Br. J. Pharmac. (1986), **89**, 493–499

The classification of peripheral 5-HT_2-like receptors using tryptamine agonist and antagonist analogues

P. Leff, G.R. Martin & J.M. Morse

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

1 In a previous study, we attempted to verify the classification of 5-hydroxytryptamine$_2$ (5-HT_2) receptors in three vascular tissues, by use of the conventional antagonists, ketanserin, spiperone, methysergide and trazodone. However, it was not possible to conclude homogeneity of the receptor type in the three tissues due to the inconsistent behaviour of these antagonists, in particular, their apparently variable affinities between the tissues. These results led to the reliability of the conventional antagonists being questioned as receptor probes.

2 In the present study, a set of tryptamine analogues were investigated in two of the tissues, the rabbit aorta and the rat jugular vein. Unlike the conventional antagonists, these compounds bear a close chemical relation to the natural agonist, 5-HT.

3 In both tissues, α,α-dimethyltryptamine demonstrated apparently simple competitive antagonism of 5-HT-induced constrictions. Its affinity was estimated to be the same in each case.

4 The affinities and relative efficacies of 5-HT, 5-cyanotryptamine, N,N-dimethyltryptamine and N-benzyl-5-methoxytryptamine were also found to be indistinguishable between the two tissues.

5 Unlike the conventional 5-HT_2 receptor antagonists, these tryptamine analogues failed to distinguish between the 5-HT receptors in the rabbit aorta and rat jugular vein implying that they truly belong to the same class. In view of this result, it is suggested that simple tryptamine analogues are more reliable probes for 5-HT receptor classification than ligands which bear little or no chemical relation to the natural agonist.

Introduction

In a previous study (Leff & Martin, 1986) we investigated the effects of some standard 5-hydroxytryptamine (5-HT) antagonists, ketanserin, trazodone, methysergide and spiperone in three isolated vascular preparations. Each of the tissues used had been claimed to contain 5-HT_2 receptors (Leysen *et al.*, 1982; Humphrey *et al.*, 1982; Cohen *et al.*, 1983; Maayani *et al.*, 1984) and the object of the study was to examine the extent to which this classification could be substantiated quantitatively. Applying the established criteria for the pharmacological analysis of antagonism (Schild, 1973; Black *et al.*, 1983) we found that only spiperone and trazodone behaved as simple competitive antagonists in every tissue. However, affinity estimates for trazodone varied between the tissues by over an order of magnitude and smaller but significant discrepancies were also noted for spiperone. Although ketanserin produced surmountable competitive antagonism in two of the three tissues it too expressed significantly different affinities in these cases. The non-surmountable type of antagonism obtained with ketanserin in the third tissue was also obtained with methysergide in two of the three tissues used.

The variable effects of these antagonists meant that the receptors in the different tissues could not be reliably classified. The data involving non-surmountable antagonism could not contribute meaningful information towards receptor classification and the variable affinities expressed by the same antagonist could be interpreted either as evidence for receptor heterogeneity or as an indication that the antagonists used were inadequate receptor probes. The latter argument would imply that the antagonists interact with binding sites in the receptor which are accessory to those with which the natural agonist, 5-HT, interacts. These accessory sites could vary between the different tissues (Ariëns *et al.*, 1979; Kenakin, 1984).

In an attempt to resolve this question we have now studied a series of tryptamine analogues which, unlike the antagonists used previously, retain a close chemical relation to the natural agonist. The object of

the study was to determine whether such compounds would provide more meaningful information for the classification of the receptors in question.

Methods

Vascular tissue preparations

Rabbit aorta: The thoracic aorta was removed from male New Zealand white rabbits (2.0–2.5 kg) which had been killed by injection of pentobarbitone sodium (Sagatal: 60 mg kg^{-1}) into a marginal ear vein. The vessel was cleared of adhering connective tissue after mounting on a polypropylene cannula (external diameter = 2.5 mm). For each experiment, six ring segments, approximately 3 mm wide, were prepared as described by Stollak & Furchgott (1983), preserving the plane of the circular smooth muscle.

Rat jugular veins: Male Wistar rats (375–425 g) were sacrificed by CO_2 suffocation. Right and left external jugular veins were exposed and cannulated *in situ* with polypropylene tubing (external diameter = 1.0 mm). Two ring preparations, each approximately 5 mm wide, were obtained from each vein. Since each experiment required six preparations, tissues were taken from two animals. Vascular rings were carefully transferred from the cannula onto two fine stainless steel hooks (27-gauge) according to the method of Hooker *et al.* (1977).

Tissues were mounted in 20 ml organ baths containing Krebs solution 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 continuously gassed with 95% O_2:5% CO_2. Ring preparations were suspended between two wire hooks (platinum or stainless steel), one attached to a Grass FT03C force displacement transducer and the other to a stationary support in the organ bath.

Experimental protocols

At the beginning of each experiment, a force was applied to the tissue preparations (aortic rings, 3.0 g; jugular vein rings, 0.4 g). During a subsequent stabilization period of 30 min, the force was re-established twice and tissues were exposed to pargyline (500 μM) in order to inhibit monoamine oxidase irreversibly. 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. Tissues were then challenged with a near-maximally effective concentration of 5-HT (rabbit aorta 10 μM, rat jugular vein 1 μM) to establish viability. Also, in antagonism experiments, this treatment provided a reference contraction by which subsequent responses could be normalized.

In six replicate antagonist experiments with α,α-dimethyltryptamine, tissues were exposed to drug or the vehicle for 60 min before the construction of a 5-HT concentration-effect (E/[A]) curve. In a single experiment each of the six vascular ring preparations were treated with different concentrations, including zero, of the antagonist. Therefore replicate numbers and errors refer to the number of preparations. When the agonists 5-HT, 5-cyanotryptamine, N,N-dimethyltryptamine and N-benzyl-5-methoxytryptamine were studied in four experiments, single E/[A] curves were obtained in different preparations; two additional 5-HT E/[A] curves were obtained at the same time in tissues previously exposed for 30 min to phenoxybenzamine (rabbit aorta: 5×10^{-8} M and 10^{-7} M; rat jugular vein: 10^{-8} M and 2×10^{-8} M). Thus, each of the six ring segments was treated with a different agonist or subjected to phenoxybenzamine treatment. As with antagonism experiments, replicate numbers for each condition refer to the number of preparations used. The concentration of agonist in the organ bath was always increased cumulatively in 0.5 $\log_{10}$ unit increments.

Analysis of data

Antagonist experiments: Each E/[A] curve data set was fitted to a logistic function of the form;

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

in which α,$[A_{50}]$ and m are the asymptote, location and slope parameters respectively. Location parameters were actually estimated as logarithms ($-\log_{10}[A_{50}]$). Six individual experiments were conducted with α,α-dimethyltryptamine used as antagonist. On each occasion, a one-way analysis of variance tested for treatment effects on the computed estimates of α and m. If the treatments did not significantly ($P < 0.05$) modify these parameter estimates, then computed $\log_{10}$ $[A_{50}]$ values were fitted to a linear form of the Schild equation:

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

where $[A_{50}^c]$ is a control A_{50} value, [B] is the concentration of antagonist, K_B is its equilibrium dissociation constant and n is its order of reaction with the receptor. If the average value of n from the six analyses was not significantly different from unity it was

constrained to this value in order to obtain an estimate of pK_B ($-\log_{10} K_B$) in each experiment. These values were then averaged to provide a mean estimate with standard error. [A_{50}] values, obtained in these experiments are displayed in Clark plot form (Stone & Angus, 1978). This plot has advantages over the Schild plot in that it displays control [A_{50}]s and avoids the calculation of concentration-ratios which tend to over-weight the control values.

Operational model fitting: The averaged E/[A] curve data measured in grams force were fitted directly to the operational model of agonism (Black & Leff, 1983; Black *et al.*, 1985; Barrett *et al.*, 1986):

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

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 and solutions

The following drugs were used: 5-hydroxytryptamine hydrochloride (Sigma Chemical Company, St. Louis, MO., U.S.A.); pargyline hydrochloride (Sigma): benextramine tetrahydrochloride monohydrate (Aldrich Chemical Company Ltd., Dorset, England); phenoxybenzamine hydrochloride (Smith, Kline and French, Welwyn Garden City, Herts, England).

α,α-Dimethyltryptamine hydrochloride, N,N-dimethyltryptamine hydrogen oxalate, N-benzyl-5-methoxytryptamine hydrochloride and 5-cyanotryptamine hydrochloride were synthesized by Dr H.F. Hodson, Medicinal Chemistry Department, Wellcome Research Laboratories, Beckenham, Kent.

Phenoxybenzamine was dissolved in absolute ethanol which attained a concentration in the organ bath of 0.01% v/v. This did not influence tissue responsiveness. All other drugs were dissolved and diluted in distilled water.

Results

Analysis of agonist action

The agonist effects of 5-HT and three tryptamine analogues, 5-cyanotryptamine, N,N-dimethyltryptamine and N-benzyl-5-methoxytryptamine in the rabbit aorta and rat jugular vein preparations are shown in Figure 1. The other analogue, α,α-dimethyltryptamine, showed no agonism in either preparation. The data are the average increases in force produced in four separate experiments.

The lines drawn through the data were produced by fitting both sets of data simultaneously to the operational model using equation (3), assuming that the same value of K_A and the same relative value of τ could be estimated for each agonist in the two tissues, in other words, assuming that the receptors in the two tissues were of the same type. The maximum possible response, E_m, and n, the sensitivity of the occupancy-effect, E/[AR], relation were assumed to be tissue-dependent. In order to estimate K_A and τ for partial agonists it is necessary that E_m be known (Black & Leff, 1983; Leff *et al.*, 1985; Leff, 1986). When a full agonist is available its maximal effect, by definition, provides an estimate of E_m. In the absence of a full agonist, E_m can be estimated when Furchgott's

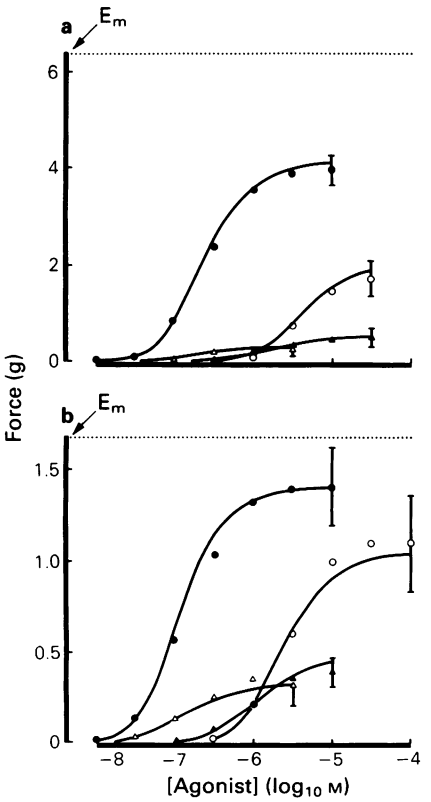

Figure 1 The average constrictor effects produced by 5-hydroxytryptamine (●), 5-cyanotryptamine (○), N,N-dimethyltryptamine (▲) and N-benzyl-5-methoxytryptamine (△) in the rabbit aorta (a) and in the rat jugular vein (b). In each case data are the averages of 4 replicate E/[A] curves. Standard error bars are shown on the maximal effects. The lines drawn through the averaged data are the results of fitting them to the operational model, equation (3). Each value of E_m was estimated as described in the text.

method (1966) of irreversible antagonism is applied to partial agonists. As shown previously (Black *et al.*, 1985; Barrett *et al.*, 1986) 5-HT itself acts as a partial agonist in the rabbit aorta and E_m must be estimated in this system. Therefore, Furchgott's method was applied in this study. The effects of phenoxybenzamine on 5-HT responses were measured in each tissue and the resulting E/[A] curves were fitted simultaneously with those of the other agonists. For clarity, the phenoxybenzamine-treatment data are shown separately in Figure 2. The results demonstrate that in the rat jugular vein as well as the rabbit aorta, 5-HT behaves partially.

Estimates of pK_A and τ for each agonist together with estimates of E_m and n in each tissue are shown in Table 1. Comparison of the data with the fitted lines in Figure 1 indicates that the assumption of receptor homogeneity in the two tissues was reasonable. Separate analyses of the data shown in Figure 2 gave estimates of pK_A for 5-HT of 6.83 and 6.70 in the rabbit aorta and rat jugular vein respectively. These values are comparable with the value obtained by fitting all the data simultaneously.

Analysis of antagonism

The tryptamine analogue, α,α-dimethyltryptamine, which showed no agonism in either preparation, was analysed as an antagonist of 5-HT. In both tissues, this compound produced parallel displacements of E/[A] curves (Figures 3a and b). Analysis of computed $[A_{50}]$ values by equation (2) gave estimates of n (equivalent to the Schild plot slope) of 0.97 ± 0.03 in the rabbit aorta and 1.00 ± 0.04 in the rat jugular vein. Each estimate was not significantly different from unity. The estimated pK_B values (estimated with n constrained to unity) were 5.67 ± 0.16 in the rabbit aorta and 5.53 ± 0.07 in the rat jugular vein. These two estimates were not significantly different. The effect of α,α-dimethyltryptamine on computed $[A_{50}]$ values is shown for each tissue in the form of Clark plots (Figures 3c and d).

Discussion

In our previous study (Leff & Martin, 1986) the classification of 5-HT receptors in three different vascular tissues was made equivocal because of variable qualitative and quantitative effects of the putative 5-HT_2-receptor antagonists used. In this study we used some simple analogues of tryptamine to re-examine the classification of 5-HT receptors in two of these tissues, the rabbit aorta and rat jugular vein. The results obtained with agonist and antagonist analogues demonstrate that the 5-HT receptors in these two tissues are quantitatively indistinguishable.

Unlike any of the conventional antagonists, α,α-dimethyltryptamine demonstrated simple competition in both tissues and expressed the same affinity. Analysis of the agonist action of 5-HT, 5-cyanotryptamine, N,N-dimethyltryptamine and N-benzyl-5-methoxtryptamine showed that each agonist expressed the same affinity and relative efficacy in the two tissues. In general, model-fitting indicated that each

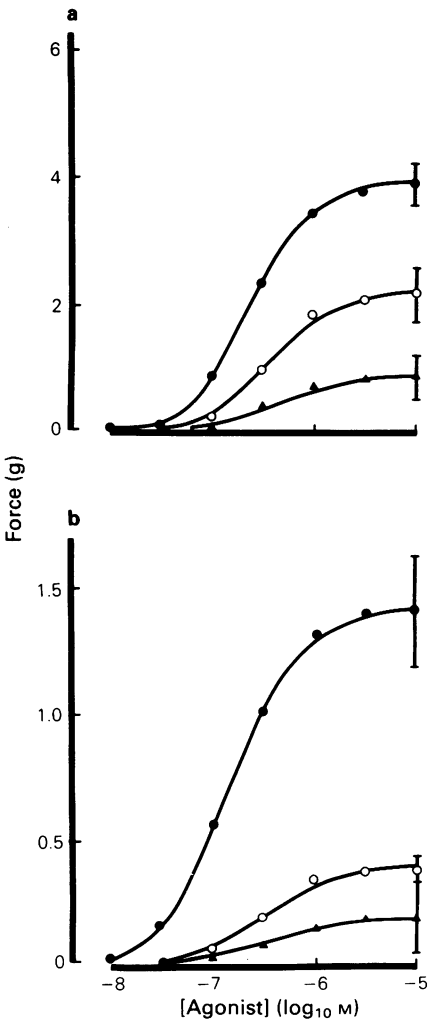

Figure 2 The average constrictor effects produced by 5-hydroxytryptamine in the absence (●) and presence of phenoxybenzamine treatment: 5×10^{-8}M (○) and 10^{-7}M (▲) in the rabbit aorta (a); 10^{-8}M (○) and 2×10^{-8}M (▲) in the rat jugular vein (b). The lines drawn through the averaged data are the results of fitting them to the operational model, equation (3). Data are the averages of 4 replicate E/[A] curves in the rabbit aorta and 3 or 4 replicate curves in the rat jugular vein.

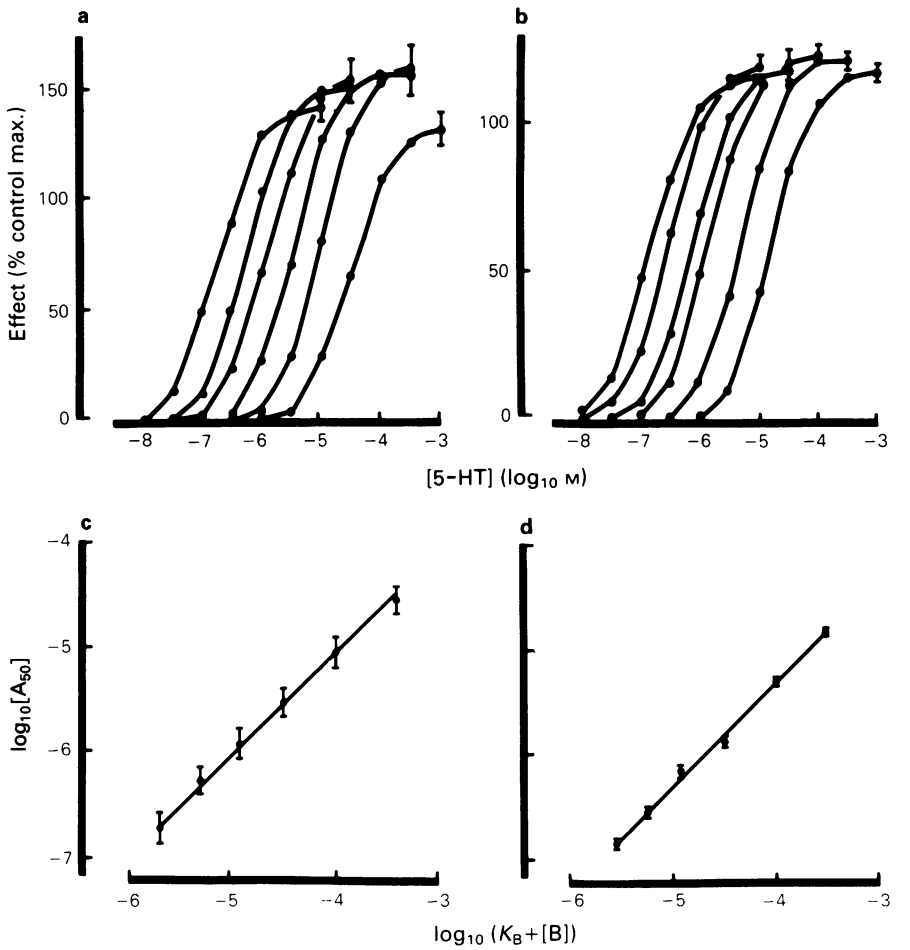

Figure 3 Panels (a) and (b) show the antagonist effects of α,α-dimethyltryptamine on 5-hydroxytryptamine E/[A] curves in the rabbit aorta and the rat jugular vein respectively. In each case data are the averages of 6 replicate E/[A] curves. The concentrations of the antagonist were 3×10^{-6}M, 10^{-5}M, 3×10^{-5}M, 10^{-4}M and 3×10^{-4}M. Panels (c) and (d) show the effects of α,α-dimethyltryptamine on the $[A_{50}]$s of 5-hydroxytryptamine curves. The Clark plot (Stone & Angus, 1978) is used to display the data for reasons given in the text. The adherence of the data with the unit slope line drawn through them indicates consistency with simple competition. The pK_B values, estimated by fitting equation (2) to the $[A_{50}]$ values, were 5.67 in the rabbit aorta and 5.53 in the rat jugular vein.

agonist was about 1.5 times more 'efficacious' in the jugular vein preparation than in the rabbit aorta (Table 1). This difference can be explained by a higher functional receptor concentration in the former tissue compared with the latter and/or a more efficient transducer system (Black & Leff, 1983; Kenakin, 1984).

The present results contradict those previously obtained with the antagonists ketanserin, spiperone and trazodone, each of which displayed variable affinities for the receptors in question (Leff & Martin, 1986). Whereas the previous data allowed two possible interpretations, namely, dissimilarity between the receptors or unreliability of the antagonists as probes, the present data permit only one conclusion, that is, receptor homogeneity. The use of agonists as well as a competitive antagonist in the present study lends weight to this conclusion since it is based on efficacy and affinity information and not merely the latter.

Since, evidently, the chemical interactions between the tryptamine analogues and the receptors in each tissue appear to be the same, as judged by affinity and efficacy, but the affinities of the conventional 5-HT_2-antagonists are variable, the simple interpretation is

Table 1 Parameters corresponding to the fitted lines in Figure 1: the data could evidently be fitted assuming that each agonist expressed the same affinity and relative efficacy in the two tissues

Parameter		*Rabbit aorta*		*Rat jugular vein*
E_m		6.40g		1.67g
n		2.95		2.25
pK_a	5-HT:	6.88	5-HT:	6.88
	5-CN:	5.80	5-CN:	5.80
	NND:	6.34	NND:	6.34
	NBT:	7.30	NBT:	7.30
τ	5-HT:	1.20	5-HT:	1.93
	5-CN:	0.77	5-CN:	1.24
	NND:	0.44	NND:	0.71
	NBT:	0.36	NBT:	0.58

Abbreviations: 5-HT = 5-hydroxytryptamine; 5-CN = 5-cyanotryptamine; NND = N,N-dimethyltryptamine; NBT = N-benzyl-5-methoxytryptamine. For each agonist the value of τ was 1.61 times higher in the jugular vein compared with the rabbit aorta.

that the latter group of compounds interact with sites on the receptor that are not utilised by the natural agonist and its congeners. As discussed elsewhere (Leff & Martin, 1986) the relevance of such chemical information to receptor classification is arguable, depending on the use of the term 'receptor'.

If, by this term, we mean sites for drug interaction, then we might conclude that the receptors examined here are 'similar' but 'different'; 'similar' because they cannot be distinguished by the natural agonist or close chemical analogues but 'different' because selectivity is expressed by chemically heterogeneous compounds that can apparently still compete with agonists. For example, with ketanserin used as the receptor probe, the following tissues could be concluded to contain subtypes of 5-HT_2-like receptors (pA_2 in parentheses): rat aorta (8.4) (Bradley *et al.*, 1983); rat caudal artery (8.8) (Bradley *et al.*, 1983); bovine coronary artery (9.2) (Kaumann, 1983); rat portal vein (9.7) (Lemberger *et al.*, 1984); rat femoral artery (10.4) (Mecca & Webb, 1984). In the authors' opinion, such subtyping serves no genuine purpose in pharmacological classification as it tends to create disorder rather than order. By implication the definition of the term 'receptor' upon which it is based must be regarded as too plastic.

If, as we prefer, the term is reserved for sites at which hormones and transmitters act in physiologically intact systems then the receptors in the present study would be classified as the same. Adopting Stephenson's definition of a receptor (Stephenson, 1975), namely 'that small spatial arrangement of atoms to which a substance endogeneous to the organism attaches itself as an essential step in modifying cellular functions', subtyping of receptors would only be possible when there is evidence that the chemical information in a hormone is processed differently by different sites. As well as being more conservative, this definition and the tests for receptor homogeneity or heterogeneity that it entails, provides a means of understanding the ways in which physiological systems are ordered. This must be one of the objects of pharmacological classification.

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.

ARIËNS, E.J., BELD, A.J., RODRIGUES DE MIRANDA, J.F. & SIMONIS, A.M. (1979). The pharmacon-receptor-effector concept: a basis for understanding the transmission of information in biological systems. In *The Receptors. A Comprehensive Treatise.* Volume I, General Principles and Procedures. ed. O'Brien, R.D. pp. 33–91. New York, London: Plenum Press.

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. (1983). Operational models of pharmacological agonism. *Proc. R. Soc. B.*, **220**, 141–162.

BLACK, J.W., GERSKOWITCH, V.P. & LEFF, P. (1983). Reflections on the classification of histamine receptors. In *Pharmacology of Histamine Receptors.* ed. Ganellin,

C.R. & Parsons, M.E. pp. 1–9, Bristol, London, Boston: John Wright.

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. (1983). Are vascular 'D' and '5-HT_2' receptors for 5-hydroxytryptamine the same? *Br. J. Pharmac.*, **79,** 295P.

COHEN, M.L., MASON, N., WILEY, S.K. & FULLER, R.W. (1983). Further evidence that vascular serotonin receptors are of the 5-HT_2 type. *Biochem. Pharmac.*, **32,** 567–570.

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.

HOOKER, C.W., CALKINS, P.J. & FLEISCH, J.H. (1977). On the measurement of vascular and respiratory smooth muscle responses *in vitro*. *Blood Vessels*, **14,** 1–11.

HUMPHREY, P.P.A., FENIUK, W. & WATTS, A.D. (1982). Ketanserin – a novel antihypertensive drug? *J. Pharm. Pharmac.*, **34,** 541.

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

KAUMANN, A.J. (1983). Yohimbine and rauwolscine inhibit 5-hydroxytryptamine contraction of large coronary arteries of calf through blockade of 5-HT_2 receptors. *Naunyn-Schmiedebergs Arch. Pharmac.*, **323,** 149–154.

KENAKIN, T.P. (1984). The classification of drug and drug receptors in isolated tissues. *Pharmac. Rev.*, **36,** 165–222.

LEFF, P. (1986). Can operational models of agonism provide a framework for classifying hormone receptors. In *Towards the Classification of 'Hormone' Receptors.* ed. Black, J.W., Gerskowitch, V.P. & Jenkinson, D., (in press).

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. (1985). Application of the operational model of agonism to establish conditions when functional antagonism may be used to estimate agonist dissociation constants. *Br. J. Pharmac.*, **85,** 655–663.

LEMBERGER, H.F., MASON, N. & COHEN, M.L. (1984). 5-HT_2-receptors in the rat portal vein: desensitisation following cumulative serotonin addition. *Life Sci.*, **35,** 71–77.

LEYSEN, J., NIEMEGEERS, C.J.E., VAN NUETEN, J.M. & LADURON, P.M. (1982). [^{3}H] Ketanserin (R 41468), a selective ^{3}H-ligand for Serotonin$_2$ receptor binding sites. Binding properties, brain distribution and functional role. *Mol. Pharmac.*, **21,** 301–314.

MAAYANI, S., WILKINSON, W.C. & STOLLAK, J. (1984). 5-HT receptor in the rabbit aorta: characterisation with some butyrophenone analogs. *J. Pharmac. exp. Ther.*, **229,** 346–350.

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.

MECCA, T.E. & WEBB, R.C. (1984). Vascular responses to serotonin in steroid hypertensive rats. *Hypertension*, **6,** 887–892.

SCHILD, H.O. (1973). Receptor classification with special reference to beta-adrenergic receptors. In *Drug Receptors.* ed. Rang, H.P. pp. 29–36. London: Macmillan.

STEPHENSON, R.P. (1975). Interactions of agonists and antagonists with their receptors. In *Les Colloques de l'institut National de la Santé et de la Recherche Medicale.* Smooth muscle pharmacology and physiology. Volume 50, ed. Warcel, M. & Vassort, G. pp. 15–28.

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.

STONE, M. & ANGUS, J.A. (1978). Developments of computer-based estimation of pA_2 values and associated analysis. *J. Pharmac. exp. Ther.*, **207,** 705–718.

(Received March 10, 1986.
Revised June 23, 1986.
Accepted June 30, 1986.)