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Characterisation of the 5-HT receptor binding profile of eletriptan and kinetics of [^{3}H]eletriptan binding at human 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors

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Abstract

The affinity of eletriptan ((*R*)-3-(1-methyl-2-pyrrolidinylmethyl)-5-[2-(phenylsulphonyl)ethyl]-1*H*-indole) for a range of 5-HT receptors was compared to values obtained for other 5-HT$_{1B/1D}$ receptor agonists known to be effective in the treatment of migraine. Eletriptan, like sumatriptan, zolmitriptan, naratriptan and rizatriptan had highest affinity for the human 5-HT$_{1B}$, 5-HT$_{1D}$ and putative 5-ht$_{1f}$ receptor. Kinetic studies comparing the binding of [^{3}H]eletriptan and [^{3}H]sumatriptan to the human recombinant 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors expressed in HeLa cells revealed that both radioligands bound with high specificity (> 90%) and reached equilibrium within 10–15 min. However, [^{3}H]eletriptan had over 6-fold higher affinity than [^{3}H]sumatriptan at the 5-HT$_{1D}$ receptor (K_D: 0.92 and 6.58 nM, respectively) and over 3-fold higher affinity than [^{3}H]sumatriptan at the 5-HT$_{1B}$ receptor (K_D: 3.14 and 11.07 nM, respectively). Association and dissociation rates for both radioligands could only be accurately determined at the 5-HT$_{1D}$ receptor and then only at 4°C. At this temperature, [^{3}H]eletriptan had a significantly ($P < 0.05$) faster association rate (K_{on} 0.249 min^{-1} nM^{-1}) than [^{3}H]sumatriptan (K_{on} 0.024 min^{-1} nM^{-1}) and a significantly ($P < 0.05$) slower off-rate (K_{off} 0.027 min^{-1} compared to 0.037 min^{-1} for [^{3}H]sumatriptan). These data indicate that eletriptan is a potent ligand at the human 5-HT$_{1B}$, 5-HT$_{1D}$ and 5-ht$_{1f}$ receptors and are consistent with its potent vasoconstrictor activity and use as a drug for the acute treatment of migraine headache.

Keywords: 5-HT (5-hydroxytryptamine, serotonin); 5-HT$_{1B}$ receptor; 5-HT$_{1D}$ receptor; 5-ht$_{1f}$ receptor; [^{3}H]Eletriptan; [^{3}H]Sumatriptan

1. Introduction

Eletriptan ((*R*)-3-(1-methyl-2-pyrrolidinylmethyl)-5-[2-(phenylsulphonyl)ethyl]-1*H*-indole) has high affinity for the human recombinant serotonin (5-hydroxytryptamine, 5-HT) 1B and 1D receptor subtypes (Gupta et al., 1996). These receptors belong to the G-protein-coupled 5-HT$_1$ family of 5-HT receptors which also includes the 5-HT$_{1A}$ and putative 5-ht$_{1e}$ [1] and 5-ht$_{1f}$ receptor subtypes (Hoyer and Martin, 1997). Evidence suggests that activation of 5-HT$_{1B/1D}$ receptors, by selective agonists, such as eletriptan, may provide effective treatment for migraine (Saxena and Ferrari, 1996; Meng, 1997). Indeed, eletriptan has been shown to be clinically effective in the treatment of migraine (Jackson et al., 1996). Although the precise site of action of the 5-HT$_{1B/1D}$ agonists is not entirely clear, the effectiveness of these agents in migraine is generally attributed to two mechanisms: constriction of the cerebral vasculature via post-synaptic 5-HT$_{1B}$ receptors (Humphrey and Feniuk, 1991) and blockade of dural inflammation by modulation of trigeminal afferents via pre-synaptic 5-HT$_{1B/1D}$ receptors (Moskowitz, 1992). These mechanisms are supported by the observation that the mRNA coding for the 5-HT$_{1B}$ receptor is the more abundant than that for 5-HT$_{1D}$ receptor in human blood vessels, including cerebral vasculature while mRNA for 5-HT$_{1D}$ appears to be expressed in neural tissues (Hamel et al., 1993; Ullmer et al., 1995; Bouchelet et al., 1996). Furthermore, 5-HT$_{1B}$ receptor protein, but not 5-HT$_{1D}$ receptor protein, was expressed on smooth muscle cells in human meningeal

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[1] Lower case letters (i.e., 5-ht$_{1e}$) are used in accordance with NC-IUPHAR Recommendations for Nomenclature of Receptors (Martin, 1998).

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blood vessels, while only 5-HT_{1D} receptor protein was expressed on trigeminal sensory neurones (Longmore et al., 1997). Studies have also shown mRNA coding for the 5-ht_{1f} receptor is expressed in human trigeminal ganglia and cerebral blood vessels (Bouchelet et al., 1996) and that selective 5-ht_{1f} agonists, such as LY 344864 and LY 334370 are effective in the animal models of neurogenic dural inflammation (Phebus et al., 1996, 1997) implying that affinity at 5-ht_{1f} receptors may contribute to an anti-migraine action.

The expression of genes coding 5-HT receptors in separate cell lines provides the opportunity to characterise the binding properties and assess the selectivity of novel 5-$HT_{1B/1D}$ agonists. In this study the binding profile of eletriptan at a range of recombinant 5-HT receptors was compared to the binding profile of sumatriptan, zolmitriptan, naratriptan and rizatriptan. In addition, we compared the receptor binding kinetics of [^{3}H]eletriptan with [^{3}H]sumatriptan at the human 5-HT_{1B} and 5-HT_{1D} receptors stably expressed in HeLa cells. In particular, the affinity (K_D) of [^{3}H]eletriptan and [^{3}H]sumatriptan at these receptors was determined and rates of association and dissociation were measured.

2. Materials and methods

2.1. Cell culture and membrane preparations

Separate plasmids containing the human putative 5-ht_{1e}, 5-ht_{1f} or 5-ht_{5A} receptor gene were transiently transfected, using a lipofectamine kit (Life Sciences, Paisley, Scotland), in either COS-7 cells (1e, 5a) or Human Embryonic Kidney (HEK)-293 cells (1f) which were then grown to confluency in Dulbecco's minimum essential medium (DMEM) supplemented with 10% foetal calf serum (FCS), 2 mM L-glutamine and 0.8 mg/ml geneticin. HeLa cells stably transfected with either the human 5-HT_{1D} or 5-HT_{1B} receptor gene (obtained from Dr. M. Hamblin, Seattle Veterans Affairs Medical Centre, USA) were grown to confluency in minimum essential medium (Earle's salts) supplemented with 10% FCS, 2 mM L-glutamine, 1% non-essential amino acids and geneticin (0.48 mg/ml 5-HT_{1B} and 0.8 mg/ml 5-HT_{1D}). HeLa cells stably transfected with the human 5-HT_{1A} receptor gene (obtained from Dr. Mark Caron, NC, USA) were grown to confluency in DMEM supplemented with 10% FCS and 2 mM L-glutamine and 0.8 mg/ml geneticin. NE-115 cells which express the mouse 5-HT_3 receptor (obtained from the ATCC, USA) were grown to confluency in DMEM supplemented with 10% FCS and 2 mM L-glutamine. Cells were grown in an humidified incubator at 37°C in 5% CO_2 and washed twice in phosphate buffered saline (PBS) prior to harvesting by scraping. Scraped cells were resuspended in PBS, pelleted at $1000 \times g$ for 10 min and stored at −80°C until used.

Membrane homogenates of Chinese Hamster Ovary (CHO) cells expressing the human putative 5-ht_6 and 5-HT_7 receptors were purchased from Receptor Biology (Baltimore, USA) and Biosignal (Montreal, Canada), respectively. Membrane homogenates of CHO cells expressing human 5-HT_{2A} or 5-HT_{2C} receptors were obtained from Euroscreen (Brussels, Belgium). Membrane homogenates of guinea pig brain striatum for 5-HT_4 receptor binding were prepared as previously described (Grossman et al., 1993).

Thawed cell pellets and membranes were resuspended in cold binding buffer (50 mM Tris–HCl, 4 mM $CaCl_2$, 10 μM pargyline and 0.1% ascorbic acid, pH 7.5 at 4°C) using a Polytron homogeniser (maximum speed for 2 min) and were washed three times by centrifugation at $38{,}000 \times g$ for 20 min at 4°C. After each centrifugation, the pellet was resuspended and incubated for 10 min at 37°C (to metabolise endogenous 5-HT). Final pellets were resuspended in 10 ml of buffer, aliquoted and stored at −80°C until use. The protein concentration of the resuspended membranes was determined using a Coomassie blue-based microtitre protein assay reagent (Sigma, Poole, Dorset).

2.2. [^{3}H]Eletriptan and [^{3}H]sumatriptan binding.

Binding studies were performed in a total assay volume of 500 μl containing final protein concentrations of 50–150 μg/ml. Time to equilibrium was determined by incubating 50 μl of either [^{3}H]eletriptan (85 Ci/mmol, Amersham International, Buckinghamshire, UK) or [^{3}H]sumatriptan (75–81 Ci/mmol, Amersham) with 400 μl of either 5-HT_{1B} or 5-HT_{1D} membrane homogenate for time points ranging from 1 to 60 min. These experiments were performed at either 4°C or 22°C using a K_D, or lower, concentration of the appropriate radioligand, as determined from saturation assays. Total and non-specific binding were determined in duplicate at each time point. Non-specific binding was defined by 10 μM 5-HT. Following an initial incubation period of 30 min at either 4°C or 22°C, dissociation was initiated by addition of 10 μM 5-HT, an excess concentration which over time would effectively displace all radioligand from the receptors. Total and non-specific binding were determined in duplicate at time points ranging from 1–90 min. Saturation analysis was performed over the concentration range: 0.1–50 nM for [^{3}H]eletriptan and 1–80 nM (5-HT_{1D}) or 1–100 nM (5-HT_{1B}) for [^{3}H]sumatriptan with total and NSB determined in duplicate at each radioligand concentration.

Competition assays were performed with either 0.5–1 nM [^{3}H]eletriptan or 2–5 nM [^{3}H]sumatriptan using 12 half-log concentrations of test compound and were carried out for 30 min at 22°C. Incubations were started by the addition of receptor membranes and were terminated by

rapid filtration using a Brandel Cell harvester through Wallac or Whatman GF/B filters pre-soaked in 0.5% polyethylenimine. Filters were washed 3 times with ice-cold Tris–HCl (50 mM pH 7.5). Each filter was dried and receptor bound radioligand was quantified by liquid scintillation counting either on Wallac 1404 LS counters or by using Meltilex™-coated filters on Wallac 1204 Beta counters.

2.3. 5-HT receptor binding profile

Experiments to determine the affinity of eletriptan, sumatriptan, naratriptan, zolmitriptan and rizatriptan at several 5-HT receptors were carried out using the same filtration method as described above using the conditions shown in Table 1.

2.4. Data analysis

Binding data from saturation experiments were analysed by non-linear regression using the program PRISM™ (GraphPad Software, San Diego, CA, USA). The equilibrium dissociation constant (K_D) and the maximal number of binding sites (B_{max}) for each radioligand were derived from the Langmuir equation $RL = R_t L/(K_D + L)$ where L is the concentration of free ligand concentration, RL is the concentration of receptor-bound ligand at equilibrium and R_t is the total receptor concentration.

IC_{50} (the concentration producing 50% inhibition of specific binding) and Hill coefficients were derived from 12 point curves using an in-house data fitting programme, where each inhibitor was tested in at least three individual experiments. Apparent K_i values were derived using the equation of Cheng and Prusoff (1973): $K_i = IC_{50}/(1 + L/K_D)$, where L is the concentration of radioligand. The association rate constant (K_{on} in units of min^{-1} nM^{-1}) was derived by the pseudo first-order method (Weiland and Molinoff, 1981) which takes into account the concentration (nM) of radioligand used. This method also accounts for the observed association rate (K_{obs} in units of min^{-1}) and the dissociation rate constant (K_{off} in units of min^{-1}) which were both derived directly from the association and dissociation curves, i.e., $K_{on} = (K_{obs} - K_{off})/L$. The dissociation half-life ($T_{0.5}$) was derived directly from the first-order dissociation curve generated using PRISM. Kinetic parameters were compared using a two sample, unpaired *t*-test (Microsoft Excel, Version 7.0a).

2.5. Drugs

[^{3}H]5-HT (100–130 Ci/mmol) and [^{3}H]8-hydroxy-2-(di-*n*-propylamino)tetralin ([^{3}H]8-OH-DPAT; 132 Ci/mmol)were purchased from Amersham International. Eletriptan hemisulphate, sumatriptan succinate, zolmitriptan maleate, naratriptan hydrochloride (HCl) and rizatriptan hemisulphate were synthesised at Pfizer Central Research (Sandwich, U.K). 5-HT (5-hydroxytryptamine HCl) and dihydroergotamine tartrate were purchased from Sigma (Poole, UK). Ketanserin tartrate, methysergide maleate, metergoline, methiothepin mesylate, yohimbine HCl and 8-OH-DPAT hydrobromide were purchased from Research Biochemicals International (Natick, MA, USA). All compounds were dissolved in 0.2 ml dimethyl sulfoxide (DMSO) and made to 1 mM stock solutions with distilled water, except for ketanserin which was dissolved in 50% DMSO/water and metergoline which was dissolved in 100% DMSO. All subsequent dilutions were made in distilled water.

Table 1
Conditions for 5-HT receptor binding profile experiments

Receptor	Ligand concentration (nM)	Concentration (nM)	NSB	Incubation time (min)	Temperature (°C)	Ref.
h 5-HT_{1A}	[^{3}H]8-OH-DPAT	1.5	8-OH-DPAT 10 μM	45	25	(1)
h 5-HT_{1B}	[^{3}H]5-HT	6	5-HT 10 μM	30	25	(2)
h 5-HT_{1D}	[^{3}H]5-HT	6	5-HT 10 μM	30	25	(2)
h 5-ht_{1e}	[^{3}H]5-HT	6	5-HT 10 μM	30	25	(2)
h 5-ht_{1f}	[^{3}H]5-HT	6	5-HT 10 μM	30	25	(2)
h 5-HT_{2A}	[^{3}H]ketanserin	2	ketanserin 1 μM	15	37	(3)[a]
h 5-HT_{2B}	[^{125}I]-DOI	25	DOI 1 μM	30	37	(4)[b]
h 5-HT_{2C}	[^{3}H]mesulergine	0.7	mesulergine 1 μM	30	37	(3)[a]
m 5-HT_3	[^{3}H]BRL43694	1	metoclopromide 100 μM	180	4	(5)[a]
gp 5-HT_4	[^{3}H]GR113808	0.1	5-HT 10 μM	30	37	(6)[a]
r 5-HT_{5A}	[^{3}H]5-HT	6	5-HT 10 μM	30	25	(2)
h 5-ht_6	[^{3}H]LSD	3	5-HT 10 μM	30	37	(7)[a]
h 5-HT_7	[^{3}H]5-HT	1.5	5-HT 10 μM	30	25	(2)

Experiments performed by: [a]Cerep, Celle L'Evescault, France, [b]Prof. L. Maroteaux, IGBMC, Strasbourg, France.
References: (1) Mulheron et al., 1994; (2) Zgombick et al., 1991; (3) Bonhaus et al., 1995; (4) Loric et al., 1995; (5) Hoyer and Neijt, 1988; (6) Grossman et al., 1993; (7) Monsma et al., 1993.
DOI = 2,5-dimethoxy-4-iodophenyl-2-aminopropane.

3. Results

3.1. 5-HT receptor binding profile of eletriptan

The binding affinities (pK_i) of eletriptan, sumatriptan, zolmitriptan, naratriptan and rizatriptan are shown in Table 2. Overall the binding profiles were qualitatively similar, although there were differences in absolute selectivity. Eletriptan, like the other 5-$HT_{1B/1D}$ agonists tested, consistently exhibited three principle affinities for the 5-HT_{1B}, 5-HT_{1D} and 5-ht_{1f} receptors and had similar low affinity for 5-HT_{2A}, 5-HT_{2C}, 5-HT_3, 5-HT_4 ($pK_i < 5.5$, not shown in Table 2) 5-ht_{5a} and 5-ht_6 receptors. A comparison of the selectivity ratios indicates that none of the compounds has selectivity for the 5-HT_{1B} over the 5-ht_{1f} receptor, while 5-HT_{1B} versus 5-HT_{1A} receptor selectivity varied from only 3-fold (rizatriptan) and 4.5-fold (eletriptan) to over 10-fold (zomitriptan, naratriptan and sumatriptan).

3.2. Kinetics of [^{3}H]eletriptan and [^{3}H]sumatriptan binding

Specific binding of [^{3}H]eletriptan and [^{3}H]sumatriptan to the human 5-HT_{1D} and 5-HT_{1B} receptor subtypes was time dependent with equilibrium established rapidly, typically within 10–15 min at 22°C (data not shown). Specific binding remained stable for up to 60 min (longest time point tested). All subsequent saturation and competition assays used a 30 min incubation time. Dissociation of [^{3}H]eletriptan from the human 5-HT_{1D} receptor at 22°C was rapid (data not shown) with over 50% of specific binding having dissociated within the first minute and complete dissociation achieved within 15–20 min after addition of a saturating concentration of 5-HT (10 μM). As the association and dissociation rates of both radioligands at 22°C was too rapid to determine accurately, and given that rate constants of ligand–receptor interactions are generally highly temperature sensitive (Weiland and Molinoff, 1981), experiments to determine kinetic parameters were repeated at 4°C in order to slow the approach to equilibrium and the dissociation rate (Fig. 1). Under these conditions, the specific binding of [^{3}H]eletriptan to the human 5-HT_{1D} receptor displayed a statistically significant ($P < 0.05$) faster on-rate constant than [^{3}H]sumatriptan, with K_{on} values (95% CI) of 0.249 (0.132–0.47) min^{-1} nM^{-1} and 0.024 (0.012–0.05) min^{-1} nM^{-1}, respectively. In addition, [^{3}H]eletriptan displayed a statistically significant ($P < 0.05$) slower off-rate constant than [^{3}H]sumatriptan with K_{off} values (95% CI) of 0.027 (0.023–0.031) min^{-1} and 0.037 (0.029–0.046) min^{-1}, respectively. [^{3}H]Eletriptan also displayed a statistically significant ($P < 0.05$) slower dissociation half-life compared to [^{3}H]sumatriptan with $T_{0.5}$ values (95% CI) of 26.02 (22.2–30.49) and 18.82 (15.0–23.62) min, respectively. In contrast to the 5-HT_{1D} receptor, reliable estimates of the kinetic parameters for [^{3}H]eletriptan binding to the 5-HT_{1B} receptor could not be obtained as the data for both association and dissociation was not adequately described by either a one- or two-site model.

3.3. Saturation experiments

In some of the saturation experiments, there was evidence for two binding sites with [^{3}H]eletriptan binding to the 5-HT_{1B} cell line with a high affinity component, $K_D = 0.6–1.22$ nM and a low affinity component, $K_D =$ 16–70 nM (data from three experiments). In contrast, both [^{3}H]5-HT and [^{3}H]sumatriptan labelled only a single site in this cell line. It is unlikely that this represents binding to an endogenous 5-HT receptor as there was no binding of [^{3}H]5-HT, [^{3}H]eletriptan or [^{3}H]sumatriptan to untransfected HeLa cells (data not shown). As the concentration of [^{3}H]eletriptan used in competition and kinetic experiments was < 3 nM it was assumed that negligible binding to the low affinity site would occur and that data generated in these experiments represented binding to the high affinity site. When the saturation binding data for the 5-HT_{1B} was fitted to a single site [^{3}H]eletriptan bound with a K_D of 3.14 nM compared to [^{3}H]sumatriptan which had a K_D of 11.07 nM (Table 3, Fig. 2). At the 5-HT_{1D} receptor [^{3}H]eletriptan labelled a single site which had a K_D of 0.92 nM compared to [^{3}H]sumatriptan which had a K_D of 6.58 nM. At these concentrations specific binding typically represented 90% to 95% of total binding for both radioli-

Table 2
Radioligand binding affinity (pK_i) of 5-$HT_{1B/1D}$ agonists at 5-HT receptors

Receptor	Eletriptan	Sumatriptan	Zolmitriptan	Rizatriptan	Naratriptan
h 5-HT_{1A}	7.35 ± 0.08	5.96 ± 0.06	6.64 ± 0.06	6.37 ± 0.04	7.12 ± 0.08
h 5-HT_{1D}	8.94 ± 0.04	8.04 ± 0.02	8.88 ± 0.06	7.88 ± 0.07	8.41 ± 0.07
h 5-HT_{1B}	8.0 ± 0.04	7.37 ± 0.04	7.69 ± 0.12	6.86 ± 0.13	8.09 ± 0.07
h 5-ht_{1e}	7.25 ± 0.04	5.79 ± 0.07	7.73 ± 0.05	6.77 ± 0.07	7.69 ± 0.04
h 5-ht_{1f}	7.99 ± 0.03	7.88 ± 0.06	7.54 ± 0.08	6.81 ± 0.06	8.18 ± 0.07
r 5-ht_{5a}	5.82 ± 0.14	< 5.5	6.4 ± 0.02	5.26 ± 0.03	5.47 ± 0.03
h 5-ht_6	6.28 ± 0.04	< 5.5	< 5.5	< 5.5	< 5.5
h 5-HT_7	6.7 ± 0.06	5.86 ± 0.11	7.02 ± 0.07	5.73 ± 0.13	< 5.5

h: human; r: rat.
Values represent the mean ± S.E.M. of at least three independent experiments. Hill slopes were not different from unity.

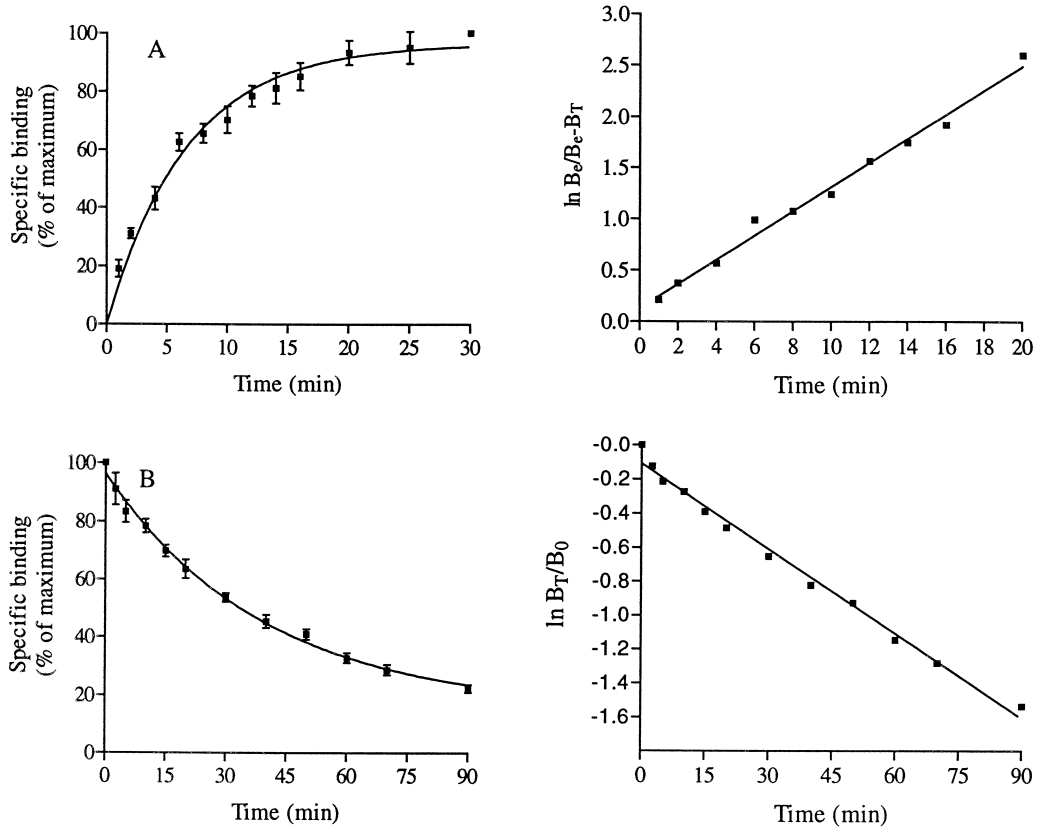

Fig. 1. Association (A) and dissociation (B) kinetics of [^{3}H]eletriptan at cloned human 5-HT$_{1D}$ receptors at 4°C. Each point on the raw association and dissociation curves represents the mean ± S.E.M. from at least three independent experiments. The linear transformations are from a representative experiment where each point is the mean of duplicate determinations and where B_e represents specific binding obtained at equilibrium, B_T is specific binding obtained at each time point tested and B_0 represents specific binding at time point 0.

gands at either receptor subtype. As Table 3 shows, estimations of receptor densities (B_{max}) were similar regardless of the radioligand used suggesting that the same population of receptors was being labelled.

Estimates of K_D for [^{3}H]eletriptan and [^{3}H]sumatriptan binding to the 5-HT$_{1D}$ receptor derived from saturation experiments were 5- and 10-fold higher than when derived from the ratio K_{off}/K_{on}. This discrepancy relates to the fact that K_{on} and K_{off} were derived from experiments performed at 4° and K_D was derived from experiments performed at 22°C. Rate constants and equilibrium constants are known to be temperature dependent (Weiland and Molinoff, 1981; Treherne and Young, 1988) and therefore in these studies it was not an unexpected finding that the K_D derived from saturation experiments differed from the ratio K_{off}/K_{on}.

3.4. Competition studies

Table 4 compares the affinities (pK_i) of a range of compounds for the human 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors determined using [^{3}H]eletriptan and [^{3}H]sumatriptan. The K_i values of both unlabelled eletriptan and sumatriptan at these receptors were in good agreement with the K_D values obtained for the radiolabelled forms shown in Table 3. In general, affinity values were similar regardless of

Table 3
Apparent equilibrium dissociation constants (K_D) and total number of binding sites (B_{max}) for [^{3}H]eletriptan and [^{3}H]sumatriptan binding to the human 5-HT$_{1D}$ and 5-HT$_{1B}$ receptors

	[^{3}H]Eletriptan		[^{3}H]Sumatriptan	
	K_D (nM)	B_{max} (fmol (mg protein)$^{-1}$)	K_D (nM)	B_{max} (fmol (mg protein)$^{-1}$)
5-HT$_{1B}$	3.14 (2.69–3.66)	2478 (1655–3711)	11.07 (6.74–16.99)	2460 (1224–4944)
5-HT$_{1D}$	0.92 (0.4–2.11)	1576 (1171–2122)	6.58 (4.06–10.66)	1493 (997–2237)

Values represent the geometric and associated 95% confidence intervals of four independent experiments. All associated Scatchard plots were linear indicating the presence of a homogenous receptor population hence all results derived from one-site fitting.

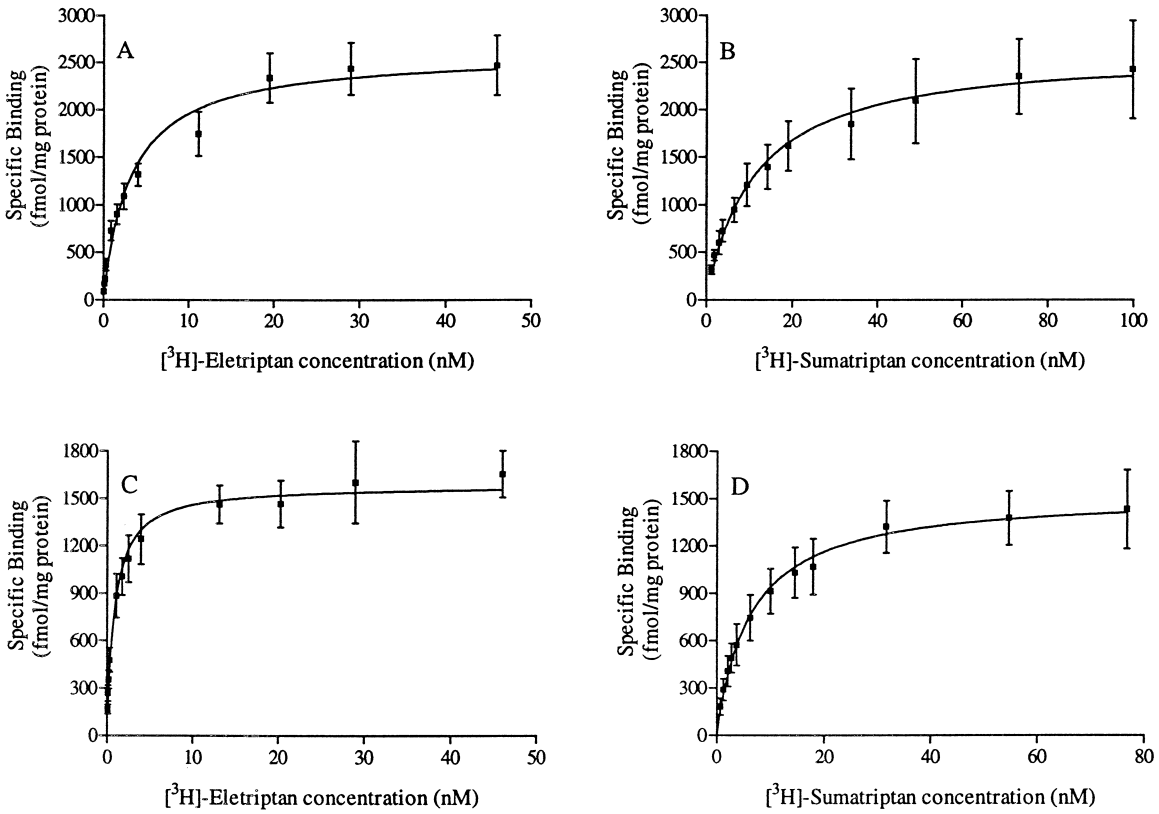

Fig. 2. Mean saturation isotherms of [^{3}H]eletriptan and [^{3}H]sumatriptan specific binding to cloned human 5-HT$_{1B}$ (A and B) and 5-HT$_{1D}$ (C and D) receptors stably expressed in HeLa cells. Each point represents the mean ± S.E.M. from four independent experiments.

radioligand used. In agreement with the published literature (Leysen et al., 1996; Pauwels et al., 1997), ketanserin was the only compound capable of reliably distinguishing between the two receptor subtypes with at least 50-fold higher affinity (based upon one-site fitting) for 5-HT$_{1D}$ over the 5HT$_{1B}$ receptor.

With the exception of ketanserin and methiothepin all competition curves generated were adequately described by a one-site model. In some assays, several agents consistently yielded slopes markedly higher than unity, e.g., naratriptan and metergoline against both radioligands at the 5-HT$_{1D}$ subtype, the most likely explanation being that

Table 4
Apparent equilibrium inhibition constants (pK_i) and Hill slopes for compounds at the human 5-HT$_{1B}$ receptor subtype using [^{3}H]eletriptan and [^{3}H]sumatriptan

	5-HT$_{1B}$				5-HT$_{1D}$			
	[^{3}H]Eletriptan		[^{3}H]Sumatriptan		[^{3}H]Eletriptan		[^{3}H]Sumatriptan	
	pK_i	Hill slope	pK_i	Hill slope	pK_i	Hill slope	pK_i	Hill slope
5-HT	8.49 ± 0.11	0.88 ± 0.02	8.61 ± 0.06	0.82 ± 0.03	8.23 ± 0.08	0.96 ± 0.02	8.30 ± 0.17	0.95 ± 0.03
Eletriptan	8.35 ± 0.05	1.00 ± 0.04	8.49 ± 0.05	1.09 ± 0.09	8.79 ± 0.03	1.08 ± 0.07	8.77 ± 0.14	1.14 ± 0.16
Sumatriptan	7.65 ± 0.05	0.95 ± 0.06	8.06 ± 0.08	1.00 ± 0.07	8.00 ± 0.02	1.20 ± 0.04	7.99 ± 0.16	1.11 ± 0.12
Zolmitriptan	8.15 ± 0.03	1.02 ± 0.09	8.54 ± 0.15	1.07 ± 0.14	8.67 + 0.06	1.30 ± 0.12	8.91 ± 0.18	1.01 ± 0.07
Naratriptan	8.37 ± 0.05	1.20 ± 0.11	8.73 ± 0.11	1.16 ± 0.10	8.29 ± 0.09	1.35 ± 0.09	8.34 ± 0.13	1.27 ± 0.05
Rizatriptan	7.56 ± 0.02	0.92 ± 0.04	7.86 ± 0.05	0.97 ± 0.09	7.73 ± 0.11	1.09 ± 0.07	7.94 ± 0.20	0.98 ± 0.12
Ketanserin	4.79 ± 0.06	0.68 ± 0.05	5.01 ± 0.04	0.90 ± 0.16	6.90 ± 0.06[a]	0.43 ± 0.02	6.70 ± 0.27[a]	0.40 ± 0.02
Dihydroergotamine	8.57 ± 0.03	1.40 ± 0.06	8.78 ± 0.04	1.22 ± 0.14	9.05 ± 0.06	1.05 ± 0.08	8.65 ± 0.14	1.12 ± 0.16
Methysergide	7.24 ± 0.03	0.98 ± 0.03	7.22 ± 0.06	0.98 ± 0.07	8.08 ± 0.10	1.41 ± 0.03	8.08 ± 0.23	1.35 ± 0.17
Metergoline	7.76 ± 0.08	1.21 ± 0.08	8.07 ± 0.09	1.16 ± 0.03	8.41 ± 0.07	1.47 ± 0.08	8.13 ± 0.12	1.43 ± 0.17
Methiothepin	7.04 ± 0.05[a]	0.62 ± 0.02	7.36 ± 0.06[a]	0.59 ± 0.04	8.29 ± 0.24[a]	0.36 ± 0.03	7.69 ± 0.30[a]	0.38 ± 0.02
Yohimbine	7.01 ± 0.05	0.95 ± 0.02	7.16 ± 0.03	0.89 ± 0.08	7.48 ± 0.05	1.04 ± 0.04	7.45 ± 0.07	0.91 ± 0.03

Values represent the mean ± S.E.M. of at least three independent experiments. All results generated from curve fitting to a one-site model.
[a]Curves were shallow and best fitted to a two-site model.

Table 5
Comparison of the apparent equilibrium inhibition constants (pK_i) for the low and high affinity ketanserin and methiothepin binding components obtained using [^{3}H]eletriptan and [^{3}H]sumatriptan at the human 5-HT_{1D} and 5-HT_{1B} receptors

	Site 1		Site 2	
	pK_i	%	pK_i	%
5-HT_{1D}				
Methiothepin vs. [^{3}H]eletriptan	8.73 ± 0.18	64.99 ± 2.30	6.14 ± 0.13	35.01 ± 2.30
Methiothepin vs. [^{3}H]sumatriptan	8.18 ± 0.07	60.29 ± 4.46	5.65 ± 0.08	39.71 ± 4.46
Ketanserin vs. [^{3}H]eletriptan	7.87 ± 0.03	55.67 ± 3.03	5.95 ± 0.14	44.33 ± 3.03
Ketanserin vs. [^{3}H]sumatriptan	7.87 ± 0.10	59.99 ± 4.72	5.55 ± 0.14	40.01 ± 4.72
5-HT_{1B}				
Methiothepin vs. [^{3}H]eletriptan	7.75 ± 0.01	54.79 ± 1.46	6.17 ± 0.04	45.21 ± 1.46
Methiothepin vs. [^{3}H]sumatriptan	7.99 ± 0.11	57.98 ± 2.69	6.36 ± 0.25	42.02 ± 2.69

Values represent arithmetic mean ± S.E.M. of at least three independent experiments.

the compounds had not reached equilibrium (Motulsky and Mahan, 1983).

Ketanserin and methiothepin competition curves consistently displayed low Hill slopes which could be resolved into a high affinity and a low affinity component against both [^{3}H]eletriptan and [^{3}H]sumatriptan binding to the 5-HT_{1D} receptor (Table 5). In addition, methiothepin competition curves with both [^{3}H]eletriptan and [^{3}H]sumatriptan binding to the 5-HT_{1B} receptor were also biphasic, although the separation between the affinities of these components was smaller than that observed at the 5-HT_{1D} receptor (Table 5). It was not possible to determine if ketanserin competition curves were also biphasic at the 5-HT_{1B} receptor as the binding of [^{3}H]eletriptan and [^{3}H]sumatriptan were not completely inhibited by ketanserin, even at the highest concentration tested (10 μM).

4. Discussion

This study has demonstrated that the 5-HT receptor binding profile of eletriptan is qualitatively similar to the binding profile of sumatriptan, zolmitriptan, naratriptan and rizatriptan. As expected these compounds demonstrated high affinity for the human 5-HT_{1B} and 5-HT_{1D} receptors which is consistent with their known vasoconstrictor properties in isolated vascular tissues (Ferro et al., 1995; Connor et al., 1997; Martin, 1997; Gupta et al., 1999), effects which are generally accepted to be mediated through smooth muscle 5-HT_{1B} receptors (Hamel et al., 1993; Longmore et al., 1997; Razzaque et al., 1997; Verheggen et al., 1998). In addition to high affinity for 5-$HT_{1B/1D}$ receptors, all of the 5-$HT_{1B/1D}$ receptor agonists consistently had high affinity for the 5-ht_{1f} receptor, indeed a comparison of potencies at the 5-HT_{1B} versus the 5-ht_{1f} receptor indicated that none of the compounds were selective, suggesting that affinity at this receptor subtype may be important in the antimigraine action of these drugs. A recent study by Wainscott et al. (1998) has shown that for 5-$HT_{1B/1D}$ agonists a statistically significant correlation exists between the affinity and potency for the human 5-ht_{1f} receptor and inhibition of trigeminal nerve-stimulated plasma protein extravasation, a model of migraine. Thus, if the 5-ht_{1f} receptor is implicated in inhibition of dural plasma protein extravasation, then high affinity for the 5-ht_{1f} receptor may be an advantage for antimigraine drugs such as eletriptan. As yet, the efficacy of highly selective 5-ht_{1f} receptor agonists, such as LY334370, in the treatment of migraine remains to be demonstrated.

Selectivity for the 5-HT_{1A} receptor ranged from 3-fold (rizatriptan) and 4.5-fold (eletriptan) to over 10-fold (zolmitriptan, naratriptan and sumatriptan). Agonist activity at the human recombinant 5-HT_{1A} receptor has been observed for drugs used in migraine therapy including the 5-$HT_{1B/1D}$ receptor agonists (Newman-Tancredi et al., 1997; Dupuis et al., 1998; Pauwels et al., 1998) and also eletriptan (unpublished data). However, effects in vivo have not been consistently demonstrated, for example, sumatriptan and rizatriptan elicit hypotension and bradycardia in the anaesthetised normotensive rat, an effect attributed to activation of central 5-HT_{1A} receptors and reduction in sympathetic outflow, but major effects on haemodynamics were not observed with the more brain-penetrant zolmitriptan (Paignez et al., 1998). Furthermore, the 5-ht_{1f}-selective agonist LY334370 which shows nanomolar affinity for the 5-HT_{1A} receptor and stimulation of [^{35}S]-GTP-γ-S binding in vitro, also failed to produce any 5-HT_{1A} activity in rats in vivo (Overshiner et al., 1996). Nevertheless, given the evidence of involvement of 5-HT_{1A} receptors in the modulation of sleep, emesis, anxiety and depression (Lucot and Crampton, 1989; Baldwin and Rudge, 1995; De Vry, 1995; Gale, 1995; Wolff et al., 1997) agonist activity at this receptor might have potential implications in terms of the ancillary properties of drugs used for migraine treatment.

With the exception of modest affinity for the 5-HT_7 and 5-ht_{1e} receptors, and for which there is no evidence that activity at these receptors contributes to the antimigraine properties of 5-$HT_{1B/1D}$ agonists, eletriptan showed no significant activity at other 5-HT receptors. In addition,

concentrations up to 10 μM eletriptan displayed little affinity for β_1- and β_2-adrenoceptors, adenosine (A_1), dopamine (D_1 and D_2) and opioid receptors and dihydropyridine calcium channel binding sites (unpublished data).

A comparison of the affinity estimates using [^{3}H]eletriptan and [^{3}H]sumatriptan indicated that eletriptan had a 6-fold higher affinity for the human 5-HT_{1D} receptor compared to sumatriptan. This is in good agreement with the data generated using [^{3}H]5-HT as the ligand (Table 2) in which eletriptan displayed 8-fold higher affinity. At the human 5-HT_{1B} receptor eletriptan was 5-fold more potent than sumatriptan using [^{3}H]eletriptan as the ligand and 3-fold more potent when using [^{3}H]sumatriptan. Again, this result agrees with that generated using [^{3}H]5-HT in which eletriptan had 4-fold higher affinity for the 5-HT_{1B} receptor compared to sumatriptan. The observation of two binding sites in the 5-HT_{1B} receptor cell line with [^{3}H]eletriptan was an unexpected finding, as we did not observe this with either [^{3}H]5-HT or [^{3}H]sumatriptan when tested against the same batch of cell membranes. However, it is consistent with the report by Selkirk et al. (1998) that [^{3}H]5-HT labels two binding sites in a CHO cell line expressing the human 5-HT_{1B}, but not the 5-HT_{1D} receptor. In addition, these workers have also shown that the 5-$HT_{1B/1D}$ receptor antagonist [^{3}H]GR125743 labels both high and low affinity states of the 5-HT_{1B} receptor, but predominantly the high affinity state in the 5-HT_{1D} receptor (Selkirk et al., 1997). As this effect was only observed at high concentrations and given that the concentration of [^{3}H]eletriptan used in the competition and kinetic studies was < 3 nM it was assumed that binding to the low affinity site was negligible.

Both [^{3}H]eletriptan and [^{3}H]sumatriptan displayed high affinity, high selectivity, and rapid association. Eletriptan is more lipophilic than sumatriptan as reflected by the $\log D_{\text{pH 7.4}}$ (octanol:buffer distribution coefficients at pH 7.4) for the two compounds of -1.6 and $+0.5$, respectively (Rance et al., 1996) which would suggest that eletriptan and sumatriptan may show differences in their ability to cross biological membranes and might have been predicted to influence the properties of these radioligands. However, this was not apparent as both radioligands displayed high levels ($> 90\%$) of specific binding. A rapid approach to equilibrium was not unexpected, as we have observed this in the same cell lines using [^{3}H]5-HT (data not shown). Similar findings have been reported for [^{3}H]alnitidan (Leysen et al., 1996) which, unlike eletriptan and sumatriptan, is a benzopyran derivative, indicating that this rapid kinetic profile is not restricted to agonists possessing an indole group.

As the kinetics of [^{3}H]eletriptan were so rapid, it was not possible to accurately determine on/off rates at 22°C. However, at 4°C [^{3}H]eletriptan was found to have a faster on-rate and a slower off-rate than [^{3}H]sumatriptan at the 5-HT_{1D} receptor (both differences were statistically significant). Although we accept that studies in recombinant cell lines are far removed from the clinical situation, this may contribute to the rapid onset, longer duration of action and lower frequency of headache recurrence that has been reported for eletriptan in man (Steiner, 1998). Reliable estimation of kinetic parameters for [^{3}H]eletriptan at the 5-HT_{1B} receptor subtype could not be obtained as the data for both association and dissociation was not adequately described by either a one or two site model. This may have been due to the low affinity component which was occasionally detected in [^{3}H]eletriptan saturation assays.

As expected pK_i values for a range of compounds were similar regardless of radioligand and the affinities were in good agreement with published data (Bard et al., 1996; Leysen et al., 1996; Saxena and Ferrari, 1996; Meng, 1997; Pauwels et al., 1997; Wurch et al., 1997), with any differences in absolute affinity probably reflecting the expression of receptors in different cell lines. The biphasic competition curves with ketanserin and methiothepin at the 5-HT_{1D} receptor and methiothepin at the 5-HT_{1B} receptor were an unexpected observation, given that biphasic competition curves are more commonly observed when using an antagonist radioligand, although similar findings have been reported with ketanserin at the rat recombinant 5-HT_{1D} and the human recombinant 5-HT_{1D} receptor using [^{3}H]5-HT (Bach et al., 1993; Zgombick et al., 1995). In the present study the agonist radioligands were used a single low concentration equal to or less than their respective K_D values such that pK_i values were assumed represent binding to a high affinity agonist state. This contrasts the situation with the saturation studies where a second low affinity [^{3}H]eletriptan binding site in the 5-HT_{1B} receptor cell line was detected at high concentrations. As there was no [^{3}H]eletriptan or [^{3}H]sumatriptan binding to untransfected HeLa cells, the possibility of a second population of endogenously expressed receptors can be ruled out. These data suggest that in these cell lines both high and low affinity states of the receptor exist for which the 5-$HT_{1B/1D}$ receptor agonists have equal affinity but which ketanserin and methiothepin are able to discriminate.

In summary, these data indicate that eletriptan is a potent and selective ligand for the 5-HT_{1B}, 5-HT_{1D} and 5-ht_{1f} receptor subtypes. The binding characteristics of eletriptan and [^{3}H]eletriptan described in this study are consistent with the reported potent vasoconstrictor activity in isolated vascular tissues (Gupta et al., 1999) also with the clinical findings that eletriptan is an effective treatment for migraine in man (Jackson et al., 1996).

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