terminated (inset to Figure 4). In contrast, the vasodilator response to nitroprusside was unchanged after CHAPS treatment in the perfused rat kidney (EC_{50} = 2.6 ± 0.5 nmol, n = 8, c.f. 2.3 ± 0.03 nmol, n = 8) and perfused rat mesentery (EC_{50} = 7.6 ± 0.9 nmol, n = 5, c.f. 7.1 ± 0.9 nmol, n = 8, both $P > 0.05$).

No vasoconstrictor response to acetylcholine or histamine was ever observed in CHAPS-treated rat kidney or mesentery. In contrast, the vasoconstrictor response to noradrenaline and vasopressin in the perfused rat mesentery (Figure 5) and kidney (data not shown) was significantly enhanced after CHAPS treatment. EC_{50} values for noradrenaline in the rat mesentery before and after CHAPS treatment are shown in the legend to Figure 5.

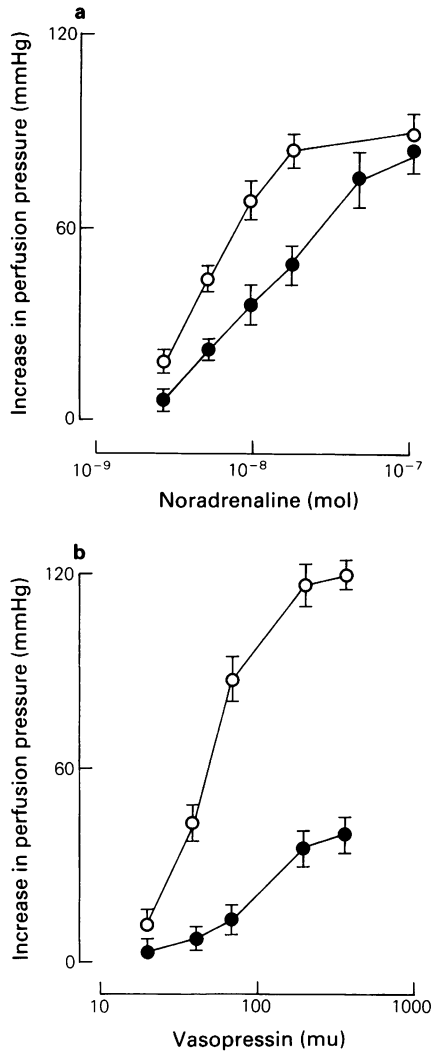

Figure 5 Vasoconstrictor effect of noradrenaline (a) and vasopressin (b) in the isolated perfused mesentery of the rat before (closed symbols) and after (open symbols) removal of endothelium by CHAPS. Results show increase in perfusion pressure in mmHg and are mean with s.e.mean shown by vertical bars; n = 7. Calculated EC_{50} values for noradrenaline are 29.2 ± 4.6 nmol before and 6.0 ± 1.1 nmol (both n = 7) after CHAPS administration.

Effect of drugs which influence EDRF biosynthesis or stability on response to vasoactive drugs

Gossypol (3 μM), mepacrine (10 μM), metyrapone (10 μM), methylene blue (100 μM) or NDGA (30 μM) reduced, or more usually abolished, the vasodilator effect of acetylcholine and histamine in the perfused 'high tone' rat kidney and mesentery. In both organs the effect of these inhibitors was reversible within 30 min. In addition, both methylene blue and mepacrine reduced the vasodilator response to nitroprusside in both vascular beds (Table 1). Superoxide dismutase (15 u ml^{-1}) perfused through either kidney or mesentery for a minimum of 30 min did not affect the vasodilator response to acetylcholine, histamine

Table 1 Effect of drugs on the vasodilator response to standard EC_{50} doses of acetylcholine (ACh, 0.18 nmol and 3.1 nmol), histamine (Hist, 31.2 nmol and 46.2 nmol) and nitroprusside (NP, 2.3 nmol and 7.1 nmol) in the perfused rat kidney and mesentery.

	Vasodilator response (mmHg)					
	Kidney			*Mesentery*		
	ACh	*Hist*	*NP*	*ACh*	*Hist*	*NP*
Control	38.7 ± 5.2	35.0 ± 2.5	42.3 ± 5.3	31.2 ± 3.2	24.6 ± 2.3	34.5 ± 3.4
Metyrapone	3.4 ± 1.2*	2.5 ± 1.2*	40.6 ± 4.3	2.3 ± 0.9*	2.3 ± 0.6*	33.3 ± 1.2
NDGA	1.2 ± 0.6*	3.4 ± 1.1*	38.7 ± 6.5	4.5 ± 0.6*	2.3 ± 0.5*	32.6 ± 5.6
Mepacrine	0	0	23.5 ± 3.4*	0	0	23.4 ± 3.3*
Gossypol	0	0	43.2 ± 2.4	0	0	34.9 ± 3.1*
Methylene blue	1.4 ± 0.5*	0.5 ± 0.1*	24.5 ± 3.5*	1.2 ± 0.2*	0.5 ± 0.1*	22.6 ± 2.3*
SOD	36.7 ± 4.5	34.6 ± 3.4	40.5 ± 5.4	36.4 ± 1.5	23.9 ± 2.9	36.4 ± 4.4

Preparations were perfused with Krebs solution containing metyrapone (10 μM), NDGA (30 μM), mepacrine (10 μM), gossypol (3 μM), methylene blue (100 μM) or superoxide dismutase (SOD, 15 u ml^{-1}) for 30 min before challenge with vasodilator drugs injected in random order. For control experiments, preparations were perfused with Krebs solution containing saline (0.5 ml added to 200 ml Krebs) as diluent. Results show fall in perfusion pressure in mmHg and are mean ± s.e.mean, $n = 6$–8, $^*P < 0.01$.

or nitroprusside. Neither gossypol nor metyrapone influenced vasoconstriction due to noradrenaline or vasopressin in perfused 'low tone' rat kidney or mesentery (Table 2).

Release of 6-oxo-prostaglandin $F_{1\alpha}$ from perfused organs

Release of PGI_2, measured by radioimmunoassay of 6-oxo-$PGF_{1\alpha}$, from perfused rat kidney and mesentery in the presence and absence of indomethacin (8 μM) is shown in Table 3. Release of 6-oxo-$PGF_{1\alpha}$ was reduced by 90% or more at this concentration. No significant increase in 6-oxo-$PGF_{1\alpha}$ release in the effluent was observed in indomethacin-pretreated perfused rat kidney challenged with maximally effective concentrations of either acetylcholine (10 nmol, 2.5 ± 0.3 ng min^{-1}, $n = 6$) or noradrenaline (50 nmol, 2.6 ± 0.5 ng min^{-1}, $n = 6$).

Discussion

EDRF mediates relaxation of vascular smooth muscle by vasodilator drugs such as acetylcholine and histamine. Responses to these and similar endothelium-dependent vasodilator drugs are characterised by, (a) a strict requirement for an intact vascular endothelium, (b) inhibition by drugs which either inactivate or prevent the synthesis or activity of EDRF and (c) conclusive evidence that PGI_2 release does not contribute to the vasodilator responses observed. Using these criteria, we provide

Table 3 Effect of indomethacin (8 μM) on 6-oxo-prostaglandin $F_{1\alpha}$ (6-oxo-$PGF_{1\alpha}$) release from perfused rat kidney and mesentery

	6-oxo-$PGF_{1\alpha}$ release (ng min^{-1})	
	No indomethacin	*With indomethacin*
Kidney	2.3 ± 0.5	0.21 ± 0.04*
Mesentery	1.1 ± 0.3	0.10 ± 0.01*

6-oxo-$PGF_{1\alpha}$ was measured by radioimmunoassay in extracts of Krebs effluent from each organ as described in Methods. Results show 6-oxo-$PGF_{1\alpha}$ release in ng min^{-1} and are mean ± s.e.mean, $n = 6$; $^*P < 0.001$.

Table 2 Effect of gossypol (3 μM) and metyrapone (10 μM) on vasoconstrictor responses to noradrenaline (NA) and vasopressin (VP) in perfused 'low tone' rat kidney and mesentery

	Kidney		*Mesentery*	
	NA (nmol)	*VP* (mu)	*NA* (nmol)	*VP* (mu)
Control	0.6 ± 0.1	4.0 ± 0.7	2.4 ± 0.5	62.0 ± 6.2
Gossypol	0.4 ± 0.2	5.1 ± 1.2	2.0 ± 0.8	59.5 ± 5.9
Metyrapone	0.9 ± 0.4	4.1 ± 0.9	2.1 ± 1.0	58.2 ± 9.2

Values indicate EC_{50}s and are the mean ± s.e.mean obtained from dose-response curves in which 5 different concentrations of drug were tested in each of 6 tissues. Preparations were perfused with Krebs solution containing test drug for 30 min before challenge with noradrenaline or vasopressin. Control preparations were perfused with Krebs solution containing saline as described in the legend to Figure 1. All results are $P < 0.05$.

evidence in this paper that EDRF mediates the vasodilator effect of acetylcholine and histamine but not that of nitroprusside in resistance vessels of the perfused rat kidney and mesentery.

Removal of vascular endothelial cells from isolated, perfused organs has provided a major problem for researchers investigating the effect of EDRF on resistance blood vessels. A suitable procedure which consistently and selectively removes endothelial cells from such vessels without damaging the underlying smooth muscle is required. Previously reported techniques include treatment with collagenase (Furchgott *et al.*, 1987), hypotonic buffer (Criscione & DeScott, 1984), saponin (Chiba & Tsukada, 1984) or electrical stimulation which is presumed to release, as yet unidentified, oxygen radicals toxic to endothelial cells (Lamb *et al.*, 1987). All of these procedures produce some damage of the underlying vascular smooth muscle as shown by reduced responsiveness to endothelium-independent drugs such as nitroprusside and the development of oedema.

In the present study we propose that CHAPS treatment provides a simple and efficient alternative to the above procedures for the selective removal of endothelial cells lining blood vessels of perfused organs. This conclusion is based upon several experimental observations in CHAPS-treated perfused rat kidney and mesentery including (A) histological evidence indicating reduced endothelium-associated staining of intra-renal blood vessels, (B) the failure of CHAPS to cause significant oedema in perfused rat organs, (C) an unchanged vasodilator effect of nitroprusside, and (D) the enhanced vasoconstrictor effect of both noradrenaline and vasopressin.

Of these individual pieces of evidence, histological data revealing CHAPS-mediated stripping of vascular endothelial cells within the kidney is probably the most convincing. This immunocytochemical staining procedure relies upon the selectivity of MRC OX-45 for components of the endothelial cell membrane. As yet, the identity of the antigen(s) bound by MRC OX-45 is (are) not clear. However, the likely antigens are glycoproteins (molecular weight, approximately 45,000) related to lymphocyte function associated (LFA-3) antigens. According to Arvieux *et al.* (1986), MRC OX-45 reacts to varying extents with such antigens on the surface of thymocytes, macrophages and erythrocytes. These cell types would not be expected to occur in significant amounts in Krebs-perfused rat organs and thus cannot account for the localised staining of blood vessels observed. According to a recently published report, MRC OX-45 also binds to LFA-3-like glycoproteins on vascular endothelial cells of several rat organs including the kidney (Arvieux *et al.*, 1986). For this reason, it seems likely that MRC OX-45 binding in rat kidney slices in the present experiments is selective for vascular endothelial cells. Although not determined histologically in the present study it seems reasonable to suggest that CHAPS also removes endothelial cells from the perfused rat mesentery in these experiments.

The precise mechanism of action of CHAPS is not clear but probably involves osmotic lysis of cells with which it comes in contact. In our experience, exposure of perfused rat organs to CHAPS for longer periods (i.e. 1 min) reduces vasodilator responses to nitroprusside as well as acetylcholine suggesting that removal of endothelial cells which occurs within the first 30 s allows CHAPS to attack and lyse underlying smooth muscle cells. In this respect, CHAPS mimics the effect of another detergent, 2-deoxycholate, which has been reported to deplete endothelial cells from the perfused rat mesentery preparation (Byfield *et al.*, 1986).

Additional evidence for the involvement of EDRF in the vasodilator response to acetylcholine and histamine in perfused rat organs was obtained using drugs previously reported to prevent responses due to EDRF. For example, NDGA, mepacrine and metyrapone diminish the response to acetylcholine and histamine most probably by promoting EDRF catabolism following generation of superoxide anions (Luckhoff *et al.*, 1987). In contrast, gossypol has been reported to prevent EDRF-mediated vasodilatation by inhibiting the biosynthesis and/or release of this mediator (Alheid *et al.*, 1987) while methylene blue is believed to act intracellularly by oxidation of the haemoprotein component of guanylate cyclase thereby preventing activation of this enzyme by EDRF (Martin *et al.*, 1985). Whatever their precise mechanisms of action, each of these inhibitors selectively and reversibly inhibited vasodilatation due to acetylcholine and histamine in the perfused rat kidney and mesentery. Mepacrine and methylene blue also reduced responses to nitroprusside in both organs and thus cannot be recommended for studying the effect of EDRF, at least in perfused organs.

Interestingly, SOD did not potentiate the vasodilator effect of acetylcholine or histamine in either perfused rat organ studied. In this context, Silin *et al.* (1985) also failed to observe an effect of SOD on relaxation of the rabbit isolated aorta to acetylcholine. The inability of SOD to potentiate/prolong the effect of EDRF in the present study may be explained by a high concentration of endogenous SOD and/or other superoxide anion scavenging enzymes in the kidney and mesentery.

Finally, preparations in this study were routinely perfused with a concentration of indomethacin sufficient to produce a substantial reduction in 6-oxo-$PGF_{1\alpha}$ efflux into the Krebs perfusate of both organs. Thus, PGI_2 can be presumed to play no part

in the vasodilator effect of acetylcholine and histamine and does not modify the vasoconstrictor response to noradrenaline and vasopressin in the perfused rat kidney and mesentery in these experiments.

The present results add to the growing body of evidence that EDRF mediates the vasodilator effect of acetylcholine and like drugs in resistance blood vessels. A similar conclusion has been reached following experiments in perfused rat lung (Cherry & Gillis, 1987) and rabbit heart (Stewart *et al.*, 1987). Thus, EDRF may have a physiological role in controlling the calibre of resistance blood vessels although the stimulus for EDRF release from such vessels remains to be identified. The observation that endothelial cells possess choline acetyltransferase activity (Parnavelas *et al.*, 1985) suggests that acetylcholine, formed in the endothelium, acts on neighbouring cells to cause EDRF release. In this way the vascular endothelium may 'sense' changes in the force of flowing blood on the vessel wall and adjust (possibly in concert with other endogenous mediators, such as PGI_2) the diameter of the blood vessel accordingly. In this context pulsatile flow of physiological buffer through isolated blood vessels has been reported to enhance EDRF release (Rubanyi *et al.*, 1986).

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