Br. J. Pharmac. (1987), **91**, 321–331

Differential classification of vascular smooth muscle and endothelial cell 5-HT receptors by use of tryptamine analogues

P. Leff, [1]G.R. Martin & J.M. Morse

Analytical Pharmacology Group, Department of Pharmacology I, Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS

1 In ring preparations of the rabbit external jugular vein contracted with the thromboxane-mimetic U-46619, submicromolar concentrations of 5-hydroxytryptamine (5-HT) and chemically related analogues produced relaxations that were dependent on the integrity of the vascular endothelium.

2 The receptor mediating endothelium-dependent relaxations was evidently similar to previously described endothelial 5-HT receptors since relaxation responses to α-methyl-5-HT were not blocked by atropine, (±)-propranolol, yohimbine, indomethacin, ketanserin or MDL-72222, but were non-competitively antagonized by methysergide, methiothepin and cyproheptadine.

3 The activities of some tryptamine agonists and antagonists at the endothelial 5-HT receptor in rabbit jugular vein were compared with their activities at the smooth muscle 5-HT_2-receptor in rabbit aortic rings. Differences in the tryptamines' affinities and relative efficacies showed that the endothelial 5-HT receptor was not of the 5-HT_2-type.

4 The high agonist potencies of 5-HT and 5-carboxamidotryptamine, the susceptibility to antagonism by both methiothepin and methysergide and the resistance to blockade by selective 5-HT_2 and 5-HT_3 ('M') receptor antagonists implies that the endothelial receptor belongs to the '5-HT_1-like' class. However, the agonist potency order 5-HT = α-methyl-5-HT > 5-carboxamidotryptamine suggested that the receptor is not the same as the peripheral '5-HT_1-like' receptors reported to mediate directly contraction of the dog saphenous vein or relaxation of vascular and non-vascular smooth muscles. At these receptors, the potency order is 5-carboxamidotryptamine > 5-HT > α-methyl-5-HT.

5 These results constitute preliminary evidence that peripheral '5-HT_1-like' receptors, like central 5-HT_1 recognition sites, are a heterogeneous population. Further comparative studies with a wider range of receptor probes are necessary to establish whether or not these receptors represent functional counterparts of the ligand binding sites in the brain.

Introduction

In addition to its well-characterized action at the 5-hydroxytryptamine (5-HT_2) receptor mediating vascular smooth muscle contraction, 5-HT has been shown to elicit vasorelaxation by an endothelium-dependent mechanism. Using isolated, endothelium-intact canine and porcine coronary arteries, Cocks & Angus (1983) showed that 5-HT_2 receptor-mediated contractions were augmented when the endothelium was physically removed. In the presence of the 5-HT_2 receptor antagonist ketanserin, 5-HT produced endothelium-dependent relaxation of the porcine coronary artery. Similar findings in other isolated blood vessels have also been reported (Cocks & Angus, 1984; Griffith *et al.*, 1984; Imaizumi *et al.*, 1984).

The lack of effect of ketanserin on endothelium-dependent relaxations implies that the 5-HT receptor involved is not 5-HT_2-like. Indeed it has been suggested that the receptor is of the 5-HT_1 type, because both methysergide and methiothepin antagonize 5-HT-induced relaxations in canine coronary arteries (Cohen *et al.*, 1983a, b; Houston *et al.*, 1985). These agents, but not ketanserin, express a high affinity for 5-HT_1 as well as 5-HT_2 binding sites in rat brain cortex (Leysen *et al.*, 1981). However, Imaizumi *et al.* (1984) showed that 5-HT-induced relaxation of the endoth-

[1] Author for correspondence.

elium-intact chick jugular vein could be blocked with cyproheptadine, a potent 5-HT_2 receptor antagonist with only negligible affinity for 5-HT_1 binding sites (Peroutka & Snyder, 1979). It is conceivable that such conflicting results reflect either venous and arterial or species differences in the endothelial cell 5-HT receptor. Alternatively, the ligands used might be unreliable receptor probes since, as we have previously shown (Leff & Martin, 1986; Leff *et al.*, 1986), antagonists which bear little chemical relation to the endogenous agonist can provide misleading information for 5-HT receptor classification.

In our laboratory, we recently identified a vascular tissue, the rabbit external jugular vein, in which 5-HT elicits endothelium-dependent relaxations which are not confounded by concomitant 5-HT_2 receptor-mediated smooth muscle contraction. We have now used ring preparations of this tissue to compare the action of some simple tryptamine analogues at the endothelial 5-HT receptor with their activity at the 5-HT_2 receptor in the rabbit isolated aorta. The aim of the study was to determine whether mimetics and antagonists which possess some chemical identity with the endogenous agonist 5-HT could differentiate 5-HT_2 and endothelial cell 5-HT receptors and thereby provide a quantitative basis for their classification.

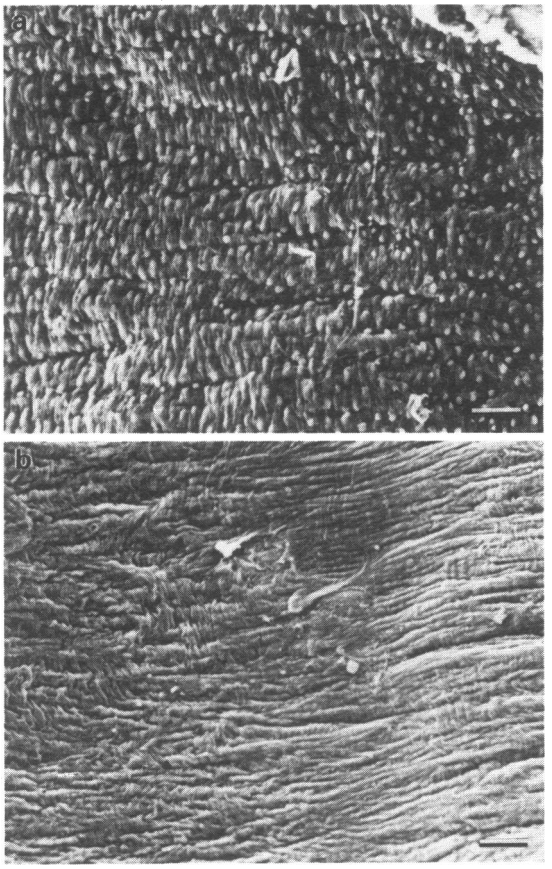

Figure 1 Scanning electron micrographs showing the luminal surface of the rabbit jugular vein (a) with endothelium intact and (b) after mechanical denudation as described in the text. The bar represents 10 μm.

Methods

Vascular tissues were obtained from male New Zealand White rabbits (2.4–2.9 kg) killed by injecting pentobarbitone sodium (Sagatal: 60 mg kg^{-1}) into a marginal ear vein.

Rabbit aorta: The thoracic aorta was isolated and the vessel cleared of adhering connective tissue after mounting on a polypropylene cannula (external diameter = 2.5 mm). Cannulation abolished acetylcholine-induced endothelium-dependent relaxations. Ring segments, approximately 3 mm wide, were prepared as described by Stollak & Furchgott (1983), preserving the plane of the circular smooth muscle.

Rabbit jugular vein: Right and left external jugular veins were removed, cleared of adhering connective tissue without cannulation and each cut into 3 ring preparations 3–5 mm wide.

Vascular ring preparations were suspended between two wire hooks and immersed in 20 ml organ baths containing Krebs solution (pH 7.4) of the following composition (mM); NaCl 118.41, $NaHCO_3$ 25.00, KCl 4.75, KH_2PO_4 1.19, $MgSO_4$ 1.19, glucose 11.10 and $CaCl_2$ 2.50. This was maintained at 37°C and continually gassed with 95% O_2:5% CO_2. Changes in tissue isometric force were measured with Grass FTO3C force displacement transducers and recorded on Gould BS-212 pen recorders.

Endothelial denudation of jugular veins

In some experiments, endothelium-intact and -denuded jugular veins were compared using vessels from the same animal. Denudation was achieved mechanically, by inserting into a vein a shortened plastic disposable pipette tip which had been serrated using a scalpel blade. The vein was then rolled back and forth on tissue paper moistened with Krebs solution. Tissues treated in this way were examined histologically by the method of Malick & Wilson (1975) to confirm that the endothelium had been effectively removed (Figure 1).

Definition of endothelium-dependent relaxations

In order to measure endothelium-dependent relaxant responses, jugular vein rings were contracted with the

thromboxane A_2-mimetic, U-46619. As in isolated coronary vasculature (Cocks & Angus, 1983), this agent appeared to be devoid of any endothelium-dependent component in the jugular vein since cumulative concentration-effect curves for U-46619 obtained in endothelium-intact and denuded preparations were not statistically different (Figure 2). From this study, a concentration of 10 nM U-46619 was chosen for inducing tissue contracture in subsequent experiments.

Preliminary experiments in endothelium-intact jugular veins showed that both 5-HT and α-methyl-5-HT produced concentration-dependent relaxations in the range 1–100 nM. However, higher concentrations of 5-HT, but not α-methyl-5-HT, also elicited endothelium-independent relaxant responses. On the basis of tryptamine agonist orders of potency obtained in the endothelium-denuded rabbit jugular vein (i.e. 5-carboxamidotryptamine > 5-HT >> α-methyl-5-HT; unpublished observations), we concluded that this tissue possesses not only specific endothelial 5-HT receptors, but also smooth muscle relaxant 5-HT receptors similar to those previously described by Feniuk *et al.* (1983, 1984). Therefore, in order to avoid the problems associated with receptor heterogeneity, α-methyl-5-HT was used instead of 5-HT to study antagonist interactions at the endothelial cell 5-HT receptor.

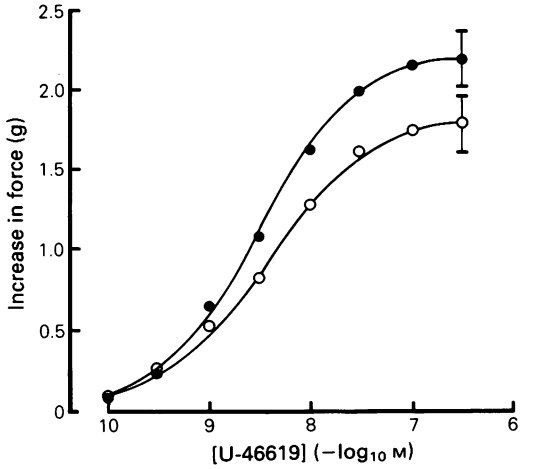

Figure 2 Computer-fitted semi-logarithmic concentration-effect curves for the thromboxane-mimetic U-46619 obtained by cumulative addition in endothelium-intact (●) and -denuded (○) rings of rabbit jugular vein. The slope, $p[A_{50}]$ and asymptote estimates for the two curves were not significantly different ($P > 0.05$). The data are the average increases in g force obtained from 12 replicate curves in endothelium-intact tissues and 11 replicate curves in endothelium-denuded tissues. Vertical lines show s.e.mean on the maximum response.

Experimental protocols

At the beginning of each experiment a force was applied to each preparation (aortic rings, 3.0 g: jugular vein rings, 0.75 g). During a subsequent stabilisation period, the force was re-established once and tissues were exposed to pargyline (500 μM) in order to inhibit monoamine oxidase irreversibly. In experiments with aortic rings, concomitant 30 min exposure to benextramine tetrahydrochloride monohydrate (BHC: 10 μM) also inactivated α_1-adrenoceptors, thereby preventing direct or indirect α_1-adrenoceptor stimulation by 5-HT (Innes, 1962; Apperley *et al.*, 1976; Fozard & Mwaluko, 1976; Marin *et al.*, 1981). At the end of the stabilization period, the inhibitors were removed by several exchanges of the organ bath Krebs solution. Only a single concentration-effect curve was obtained in each tissue preparation, therefore the number of replicates refers to the number of preparations.

Agonist experiments in rabbit aorta: Each preparation was challenged with 5-HT (10 μM) to establish viability. Then, following washing and restabilization, a cumulative concentration-effect curve was obtained for one of the following tryptamine agonists: 5-HT, N-ethyl-5-methoxytryptamine, N-isopropyl-5-methoxytryptamine or 5-carboxamidotryptamine. 5-HT curves were also produced in tissues previously exposed for 30 min to phenoxybenzamine (0.1 μM). Contractile responses were measured as increases in grams force.

Agonist experiments in rabbit jugular vein: Agonist additions were made according to a single exposure design, because the transient nature of 5-HT and α-methyl-5-HT responses made cumulative additions impossible (See Figure 3). Contracture was induced in each tissue by introducing Krebs solution containing U-46619 (10nM). When a steady contracture was obtained the agonist under study was added, the maximal response recorded and the agonist removed by replacing the organ bath buffer with fresh solution. This cycle was repeated at intervals of 30 min, an interval which was shown independently to avoid problems of tachyphylaxis.

In each experiment, tissues were challenged initially with α-methyl-5-HT (0.1 μM) until consecutive responses were consistent (usually 3 or 4 challenges). Thereafter, the test agonist was applied, successive concentrations increasing in 0.5 $\log_{10}$ increments until the full concentration-effect curve was defined. In this way, curves for 5-HT, tryptamine, 5-carboxamidotryptamine, RU-24969, N-ethyl-5-methoxytryptamine and N-isopropyl-5-methoxytryptamine were obtained. 5-HT, N-ethyl-5-methoxytryptamine and 5-carboxamidotryptamine curves were also obtained

after partial occlusion of the receptor population with phenoxybenzamine (3 μM for 30 min). Data used in operational model-fitting procedures were expressed as changes in grams force. For potency comparisons, agonist responses were normalized in each tissue by scaling them to the average of the last two responses obtained during the initial challenges with α-methyl-5-HT (0.1 μM).

Antagonist experiments in rabbit jugular vein: In antagonist experiments, tissues were exposed to the drug or vehicle for 60 min before the construction of an α-methyl-5-HT concentration-effect curve. In each tissue, agonist responses were normalized, as described above, by scaling them to the average of the last two responses obtained during the initial challenges with α-methyl-5-HT (0.1 μM).

Data analysis

Analysis of concentration-effect curves: Individual concentration-effect curves (rabbit aorta) or the average data from replicate curves (rabbit jugular vein) were fitted to a logistic function of the form:

$$E = \frac{\alpha [A]^m}{[A_{50}]^m + [A]^m} \quad \ldots .(1)$$

in which E is the effect, [A] is the agonist concentration and α, $[A_{50}]$ and m are the asymptote, location and slope parameters respectively. Location parameters were actually estimated as logarithms ($-\log_{10}[A_{50}]$). For the analysis of competitive antagonism this fitting procedure also performed a one-way analysis of variance comparing cumputed estimates of α and m between and within treatment groups. Further analysis of competitive antagonism was performed by fitting computed $\log_{10}[A_{50}]$ values to the following linear form of the Schild equation (Trist & Leff, 1985; Leff *et al.*, 1986):

$$\log_{10}[A_{50}] = \log_{10}[A_{50}^c] + \log_{10}(1 + [B]^n/K_B). \quad .(2)$$

in which $[A_{50}^c]$ is a control $[A_{50}]$ value, [B] is the concentration of antagonist, K_B its dissociation constant and n its order of reaction with the receptor (unity for simple competition). If n was not significantly different from unity it was constrained to this value in order to estimate pK_B ($-\log_{10} K_B$).

Operational model fitting: Concentration-effect curve data measured in grams force were fitted directly to the operation model of agonism, (Black & Leff, 1983; Black *et al.*, 1985; Barrett *et al.*, 1986):

$$E = \frac{E_m \tau^n [A]^n}{(K_A + [A])^n + \tau^n [A]^n} \quad \ldots .(3)$$

in which K_A is the agonist dissociation constant, τ is the efficacy of the agonist in a particular tissue, E_m is the maximum possible effect in the receptor system and n determines the sensitivity of the occupancy-effect relation.

Drugs

5-Hydroxytryptamine creatinine sulphate (Sigma Chemical Co., St Louis, MO, U.S.A.); pargyline hydrochloride (Sigma); benextramine tetrahydrochloride monohydrate (Aldrich Chemical Co. Ltd, Dorset); phenoxybenzamine hydrochloride (Smith, Kline and French, Welwyn Garden City, Herts.); ketanserin tartrate (Janssen Pharmaceutica, Beerse, Belgium); 9, H-dideoxy, 9α, 11α-methanoepoxy $PGF_{2\alpha}$ (U-46619: Cayman Chemical, Denver, Colorado, U.S.A.); indomethacin (Sigma); atropine sulphate (Sigma); (±) - propranolol hydrochloride (Sigma); yohimbine hydrochloride (Sigma); tryptamine hydrochloride (Sigma); 1αH,3α,5αH-tropan-3-yl-3, 5-dichlorobenzoate methane sulphonate (MDL-72222: Merrell-Dow, Strasbourg, France); 5-methoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1H-indole succinate (RU-24969: Roussel-Uclaf, Paris, France).

(±)α-methyl-5-hydroxytryptamine hydrogen maleate, 5-carboxamidotryptamine hydrochloride, N-ethyl-5-methoxytryptamine hydrochloride, N-isopropyl-5-methoxytryptamine hydrochloride and N-benzyl-5-methoxytryptamine hydrochloride were synthesized by Dr H.F. Hodson, Medicinal Chemistry Department, Wellcome Research Laboratories, Beckenham, Kent.

Phenoxybenzamine and U-46619 were dissolved in absolute ethanol. Indomethacin was dissolved initially in Tris buffer (1M, pH 8.5) and diluted in distilled water. At their final concentration in the organ bath (<0.01% v/v) these drug vehicles did not influence tissue responsiveness. All other drugs were dissolved and diluted in distilled water.

Results

Characterization of endothelium-dependent relaxations in rabbit jugular vein

Figure 3 illustrates typical endothelium-dependent relaxations to α-methyl-5-HT (1–100 nM) obtained in rings of rabbit jugular vein. Qualitatively similar responses (not shown) were obtained with 5-HT (1–100 nM). In endothelium-denuded preparations responses to α-methyl-5-HT were abolished, but 5-HT (>30 nM) produced endothelium-independent relaxations. Contractile responses were not observed with either agonist at concentrations up to 30 μM.

The lack of any involvement of muscarinic receptors, β- or α_2-adrenoceptors, 5-HT_2 receptors, 5-HT_3

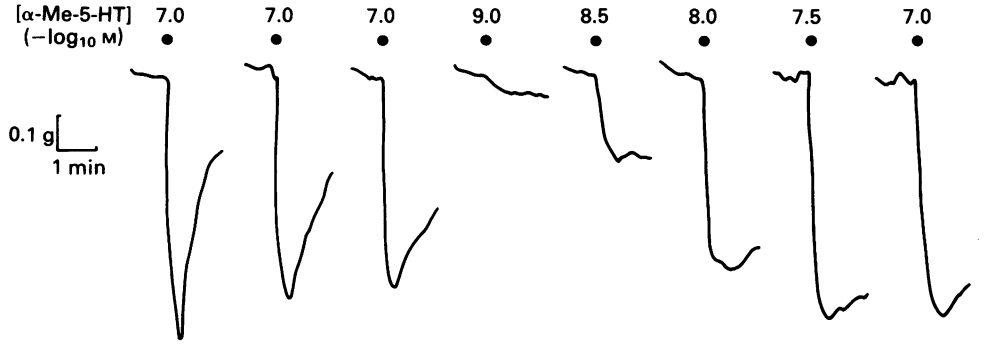

Figure 3 The tracing illustrates typical endothelium-dependent relaxations obtained with α-methyl-5-HT (α-Me-5-HT) in rings of rabbit jugular vein contracted with U-46619 (10 nM). The initial challenges with a maximally-effective concentration (0.1 μM) of α-methyl-5-HT established a consistent response before the agonist under study, in this case α-methyl-5-HT itself, was added. Successive agonist additions were made at intervals of 30 min.

('M') receptors or cyclo-oxygenase products was demonstrated by the failure of the following to modify the α-methyl-5-HT concentration-effect curve in 2–4 preparations ($\Delta p[A_{50}]$, control-test; $\alpha_{test}/\alpha_{control}$ shown in parentheses): 0.1 μM atropine (− 0.12; 0.96), 0.3 μM (±)-propranolol (0.08; 1.11), 1.0 μM yohimbine (− 0.12; 0.96), 0.1 μM ketanserin (− 0.08; 0.85), 0.1 μM MDL-72222 (− 0.03; 1.06) and 2.8 μM indomethacin (− 0.21; 0.97). In contrast, α-methyl-5-HT responses were non-competitively antagonised by methysergide (0.03–0.30 nM), methiothepin (0.3–3.0 nM) and cyproheptadine (0.03–1.00 μM). In each case the antagonism was essentially of the type illustrated for methiothepin in Figure 4, although the degree of rightward shift and asymptote depression differed. Thus, in four preparations exposed to only 0.1 nM methysergide, the α-methyl-5-HT concentration-effect curve asymptote was decreased to 55% of the control and the $\Delta p[A_{50}]$, as defined above, was 0.36. As shown in Figure 4, a ten fold higher concentration (1 nM) of methiothepin reduced the agonist curve asymptote to a similar extent (56%), but increased $\Delta p[A_{50}]$ to 0.64. In three preparations, cyproheptadine produced proportionately more rightward displacement than asymptote depression, a concentration of 0.3 μM shifting the agonist concentration-effect curve with a $\Delta p[A_{50}]$ of 1.46 and depressing the asymptote to 70% of the control.

Analysis of agonism with tryptamine analogues in rabbit aorta and rabbit jugular vein

Changes in tissue isometric force induced by 5-HT and the agonist analogues N-ethyl-5-methoxytryptamine, N-isopropyl-5-methoxytryptamine and 5-carboxamidotryptamine in the rabbit aorta and endothelium-intact rabbit jugular vein are shown in Figure 5. Increases in force in aortic rings are the averages from 4–9 separate experiments and decreases in force in jugular vein rings are averages from 6–13 separate experiments.

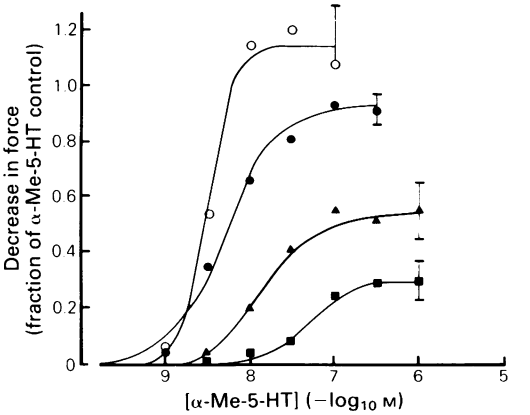

Figure 4 Antagonism by methiothepin of relaxation responses to α-methyl-5-HT (α-Me-5-HT) in rings of endothelium-intact rabbit jugular vein. The lines through the data were fitted by computer to the averages of 3–5 replicate concentration-effect curves. Vertical lines show s.e.mean. Open symbols denote control concentration-effect curve and solid symbols denote responses in the presence of methiothepin (● 0.3 nM, ▲ 1.0 nM and ■ 3.0 nM). Increasing concentrations of antagonist caused progressive rightward displacement of the agonist concentration-effect curves and concomitant depression of their asymptotes.

For each tissue, agonist affinity (K_A) and efficacy (τ) estimates were obtained by fitting each tissue agonist data set directly to the operational model of agonism (equation 3). The lines drawn through the data in Figure 5 are the best-fit lines in each case. In the rabbit aorta, each of the tryptamine analogues expressed partial agonism with respect to 5-HT (Figure 5b). However, 5-HT itself behaves as a partial agonist in this tissue (Black *et al.*, 1985; Barrett *et al.*, 1986). Therefore, as discussed previously (Leff *et al.*, 1986), E_m had to be estimated concomitantly with K_A and τ values. This was achieved using 5-HT response data obtained after phenoxybenzamine treatment, according to the method of Furchgott (1966). 5-HT concentration-effect curves obtained in this way were fitted simultaneously with the agonist curves shown in Figure 5b.

In the rabbit jugular vein, N-ethyl-5-methoxytryptamine, 5-carboxamidotryptamine and 5-HT concentration-effect curve data were used to estimate E_m, the phenoxybenzamine-treatment curves obtained for

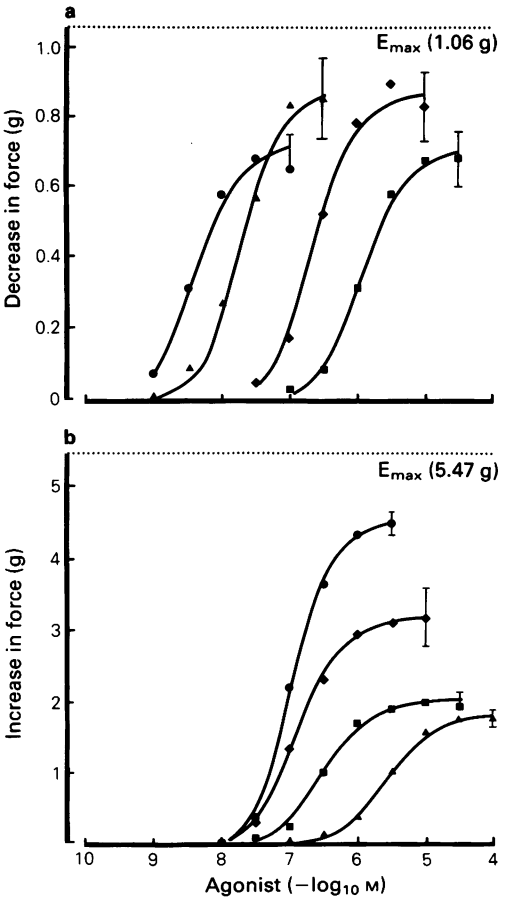

Figure 5 Changes in g force produced by 5-HT (●), N-ethyl-5-methoxytryptamine (◆), N-isopropyl-5-methoxytryptamine (■) and 5-carboxamidotryptamine (▲) in the endothelium-intact rabbit jugular vein (a) and in the rabbit aorta (b). Relaxant responses in the jugular vein are the averages of 6–13 replicate concentration-effect curves and constrictor responses in the aorta are the averages of 4–9 replicate curves. Vertical lines show s.e.mean on the maximum response. The lines through the data are the results of fitting them to the operational model (equation 3).

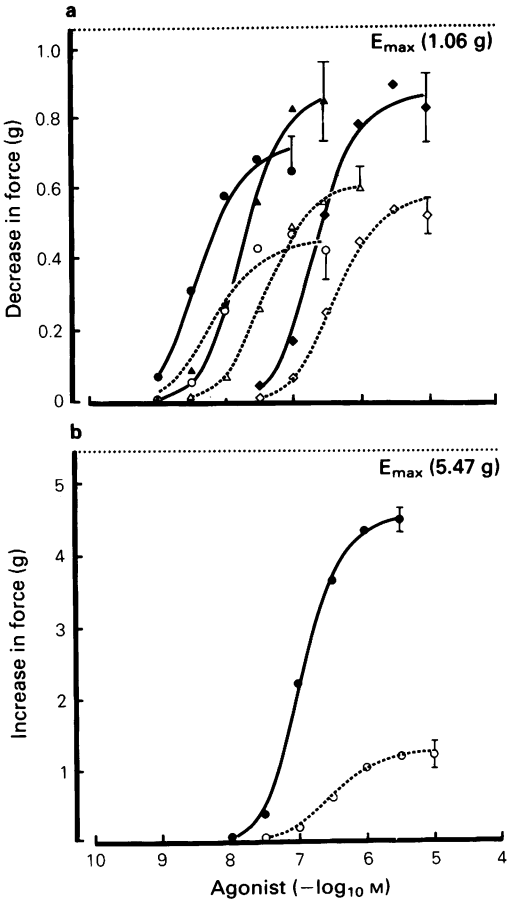

Figure 6 (a) Decreases in g force produced by 5-HT (circles), N-ethyl-5-methoxytryptamine (diamonds) and 5-carboxamidotryptamine (triangles) before (solid symbols) or after (open symbols) 30 min exposure of endothelium-intact rabbit jugular vein preparations to phenoxybenzamine (3 μM). (b) Increases in g force produced by 5-HT before (●) or after (○) 30 min exposure of rabbit aortic rings to phenoxybenzamine (0.1 μM). Data are the averages of 9 replicate curves in the aorta and 6 to 13 replicate curves in the jugular vein. Vertical lines show s.e.mean on the maximum response. The lines through the data are the results of fitting them to the operational model (equation 3).

each of three agonists being fitted simultaneously with the curves shown in Figure 5a. For both tissues the phenoxybenzamine treatment curves are shown separately in Figure 6. A comparison of the pK_A and τ values estimated in the two tissues is given in Table 1. On the basis of both affinity and relative efficacy differences it is apparent that the 5-HT receptor type located on the vascular endothelium is different from the 5-HT_2 receptor in the rabbit aorta.

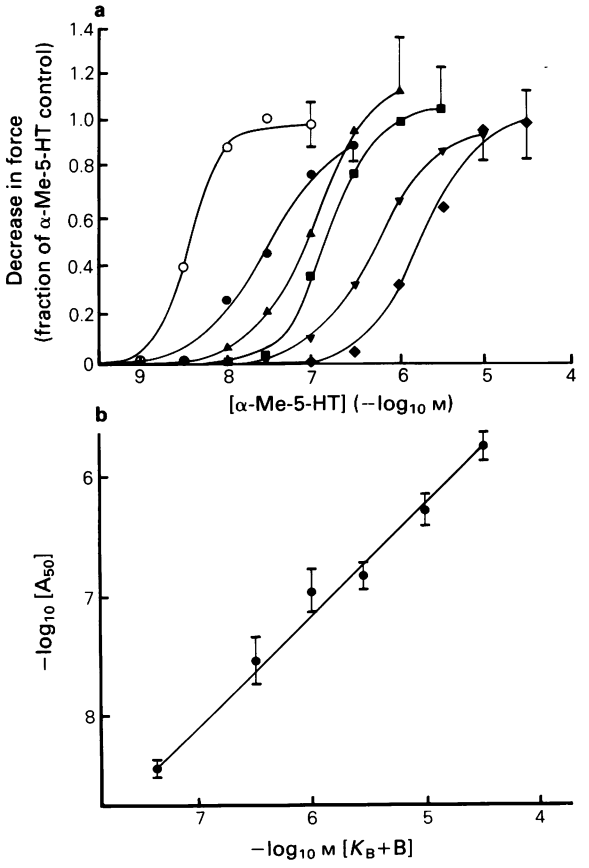

Figure 7 (a) The antagonism by N-benzyl-5-methoxytryptamine of relaxation responses to α-methyl-5-HT (α-Me-5-HT) in rings of endothelium-intact rabbit jugular vein. The lines through the data were fitted by computer to the averages of 4 replicate concentration-effect curves at each antagonist concentration. Vertical lines show s.e.mean on the maximum response. Open symbols denote control concentration-effect curve and closed symbols denote responses in the presence of antagonist (● 0.3 μM, ▲ 1.0 μM, ■ 3.0 μM, ▼ 10.0 μM and ◆ 30.0 μM). (b) Shows, in the form of a Clark plot, the effect of the antagonist on the $p[A_{50}]$ values of α-methyl-5-HT curves. The adherence of the data with the unit slope line drawn through them is consistent with simple competitive antagonism. The pK_B value estimated using equation (2) was 7.27 ± 0.16 (4 d.f.).

Analysis of antagonism with N-benzyl-5-methoxytryptamine in the rabbit jugular vein

We have previously shown that, in rabbit aortic rings, N-benzyl-5-methoxytryptamine expresses agonism with a low efficacy but high affinity (pK_A = 7.30) (Leff *et al.*, 1986). In the present study, this tryptamine analogue showed no agonism in endothelium-intact jugular vein rings, but behaved as a simple, competitive antagonist of α-methyl-5-HT (Figure 7). Using equation (2) the Schild plot slope parameter, n, was estimated to be 0.89 ± 0.08 which was not significantly different from unity. When n was constrained to unity, the pK_B estimate was 7.27 ± 0.16 (4 d.f.).

Potency order of agonists in the rabbit jugular vein

Average concentration-effect data for some tryptamines and the indole RU-24969 are shown in Figure 8. The agonist potencies, ranked according to computed $p[A_{50}]$ values, were (number of replicate curves in parentheses): 5-HT 8.48 ± 0.05 (9); α-methyl-5-HT 8.42 ± 0.04 (7); 5-carboxamidotryptamine 7.90 ± 0.06 (5); tryptamine 6.94 ± 0.04 (7) and RU-24969 6.64 ± 0.09 (4). In each case the agonist concentration-effect curve was steeper than a rectangular hyperbola,

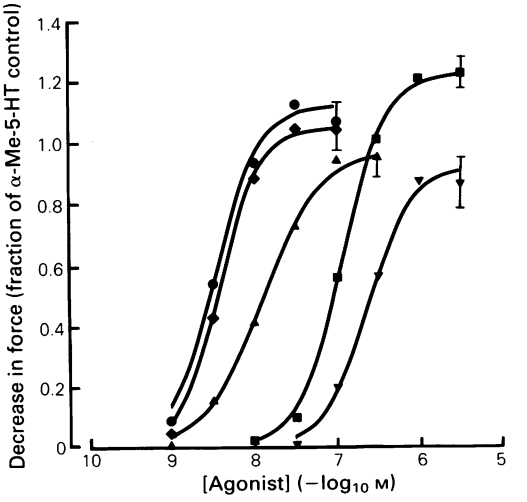

Figure 8 Decreases in tissue isometric force produced by 5-HT (●; n = 9), α-methyl-5-HT (◆; n = 7), 5-carboxamidotryptamine (▲; n = 5), tryptamine (■; n = 7) and RU-24969 (▼; n = 4) in rings of endothelium-intact rabbit jugular vein contracted with U-46619 (10 nM). For each agonist a line was fitted by computer to the averages of n replicate concentration-effect curves. Computed $p[A_{50}]$ values are given in the text. Vertical lines show s.e.mean on the maximum response.

but estimates of the slope parameter, m, which varied between 1.32 and 1.92 were not statistically different ($P > 0.05$). Furthermore, the maximum response to each agonist was similar, implying that like 5-HT and 5-carboxamidotryptamine (see Figure 5), these compounds behaved as partial agonists.

Discussion

The results presented here confirm and extend previous reports concerning the existence of an endothelial cell 5-HT receptor which mediates, indirectly, relaxation of arterial and venous smooth muscle (Cocks & Angus, 1983; 1984; Cohen *et al.*, 1983a; Imaizumi *et al.*, 1984). Like endothelium-dependent responses to 5-HT measured in canine and porcine coronary arteries (Cocks & Angus, 1983; 1984; Cohen *et al.*, 1983a, b), relaxations of the rabbit jugular vein were not affected by ketanserin implying that the receptor is not 5-HT_2-like. Nor were the responses blocked by MDL-72222 suggesting that the receptor does not belong to the 5-HT_3 ('M') receptor class. On the other hand, methysergide, cyproheptadine and methiothepin each produced non-surmountable antagonism of endothelium-dependent relaxations. These results are qualitatively consistent with those reported by Cohen *et al.* (1983a, b) for methysergide, Imaizumi *et al.* (1984) for cyproheptadine and Houston *et al.* (1985) for methiothepin. Evidently the 5-HT receptor associated with the rabbit jugular vein endothelium is similar to the receptor described previously in a variety of other venous and arterial preparations.

The different affinity and relative efficacy estimates (Table 1) obtained for a series of simple tryptamine analogues in the rabbit aorta and rabbit jugular vein provide quantitative evidence that the endothelial cell 5-HT receptor is not of the 5-HT_2 type. Clancy & Maayani (1985) have already demonstrated that the molecular determinants of efficacy and affinity at the rabbit aorta 5-HT_2 receptor are different. For tryptamines, efficacy alone is modified by ethylamine N-alkyl substitution. We have confirmed this finding in this and a previous study using aortic rings (Leff *et al.*, 1986), efficacy decreasing in the order 5-HT (1.0) > N-ethyl-5-methoxytryptamine (0.62) > N-isopropyl-5-methoxytryptamine (0.44) > N-benzyl-5-methoxytryptamine (0.30), while the affinities of these compounds were similar. At the endothelial cell 5-HT receptor also, efficacy showed some dependence on the size of the N-alkyl substituent, N-benzyl-5-methoxytryptamine being a silent competitive antagonist. However, the overall order and the estimated values of the relative efficacies were different from those in the aorta. Furthermore, in the jugular vein preparation, clear differences in affinity were demonstrated by the compounds. The distinction between the aortic smooth muscle receptor and the jugular vein endothelial receptor on the basis of affinities is emphasised if the antagonist affiny of N-benzyl-5-methoxytryptamine at the endothelial receptor and the compounds' agonist affinity at the aortic smooth muscle receptor (Leff *et al.*, 1986) are included in the comparison. The affinity orders then read (pK_A or pK_B): 5-HT (8.36) > 5-carboxamidotryptamine (7.51) > N-benzyl-5-methoxytryptamine (7.27) > N-ethyl-5-methoxytryptamine (6.47) > N-isopropyl-5-methoxytryptamine (5.93) in the rabbit jugular vein and N-benzyl-5-methoxytryptamine (7.30) > N-ethyl-5-methoxytryptamine (7.11) > 5-HT (6.92) = N-isopropyl-5-methoxytryptamine (6.86) > 5-carboxamidotryptamine (5.96) in the rabbit aorta. Evidently, while N-benzyl-5-methoxytryptamine has *relatively* greater affinity in the latter case, it is non-selective in the absolute sense. Unfortunately this limits its utility as an antagonist probe for the differential classification of 5-HT receptors.

In both vascular and non-vascular tissues, a 5-HT receptor mediating directly smooth muscle relaxation has been described (Feniuk *et al.*, 1983; 1984; Connor *et al.*, 1986). Like the endothelial 5-HT receptor, this receptor is resistant to blockade by ketanserin but is potently antagonized by methiothepin (Connor *et al.*, 1986). However, in the present study, the potency order of tryptamine agonists mediating endothelium-dependent relaxation was 5-HT = α-methyl-5-HT > 5-carboxamidotryptamine. At the receptor mediating relaxation of the cat saphenous vein and guinea-pig ileum, Feniuk *et al.* (1983; 1984) reported that 5-carboxamidotryptamine was 30 to 100 fold more potent as an agonist than 5-HT, while α-methyl-5-HT was at least 200 times less active. Such discontinuities in tryptamine agonist potency orders cannot be attributed to between-tissues variability in receptor density and/or efficiency of occupancy-effect coupling and, therefore, constitute evidence for a genuine difference between the endothelial and smooth muscle

Table 1 Affinity (pK_A) and efficacy (τ) estimates for 5-hydroxytryptamine (5-HT), N-ethyl-5-methoxytryptamine (NEMT), N-isopropyl-5-methoxytryptamine (NIMT) and 5-carboxamidotryptamine (5-CT) in the rabbit aorta and endothelium-intact rabbit jugular vein

	Rabbit aorta		*Rabbit jugular vein*	
	pK_A	τ	pK_A	τ
5-HT	6.92	1.85 (1.00)	8.36	1.48 (1.00)
NEMT	7.11	1.14 (0.62)	6.47	2.13 (1.44)
NIMT	6.86	0.82 (0.44)	5.93	1.44 (0.97)
5-CT	5.96	0.77 (0.42)	7.51	2.26 (1.53)
	$E_m = 5.47$ g, n = 2.63		$E_m = 1.06$ g, n = 2.04	

relaxant 5-HT receptors.

Yet a further 5-HT receptor, which is clearly not of the 5-HT_2 type (Feniuk *et al.*, 1985), has been shown to mediate contraction of certain cutaneous and cerebral blood vessels (Apperley *et al.*, 1980; Müller-Schweinitzer, 1981; Bradley *et al.*, 1986a) and to inhibit release of noradrenaline from sympathetic nerve terminals (Feniuk *et al.*, 1979; Engel *et al.*, 1983; Cohen, 1985). This receptor shares in common with the endothelial and smooth muscle relaxant 5-HT receptors a susceptibility to blockade by low concentrations of methiothepin (Apperley & Humphrey, 1986) and a resistance to blockade by ketanserin (Cohen, 1985; Bradley *et al.*, 1986a; Feniuk *et al.*, 1985). Using the canine saphenous vein as a representative bioassay for this receptor type, Feniuk *et al.* (1985) recently demonstrated that the potency of tryptamines mediating smooth muscle contraction decreased in the order 5-carboxamidotryptamine > 5-HT > α-methyl-5-HT. Once again, comparison of this result with the potency order obtained in the endothelium-intact rabbit jugular vein implies that different 5-HT receptors mediate these two types of response.

The increasing evidence for a multiplicity of peripheral 5-HT receptors has resulted in attempts to identify functional correlates of the 5-HT binding sites which have been found in mammalian brain tissue. Four apparently distinct binding sites exist, 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} and 5-HT_2 (Peroutka & Snyder, 1979; Pedigo *et al.*, 1981; Pazos *et al.*, 1984), but only the 5-HT_2 site has a well established functional identity in the periphery (Leysen *et al.*, 1984). In a number of isolated tissues (Feniuk *et al.*, 1983; 1985; Apperley & Humphrey, 1986; Bradley *et al.*, 1986a) and *in vivo* (Kalkman *et al.*, 1984; Sazena *et al.*, 1985; Connor *et al.*, 1986), the classification of 5-HT receptors as '5-HT_1-like' has so far been based on the high potency of 5-HT, an equally high or even higher potency of 5-carboxamidotryptamine, the high antagonist potency of methiothepin and the generally low affinity or inactivity of conventional 5-HT_2 receptor antagonists (Bradley *et al.*, 1986b). According to these criteria the endothelial 5-HT receptor could also be provisionally classified as '5-HT_1-like'. However, it remains unclear whether this receptor can be reconciled with any of the identified 5-HT_1 binding sites. None of the indolamines studied in the rabbit jugular vein appeared to behave as a 'full' endothelial receptor agonist, therefore the potency order 5-HT = α-methyl-5-HT > 5-carboxamidotryptamine > tryptamine > RU-24969 should reflect the rank order of these compounds' affinities. Accepting this, the endothelial receptor is unlikely to be either the 5-HT_{1A} or 5-HT_{1B} type since, for the former, the indolamines affinities decrease in the order 5-carboxamidotryptamine > 5-HT > RU24969 > α-methyl-5-HT > tryptamine and for the latter, affinities decrease in the order RU-24969 = 5-carboxamidotryptamine > 5-HT > α-methyl-5-HT > tryptamine (see Engel *et al.*, 1986). Furthermore, spiperone, which exhibits a high affinity for the 5-HT_{1A} binding site (Pedigo *et al.*, 1981), is inactive at the endothelial receptor at a concentration of 0.1 μM (unpublished observation). Only the 5-HT_{1C} binding site appears to have properties in common with the endothelial 5-HT receptor, the rank order of indolamine affinities for this site decreasing in the order 5-HT = α-methyl-5-HT = tryptamine > 5-carboxamidotryptamine = RU24969 (Engel *et al.*, 1986). Obviously, in order to establish whether or not the endothelial 5-HT receptor shares a common identity with one of the 5-HT_1 binding sites in the brain, a more extensive range of receptor probes should be studied comparatively in the endothelial assay, 5-HT_1 binding assays and claimed functional correlates of these recognition sites.

In this study we have shown that ring preparations of the endothelium-intact rabbit external jugular vein serve as a reliable bioassay for the endothelial 5-HT receptor which mediates indirectly the relaxation of vascular smooth muscle. Affinity and relative efficacy estimates obtained for some analogues of 5-HT provided quantitative evidence that the endothelial 5-HT receptor is not the same as the 5-HT_2 receptor in the rabbit aorta. Indeed, excepting the lower potency of 5-carboxamidotryptamine with respect to 5-HT, the receptor satisfies the current criteria for a '5-HT_1-like' classification (Bradley *et al.*, 1986b). However, the results suggest that the endothelial 5-HT receptor is different from other reported '5-HT_1-like' receptors implying that, like the central 5-HT_1 recognition sites, functional '5-HT_1-like' receptors are a heterogeneous population. Whether or not these receptors represent functional counterparts of the binding sites in the brain remains to be elucidated.

References

APPERLEY, E., HUMPHREY, P.P.A. & LEVY, G.P. (1976). Receptors for 5-HT and noradrenaline in rabbit isolated ear artery and aorta. *Br. J. Pharmac.*, **58**, 215–224.

APPERLEY, E., FENIUK, W., HUMPHREY, P.P.A. & LEVY, G.P. (1980). Evidence for two types of excitatory receptor for 5-hydroxytryptamine in dog isolated vasculature. *Br. J. Pharmac.*, **68**, 215–224.

APPERLEY, E. & HUMPHREY, P.P.A. (1986). The interaction of 5-hydroxytryptamine and methysergide with methiothepin at '5-HT_1-like' receptors in dog saphenous vein. *Br. J. Pharmac.*, **87**, 131P.

BARRETT, V.J., LEFF, P., MARTIN, G.R. & RICHARDSON, P.J. (1986). Pharmacological analysis of the interaction between Bay K 8644 and 5-HT in the rabbit aorta. *Br. J. Pharmac.*, **87**, 487–494.

BLACK, J.W. & LEFF, P. (1982). Operational models of

pharmacological agonism. *Proc. R. Soc. B.*, **220**, 141–162.

BLACK, J.W., LEFF, P., SHANKLEY, N.P. & WOOD, J. (1985). An operational model of agonism: the effect of E/[A] curve shape on agonist dissociation constant estimation. *Br. J. Pharmac.*, **84**, 561–571.

BRADLEY, P.B., HUMPHREY, P.P.A. & WILLIAMS, R.H. (1986a). Evidence for the existence of 5-hydroxytryptamine receptors, which are not of the 5-HT_2 type, mediating contraction of rabbit isolated basilar artery. *Br. J. Pharmac.*, **87**, 3–4.

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. (1986b). Proposals for the classification and nomenclature of functional receptors for 5-hydroxytryptamine. *Neuropharmac.*, **25**, 563–576.

CLANCY, B.M. & MAAYANI, S. (1985). 5-Hydroxytryptamine receptor in isolated rabbit aorta: Characterisation with tryptamine analogs. *J. Pharmac. exp. Ther.*, **233**, 761–769.

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

COCKS, T.M. & ANGUS, J.A. (1984). Endothelium-dependent modulation of blood vessel reactivity. In *The Peripheral Circulation*. ed. Hunyor, S., Ludbrook, J., Shaw, J. & McGrath, M., Int. Cong. Ser. *Excerpta Med.*, Vol. **630**, pp. 9–21 Amsterdam: Elsevier Science Publishers B.V.

COHEN, R.A. (1985). Serotonergic prejunctional inhibition of canine coronary adrenergic nerves. *J. Pharmac. exp. Ther.*, **235**, 76–80.

COHEN, R.A., SHEPHERD, J.T. & VANHOUTTE, P.M. (1983a). Inhibitory role of the endothelium in the response of isolated coronary arteries to platelets. *Science*, **221**, 273–274.

COHEN, R.A., SHEPHERD, J.T. & VANHOUTTE, P.M. (1983b). 5-Hydroxytryptamine can mediate endothelium-dependent relaxation of coronary arteries. *Am. J. Physiol.*, **245**, (Heart Circ. Physiol., 14), H1077–H1080.

CONNOR, H.E., FENIUK, W., HUMPHREY, P.P.A. & PERREN, M.J. (1986). 5-Carboxamidotryptamine is a selective agonist at 5-hydroxytryptamine receptors mediating vasodilatation and tachycardia in anaesthetized cats. *Br. J. Pharmac.*, **87**, 417–426.

ENGEL, G., GÖTHERT, M., HOYER, D., SCHLICKER, E. & HILLENBRAND, K. (1986). Identity of inhibitory presynaptic 5-hydroxytryptamine (5-HT) autoreceptor in the rat brain cortex with 5-HT_{1B} binding sites. *Naunyn-Schmiedebergs Arch. Pharmac.*, **332**, 1–7.

ENGEL, G., GÖTHERT, M., MÜLLER-SCHWEINITZER, E., SCHLICKER, E., SISTONEN, L. & STADLER, P.A. (1983). Evidence for common pharmacological properties of [^{3}H]5-hydroxytryptamine binding sites, presynaptic 5-hydroxytryptamine autoreceptors in CNS and inhibitory presynaptic 5-hydroxytryptamine receptors on sympathetic nerves. *Naunyn-Schmiedebergs Arch. Pharmac.*, **324**, 116–124.

FENIUK, W., HUMPHREY, P.P.A. & WATTS, A.D. (1979). Presynaptic inhibitory action of 5-hydroxytryptamine in dog isolated saphenous vein. *Br. J. Pharmac.*, **67**, 247–254.

FENIUK, W., HUMPHREY, P.P.A. & WATTS, A.D. (1983). 5-Hydroxytryptamine-induced relaxation of isolated mammalian smooth muscle. *Eur. J. Pharmac.*, **96**, 71–78.

FENIUK, W., HUMPHREY, P.P.A. & WATTS, A.D. (1984). 5-Carboxamidetryptamine a potent agonist at 5-hydroxytryptamine receptors mediating relaxation. *Br. J. Pharmac.*, **82**, 209P.

FENIUK, W., HUMPHREY, P.P.A., PERREN, M.J. & WATTS, A.D. (1985). A comparison of 5-hydroxytryptamine receptors mediating contraction in rabbit aorta and dog saphenous vein: evidence for different receptor types obtained by use of selective agonists and antagonists. *Br. J. Pharmac.*, **86**, 697–704.

FOZARD, J.R. & MWALUKO, G.M.P. (1976). Mechanism of the indirect sympathomimetic effect of 5-hydroxytryptamine on the isolated heart of the rabbit. *Br. J. Pharmac.*, **57**, 155–175.

FURCHGOTT, R.F. (1966). The use of β-haloalkylamines in the differentiation of receptors and determination of dissociation constants of receptor-agonist complexes. *Adv. Drug Res.*, **3**, 21–55.

GRIFFITH, T.M., HENDERSON, A.H., HUGHES EDWARDS, D. & LEWIS, M.J. (1984). Isolated perfused rabbit coronary artery and aortic strip preparations: the role of endothelium-derived relaxant factors. *J. Physiol.*, **351**, 13–24.

HOUSTON, D.S., SHEPHERD, J.T. & VANHOUTTE, P.M. (1985). Adenine nucleotides, serotonin and endothelium-dependent relaxations to platelets. *Am. J. Physiol.*, **248** (Heart Circ. Physiol., 17), H389–H395.

IMAIZUMI, Y., BABA, M., IMAIZUMI, Y. & WATANABE, M. (1984). Involvement of endothelium in the relaxation of isolated chick jugular vein by 5-hydroxytryptamine. *Eur. J. Pharmac.*, **97**, 335–336.

INNES, I.R. (1962). An action of 5-hydroxytryptamine on adrenaline receptors. *Br. J. Pharmac. Chemother.*, **19**, 427–441.

KALKMAN, H.O., ENGEL, G. & HOYER, D. (1984). Three distinct subtypes of serotonergic receptors mediate the triphasic blood pressure response to serotonin in rats. *J. Hypertension*, **2**, (Suppl, 3), 143–145.

LEFF, P. & MARTIN, G.R. (1986). Peripheral 5-HT_2-like receptors. Can they be classified with the available antagonists? *Br. J. Pharmac.*, **88**, 585–593.

LEFF, P., MARTIN, G.R. & MORSE, J.M. (1986). The classification of peripheral 5-HT_2-like receptors using tryptamine agonist and antagonist analogues. *Br. J. Pharmac.*, **89**, 493–499.

LEYSEN, J.E., AWOUTERS, F., KENNIS, L., LADURON, P.M., VANDENBERK, J. & JANSSEN, P.A.J. (1981). Receptor binding profile of R 41468, a novel antagonist of 5-HT_2 receptors. *Life Sci.*, **28**, 1015–1022.

LEYSEN, J.E., de CHAFFOY de COURCELLES, D., DE CLERCK, F., NIEMEGEERS, C.J.E. & VAN NUETEN, J.M. (1984). Serotonin-S_2 receptor binding sites and functional correlates. *Neuropharmac.*, **23**, 1493–1501.

MALICK, L. & WILSON, R.B. (1975). Modified thiocarbohydrazide procedure for scanning electron microscopy. *Stain Technology*, **50**, 4, 265–269.

MARIN, J., SALAICES, M., GOMEZ, B. & LLUCH, S. (1981). Noradrenergic component in the vasoconstriction induced by 5-hydroxytryptamine in goat cerebral arteries. *J. Pharm. Pharmac.*, **33**, 715–719.

MÜLLER-SCHWEINITZER, E. (1981). Agonist potencies of

tryptamine derivatives at pre- and post-junctional receptors in canine saphenous vein. *Prostgrad. Med. J.*, **57** (Suppl. 1), 36–44.

PAZOS, A., HOYER, D. & PALACIOS, J.M. (1984). The binding of serotonergic ligands to the porcine choroid plexus: Characterisation of a new type of serotonin recognition site. *Eur. J. Pharmac.*, **106,** 539–546.

PEDIGO, N.W., YAMAMURA, H.I. & NELSON, D.L. (1981). Discrimination of multiple [^{3}H]5-hydroxytryptamine binding site by the neuroleptic spiperone in rat brain. *J. Neurochem.*, **36,** 220–226.

PEROUTKA, S.J. & SNYDER, S.H. (1979). Multiple serotonin receptors: differential binding of [^{3}H] 5-hydroxytryptamine, [^{3}H] lysergic acid diethylamide and [^{3}H] spiroperidol. *Mol. Pharmac.*, **16,** 687–699.

SAXENA, P.R., MYLECHARANE, E.J. & HEILIGERS, J. (1985). Analysis of the heart rate effects of 5-hydroxytryptamine in the cat; mediation of tachycardia by 5-HT$_1$-like receptors. *Naunyn-Schmiedebergs Arch. Pharmac.*, **330,** 121–129.

STOLLAK, J.S. & FURCHGOTT, R.F. (1983). Use of selective antagonists for determining the types of receptors mediating the action of 5-hydroxytryptamine and tryptamine in the isolated rabbit aorta. *J. Pharmac. exp. Ther.*, **224,** 215–221.

TRIST, D.G. & LEFF, P. (1985). Quantification of H_2-agonism by clonidine and dimaprit in an adenylate cyclase assay. *Agents and Actions*, **16,** 222–226.

(Received November 17, 1986.
Revised February 9, 1987.
Accepted February 17, 1987.)