Br. J. Pharmacol. (1991), **102**, 938–942

Characterization of the 5-HT receptor mediating endothelium-dependent relaxation in porcine vena cava

M.J. Sumner

Peripheral Pharmacology Department, Glaxo Group Research, Ware, Hertfordshire SG12 0DP

1 5-Hydroxytryptamine (5-HT) relaxes rings of neonatal porcine isolated vena cava by both an endothelium-dependent and an endothelium-independent mechanism. The receptor mediating the latter response has been shown to be a 5-HT_1-like receptor (positively coupled to adenylyl cyclase) located on the vascular smooth muscle. The features of the endothelium-dependent response to 5-HT in this preparation are now described.

2 In ring preparations contracted with the stable thromboxane-A_2-mimetic, U-46619 (10 nM), and in the presence of the 5-HT_2 receptor antagonist ketanserin (1 μM), low concentrations of 5-HT (1–100 nM) evoked an endothelium-dependent, rapid, 'spike-like' relaxation. Higher concentrations of 5-HT (0.1–10 μM) elicited a more sustained, but endothelium-independent relaxation.

3 Relaxation induced by low concentrations (1–100 nM) of 5-HT was abolished by endothelium removal, and was markedly (but not totally) inhibited by the guanylate cyclase inhibitor, methylene blue (10 μM) or by the inhibitor of endothelium-derived nitric oxide (NO) synthesis, L-N^G-monomethylarginine (L-NMMA; 100–500 μM).

4 The endothelium-dependent response to 5-HT was mimicked by α-methyl-5-HT, 5-methoxytryptamine, tryptamine and 2-methyl-5-HT, but not by sumatriptan or 8-hydroxy-di-n-propylaminotetralin (8-OH-DPAT) at concentrations up to 10 μM. In contrast, relaxation evoked by 5-carboxamidotryptamine (5-CT) was endothelium-independent.

5 The endothelium-dependent relaxation induced by 5-HT or α-methyl-5-HT was antagonized by methysergide, methiothepin, cyproheptadine and metergoline, but not by ketanserin, spiperone, ondansetron, verapamil, cyanopindolol, mesulergine, ICS 205-930, or indomethacin.

6 These results suggest that the endothelium-dependent relaxation of porcine vena cava induced by 5-HT is largely mediated by the release of NO (although other endothelium-derived relaxing factors may also be involved) and that 5-HT is acting at a receptor which is not '5-HT_1-like', 5-HT_2, 5-HT_3 or 5-HT_4 and is not comparable to recognised 5-HT receptor ligand binding sites. The characteristics of this receptor are discussed in relation to the endothelial 5-HT receptor types in other blood vessels.

Keywords: Endothelium; EDRF; 5-HT receptor; α-methyl-5-HT; porcine vena cava

Introduction

Relaxation of vascular smooth muscle by 5-hydroxytryptamine (5-HT) can be brought about by at least two mechanisms. We have previously described an endothelium-independent relaxation of neonatal porcine isolated vena cava by 5-HT and have shown this to be mediated through a '5-HT_1-like' receptor located on the vascular smooth muscle, activation of which leads to increases in intra-cellular adenosine 3':5'-cyclic AMP (cyclic AMP) and smooth muscle relaxation (Sumner *et al.*, 1989).

Relaxation of vascular smooth muscle by 5-HT can also be elicited through an endothelium-dependent mechanism. The 5-HT receptors mediating such responses, which have been studied extensively in coronary artery preparations, have been designated '5-HT_1-like'. Such a classification has largely been based on the lack of effect of 5-HT_2 and 5-HT_3 receptor antagonists (Cocks & Angus, 1983; Leff *et al.*, 1987; Molderings *et al.*, 1989; reviewed in Angus & Cocks, 1989). An endothelium-dependent relaxation to 5-HT in the neonatal porcine isolated vena cava preparation has previously been described (Sumner & Humphrey, 1988). The characteristics of this response are now described in more detail.

Methods

Measurement of vascular relaxation

This was performed as previously described (Sumner *et al.*, 1989). Briefly, lengths of abdominal vena cava were removed from neonatal pigs (Large White variety, 6–10 days of age, killed by captive-bolt pistol and exsanguination) and were divided into 4 rings (each 2–3 mm in length). These were mounted for isometric recording of tension changes and were maintained at 37°C in a gassed Krebs solution under a resting tension of 0.2–0.5 g, and allowed to equilibrate for 60 min, with changes of Krebs solution every 15 min. After this period, preparations were 'primed' with 80 mM KCl which was then washed out with fresh Krebs solution. After a further 30 min, preparations were contracted with 10 nM U-46619, a concentration that had previously been shown to produce 70–90% of its maximal response. When a stable tone had been established, cumulative concentration-effect curves to 5-HT were produced in all preparations. Throughout this study, the thromboxane A_2 receptor agonist, U-46619 was used to contract porcine vena cava ring preparations in the presence of the 5-HT_2 receptor antagonist, ketanserin (1 μM) to prevent the contractile effects of 5-HT_2 receptor agonists (Cocks & Angus, 1983).

Agonist potency measurements

Cumulative concentration-effect curves (over the range 0.1 nM to 10 μM) to agonists were constructed in preparations contracted with U-46619 (10 nM), each successive concentration being applied when the response to the previous concentration had reached a plateau. Agonist potencies were assessed relative to 5-HT. Thus, on one ring preparation (control), cumulative concentration-effect curves were repeated to assess changes in tissue sensitivity to 5-HT (geometric mean agonist concentration-ratio, with 95% confidence intervals, was 1.23, 0.38–1.97, $n = 28$). On three other ring preparations from the same vessel, concentration-effect curves to 5-HT were followed

by curves for test agonists. Relaxant responses were expressed as a percentage of U-46619-induced tone, this being the difference between resting and sustained tensions (0.5–1 g). Relaxation was measured at the peak response and expressed as a percentage relative to the initial tone preceding agonist addition. The concentration of an agonist required to produce 50% of its own maximal relaxation represented the EC_{50} value; where quoted, the pEC_{50} is the negative logarithm$_{10}$ of the molar EC_{50} value.

Antagonist potency measurements

Potential antagonists of the relaxation induced by 5-HT or α-methyl-5-HT were assessed in preparations contracted with U-46619 in the presence of ketanserin (1 μM).

For these experiments, four ring preparations from the same blood vessel were used. Cumulative concentration-effect curves to 5-HT or α-methyl-5-HT were produced for each preparation. After washing, three rings were exposed to antagonist or vehicle, whilst the fourth ring remained untreated (control). After 30 min, the preparations were contracted to a constant tone and cumulative concentration-effect curves to 5-HT or α-methyl-5-HT were repeated.

After correcting for any sensitivity changes over successive agonist curves, concentration-ratios were calculated by dividing the EC_{50} for the agonist in the presence of antagonist (second curve) by the EC_{50} for the agonist in the absence of antagonist (first curve). In control preparations, the geometric mean concentration-ratios (with 95% confidence intervals) over two consecutive 5-HT or α-methyl-5-HT concentration-effect curves were 1.53 (0.51–2.13) and 1.71 (0.29–2.39) respectively ($n = 26$). Concentration-ratios were used to calculate pA_2 (slope) values by linear regression analyses of Schild plots (Arunlakshana & Schild, 1959).

Endothelium removal

In experiments to examine the endothelium dependency of 5-HT-induced relaxant responses, lengths of porcine vena cava were divided in half. One half was endothelium-denuded by scraping the lumen with an inflated balloon catheter (Swan-Ganz, 0.5 ml capacity), whilst the other was left intact (control). The vessel sections were then divided into rings and mounted in organ baths. Absence of the endothelium was confirmed by loss of relaxant response to acetylcholine or carbachol (1 μM).

Drugs

The following compounds were purchased or generously donated: 5-hydroxytryptamine creatinine sulphate, tryptamine hydrochloride, methylene blue, 5-methoxytryptamine hydrochloride and verapamil hydrochloride (Sigma), spiperone, ketanserin (Janssen), methiothepin maleate (Hoffman-La Roche), metergoline (Farmitalia), methysergide hydrogen maleate, mesulergine (a kind gift from Dr G. Engel, Sandoz), L-N^G-monomethylarginine citrate (Salford Ultrafine Chemicals, Manchester), and ICS 205–930 (Research Biochemicals).

The following compounds were synthesized by Chemistry Research, Glaxo Group Research, Ware, Herts: 5-carboxamidotryptamine, α-methyl-5-HT maleate, 2-methyl-5-HT sulphate, 8-hydroxy-2(di-n-propylamino)tetralin hydrobromide, (±)-cyanopindolol, U-46619 (11,9-epoxymethanoprostaglandin), sumatriptan and ondansetron.

Statistics

Values are expressed as arithmetic means (± s.e.mean) or geometric means (with 95% confidence limits) from n observations, which is also the number of animals used.

Results

In ring preparations of neonatal isolated vena cava of the pig contracted with U-46619 (10 nM), and in the presence of the 5-HT_2 receptor antagonist ketanserin (1 μM), low concentrations of 5-HT (1–100 nM) elicited a complex relaxant response (Figure 1). This response (exemplified by 10 nM 5-HT) was comprised of an initial, rapid 'spike-like' phase of relaxation, followed by a less marked but more sustained reduction in tone (Figure 1a). The full response required the continued presence of 5-HT, since washout with Krebs solution containing U-46619 (10 nM) restored the level of contraction to that prior to the addition of 5-HT (Figure 1b). Removal of the endothelium abolished all responses elicited by 5-HT at concentrations below 100 nM (Figure 1c).

Agonist potency measurements

The 'spike-like', endothelium-dependent relaxation induced by 5-HT ($pEC_{50} = 8.4$) was reproduced, both qualitatively and quantitatively by α-methyl-5-HT ($pEC_{50} = 8.8$). Similar responses were obtained with 5-methoxytryptamine, tryptamine and 2-methyl-5-HT (Table 1), but not with 8-hydroxy-di-n-propylaminotetralin (8-OH-DPAT) or sumatriptan at concentrations up to 10 μM. Concentration-effect curves for 5-HT receptor agonists were bell-shaped (Figure 2), with responses decreasing at higher concentrations.

At concentrations greater than 30–100 nM, 5-HT also elicited a relaxation which was endothelium-independent (Figure 3), a response that was mimicked by the 5-HT_1-like receptor agonist, 5-carboxamidotryptamine (5-CT; 1–100 nM). Thus, endothelium removal did not affect either the sensitivity or the pattern of relaxation to higher concentrations (30 nM–10 μM) of 5-HT or to 5-CT (no evidence for an endothelium-dependent relaxation to 5-CT could be obtained over the concentration range 1 pM–1 μM). It should be noted, however, that the endothelium-independent relaxation to 5-CT was maximal at 100 nM, and therefore any activity of 5-CT on the endothelial

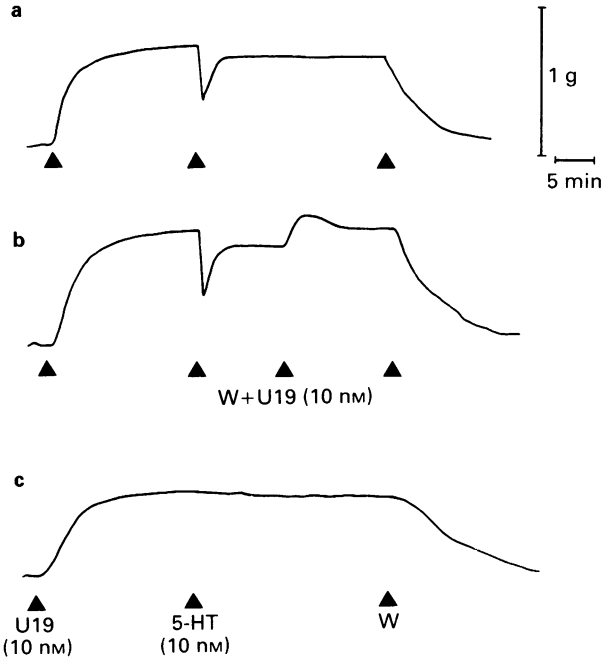

Figure 1 Relaxation to a single concentration of 5-hydroxytryptamine (5-HT, 10 nM) in endothelium-intact (a and b) or endothelium-denuded (c) ring preparations of neonatal porcine isolated vena cava contracted with U-46619 (U19; 10 nM) in the presence of ketanserin (1 μM). The sustained phase of relaxation induced by 5-HT (a) was reversed by washout (W) with Krebs solution containing U19 (10 nM, b). No response to 5-HT was evident in an endothelium-denuded preparation (c).

Table 1 Endothelium-dependent relaxant activity of 5-HT receptor agonists in neonatal porcine isolated vena cava ring preparations contracted with U-46619 (10 nM) in the presence of ketanserin (1 μM)

Agonist	pEC_{50}	% *maximal relaxation*	n
α-Methyl-5-HT	8.8 ± 0.1	70 ± 10	13
5-HT	8.4 ± 0.6	85 ± 3	20
5-Methoxytryptamine	8.4 ± 0.7	81 ± 33	4
Tryptamine	8.0 ± 0.2	91 ± 15	4
2-Methyl-5-HT	7.2 ± 0.1	62 ± 11	4
5-CT	*		
8-Hydroxy-DPAT	< 5.0	0	3
Sumatriptan	< 5.0	0	3

Results are means ± s.e.mean from *n* experiments.
* At a concentration of 100 nM, 5-CT produced a maximal relaxation through an endothelium-independent mechanism which precluded an assessment of any activity of this agonist at the endothelial cell 5-HT receptor.

5-HT receptor at higher concentrations could not be determined. In an attempt to unmask endothelium-dependent effects of 5-CT, responses were examined in the presence of spiperone (1 μM), an antagonist at the smooth muscle cell 5-HT receptor (Sumner *et al.*, 1989). This concentration produced a 10 fold rightward shift in the concentration-effect curve to 5-CT, but did not reveal any endothelium-dependent activity at 5-CT concentrations up to 1 μM. Attempts to use higher concentrations of spiperone were unsuccessful due to an action at the endothelial cell 5-HT receptor at concentrations of 3 μM or greater (results not shown). In contrast to 5-CT, α-methyl-5-HT evoked only an endothelium-dependent relaxation (Figure 3), producing no response (at concentrations up to 1 μM) in the absence of an intact endothelium.

Antagonist studies

Endothelium-dependent relaxations induced by 5-HT or α-methyl-5-HT were abolished in preparations treated with 1 μM methiothepin, methysergide, or cyproheptadine. Antagonism by lower concentrations of methysergide (1 and 10 nM) was

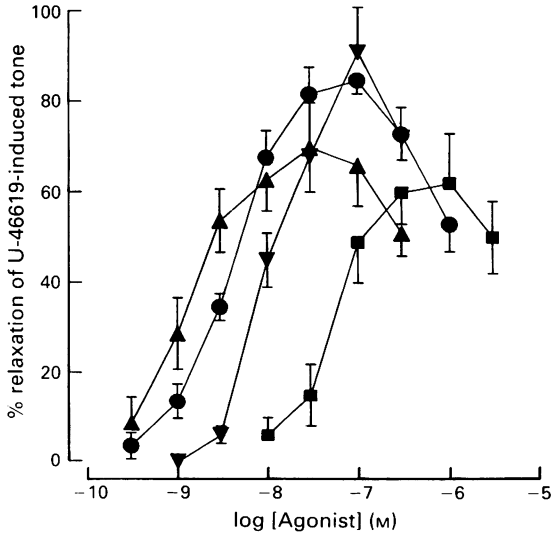

Figure 2 Concentration-effect curves for the endothelium-dependent relaxation of neonatal porcine isolated vena cava by 5-hydroxytryptamine (5-HT) (●), α-methyl-5-HT (▲), tryptamine (▼) and 2-methyl-5-HT (■). Ring preparations were contracted with U-46619 (10 nM), in the presence of ketanserin (1 μM), and cumulative curves established. Results are means from at least four separate determinations, with relaxation being measured at the peak response ('spike') in each case; vertical bars show s.e.mean.

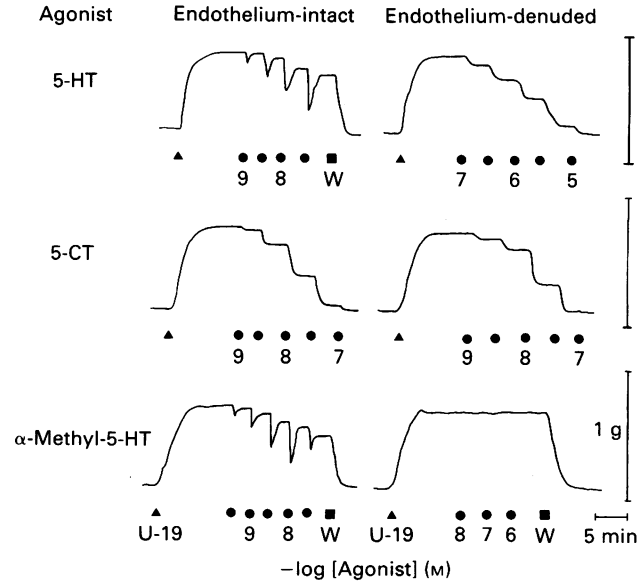

Figure 3 Effect of endothelium removal on relaxant responses to 5-HT, 5-carboxamidotryptamine (5-CT) and α-methyl-5-HT. Ring preparations of neonatal porcine isolated vena cava were contracted with U-46619 (U-19; 10 nM), in the presence of ketanserin (1 μM), and cumulative concentration-effect curves (3 fold increments) were constructed for each of the 5-HT receptor agonists. Note that although, unlike the other two agonists, 5-HT produced both endothelium-dependent and independent relaxations, the endothelium-independent relaxation occurred at considerably higher relative concentrations of 5-HT (0.1–10 μM).

accompanied by a depression in the maximum response to the relaxant agonist (Figure 4a). Only metergoline appeared to behave as a competitive antagonist (Figure 4b; pA_2 7.2 ± 0.3, slope 1.22 ± 0.18 versus α-methyl-5-HT). Specificity of antagonism was assessed at 1 μM versus carbachol. Carbachol produced a concentration-dependent relaxation (pEC_{50} 7.7 ± 0.2) of the porcine vena cava preparation, eliciting 100% relaxation at 1 μM. This response was totally dependent upon an intact endothelium, there being no relaxation in endothelium-denuded preparations. Methiothepin (geometric mean concentration-ratio (CR) with [95% confidence limits] of 10.2[2.1–17.3]) and methysergide (CR = 5.7, [2.3–9.3]), but not metergoline (CR < 2), produced some antagonism (at 1 μM) of the relaxant responses to carbachol (1 nM–1 μM). Antagonists which failed to modify endothelium-dependent relaxation to either 5-HT or α-methyl-5-HT included mesulergine, cyanopindolol, ketanserin, spiperone and ondansetron, all at 1 μM, and ICS 205-930 at 10 μM. In addition, sumatriptan and 8-OH-DPAT were not antagonists (at 10 μM).

Mechanistic studies

Relaxation of porcine vena cava induced either by 5-HT (1–100 nM) or α-methyl-5-HT (0.3–100 nM) was clearly abolished by endothelium removal (Figure 3). Responses evoked by these agonists were not mediated by the release of prostacyclin, since relaxation was unaffected by the cyclo-oxygenase inhibitor, indomethacin (2.8 μM); furthermore, prostacyclin produced only a contractile response in this preparation (Sumner, unpublished observation). The calcium channel blocker verapamil (1 μM) also failed to inhibit responses to α-methyl-5-HT (the effect on responses to 5-HT was not investigated). However, relaxation induced either by 5-HT or α-methyl-5-HT was markedly, but not totally, attenuated (Figure 5) by the inhibitor of soluble guanylate cyclase, methylene blue (10 μM; Ignarro & Kadowitz, 1985) and by the inhibitor of endothelium-derived nitric oxide (NO) synthesis, L-N^G-monomethylarginine (L-NMMA, 500 μM; Rees *et al.*, 1989) with mean CR values (calculated at the 20% level) of 250[98–288] and 56[10–228] respectively. Both of these inhibitors

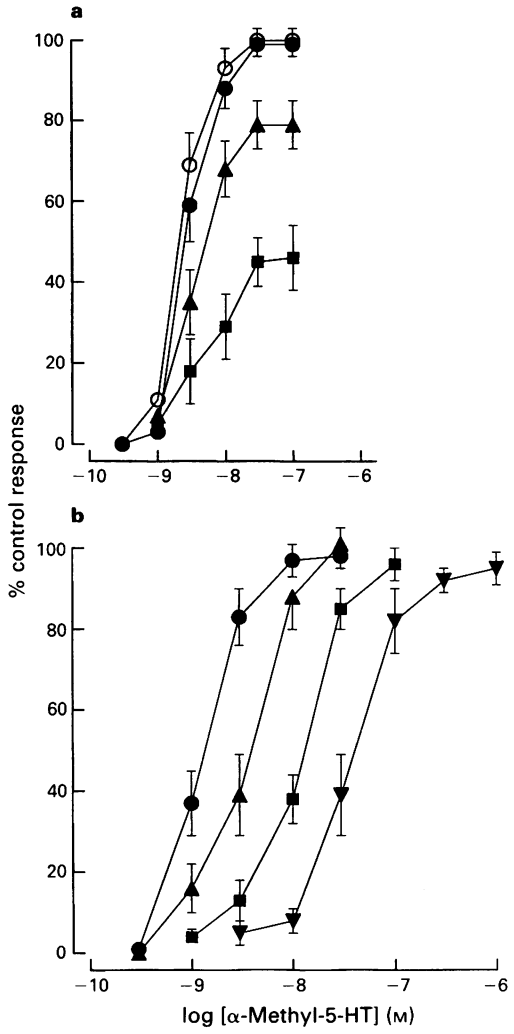

Figure 4 Antagonism of relaxant responses to α-methyl-5-hydroxytryptamine (α-methyl-5-HT) in ring preparations of neonatal porcine isolated vena cava by methysergide (a) and metergoline (b). Control responses to α-methyl-5-HT were repeated in preparations which had been pretreated (30 min) with either Krebs solution (○, ●) or antagonist solution. Methysergide at 1 nM (▲) or 10 nM (■); metergoline 0.1 μM (▲), 0.3 μM (■) or 1 μM (▼). Results are means of 3 determinations (with s.e.mean shown by vertical bars), and are expressed relative to the control response to α-methyl-5-HT in each preparation. Ketanserin (1 μM) was present throughout.

caused a small contraction (about 15% of the U-46619 response) of resting ring preparations.

Discussion

The neonatal porcine isolated vena cava preparation appears to contain two types of 5-HT receptor mediating smooth muscle relaxation. A 5-HT_1-like receptor is located on the vascular smooth muscle and is coupled to adenylyl cyclase (Sumner *et al.*, 1989). This receptor is stimulated by 5-HT and 5-CT, but does not recognise either α-methyl-5-HT or tryptamine. Furthermore, the smooth muscle receptor can be antagonized by methiothepin, methysergide, metergoline, mesulergine and spiperone (Sumner *et al.*, 1989).

The results of the present study suggest that relaxation of the porcine vena cava by 5-HT can also be elicited through an action at an endothelial cell 5-HT receptor. Activation of this receptor appears to evoke the release of an endothelium-derived relaxing factor (EDRF), principally NO, which in turn causes smooth muscle relaxation by elevating intracellular guanosine 3':5'-cyclic monophosphate (cyclic GMP) levels (reviewed in Ignarro & Kadowitz, 1985). In keeping with this

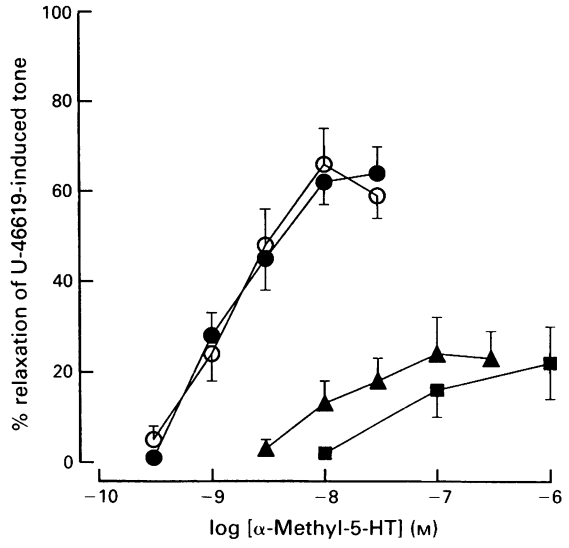

Figure 5 Effects of L-N^G-monomethylarginine and methylene blue on relaxant responses to α-methyl-5-hydroxytryptamine (α-methyl-5-HT). Ring preparations of neonatal porcine vena cava were exposed to Krebs solution (○, ●), monomethylarginine (▲, 500 μM) or methylene blue (■, 10 μM) for 15 min prior to contraction with U-46619 (10 nM) and, in the presence of ketanserin (1 μM), cumulative concentration-effect curves were constructed for α-methyl-5-HT (results are means from 3 determinations; s.e.mean shown by vertical bars).

mechanism, relaxation evoked by 5-HT or its analogue, α-methyl-5-HT, was attenuated by the guanylate cyclase inhibitor, methylene blue (Ignarro & Kadowitz, 1985) or by the inhibitor of EDNO synthesis, L-NMMA (Rees *et al.*, 1989). It should be noted, however, that these compounds did not totally abolish responses to α-methyl-5-HT, thus raising the possibility that more than one EDRF could be involved.

The two types of 'relaxant' 5-HT receptors in this preparation can readily be distinguished by removal of the endothelium. In addition, these receptors can also be differentiated on the basis of their pharmacology. Thus, the smooth muscle relaxant 5-HT_1-like receptor is selectively stimulated by 5-CT and antagonized by spiperone or mesulergine (Sumner *et al.*, 1989), whereas the endothelial cell 5-HT receptor is activated by α-methyl-5-HT and tryptamine, does not appear to recognise 5-CT, and is not antagonized by spiperone or mesulergine (at concentrations up to 1 μM). Higher concentrations of spiperone also blocked the endothelial cell 5-HT receptor, and hence could not be used to reveal any endothelium-dependent relaxation to 5-CT at concentrations above 1 μM.

An endothelium-dependent relaxation to 5-HT has been widely reported (Cocks & Angus, 1983; Molderings *et al.*, 1989; reviewed in Angus & Cocks, 1989). In the majority of cases, responses have been studied in porcine or canine coronary blood vessels and have demonstrated both equipotent agonist activity for 5-HT and 5-CT, and antagonism by methiothepin or methysergide, but not by ketanserin. This has prompted the classification of this type of receptor as 5-HT_1-like (see Bradley *et al.*, 1986). An extensive receptor characterization has also been undertaken for the endothelium-dependent response to 5-HT in rabbit jugular vein (Leff *et al.*, 1987), the pharmacology of which appears similar to that described here. Interestingly, the pattern of response ('spike-like') and the relative agonist potencies, particularly with respect to α-methyl-5-HT and 5-CT in the studies using venous tissue, are somewhat different from those seen with coronary artery preparations. Furthermore, recent publications have reported significant agonist activity with sumatriptan (Schoeffter & Hoyer, 1989; 1990) and antagonism by cyanopindolol (Molderings *et al.*, 1989) in the porcine coronary artery, observations that contrast with those made

in this study, in which sumatriptan and cyanopindolol were without effect either as agonists or antagonists. It is tempting, therefore, to speculate that these differences reflect endothelial cell 5-HT receptor heterogeneity, a possibility which is under investigation at present. Whether or not this proves to be the case, the apparent lack of response (this study) or the weaker activity, relative to 5-HT (Leff *et al.*, 1987), of the 5-HT_1-like receptor agonist, 5-CT, raises questions about including the venous endothelial cell receptor in the 5-HT_1-like class. By contrast, the coronary artery endothelial cell 5-HT receptor is clearly of the 5-HT_1-like class, and shows similarities to the 5-HT_{1D} receptor (Schoeffter & Hoyer, 1989; 1990).

In conclusion, the neonatal porcine vena cava contains an endothelial cell 5-HT receptor, activation of which leads to EDRF release and subsequent vascular relaxation. The potent agonist, α-methyl-5-HT may provide a useful tool for further investigating the significance of this receptor, which does not appear to correspond to reported 5-HT receptors or to radioligand binding sites. Thus the lack of effect (as agonists or antagonists) of 5-CT, 8-OH-DPAT, spiperone, cyanopindolol, mesulergine, sumatriptan, ondansetron, ketanserin and ICS 205–930 would rule out a role for 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_{1D}, smooth muscle contractile and relaxant 5-HT_1-like, 5-HT_2, 5-HT_3 and 5-HT_4 receptors.

References

ANGUS, J.A. & COCKS, T.M. (1989). Endothelium-derived relaxing factor. *Pharmacol. Ther.*, **41**, 303–351.

ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmacol. Chemother.*, **14**, 48–58.

BRADLEY, P.B., ENGEL, G., FENIUK, W., FOZARD, J.R., HUMPHREY, P.P.A., MIDDLEMISS, D.N., MYLECHARANE, E.J., RICHARDSON, B.P. & SAXENA, P.R. (1986). Proposals for the classification and nomenclature of functional receptors for 5-hydroxytryptamine. *Neuropharmacology*, **25**, 563–576.

COCKS, T.M. & ANGUS, J.A. (1983). Endothelium-dependent relaxation of coronary arteries by noradrenaline and serotonin. *Nature*, **305**, 627–630.

IGNARRO, L.J. & KADOWITZ, P.J. (1985). The pharmacological and physiological role of cyclic GMP in vascular smooth muscle relaxation. *Annu. Rev. Pharmacol. Toxicol.*, **237**, 893–900.

LEFF, P., MARTIN, G.R. & MORSE, J.M. (1987). Differential classification of vascular smooth muscle and endothelial cell 5-HT receptors by use of tryptamine analogues. *Br. J. Pharmacol.*, **91**, 321–331.

MOLDERINGS, G.J., ENGEL, G., ROTH, E. & GÖTHERT, M. (1989). Characterization of an endothelial 5-hydroxytryptamine (5-HT) receptor mediating relaxation of the porcine coronary artery. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **340**, 300–308.

REES, D.D., PALMER, R.M.J., HODSON, H.F. & MONCADA, S. (1989). A specific inhibitor of nitric oxide formation from L-arginine attenuates endothelium-dependent relaxation. *Br. J. Pharmacol.*, **96**, 418–424.

SCHOEFFTER, P. & HOYER, D. (1989). Is the sumatriptan (GR43175)-induced endothelium-dependent relaxation of pig coronary arteries mediated by 5-HT_{1D} receptors? *Eur. J. Pharmacol.*, **166**, 117–119.

SCHOEFFTER, P. & HOYER, D. (1990). 5-Hydroxytryptamine (5-HT)-induced endothelium-dependent relaxation of pig coronary arteries is mediated by 5-HT receptors similar to the 5-HT_{1D} receptor subtype. *J. Pharmacol. Exp. Ther.*, **252**, 387–395.

SUMNER, M.J. & HUMPHREY, P.P.A. (1988). Characterization of the endothelial-dependent relaxation of neonatal porcine vena cava evoked by 5-hydroxytryptamine. *Br. J. Pharmacol.*, **95**, 788P.

SUMNER, M.J., FENIUK, W. & HUMPHREY, P.P.A. (1989). Further characterization of the 5-HT receptor mediating vascular relaxation and elevation of cyclic AMP in porcine isolated vena cava. *Br. J. Pharmacol.*, **97**, 292–300.

(Received August 27, 1990
Revised November 23, 1990
Accepted November 26, 1990)