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Pharmacological characterization of almotriptan: an indolic 5-HT receptor agonist for the treatment of migraine

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Abstract

Almotriptan (3-[2-(dimethylamino)ethyl]-5-(pyrrolidin-1-ylsulfonylmethyl)-1*H*-indole) has been studied in several models predictive of activity and selectivity at 5-HT receptors. Almotriptan showed low nanomolar affinity for the 5-HT_{1B} and 5-HT_{1D} receptors in several species, including the human, while affinity for 5-HT receptors other than 5-$HT_{1B/1D}$ was clearly less. Affinity for 5-HT_7 and 5-HT_{1A} receptors was approximately 40 and 60 times lower than that for 5-$HT_{1B/1D}$ receptors, respectively. Almotriptan did not exhibit significant affinity for several non-5-HT receptors studied up to 100 μM. Almotriptan inhibited forskolin-stimulated cyclic AMP accumulation in HeLa cells transfected with 5-HT_{1B} or 5-HT_{1D} human receptors. In this model, almotriptan had the same efficacy as serotonin and an affinity in the low nanomolar range. It induced vasoconstriction in several vessels in which it was compared with sumatriptan. In isolated dog saphenous veins, almotriptan elicited concentration-dependent contractions with an EC_{50} of 394 nM. In both these systems, almotriptan behaved as a full agonist. Infusion of almotriptan into the porcine meningeal vasculature induced vasoconstriction. In contrast, in the pig renal and rabbit mesenteric arteries, it had a very low maximal efficacy even at 100 μM, with similar results obtained in the rabbit renal artery. The results suggest that almotriptan is a potent and selective 5-$HT_{1B/1D}$ receptor agonist, with selectivity for the cranial vasculature as compared with peripheral vessels.

Keywords: 5-HT receptor; cAMP accumulation; Vasoconstriction

1. Introduction

Several observations indicate that serotonin (5-hydroxytryptamine, 5-HT) is involved in the etiopathogenesis of migraine, suggesting that compounds mimicking 5-HT at craniovascular receptors should abort a migraine attack (see Humphrey et al., 1990, for a review). This led to the synthesis and development of sumatriptan for the treatment of migraine and to the discovery that sumatriptan was acting on a selective sub-population of 5-HT receptors (Humphrey et al., 1988), at least 14 different subtypes which have been described (Hoyer et al., 1994). In particular, sumatriptan has been shown to possess a high degree of selectivity for at least two different receptor subtypes, currently named 5-HT_{1B} and 5-HT_{1D} (Hartig et al., 1996). Both receptors have been found to be present in humans and laboratory animals, particularly in the brain, although at different levels of expression (Hoyer et al., 1994).

While sumatriptan has been found to be highly efficacious when given intravenously, its oral administration resulted in less effect, probably due to its low oral absorption. However, sumatriptan has been found to produce some cardiovascular side effects, which (although present in a minority of patients) complicate its use. Additionally, probably due to the relatively short half-life of the compound, the percentage of patients experiencing a recurrent attack after oral sumatriptan administration has been found to be important (ranging from 21% to 51%) (Perry and Markham, 1998).

In the search for safer and longer-acting compounds, a number of second generation triptans have been investigated, developed and made available on the market, whilst others are in different stages of clinical development (Saxena and Ferrari, 1996). Even though few comparative

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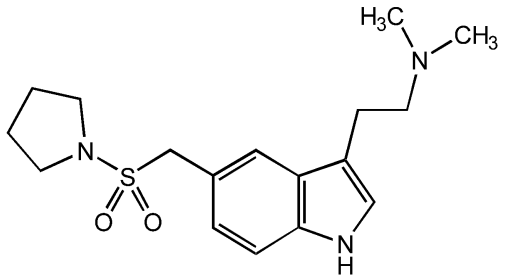

Fig. 1. Chemical structure of almotriptan.

studies have been performed concerning their efficacy and safety, their profiles do not seem to differ much from that of sumatriptan.

We now describe the preclinical properties of a new 5-$HT_{1B/1D}$ receptor agonist almotriptan (Fig. 1) which was selected on the basis of its preclinical properties predictive of a clinical profile better than that of sumatriptan.

2. Methods

2.1. Radioligand displacement assays

Post-mortem human brain tissues were provided by the Department of Neurology of the Hospital Clínic i Provincial (Barcelona, Spain). These tissues, obtained at autopsies, were immediately dissected and frozen at −70°C until use. Fresh bovine brains were obtained from Decoinsa (Barcelona, Spain). They were dissected at the laboratory and frozen at −70°C until the membranes were prepared. Tissues from male Wistar rats (Harlan Interfauna Ibérica, Sant Feliu de Codines, Spain) weighing from 200 to 250 g were used.

HeLa cells transfected with either the human 5-HT_{1D} (MA6A clone) or the human 5-HT_{1B} (5AET14 clone) receptors (Domènech et al., 1997) were cultured at 37°C in Minimum Essential Medium with Earle's salts (EMEM medium) containing 10% foetal calf serum and 600 μg/ml of gentamicin under a O_2/CO_2 (95:5) atmosphere. For binding experiments the medium was replaced 1 day before collection (90% confluence), by a medium containing dialysed serum. Cells were harvested by scraping the dish and centrifugation. The pellets were frozen at −70°C until processing.

For the different receptor preparations, membranes were obtained from the corresponding tissues by homogenisation and centrifugation and were resuspended in binding buffer according to the procedures described in the published methods. The experimental details of such methods and their references are summarised in Table 1.

In order to determine the displacement of the different radioligands, serial dilutions of the products were transferred directly to test tubes with the corresponding radioligand and membrane preparation, and incubated at the appropriate temperature.

At least seven concentrations of each product were tested in triplicate to obtain an individual displacement curve. The mean of these values was used to either determine the concentration of compound inhibiting by 50% the maximum binding of the radioligand to the receptor (IC_{50}), using non-linear regression, or the percentage displacement obtained at the highest concentration tested, when 50% displacement was not reached. The programme PRISM (GraphPad Software, version 2.0, San Diego, CA, USA) was used for this purpose. The results are expressed as mean IC_{50} or percentage displacement. Dispersion is indicated as S.E.M. and the number of independent assays was used as n. to calculate these. Protein concentrations were determined according to Bradford (1976).

In addition, competition for the binding to the following panel of non-5-HT receptors was tested: rat α_1- and α_2-adrenoceptors (Greengrass and Bremner, 1979; Uhlen

Table 1
Experimental conditions used in the radioligand displacement assays

Receptor subtype	Source	Radioligand		Non-specific binding agent		Reference
		Name	Concentration (nM)	Name	Concentration	
5-HT_{1A}	Rat hippocampus	[^{3}H]8-OH-DPAT	0.5	5-HT	1 mM	Deliganis et al. (1991)
5-HT_{1B}	Human recombinant (HeLa cells)	[^{125}I]GTI	0.1	5-HT	1 mM	Bruinvels et al. (1991)
5-HT_{1D}	Human recombinant (HeLa cells)	[^{125}I]GTI	0.1	5-HT	1 mM	Bruinvels et al. (1991)
5-$HT_{1B/1D}$	Calf caudate	[^{125}I]GTI	0.1	5-HT	1 mM	Bruinvels et al. (1991)
5-HT_{2A}	Human cortex	[^{3}H]ketanserin	2	Mianserin	10 μM	Pazos et al. (1984b)
5-HT_{2C}	Pig choroid plexus	[^{3}H]mesulergine	1	5-HT	1 μM	Pazos et al. (1984a)
5-HT_3	N1E-115 cells	[^{3}H]BRL 43694	1	Metoclopramide	100 μM	Hoyer and Neijt (1988)
5-HT_4	Human caudate	[^{3}H]GR 113808	0.12	5-HT	10 μM	Domènech et al. (1994)
5-HT_6	Rat recombinant (HEK 293 cells)	[^{3}H]LSD	1	5-HT	10 μM	Monsma et al. (1993)
5-HT_7	Rat recombinant (HEK 293 cells)	[^{3}H]LSD	1	5-HT	10 μM	Shen et al. (1993)

and Wikberg, 1991), rat β_1- and β_2-adrenoceptors (Rimele et al., 1986), rat adenosine A_1, bovine A_{2A} and guinea-pig adenosine uptake site (Borea et al., 1991; Jarvis et al., 1989; Verma and Marangos, 1985), rat angiotensin AT_1 and bovine AT_2 (Chiu et al., 1990; Bottari et al., 1991), guinea-pig histamine H_1 and H_2 (Gajtkowski et al., 1983), human dopamine D_1 and D_2 (Zhou et al., 1990; Grandy et al., 1989), human endothelin ET_A and ET_B (Buchan et al., 1994), rat CGRP (Mimeault et al., 1992), human muscarinic M_1, M_2 and M_3 (Waelbroek et al., 1990), rat non-selective opiate (Childers et al., 1979) and human tachykinin NK_1, NK_2 and NK_3 (Heuillet et al. 1993; Aharony et al., 1993; Guard et al., 1990).

2.2. Modulation of the forskolin-induced cAMP accumulation on human 5-HT_{1B} or human 5-HT_{1D} receptor transfected HeLa cells

Cells were plated in 24-well plates at a density of 2×10^4 cells/well and allowed to grow for 96 h. For the last 24 h before the assay, the medium was replaced by fresh medium containing dialyzed serum. Cells, 90% confluent, were washed twice with Hank's buffered salt solution containing 10 mM HEPES, pH 7.55 at room temperature and preincubated in the same solution containing 100 μM of the phosphodiesterase inhibitor, 3-isobutyl-1-methylxanthine (IBMX), for 20 min at 37°C. After that, the cells were incubated with 10 μM forskolin and seven or eight different concentrations of the corresponding drug for an additional 10-min period at 37°C. The reaction was terminated by aspiration of the medium and addition of 500 μM of ice-cold absolute ethanol per well. After 30 min, the contents of the wells were transferred to tubes and the wells were rinsed with an additional 250 μM ethanol, which was added to the first extract. The samples were then dried and frozen until cAMP was quantified, using a commercial enzyme immunoassay kit (Biotrak, Amersham, Buckinghamshire, UK).

The cAMP levels obtained were used to calculate the IC_{50} values for various compounds, using non-linear regression and the programme, PRISM (version 2.0). The cAMP levels obtained in the presence of forskolin and in the absence of compound were taken as 100%. Values are given as the means of three independent experiments run in duplicate ± S.E.M.

2.3. Contractile effects on dog saphenous veins

Tissue was obtained from Beagle dogs (Biocentre, Sant Feliu de Codines, Spain) of either sex weighing 10–12 kg. After being fasted with free access to water for 16 h, the animals were anaesthetised with sodium pentobarbital (35 mg/kg, i.v.). The saphenous vein of each hindlimb was dissected out and maintained in Krebs solution (composition in mM concentration: NaCl 118, KCl 4.7, $NaHCO_3$ 25, $MgCl_2$ 1.2, NaH_2PO_4 1, $CaCl_2$ 2.6 and glucose 11.1) at room temperature, supplemented with 1 μM ketanserin, pyrilamine and atropine.

Rings of 2–3 mm in length were cut and suspended isometrically in 30-ml organ baths containing the supplemented Krebs solution at 37°C, gassed with 5% CO_2 in O_2. After a 30-min stabilisation period, during which tension was adjusted to 2 g, contractions were induced with 5-HT (1 μM). When uniformity of isometric tension was achieved, compounds were added at increasing concentrations from 0.01 to 10 μM and the contractions induced were recorded. The values obtained were expressed as percentages of the last response to 1 μM of 5-HT and used to calculate the EC_{50} (50% of maximum effect) using linear regression.

2.4. Contractile effects on pig meningeal arteries

Complete skulls from Pietrain pigs of either sex, weighing 80–90 kg, were obtained from a local slaughterhouse (Vic, Spain) and transferred to the laboratory at 4°C. A bone portion of 20 cm^2 was removed from the temporal part of the skull and the meningeal artery was exposed and cannulated using a round-pointed needle 21 gauge (0.8×2.5 mm) connected through polyethylene tubing to a perfusion pump. The prepared specimen was placed in a thermostatic chamber at 37°C and the meningeal vascular bed was perfused at a constant flow of 3.3 ml/min with 10 μM N^G-nitro-L-arginine methyl ester (L-NAME) supplemented, 5% CO_2 in O_2 equilibrated, Krebs solution at 37°C. A pressure transducer (HP transducer, type # 1146-DPT-100) inserted in the circuit allowed measurement of the changes in perfusion pressure that were continuously recorded on a polygraph (7758-B Hewlett Packard, Palo Alto, CA, USA).

After an initial 30-min stabilisation period, functional activity of the vascular tissue was assessed by its contractile response to 40 mM KCl. Positive responders were then exposed, at 30-min intervals, to successive challenges with 0.1 μM 5-HT dissolved in the perfusion fluid until a stable response was obtained. Study compounds were then administered in the same way at 7-min intervals at concentrations ranging from 0.001 to 100 μM. The concentration eliciting a contractile response equivalent to that caused by 0.1 μM 5-HT, calculated using linear regression, was defined as the intrinsic activity relative to 5-HT. The maximal agonist effect was expressed as a percentage of the contractile response obtained with 0.1 μM 5-HT.

2.5. Vasoconstrictor effects on other vessels

Pig kidneys were obtained from a local slaughterhouse and maintained at 4°C until transfer to the laboratory. Male New Zealand White rabbits (weight range 1.5–2.0 kg) were obtained from Biocentre (Sant Feliu de Codines,

Spain) and were fasted for 16 h, but with free access to water before being sacrificed by concussion. Proximal pig renal and rabbit upper mesenteric and renal arteries were dissected and maintained in Krebs solution at room temperature. Rings of 2–3 mm in length were cut and suspended isometrically in 30-ml organ baths filled with 5% CO_2 in O_2 equilibrated Krebs solution.

After a 30-min stabilisation period, during which isometric tension was recorded and adjusted to 2 g, contractions were induced with 5-HT (10 μM). When uniformity of response had been achieved, the study compounds were added at increasing concentrations ranging from 0.01 to 100 μM. The contractions induced by the compounds were recorded and expressed as percentages of the initial response to 10 μM of 5-HT, EC_{50} was calculated using linear regression.

All experimental procedures relating to animals described in this paper were previously submitted to the regulatory authorities, and guidelines approved by the Catalan Parliament were strictly followed.

2.6. Data analysis

For the isolated organ studies, statistical significance of differences between compounds was evaluated, using a two-tailed Student's *t*-test for unpaired data.

2.7. Drugs and reagents

The drugs and reagents used were: atropine sulphate, 2-chloroadenosine, forskolin, 5-HT creatine sulphate, IBMX, isoprenaline, L-NAME hydrochloride, prazosin, pyrilamine maleate, [*R*]-N^6-(2-Phenylisopropyl)-adenosine, [Sar^1-Ile^8]angiotensin II and yohimbine were from Sigma-Aldrich Química (Alcobendas, Spain). Ketanserin tartrate, 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) and mianserin were from RBI (Natick, MA, USA). HEPES was from Merck Farma y Química (Mollet del Vallés, Spain). Almotriptan hydrochloride, sumatriptan, 5-carboxamidoytryptamine (5-CT), 2-methyl-4-(5-methyl-[1,2,4]oxadiazol-3-yl)-biphenyl-4-carboxylic acid[4-methoxy-3-(4-methyl-piperazin-1-yl)-phenyl]-amide (GR127935), [1-[2-[(methylsulphonyl)amino]ethyl]-4-piperidinyl]methyl 1-methyl-1*H*-indole-3carboxylate (GR-113808) and losartan were synthesised at the Medicinal Chemistry Department of Almirall Prodesfama. Cell culture reagents were from Gibco (Life Technologies, Paisley, UK).

3. Results

3.1. Affinity for serotoninergic receptors

The affinity of almotriptan and sumatriptan in comparison to that of appropriate reference compounds was determined using specific radioligand displacement assays for the different serotonin receptor subtypes. As shown in Table 2, almotriptan exhibited a high affinity ($IC_{50} = 13 \pm 1$ nM) for calf caudate 5-$HT_{1B/1D}$ receptors. Equivalent values were obtained for the cloned human 5-HT_{1B} and 5-HT_{1D} receptor subtypes ($IC_{50} = 12 \pm 1$ and 13 ± 1 nM, respectively). The affinity of sumatriptan for these receptors was similar to that found for almotriptan. Almotriptan had lower affinity for all the other serotoninergic receptors tested. In particular it showed selectivity in excess of 60-fold for the closely related 5-HT_{1A} receptor, a value similar to the one found for sumatriptan.

3.2. Affinity for different non-serotoninergic receptors

When tested against a panel of receptors in radioligand binding assays, almotriptan showed less than 50% displacement at a concentration of 100 μM for the following receptor subtypes: adenosine A_1, A_{2A} and uptake site, angiotensin AT_1 and AT_2, histamine H_1 and H_2, dopamine D_1 and D_2, endothelin ET_A and ET_B, CGRP, muscarinic M_1, M_2 and M_3 and tachykinin NK_1, NK_2 and NK_3.

Table 2
Affinity of almotriptan, sumatriptan and reference compounds for different 5-HT receptors

Receptor subtype	Species	Almotriptan	Sumatriptan	Reference compound	
		IC_{50}	IC_{50}	Name	IC_{50}
5-HT_{1A}	Rat	$8.5 \pm 0.7 \times 10^{-7}$	$4.6 \pm 0.7 \times 10^{-7}$	8-OH-DPAT	$1.8 \pm 0.2 \times 10^{-9}$
5-HT_{1B}	Human	$1.2 \pm 0.7 \times 10^{-8}$	$3.6 \pm 0.8 \times 10^{-9}$	5-CT	$6.0 \pm 0.1 \times 10^{-10}$
5-HT_{1D}	Human	$1.3 \pm 0.1 \times 10^{-8}$	$7.83 \pm \times 10^{-9}$	5-CT	$2.8 \pm 1.8 \times 10^{-9}$
5-$HT_{1B/1D}$	Calf	$1.3 \pm 0.1 \times 10^{-8}$	$7.8 \pm 1.6 \times 10^{-9}$	5-CT	$9.8 \pm 0.5 \times 10^{-10}$
5-HT_{2A}	Human	$2.51. \pm 1 \times 10^{-5}$	$8.8 \pm 2.5 \times 10^{-5}$	Ketanserin	$1.6 \pm 0.1 \times 10^{-8}$
5-HT_{2C}	Pig	54% at 100 μM	n.t.	5-HT	3.2×10^{-8}
5-HT_3	Mouse	37% at 100 μM	n.t.	MDL72222	1.46×10^{-8}
5-HT_4	Human	$1.4 \pm 1.1 \times 10^{-4}$	n.t.	GR113808	$2.3 \pm 0.1 \times 10^{-8}$
5-HT_6	Rat	23% at 1 μM	n.t.	5-HT	3.19×10^{-7}
5-HT_7	Rat	63% at 1 μM	n.t.	5-HT	5.3×10^{-9}

Data from at least three independent experiments are expressed as the means ± S.E.M. of either IC_{50} values in molar concentrations or the percentage inhibition obtained at the indicated concentration. n.t. indicates not tested.

Table 3
Comparison of drug potency and efficacy values as inhibitors of forskolin-induced cAMP accumulation in 5-HT_{1B} and 5-HT_{1D} receptor-transfected HeLa cells

	5-HT_{1B}		5-HT_{1D}	
	EC_{50} (nM)	E_{max} (%)	EC_{50} (nM)	E_{max} (%)
5-HT	0.45 (0.29–0.69)	79 (82–76)	0.49 (0.30–0.82)	67 (70–63)
Almotriptan	1.6 (0.52–4.9)	83 (91–74)	3.1 (0.73–13)	69 (81–57)
Sumatriptan	2.3 (0.65–8.1)	81 (92–69)	2.0 (0.24–16)	54 (69–39)

E_{max} indicates the maximum percentage of reversal in cAMP increase induced by forskolin. Data are presented as the means of at least three independent experiments with 95% confidence interval between brackets.

Almotriptan showed minimal affinity (IC_{50} ranging from 10 to 100 μM) for the non-selective opiate receptors and for the α- and β-adrenoceptors (data not shown).

3.3. Modulation of forskolin-induced cAMP accumulation in HeLa cells transfected with human 5-HT_{1B} or 5-HT_{1D} receptors

The basal levels of cAMP were similar for HeLa cells stably transfected with either the 5-HT_{1B} or the 5-HT_{1D} human receptor (9.92 ± 0.8 and 9.74 ± 0.5 pmol/well, respectively, mean ± S.E.M.). Forskolin (10 μM) produced increases over basal cAMP levels of 27.3 ± 1.9-fold for the h5-HT_{1B} transfected cells and of 7.8 ± 0.45-fold for the h5-HT_{1D} transfected cells. These increases were reversed in a concentration-dependent fashion by 5-HT, with EC_{50} values of 0.45 nM and 0.49 nM for the 5-HT_{1B} and 5-HT_{1D} receptors, respectively. 5-HT only partially reversed the increase of cAMP induced by forskolin, showing efficacies of 79% and 67%, respectively for both receptors. Almotriptan also potently and concentration-dependently reduced the accumulation of cAMP in these systems (EC_{50}s = 1.6 nM and 3.1 nM at the 5-HT_{1B} and 5-HT_{1D} receptors, respectively). As shown in Table 3, the affinity found for sumatriptan was very similar at both receptors. In terms of maximal effect, both compounds were equivalent, with an efficacy comparable to that of 5-HT. The effects of almotriptan and sumatriptan on forskolin-induced cAMP accumulation in the 5-HT_{1B} and the 5-HT_{1D} transfected HeLa cells are presented in Fig. 2 in comparison with those of 5-HT. None of the agonists affected the basal levels of cAMP in the absence of forskolin pretreatment (data not shown).

3.4. Contractile effects in the dog isolated saphenous vein

Almotriptan and sumatriptan produced potent concentration-dependent contractions in the dog isolated saphenous vein. (EC_{50}s of 394 ± 40 and 574 ± 99 nM, respectively). Both compounds were significantly less potent than 5-HT (EC_{50} of 135 ± 14 nM, $P < 0.05$). Fig. 3 shows that both almotriptan and sumatriptan behaved as full

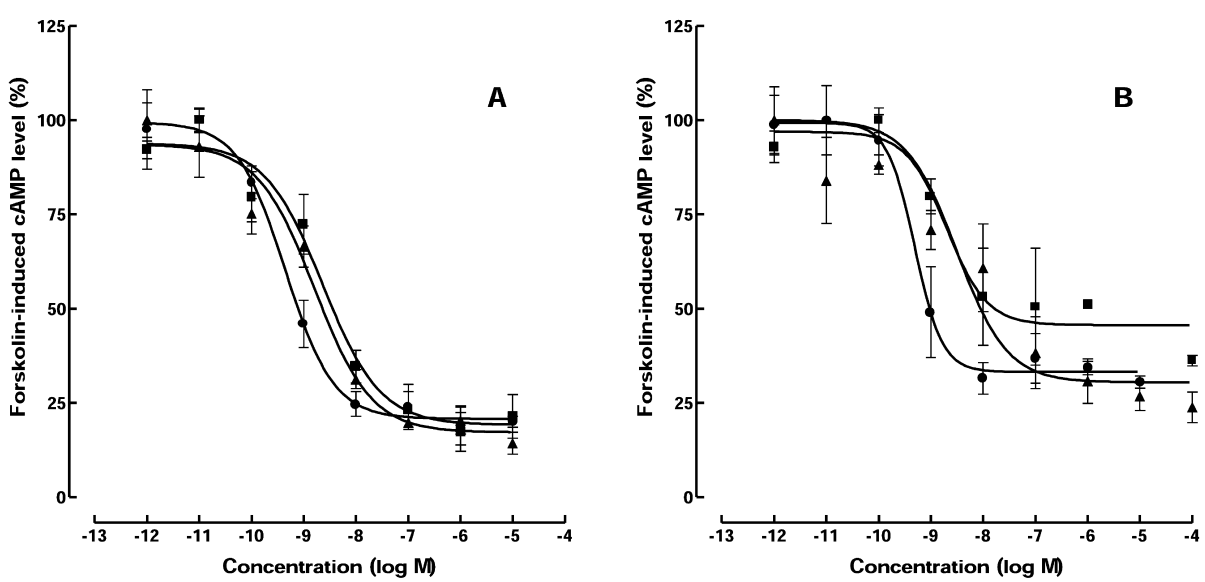

Fig. 2. Concentration–response curves for the inhibition of forskolin-stimulated cAMP accumulation by 5-HT (●), almotriptan (▲) or sumatriptan (■) in intact cells stably expressing recombinant human 5-HT-1B (panel A) or 5-HT-1D (panel B) receptors.

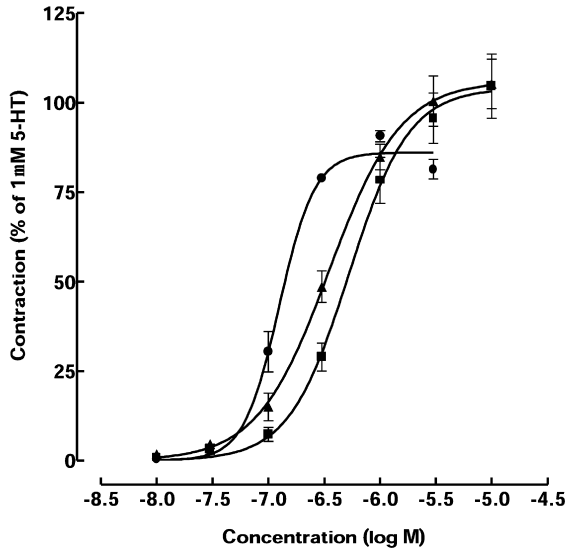

Fig. 3. Cumulative concentration–response curves for 5-HT (●), almotriptan (▲) and sumatriptan (■) in the dog isolated saphenous vein. Data are shown as percentages of the contractile response obtained initially with 1 μM 5-HT, and represent the means of between 5 and 13 experiments, with S.E.M. indicated by the vertical bars.

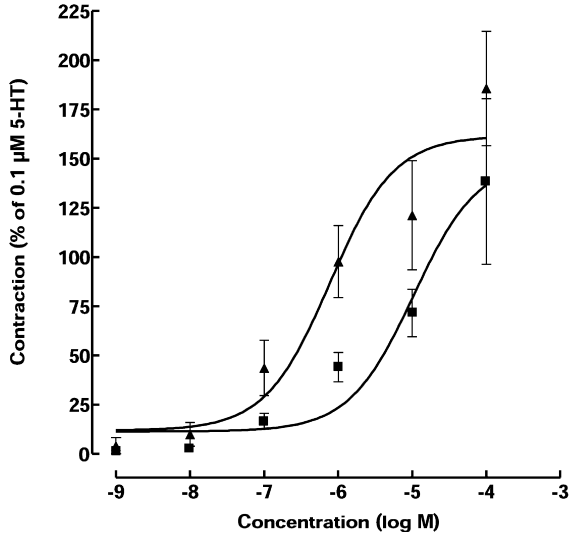

Fig. 4. Cumulative concentration–response curves for almotriptan (▲) and sumatriptan (■) in the perfused pig meningeal artery. Data are shown as percentages of the response obtained initially with 0.1 μM 5-HT, and represent the means of seven experiments, with S.E.M. indicated by the vertical bars.

agonists in this preparation. A summary of agonist potency and efficacy estimates is shown in Table 4. The interaction of almotriptan with 5-$HT_{1B/1D}$ receptors was confirmed by looking at the effect of GR127935, a selective antagonist for these receptors (Skingle et al., 1993), on the contractile response induced by almotriptan. Preincubation of the preparations with varying concentrations of GR127935 for 30 min before addition of almotriptan produced a concentration-dependent inhibition of the response to almotriptan, with an IC_{50} of 0.6 nM.

3.5. Contractile effects on pig meningeal artery preparations

Both almotriptan and sumatriptan produced concentration-dependent contractions in this preparation. However, almotriptan showed an intrinsic activity relative to 5-HT more than 10-fold greater than that of sumatriptan. In addition, the maximal agonist effect of almotriptan was significantly higher than sumatriptan in this preparation (Fig. 4). A summary of agonist potency and efficacy estimates is shown in Table 5.

3.6. Contractile effects on other vessels

The in vitro contractile response to almotriptan, sumatriptan and 5-HT in other vascular territories was determined. In all cases the efficacy of both drugs was much less than that of the endogenous agonist, 5-HT. With pig renal and rabbit mesenteric arteries, both almotriptan and sumatriptan exhibited maximal efficacy at the highest concentration tested (100 μM) in the range of 5–10% that of 5-HT. On rabbit renal artery, the effect of almotriptan was similar to that on the other vessels, whereas sumatriptan stimulated this preparation with low potency, reaching an

Table 4
Comparison of drug potency and efficacy values obtained in isolated blood vessels

Vessel	Compounds					
	Almotriptan		Sumatriptan		5-HT	
	EC_{50} (μM)	E_{max} (%)	EC_{50} (μM)	E_{max} (%)	EC_{50} (μM)	E_{max} (%)
Dog saphenous vein	0.394[a] ± 0.04	105 ± 7	0.574[a] ± 0.1	105 ± 9	0.135 ± 0.014	91 ± 2
Pig renal artery	n.r.	12 ± 3	n.r.	9 ± 3	1.33 ± 0.38	125 ± 13
Rabbit renal artery	n.r.	10[b] ± 4	10.5 ± 3.3	41 ± 7	0.31 ± 0.06	99 ± 6
Rabbit mesenteric artery	n.r.	5 ± 1	n.r.	12 ± 1	0.61 ± 0.11	105 ± 2.7

E_{max} indicates the maximal contractile response reached by each compound as a percentage of the contraction initially obtained with 1 μM 5-HT (dog saphenous vein) or 10 μM 5-HT (all other vessels). Data are presented as the means ± S.E.M. of 5–14 experiments. n.r.: Non-relevant value due to marginal vasoconstriction.

[a] $P < 0.05$ compared to 5-HT.

[b] $P < 0.05$ compared to sumatriptan.

Table 5
Characterization of the contractile effects of almotriptan and sumatriptan in the perfused pig meningeal artery

Compound	Intrinsic activity[a] (μM)	E_{max} (%)[b]
Almotriptan	1.6	185.6 ± 29[c]
Sumatriptan	21.0	138.4 ± 42

Data are presented as the means of seven experiments.

[a] Intrinsic activity, relative to 5-HT, was defined as the concentration eliciting a contractile response equivalent to 0.1 μM 5-HT.

[b] Maximal agonist effect (E_{max}) is expressed as a percentage, taking the contractile response obtained with 0.1 μM 5-HT as 100%.

[c] $P < 0.05$ when compared to sumatriptan and 5-HT using Student's *t*-test for unpaired data.

efficacy of 41% compared with that of 5-HT. A summary of agonist potency and affinity estimates for these preparations is shown in Table 4.

4. Discussion

The present binding and in vitro studies showed that almotriptan is a potent and selective 5-$HT_{1B/1D}$ receptor agonist. It has high affinity (in the low nM range) and high selectivity for serotonin 5-$HT_{1B/1D}$ human cloned receptors and for those in animal tissues. We have not detected major differences in the affinity of almotriptan for the cloned receptors. Almotriptan shows much lower affinities for other 5-HT receptors. The affinity of almotriptan for 5-HT_{1A} receptors is more than 60-fold lower than that for 5-$HT_{1B/1D}$ receptors, and about 40-fold lower for 5-HT_7 receptors. Similarly, significant affinity was found for 5-HT_6 receptors, although the clinical potential of ligands for 5-HT_6 and 5-HT_7 receptors is still unknown. Almotriptan has been studied in human cloned transfected 5-HT_{1F} receptors, for which it also shows significant affinity, about 20 nM (Mengod et al., unpublished results).

An effect of almotriptan on central receptors in humans is unlikely, as the data on brain penetrability of C^{14}-labelled-almotriptan in animals suggest that it is very low (J. Aubets et al., unpublished results; Almirall Prodesfarma). Consequently, it is possible to hypothesise that most, if not all, of the therapeutic effects of almotriptan should be mediated by the interaction of this compound with 5-$HT_{1B/1D}$ and, possibly, 5-HT_{1F} receptors in the meningeal vascular musculature and trigeminal ganglia in humans, a profile that is very similar to that observed for sumatriptan (Ferrari, 1998).

We have also characterised almotriptan as an agonist on these receptors, as shown by its ability to induce inhibition of forskolin-stimulated cyclic AMP formation in HeLa cells transfected with human 5-HT_{1B} or 5-HT_{1D} receptors. In these cellular systems, almotriptan exhibited the characteristics of a full agonist, and a very high affinity in the low nanomolar range. This agonistic effect was also demonstrated in several other vessels, in which almotriptan showed vasoconstrictor activity. In the isolated dog saphenous vein, concentration-dependent vasoconstriction induced by almotriptan was mediated by an agonistic effect on 5-$HT_{1B/1D}$ receptors, as demonstrated by its sensitivity to inhibition by the selective antagonist, GR127935. The EC_{50} values in this preparation were 394 and 574 nM for almotriptan and sumatriptan, respectively. It has been demonstrated that both 5-HT_{1D} and 5-HT_{1B} receptor mRNA are present in the canine saphenous vein (Cushing et al., 1994), even though other authors (Sgard et al., 1996) have not detected 5-HT_{1D} receptor mRNA in this tissue. According to the literature, a good correlation exists between agonist potency to contract human cerebral arteries and contractile potency in the canine saphenous vein (Cohen et al., 1997).

5-$HT_{1B/1D}$ receptor agonists produce vasoconstriction in the pig carotid vascular bed as a result of the reduction of cranial arteriovenous shunting (Den Boer et al., 1991). In the present experiments, almotriptan perfused through the isolated meningeal vascular bed of the pig elicited contractile responses with a potency and efficacy clearly higher than that of sumatriptan.

In other vascular beds, such as pig and rabbit renal arteries and rabbit mesenteric arteries, almotriptan is either ineffective or much less active, suggesting a profile of vascular selectivity. Although there is no mention in recent reviews (Plosker and MacTavish, 1994; Perry and Markham, 1998) of any adverse effect of sumatriptan on renal function, some contractile effects of this drug have been reported on rabbit renal artery (Tadipatri et al., 1991) and on prostaglandin $F_{2\alpha}$-pre-treated rabbit mesenteric rabbit arteries (Yildiz and Tuncer, 1995).

The activity of almotriptan as a 5-$HT_{1B/1D}$ receptor agonist has also been established in in vivo models that have been proved to be predictive of migraine activity, at least for drugs acting through 5-HT receptors. These models include assessment of the selective increase of carotid vascular resistance, and inhibition of the neurogenically evoked plasma protein extravasation of the dura mater (Gras et al., 2000). Together, results of these experiments and the data from the present experiments suggest that almotriptan, through its selective activation of 5-$HT_{1B/1D}$ receptors, is active in the neuronal and vascular components of the trigeminal system, which have been implicated in the action of serotoninergic compounds in migraine treatment. This has been confirmed by clinical trials that showed the efficacy and safety of almotriptan in the treatment of migraine attacks (Cabarrocas and Zayas, 1998).

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