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GR43175, a selective agonist for the 5-HT_1-like receptor in dog isolated saphenous vein

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1 We describe the actions of a novel and selective 5-HT_1-like receptor agonist, GR43175, in a range of isolated tissue preparations containing different 5-hydroxytryptamine (5-HT) receptor types.

2 GR43175 was a potent agonist at 5-HT_1-like receptors mediating contraction of the dog isolated saphenous vein and also at those inhibiting neuronally mediated contractions in the same preparations. For both actions, GR43175 was approximately four times weaker than 5-HT.

3 GR43175 was devoid of agonist properties at 5-HT_1-like receptors mediating relaxation of the cat isolated saphenous vein.

4 GR43175 was devoid of agonist properties at 5-HT_2 receptors mediating contraction of the rabbit isolated aorta, pig coronary artery, greyhound coronary artery and beagle femoral artery.

5 GR43175 was devoid of agonist properties at 5-HT_3 receptors mediating depolarization of the rat isolated vagus nerve.

6 The contractile response to GR43175 in the dog isolated saphenous vein was selectively antagonized by methiothepin but was resistant to antagonism by the 5-HT_2 receptor blocking drug ketanserin and the 5-HT_3 receptor blocking drug MDL 72222. Methiothepin antagonized the contractile action of 5-HT and GR43175 to an equal extent suggesting that both agonists act at the same receptor.

7 The results demonstrate that GR43175 is a highly selective agonist for the 5-HT_1-like receptors found in the dog saphenous vein. The absence of an action of GR43175 at 5-HT_1-like receptors mediating relaxation of the cat isolated saphenous vein provides further evidence that 5-HT_1-like receptors are heterogeneous.

Introduction

The receptors mediating the many and varied biological effects of the naturally occurring indole, 5-hydroxytryptamine (5-HT) have recently been classified according to the actions of selective agonists such as 5-carboxamidotryptamine and 2-methyl 5-HT and specific antagonists such as ketanserin and MDL 72222. The three distinct classes of receptor identified have been designated the nomenclature, 5-HT_1-like, 5-HT_2 and 5-HT_3 (see Bradley *et al.*, 1986). 5-HT_1-like receptors have been so designated because many examples of the class, although having similarities with the 5-HT_1 recognition site in rat brain, are not the same as the currently known 5-HT_1 binding site sub-types. Although these 5-HT_1-like receptors can all be characterized by the high agonist potency of 5-carboxamidotryptamine (5-CT), we and others have argued that 5-HT_1-like receptors are heterogeneous (Bradley *et al.*, 1986; Trevethick *et al.*, 1986; Martin *et al.*, 1987; Humphrey & Feniuk, 1987) and their definitive classification awaits the identification of good selective drug tools.

We now describe the actions of a novel and highly selective 5-HT receptor agonist, GR43175 (3-[2-dimethylamino]ethyl-*N*-methyl-1H-indole-5 methane sulphonamide), in functional studies in a range of isolated tissue preparations containing different 5-HT receptor types. The data provide further evidence to support the view that 5-HT_1-like receptors can be sub-classified. The chemical structure of GR43175 is shown in Figure 1.

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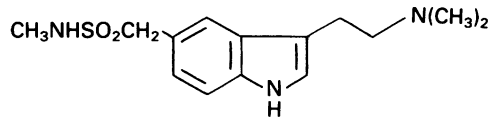

Figure 1 Structure of GR43175 (3-[2-dimethylamino] ethyl-N-methyl-1H-indole-5-methane sulphonamide).

A preliminary account of these findings has been presented to the British Pharmacological Society (Humphrey *et al.*, 1987a).

Methods

Preparation of vascular strips

Contraction of dog isolated saphenous vein and rabbit aorta Spirally cut strips of rabbit thoracic aorta and beagle isolated saphenous vein were prepared essentially as described by Apperley *et al.* (1976; 1980) and suspended in a modifed Krebs solution (Apperley *et al.*, 1976), bubbled with 95% O_2: 5% CO_2 under an original resting tension of 0.5 g. Isometric contractions were recorded by use of Statham transducing cells. All tissues were allowed to equilibrate for a period of at least 1 h and were then 'primed' with potassium chloride to give a final bath concentration of 30 mM. Agonists were not administered for periods of at least 30 min following washout of potassium chloride.

Agonist studies in the rabbit aorta were conducted in the continuous presence of atropine (1 μM), benextramine (3 μM) and mepyramine (1 μM), whilst those in the dog isolated saphenous vein were conducted in the continuous presence of atropine (1 μM), ketanserin (1 μM) and mepyramine (1 μM). The antagonists were included in order to exclude an action of 5-HT and GR43175 at a variety of other receptor sites.

Inhibition of neurogenically mediated contractions of dog isolated saphenous vein Beagle isolated saphenous vein strips were prepared essentially as described by Feniuk *et al.* (1979). The strips were suspended in Krebs solution between platinum electrodes and stimulated electrically (2 Hz for 10 s every 180 s, 0.1 ms pulse width, supramaximal voltage). The contraction produced under these conditions has been shown to be due to stimulation of noradrenergic nerves (Feniuk *et al.*, 1979; Watts *et al.*, 1981). Experiments were performed in the continuous presence of indomethacin (2.8 μM); cocaine (30 μM), atropine, mepyramine and cyproheptadine (all at 1 μM) which were included to optimize the experimental conditions and to exclude possible effects of 5-HT and GR43175 at a variety of other receptor sites.

Relaxation of cat isolated saphenous vein Spirally cut strips of cat isolated saphenous vein were prepared as described previously (Feniuk *et al.*, 1983) and suspended in Krebs solution under a resting tension of 0.4 g. Recordings of isometric tension changes were made by use of Statham transducing cells. All preparations were allowed to equilibrate for a period of 1 h before beginning an experiment. Tone in each preparation was increased by the addition of α-methyl 5-HT (10 μM) and, once the contraction had stabilised (normally 10–15 min), 5-HT was added to the tissue (see below).

Depolarization of rat isolated vagus nerve

Rat isolated vagus nerves were prepared as described in detail by Ireland & Tyers (1987) and transferred to a two compartment perspex bath to permit extracellular recordings of 5-HT-induced potential changes. Tissues were continually superfused with Krebs-Henseleit solution (see Ireland & Tyers, 1987) at a constant rate of approximately 1 ml min^{-1} and drugs applied at a known concentration via the superfusion stream into the first compartment only. Non-cumulative applications of agonists were used to construct concentration-effect curves. The period of exposure to each concentration of 5-HT was sufficient for the evoked potential change to have stabilized. Contact times were 3 min or less and preparations were allowed to repolarize fully between agonist applications.

Measurement of agonist potencies in rabbit aorta, dog and cat saphenous veins

Cumulative concentration-effect curves to 5-HT were obtained on all tissues (see above) and the 5-HT was then washed from the bath. Sixty minutes later a cumulative concentration-effect curve to GR43175 was then determined in one preparation, whilst a second preparation was again dosed with 5-HT; this acted as a control to monitor any spontaneous changes in sensitivity to 5-HT. Relative potencies were determined by dividing the EC_{50} (molar concentration of each compound to produce 50% of its maximum 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 strip. This value varied by less than two fold. The maximum response to GR43175 was also compared to the maximum response to 5-HT in the same preparation and corrected for time-related changes by dividing the ratio of the relative maximum responses to 5-HT in the control concentration-effect curves.

In those preparations where GR43175 was devoid of agonist effects, a concentration-effect curve to 5-HT was re-established in the presence of the maximum concentration of GR43175 examined, 15 min after its addition.

Agonist potencies in rat isolated vagus nerve

Concentration-effect curves to 5-HT and GR43175 were obtained by non-cumulative addition of doses in a pre-determined randomised order and the EC_{50} value for the agonists determined.

Effect of antagonists on the contractile effect of GR43175 in the dog isolated saphenous vein

Concentration-effect curves to GR43175 in the dog isolated saphenous vein were established in the absence of antagonists in the Krebs solution and then repeated in the presence of a single fixed antagonist concentration. The interval between the start of each concentration-effect curve was 1 h and the antagonist contact time was 30 min. Agonist equiactive concentration-ratios were determined by comparing the EC_{50} values for 5-HT in the presence and absence of antagonist and corrections made for spontaneous changes in agonist sensitivity as previously described (Apperley *et al.*, 1976).

Effect of methiothepin and cyanopindolol on the contractile action of 5-HT and GR43175

In view of our previous finding (Feniuk *et al.*, 1985) that a small fraction of the 5-HT-induced contraction of the dog isolated saphenous vein is due to the activation of 5-HT$_2$ receptors, these experiments were conducted in the continuous presence of ketanserin (1 μM). Concentration-effect curves to 5-HT or GR43175 were established and then repeated in the presence of methiothepin (0.1 μM) or cyanopindolol (1 μM) or mesulergine (1 μM) or metergoline (0.1 μM). Agonist equiactive concentration-ratios were calculated as described above. Specificity studies were carried out with the thromboxane A_2 receptor agonist, U-46619.

Other vascular preparations

Dog femoral artery Isolated strips of beagle femoral artery were prepared as described by Apperley *et al.* 1980.

Pig and greyhound coronary arteries Isolated ring segments of pig anterior descending coronary artery and greyhound circumflex coronary artery were prepared and the intimal surface rubbed to remove the vascular endothelium. The ring segments were placed in separate 10 ml organ baths containing modified Krebs solution (Apperley *et al.*, 1976) and the segments suspended between two L-shaped stainless steel wire supports (0.2 mm diameter) inserted into the lumen to record isometric tension changes. Preparations were maintained at a resting tension of 0.5–1 g and allowed to equilibrate for periods of at least 1 h.

Measurement of agonist potencies (5-HT and GR43175) were determined as already described but in the absence of antagonists.

Statistics

Unless otherwise stated values are the arithmetic means $\pm$ s.e.mean or geometric means with 95% confidence limits in parentheses.

Drugs used

The following compounds were purchased: atropine sulphate (Sigma), benextramine tetrahydrochloride (Sigma), cocaine hydrochloride (May & Baker), cyproheptadine hydrochloride (Merck, Sharp & Dohme), 5-hydroxytryptamine creatinine sulphate (Sigma), indomethacin (Sigma), mepyramine maleate (May & Baker), metergoline (Farmitalia) and methiothepin maleate (Roche).

The following compounds were gifts: cyanopindolol (Sandoz); MDL 72222 (1αH,3α,5αH-tropan-3yl-3,5 dichlorobenzoate Merrell-Dow); mesulergine (Sandoz); ketanserin (Janssen), and we acknowledge the generosity of the Companies.

GR43175, α-methyl 5-HT, U-46619 (11,9 epoxymethano PGH$_2$) and AH23848 ([1α(z),2β,5α]-($\pm$)-7-[5-[[(1,1'-biphenyl)-4-yl] methoxy]-2-(4-morpholinyl)-3-oxocyclopentyl]-4-heptenoic acid) were synthesized by the Chemistry Research Department at Glaxo Group Research.

Results

The agonist effects of 5-HT and GR43175 in a range of isolated tissue preparations containing different 5-HT receptor types are shown in Figure 2.

Agonist studies

Contraction of dog isolated saphenous vein Both 5-HT (10 nM–10 μM) and GR43175 (10 nM–10 μM) produced concentration-dependent contractile responses of the dog isolated saphenous vein, GR43175 being approximately four times weaker than 5-HT. The maximum response produced by 5-HT and GR43175 was similar (Table 1).

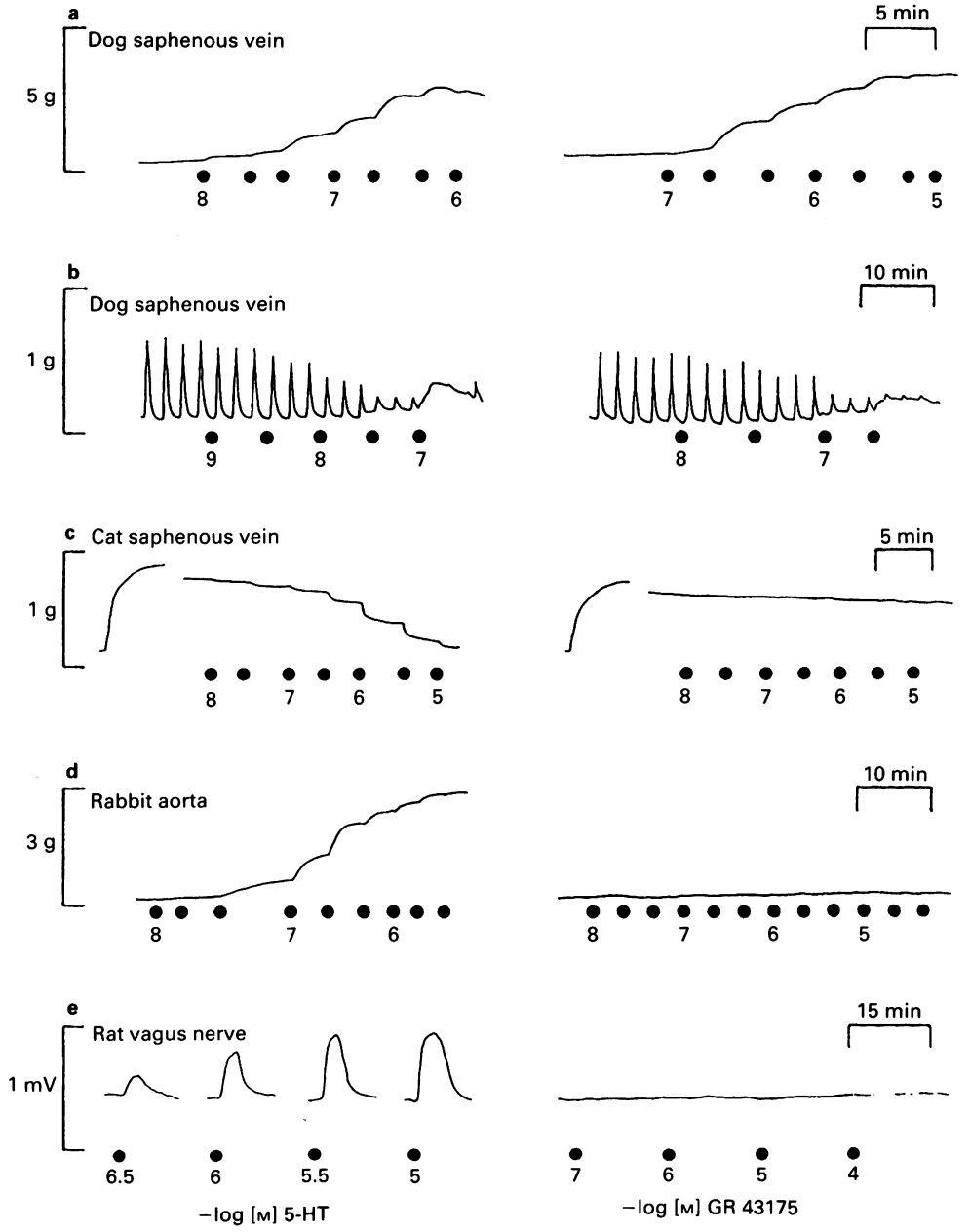

Figure 2 Experimental recordings illustrating the effects of 5-hydroxytryptamine (5-HT) and GR43175 in a range of isolated tissues containing different functional 5-HT receptor types. (a) Contractile effect of 5-HT and GR43175 in dog isolated saphenous vein, (b) inhibition of neurogenically (2 Hz for 10 s, 0.1 ms pulse width, every 3 min) mediated contractions of dog isolated saphenous vein, (c) relaxant effect of 5-HT in cat isolated saphenous vein contracted with α-methyl 5-HT, (d) contractile effect of 5-HT in rabbit isolated aorta and (e) depolarization induced by 5-HT in the rat isolated vagus nerve. Intermediate doses of 5-HT and GR43175 are 2.5 to 5 fold incremental increases in concentration.

Note the agonist activity of GR43175 at post- and pre-junctional 5-HT_1-like receptors in the dog isolated saphenous vein and the absence of agonist activity at 5-HT_1-like receptors mediating relaxation of the cat isolated saphenous vein. GR43175 was also devoid of agonist activity at 5-HT_2 receptors mediating contraction of the rabbit aorta and 5-HT_3 receptors mediating depolarization of the rat vagus nerve.

Table 1 Agonist potencies of 5-hydroxytryptamine (5-HT) and GR43175 in a range of isolated tissue preparations containing different 5-HT receptors

Receptor	*Preparation*	*Response*	*EC_{50} 5-HT* (nM)	*EC_{50} GR43175* (nM)	*EPMR*	*GR43175 as % 5-HT max*
5-HT$_1$-like	Dog saphenous vein	Contraction	44 (33–59)	302 (117–783)	4.6 (2.5–8.2)	106 ± 8
5-HT$_1$-like	Dog saphenous vein	Inhibition of neurotransmission	10 (3–28)	40 (19–87)	4.2 (3.1–5.6)	105 ± 6
5-HT$_1$-like	Cat saphenous vein	Relaxation	117 (38–363)	> 10,000	—	—
5-HT$_2$	Rabbit aorta	Contraction	160 (61–416)	> 50,000	—	—
5-HT$_3$	Rat vagus nerve	Depolarization	2260 (1370–3740)	> 100,000	—	—

Values shown are geometric mean (95% confidence limits) or arithmetic mean ± s.e.mean from at least four experiments.
EC_{50} values were determined as described in Methods.
EPMR = Equipotent molar concentration ratio.

Inhibition of neurogenically mediated contraction in the dog isolated saphenous vein Both 5-HT (1 nM–1 μM) and GR43175 (1 nM–1 μM) inhibited the contractile response of the dog isolated saphenous vein to electrical field stimulation (2 Hz for 10 s, 0.1 ms pulse width, every 3 min) in a concentration-dependent manner. GR43175 was approximately four times weaker than 5-HT and produced a similar maximum response (Table 1).

Relaxation of cat isolated saphenous vein Although 5-HT (10 nM–10 μM) produced a concentration-related relaxation of the cat isolated vein contracted with α-methyl 5-HT (10 μM), GR43175 in concentrations up to 10 μM had no effect (Table 1). Furthermore, GR43175 (10 μM) had no effect on the relaxant action of 5-HT, the 5-HT concentration-ratio being 1.1 (0.5–2.5). Values are geometric mean (95% confidence limits) from four experiments.

Contraction of rabbit isolated aorta 5-HT (10 nM–10 μM) produced a concentration-dependent contraction of the rabbit isolated aorta, whilst GR43175 in concentrations up to 50 μM was devoid of agonist activity. These results are summarised in Table 1. GR43175 (50 μM) did not modify the contractile action of 5-HT in the rabbit isolated aorta, the 5-HT concentration-ratio being 0.8(0.4–1.7). Values are geometric mean (95% confidence limits) from four experiments.

Depolarization of rat isolated vagus nerve 5-HT (0.1 μM–30 μM) caused a rapid concentration-dependent depolarisation of the rat isolated vagus nerve. In concentrations up to 100 μM, GR43175 had no agonist activity. These results are summarised in Table 1.

Antagonist studies in dog isolated saphenous vein

The contractile action of GR43175 was resistant to antagonism by the 5-HT$_2$ antagonist, ketanserin (1 μM), and the 5-HT$_3$ antagonist, MDL 72222 (1 μM). The results are shown in Figure 3.

In marked contrast, methiothepin (0.1 μM) caused a rightward displacement of the concentration-effect curves to both 5-HT and GR43175, such that the agonist concentration-ratios were similar (Figure 4). The corresponding pA_2 values for methiothepin against 5-HT- and GR43175-induced contractions of the dog isolated saphenous vein were 7.82 (7.32–8.34) and 8.09 (7.34–8.91), respectively. Values are geometric mean (95% confidence limits) from four experiments. The antagonist action of methiothepin was specific, since concentration-effect curves to the thromboxane A_2 receptor agonist, U-46619, were not affected (Figure 4).

Cyanopindolol (1 μM), which has a high affinity at 5-HT$_{1A}$ and 5-HT$_{1B}$ recognition sites in brain ligand binding studies, did not modify the contractile action of either 5-HT or GR43175 in the dog isolated saphenous vein (Figure 5).

The agonist effects of GR43175 were also resistant to antagonism by mesulergine (1 μM) and metergoline (0.1 μM), which have high affinities at 5-HT$_{1C}$ and 5-HT$_{1D}$ recognition sites, respectively. The GR43175 concentration-ratios in the presence of mesulergine and metergoline were 0.7 (0.2–2.2) and 1.5 (0.5–4.2), respectively. Values are geometric mean (95% confidence limits) from four and three experiments, respectively.

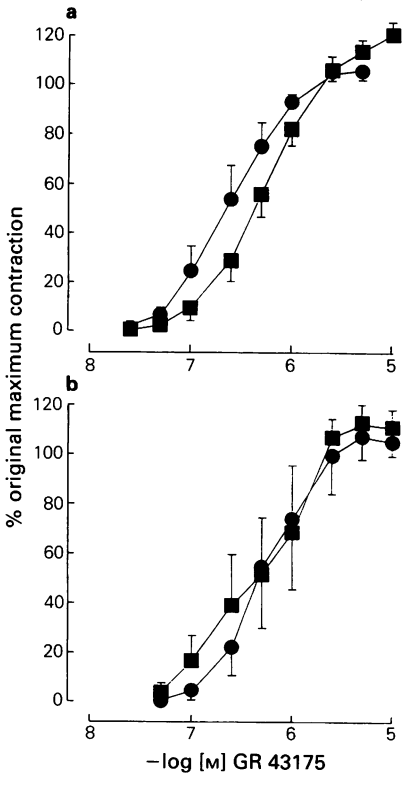

Figure 3 Concentration-effect curves to GR43175 in the dog isolated saphenous vein in the absence (●) and presence (■) of ketanserin (1 μM) in (a) or MDL 72222 (■) (1 μM) in (b). Curve in the absence of antagonist is the second GR43175 concentration-effect curve in the control preparation and served as a time-matched control. Values are mean from four experiments, with s.e.mean shown by vertical bars. In (a) dose-ratio = 1.1 (0.6–1.8) and in (b) dose-ratio = 1.1 (0.3–4.4).

Other antagonists in dog isolated saphenous vein

The contractile effect of GR43175 was also resistant to antagonism by a range of antagonists, including phentolamine (1 μM), mepyramine (1 μM), the thromboxane A_2 receptor blocking drug, AH23848 (1 μM), and atropine (1 μM). The GR43175 concentration-ratios were: 1.8 (1.6–2.0); 0.6 (0.3–1.2); 0.8 (0.6–1.3); 1.0 (0.7–1.4), respectively (geometric mean values and 95% confidence limits for minimum of three determinations for each value).

Other vascular preparations

In isolated vascular preparations of dog femoral artery, pig anterior descending coronary artery and

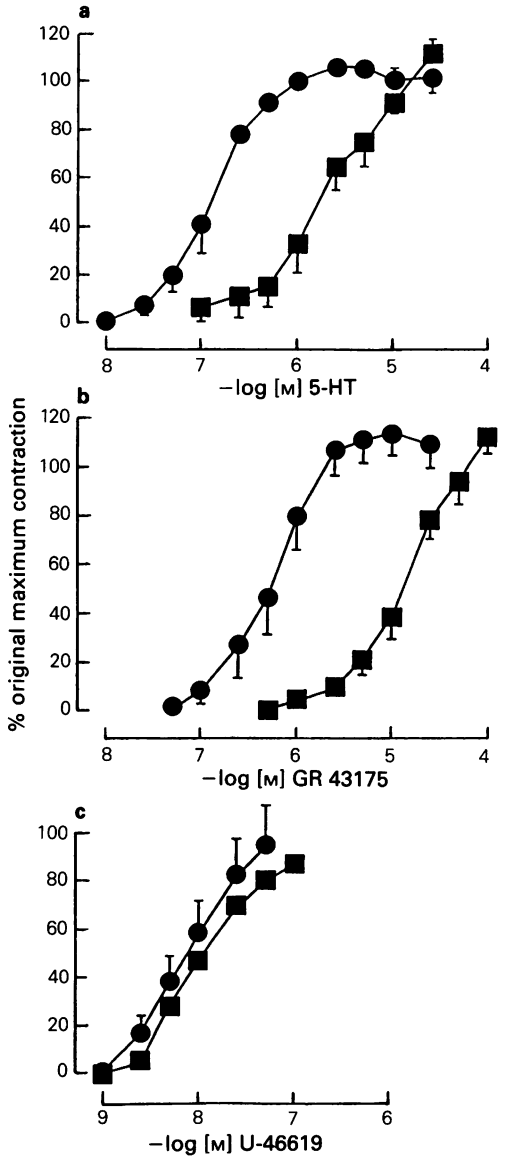

Figure 4 Concentration-effect curves to (a) 5-hydroxytryptamine (5-HT), (b) GR43175 and (c) U-46619 in the dog isolated saphenous vein in the absence (●) and presence (■) of methiothepin (0.1 μM). Curve in the absence of antagonist is the second agonist concentration-effect curve in the control preparation and served as a time matched control. Values are mean from four experiments with s.e.mean shown by vertical bars. Note the similarity in the degree of antagonism produced by methiothepin against 5-HT- and GR43175-induced contractile responses. In (a) 5-HT dose-ratio = 11 (2.5–50); in (b) GR43175 dose-ratio = 14 (2.8–72) and in (c) U-46619 dose-ratio = 1.1 (0.5–2.6).

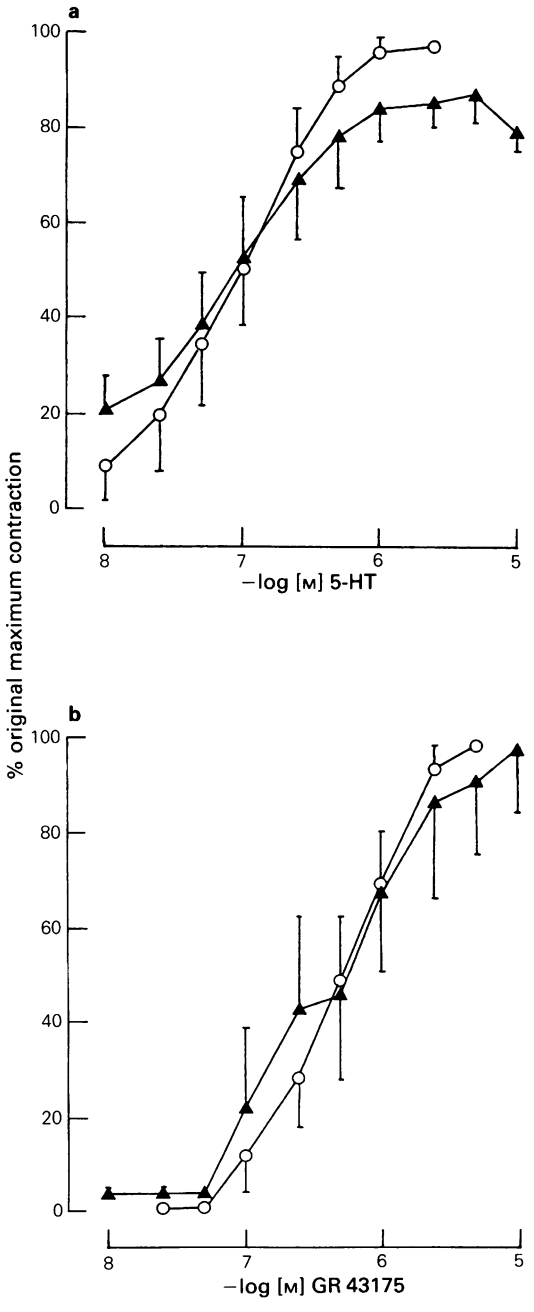

Figure 5 Concentration-effect curves to (a) 5-hydroxytryptamine (5-HT) and (b) GR43175 in the dog isolated saphenous vein in the absence (○) and presence (▲) of cyanopindolol (1 μM). Curve in the absence of antagonist is the second agonist concentration-effect curve in the control preparation and served as a time matched control. Values are mean from four experiments; s.e.mean shown by vertical bars.

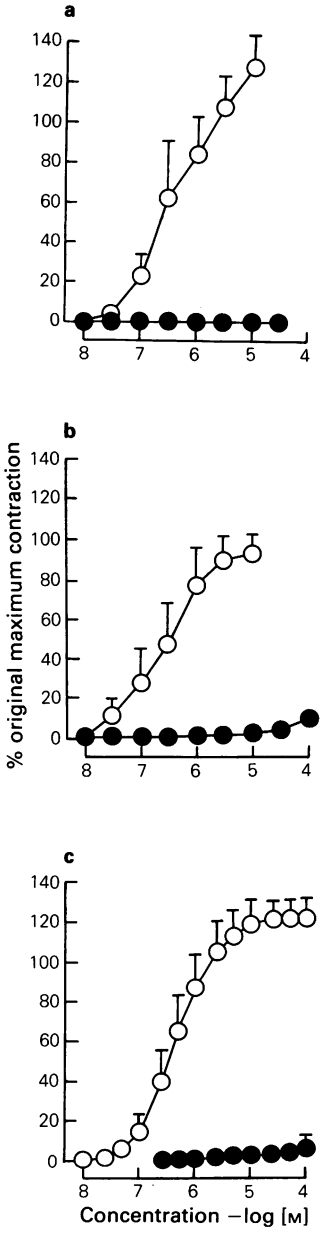

Figure 6 Concentration-effect curves to 5-hydroxytryptamine (5-HT) (○) and GR43175 (●) in (a) pig isolated coronary artery, (b) greyhound isolated coronary artery and (c) beagle isolated femoral artery. Curves shown are the second agonist concentration-effect curves in the control and GR43175-treated preparations. Values are mean from at least four experiments with s.e.mean shown by vertical bars. Note high agonist potency of 5-HT, yet GR43175 was virtually devoid of agonist activity in concentrations up to 30 μM.

greyhound circumflex coronary artery, 5-HT produced concentration-dependent contractile responses with EC_{50} values of approximately 0.3 μM (Figure 6). GR43175 was virtually devoid of agonist properties in these preparations in concentrations up to 30 μM and was at least 300 times weaker than 5-HT in this respect (Figure 6).

Discussion

The aim of this study was to determine the pharmacology *in vitro* of a novel 5-HT receptor agonist, GR43175, which potently and selectively activates those 5-HT_1-like receptors known to exist both pre- and post-junctionally in the dog isolated saphenous vein (Feniuk *et al.*, 1985; Humphrey & Feniuk, 1987). GR43175 was approximately four times weaker than 5-HT at causing contraction of the dog isolated saphenous vein and at inhibiting the contractile response of the dog isolated saphenous vein to electrical field stimulation. The ability of methiothepin to antagonize specifically the contractile effect of both 5-HT and GR43175 to the same degree suggests that GR43175 activates the same receptor mechanism as 5-HT. This agonist activity of GR43175 was resistant to antagonism by the selective 5-HT_2 and 5-HT_3 receptor blocking drugs, ketanserin and MDL 72222, respectively. A possible action of GR43175 at non-5-HT receptors can be excluded by the absence of its antagonism by a wide range of specific receptor blocking drugs, including atropine, mepyramine, phentolamine and the thromboxane A_2 receptor antagonist, AH23848 (Brittain *et al.*, 1985).

The remarkable selectivity of action of GR43175 for the 5-HT_1-like receptors in the dog saphenous vein was further demonstrated by the absence of agonist activity at 5-HT_1-like receptors mediating relaxation of the cat isolated saphenous vein, 5-HT_2 receptors mediating contraction of the rabbit isolated aorta and 5-HT_3 receptors mediating depolarization of the rat vagus nerve. Furthermore, the absence of agonist properties of GR43175 in these isolated tissue preparations did not merely reflect differences in receptor concentration or stimulus-response coupling processes (e.g. see Kenakin & Beek, 1980), since GR43175 was also devoid of 5-HT antagonist activity in these preparations. Additionally we carried out experiments with GR43175 in a range of other isolated vascular preparations containing 5-HT_2 receptors, such as the dog femoral artery and pig and greyhound coronary artery (Apperley *et al.*, 1976; Cocks & Angus, 1983) and in all these tissues GR43175 was devoid of agonist properties in concentrations as high as 30 μM.

The similarity in the relative potency of GR43175, compared with 5-HT, at pre- and post-junctional 5-HT receptors in the dog isolated saphenous vein provides further evidence that the 5-HT_1-like receptors mediating both effects are similar (Feniuk *et al.*, 1981; Muller-Schweinitzer, 1981; Watts *et al.*, 1981). Previous studies have suggested that the 5-HT receptor mediating inhibition of sympathetic neurotransmission in the dog isolated saphenous vein bears similarities with the [^{3}H]-5-HT binding sites identified in rat brain cortex (Engel *et al.*, 1983). Furthermore, we have suggested (Feniuk *et al.*, 1985) that this same 5-HT receptor mediating contraction of the dog isolated saphenous vein may be similar to a subpopulation of these binding sites that can be characterized by a high affinity for cyanopindolol, namely the 5-HT_{1B} binding site (Hoyer *et al.*, 1985). The results from the present study unequivocally demonstrate that the agonist properties of both 5-HT and GR43175 are not a consequence of activation of 5-HT_{1B} receptors since a high concentration of cyanopindolol did not modify the actions of either agonist. However, despite this clear difference, GR43175 has the ability to displace I^{125}-cyanopindolol from its binding site in rat brain cortex (pKi = 6.48, M.J. Sumner, unpublished observation) with a potency which is almost the same as its $-\log EC_{50}$ value for causing contraction of the dog saphenous vein, suggesting that similarities exist. Nevertheless, the 5-HT receptor mediating smooth muscle contraction and inhibition of sympathetic neurotransmission in the dog isolated saphenous vein appears more like the inhibitory prejunctional 5-HT_1-like receptor identified in the rat isolated perfused kidney which is also similar to, but not the same as, any of the 5-HT_1 sub-types of brain ligand binding site yet identified (Charlton *et al.*, 1986; Humphrey & Feniuk, 1987). Thus, the effects of GR43175 in dog saphenous vein are not only resistant to antagonism by the 5-HT_{1A} and 5-HT_{1B} ligand, cyanopindolol, but are also resistant to antagonism by both mesulergine and metergoline, which have high affinities at 5-HT_{1C} and 5-HT_{1D} recognition sites, respectively (see Heuring & Peroutka, 1987). However, one major difference between the two studies is that in the rat kidney metergoline is an antagonist (Charlton *et al.*, 1986).

One of the most important characteristics of a 5-HT_1-like receptor, as defined by Bradley *et al.*, is the high agonist potency of 5-CT (Bradley *et al.*, 1986). We have shown that 5-CT is a highly active agonist at both pre- and post-junctional 5-HT receptors in the dog isolated saphenous vein as well as 5-HT receptors mediating relaxation of vascular smooth muscle (Feniuk *et al.*, 1981; 1984; 1985; Trevethick *et al.*, 1986; Humphrey & Feniuk 1987). The most remarkable finding from the present study was

that GR43175 has a more selective effect than 5-CT, retaining high agonist potency at 5-HT$_1$-like receptors in the dog saphenous vein but being devoid of both agonist and antagonist properties at 5-HT$_1$-like receptors mediating relaxation of vascular smooth muscle. Indeed, this difference in the pharmacological profile of 5-CT and GR43175 is further exemplified by studies *in vivo*, where 5-CT produces profound vasodilatation in the carotid artery bed of anaesthetized dogs and cats (see Feniuk *et al.*, 1984; Connor *et al.*, 1986), whilst GR43175 produces a highly localised vasoconstriction in the same arterial bed (Brittain *et al.*, 1987). This vasoconstrictor effect is largely associated with a decrease in blood flow through carotid arterio-venous anastomoses (Feniuk *et al.*, 1987) and clearly supports the view that the 5-HT$_1$-like receptor mediating contraction of the dog isolated saphenous vein also mediates vasoconstriction of carotid arterio-venous anastomoses (see Apperley *et al.*, 1980; Saxena & Verdouw, 1985; Verdouw *et al.*, 1985; Humphrey *et al.*, 1987b).

In conclusion, the results from the present study demonstrate that GR43175 is a highly selective 5-HT$_1$-like receptor agonist. Furthermore, the absence of an action of GR43175 at 5-HT$_1$-like receptors mediating relaxation of vascular smooth muscle provides evidence that 5-HT$_1$-like receptors are heterogeneous. GR43175 should be a useful tool for the further characterization of 5-HT$_1$-like receptors, being even more selective than 5-CT. Indeed, using GR43175 we have recently shown that dog and primate cerebral blood vessels contain 5-HT receptors mediating contractions which are similar to those occurring in the dog isolated saphenous vein (Connor *et al.*, 1987).

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