

Endothelium-derived relaxing factor (EDRF) and resistance vessels in an intact vascular bed: a microangiographic study of the rabbit isolated ear

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1 Microradiographic techniques have been used to show that endothelium-derived relaxing factor (EDRF), which is believed to be nitric oxide, influences vasomotor responses in small arteries and arterioles down to 25 μm in diameter in an isolated, intact, buffer-perfused ear preparation of the rabbit. Arteries down to 75 μm in diameter, i.e. the central ear artery (G0) and its first three generations of branch vessels (G1, G2 and G3) were studied quantitatively.

2 Relative constrictor responses to 1 μM 5-hydroxytryptamine (5-HT) and the combination of 1 μM 5-HT and 1 μM histamine diminished progressively from G0 to G3. Constrictor responses to 5-HT were doubled in all generations by 1 μM haemoglobin which abolishes EDRF activity.

3 Relative dilator responses to acetylcholine or to substance P in precontracted arteries were, in contrast, equal in the different generations. Mean $-\log(\text{IC}_{50})$ values calculated from diameter measurements were $7.63 \pm 0.10 \text{ M}$ and $9.80 \pm 0.11 \text{ M}$, respectively. These dilator responses were abolished by 1 μM haemoglobin, implying that they were EDRF-mediated. Spatial homogeneity of relative dilator responses was found also with glyceryl trinitrate (10 or 50 μM) whose activity is thought to depend on biotransformation to nitric oxide, in both the presence and the absence of haemoglobin.

4 This finding of spatial homogeneity of the diameter response to changes in EDRF activity (or to glyceryl trinitrate) implies that EDRF influences hydrodynamic resistance more in vessels where constrictor tone is high.

Introduction

Endothelium-dependent relaxation is mediated through the release of an unstable endogenous vasodilator, endothelium-derived relaxing factor (EDRF) (Furchgott, 1983; Griffith *et al.*, 1984a), which has recently been shown to be nitric oxide (Palmer *et al.*, 1987). Two mechanisms of release may be important in the regulation of resistance vessel tone since EDRF is known to be released from the endothelium of conduit vessels either basally (Griffith *et al.*, 1984a,b) or as a result of pharmacological stimulation (Furchgott & Zawadzki, 1980; Furchgott, 1983). Basal EDRF activity is enhanced by fluid flow (Rubanyi *et al.*, 1986; Pohl *et al.*, 1986) and influences constrictor and relaxant responses in conduit vessels (Griffith *et al.*, 1984a,b; Shirasaki & Su, 1985; White *et al.*, 1986; Pohl & Busse, 1987). We have

previously shown that such basal activity modulates myogenic tone in resistance vessels of the rabbit isolated perfused ear (Griffith *et al.*, 1987). In the present study we have investigated its influence on pharmacological constrictor and dilator responses in the same preparation. We have also studied responses to acetylcholine and substance P which can mediate relaxation in isolated conduit vessel preparations through an endothelium-dependent mechanism (Furchgott, 1983; D'Orleans-Juste *et al.*, 1985). In addition, we studied responses to glyceryl trinitrate (GTN), whose activity is thought to depend on biotransformation to nitric oxide (Schröder *et al.*, 1985). EDRF and GTN relax vascular smooth muscle by activating soluble guanylate cyclase, elevating tissue levels of guanosine 3':5'-cyclic monophosphate (cyclic GMP; Holzmann, 1982; Rapoport *et al.*, 1983; Griffith *et al.*, 1985; Forstermann *et al.*,

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1986; Ignarro *et al.*, 1986), and thereby reducing calcium influx and intracellular calcium release (Collins *et al.*, 1986).

A novel X-ray microscopic technique was used to perform these studies. It obviates the necessity for dissection and isolation of blood vessels, the trauma of which can potentially alter their responsiveness, and allows simultaneous imaging of vessels over a wide range of sizes (25–800 μm) in an intact vascular bed. The isolated, controlled-flow buffer-perfused ear preparation from the rabbit (Griffith *et al.*, 1987) is two-dimensional, which simplifies analysis, and relatively free of metabolic effects which might secondarily influence vascular tone; it also avoids the further complexity of pulsatile flow.

Methods

Rabbit isolated ear preparation

Male New Zealand White rabbits (2.5 kg) were killed by a blow to the neck. Isolated ear preparations were perfused with oxygenated Holman's buffer (composition, μM : NaCl 120, KCl 5, CaCl_2 2.5, NaH_2PO_4 1.3, NaHCO_3 25, glucose 11, sucrose 10, pH 7.2–7.4) and microangiograms made with a 4 μm microfocal X-ray source as described previously (Griffith *et al.*, 1987). Dextran (5%, mol. wt. 80,000) was dissolved in the buffer to prevent the development of oedema and to increase viscosity to 2.23 mPas so as to eliminate artefactual rises in perfusion pressure when contrast medium entered the perfusion circuit. Each ear was mounted vertically in front of the X-ray source so that contrast medium did not overflow onto its surface and obscure the field of view. The static pressure head (ca. 10 mmHg) resulting from this vertical orientation and the pressure drop occurring across the input cannula were subtracted before analysing perfusion pressure data. Geometrically magnified images of the vascular network of the rabbit ear were obtained on conventional radiographic film. Iodinated contrast medium possesses intrinsic vasodilator properties and a low concentration (100 mg iodine ml^{-1}) of non-ionic contrast medium (iohexol 350 mg iodine ml^{-1} diluted in Holman's buffer) was chosen to minimize this effect. The perfusion circuit was designed so that a short pulse (0.5 ml) of this diluted contrast medium was washed out by Holman's solution immediately after each X-ray exposure. Preliminary real-time experiments were performed with an image intensification system to synchronize the exposures with the transit of contrast medium. The maximal pressure fall induced by the contrast medium occurred towards the end of, or after, termination of the radiographic exposure and was on average <2 mmHg in

resting preparations (perfusion pressure ca. 30–40 mmHg), and <8 mmHg in constricted preparations (perfusion pressure ca. 100–150 mmHg). Estimated diameter changes corresponding to these pressure falls were made by eliminating flow from the diameter-flow and pressure-flow data of Figures 2 and 3, and indicated that systematic errors would be most significant in highly activated beds, and then be of the order of $\approx 5\%$. The vasodilator properties of the contrast medium were therefore ignored, particularly in view of the fact that it would affect all vessels simultaneously.

Quantitation

Vascular diameters were measured from the static radiographic images by using an IBAS Kontron Semi-interactive Image Analysis System (Kontron Electronics, Munich, F.R.G.). Briefly, the edges of vessels under study were first identified visually on a display monitor and the analysis system then computed absolute diameters. Each vessel selected for study was measured 6 times and the results meaned. The accuracy of the method was shown to be better than 5% for vessels in the range of 70 μm , and higher for larger vessels such as the central ear artery, by using fine cylindrical tungsten wires calibrated by electron microscopy. Experiments were performed at geometric magnifications of approximately 4 which were determined exactly by placing a calibrated 3 mm gold grid on the rabbit ear within the field of interest. Further magnification was provided electronically by the image analysis system. Although vessels >25 μm diameter could be visualized, accurate quantitation was possible only in those of diameter >70 μm , that is, in the central ear artery, (G0) and its first three generations of branch vessels (G1–G3). In order to obtain a spatially 'averaged' diameter estimate for a typical vessel in G1 to G3, five representative vessels from each generation were selected in each preparation and their diameters meaned. G0 was measured midway between the base and the tip of each ear. Data from all preparations were then pooled and further analysed. Small veins and venules were studied by an identical protocol. If experiments were repeated after 60 min, diameter measurements were then always within 10% of each other and in many cases agreement was within 5%. Intermittent exposure to iohexol did not therefore appear to impair endothelium-dependent dilatation or pharmacological responses significantly.

Pharmacological agents

Microradiographs were obtained at steady-state after addition of pharmacological agents as determined by observation of the pressure trace. In

experiments studying basal EDRF activity or responses to GTN, tone was induced in the bed by $1\text{ }\mu\text{M}$ 5-hydroxytryptamine (5-HT) which produced approximately half-maximal constriction for this agent. This constriction was well maintained for 20–30 min. However, in order to construct cumulative concentration-response curves to acetylcholine or substance P, the combination of $1\text{ }\mu\text{M}$ 5-HT and $1\text{ }\mu\text{M}$ histamine was found necessary to provide stable pre-constriction for up to 50 min. This combination of agents produced a higher degree of constriction than maximal concentrations of 5-HT alone. Higher concentrations of histamine generally resulted in phasic activity and therefore an unsteady perfusion pressure.

At concentrations of $1\text{ }\mu\text{M}$ 5-HT and histamine do not stimulate EDRF release from rabbit aortic endothelium (Griffith *et al.*, 1984b; Griffith, 1985), and do not appear to induce endothelium-dependent relaxation in the rabbit ear vasculature: after constriction by 5-HT ($1\text{ }\mu\text{M}$), low concentrations ($0.001\text{ }\mu\text{M}$ to $0.1\text{ }\mu\text{M}$) of histamine produced further constriction only and vice versa. Furthermore, endothelium-dependent dilatation by 5-HT was not unmasked by the S2 antagonist ketanserin ($3\text{ }\mu\text{M}$), as is the case in canine coronary artery (Cocks & Angus, 1983). Histamine can mediate endothelium-dependent relaxation of precontracted rabbit middle cerebral arteries through stimulation of an H_2 receptor but only at concentrations higher than those used in the present study (Sercombe *et al.*, 1986).

EDRF activity was inhibited by haemoglobin ($1\text{ }\mu\text{M}$) prepared by lysis of washed human red cells and purified by Sephadex G200 gel chromatography as described previously (Edwards *et al.*, 1986). Its action appears to be specific against EDRF in buffer perfused arteries where the normal endothelial permeability barrier is presumably lost, and it has no direct constrictor action at this concentration (Martin *et al.*, 1985; Edwards *et al.*, 1986). In preliminary experiments with isolated segments of central ear artery, dilatation by acetylcholine and constriction by haemoglobin (up to $1\text{ }\mu\text{M}$) were lost after reaming with thread, and were reduced or absent in intact preparations which had been intermittently perfused with air, which is also known to damage endothelium. These findings provide confirmatory evidence that their action was due to stimulation and inhibition of EDRF activity.

Materials

5-HT, histamine, acetylcholine and substance P were purchased from Sigma Chemical Co., Poole. Glyceryl trinitrate was purchased from Napp Laboratories, Cambs., Iohexol was obtained from Nyegaard.

Statistical analysis

Logarithmic concentration-response curves were constructed for dilatation produced by acetylcholine and substance P in terms of diameter increases in the separate generations of branch vessels (G0 to G3) and also for the corresponding fall in perfusion pressure of the whole network. Concentrations producing 50% reversal of constrictor responses (i.e. median effective concentrations, IC_{50}) and maximum % dilatation (D_{max}) (expressed relative to the constrictor response) were determined for individual preparations, and geometric mean IC_{50} values and mean D_{max} determined from these values. The geometric mean IC_{50} and D_{max} of the various data groups were compared by one-way analysis of variance. Dilator responses to GTN were also compared by one-way analysis of variance. The effect of haemoglobin on constrictor responses in each generation was evaluated by a paired Student's *t* test. $P < 0.05$ was taken as the significance level.

Results

Constrictor responses

There was spatial heterogeneity in the responsiveness of different generations of branch vessels to the constrictor stimulus of $1\text{ }\mu\text{M}$ 5-HT. Constriction was greatest in the central artery (G0) and diminished progressively through successive generations of branch vessels (Figure 1, Table 1). Constriction expressed as decrease in diameter relative to uncontracted diameter ($\Delta\text{D}/\text{D}\%$, mean from Table 1a,b) was 26% in G0, 16% in G1, 11% in G2 and –2% in G3. The mean pressure drop across the bed increased from 33 to 51 mmHg (Table 1). A similar pattern of spatial heterogeneity was also found with the greater constrictor stimulus of $1\text{ }\mu\text{M}$ 5-HT plus $1\text{ }\mu\text{M}$ histamine, $\Delta\text{D}/\text{D}\%$ being 34%, 23%, 16% and 9% in G0–G3 respectively. With this combination of constrictor agents the pressure drop across the bed increased from 34 mmHg to 136 mmHg (Table 1c).

When the influence of basal EDRF activity was abolished by adding $1\text{ }\mu\text{M}$ haemoglobin to the perfusate containing $1\text{ }\mu\text{M}$ 5-HT, constriction relative to the resting state ($\Delta\text{D}/\text{D}$) was significantly greater than in the absence of haemoglobin and was increased by factors of 2.33, 2.20 and 2.14 in generations G0, G1 and G2, respectively. Responses also became more consistently constrictor in generation G3 (Table 1b). Since the relative constrictor response approximately doubled in generations G0–G2 the spatial heterogeneity of the response to 5-HT therefore persisted. The mean pressure drop across the

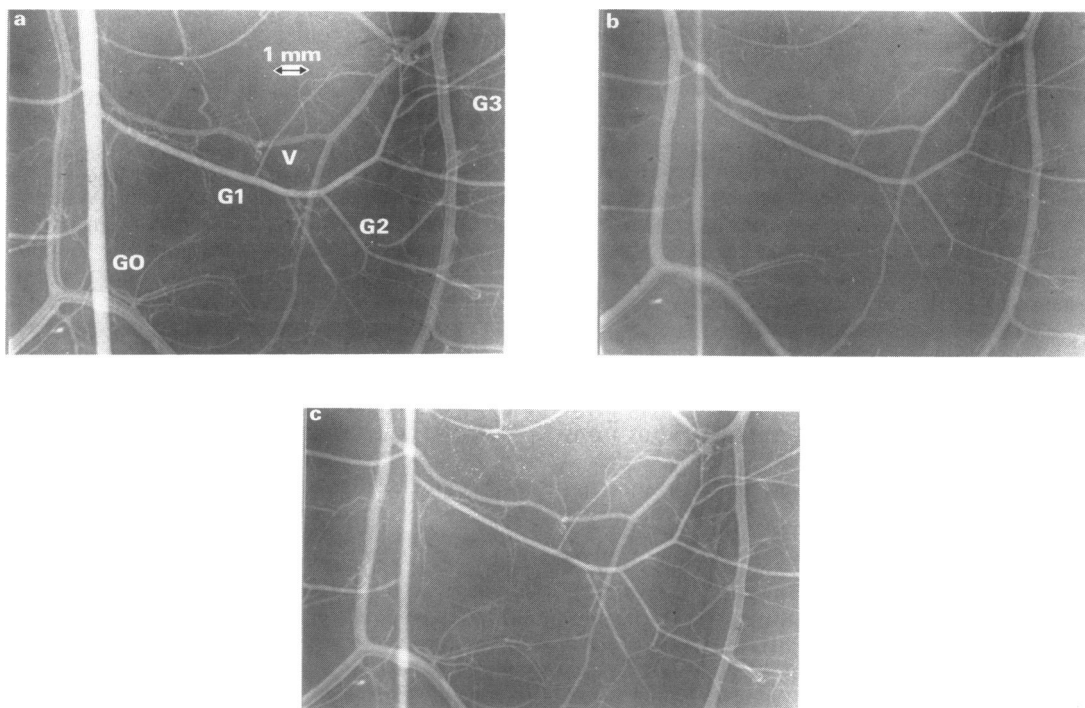


Figure 1 Representative microradiographs illustrating diameter changes in part of the vascular bed of an isolated ear preparation perfused at a constant flow rate of 2.00 ml min^{-1} . (a) Control situation. The central artery is labelled G0, and representative vessels of the next three generations also indicated (G1–G3). (b) 5-

Hydroxytryptamine (5-HT, $1 \mu\text{M}$) constricted the central artery and in this particular example all vessels down to about $25 \mu\text{m}$. (c) Subsequent addition of $1 \mu\text{M}$ acetylcholine dilated vessels down to $25 \mu\text{m}$. There was an apparent lack of responsiveness to 5-HT and acetylcholine in veins of any size (V).

bed on adding haemoglobin in the presence of 5-HT increased from 48 mmHg to 81 mmHg.

Dilatation by glyceryl trinitrate

Dilator responses to GTN in preparations constricted by $1 \mu\text{M}$ 5-HT, in contrast, showed spatial homogeneity when expressed as dilatation (increase in diameter) relative to the preceding constriction (decrease in diameter), i.e. $\Delta d/\Delta D$ (Table 1a,b). This calculation could not be made for generation G3 because 5-HT alone did not consistently induce constrictor responses. The relative dilatation of G0, G1 and G2 was the same in each case, whether to $10 \mu\text{M}$ or $50 \mu\text{M}$ GTN. Furthermore, the magnitude of these relative dilator responses was not affected by addition of $1 \mu\text{M}$ haemoglobin to the buffer, although absolute diameter increases were larger because of the greater constriction which was then obtained.

Stimulated release of EDRF

Arteries precontracted by the combination of $1 \mu\text{M}$ 5-HT and histamine were dilated both by acetylcholine and by substance P in a concentration-dependent manner (Figures 2 and 3). This dilatation was reversed by the subsequent addition of haemoglobin ($1 \mu\text{M}$) as was the fall in perfusion pressure across the bed. $\text{Log}(\text{IC}_{50})$ values either for acetylcholine or for substance P were the same whether derived from G0, G1, G2, G3 or the pressure drop across the bed (Figures 2 and 3; Table 2): mean values calculated from diameter measurements were $7.63 \pm 0.10 \text{ M}$ for acetylcholine and $9.80 \pm 0.11 \text{ M}$ for substance P (Table 2). The maximum dilatation with substance P ($83 \pm 7\%$) was slightly less than that with acetylcholine ($95 \pm 4\%$) (Table 2). In some preparations $1 \mu\text{M}$ 5-HT or the combination of $1 \mu\text{M}$ 5-HT and histamine produced demonstrable constriction in arterioles down to $25 \mu\text{m}$ in size. Acetyl-

Table 1 Relative constrictor and dilator responses in generations G0–G3

		<i>Constriction</i>		<i>Dilatation</i>	
		$\frac{\Delta D}{D}$	$\frac{\Delta D}{D}$	$\frac{\Delta d}{\Delta D}$	$\frac{\Delta d}{\Delta D}$
a		<i>5-HT</i> (1 μ M)	—	+ <i>GTN</i> (10 μ M)	+ <i>GTN</i> (50 μ M)
	G0	0.31 \pm 0.06	—	0.64 \pm 0.14**	0.79 \pm 0.09***
	G1	0.18 \pm 0.05	—	0.62 \pm 0.14**	0.67 \pm 0.13***
	G2	0.16 \pm 0.04	—	0.63 \pm 0.15**	0.89 \pm 0.10***
	G3	-0.03 \pm 0.03	—	—	—
Pressure drop (mmHg) (resting = 33 \pm 3)		55 \pm 5	—	35 \pm 6	31 \pm 7
b		<i>5-HT</i> (1 μ M)	+ <i>Hb</i>	+ <i>Hb</i> + <i>GTN</i> (10 μ M)	+ <i>Hb</i> + <i>GTN</i> (50 μ M)
	G0	0.21 \pm 0.07	0.50 \pm 0.06*	0.50 \pm 0.14**	0.78 \pm 0.15***
	G1	0.15 \pm 0.04	0.33 \pm 0.08*	0.56 \pm 0.14**	0.83 \pm 0.09***
	G2	0.07 \pm 0.03	0.15 \pm 0.03*	0.66 \pm 0.16**	0.84 \pm 0.16***
	G3	-0.01 \pm 0.07	0.03 \pm 0.05	—	—
Pressure drop (mmHg) (resting = 32 \pm 4)		48 \pm 6	81 \pm 10	65 \pm 7	48 \pm 7
c		<i>5-HT plus histamine</i> (both 1 μ M)			
	G0	0.34 \pm 0.06			
	G1	0.23 \pm 0.06			
	G2	0.16 \pm 0.04			
	G3	0.09 \pm 0.05			
pressure drop (mmHg) (resting = 34 \pm 3)		136 \pm 13			

* Constrictor responses of the central ear artery (G0) and its first three branch generations (G1–G3) to 5-hydroxytryptamine (5-HT, 1 μ M) in the absence (a, n = 9) and presence (b, n = 9) of 1 μ M haemoglobin (Hb) expressed as decrease in diameter/resting diameter, $\Delta D/D$. Constrictor responses to the combination of 1 μ M 5-HT and 1 μ M histamine are also given (c, n = 9). Dilator responses to glyceryl trinitrate (GTN) 10 μ M and 50 μ M in preparations constricted by 5-HT are expressed as increase in diameter from constricted state/decrease in diameter, $\Delta d/\Delta D$. Vessels in generation G3 did not always exhibit constrictor responses to 5-HT so that it was not possible consistently to calculate relative dilator responses to GTN. The total pressure drop across the bed at steady state was increased by haemoglobin (b). Veins and venules did not appear to react actively to 5-HT, haemoglobin or GTN.

* Denotes constrictor responses significantly increased by the presence of haemoglobin compared to its absence (P < 0.05).

Mean relative dilator responses at each concentration of GTN (10 μ M GTN (***) or 50 μ M GTN (**)) in generations G0–G2 were not significantly different from each other, and were not significantly altered by the presence of haemoglobin (P < 0.05).

Table 2 Comparison of acetylcholine- and substance P-induced dilatation

	<i>Acetylcholine</i>		<i>Substance P</i>	
	$-\log_{10} (IC_{50})$	D_{max}	$-\log_{10} (IC_{50})$	D_{max}
G0	7.89 \pm 0.27	96 \pm 3	9.55 \pm 0.38	82 \pm 10
G1	7.51 \pm 0.22	93 \pm 5	9.70 \pm 0.20	73 \pm 11
G2	7.67 \pm 0.16	92 \pm 8	10.03 \pm 0.15	93 \pm 4
G3	7.46 \pm 0.25	90 \pm 10	9.94 \pm 0.14	82 \pm 10
Mean	7.63 \pm 0.10	93 \pm 1	9.80 \pm 0.11	83 \pm 4
value				
Pressure responses	7.84 \pm 0.20	95 \pm 4	9.80 \pm 0.23	83 \pm 7*

Molar $\log_{10} (IC_{50})$ values for reversal of constriction, induced by the combination of 1 μ M 5-hydroxytryptamine (5-HT) and 1 μ M histamine, by acetylcholine and substance P (n = 9 in each case). These did not differ significantly in terms of diameter changes in G0 to G3, or the corresponding pressure response of the whole network. The maximum % dilatation (D_{max}) produced by these agents is also given, and was significantly less for substance P than for acetylcholine.

* Significantly different from maximum % response to acetylcholine (P < 0.01). Values within each column are not significantly different from each other.

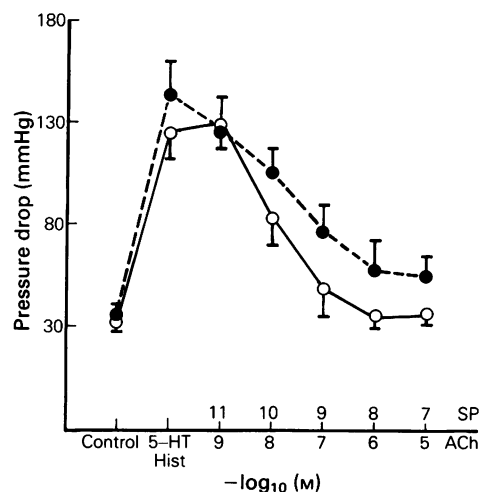
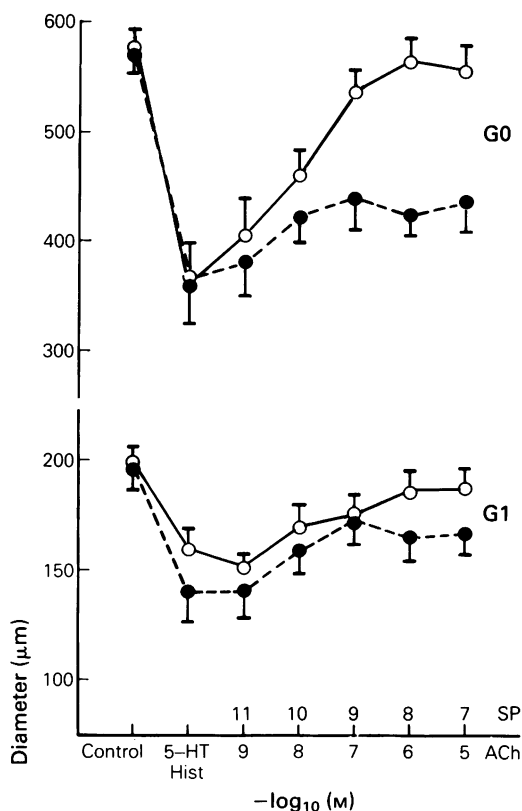


Figure 3 Log concentration-response curves showing mean pressure drop across the ear network after constriction by $1 \mu\text{M}$ 5-hydroxytryptamine (5-HT) plus $1 \mu\text{M}$ histamine (Hist) and dilatation by acetylcholine (\circ — \circ) or substance P (\bullet — \bullet) in the preparations used to obtain the diameter data of Figure 2. Log_{10} (IC_{50}) values were $7.84 \pm 0.20 \text{ M}$ and $9.80 \pm 0.23 \text{ M}$, respectively (see Table 2).

choline and substance P were then similarly able to dilate such vessels although their effect was not studied quantitatively (Figure 1).

Responses of small veins and venules

Veins visualized down to $25 \mu\text{m}$ diameter showed no change of diameter in response to 5-HT, histamine, haemoglobin, acetylcholine, substance P or GTN (Figure 1). In the absence of a measurable constrictor response, dilator responses could not be studied. Lack of measurable constriction in this model does not exclude a constrictor response occurring in a direction parallel to the X-ray beam. Veins fixed by 1% glutaraldehyde during perfusion (which did not

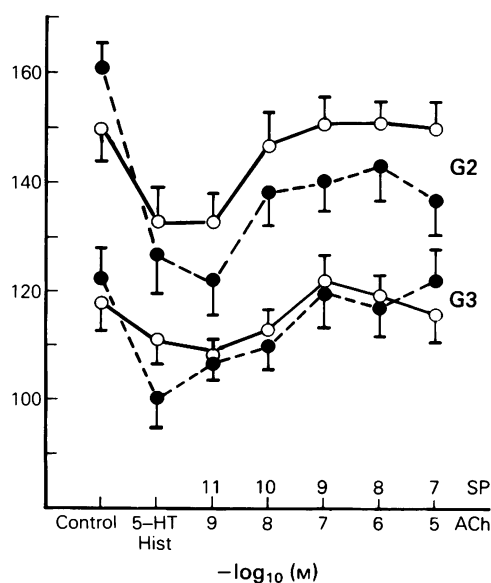


Figure 2 Logarithmic concentration-response curves showing diameter changes in G0–G3 induced by cumulative addition of acetylcholine (ACh) 10^{-9} to 10^{-5} M (\circ — \circ , $n = 9$) or substance P (SP) 10^{-11} to 10^{-7} M (\bullet — \bullet , $n = 9$) in preparations precontracted by the combination of $1 \mu\text{M}$ 5-hydroxytryptamine (5-HT) and $1 \mu\text{M}$ histamine (Hist). Measurements were made when the pressure response following each addition of drug was steady. Log (IC_{50}) values for acetylcholine and substance P were identical in G0 to G3, the mean values being $7.63 \pm 0.10 \text{ M}$ and $9.80 \pm 0.11 \text{ M}$, respectively (see Table 2).

change perfusion pressure) and then stained histologically were elliptical in cross-section with their short axis parallel to the X-ray beam and, *a priori*, constriction would probably occur predominantly along the short axis.

Discussion

The data show that relative constrictor responses to either 1 μ M 5-HT, which increased the pressure drop across the bed by approximately 20 mmHg, or 1 μ M 5-HT plus 1 μ M histamine, which increased the pressure drop by approximately 100 mmHg, exhibit spatial heterogeneity in the rank order $G0 > G1 > G2 > G3$ in these rabbit ear preparations. Constrictor responses to 5-HT were significantly attenuated by basal EDRF activity, since they were amplified by freshly prepared haemoglobin at a concentration which abolishes EDRF activity without producing vascular smooth muscle constriction (Edwards *et al.*, 1986). We have previously shown that in non-constricted rabbit ear preparations basal EDRF activity inhibits myogenic tone maximally in generation G1 (Griffith *et al.*, 1987). This contrasts with the present data in which relative constrictor responses were doubled by haemoglobin in each generation of vessels, so that basal EDRF activity was spatially 'uniform' in these constricted preparations. Pharmacological constriction therefore appears to be able to 'override' the intrinsic myogenic properties of the individual vessels. Preservation of the spatial heterogeneity of constrictor responses in the presence of haemoglobin implies that it is primarily determined by smooth muscle responsiveness and not by EDRF activity.

The effects of GTN are relevant in that its activity is dependent on biotransformation to nitric oxide (Schröder *et al.*, 1985), so that it can therefore be regarded as an exogenous counterpart of EDRF. GTN (at two different concentrations) dilated all generations of the precontracted bed to an equivalent relative extent ($\Delta d/\Delta D$, Table 1a,b), either in the presence or in the absence of basal EDRF activity. The dilator effect of GTN, like that of basal EDRF activity, was therefore spatially homogeneous. Haemoglobin partially inhibits vascular smooth muscle responses to GTN (Martin *et al.*, 1985), and therefore IC_{50} values in the presence and absence of haemoglobin could not be formally compared. Under various experimental conditions EDRF may depress (Shirasaki & Su, 1985; White *et al.*, 1986; Pohl & Busse, 1987), enhance (White *et al.*, 1986) or not affect (Pohl & Busse, 1987) vascular responsiveness to nitrovasodilators. In this intact rabbit ear preparation, dilator responses to GTN (expressed with

respect to constrictor responses) appear to be independent of EDRF activity.

The finding that acetylcholine- and substance P-induced dilatation was inhibited by haemoglobin in all generations suggests that they influence tone in resistance vessels by stimulating EDRF release as in large arteries. Substance P was less effective in absolute terms than acetylcholine in reversing constrictor tone, probably due to the rapid development of tachyphylaxis to its action (Furchgott, 1983). The dilator response both to acetylcholine and to substance P showed spatial homogeneity in respect of changes of diameter, as confirmed by similar IC_{50} values through all generations. Thus, either inhibition or stimulation of basal EDRF activity resulted in relative changes in diameter which were independent of vessel size and degree of constriction.

Previous workers have shown increased potency of acetylcholine and other endothelium-dependent dilators with diminishing vessel size in resistance vessels from the rabbit ear and rat mesentery, iso-

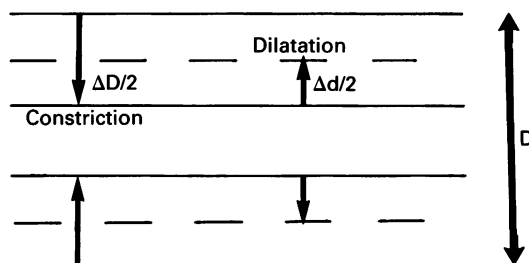


Figure 4 Schematic diagram showing constriction (ΔD = decrease in diameter) and subsequent dilatation (Δd = increase in diameter) in a vessel of resting diameter D . Since hydraulic resistance (R) is proportional to $1/(\text{diameter})^4$, relative changes in resistance ($\Delta R/R$) are directly proportional to relative changes in diameter. For dilator responses therefore,

$$\Delta R/R \propto \frac{\Delta d}{D - \Delta D} = \frac{\frac{\Delta d}{\Delta D} \times \frac{\Delta D}{D}}{\left(1 - \frac{\Delta D}{D}\right)}$$

where $\Delta d/\Delta D$ may be taken as a constant which is the same in each generation at a given concentration of dilator agent. This applies to glyceryl trinitrate (GTN, Table 1) and also acetylcholine and substance P because of the similar IC_{50} values in each generation (Table 2). It follows that $\Delta R/R = 0$ in vessels in which there is no constrictor response (i.e. $\Delta D = 0$), and conversely that $\Delta R/R \rightarrow \infty$ as relative constriction increases and $\Delta D \rightarrow D$. Dilator agents therefore affect hydraulic resistance most in highly constricted vessels, in spite of identical values of $\Delta d/\Delta D$ in each generation.

lated and studied *in vitro* at resting tensions adjusted to achieve maximum active tension development (de Mey & Gray, 1985; Owen & Bevan, 1985). This may be attributed to the fact that the endothelium/smooth muscle volume ratio is higher and effective diffusion distance shorter in small arteries. In an intact perfused bed such as we have studied here, the situation will be more complex. Vasomotion in one part of the bed will secondarily influence vasomotion elsewhere in that bed, as any agent which produces constriction or dilatation of 'upstream' vessels will also influence downstream vessel calibre through alterations in 'downstream' intraluminal pressure. Furthermore, pharmacological responsiveness is dependent on circumferential wall stress (Gore, 1972; Price *et al.*, 1983; Nilsson & Sjöblom, 1985). The regional variation in contractile responses may thus be explained by the fact that different sized vessels will be under different levels of wall stress in a perfused vascular network (Gore, 1972). Indeed, contractile responses of resistance vessels from the rabbit ear exhibit a different pattern of spatial heterogeneity to that described here when studied in isolation (Owen *et al.*, 1983). Further complexity is introduced by the fact that fluid flow stimulates EDRF release through shear stress on the endothelium (Rubanyi *et al.*, 1986; Pohl *et al.*, 1986). In a constant flow system, as used in the present study, EDRF activity will thus be enhanced in any vessel by constriction, which will increase shear stress, and conversely be reduced by dilatation.

The hydraulic resistance of any vessel is related to its calibre by an inverse 4th power relationship ($R \propto 1/D^4$). Although the influence of EDRF and GTN on diameter changes was spatially homogeneous, it follows that they will nevertheless influence hydraulic resistance in each branch generation in a manner which parallels the spatial heterogeneity of the constrictor responses, namely in the rank order $G_0 > G_1 > G_2 > G_3$ (Figure 4). Since EDRF activity inhibits myogenic tone to the greatest extent in generation G_1 in these preparations (Griffith *et al.*, 1987), its influence on hydraulic resistance therefore depends on both the degree and the mode of smooth muscle activation. In an intact bed subject to a combination of humoral, neurogenic and metabolic vasomotor influences, such as will be the case *in vivo*, EDRF may play a very complex role in the modulation of the relative resistance of vessels of different sizes.

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