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Peripheral 5-HT_2-like receptors. Can they be classified with the available antagonists?

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1 Interactions between 5-hydroxytryptamine (5-HT) and the so-called 5-HT_2 receptor antagonists ketanserin, spiperone, trazodone and methysergide were studied in isolated preparations of the rabbit aorta, rat jugular vein, and rat caudal artery.

2 Trazodone and spiperone were apparently simple competitive antagonists since they produced antagonism that was surmountable over the concentration range studied and, in each tissue, their apparent affinity appeared to be independent of the antagonist concentration. Furthermore, concentration-ratios obtained with the two antagonists in combination suggested that antagonism was additive, implying mutual competition with a single population of 5-HT receptors.

3 Ketanserin was a non-surmountable antagonist of 5-HT in the rat caudal artery and methysergide demonstrated surmountable, competitive antagonism only in the rabbit aorta.

4 Antagonist dissociation constants estimated for apparently competitive interactions showed that ketanserin, spiperone and trazodone expressed affinities which differed according to the tissue used. In the case of trazodone, affinity estimates differed by as much as 12 fold. These discrepancies were independent of the 5-HT receptor agonist used and could not be attributed to an inadequate equilibration of the antagonist.

5 These results can be interpreted in two ways: either the receptors in the different tissues are heterogeneous or the antagonists used here must be considered as unreliable probes for the classification of 5-HT_2-like receptors.

Introduction

It is generally accepted that hormone receptors can be quantitatively classified by the equilibrium dissociation constants (K_Bs) of competitive antagonists (Schild, 1968; Waud, 1968). For the reliable estimation of these dissociation constants a number of necessary, although not sufficient, criteria must be satisfied (Schild, 1973; Black *et al.*, 1983a). Firstly, a competitive antagonist should produce parallel displacements of semilogarithmic agonist concentration-effect curves. Secondly, the estimated antagonist dissociation constant should be independent of the agonist used and thirdly, the fractional increase in agonist concentration needed to overcome the antagonism should be a linear function of antagonist concentration in accordance with the Schild equation (Schild, 1947). In addition, if identical receptors exist in different tissues, the same dissociation constant should be obtained in those tissues.

There are, however, problems in applying these criteria that involve: (i) the inevitable circularity relating the object of classification, the receptor, and the tool for the process, the antagonist, (ii) the insufficiency of the criteria, meaning that we can never prove simple competition, but only disprove it.

In this paper we have examined the extent to which the classification of peripheral 5-hydroxytryptamine (5-HT) receptors using antagonists might be compromised by problems of circularity and insufficiency in the analytical criteria. Various smooth muscle 5-HT receptors have been claimed to exist (Leysen *et al.*, 1982; Feniuk *et al.*, 1983a, b; Wrigglesworth, 1983; Humphrey, 1984; Cohen *et al.*, 1985)., but only in the case of the so-called 5-HT_2 receptor are potent and selective competitive antagonists reported to be available. Many antagonists, notably spiperone and ketanserin, have emerged from radioligand binding studies where they demonstrated high affinity for 5-HT_2 binding sites in the brain (Peroutka & Snyder, 1979, 1981; Leysen *et al.*, 1982; Engel *et al.*, 1984). The binding affinity of these and a range of other chemically diverse ligands appeared to correlate with their antagonist potencies measured in isolated tissues

(Leysen *et al.*, 1982; Van Nueten *et al.*, 1982; Cohen *et al.*, 1983; Müller-Schweinitzer & Engel, 1983; Engel *et al.*, 1984) leading to their widespread application as peripheral 5-HT_2 receptor probes (Leysen *et al.*, 1982; Feniuk *et al.*, 1983a; Frenken & Kaumann, 1984; Cohen *et al.*, 1985).

However, the affinity estimates for these ligands measured in different tissues covers an inordinately large range. In the case of ketanserin these estimates, expressed as pA_2 values, differ by as much as 100 fold (e.g. Bradley *et al.*, 1983 cf. Mecca & Webb, 1984). This is incompatible with the previously stated criteria for simple competition with a single class of 5-HT_2-like receptors. It is conceivable that such discrepancies might be attributable to different experimental conditions or analytical methods, but it is also possible that either the receptors in these systems are genuinely different or the antagonists used are unreliable receptor probes. In an attempt to resolve this problem, we have conducted a between-tissues study of the 5-HT receptor antagonism obtained with the putative 5-HT_2 antagonists ketanserin, spiperone, trazodone and methysergide. Experiments were performed in the rabbit aorta, the rat jugular vein and the rat caudal artery, each of which is claimed to contain 5-HT_2 receptors on the basis of affinity correlations with the central 5-HT_2 binding site (Leysen *et al.*, 1982; Humphrey *et al.*, 1982; Cohen *et al.*, 1983; Maayani *et al.*, 1984). Data were analysed strictly according to the established criteria for simple competition. Agonist-independence of antagonism was investigated using α-methyl-5-HT as well as 5-HT. In addition, interactions between antagonists were analysed according to the concentration-ratio method of Paton & Rang (1965). The results are discussed in terms of their implications for 5-HT receptor classification and for the development of selective 5-HT receptor based drugs.

Methods

Vascular tissue preparations

Rabit aorta The thoracic aorta was removed from male New Zealand White rabbits (2.0–2.5 kg) killed by injecting pentobarbitone sodium (Sagatal: 60 mg kg^{-1}) into a marginal ear vein. The vessel was cleared of adhering connective tissue after being mounted on a polypropylene cannula (external diameter = 2.5 mm). 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 (350–425 g) were killed by CO_2 suffocation. Right and left external jugular veins were exposed and cannulated *in situ* using polypropylene tubing (external diameter = 1.0 mm). Two ring preparations, each approximately 5 mm wide, were obtained from each vein. These were carefully transferred from the cannula onto two fine stainless steel hooks (27 gauge) according to the method of Hooker *et al.* (1977).

Rat caudal arteries Male Wistar rats (200–250 g) were killed by a blow to the head and the proximal caudal artery exposed by a ventral incision in the tail. A stainless steel wire (27 gauge) was inserted into the lumen of the vessel to facilitate removal and the subsequent preparation of spiral strips. Two strips, approximately 25 mm long and 2 mm wide were prepared from each vessel.

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. Spiral strips were suspended between the transducer and stationary support using cotton thread. Tissue responses were measured as changes in isometric force and recorded on Gould BS 272 pen recorders.

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; caudal artery strips, 1.0 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 irreversibly inhibit monamine oxidase. 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.

In experiments with antagonists tissues were challenged with a near-maximally effective concentration of 5-HT (rabbit aorta 10 μM, otherwise 1 μM) to establish viability and provide a reference contracture that was used to normalise subsequent agonist responses. Antagonist, or the vehicle, was then added to the organ bath 60 min prior to cumulative additions of agonist (0.5 $\log_{10}$ unit increments). Only a single agonist concentration-effect (E/[A]) curve was obtained from each preparation.

Analysis of data

Treatment of E/[A] curves 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 $-\log_{10}[A_{50}]$.

Estimation of antagonist K_Bs In antagonist experiments 3–6 replicates were usually obtained at each antagonist concentration using preparations from at least three animals. A one-way analysis of variance tested for treatment effects on the computed estimates of agonist E/[A] curve asymptotes and slopes. 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 which, unlike the conventional Schild analysis, gives equal weights to control and treatment data (Black *et al.*, 1985a, b). The expression used was:

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

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 n was not significantly different from unity, indicating simple competition, it was constrained to this value in order to obtain an estimate of K_B. The dissociation constant was actually estimated as pK_B ($-\log_{10} K_B$). For display purposes only, the parameters estimated were used to generate a Schild plot which was superimposed upon dose-ratios calculated using average $[A_{50}]$ values.

All fitting procedures were unweighted, iterative least squares minimisation routines which gave standard error estimates based on the second derivative. For each antagonist, the pK_B value obtained in different tissues were compared by unpaired *t* tests using these standard errors.

Drugs

5-Hydroxytryptamine creatinine sulphate (Koch-Light Laboratories Ltd., Haverhill, Suffolk); 5-hydroxytryptamine hydrochloride (Sigma Chemical Co. St. Louis, MO, U.S.A.); α-methyl-5-hydroxytryptamine hydrogen maleate (prepared by Dr. H.F. Hodson, Wellcome Research Laboratories, Beckenham, Kent); pargyline hydrochloride (Sigma); benextramine tetrahydrochloride monohydrate (Aldrich

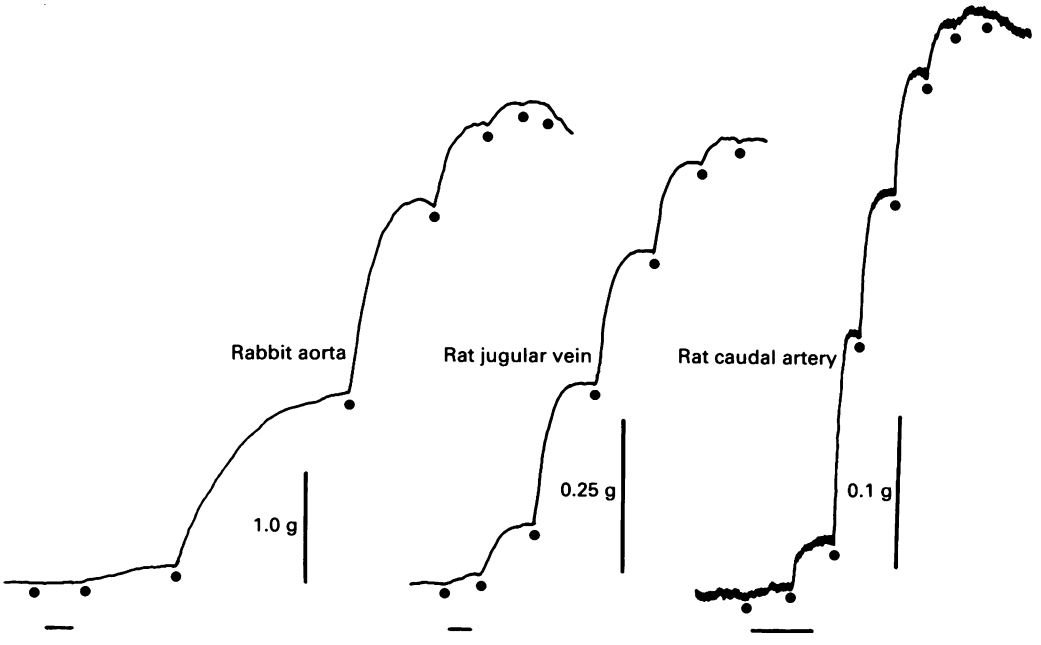

Figure 1 Representative recordings showing 5-hydroxytryptamine (5-HT)-induced increases in force in isolated preparations of the rabbit aorta, rat jugular vein and rat caudal artery. Additions of 5-HT (denoted by ●) were cumulative and the organ-bath concentration of drug was increased in 0.5 $\log_{10}$ unit increments from a starting concentration of 10 nM. In each case the horizontal bar represents 3 min.

Chemical Company Ltd., Dorset); spiperone (Janssen Pharmaceutica, Beerse, Belgium); ketanserin tartrate (Janssen); trazodone hydrochloride (Roussel Laboratories Ltd., Middlesex); methysergide bimaleate (Sandoz Ltd, Basle, Switzerland).

Spiperone was dissolved initially in dimethylsulphoxide and diluted in distilled water. At the final concentration in the organ bath (0.01% v/v), the vehicle did not influence tissue responsiveness. Solutions of all other drugs were prepared using fresh physiological medium or distilled water.

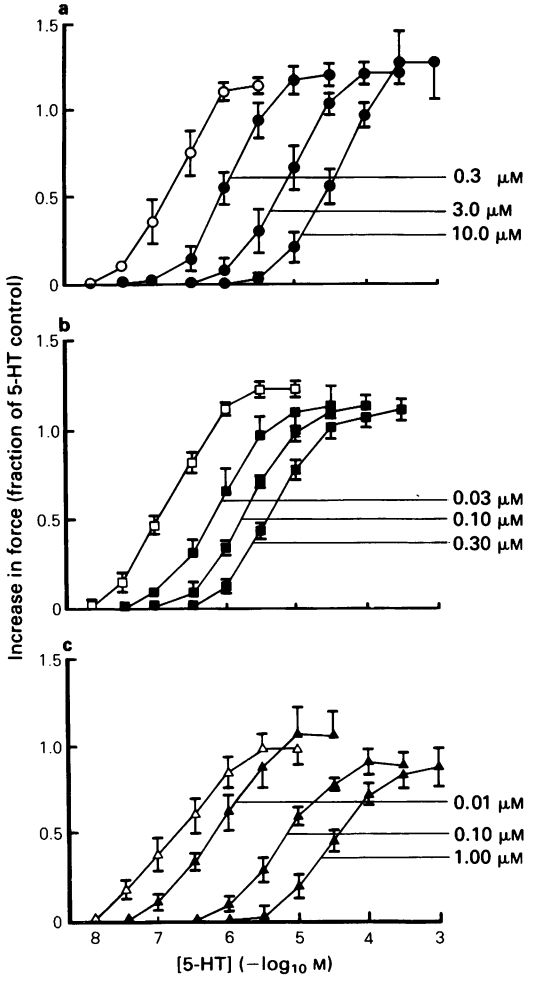

Figure 2 Competitive antagonism by trazodone of 5-hydroxytryptamine (5-HT) responses in (a) the rabbit aorta, (b) rat jugular vein and (c) rat caudal artery. In each case, increasing concentrations of the antagonist produced successive parallel rightward displacement of the 5-HT E/[A] curve (open symbols denote control and solid symbols denote responses in presence of trazodone, at concentrations indicated). Analysis of variance on E/[A] curve slopes and asymptotes revealed no significant differences. Each point is the mean of 4–6 values. Vertical lines show s.e. Note that in (a), data obtained in the presence of 0.1 μM and 1.0 μM trazodone are omitted for clarity.

Results

5-HT response characteristics

In each tissue preparation smooth, monophasic E/[A] curves were produced by cumulative additions of 5-HT. Tracings from original recordings of the 5-HT responses in each preparation are shown in Figure 1.

Antagonist experiments

The interaction between 5-HT and the antagonists trazodone, ketanserin, spiperone and methysergide was examined in each preparation. Figures 2 and 3 illustrate the antagonism by trazodone and methysergide respectively.

In rabbit aortic rings, antagonism of 5-HT responses was always completely surmountable and n, equivalent to the slope of a Schild regression, was not significantly different from unity (Table 1). Thus, each compound behaved as a simple competitive antagonist of 5-HT in this preparation.

In the rat jugular vein, apparent competitive antagonism was obtained with trazodone (Figure 2b), ketanserin and spiperone, but the right-shift caused by methysergide ($>$ 1 nM) was accompanied by depression of the 5-HT E/[A] curve asymptote (Figure 3b). Similar non-surmountable antagonism was obtained with both methysergide ($>$ 1 nM) (Figure 3c) and ketanserin ($>$ 3 nM) in the caudal artery. Thus, only trazodone and spiperone displayed surmountable antagonism in all three isolated tissues. Schild plots for each competitive interaction are shown in Figure 4.

Antagonist pK_Bs estimated in each vascular tissue are compared in Table 1. The values of P calculated from comparisons of trazodone affinity estimates in each tissue show that, regardless of the agonist used, estimates obtained in the rabbit aorta were clearly different ($P < 0.001$) from those measured in the rat vascular tissues. In contrast, the differences between pK_B values measured in the rat jugular vein and caudal artery preparations were much smaller and, although still statistically significant ($0.02 < P < 0.05$) when 5-HT was the agonist, there was no difference in the pK_B estimates when α-methyl-5-HT was used. This suggests that such small differences are probably not meaningful. A similar result was also obtained with spiperone, affinity estimates being similar in the two rat tissues, but each of these differing significantly ($P < 0.02$) from the estimate in the rabbit aorta. For

ketanserin, the affinity estimate in the rabbit aorta was different ($P < 0.02$) from that in the rat jugular vein, but the discrepancy was not as profound as that obtained with trazodone.

Influence of incubation time on trazodone pK_B estimates

The influence of incubation time on the antagonism of 5-HT by trazodone (Kenakin, 1980) was investigated in the rabbit aorta by exposing the tissues to trazodone (0.3, 3.0 and 10 μM) for 120 min before agonist E/[A] curves were obtained. The pK_B estimate obtained from 3 experiments (11 preparations) was 7.45 ± 0.07 ($n = 1.07 \pm 0.05$) which was not statistically different ($0.2 < P < 0.3$) from that estimated after 60 min incubation (Table 1), implying that antagonist and receptor were in equilibrium after 60 min.

Agonist-dependence of trazodone pK_B estimates

In each of the three tissues, α-methyl-5-HT was used to test the agonist-dependence of antagonism by trazodone. This antagonist was chosen because it provided the most discrepant affinity estimates when 5-HT was the agonist. In each preparation, trazodone competitively antagonized α-methyl-5-HT and the pK_B estimates obtained, shown in Table 1, were comparable to those obtained when 5-HT was the agonist.

Antagonism by trazodone and spiperone in combination

The mutual competition between trazodone and spiperone was investigated on the basis of concentration-ratios analysis (Paton & Rang, 1965). Table 2 shows the 5-HT concentration-ratio (average [A_{50}] in presence of antagonist(s)/average [A_{50}] of control) obtained in each vascular preparation following 60 min exposure to trazodone, spiperone or the two antagonists in combination. Obviously, the results are not consistent with a multiplication of concentration-ratios which would be expected if the two antagonists interacted at independent sites. Indeed, the data more closely agree with an addition of concentration-ratios which would be anticipated for a mutually competitive interaction of the antagonists in each preparation.

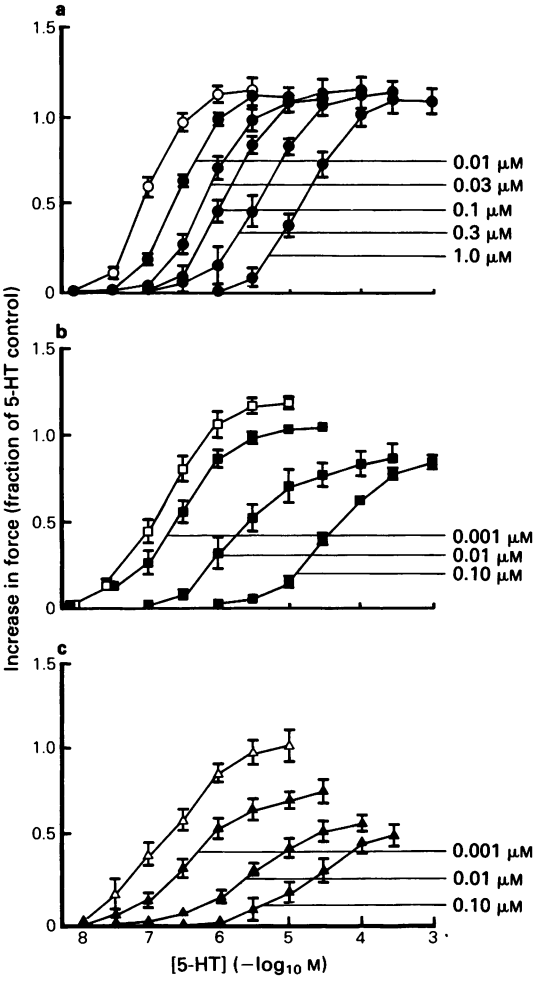

Figure 3 Methysergide antagonism of 5-hydroxytryptamine (5-HT) responses in (a) the rabbit aorta, (b) rat jugular vein and (c) rat caudal artery. Open symbols denote control E/[A] curve and solid symbols denote responses in presence of methysergide at concentrations indicated. In the rabbit aorta (a), E/[A] curve slopes and asymptotes were not different. In the rat jugular vein (b) and caudal artery (c) E/[A] curve slopes were unchanged but their asymptotes were significantly ($P < 0.01$) depressed. Each point is the mean of 4–6 values. Vertical lines show s.e.

Discussion

The 5-HT receptors mediating contraction of the rabbit aorta, the rat caudal artery and the rat jugular vein have been claimed to belong to the same class, namely the 5-HT$_2$ type (Leysen *et al.*, 1982; Humphrey *et al.*, 1982; Cohen *et al.*, 1983; Maayani *et al.*, 1984). In this paper we have investigated the extent to which this classification can be sustained when these receptors, and the ligands which have been used to classify them, are analysed quantitatively using well-established pharmacological criteria (Schild, 1968; Black *et al.*, 1983a). Equivalence of the experimental conditions in each vascular tissue was attempted by inhibiting oxidative deamination, a principle route of metabolic disposition of 5-HT (Paiva *et al.*, 1984).

Table 1 Equilibrium dissociation constants (pK_Bs) and Schild plot slope parameters (in parentheses) measured for some 5-hydroxytryptamine (5-HT) receptor antagonists in different isolated vascular smooth muscles

a

Antagonist	*Agonist*	*Vascular tissue* *Rabbit aorta*	*Rat jugular vein*	*Rat caudal artery*
Trazodone	5-HT	7.23 ± 0.19 (1.00 ± 0.13)	8.02 ± 0.07 (0.90 ± 0.08)	8.32 ± 0.12 (0.91 ± 0.08)
	α-me-5-HT	7.39 ± 0.11 (1.09 ± 0.09)	8.33 ± 0.11 (1.17 ± 0.09)	8.06 ± 0.09 (1.03 ± 0.09)
Ketanserin	5-HT	8.56 ± 0.09 (0.91 ± 0.09)	9.05 ± 0.16 (1.12 ± 0.10)	NSA
Spiperone	5-HT	9.28 ± 0.10 (1.10 ± 0.06)	9.66 ± 0.11 (0.98 ± 0.14)	9.63 ± 0.09 (0.91 ± 0.07)
Methysergide	5-HT	8.25 ± 0.06 (0.99 ± 0.04)	NSA	NSA

b

	RA v RJV	*RA v RCA*	*RCA v RJV*
5-HT/trazodone	$P<0.001$ (48 d.f.)	$P<0.001$ (49 d.f.)	$0.02<P<0.05$ (39 d.f.)
α-me-5-HT/trazodone	$P<0.001$ (25 d.f.)	$P<0.001$ (22 d.f.)	$0.05<P<0.1$ (29 d.f.)
5-HT/ketanserin	$P<0.02$ (45 d.f.)	—	—
5-HT/spiperone	$P<0.02$ (34 d.f.)	$P<0.02$ (47 d.f.)	$0.08<P<0.9$ (39 d.f.)

(a) Each agonist-antagonist data set comprised 3–6 $\log_{10}[A_{50}]$ values at each antagonist concentration and these were fitted directly to equation (2) to yield the parameter estimates shown. For each antagonist unpaired t tests were used to ascertain the statistical significance of between-tissue differences in pK_B estimates. (b) Shows probability values and the total number of degrees of freedom (d.f.) associated with each comparison (= sum of the degrees of freedom for each pK_B estimate − 2). Key: NSA = non-surmountable antagonism; RA = rabbit aorta; RJV = rat jugular vein; RCA = rat caudal artery; α-me-5-HT = α-methyl-5-HT.

Table 2 Concentration-ratios analysis of the interaction between trazodone and spiperone

	Observed r_B	r_C	r_{B+C}	*Expected* $r_B + r_C - 1$	$r_B \times r_C$
Rabbit aorta	128	110	183	237	14080
Rat jugular vein	63	66	107	128	4158
Rat caudal artery	55	21	69	75	1155

The observed ratios (r) are those calculated from the mean $[A_{50}]$ values of at least three 5-HT E/[A] curves performed in the absence and presence of: r_B, trazodone (rabbit aorta 5 μM; rat caudal artery and jugular vein 0.6 μM); r_C, spiperone (rabbit aorta 0.05 μM; rat caudal artery and jugular vein 0.01 μM) or r_{B+C}, the two antagonists in combination.

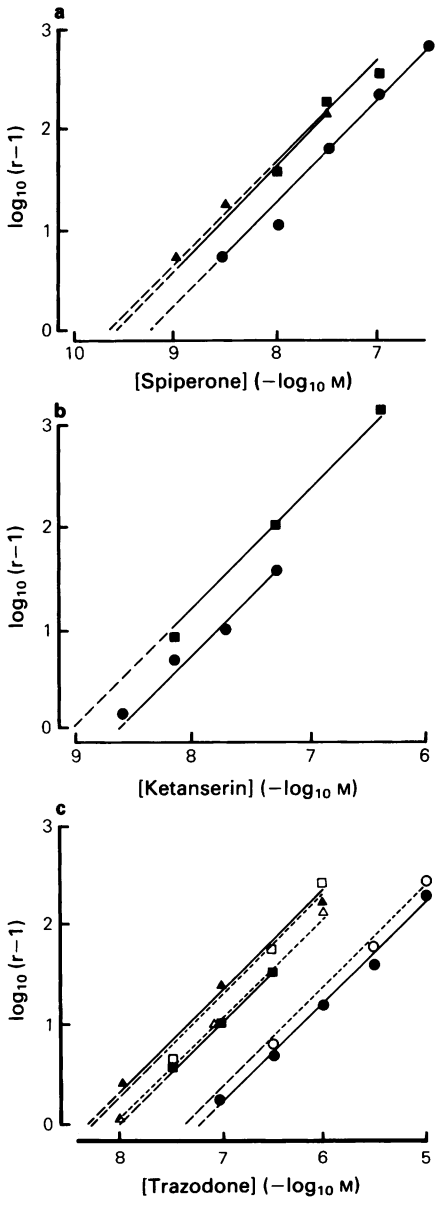

Figure 4 Competitive antagonism of 5-hydroxytryptamine (5-HT) by (a) trazodone; (b) spiperone and (c) ketanserin in the rabbit aorta (circles), rat jugular vein (squares) and rat caudal artery (triangles). Concentration-ratios (r) were calculated using the mean [A_{50}] values in each data set. The line drawn through each data set was obtained by fitting equation (2), with n constrained to unity, directly to $\log_{10}[A_{50}]$ values. In (c) the broken lines (open symbols) are those obtained when α-methyl-5-HT was the agonist instead of 5-HT. Antagonist equilibrium dissociation constants (pK_Bs) and Schild plot slope parameters are summarized in Table 1.

Irreversible blockade of α_1-adrenoceptors also prevented their direct (Apperley *et al.*, 1976; Stollak & Furchgott, 1983) or indirect (Innes, 1962; Marin *et al.*, 1981) activation by either 5-HT or α-methyl-5-HT.

Of the four antagonists examined in this study, only trazodone and spiperone maintained surmountable, apparently simple competitive antagonism in all three assay preparations. Both of these compounds selectively displace [^{3}H]-spiperone from central 5-HT$_2$ binding sites and both exhibit a high affinity for these sites (Riblet & Taylor, 1981; Leysen *et al.*, 1981). The compounds have also been shown to potently antagonize 5-HT contractions in a variety of isolated smooth muscles (Brazenor & Angus, 1982; Humphrey *et al.*, 1982; Cohen *et al.*, 1983; Ichida *et al.*, 1983; Wrigglesworth, 1983; Clancy & Maayani, 1985). In the present experiments, spiperone expressed a similar potency in the rat jugular vein, rat caudal artery and rabbit aorta although the pK_B value measured in the aorta was significantly different from the pK_Bs obtained in either of the other two. More emphatically, the pK_Bs for trazodone varied by an order of magnitude between the rabbit aorta estimate and the rat caudal artery value, the rat jugular vein estimate being intermediate. The apparently low affinity of trazodone for 5-HT receptors in the rabbit aorta was not the result of inadequate antagonist incubation resulting in non-equilibrium conditions (Kenakin, 1980) because doubling the antagonist exposure time failed to modify the affinity estimate. Also, this discrepancy was not a particular feature of the interaction of trazodone with 5-HT, because the same affinity estimates were made in each biossay using α-methyl-5-HT as agonist. Furthermore, the apparent addition of antagonist concentration-ratios produced by spiperone and trazodone is consistent with mutual competition between these two ligands and 5-HT. Thus, in each preparation, the effects of trazodone and spiperone were indistinguishable from simple competition, but evidently the antagonists expressed different affinities for the receptors in each tissue, the most notable differences being expressed by trazodone.

Consistent with previous findings, methysergide and ketanserin behaved as surmountable, competitive antagonists in the rabbit aorta (Apperley *et al.*, 1976; Humphrey *et al.*, 1982; Black *et al.*, 1983b), but in the rat caudal artery both compounds depressed the agonist maximum response (Hicks & Langer, 1983; Marwood & Stokes, 1983). This type of antagonist behaviour cannot be ascribed to any single mechanism and, therefore, data obtained with compounds exhibiting non-surmountable antagonism do not contribute usefully to receptor classification. The propensity of methysergide and ketanserin to demonstrate non-surmountable antagonism must limit their utility as 5-HT receptor probes.

The reliability of ketanserin can be further questioned on the grounds that the antagonist produced surmountable competitive antagonism in both the rabbit aorta and rat jugular vein, but, like trazodone and spiperone, the compound expressed different affinities for the 5-HT receptors in these tissues. These discrepancies appear to be real, because antagonist dissociation constants were measured under circumstances where differences in analytical methods and experimental conditions were minimized.

Evidently, trazodone, ketanserin and spiperone can each behave as simple competitive antagonists of 5-HT, but their expressed affinities change according to the bioassay used. The conventional interpretation of these results would be that they indicate subtypes of the general 5-HT_2-receptor class. This would mean that trazodone represents the most selective probe amongst the antagonists studied here. However, the chemical, structural relation between the antagonists and the natural agonist, 5-HT, is relevant to this interpretation. It has been suggested that antagonists may be more selective receptor probes than agonists due to their ability to interact with sites which are not utilized by the natural agonist (Ariëns *et al.*, 1979; Kenakin, 1984). If this argument is taken to extremes, compounds which bear little or no chemical relation to the natural agonist could be regarded as the most useful in receptor classification. Depending on the meaning attached to the term 'receptor' this may or may not be viewed as a rather perverse conclusion. Assuming that 'receptor' is used to define a cellular constituent whose function is to convert the chemical information in a hormone or transmitter into a physiological change, then the most appropriate evidence for distinguishing between receptor subtypes logically derives from the use of compounds related chemically to the natural agonist. Classification of receptors made on the basis of chemical interactions in which the natural agonist cannot participate appears to be illogical. For these reasons, in the present case, the lack of any obvious chemical resemblance between the antagonists used and 5-HT may mean that the results obtained with them are of limited relevance to the classification of the 5-HT receptors under study. By implication, these antagonists would be concluded to be unreliable probes of the 5-HT_2-like receptor. A more reliable antagonist probe may result from simple chemical alterations in the 5-HT molecule to eliminate efficacy but retain affinity. As Clancy & Maayani (1985) recently showed, this may be possible by N-alkyl substitutions. The analysis of potential 5-HT related antagonists of this kind is intended to be the subject of a further paper.

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