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Analysis of the 5-HT receptor in rabbit saphenous vein exemplifies the problems of using exclusion criteria for receptor classification

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Summary. 5-Hydroxytryptamine (5-HT) contracts ring preparations of rabbit saphenous vein via direct and indirect components, the latter being compatible with a "tyramine-like" action at sympathetic nerve terminals. Here an attempt was made to establish the identity of the receptor mediating contraction directly, in terms of the currently accepted proposals (Bradley et al. 1986).

Results with agonists suggested 5-HT_1-like receptor activation: methysergide behaved as a partial agonist with microcolar affinity and 5-HT effects were mimicked by 5-carboxamidotryptamine (5-CT) and GR43175. The agonist potency order was 5-CT > 5-HT > methysergide $\geq$ GR43175, the same as that reported at the 5-HT_1-like receptor in dog saphenous vein (Feniuk et al. 1985; Humphrey et al. 1988). Consistent with this, 5-HT effects were resistant to blockade by the selective 5-HT_3 receptor antagonist MDL72222 (1.0 μmol/l). In contrast, methiothepin (0.01–0.3 μmol/l), ketanserin (0.3–30.0 μmol/l) and spiperone (0.3–30.0 μmol/l) each produced surmountable antagonism which, although competitive in nature only for methiothepin (pK_B = 9.45 ± 0.09, 17 d.f.), implied 5-HT_2 receptor involvement. The possibility that these discrepancies resulted from mixed populations of 5-HT_1-like and 5-HT_2 receptors can be excluded because; 1). Ketanserin and spiperone blocked the actions of 5-HT and the selective 5-HT_1-like receptor agonist GR43175 with equal facility and 2). Responses to all of the agonists studied were similarly antagonised by flesinoxan (pK_B ~ 6.4), a simple competitive antagonist at the receptor in rabbit saphenous vein. This novel result with flesinoxan demonstrates that the ligand displays affinity at 5-HT receptors other than the 5-HT_{1A} subtype.

These data show that the 5-HT receptor in rabbit saphenous vein shares features in common with, and may be identical to, the 5-HT_1-like receptor in dog saphenous vein. However, unlike the latter it demonstrates qualities evident in both 5-HT_1-like and 5-HT_2 receptors and for this reason fails to meet the currently accepted criteria for admission into any of the recognised classes. It is suggested that this sort of problem reflects the generally unreliable behaviour of the available receptor antagonists and the emphasis which the Bradley et al. (1986) scheme places upon them for classification by exclusion. A complementary approach which provides a rigorous, quantitative basis for receptor differentiation uses "fingerprints" comprising affinity and relative efficacy estimates for a set of tryptamines. This study illustrates the power and economy of this approach by showing how affinity and relative efficacy "fingerprints" obtained using 5-HT, 5-CT, (±) α-methyl-5-HT, 5-methyltryptamine and N,N-dimethyltryptamine establish a positive identity for the 5-HT receptor in rabbit saphenous vein and at the same time enable it to be distinguished from other 5-HT receptor types presently allocated to the 5-HT_1-like, 5-HT_2 and so-called "orphan" receptor classes.

Key words: 5-HT receptors – Classification – Rabbit saphenous vein

Introduction

The operational classification of 5-HT receptors as 5-HT_1-like, 5-HT_2 or 5-HT_3 is presently made according to recommendations suggested by Bradley and colleagues in 1986. The widespread acceptance of these authors' proposals has usefully consolidated 5-HT receptor research but the significant advances stimulated by them means that the criteria by which 5-HT receptors are defined now need to be re-appraised (Humphrey and Richardson 1989).

For example, multiple subtypes of the 5-HT_1-like class have been identified, principally in the central nervous system, and these have been nominated 5-$HT_{1A, 1B, 1C}$ and $_{1D}$. The distinctions between these subtypes arose initially from radioligand binding studies using a comprehensive series of chemically diverse ligands which sub-

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sequently provided a positive correlation between binding affinities and estimates of potency made in various functional assays (e.g. Hoyer 1989). However, these techniques have not enabled the identity or otherwise of these receptors with the various 5-HT_1-like receptors in the periphery to be established. Hence, in the absence of selective, competitive receptor antagonists peripheral 5-HT_1-like receptors remain a heterogeneous and ill-defined class.

A second problem relates to the increasing number of reports which describe 5-HT receptor types that cannot be allocated to any of the currently recognized classes. The 5-HT receptor on vascular endothelium (Leff et al. 1987), the receptor subserving contraction of the rat stomach fundus (Clineschmidt et al. 1985; Cohen and Fludzinski 1987) and that mediating depolarisation of rat spinal motoneurones (Connell and Wallis 1988) are just three examples of such "orphan" receptor types. Indeed Dumuis and colleagues (1988) recently introduced the notation 5-HT_4 to describe one such "orphan" receptor type in the absence of any formal criteria for its classification. As pointed out by Clarke et al. (1989), unilateral designation of this sort hinder rather than help in establishing the minimum safe criteria for receptor classification.

In our view, this failure of the present scheme to readily accommodate novel 5-HT receptor types results from the nature of the classification criteria. These are essentially descriptive and emphasise the use of available antagonists for classification by exclusion (as with the 5-HT_1-like class). For a number or reasons we feel that this quality of information is inadequate for classification purposes. Firstly, it describes what the receptor is not and fails to provide a quantitative basis for its positive indentification. Secondly, it presumes the selectivity of the available antagonists, a dangerous presumption in view of the known tendency for some of these ligands to display affinities which vary at the same receptor type by as much as 2-orders of magnitude (Leff and Martin 1986; Mylecharane 1990). Finally, it emphasises the use of agents chemically remote from the endogenous receptor substrate 5-HT raising the possibility that receptor subtypes indentified in this way reflect differences in drug recognition sites. These would not necessarily correspond to distinctions perceived by 5-HT itself.

These considerations appear to pertain in the case of a 5-HT receptor mediating contraction of isolated rings of rabbit saphenous vein. Here we show that an attempt to classify this receptor using the criteria proposed by Bradley et al. (1986) left its classification equivocal because responses to selective agonists and antagonists suggested qualities evident in both 5-HT_1-like and 5-HT_2 receptors. Consequently a positive identity was established for the receptor in the form of "fingerprints" comprising affinity and efficacy estimates for a set of five tryptamines. This enabled at the same time a quantitative comparison with three other peripheral 5-HT receptor types shown previously to be distinguished by these hormone analogues (Martin et al. 1987; 1988). The results are discussed in terms of their implications for the differential classification of 5-HT receptors.

Methods

Right and left lateral saphenous veins were obtained from male New Zealand White rabbits (2.5–3.0 kg) killed by intravenous administration of pentobarbitone sodium (Sagatal, 60 mg/kg). Vessels were cannulated in situ using polypropylene tubing (o.d. = 1.0 mm), cleared of adhering connective tissue and cut into ring segments 3–4 mm in length. These were carefully transferred from the cannula onto two tungsten wire hooks (diameter 250 μm) using the method of Hooker et al. (1977).

Changes in tissue isometric force were recorded from vascular rings suspended in 20 ml organ baths containing Krebs solution of the following composition (mmol/l): 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.

Experimental protocols. Following a stabilisation period of 20–30 min a force of 2 g was applied to each tissue and subsequently re-applied twice at intervals of 15 min. During this 30 min period, tissues were exposed to pargyline (500 μmol/l) which irreversibly inhibited MAO. Since preliminary experiments indicated that 5-HT produced a "tyramine-like" effect in the rabbit saphenous vein (see results), tissues were exposed at the same time to phenoxybenzamine (0.3 μmol/l for 30 min). This treatment served the dual purpose of inactivating intraneuronal transport of 5-HT and also preventing the direct activation of post-junctional α-adrenoceptors (Iversen 1965; Apperley et al. 1976).

After washout of excess inhibitors tissues were challenged with a near maximally-effective concentration of 5-HT (1.0 μmol/l) in order to establish viability and to provide a reference contracture by which subsequent concentration-effect curves could be normalised.

Experiments with agonists. After recovery from the initial challenge with 5-HT, a cumulative concentration-effect curve was constructed, successive agonist concentrations increasing in 0.5 $\log_{10}$ units until the maximum effect was defined. Following washout of the agonist, tissues were incubated for 30 min with benextramine tetrahydrochloride (BHC: 1.0 μmol/l) or with vehicle (distilled water). At the end of this period unreacted BHC was removed by five exchanges of the organ bath Krebs solution over a period of 15 min. A second cumulative concentration-effect curve was then constructed. Only a single agonist was tested in any one tissue, therefore the number of replicates refers to the number of tissue preparations. Contractions were measured as increases in grams force.

Experiments with reversible antagonists. Tissues were exposed to reversible antagonists for a period of 60 min between successive concentration-effect curves. In each tissue, responses were normalised by scaling them to the response obtained with the initial 5-HT (1.0 μmol/l) challenge.

In experiments using methiothepin, only a single curve was obtained in each preparation following 120 min exposure to antagonist. With shorter incubation times (60 min), low concentrations of antagonist failed to achieve equilibrium with the receptor resulting in steep Schild regressions (data not shown).

Data analysis

Logistic curve fitting and the analysis of antagonism. Two types of experiment were performed; those in which only a single concentration-effect curve was obtained in each preparation and those in which two successive curves were obtained before and after a specific intervention. In both cases concentration-effect curve data were fitted to the following logistic function:

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

which provided estimates of α, $[A_{50}]$ and m, the asymptote, mid-point location and slope parameters respectively. Location parameters were actually estimated as negative logarithms ($p[A_{50}]$). Parameter estimates obtained for first and second curves (obtained either in the absence or presence of antagonist), were compared using a paired t-test. In antagonist experiments using a paired curve design a mid-point concentration-ratio ($\Delta p[A_{50}]$) was calculated from each curve pair as control $p[A_{50}]$ − test $p[A_{50}]$. Arithmetic mean values of $\Delta p[A_{50}]$ were calculated together with the standard error. When antagonists were studied using a single curve/preparation design a one-way analysis of variance compared computed estimates of curve slope and maximum within and between treatment groups. If the antagonist produced parallel curve displacements suggesting competitive antagonism, computed $p[A_{50}]$ values were fitted to the following form of the Schild equation (Trist and Leff 1985; Leff et al. 1986):

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

where $[A_{50}{}^{C}]$ is a control curve $[A_{50}]$ value, [B] is the concentration of antagonist, K_B its dissociation constant and n a value equivalent to the slope of the Schild regression. If n was not significantly different from unity it was constrained to this value in order to estimate K_B.

Operational model fitting. The concentration-effect curve data obtained with tryptamines were fitted directly to the operational model of agonism (Black and 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 defines the slope of the occupancy-effect relation.

Drugs

The following drugs were used (source in parentheses): pargyline hydrochloride (Sigma, St. Louis, MO, USA); phenoxybenzamine hydrochloride (dibenzyline: Smith, Kline and French, Welwyn Garden City, UK); 1αH,3α,5αH-tropan-3-yl-3,5-dichlorobenzoate methane sulphonate (MDL 72222; Merrell-Dow, Strasbourg, France); (+)p-fluoro-N-[2-[4-[2-(hydroxymethyl)-1, 4-benzodioxan-5-yl]-1-piperazinyl]ethyl] benzamide hydrochloride (flesinoxan: Duphar, Weesp, The Netherlands); spiperone (Janssen Pharmaceutica, Beerse, Belgium); ketanserin tartate (Janssen Pharmaceutica); methysergide bimaleate (Sandoz, Basel, Switzerland); prazosin hydrochloride (Pfizer Central Research, Sandwich, UK); idazoxan hydrochloride (Reckitt and Colman, Kingston-upon-Hull, UK); mepyramine maleate (May and Baker, Dagenham, UK); atropine sulphate (Sigma); 5-hydroxytryptamine creatinine sulphate (Sigma); 5-methyltryptamine hydrochloride (Sigma); cocaine hydrochloride (MacFarlan Smith, Edinburgh, UK). (±)-α-Methyl-5-hydroxytryptamine hydrogen maleate, 5-carboxamidotryptamine hydrochloride and GR43175 (3-[2-dimethylamino]ethyl-N-methyl-1H-indole-5 methane sulphonamide hydrochloride) were synthesised by Dr. A. D. Robertson, Medicinal Chemistry Department, Wellcome Research Laboratories, Kent, UK.

Phenoxybenzamine was dissolved and diluted in absolute ethanol. Spiperone was dissolved in dimethylsulphoxide and diluted in distilled water. The final concentration of vehicle in the organ bath ($\leq 0.01\%$ v/v) did not affect tissue responsiveness. All other drugs were dissolved and diluted in distilled water.

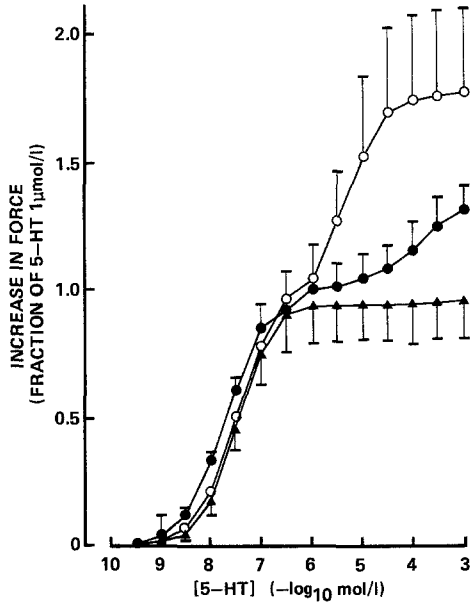
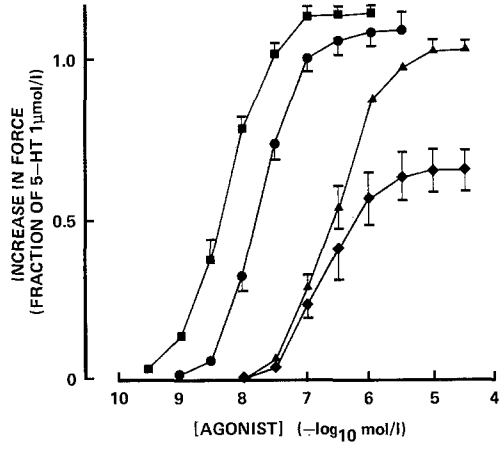


Fig. 1. Concentration-effect curves to 5-HT obtained in rings of rabbit saphenous vein. Responses were obtained in untreated tissues (○), in the presence of cocaine (30 μmol/l: ●) or following treatment with phenoxybenzamine (0.3 μmol/l for 30 min; ▲). In each case data are the averages of 6 concentration-effect curves. Vertical bars how SEM. *Ordinate*: Increase in tissue force, expressed as a fraction of the initial challenge with 5-HT (1 μmol/l). *Abscissa*: $-\log_{10}$ molar concentration of 5-HT



Agonist	p[A_{50}]	Rel. potency	Max.	Flesinoxan pK_B
5-HT	7.75 ± 0.05	1.0	1.10 ± 0.05	6.44 ± 0.11
5-CT	8.28 ± 0.06	0.3	1.16 ± 0.02	6.56 ± 0.06
GR43175	6.60 ± 0.07	14.1	1.04 ± 0.03	6.51 ± 0.08
Methysergide	6.68 ± 0.10	11.7	0.68 ± 0.08	6.43 ± 0.14

Fig. 2. Increases in tissue force produced by 5-HT (●, $n = 6$), 5-CT (■, $n = 5$), GR43175 (▲, $n = 6$) and methysergide (◆, $n = 6$) in rings of rabbit saphenous vein. Vertical bars show SEM. *Ordinate*: Increase in tissue force expressed as a fraction of the initial challenge with 5-HT (1 μmol/l). *Abscissa*: $-\log_{10}$ molar concentration of agonist. The Table shows that average (± SEM) computed estimates of the mid-point location (p[A_{50}]), the maximum effect obtained for each agonist and mean estmates of pK_B (± SEM, $n = 3-4$) obtained when flesinoxan (10 μmol/l) was used as an antagonist

Results

5-HT contractions of rabbit saphenous vein

In rings of rabbit saphenous vein treated only with pargyline, 5-HT (1 nmol/l – 1 mmol/l) produced a biphasic concentration-effect curve with a clear inflection at 0.3 – 1.0 μmol/l (Fig. 1). Over the first phase of the curve 5-HT responses quickly attaind a steady state (3 – 5 min) whereas at high concentrations the contractions were of a different quality, developing more slowly. As shown in Fig. 1, cocaine (30 μmol/l) attenuated the second phase of contraction but without significantly modifying the first phase. Similar results were obtained in the presence of the α_1-adrenoceptor antagonist prazosin (0.3 μmol/l: data not shown). These results indicated that the second phase of contraction might be indirect and result from the sympathetic neuronal uptake of 5-HT followed by displacement of noradrenaline. In order to eliminate this complexity, tissues used in subsequent experiments were also treated with phenoxybenzamine as described in the methods. Following this treatment 5-HT produced only a monophasic concentration-effect curve as illustrated in Fig. 1.

5-HT receptor characteristics in rabbit saphenous vein

Studies with agonists. Figure 2 shows that, like 5-HT, the selective 5-HT_1-like receptor agonist GR43175 produced concentration-dependent contractions of the rabbit saphenous vein, but with lower potency. 5-CT, in contrast, mimicked the effect of 5-HT with higher potency. Although methysergide is regarded conventionally as an antagonist of 5-HT receptors, in these experiments it behaved as a partial agonist, lower in intrinsic activity than any of the simple tryptamine analogues studied. The agonist potency order was: 5-CT > 5-HT > methysergide ≥ GR43175 and maximum effects relative to 5-HT were: 5-HT = 5-CT = GR43175 > methysergide. Computed estimates of the midpoint location ($p[A_{50}]$) and maximum for each agonist are summarised in the Table shown in Fig. 2. The Table also shows that when interacted with flesinoxan (10 μmol/l), a simple competitive antagonist in this tissue (see below), each agonist produced essentially the same estimate of pK_B consistent with the activation of a single population of 5-HT receptors.

Studies with antagonists. 5-HT contractions of saphenous vein rings were unaffected by mepyramine (0.3 μmol/l), atropine (0.3 μmol/l), prazosin (0.3 μmol/l) or idazoxan

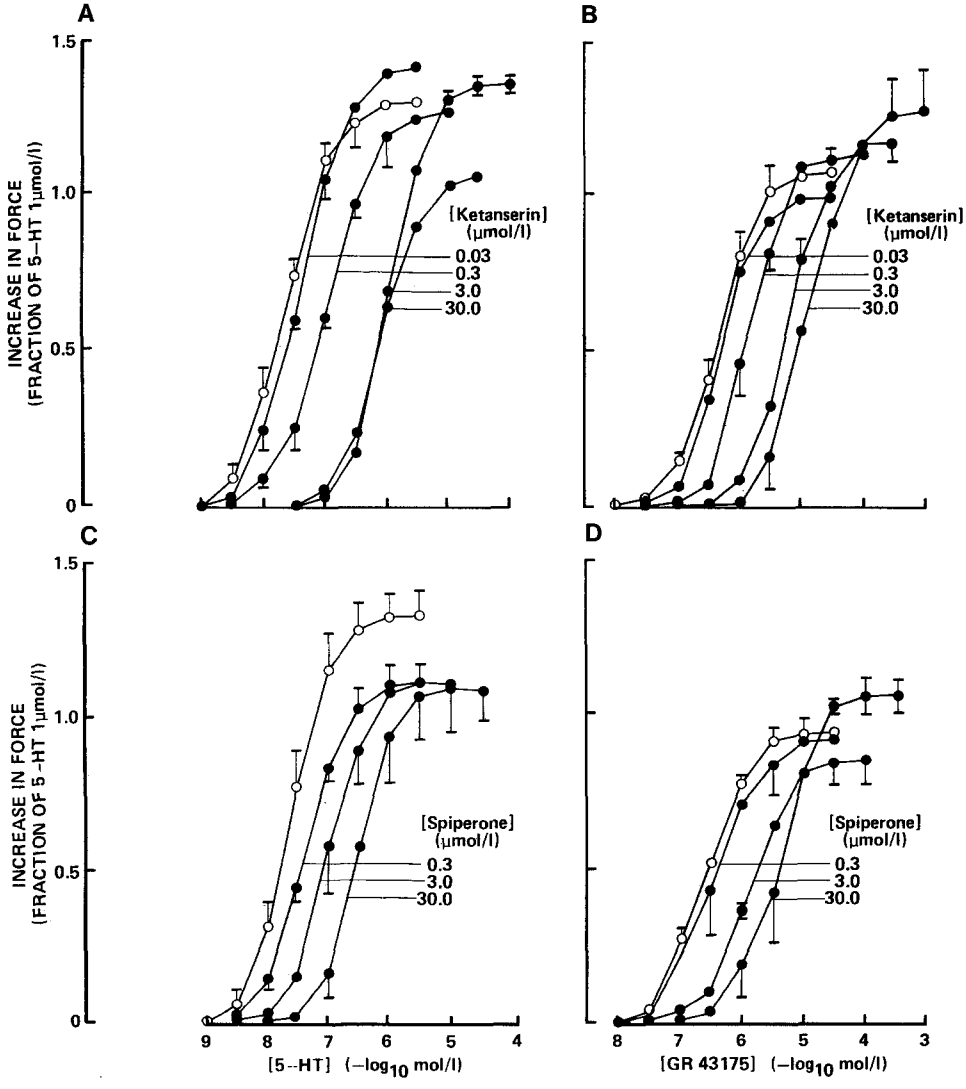


Fig. 3. Antagonistm by ketanserin (*panels* **A** + **B**) and spiperone (*panels* **C** + **D**) of contractions produced by 5-HT (*left hand panels*) and GR43175 (*right hand panels*) in rings of rabbit saphenous vein. *Open symbols* denote control concentration-effect curves, *closed symbols* the curves obtained in the presence of antagonist at the concentrations shown. In each case data are the averages of 3 – 4 replicate concentration-effect curves. *Vertical bars* show SEM. *Ordinate*: Increase in tissue force expressed as a fraction of the initial challenge with 5-HT (1 μmol/l) *Abscissa*: $-\log_{10}$ molar concentration of agonist

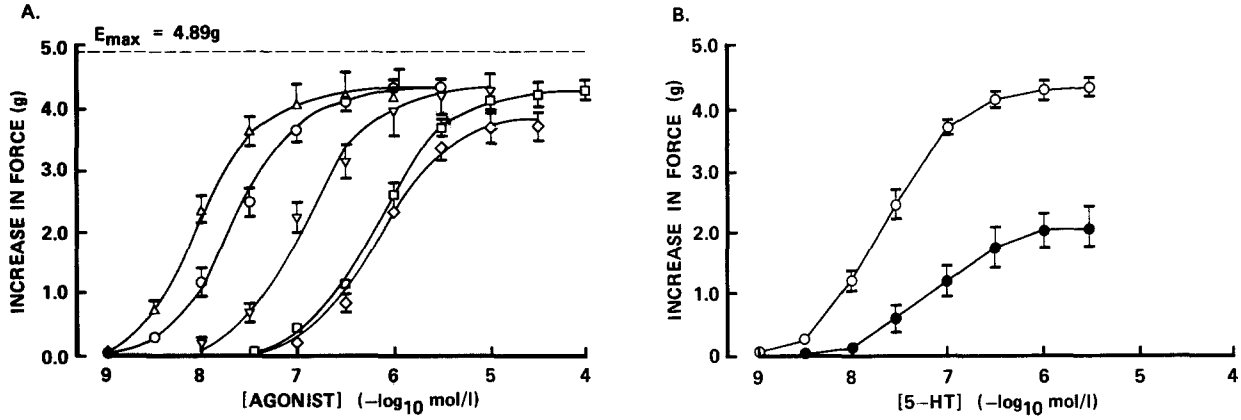


Fig. 4. *Panel* **A** shows increases in grams force produced by 5-HT (○), 5-MeT (▽), 5-CT (△), α-Me-5HT (□) and N,N-DMT (◇) in rings of rabbit saphenous vein. *Panel* **B** illustrates concentration-effect curves to 5-HT only obtained before (○) and after (●) irreversible receptor occlusion using BHC (1.0 μmol/l for 30 min). Data are the average of replicate concentration-effect curves. *Ordinate*: Increase in tissue isometric force in grams. *Abscissa*: $-\log_{10}$ molar concentration of agonist. The *lines* through the data were produced by fitting them to the operational model (equation 3)

Table 1. Tryptamine affinity (pK_A) and efficacy (τ) "fingerprints" obtained for the 5-HT receptors in rabbit saphenous vein (RbSV), rabbit aorta (RbA) and rabbit jugular vein with (RbJV + E) and without (RbJV-E) endothelium

Assay	Receptor type		Affinity order								
RbSV	5-HT_1-like?	pK_A	5-CT	>	5-HT	>	5-MeT	>	N,N-DMT	>	α-Me-5-HT
		τ	7.53		7.12		6.43		5.82		5.68
			0.98		1.00		1.00		0.65		0.93
			5-HT	>	α-Me-5-HT	>	5-MeT	>	N,N-DMT	>	5-CT
RbA*	5-HT_2	pK_A	6.92		6.59		6.28		6.09		5.90
		τ	1.00		0.79		0.59		0.18		0.35
			5-HT	>	α-Me-5-HT	>	5-CT	>	5-Met	>	N,N-DMT
RbJV + E†	"orphan"	pK_A	8.36		8.14		7.51		7.17		6.57
		τ	1.00		1.04		0.95		1.14		0.50
			5-CT	>	5-HT	>	N,N-DMT	>	5-MeT	>	α-Me-5-HT
RbJV-E†	5-HT_1-like	pK_A	6.66		6.20		5.94#		5.67		< 4.50
		τ	4.70		1.00		< 0.10		0.53		–

pK_B by Schild analysis * from Leff and Martin 1986, 1988 † from Martin et al. 1987; 1988 KEY: 5-CT = 5-carboxamidotryptamine; 5-MeT = 5-methyltryptamine; N,N-DMT = N,N-dimethyltryptamine; α-Me-5-HT = (±) α-methyl-5-HT

(1.0 μmol/l) eliminating the possible involvement of histamine H_1 receptors, muscarinic receptors or either α_1- or α_2-adrenoceptors. Responses were likewise resistant to blockade by the selective 5-HT_3 receptor antagonist MDL72222 (1.0 μmol/l). In contrast, when the tissues were exposed for 120 min to the non-selective antagonist methiothepin (0.003 μmol/l – 0.30 μmol/l) simple competitive antagonism was obtained. The slope of the Schild regression (1.07 ± 0.07, 18 d.f.) was not different from unity and when constrained to one, gave an estimated pK_B of 9.45 ± 0.09 (17 d.f.).

Surprisingly, ketanserin (0.3 μmol/l) also produced a small but significant ($p < 0.05$) parallel rightward displacement of the 5-HT curve ($\Delta p[A_{50}] = 0.44 \pm 0.07$, $n = 4$). Further analysis of this interaction (Fig. 3) showed that although antagonism was surmountable, it was non-competitive in nature since the fractional increase in agonist concentration required to maintain the effect was antagonist concentration-independent (Fig. 3A). Moreover the antagonism was agonist-independent, similar results being obtained when the agonist was GR43175. (Fig. 3B). Panels C and D of Fig. 3 show that ketanserin was not alone in producing these effects; the butyrophenone spiperone also produced surmountable non-competitive blockade of both 5-HT and GR43175-induced contractions with equal facility.

Tryptamine fingerprinting

Figure 4A shows the increases in tissues isometric force produced by 5-HT, 5-CT, α-methyl-5-HT(α-Me-5-HT), 5-methyltryptamine (5-MeT) and N,N-dimethyltryptamine (N,N-DMT) in rings of rabbit saphenous vein. As was the case for 5-HT and 5-CT (see Fig. 2), flesinoxan (10 μmol/l) produced similar antagonism of effects obtained with α-me-5-HT ($pK_B = 6.27 \pm 0.07$, $n = 4$), 5-MeT ($pK_B = 6.52 \pm 0.05$, $n = 3$) and N,N-DMT ($pK_B = 6.64 \pm 0.05$, $n = 4$), again supporting an action at a single

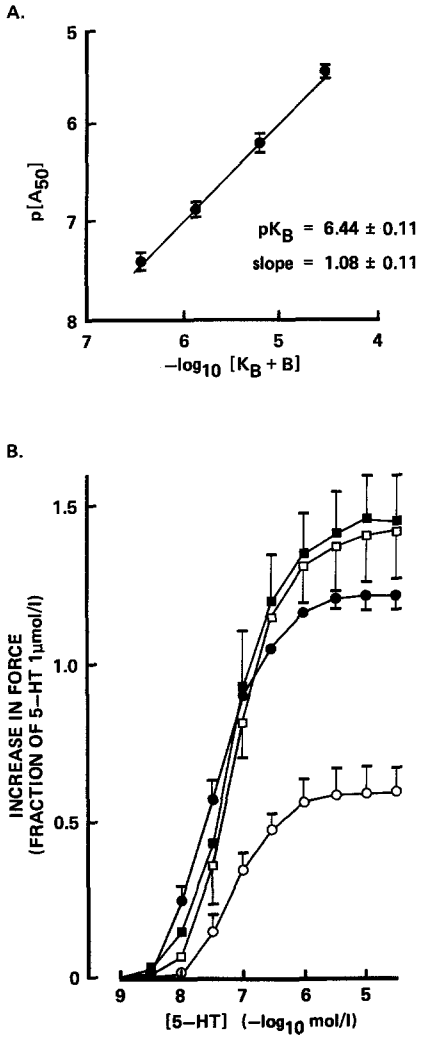


Fig. 5. Effect of the reversible, competitive antagonist flesinoxan on 5-HT receptor inactivation by BHC. *Panel* **A** illustrates the antagonism of 5-HT by flesinoxan in the form of a Clark plot. Identity of the data with a unit slope line drawn through them is consistent with simple competitive antagonism. *Panel* **B** shows the results obtained when rings of rabbit saphenous vein were exposed for 30 min to; BHC alone (1.0 μmol/l, ○), flesinoxan alone (30 μmol/l, ■), BHC + flesinoxan (□), or drug vehicle (●) followed by washout of the treatment. Each point is the average of 4 replicate concentration-effect curves. Vertical bars show SEM. *Panel* **B** *ordinate*: Increase in tissue force expressed as a fraction of the initial challenge with 5-HT (1 μmol/l). *Abscissa*: $-\log_{10}$ molar concentration of 5-HT

5-HT receptor type. In order to obtain affinity (pK_A) and efficacy (τ) estimates for each agonist, concentration-effect curves were obtained before and after fractional, irreversible receptor occlusion using BHC (1.0 μmol/l for 30 min) according to the method of Furchgott (1966). Without exception this treatment resulted in an immediate decrease in agonist curve maximum with only a small rightward displacement in the location. This is illustrated in Fig. 4B for 5-HT only, although essentially similar results were obtained with the other agonists. Pre- and post-inactivation concentration-effect curve data were fitted simultaneously to the operational model (equation 3) which produced the affinity and efficacy estimates given in Table 1 and these were used to generate the lines shown through the data in Fig. 4. The estimated values for Emax and n were 4.89 g and 1.51 respectively. As indicated in Fig. 4 it is evident from this analysis that in this receptor system 5-HT and the other tryptamines behave as partial agonists. Table 1 also shows for comparison the affinity and efficacy "fingerprints" that have been reported previously for three other types of peripheral 5-HT receptor.

Protection against receptor inactivation by BHC was achieved using flesinoxan. This ligand behaves as an agonist at the putative 5-HT_{1A} receptor (Wouters et al. 1988; Schoeffter and Hoyer 1988) but at the receptor in the rabbit saphenous vein flesinoxan behaved as a simple competitive antagonist of 5-HT. Analysis of the data using equation 2 yielded a Schild plot slope of 1.08 ± 0.11 (9 d.f.) and an equilibrium dissociation constant (pK_B) of 6.44 ± 0.11 (10 d.f.). The results are illustrated in the form of a Clark plot in Fig. 5A. Figure 5B shows that flesinoxan (30 μmol/l) afforded complete protection against the effects of BHC (1.0 μmol/l) indicating that the latter acts only to reduce the functional 5-HT receptor concentration and has no post-receptor actions.

Discussion

The finding in our laboratory that 5-HT contracts isolated rings of rabbit saphenous vein prompted the present study to establish the identity of the receptor type involved. Preliminary experiments showed that the effects of 5-HT in this tissue are complex comprising both direct and indirect components. Only low concentrations (0.001 − 1.0 μmol/l) of the amine produced smooth muscle contraction directly; at higher concentrations (3.0 − 1000 μmol/l) contraction was mediated indirectly, presumably by stoichiometric displacement of noradrenaline from sympathetic nerve terminals. This assumption of a "tyramine-like" action for 5-HT is based upon the observation that the second, but not the first phase of contraction was sensitive to blockade by the α_1-adrenoceptor antagonist prazosin as well as cocaine, a well-established inhibitor of sympathetic neuronal Uptake_1 (Iversen 1965). The ability of 5-HT to access sympathetic neuronal stores of noradrenaline via this transport process is not without precedent (Jaim-Etcheverry and Zieher 1969; Thoa et al. 1969) and has been shown to account for indirectly mediated effects of 5-HT in a variety of isolated intact tissue preparations (e.g. Trendelenburg 1960; Pluchino 1972; Humphrey 1978).

Treatment of saphenous vein rings with phenoxybenzamine abolished the direct and indirect actions of 5-HT at α-adrenoceptors and enabled an unconfounded study of the 5-HT receptor mediating smooth muscle contraction. Evidence that agonist interactions occurred at a single homogeneous population of 5-HT receptors was provided by competition studies using flesinoxan as an antagonist. In this regard flesinoxan served as a useful probe of the 5-HT receptor in the rabbit saphenous vein and, as far as we are aware, this novel finding is the first demonstration that it displays affinity at a 5-HT receptor

other than the 5-HT_{1A} subtype (Wouters et al. 1988). Interestingly, we have also found that it binds to the 5-HT_1-like receptor in dog saphenous vein, but with lower affinity ($pK_B = 5.75 \pm 0.19$, 8 d.f.).

The failure of either phenoxybenzamine or cocaine to attenuate 5-HT responses mediated by this receptor was intriguing since, historically, this would have excluded a "D" or an "M" classification (Gaddum and Picarelli 1957). This encouraged our attempts to establish its classification using the recommendations of the currently accepted scheme (Bradley et al. 1986). In this "D" and "M" receptors are broadly, but not exclusively, encompassed by the 5-HT_2 and 5-HT_3 classes at which ketanserin and MDL72222 respectively are regarded as archetypal selective receptor antagonists. According to Bradley et al. (1986) the characteristics of a 5-HT_2 receptor include a susceptibility to antagonism by ketanserin and a resistance to blockade by MDL72222, whilst the converse selectivity defines, in part, a 5-HT_3 receptor. Resistance to blockade by both types of antagonist is taken as evidence for a 5-HT_1-like receptor, since it implies, by exclusion, that the receptor type involved is neither 5-HT_2 nor 5-HT_3.

Applying these criteria to the 5-HT receptor mediating contraction of the rabbit saphenous vein suggested that it could not be classified as 5-HT_3 because 5-HT contractions were not blocked by MDL72222. Consistent with this, the selective 5-HT_3 receptor agonist 2-methyl-5-HT was at least 100-fold lower in potency ($pK_A = 4.74$) than 5-HT at this receptor (unpublished observation). However, responses were susceptible to antagonism by micromolar or lower concentrations of methiothepin, ketanserin and spiperone, whereas methysergide behaved as a partial agonist with micromolar affinity. This profile does not meet the presently accepted criteria for either a 5-HT_1-like or a 5-HT_2 receptor, but suggests instead qualities evident in both receptor types. On the one hand a 5-HT_1-like classification is endorsed by the observed agonism with GR43175 (Humphrey et al. 1988), the potent antagonism caused by methiothepin and the partial agonist behaviour of methysergide (Apperley et al. 1980); on the other, this is negated by the susceptibility of agonist responses to blockade by ketanserin and spiperone, a criterion which presently defines 5-HT_2 receptors. Admittedly, the antagonist potencies of ketanserin and spiperone were lower than expected from their respective affinities at the 5-HT_2 receptor in rabbit aorta (Humphrey et al. 1982; Leff and Martin 1986) but the difference might simply reflect the variable affinities that these compounds demonstrate at the 5-HT_2 receptor. We (Leff and Martin 1986) and others (Mylecharane 1990) have shown that affinity estimates obtained for a variety of 5-HT_2 receptor antagonists can vary by more than 2-orders of magnitude depending upon the tissue type studied. This means that in principle the affinities expressed by these ligands might cover a limitless range rendering them useless in the positive identification of different 5-HT receptor types. By the same token they cannot be relied upon to provide useful data even for classification by exclusion.

For these reasons, we have investigated an approach to 5-HT receptor classification in which "fingerprints" comprising affinity and, in the case of agonists, efficacy estimates are determined for a limited set of tryptamines. Recent studies (Leff et al. 1987; Leff and Martin 1988; Martin et al. 1987, 1988, 1990; Martin and Sparrowhawk 1989) have shown that this approach provides a sound, quantitative basis for the differential classification of 5-HT receptors and enables different sub-types to be identified positively. At the same time it minimises the risk of simply classifying drug recognition sites. Here, estimates of agonist affinity and efficacy were obtained by model-fitting concentration-effect curve data obtained before and after partial, irreversible receptor occlusion using BHC. The affinity order 5-CT > 5-HT > 5-MeT > N,N-DMT > α-Me-5-HT clearly distinguishes the receptors in rabbit saphenous vein from either the 5-HT_2 receptor in rabbit aorta or the 5-HT receptor mediating endothelium dependent relaxation. At these, α-Me-5-HT demonstrates only a slightly lower affinity than 5-HT itself, whereas the affinity of 5-CT is lower by at least an order of magnitude.

Neither is the receptor the same as the 5-HT_1-like receptor mediating vascular relaxation directly, since not only are the affinities demonstrated by N,N-DMT and 5-MeT reversed at these two sites, the relative efficacies of the agonists studied are also completely different. Interestingly, in the rabbit saphenous vein only N,N-DMT was lower in efficacy than 5-HT, suggesting that, as at the other 5-HT receptor types, alkylation of the ethylamine nitrogen influences the intrinsic efficacy of simple tryptamine analogues at this receptor. It is also worth noting that in each of these 5-HT receptor bioassays, the natural agonist 5-HT behaves as a partial agonist attaining only 75–90% of the maximum possible effect in each system (see also Maayani et al. 1984; Leff et al. 1986; Martin et al. 1987). If this observation represents a general phenomenon which is preserved in integrated tissue systems, then local changes in receptor density and/or efficiency of occupancy-effect coupling would provide a very efficient means of regulating 5-HT receptor function.

The use of tryptamine agonists to provide a positive identity for the 5-HT receptor mediating contraction of the rabbit saphenous vein enabled it to be differentiated quantitatively from a variety of other 5-HT receptors currently nominated 5-HT_1-like, 5-HT_2 or "orphan". The question therefore arises, to which class does the rabbit saphenous vein receptor belong? In our view, the most parsimonious interpretation of the data shown here is that the receptor shares a common identity with the 5-HT_1-like receptor in the saphenous vein of the dog (eg the high potency of 5-CT relative to 5-HT, the activity of GR43175 and the partial agonist behaviour of methysergide) (Apperley et al. 1980; Feniuk et al. 1985; Humphrey et al. 1988). This is further supported by unpublished experiments performed in this laboratory which show that the tryptamines studied here also contract rings of dog saphenous vein with a rank order of potency identical to the affinity ranking obtained in the rabbit saphenous vein. If, as in other 5-HT receptor sys-

tems, these tryptamines behave as partial agonists in dog saphenous vein, then these relative potencies and intrinsic activities should mirror the affinity and efficacy orders suggesting that simple hormone analogues cannot differentiate the receptors in the dog and rabbit saphenous veins. Only the results obtained with antagonists apparently distinguish between them. In contrast to the present results, 5-HT contractions of dog saphenous vein are not blocked by micromolar concentrations of either ketanserin or spiperone (Feniuk et al. 1985; unpublished observations). Differences are also evident with methiothepin; although the compound produces potent antagonism of 5-HT contractions in both types of tissue, it demonstrates at least a 20-fold higher potency in the rabbit than in the dog vessels (Apperley and Humphrey 1986 c.f. this study). Finally, the receptor in the rabbit, but not the dog saphenous vein, is irreversibly occluded with BHC (unpublished observation). In our view, such distinctions between receptors are symptomatic of ligands which bear little or no chemical relation to the natural receptor agonist, 5-HT. We (Leff and Martin 1986; Leff et al. 1986) have argued previously that the available antagonists might recognise poorly conserved sites accessory to those utilised by 5-HT and consequently provide misleading information for hormone receptor classification. The ability of antagonists to distinguish the receptors in dog and rabbit saphenous vein, when the agonists used fail to do so, might be explained in these terms.

The results obtained from the present experiments support further our contention that hormone-unrelated antagonists are unreliable probes for 5-HT receptor classification. Previous studies (Leff and Martin 1986; Leff et al. 1986; Mylecharane 1990) have shown that the affinities of available 5-HT_2 receptor antagonists vary depending upon the tissue type studied meaning that even under the best circumstances these ligands cannot be relied upon to positively identify 5-HT receptor subtypes. This study suggests that neither can they be used reliably for exclusion purposes since their selectivity for a particular receptor class cannot be guaranteed. Here, an approach using agonist and antagonist analogues of 5-HT to provide affinity and efficacy "fingerprints" appears to resolve these problems allowing different 5-HT receptors presently labelled 5-HT_1-like or "orphan" to be defined positively and providing, at the same time, a quantitative basis for their differential classification. Importantly the pitfalls inherent in the use of exclusion criteria as presently advocated are avoided in this way, emphasising that information obtained with tryptamine agonists and antagonists can usefully complement and extend the current scheme for identifying and classifying receptors for 5-HT.

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