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Vascular 5-HT_1-like receptors that mediate contraction of the dog isolated saphenous vein and carotid arterial vasoconstriction in anaesthetized dogs are not of the 5-HT_{1A} or 5-HT_{1D} subtype

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1 There is controversy about whether 5-HT_{1A} receptors mediate contraction of isolated cerebral blood vessels. We have therefore compared the vascular actions of the 5-HT_{1A} receptor agonist, 8-hydroxy-2-(di-n-propyl-amino)-tetralin (8-OH-DPAT) with those of the 5-HT_1-like receptor agonist, sumatriptan, on the dog isolated saphenous vein, which contains a 5-HT_1-like receptor similar to those on cerebral blood vessels, and in the carotid circulation of the anaesthetized dog.

2 5-Hydroxytryptamine (5-HT), sumatriptan and 8-OH-DPAT each caused contraction of dog isolated saphenous vein with a rank order of agonist potency of 5-HT > sumatriptan > 8-OH-DPAT and EC_{50} values (95% confidence limits) of 0.06 (0.04–0.08), 0.3 (0.1–0.8) and 3.9 (2.0–7.5) μM respectively. The maximum contractile effect produced by each agonist was similar.

3 The contractile effects of 5-HT, sumatriptan and 8-OH-DPAT in the dog isolated saphenous vein were resistant to antagonism by the 5-HT_{1A} receptor antagonists spiperone, spiroxatrine and pindolol (all 1 μM). The 5-HT_{1D} receptor ligands, metergoline (0.1 μM) rauwolscine (1 μM) and yohimbine (1 μM) had little or no antagonist activity. In contrast, the non-selective 5-HT_1-like receptor blocking drug, methiothepin (0.03–0.3 μM) potently antagonized the contractile effects of 5-HT, sumatriptan and 8-OH-DPAT to a similar degree, suggesting that all three agonists act at the same receptor.

4 In ganglion-blocked, anaesthetized dogs, intra-carotid administration of 8-OH-DPAT (0.3–3 $\mu g\,kg^{-1}$) and sumatriptan (0.1–1 $\mu g\,kg^{-1}$), caused dose-dependent carotid arterial vasoconstriction. The two agonists were approximately equipotent in this respect.

5 The carotid arterial vasoconstrictor actions of 8-OH-DPAT and sumatriptan were not modified by spiperone (1 $mg\,kg^{-1}$, i.v.) but were antagonized to a similar extent by the subsequent administration of methiothepin (1 $mg\,kg^{-1}$, i.v.).

6 These results suggest that 8-OH-DPAT contracts the dog isolated saphenous vein and constricts the carotid arterial circulation of anaesthetized dogs by activation of 5-HT_1-like receptors which are not of the 5-HT_{1A} subtype, nor, on the basis of data with metergoline in the dog isolated saphenous vein, of the 5-HT_{1D} subtype. The receptor involved in these actions appears to be the same as that mediating the vasoconstrictor effects of sumatriptan. This receptor does not appear to be like any known 5-HT_1 ligand binding site; hence the current description, 5-HT_1-like, remains the most appropriate.

Introduction

The existence of 5-HT_1 receptor subtypes has been proposed based on the subdivision of the 5-HT_1 binding site (Pedigo *et al.*, 1981; Pazos & Palacios, 1985; Heuring & Peroutka, 1987). However, the lack of sufficiently selective compounds to discriminate between the different 5-HT_1 recognition sites has often limited their correlation to functional subtypes of the 5-HT_1-like receptor group (Bradley *et al.*, 1986; Humphrey & Richardson, 1989). Functional 5-HT_1 and 5-HT_1-like receptor subtypes have been largely characterized by use of selective agonists. One such agonist is 8-hydroxy-2-(di-n-propyl-amino)-tetralin (8-OH-DPAT) which has both high affinity and selectivity for the 5-HT_{1A} recognition site compared to other 5-HT_1 recognition sites (Middlemiss & Fozard, 1983; Hoyer *et al.*, 1985; Heuring & Peroutka, 1987).

Amongst its many pharmacological actions, 8-OH-DPAT causes contraction of dog basilar artery by a mechanism which has been proposed to involve activation of 5-HT_{1A} receptors (Taylor *et al.*, 1986; Peroutka *et al.*, 1986). However, this interpretation is controversial since the potent and highly selective 5-HT_1-like receptor agonist, sumatriptan GR43175, causes contraction of dog isolated saphenous vein (Humphrey *et al.*, 1988) as well as primate, dog and human basilar artery (Connor *et al.*, 1989; Parsons & Whalley, 1989) by a receptor which is not of the 5-HT_{1A} type but has been termed 5-HT_1-like. Studies *in vivo* have shown that sumatriptan causes selective vasoconstriction within the carotid artery bed of anaesthetized dogs by activation of similar 5-HT_1-like receptors, with the effect being specifically antagonized by methiothepin (Feniuk *et al.*, 1989). The carotid arterial vasoconstrictor action of sumatriptan, like that recently described for 8-OH-DPAT, appears to be largely restricted to an action on carotid arteriovenous anastomoses (Perren *et al.*, 1989a; Bom *et al.*, 1989). The precise 5-HT_1-like receptor subtypes involved in these vasoconstrictor actions of sumatriptan and 8-OH-DPAT have therefore been investigated further.

A preliminary account of these findings has been presented to the British Pharmacological Society (Perren *et al.*, 1989b).

Methods

Dog isolated saphenous vein preparation

Saphenous veins were removed from anaesthetized beagle dogs, spirally cut into strips and suspended in modified Krebs solution (Apperley *et al.*, 1976) bubbled with 95% O_2, 5% CO_2 at 37°C under an initial resting tension of 0.5 g. Isometric tension changes were recorded with Dynamometer UF1 2 oz strain gauges. Preparations were allowed to equilibrate for at least 1 h and then the contractile response to a submaximal concentration of potassium chloride (30 mM) was determined.

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Preparations were then allowed to equilibrate for a further period of at least 30 min following washout of potassium chloride.

Determination of agonist potency

These experiments were conducted in the presence of atropine (1 μM), ketanserin (1 μM) and mepyramine (1 μM) to exclude any possible effects of the agonists at muscarinic cholinoceptors, 5-HT_2 receptors and α_1-adrenoceptors and histamine H_1 receptors, respectively.

Cumulative concentration-effect curves to 5-HT (10 nM–1 μM) were obtained on all preparations. 5-HT was then washed from the bath over a period of 30 min and tissues were then left for a further 30 min before re-challenging with either 5-HT (control preparation), or test agonist (10 nM–5 μM or 0.1–200 μM for sumatriptan and 8-OH-DPAT, respectively). The control preparation was used to monitor any changes in sensitivity to 5-HT.

Relative potencies were determined by dividing the EC_{50} (molar concentration required to produce 50% maximum contractile effect) for the test compound by the EC_{50} value for 5-HT in the same preparation. This value was then corrected for spontaneous change in sensitivity to 5-HT by dividing it by the ratio of the EC_{50} values for 5-HT in the control preparation. Maximum contractile responses to the test agonists were compared with the maximum contractile response to 5-HT in the same tissue and corrected for time-related changes by dividing by the ratio of the relative maximum responses to 5-HT in the control preparation.

Antagonist studies in the dog isolated saphenous vein

These experiments were conducted in the presence of ketanserin (1 μM) to negate any actions of the agonists at 5-HT_2 receptors or α_1-adrenoceptors. Agonist cumulative concentration-effect curves were obtained in each of four saphenous vein preparations from the same vessel. Concentration-effect curves were then repeated after 30 min incubation with an antagonist on three of the preparations, whilst a fourth preparation was again challenged with test agonist in the absence of antagonist, and therefore acted as a control.

Agonist concentration-ratios were determined by comparing the EC_{50} values in the absence and presence of antagonist, with corrections being made for spontaneous changes in agonist sensitivity in the control preparation as described above. Agonist sensitivity in the control preparation varied by less than two fold during each experiment.

When more than one concentration of a particular antagonist was examined, the results were analysed according to the method of Arunlakshana & Schild (1959) and pA_2 values and slopes of the regression determined.

Anaesthetized dog

Beagle dogs (7.4–9.7 kg) of either sex were anaesthetized with barbitone (300 mg kg^{-1}, i.p.) following induction with thiopentone (25 mg kg^{-1}, i.v.) and pentobarbitone (60 mg, i.v.) and artificially respired with room air at a rate of 20 strokes min^{-1} and a stroke volume of 13–16 ml kg^{-1}, adjusted to maintain arterial pH, $Paco_2$ and Pao_2 within normal physiological limits. Body temperature was maintained at 39–40°C. Arterial blood pressure and right common carotid artery blood flow were recorded as described previously (Feniuk *et al.*, 1989). In addition, the right cranial thyroid artery was cannulated retrogradely for the intra-carotid administration of sumatriptan or 8-OH-DPAT. In order to exclude any effects of the agonists mediated by the autonomic nervous system, dogs were treated with mecamylamine (5 mg kg^{-1}, i.v.) and atropine (0.5 mg kg^{-1}, i.v.) administered in small doses over approximately 30 min and then allowed a further 30 min to equilibrate. Bolus intra-carotid doses of sumatriptan (0.1–1.0 μg kg^{-1}) or 8-OH-DPAT (0.1–3.0 μg kg^{-1}) were then administered in volumes of less than 0.1 ml injected into the common carotid artery with 0.25 ml 0.9% saline solution. The interval between each dose was at least 15 min such that full recovery of each response occurred before subsequent doses were administered. In view of our previous finding, that the carotid arterial vasoconstrictor action of intravenously administered sumatriptan is long lasting (Feniuk *et al.*, 1989), carotid arterial vasoconstrictor responses produced by intra-arterial administration were studied over a narrow submaximal dose-range and reproducibility of the vasoconstrictor action of sumatriptan was assessed by repeating an intermediate dose. Reproducibility of the vasoconstrictor responses produced by 8-OH-DPAT was assessed by repeating the full dose-effect curve. Agonist dose-effect curves were then repeated 15 min after spiperone (1 mg kg^{-1}, i.v.) and subsequently, 15 min after methiothepin (1 mg kg^{-1}, i.v.).

Changes in carotid arterial vascular resistance were determined from the resting value immediately prior to each dose of agonist. Dose-ratios were calculated from the linear portion of the agonist dose-effect curves.

Statistics

Unless otherwise stated, values are the arithmetic means ± s.e.mean or geometric means with 95% confidence limits in parentheses from n observations.

The pA_2 values and slopes of the Schild regression obtained for methiothepin against 5-HT-, sumatriptan- and 8-OH-DPAT-induced contractions of dog isolated saphenous vein were compared by an unpaired Student's t test. Differences were considered significant when the P value was <0.05.

Drugs used

The following compounds were purchased: atropine sulphate (Sigma), 8-OH-DPAT (8-hydroxy-2-[di-n-propylamino]-tetralin) hydrogen bromide (RBI), 5-HT (5-hydroxytryptamine) creatinine sulphate (Sigma), mepyramine maleate (May and Baker), metergoline (Farmitalia), (±)-pindolol (Sigma), rauwolscine hydrochloride (Carl Roth) and yohimbine hydrochloride (Sigma).

The following compounds were gifts: mecamylamine hydrochloride (Merck, Sharpe and Dohme), methiothepin maleate (Hoffman La Roche), methoxamine hydrochloride (Wellcome), spiroxatrine (Janssen) and we acknowledge the generosity of the companies.

Spiperone and sumatriptan succinate (GR43175; 3-[2-(dimethylamino)ethyl] - N - methyl - 1H - indole - 5 - methane sulphonamide) were synthesized by the Chemistry Research Department at Glaxo Group Research Limited, Ware.

All drugs, with the exception of ketanserin, methiothepin, pindolol and spiperone, were initially dissolved in distilled water and diluted with 0.9% w/v saline. Ketanserin and spiperone were initially dissolved in 0.1 M tartaric acid and diluted with 0.9% w/v saline. Methiothepin was dissolved in 10% ethanol in distilled water. Pindolol was initially dissolved in 2 M hydrochloric acid and diluted with distilled water to give a final solution in approximately 0.1 M HCl.

For studies *in vivo*, all doses of drugs refer to the free base.

Results

In vitro *studies in dog isolated saphenous vein*

Agonist-relative potency determinations 5-HT (10 nM–1 μM), sumatriptan (30 nM–5 μM) and 8-OH-DPAT (1 μM–100 μM) caused concentration-dependent contractions of the dog isolated saphenous vein with EC_{50} values (95% confidence limits) of 0.06 (0.04–0.08), 0.3 (0.1–0.8) and 3.9 (2.0–7.5) μM, respectively. The maximum contractile responses produced by each agonist were similar (Figure 1). The relative potencies (5-

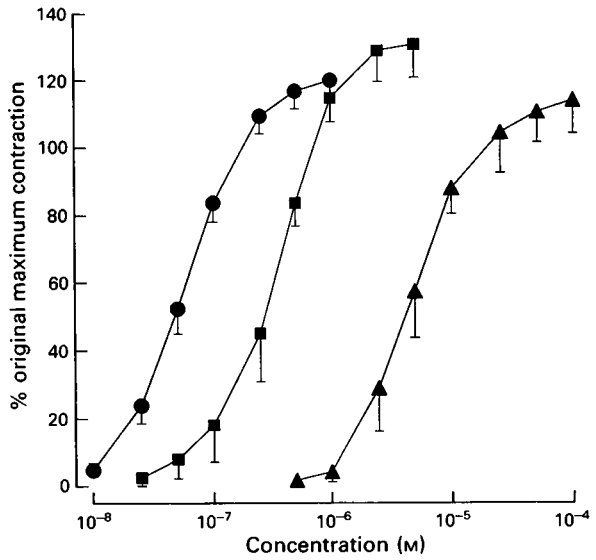

Figure 1 Concentration-effect curves to 5-hydroxytryptamine (5-HT, ●; $n = 9$), sumatriptan (■; $n = 4$) and 8-hydroxy-2-(di-n-propyl-amino)-tetralin (8-OH-DPAT, ▲; $n = 5$) in dog isolated saphenous vein in the presence of atropine, mepyramine and ketanserin (all 1 μM). Each point represents the mean from n experiments with s.e.mean shown by vertical bars. Agonist equipotent molar ratios (5-HT = 1) were 4.6 and 67 for sumatriptan and 8-OH-DPAT respectively (see text).

HT = 1) were 4.6 (2.5–8.2) and 67 (27–164) for sumatriptan and 8-OH-DPAT, respectively (mean with 95% confidence limits in parentheses).

Antagonist studies The contractile actions of 5-HT, sumatriptan and 8-OH-DPAT in the dog isolated saphenous vein were resistant to antagonism by the non-selective 5-HT_{1A} receptor antagonists, spiperone, spiroxatrine and pindolol (each at a single concentration of 1 μM). The 5-HT_{1D} receptor ligands, metergoline (0.1 μM) rauwolscine (1 μM) and yohimbine (1 μM) also had little or no antagonist activity (see Table 1). A higher concentration of metergoline (1 μM) produced a small displacement of the concentration-effect curve produced by 5-HT in the dog isolated saphenous vein. The concentration-ratio for 5-HT in the presence of this higher concentration of metergoline (1 μM) was 3.5 (1.9–6.3) corresponding to a pK_B value of 6.4 ± 0.1. However, metergoline (1 μM) also caused a similar attenuation of contractile responses elicited by the α_1-adrenoceptor agonist, methoxamine (mean concentration-ratio 3.3, $n = 2$). In five out of twelve experiments, metergoline (0.1 or 1 μM) also caused a small contraction of the dog isolated saphenous vein. However, the magnitude of these contractile responses was less than 10% of the maximum contractile response produced by the test agonists.

In contrast, methiothepin (0.03–0.3 μM) was a potent antagonist, producing concentration-dependent antagonism of the contractile effect of 5-HT, sumatriptan and 8-OH-DPAT in the dog isolated saphenous vein (Figure 2). Analysis of the data according to the method of Arunlakshana & Schild (1959) showed that the pA_2 values obtained for methiothepin against each agonist were similar in the range of 8.3–8.6 (Table 2). Additionally, the antagonism produced by methiothepin of the 5-HT- and sumatriptan-induced contractions of the dog isolated saphenous vein was competitive since slopes of the Schild regression were not significantly different from unity. The slope obtained with 8-OH-DPAT however, was significantly less than 1.0. These results are shown in Figure 2 and Table 2.

Table 1 Agonist concentration-ratios for 5-hydroxytryptamine (5-HT), sumatriptan and 8-hydroxy-2-(di-n-propyl-amino)-tetralin (8-OH-DPAT) following 30 min incubation with spiperone, pindolol, spiroxatrine, rauwolscine or yohimbine (all at 1 μM) or metergoline (0.1 μM, see text) in the dog isolated saphenous vein

	Agonist concentration-ratio		
	5-HT	*Sumatriptan*	*8-OH-DPAT*
Spiperone	1.1	1.0	0.8
	(0.8–1.4)	(0.3–3.0)	(0.4–1.6)
Pindolol	0.9	0.9	1.2
	(0.8–1.1)	(0.4–1.8)	(0.8–1.6)
Spiroxatrine	0.7	1.2	1.1
	(0.3–1.8)	(0.8–1.6)	(0.7–1.7)
Metergoline	2.5	1.5	1.3
	(1.7–3.7)	(0.5–4.2)	(0.7–2.5)
Rauwolscine	2.1	2.1	1.8
	(1.0–4.5)	(0.7–6.6)	(0.5–6.4)
Yohimbine	2.2	2.1	2.0
	(0.9–5.2)	(0.9–5.1)	(0.2–1.9)

The experiments were conducted in the presence of ketanserin (1 μM). Values shown are geometric means (95% confidence limits) from at least 3 experiments.

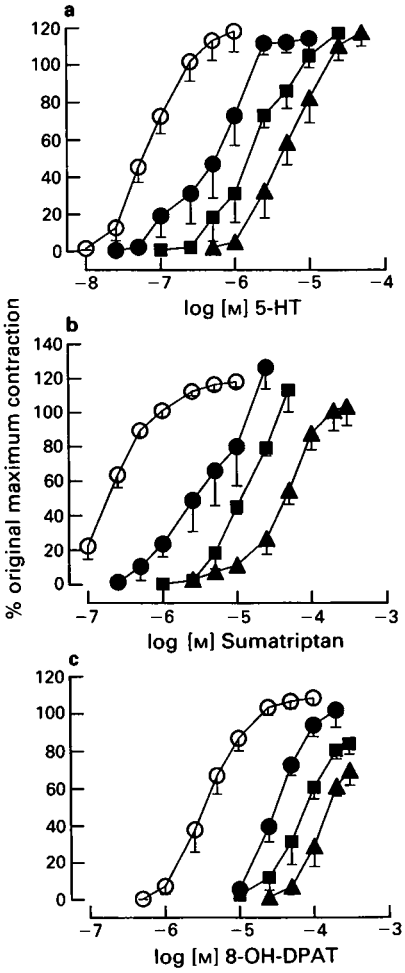

Figure 2 Effect of methiothepin on concentration-effect curves produced by (a) 5-hydroxytryptamine (5-HT), (b) sumatriptan and (c) 8-hydroxy-2-(di-n-propyl-amino)-tetralin (8-OH-DPAT) in the dog isolated saphenous vein in the presence of ketanserin (1 μM). Control concentration-effect curves are shown by (○) and concentration-effect curves in the presence of (●) 0.03, (■) 0.1 and (▲) 0.3 μM methiothepin are shown. Each point represents the mean from four experiments with s.e.mean shown by vertical bars. Calculated pA_2 values and Schild slopes from these data are shown in Table 2.

Table 2 Data from experiments in dog saphenous vein shown in Figure 2 were analysed by Schild analysis to give pA_2 values and slopes for methiothepin against 5-hydroxytryptamine, sumatriptan or 8-hydroxy-2-(di-n-propyl-amino)- tetralin (8-OH-DPAT) as agonists

Agonist	pA_2 values and slopes for methiothepin pA_2	Slope
5-Hydroxytryptamine	8.26 ± 0.20	1.03 ± 0.06
Sumatriptan	8.56 ± 0.26	1.15 ± 0.19
8-OH-DPAT	8.41 ± 0.06	0.78 ± 0.03*

Values are the mean ± s.e.mean from four experiments.
* Significantly different from unity ($P < 0.05$).

Anaesthetized dog studies

Effect of sumatriptan and 8-OH-DPAT following intra-arterial administration to the common carotid artery bed The intra-carotid administration of bolus doses of sumatriptan (0.1–1.0 $\mu g kg^{-1}$) or 8-OH-DPAT (0.3–3.0 $\mu g kg^{-1}$) to anaesthetized ganglion-blocked dogs, produced dose-dependent decreases in common carotid arterial blood flow. These reductions in blood flow were a consequence of increases in carotid arterial vascular resistance. Arterial blood pressure was not modified by either agonist. The vasoconstrictor responses produced by 8-OH-DPAT in the carotid artery bed of the anaesthetized dog were of shorter duration than those produced by sumatriptan. The percentage increase in carotid arterial vascular resistance produced by sumatriptan and 8-OH-DPAT to a bolus dose of 1 $\mu g kg^{-1}$ i.a. was 69 ± 4 and 76 ± 6% respectively, suggesting that the two agonists were approximately equipotent in this action. ED_{50} values for the agonists were not calculated since maximum vasoconstrictor responses were not achieved at the doses used (see Methods). Experimental recordings illustrating these actions of sumatriptan and 8-OH-DPAT in the anaesthetized dog are shown in Figure 3.

Antagonist studies

Effect of spiperone The intravenous administration of spiperone (1 $mg kg^{-1}$) to anaesthetized ganglion-blocked dogs caused a small fall in diastolic blood pressure and carotid arterial blood flow in both groups of dogs. However, carotid arterial vascular resistance was little changed from pre-dose levels. These effects are summarised in Table 3. Spiperone had no effect on carotid arterial vasoconstrictor responses elicited by either sumatriptan or 8-OH-DPAT; agonist dose-response curves in the presence of spiperone were superimposable with controls, with agonist dose-ratios close to unity in each case (Figure 4).

Effect of methiothepin The subsequent intravenous administration of methiothepin (1 $mg kg^{-1}$) had little effect on diastolic blood pressure or carotid arterial blood flow in either group of dogs. Consequently, carotid arterial vascular resistance was little changed from pre-dose levels. These effects are summarised in Table 3. However, methiothepin caused a marked attenuation of carotid arterial vasoconstrictor responses produced by close intra-arterial administration of both sumatriptan and 8-OH-DPAT to the anaesthetized dog. This attenuation was seen as both a rightward displacement of the agonist dose-effect curves to both sumatriptan and 8-OH-DPAT and a slight reduction in the slope (see Figure 4). The mean agonist dose-ratios (95% confidence limits, $n = 4$) produced by methiothepin for sumatriptan- and 8-OH-DPAT-induced carotid vasoconstrictor responses were similar, being 15 (8–27) and 17 (5–54) for sumatriptan and 8-OH-DPAT, respectively.

Discussion

It has been shown that 8-OH-DPAT has carotid arterial vasoconstrictor activity *in vivo* (Bom *et al.*, 1989). The aim of this study has been to investigate the effects of 8-OH-DPAT, in

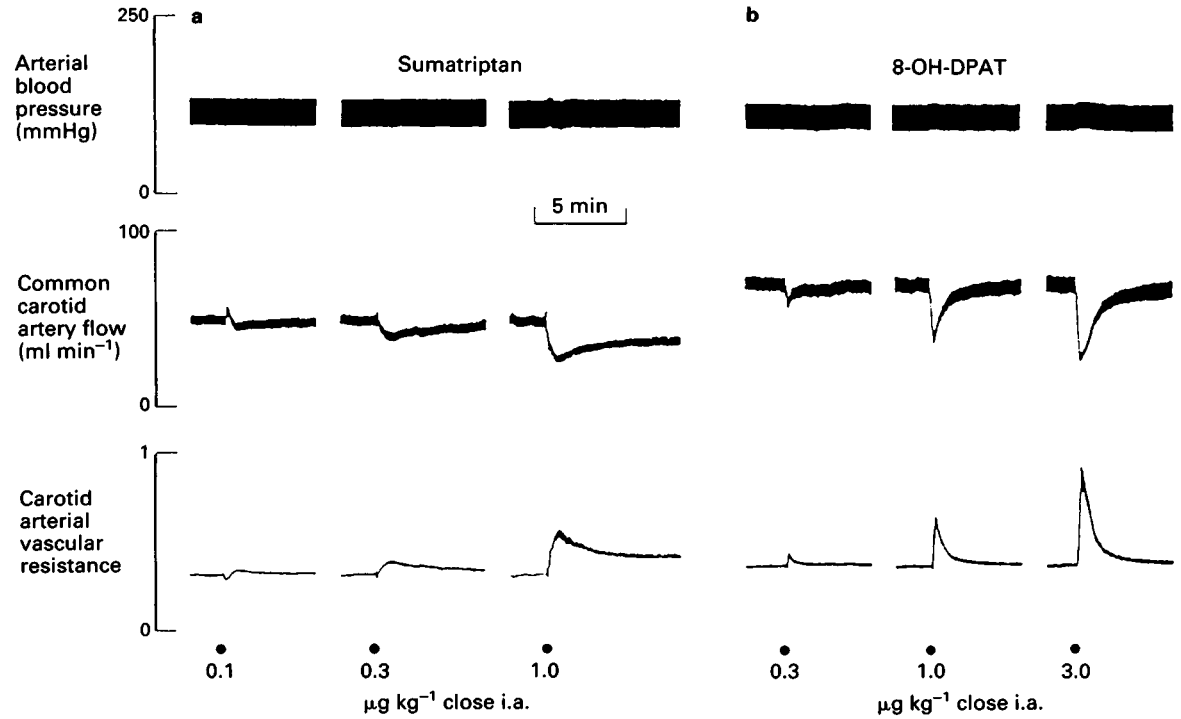

Figure 3 Experimental tracing illustrating the effects of (a) sumatriptan and (b) 8-hydroxy-2-(di-n-propyl-amino)-tetralin (8-OH-DPAT) when injected into the common carotid artery of ganglion-blocked, anaesthetized dogs. Vascular resistance in the carotid arterial bed was electronically determined and displayed continuously as arbitrary units. Both agonists produced transient carotid arterial vasoconstrictor responses (increases in vascular resistance) with little or no effect on arterial blood pressure.

Table 3 Haemodynamic effects of spiperone (1 mg kg^{-1}, i.v.) and methiothepin (1 mg kg^{-1}, i.v.) in barbitone anaesthetized, ganglion-blocked dogs

	Sumatriptan group	*8-OH-DPAT group*
Resting levels before spiperone		
Diastolic blood pressure (mmHg)	75 ± 7	75 ± 5
Carotid flow (ml min^{-1})	53 ± 12	57 ± 7
Carotid vascular resistance (mmHg min ml^{-1})	1.84 ± 0.30	1.59 ± 0.14
15 min after spiperone (1 mg kg^{-1})		
Diastolic blood pressure (mmHg)	63 ± 6	68 ± 4
Carotid flow (ml min^{-1})	49 ± 12	49 ± 6
Carotid vascular resistance (mmHg min ml^{-1})	1.78 ± 0.33	1.71 ± 0.19
Resting levels before methiothepin		
Diastolic blood pressure (mmHg)	61 ± 7	100 ± 4
Carotid flow (ml min^{-1})	38 ± 10	45 ± 6
Carotid vascular resistance (mmHg min ml^{-1})	2.17 ± 0.40	1.81 ± 0.25
15 min after methiothepin (1 mg kg^{-1})		
Diastolic blood pressure (mmHg)	56 ± 7	64 ± 4
Carotid flow (ml min^{-1})	40 ± 9	44 ± 6
Carotid vascular resistance (mmHg min ml^{-1})	1.89 ± 0.30	1.79 ± 0.24

8-OH-DPAT = 8-hydroxy-2-(di-n-propyl-amino)-tetralin.
Various haemodynamic parameters were measured as described in the text. Values are the mean ± s.e.mean from 4 dogs in each group.

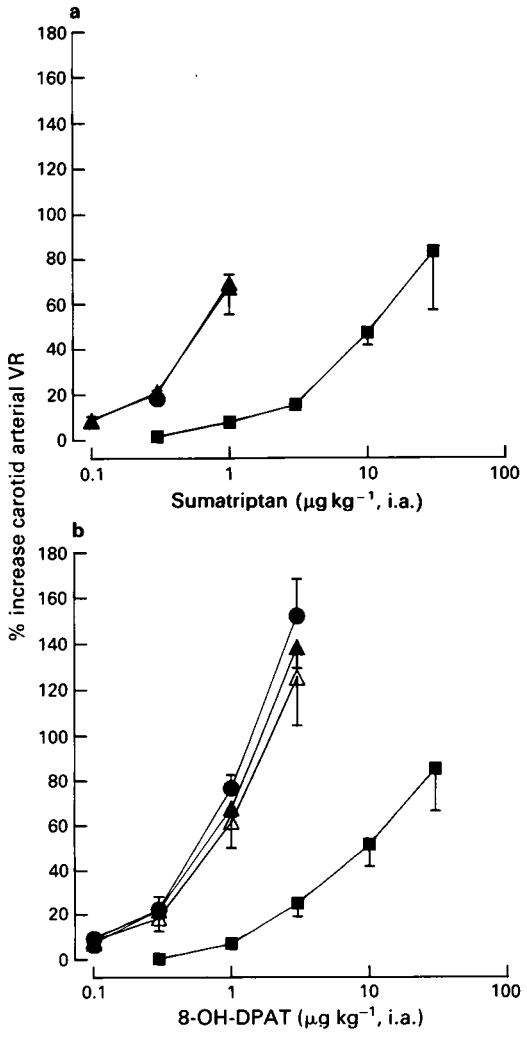

Figure 4 Carotid arterial vasoconstrictor responses to (a) sumatriptan and (b) 8-hydroxy-2-(di-n-propyl-amino)-tetralin (8-OH-DPAT) in the ganglion-blocked anaesthetized dog in the absence, (△, ●) and presence of 1 mg kg^{-1}, i.v. spiperone (▲) and finally after the subsequent administration of 1 mg kg^{-1} methiothepin (■). Note that dose-effect curves produced by both sumatriptan and 8-OH-DPAT were not affected by spiperone but were markedly displaced by methiothepin to the right to a similar extent. Values shown are mean (with s.e.mean shown by vertical bars) from four dogs.

the dog isolated saphenous vein and in the carotid artery bed of anaesthetized dogs which contain the same 5-HT$_1$-like receptor (Feniuk *et al.*, 1989). We have also compared the actions of 8-OH-DPAT with those of the selective 5-HT$_1$-like receptor agonist, sumatriptan, and attempted to characterize the receptor mechanism(s) involved in these actions by use of selective receptor antagonists.

8-OH-DPAT caused a concentration-dependent contraction of the dog isolated saphenous vein and a similar maximum contractile effect to that produced by both 5-HT and sumatriptan. 8-OH-DPAT was approximately 65 times weaker than 5-HT and 15 times weaker than sumatriptan in this respect. The ability of methiothepin to antagonize the contractile effects of 5-HT, sumatriptan and 8-OH-DPAT to the same degree suggests that all three agonists activate the same, 5-HT$_1$-like receptor mechanism. We have previously shown that at a concentration of 0.1 μM the antagonist action of methiothepin in the dog isolated saphenous vein is specific since concentration-effect curves to the thromboxane A$_2$ receptor agonist, U-46619 are unaffected (Humphrey *et al.*, 1988). The reason for the slightly shallow slope of the Schild regression obtained with 8-OH-DPAT is not known. However, it is possible that higher concentrations of 8-OH-DPAT may additionally cause contraction of the dog isolated saphenous vein by activation of receptors other than 5-HT receptors.

The contractile actions of 8-OH-DPAT, sumatriptan and 5-HT in the dog isolated saphenous vein were resistant to antagonism by the 5-HT$_{1A}$ and 5-HT$_2$ receptor antagonist, spiperone, suggesting that the 5-HT$_1$-like receptor which mediates these effects is not of the 5-HT$_{1A}$ subtype. The concentration of spiperone used (1 μM) is approximately 50 times greater than that required to inhibit 5-HT receptor-mediated inhibition of forskolin-stimulated adenylate cyclase in the guinea-pig and rat hippocampus (De Vivo & Maayani, 1985; 1986) or to inhibit 8-OH-DPAT-induced inhibition of the contraction produced by transmural electrical field stimulation of the guinea-pig whole ileum preparation (Fozard & Kilbinger, 1985). These systesms have been characterized as functional models of 5-HT$_{1A}$ receptor activation. In this study, we have also examined the effects of other putative 5-HT$_{1A}$ receptor antagonists; pindolol and spiroxatrine on 8-OH-DPAT-, sumatriptan- and 5-HT-induced contraction of dog isolated saphenous vein and failed to demonstrate any antagonistic effects, thus further reinforcing the view that 5-HT$_{1A}$ receptors are not involved.

These results support our previous findings, where the contractile effects of 5-HT and sumatriptan in the dog isolated

saphenous vein and cerebral blood vessels from dog and primate were shown to be resistant to antagonism by the 5-HT_{1A} and 5-HT_{1B} ligand, cyanopindolol (Humphrey *et al.*, 1988; Connor *et al.*, 1989). Furthermore, the results from our study seriously question the conclusions reached by others (Taylor *et al.*, 1986; Peroutka *et al.*, 1986) that 5-HT_{1A} receptors mediate contraction of canine cerebral blood vessels. The results from the present study would suggest that the contractile actions of 8-OH-DPAT and sumatriptan in such vessels are mediated via the activation of the same 5-HT_1-like receptor which is not of the 5-HT_{1A} subtype.

In the second part of the study we have compared the effects of 8-OH-DPAT with those of sumatriptan in the carotid arterial circulation of ganglion-blocked anaesthetized dogs. The animals were treated with mecamylamine and atropine in order to exclude the marked cardiovascular effects of 8-OH-DPAT which occur as a consequence of activation of central 5-HT_{1A} receptors (Ramage & Fozard, 1987). The intra-arterial administration of 8-OH-DPAT and sumatriptan to the carotid artery bed of anaesthetized dogs caused dose-dependent, reproducible vasoconstrictor responses with little or no effect on arterial blood pressure or heart rate. The carotid arterial vasoconstrictor responses produced by 8-OH-DPAT were transient in nature, whilst those produced by sumatriptan tended to be of longer duration. In marked contrast to the dog isolated saphenous vein where 8-OH-DPAT was approximately 15 times weaker than sumatriptan in causing contraction, the two agonists were approximately equipotent in causing carotid arterial vasoconstriction in anaesthetized dogs. The reason for this apparent difference in sensitivity is not known.

As was seen *in vitro*, spiperone had no antagonistic action on carotid arterial vasoconstrictor responses produced by either 8-OH-DPAT or sumatriptan in the anaesthetized dog, suggesting that the responses do not involve activation of either 5-HT_{1A} or 5-HT_2 receptors. The dose of spiperone used in our studies (1 mg kg^{-1}, i.v.) is approximately 40 times higher than that required to inhibit the flat body posture and forepaw treading components of the behavioural response to 8-OH-DPAT in the reserpinized rat, effects which have been shown to be mediated by activation of 5-HT_{1A} receptors (Tricklebank *et al.*, 1984). The carotid arterial vasoconstrictor action of 8-OH-DPAT and indeed that of sumatriptan was markedly attenuated by the subsequent administration of methiothepin. The extent of the attenuation produced by methiothepin against each agonist was similar, suggesting that both 8-OH-DPAT and sumatriptan cause carotid arterial vasoconstriction by activation of the same (5-HT_1-like) receptor. This result confirms our previous finding with sumatriptan (Feniuk *et al.*, 1989), where we also demonstrated the specificity of action of methiothepin as an antagonist of sumatriptan.

In summary, the results from the present study have clearly demonstrated that 8-OH-DPAT, like sumatriptan, causes contraction of dog isolated saphenous vein and vasoconstriction within the carotid arterial circulation of anaesthetized dogs. The receptors involved in these actions of 8-OH-DPAT are clearly 5-HT_1-like since they are antagonized by methiothepin but are not of the 5-HT_{1A} subtype since the effects of 8-OH-DPAT are unaffected by spiperone (and additionally spiroxatrine and pindolol *in vitro*). It would seem that the receptors involved in these actions of 8-OH-DPAT in the dog isolated saphenous vein and carotid artery bed of anaesthetized dogs are apparently the same as those mediating the effects of sumatriptan in the two systems. The results from our *in vitro* experiments demonstrate that 8-OH-DPAT is a relatively weak agonist (EC_{50} 3.9 (2.0–7.5) μM) in the dog isolated saphenous vein which is in marked contrast to its high potency at the 5-HT_{1A} receptor in the guinea-pig ileum (Fozard & Kilbinger, 1985). However, our studies *in vivo* show that 8-OH-DPAT activates these non 5-HT_{1A} receptors in the carotid artery bed of anaesthetized dogs at low doses, (0.3–3 μg kg^{-1}, i.a.) similar to those at which it reduces carotid arteriovenous anastomotic blood flow in anaesthetized pigs (Bom *et al.*, 1989) and causes 5-HT_{1A} receptor-mediated reductions in arterial blood pressure and heart rate in the rat (Fozard *et al.*, 1987) and cat (Ramage & Fozard, 1987). Thus, caution should be exercised in ascribing some of the *in vivo* actions of 8-OH-DPAT to 5-HT_{1A} receptor activation unless such studies are accompanied by additional experiments with selective 5-HT_{1A} receptor blocking drugs.

It has been suggested that the anti-migraine effects of sumatriptan may be mediated by the activation of 5-HT_{1D} receptors (see Hoyer, 1989; Hoyer *et al.*, 1989; Schoeffter & Hoyer, 1989; Peroutka & McCarthy, 1989). We have, therefore, considered whether the vasoconstrictor effects of 8-OH-DPAT and sumatriptan in the dog isolated saphenous vein and carotid circulation of anaesthetized dogs demonstrated in this study are mediated via the activation of 5-HT_{1D} receptors. In ligand binding studies, 8-OH-DPAT has an affinity at 5-HT_{1D} recognition sites which is some 20 fold less than that observed with sumatriptan (Hoyer *et al.*, 1989). Thus the relative affinity of the two compounds for the 5-HT_{1D} binding site is similar to their relative potency in causing contraction of the dog isolated saphenous vein. However, the 5-HT_{1D} ligand, metergoline, had no antagonistic activity in the dog saphenous vein against either 8-OH-DPAT- or sumatriptan-induced contraction even at a concentration which exceeded the estimated K_D value in calf caudate by a factor of 100 fold (Hoyer, 1989). A 10 fold higher concentration of metergoline (1 μM), caused weak antagonism of contractile responses elicited by 5-HT in the dog isolated saphenous vein (calculated pK_B value 6.4 ± 0.1). However, metergoline (1 μM) also antagonized methoxamine-induced contraction of the preparation. Evidence is emerging that there may be variations in the affinity of metergoline for 5-HT_{1D} recognition sites between different species (Waeber *et al.*, 1988; Xiong & Nelson, 1989). However, even a comparison of the lowest estimate for the 5-HT_{1D} binding affinity of metergoline (pK_i 7.24 ± 0.06, rabbit caudate nucleus; Xiong & Nelson, 1989) with its pK_B value in the dog isolated saphenous vein (6.4 ± 0.1) shows that there is still almost a 10 fold separation, suggesting that the 5-HT_1-like receptor site in the dog isolated saphenous vein is different to any 5-HT_{1D} recognition site so far identified. It would clearly be of interest to determine the affinity of metergoline for 5-HT_{1D} recognition sites in dog brain tissue.

Other 5-HT_{1D} ligands, rauwolscine and yohimbine, have been found to be similarly poor as antagonists in the dog isolated saphenous vein in concentrations as high as 1 μM which is at least ten times higher than their affinities for 5-HT_{1D} recognition sites in calf caudate (Hoyer, 1989). Admittedly these ligands have even lower affinity for the 5-HT_{1D} recognition site in human caudate, but this only serves to illustrate their limitations as drug tools (Waeber *et al.*, 1988). Importantly too, Sumner & Humphrey (1989) have provided evidence that the 5-HT_{1D} recognition site in porcine brain is heterogeneous which must confound attempts to determine the affinities of antagonists for the 5-HT_{1D} site. It would, therefore, seem reasonable to conclude, in the absence of better, more selective antagonists, that the receptor which mediates 5-HT-induced contraction of the dog isolated saphenous vein and presumably also cranial vessels from higher species, is not of the 5-HT_{1D} type.

Clearly, the definitive characterization of the receptor involved in the cranial vasoconstrictor effects of 8-OH-DPAT and sumatriptan and its relation to 5-HT_{1D} receptors identified in ligand binding studies awaits the identification of a selective receptor antagonist. Until then, the description '5-HT_1-like' still seems the most appropriate. The findings in this paper also raise questions about the degree of selectivity of 8-OH-DPAT for 5-HT_{1A} receptors, most particularly *in vivo*.

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References

APPERLEY, E., HUMPHREY, P.P.A. & LEVY, G.P. (1976). Receptors for 5-hydroxytryptamine and noradrenaline in rabbit isolated ear artery and aorta. *Br. J. Pharmacol.*, **58**, 211–221.

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

BOM, A.H., VERDOUW, P.D. & SAXENA, P.R. (1989). Carotid haemodynamics in pigs during infusions of 8-OH-DPAT: reduction in arteriovenous shunting is mediated by 5-HT_1-like receptors. *Br. J. Pharmacol.*, **96**, 125–132.

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

CONNOR, H.E., FENIUK, W. & HUMPHREY, P.P.A. (1989). Characterization of 5-HT receptors mediating contraction of canine and primate basilar artery by use of GR43175, a selective 5-HT_1-like receptor agonist. *Br. J. Pharmacol.*, **96**, 379–387.

DE VIVO, M. & MAAYANI, S. (1985). Inhibition of forskolin-stimulated adenylate cyclase activity by 5-HT receptor agonists. *Eur. J. Pharmacol.*, **119**, 231–234.

DE VIVO, M. & MAAYANI, S. (1986). Characterization of the 5-hydroxy-tryptamine$_{1A}$ receptor-mediated inhibition of forskolin-stimulated adenylate cyclase activity in guinea-pig and rat hippocampal membranes. *J. Pharmacol. Exp. Ther.*, **238**, 248–253.

FENIUK, W., HUMPHREY, P.P.A. & PERREN, M.J. (1989). The selective carotid arterial vasoconstrictor action of GR43175 in anaesthetized dogs. *Br. J. Pharmacol.*, **96**, 83–90.

FOZARD, J.R. & KILBINGER, H. (1985). 8-OH-DPAT inhibits transmitter release from guinea-pig enteric cholinergic neurones by activating 5-HT_{1A} receptors. *Br. J. Pharmacol.*, **86**, 601P.

FOZARD, J.R., MIR, A.K. & MIDDLEMISS, D.N. (1987). Cardiovascular response to 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) in the rat: Site of action and pharmacological analysis. *J. Cardiovasc. Pharmacol.*, **9**, 328–347.

HEURING, R.E. & PEROUTKA, S.J. (1987). Characterization of a novel [^{3}H]-5-hydroxytryptamine binding site subtype in bovine brain membranes. *J. Neurosci.*, **7**, 894–903.

HOYER, D., ENGEL, E. & KALKMAN, H.O. (1985). Molecular pharmacology of 5-HT_1 and 5-HT_2 recognition sites in rat and pig brain membranes: radioligand binding studies with [^{3}H]-5-HT, [^{3}H]-8-OH-DPAT, (−)[^{125}I]-iodocyanopindolol, [^{3}H]-mesulergine and [^{3}H]-ketanserin. *Eur. J. Pharmacol.*, **118**, 13–23.

HOYER, D. (1989). 5-Hydroxytryptamine receptors and effector coupling mechanisms in peripheral tissues. In *The Peripheral Actions of 5-Hydroxytryptamine*. ed. Fozard, J.R. pp. 72–99. Oxford: Oxford University Press.

HOYER, D., SCHOEFFTER, P. & GRAY, J.A. (1989). A comparison of the interactions of dihydroergotamine, ergotamine and GR43175 with 5-HT_1 receptor subtypes. *Cephalalgia*, **9** (Suppl. 10), 340–341.

HUMPHREY, P.P.A., FENIUK, W., PERREN, M.J., CONNOR, H.E., OXFORD, A.W., COATES, I.H. & BUTINA, D. (1988). GR43175, a selective agonist for the 5-HT_1-like receptor in dog isolated saphenous vein. *Br. J. Pharmacol.*, **94**, 1123–1132.

HUMPHREY, P.P.A. & RICHARDSON, B.P. (1989). Classification of 5-HT receptors and binding sites: an overview. In *Serotonin: Actions, Receptors, Pathophysiology*, ed. Mylecharane, E.J., Angus, J.A., De La Lande, I.S. & Humphrey, P.P.A. pp. 204–221. Basingstoke: Macmillan Press.

MIDDLEMISS, D.N. & FOZARD, J.R. (1983). 8-hydroxy-2-(di-n-propylamino) tetralin discriminates between subtypes of the 5-HT_1-like-recognition site. *Eur. J. Pharmacol.*, **90**, 151–153.

PARSONS, A.A. & WHALLEY, E.T. (1989). Characterization of the 5-hydroxytryptamine receptor which mediates contraction of the human isolated basilar artery. *Cephalalgia*, **9** (Suppl. 9), 47–51.

PAZOS, A. & PALACIOS, J.M. (1985). Quantitative autoradiographic mapping of serotonin receptors in the rat brain. 1. Serotonin-1 receptors. *Brain Res.*, **346**, 205–230.

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

PEROUTKA, S.J., HUANG, S. & ALLEN, G.S. (1986). Canine basilar artery contractions mediated by 5-hydroxytryptamine receptors. *J. Pharmacol. Exp. Ther.*, **237**, 3, 901–906.

PEROUTKA, S.J. & McCARTHY, B.G. (1989). Sumatriptan (GR43175) interacts selectively with 5-HT_{1B} and 5-HT_{1D} binding sites. *Eur. J. Pharmacol.*, **163**, 133–136.

PERREN, M.J., FENIUK, W. & HUMPHREY, P.P.A. (1989a). The selective closure of feline carotid arteriovenous anastomoses (AVAs) by GR43175. *Cephalalgia*, **9** (Suppl. 9), 41–46.

PERREN, M.J., FENIUK, W. & HUMPHREY, P.P.A. (1989b). Vascular actions of 8-OH-DPAT mediated via activation of non-5-HT_{1A} receptors. *Br. J. Pharmacol.*, **98**, 797P.

RAMAGE, A.G. & FOZARD, J.R. (1987). Evidence that the putative 5-HT_{1A} receptor agonists, 8-OH-DPAT and ipsapirone, have a central hypotensive action that differs from that of clonidine in anaesthetised cats. *Eur. J. Pharmacol.*, **138**, 179–191.

SCHOEFFTER, P. & HOYER, D. (1989). How selective is GR43175? Interactions with functional 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} and 5-HT_{1D} receptors. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **340**, 135–138.

SUMNER, M.J. & HUMPHREY, P.P.A. (1989). Heterogeneous 5-HT_{1D} binding sites in porcine brain can be differentiated by GR43175. *Br. J. Pharmacol.*, **97**, 410P.

TAYLOR, E.W., PIPER DUCKLES, S. & NELSON, D.L. (1986). Dissociation constants of serotonin agonists in the canine basilar artery correlate to Ki values at the 5-HT_{1A} binding sites. *J. Pharmacol. Exp. Ther.*, **236**, 118–125.

TRICKLEBANK, M.D., FORLER, C. & FOZARD, J.R. (1984). The involvement of subtypes of the 5-HT_1 receptor and of catecholaminergic systems in the behavioural response to 8-hydroxy-2-(di-n-propylamino)tetralin in the rat. *Eur. J. Pharmacol.*, **106**, 271–282.

WAEBER, C., SCHOEFFTER, P., PALACIOS, J.M. & HOYER, D. (1988). Molecular pharmacology of 5-HT_{1D} recognition sites: Radioligand binding studies in human, pig and calf brain membranes. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **337**, 595–601.

XIONG, W. & NELSON, D.L. (1989). Characterisation of a [^{3}H]-5-hydroxytryptamine binding site in rabbit caudate nucleus that differs from the 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} and 5-HT_{1D} subtypes. *Life Sci.*, **45**, 1433–1442.

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