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Specific labelling of serotonin 5-HT_{1B} receptors in rat frontal cortex with the novel, phenylpiperazine derivative, [^{3}H]GR125,743 A pharmacological characterization

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Abstract

Although several tritiated agonists have been used for radiolabelling serotonin (5-hydroxytryptamine, 5-HT)$_{1B}$ receptors in rats, data with a selective, radiolabelled antagonist have not been presented. Inasmuch as [^{3}H]GR125,743 specifically labels cloned, human and native guinea pig 5-HT_{1B} receptors and has been employed for characterization of cerebral 5-HT_{1B} receptor in the latter species [Eur. J. Pharmacol. 327 (1997) 247.], the present study evaluated its utility for characterization of native, cerebral 5-HT_{1B} sites in the rat. In homogenates of frontal cortex, [^{3}H]GR125,743 (0.8 nM) showed rapid association ($t_{1/2}$ = 3.4 min), >90% specific binding and high affinity (K_d = 0.6 nM) for a homogeneous population of receptors with a density (B_{max}) of 160 fmol/mg protein. In competition binding studies, affinities were determined for 15 chemically diverse 5-HT_{1B} agonists, including 2-[5-[3-(4-methylsulphonylamino)benzyl-1,2,4-oxadiazol-5-yl]-1*H*-indole-3-yl]ethylamine (L694,247; pK_i, 10.4), 5-carboxamidotryptamine (5-CT; 9.7), 3-[3-(2-dimethylamino-ethyl)-1*H*-indol-6-yl]-*N*-(4-methoxybenzyl)acrylamide (GR46,611; 9.6), 5-methoxy-3-(1,2,5,6-tetrahydro-4-pyridinyl)-1*H*-indole (RU24,969; 9.5), dihydroergotamine (DHE; 8.6), 5-*H*-pyrrolo [3,2-*b*]pyridin-5-one,1,4-dihydro-3-(1,2,3,6-tetrahydro-4-pyridinyl (CP93,129; 8.4), anpirtoline (7.9), sumatriptan (7.4), 1-[2-(3-fluorophenyl)ethyl]-4-[3-[5-(1,2,4-triazol-4-yl)-1*H*-indol-3-yl]propyl]piperazine (L775,606; 6.4) and (−)-1(*S*)-[2-[4-(4-methoxyphenyl)piperazin-1-yl]ethyl]-*N*-methyl-3,4-dihydro-1*H*-2-benzopyran-6-carboxamide (PNU109,291; <5.0). Similarly, affinities were established for 13 chemically diverse antagonists, including *N*-[4-methoxy-3-(4-methylpiperazin-1-yl)phenyl]-3-methyl-4-(4-pyridyl)benzamide (GR125,743; pK_i, 9.1), (−)-cyanopindolol (9.0), (−)-tertatolol (8.2), *N*-(4-methoxy-3-(4-methylpiperazin-1-yl)phenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiozol-3-yl)biphenyl-4-carboxamide (GR127,935; 8.2), *N*-[3-(1,4-benzodioxan-5-yl)piperidin-4-yl]*N*-(indan-2yl)amine (S18127; 7.9), metergoline (7.8), (−)-pindolol (7.6), 1′-methyl-5-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)-biphenyl-4-ylcarbonyl]-2,3,6,7-tetrahydro-5*H*-spiro[furo[2,3-*f*]indole-3,4′-piperidine] (SB224,289; 7.5) and ketanserin (<5.0). These rank orders of affinity correspond to the binding profile of 5-HT_{1B} rather than 5-HT_{1D} receptors. The low affinities of L775,066 and PNU109,291 versus L694,247 should be noted, as well as the low affinity of ketanserin as compared to SB224,289. Finally, in line with species differences, the affinities of several ligands including CP93,129, RU24,969, (−)-pindolol and (−)-propanolol in rat 5-HT_{1B} sites were markedly different to guinea pig 5-HT_{1B} sites labelled with [^{3}H]GR125,743. In conclusion, [^{3}H]GR125,743 is an appropriate tool for the radiolabelling of native, rat 5-HT_{1B} receptors and permitted determination of the affinities of an extensive series of ligands at these sites.

Keywords: Serotonin; 5-HT_{1B}; 5-HT_{1D}; Frontal cortex; (−)-Pindolol; GR125,743; Sumatriptan; SB224,289

1. Introduction

Currently, on the basis of their contrasting structures and coupling to transduction mechanisms, seven classes of 5-hydroxytryptamine (5-HT) receptor are recognized (Barnes and Sharp, 1999). Of these, 5-HT_{1B} receptors (formerly 5-$HT_{1D\beta}$) and closely related 5-HT_{1D} (formerly 5-$HT_{1D\alpha}$) receptors (Middlemiss and Hutson, 1990; Dickenson and Hill, 1998; Brys et al., 2000) have long attracted interest as targets for the treatment of migraine in view of their localization on primary afferent fibres, sympathetic terminals and cerebral blood vessels (Millan, 1999; Tfelt-Hansen et al., 2000). More recently, attention has been focused on the potential significance of central populations of these receptors. Here is a modest population of inhibitory 5-HT_{1D} autoreceptors on raphé-localized serotoninergic cell bodies

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(Pineyro et al., 1995; Bonaventure et al., 1998). However, these perikarya also bear colocalized, inhibitory 5-HT_{1B} autoreceptors (Evrard et al., 1999), and a high density of 5-HT_{1B} autoreceptors is present on the terminals of serotoninergic pathways (Moret and Briley, 1993; Gobert et al., 2000; Daws et al., 2000; Fabre et al., 2000; Stenfors et al., 2000).

In contrast to the relative paucity of postsynaptic 5-HT_{1D} receptors, both the rodent and human brain contain a high density of postsynaptic 5-HT_{1B} receptors, which are particularly concentrated in the hypothalamus, substantia nigra, striatum, hippocampus, frontal cortex and various other corticolimbic structures (Bruinvels et al., 1993; Neumaier et al., 1996; Bonaventure et al., 1997; Sari et al., 1999). 5-HT_{1B} receptors in these regions have been implicated in the modulation of dopaminergic, GABAergic, cholinergic and glutamatergic transmission (Galloway et al., 1993; Boeijinga and Boddeke, 1996; Maura et al., 1989; Millan et al., 2000; Morikawa et al., 2000). Correspondingly, 5-HT_{1B} receptors have been implicated in the control of mood (Geyer, 1996; Saudou et al., 1994; Dirks et al., 2001), reward (Fletcher and Korth, 1999; Belzung et al., 2000), sleep (Boutrel et al., 1999), cognition (Boulenguez et al., 1998; Malleret et al., 1999; Meneses, 1999), motor behaviour (Geyer, 1996; Skingle et al., 1996; Chaouloff et al., 1999), appetite (Lucas et al., 1998; De Vry and Schreiber, 1999), endocrine secretion (Middlemiss and Hutson, 1990) and thermoregulation (Hatcher et al., 1995; Skingle et al., 1996; Millan et al., 1999). They are thus of potential importance in the treatment of disorders such as depression, impulsive disorders, schizophrenia and Parkinson's disease (Geyer, 1996; Boulenguez et al., 1998; Barnes and Sharp, 1999; Moret and Briley, 2000; Dulawa et al., 2000; Audinot et al., 2001b). Characterization of 5-HT_{1B} receptors in various species has revealed high amino acid sequence homologies and very similar binding profiles of human (h) as compared to guinea pig 5-HT_{1B} sites. However, rodent 5-HT_{1B} receptors, due to a single amino acid substitution in the transmembrane domain (Adham et al., 1992b; Oksenberg et al., 1992; Brys et al., 2000), reveal marked differences to 5-HT_{1B} sites for certain drugs, such as the alkylarylamines, (−)-pindolol, (−)-propanolol (Gerhardt and van Heerikhuizen, 1997; Barnes and Sharp, 1999; Gobert and Millan, 1999; Hoyer and Middlemiss, 1989) and, as demonstrated more recently, a number of antipsychotic agents (Audinot et al., 2001a).

Despite the importance of transgenic strategies, from a conceptual and therapeutic perspective, it is clearly necessary to develop ligands interacting selectively at both human and rodent 5-HT_{1B} receptors. In this regard, the antagonist (weak partial agonist) *N*-(4-methoxy-3-(4-methylpiperazin-1-yl)phenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiozol-3-yl)biphenyl-4-carboxamide (GR127,933) (Skingle et al., 1995; Watson et al., 1996; Millan et al., 1999), and the novel, neutral antagonist *N*-[3-(1,4-benzodioxan-5-yl)piperidin-4-yl]*N*-(indan-2yl)amine (S18127) (Millan et al., 1999), are of considerable utility as experimental tools for exploring the role of central 5-HT_{1B} receptors. Further, 3-[3-(2-dimethylamino-ethyl)-1*H*-indol-6-yl]-*N*-(4-methoxybenzyl)acrylamide (GR46,611) and, in rodents, 5-*H*-pyrrolo[3,2-*b*]pyridin-5-one,1,4-dihydro-3-(1,2,3,6-tetrahydro-4-pyridinyl (CP93,129), have been extensively used as agonists (Yu et al., 1996; Millan et al., 1999; see Hoyer et al., 1994). However, these ligands show comparable affinity for 5-HT_{1B} and closely related 5-HT_{1D} receptors and ligands differentiating these sites are clearly important. In this regard, the selective 5-HT_{1B} antagonist, 1′-methyl-5-[2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)-biphenyl-4-ylcarbonyl]-2,3,6,7-tetrahydro-5*H*-spiro[furo[2,3-*f*]indole-3,4′-piperidine] (SB224,289), is of particular note (Gaster et al., 1998; Millan et al., 1999; Gobert et al., 2000). Further, ketanserin and ritanserin more potently recognise 5-HT_{1D} than 5-HT_{1B} receptors, though their high affinities at 5-HT_{2A} receptors compromise their utility as tools for exploring the function of 5-HT_{1D} sites (Adham et al., 1992a; Verheggen et al., 1998). As concerns 5-HT_{1B} receptors, while selective agonists do not appear to have been identified, selective 5-HT_{1D} agonists such as 1-[2-(3-fluorophenyl)ethyl]-4-[3-[5-(1,2,4-triazol-4-yl)-1*H*-indol-3-yl]propyl]piperazine (L775,606) (Chambers et al., 1999; Newman-Tancredi et al., 2000; Longmore et al., 2000) and (−)-1(*S*)-[2-[4-(4-methoxyphenyl)piperazin-1-yl]ethyl]-*N*-methyl-3,4-dihydro-1*H*-2-benzopyran-6-carboxamide (PNU109,291) (Cutrer et al., 1999; Bouchelet et al., 2000), have been described.

Radiolabelled ligands interacting specifically with cerebral population of 5-HT_{1B} receptors are of considerable importance in their characterization. In this regard, a variety of agonists have been used (Bruinvels et al., 1993; Miller and Teitler, 1992; Langlois et al., 1995; Waeber and Moskowitz, 1995), but they have the disadvantage of preferentially labelling only receptors in the active state, i.e. coupled to G-proteins. Further, [^{3}H]5-carboxamidotryptamine ([^{3}H]5-CT) requires the use of other ligands to mask non-5-HT_{1B} sites (Miller and Teitler, 1992; Nowak et al., 1993) while the potent agonist L694,247 shows only marginal selectivity versus 5-HT_{1A} sites and low specific binding (Beer et al., 1993; Yu et al., 1997; Yamada et al., 2000). Similarly, [^{3}H]alniditan displays only a modest preference for 5-HT_{1B} versus 5-HT_{1A} receptors (Leysen et al., 1996; Bonaventure et al., 1997, 1999). While the antagonist [^{125}I]iodocyanopindolol radiolabels 5-HT_{1B} receptors, its pronounced affinity for β-adrenoceptors necessitates the use of ligands for their masking (Offord et al., 1988; Zgombick and Branchek, 1998). The introduction of the novel, benzamide-substituted, phenylpiperazine derivative [^{3}H]GR125,743 as a radiolabelled antagonist is, thus, of considerable importance, and it has proven to be a suitable ligand for the characterization of cloned h5-HT_{1B} and h5-HT_{1D} receptors (Doménech et al., 1997; Grånäs and Larhammar, 1999; Newman-Tancredi et al., 2000; Audinot et al., 1997, 2001a,b). Further, although [^{3}H]GR125,743 does not discriminate 5-HT_{1B} from 5-HT_{1D}

sites, the former predominate in the CNS (vide supra) and [^{3}H]GR125,743 specifically labels central populations of 5-HT_{1B} receptors in primates, guinea pig and mice (Audinot et al., 1997; Bonaventure et al., 1997, 1999).

To date, the potential utility of [^{3}H]GR125,743 for the labelling of 5-HT_{1B} receptors in rat CNS has not been evaluated. This question was addressed in the present study, employing a broad range of chemically diverse agonists and antagonists.

2. Methods

2.1. Membrane preparation

As described previously (Audinot et al., 1997), frontal cortex from male Wistar rats (240–260 g) was dissected on ice and maintained frozen (−80 °C) until use. Structures were homogenized in 20 vol (weight/volume) of ice-cold 50 mM Tris–HCl (pH 7.7, room temperature, containing 4 mM $CaCl_2$ and 0.1% ascorbic acid) with a Kinematica Polytron and centrifuged at 48,000 × *g* and 4 °C for 25 min. The resulting supernatant was discarded, and the pellet was resuspended in the same volume of ice-cold buffer before incubation at 37 °C for 15 min to remove endogenous 5-HT. Finally, the suspension was recentrifuged at 48,000 × *g* and 4 °C for 25 min, and the pellet was resuspended in 80 vol of ice-cold buffer containing pargyline, 10 μM.

2.2. Radioligand binding

Radioligand binding assays were carried out essentially as described by Audinot et al., 1997 using the following assay buffer: 50 mM Tris–HCl (pH 7.7, room temperature, containing 4 mM $CaCl_2$, 0.1% ascorbic acid and 10 μM pargyline. 5-HT (10 μM) was used to define nonspecific binding. In kinetic and competition experiments, the concentration of [^{3}H]GR125,743 (70 Ci/mmol, Amersham) was 0.8 nM. Incubations were initiated by addition of membranes and were conducted for 60 min at room temperature for competition experiments. The reaction was terminated by rapid filtration using a Brandel Cell Harvester through Whatman GF/B filters pretreated with polyethylenimine 0.1%, followed by three rinses with ice-cold assay buffer. The addition of bovine serum albumin (0.1%) to the filtration buffer considerably lowered nonspecific binding of [^{3}H]GR125,743 to filters. Specific binding represented about 90% of total binding at concentrations of radioligand close to its K_d value.

2.3. Data analysis

Data were analyzed by nonlinear regression using the program PRISM (Graphpad Software, San Diego, CA) to yield the K_d (dissociation equilibrium constant of the radioligand) and B_{max} (total number of sites) values for saturation experiments, and IC_{50} (inhibitory concentration$_{50}$, i.e. the concentration producing 50% inhibition of binding) values for competition experiments. The inhibition constant (K_i) was calculated according to the Cheng–Prussof equation: $K_i = IC_{50}/(1 + L/K_d)$, where L is the concentration of radioligand. For correlation analyses, Pearson Product–Moment Correlation Coefficients were employed.

2.4. Drugs

5-HT and 7-trifluoromethyl-4(4-methyl-1-piperazinyl)-pyrrolol 1,2-alquinoxaline maleate (CGS12066A) were purchased from Sigma (St. Quentin, Fallavier, France); dihydroergotamine (DHE), (−)-propanolol and mesulergine were from RBI (Natick, MA, USA); 2-[5-[3-(4-methylsulphonylamino)benzyl-1,2,4-oxadiazol-5-yl]-1*H*-indole-3-yl]ethylamine (L694,247), 5-methoxy-3-(1,2,5, 6-tetrahydro-4-pyridinyl)-1*H*-indole (RU24,969) and GR46,611were from Tocris Cookson (Bristol, UK); and metergoline was

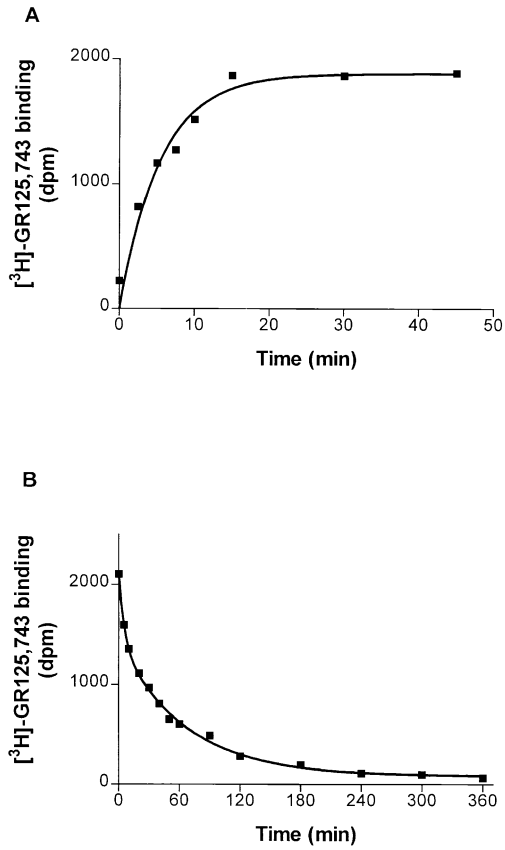

Fig. 1. Association and dissociation of [^{3}H]GR125,743 (0.8 nM) binding to homogenates of rat frontal cortex. Panels A and B indicate the time course for association and dissociation, respectively. Data are from a single, representative experiment, which was performed in triplicate and repeated on three independent occasions with very similar data.

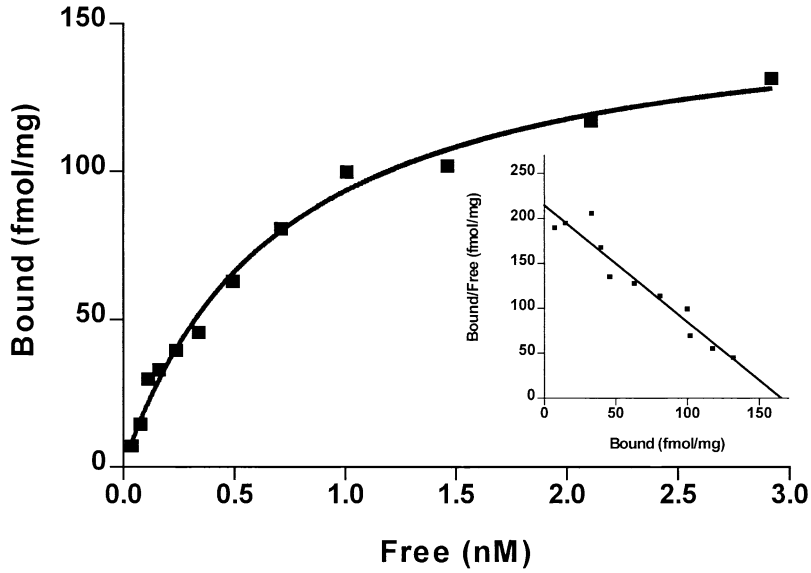

Fig. 2. Saturation isotherm of [^{3}H]GR125,743 specific binding to homogenates of rat frontal cortex. The inset indicates Scatchard analysis of the data. Data are from a single, representative experiment, which was performed in triplicate and repeated on three independent occasions with very similar data.

from Farmitalia Carlo Erba (Rodano, Italia). Ritanserin and ketanserin were obtained from Janssen (Beerse, Belgium), and methiotepin was from Hoffman-La Roche (Basle, Switzerland). Sumatriptan, 5-carboxamidotryptamine (5-CT), *N*-methyl-2-[3-(1-methylpiperidin-4-yl)-1*H*-indol-5-yl] (GR85,548), (−)-pindolol, (−)-tetratolol, 5-fluoro-3-{3-[4-(5-methoxy-pyramidin-4-yl)-piperazin-1-yl]-propyl}-1*H*-indole (BMS181,101), CP93,129, alniditan, *N*-[4-methoxy-3-(4-methylpiperazin-1-yl)phenyl]-3-methyl-4-(4-pyridyl)-benzamide (GR125,743), GR127,935, SB224,289, S18127, L775,606 and PNU109,291 were synthesised by Gilbert Lavielle or Jean-Louis Peglion (Servier).

3. Results

3.1. Kinetic analysis of [^{3}H]GR125,743 binding

[^{3}H]GR125,743 rapidly associated to rat FCX membranes with a half-time of 3.4 ± 0.6 min (Fig. 1A). Statistical analysis of these data revealed that they were better fitted ($P < .01$) by a monophasic as compared to a biphasic isotherm. Following achievement of equilibrium, binding was stable over a period of at least 2 h (not shown). For dissociation isotherms of [^{3}H]GR125,743, curves were better fitted ($P < .01$) by a biphasic as compared to a monophasic isotherm, yielding two components with half-lives of 3.1 ± 0.4 and 48 ± 4.0 min, respectively (Fig. 1B). The first component of dissociation yielded a "kinetic" K_d of 0.88 nM.

3.2. Saturation analysis

The binding of [^{3}H]GR125,743 to rat FCX membranes was fully saturable, with ~80% of sites occupied at the highest concentration (3.0 nM) examined. The Scatchard plot was clearly linear up to 3.0 nM, a concentration ~4-fold greater than the K_d, which was determined to be 0.56 ± 0.08 nM from these experiments (Fig. 2), a value close to its kinetic K_d of 0.88 nM (preceding paragraph). The number of sites labelled (B_{max}) was calculated to be 160 ± 5 fmol/mg protein from these data.

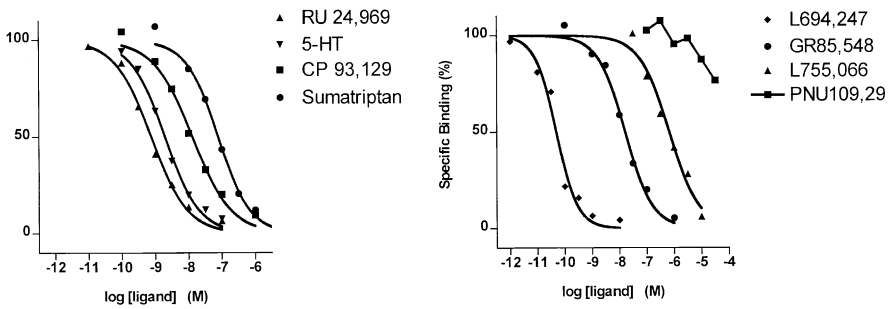

Fig. 3. Competition binding isotherms for [^{3}H]GR125,743 binding to rat frontal cortex by representative agonists. Data are from a single, representative experiment, which was performed in triplicate and repeated on three independent occasions with very similar data.

Table 1
Affinities of agonists determined by competition for [^{3}H]GR125,743 binding to rat frontal cortex, as compared to affinities for guinea pig, striatal 5-HT$_{1B}$ receptors labelled with [^{3}H]GR125,743

Ligand	Rat FCX	Guinea pig striatum
(1) L694,247	10.44 ± 0.18	10.05[a]
(2) GR46,611	9.58 ± 0.04	9.96[a]
(3) RU249,69	9.53 ± 0.06	7.49 ± 0.07
(4) 5-CT	9.47 ± 0.02	9.10[a]
(5) 5-HT	9.07 ± 0.04	8.64[a]
(6) DHE	8.58 ± 0.04	8.82[a]
(7) CP93,129	8.40 ± 0.09	< 6.0[a]
(8) GR85,548	8.10 ± 0.05	7.85[a]
(9) Anpirtoline	7.90 ± 0.01	7.98 ± 0.31
(10) Sumatriptan	7.40 ± 0.04	6.76[a]
(11) CGS12066B	7.18 ± 0.03	7.19 ± 0.05
(12) Alniditan	6.95 ± 0.04	8.38[a]
(13) BMS181,101	6.82 ± 0.15	8.20[a]
(14) L775,606	6.4 ± 0.20	6.70 ± 0.02
(15) PNU109,291	< 5.0	5.3 ± 0.2

Inhibition constants are expressed as pK_i's. Means ± S.E.M. of at least three experiments performed in triplicate are shown. Data for guinea pig 5-HT$_{1B}$ receptor are from Audinot et al., 1997[a] or from unpublished observations. FCX = frontal cortex.

Table 2
Affinities of antagonists determined by competition for [^{3}H]GR125,743 binding to rat frontal cortex, as compared to affinities for guinea pig striatal 5-HT$_{1B}$ receptors labelled with [^{3}H]GR125,743

Ligand	Rat FCX	Guinea pig striatum
(1) GR125,743	9.41 ± 0.12	9.03[a]
(2) (−)-Cyanopindolol	8.98 ± 0.04	6.64 ± 0.04
(3) (−)-Tertatolol	8.24 ± 0.26	< 6.0
(4) GR127,935	8.22 ± 0.07	8.37[a]
(5) S18127	7.88 ± 0.03	8.13 ± 0.07
(6) Metergoline	7.80 ± 0.05	7.55[a]
(7) (−)-Pindolol	7.60 ± 0.10	5.83 ± 0.17
(8) SB224,289	7.52 ± 0.08	8.44 ± 0.09
(9) (−)-Propranolol	7.44 ± 0.11	5.56 ± 0.03
(10) Methiothepin	7.00 ± 0.11	7.96[a]
(11) Methysergide	6.51 ± 0.02	6.73[a]
(12) Ritanserin	< 5.0	6.43[a]
(13) Ketanserin	< 5.0	< 5.0[a]

Inhibition constants are expressed as pK_i's. Means ± S.E.M. of at least three experiments performed in triplicate are shown. Dates for guinea pig 5-HT$_{1B}$ receptors are from Audinot et al., 1997[a] or from unpublished observations. FCX = frontal cortex.

3.3. Competition binding: displacement of [^{3}H]GR125,743 by agonists

The total of 15 chemically diverse agonists evaluated for displacement of [^{3}H]GR125,743 binding displayed a wide range of affinities (Fig. 3 and Table 1). All ligands displaced [^{3}H]GR125,743 with monophasic isotherms, indicative of a single class of binding sites. Corresponding pseudo-Hill coefficients were not significantly different from unity (not shown), with the exception of CP93,129 (0.75 ± 0.10). The rank order of affinity was as follows: L694,247 > GR46,611 > RU24,969 > 5-CT > 5-HT > DHE > CP93,129 > GR85,598 > anpirtoline > sumatriptan > CGS12066B > alniditan > BMS181,101 > L775,606 > PNU109,291.

3.4. Competition binding: displacement of [^{3}H]GR125,743 by antagonists

A total of 13 antagonists revealed a considerable range of affinities in competing with [^{3}H]GR125,743 binding. Like agonists, antagonists displayed monophasic isotherms, which were best fitted by a single class of binding sites (Fig. 4 and Table 2). Corresponding pseudo-Hill coefficients did not significantly differ to unity (not shown), with the exception of methiothepin (0.7 ± 0.01). The rank order for displacement of [^{3}H]GR125,743 was as follows: GR125,743 > (−)-cyanopindol > (−)-tertatotol > GR127,935 > S18127 > metergoline > (−)-pindololol > SB224,289 > (−)-propanolol > methiothepin > methysergide > ritanserin = ketanserin.

3.5. Comparison of binding profiles in rat versus guinea pig 5-HT$_{1B}$ receptors

As shown in Tables 1 and 2 and Fig. 5, for many ligands, affinities observed in rat 5-HT$_{1B}$ sites were similar to those determined in guinea pig 5-HT$_{1B}$ sites (Audinot et al., 1997 and unpublished observation). However, for several ligands, there were marked differences. Notably, the agonist CP93,129 was a high-affinity ligand in the rat as compared to guinea pig and human 5-HT receptors, and RU24,969 likewise displayed a preference for the former. On the other

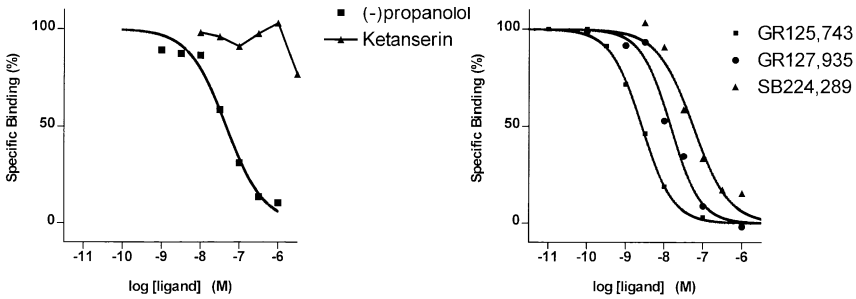

Fig. 4. Competition binding isotherms for [^{3}H]GR125,743 binding to rat frontal cortex by representative antagonists. Data are from a single, representative experiment, which was performed in triplicate and repeated on three independent occasions with very similar data.

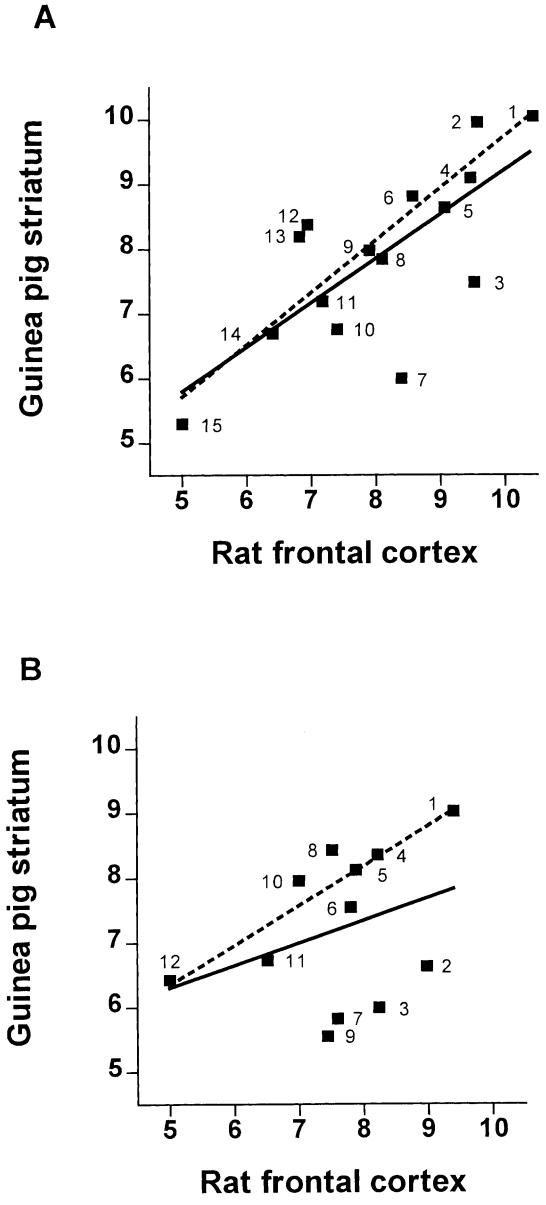

Fig. 5. Comparison of ligand affinities for displacement of [^{3}H]GR125,743 binding to rat frontal cortex as compared to guinea pig striatum. Panel A: Agonists in rat versus guinea pig 5-HT$_{1B}$ receptors. Panel B: Antagonists in rat versus guinea pig 5-HT$_{1B}$ receptors. Ligands are as indicated in Tables 1 (agonists) and 2 (antagonists). Pearson Product–Moment Correlation Coefficients were calculated. Note that while the majority of ligands lie close to the regression line, several display markedly divergent affinities. For panel A, $r=.74$, $P<.005$, $n=15$ for all ligands (continuous line), and $r=.90$, $P<.001$, $n=13$ excluding ligands 3 and 7 (dotted line). For Panel B, $r=.34$, $P>.05$, $n=12$ for all ligands (continuous line) and $r=.90$, $P<.005$, $n=8$ excluding the four arylalkylamines, compounds 2, 3, 7 and 9 (dotted line). Ketanserin (p$K_i<5.0$ in both guinea pig and rat 5-HT$_{1B}$ sites) is not shown.

hand, alniditan and BMS181,101 revealed slightly lower affinities for the rat as compared to guinea pig 5-HT$_{1B}$ receptors. Overall, there was a pronounced correlation between the affinities of agonists in guinea pig as compared to rat 5-HT$_{1B}$ receptors ($r=.74$, $P<.005$, $n=15$) (Fig. 5). This relationship was even more pronounced ($r=.90$ $P<.001$, $n=13$) upon the exclusion of the two above-mentioned agonists, CP93,129 and RU24,969, which, as outlined in the Discussion, are known to posses weak affinity for guinea pig populations of 5-HT$_{1B}$ receptor.

As regards antagonists, the arylalkylamines and potent β-adrenoceptor (AR) antagonists (−)-pindolol, (−)-cyanopindolol, (−)-propanolol and (−)-tertatolol all showed much higher affinities in rat 5-HT$_{1B}$ receptors as compared to their guinea pig counterparts, in line with previous observations considered in the Discussion. Correspondingly, upon inclusion of all antagonists, there was no overall significant correlation between affinities in guinea pig as compared to rat populations of 5-HT$_{1B}$ receptor ($r=.34$ $P>.05$, $n=12$). On the other hand, upon elimination of the four above-mentioned, structurally and pharmacologically homogeneous ligands, the correlation coefficient was highly significant ($r=.90$, $P<.005$, $n=8$).

4. Discussion

4.1. Kinetic and saturation analysis of [^{3}H]GR125,743 binding

The K_d (determined from Scatchard analysis) for binding of [^{3}H]GR125,743 to homogenates of rat FCX of 0.6 nM corresponds remarkably well to K_d estimates determined in previous studies of its binding to 5-HT$_{1B}$ receptors: notably, guinea pig FCX (0.3 nM) and striatum (0.3 nM) (Audinot et al., 1997); mouse striatum and substantia nigra (0.4 in each case, Bonaventure et al., 1997, 1999) and cloned, human h5-HT$_{1B}$ receptors expressed in CHO cells (0.5 and 0.7 nM for Newman-Tancredi et al., 2000 and Grånäs and Larhammar, 1999, respectively) or in Hela cells (1.0 nM, Doménech et al., 1997). This consistency in the K_d of [^{3}H]GR125,743 underlines its utility as a radioligand for labelling both native and cloned populations of receptors and is in line with the contention that [^{3}H]GR125,743 recognizes 5-HT$_{1B}$ receptors in rat FCX. Although the rapid phase of dissociation of [^{3}H]GR125,743 yielded a nominal, kinetic K_d of 0.88 nM close to the above-cited values, there was a second, slower component of dissociation of [^{3}H]GR125,743. This observation parallels that previously made in guinea pig FCX and appears to be an intrinsic property of this ligand, as well as the structurally related compound, GR125,937 (Skingle et al., 1996). Possible reasons underlying the unusual behaviour of GR125,743 in this regard are considered elsewhere (Audinot et al., 1997).

The density of sites labelled by [^{3}H]GR125,743 in rat FCX ($B_{max}=160$ fmol/mg), although substantially less than in several cell lines transfected with 5-HT$_{1B}$ sites (Doménech et al., 1997; Newman-Tancredi et al., 2000; Grånäs and Larhammar, 1999), bears comparison to a

value of 89 fmol/mg determined in guinea pig FCX with [^{3}H]GR125,743 (Audinot et al., 1997). Further, it is comparable to the density of 5-HT$_{1B}$ receptors documented in rat FCX using the agonist [^{125}I]GTI (319 fmol/mg, Bruinvels et al., 1993) and the nonselective antagonist [^{125}I] cyanopindolol in the presence of blocking agents (419 and 131 fmol/mg, respectively; Offord et al., 1988; Bruinvels et al., 1993). In addition, in a parallel study of rat FCX using the agonist [^{3}H]5-CT (Audinot et al., unpublished observation), a similar B_{max} of 249 fmol/mg was found. Indeed, the somewhat lower B_{max} for [^{3}H]GR125,743 versus [^{3}H]5-CT seen in rat FCX (herein) and guinea pig FCX (Audinot et al., 1997) likely reflects the lesser selectivity of 5-CT which, as discussed elsewhere (Audinot et al., 1997), labels 5-HT$_7$ receptors in rat FCX (To et al., 1995). Thus, it is likely that [^{3}H]GR125,743 versus [^{3}H]5-CT labels a pure population of 5-HT$_{1B}$ sites. Inasmuch as [^{3}H]GR125,743 behaves as an antagonist, it would be expected to recognise both coupled and uncoupled populations of 5-HT$_{1B}$ sites in contrast to the agonist, [^{3}H]5-CT, which would only bind to the former. The relative proportions of these populations in rat FCX is not known. However, the present observation that the B_{max} for [^{3}H]GR125,743 is not higher than that of [^{3}H]5-CT suggests that there is unlikely to be a major proportion of uncoupled sites.

4.2. Competition binding profiles

As discussed above, Scatchard analysis suggests that [^{3}H]GR125,743 binds to a single homogenous population of sites in rat FCX with a K_d consistent with the labelling of 5-HT$_{1B}$ receptors. Indeed, GR125,743 manifests pronounced selectivity for 5-HT$_{1B}$ receptors as compared to other classes of 5-HT receptor and a diversity of binding sites (Audinot et al., 1997; Newman-Tancredi et al., unpublished observation). Further, though [^{3}H]GR125,743 does not differentiate 5-HT$_{1B}$ from 5-HT$_{1D}$ receptors, the following observations strongly support identity of binding sites recognised by [^{3}H]GR125,743 in rat FCX with 5-HT$_{1B}$ receptors.

First, although no study to date has evaluated such an extensive series of ligands, the present findings correspond well to previous reports of drug affinities in rat 5-HT$_{1B}$ sites labelled with other radioligands. For example, the absolute and rank order of affinities of 5-CT, CP93,129 and sumatriptan determined in this study are close to those documented by Bruinvels et al., 1993. Affinities for RU28969, 5-HT and metergoline are comparable to those presented by Offord et al. (1988) with [^{125}I]cyanopindolol, while values for 5-CT, GR127,935 and DHE are similar to those reported by Waeber and Moskowitz, 1995 with [^{3}H]5-CT. Also of note is the striking affinity of L694,247 for [^{3}H]GR125,743-labelled rat FCX sites, in agreement with its very high affinity for native, rat 5-HT$_{1B}$ sites and 5-HT$_{1B}$ receptors in other species (Beer et al., 1993; Brüss et al., 1999; Grånäs and Larhammar, 1999).

Second, the sulphonamide-indole derivative (and partial agonist) L775,066 possesses marked affinity for h5-HT$_{1D}$ sites, whereas its affinity for h5-HT$_{1B}$ receptors is modest (Chambers et al., 1999; Newman-Tancredi et al., 2000; Longmore et al., 2000). A further selective agonist at 5-HT$_{1D}$ versus 5-HT$_{1B}$ receptors, PNU109,291 (Cutrer et al., 1999; Bouchelet et al., 2000), similarly revealed low affinity for rat FCX sites. In addition, ketanserin and ritanserin, which display low affinity for 5-HT$_{1B}$ as compared to 5-HT$_{1D}$ receptors (Adham et al., 1992a; Bonaventure et al., 1997, 1999), failed to displace [^{3}H]GR125,743.

Third, the indolepiperidine derivative and antagonist, SB224,289, which shows higher activity at rat, guinea pig and human 5-HT$_{1B}$ versus 5-HT$_{1D}$ receptors (Gaster et al., 1998; Gobert et al., 2000; Newman-Tancredi et al., 2000; Audinot et al., 2001a,b), displayed marked affinity for [^{3}H]GR125,743-labelled sites in rat FCX.

Fourth, for diverse ligands known not to distinguish rodent versus guinea pig/human 5-HT$_{1B}$ receptors, absolute and rank orders of affinities for [^{3}H]GR125,743-labelled 5-HT$_{1B}$ sites in rat FCX were very similar to those previously determined with [^{3}H]GR125,743 in guinea pig (see Table 1 and Fig. 5). This was the case for the agonists, CGS12066, DHE, 5-CT, 5-HT, GR46,611, GR85,548, anpirtoline and sumatriptan, as well as the antagonists, GR125,743 itself, GR127,935, S18127, methysergide, metergoline (see Audinot et al., 1997).

Nevertheless, the present study also incorporated several ligands known to possess contrasting affinities for rodent versus guinea pig and human 5-HT$_{1B}$ receptors (Oksenberg et al., 1992; see Tables 1 and 2 and Fig. 5). Most notably, the arylalkylamines, (−)-pindolol, (−)-propranolol, (−)-tertatolol and (−)-cyanopindolol, which display higher affinity for rodent 5-HT$_{1B}$ receptors as compared to human and guinea pig receptors (Adham et al., 1992a,b; Oksenberg et al., 1992; Brüss et al., 1999; Gobert and Millan, 1999; Audinot et al., 1997; Bonaventure et al., 1999). Similarly, in line with previous work (Yu et al., 1996; Audinot et al., 1997; Bonaventure et al., 1999), the agonists CP93,129 and RU24,969 show higher affinity in rat and mouse as compared to guinea pig and human 5-HT$_{1B}$ receptors. On the other hand, while alnitidan shows high affinity for guinea pig and h5-HT$_{1B}$ receptors, it showed relatively low affinity for 5-HT$_{1B}$ receptors in the rat (Table 1) and mouse (Bonaventure et al., 1999; Leysen et al., 1996; Audinot et al., 1997). Interestingly, the potential antidepressant, BMS181,101 revealed a similar preference for guinea pig as compared to rat 5-HT$_{1B}$ sites (Nowak et al., 1994; Table 1). In this light, we recently showed that a wide range of antipsychotics, including clozapine, ziprasidone, risperidone and S16924, despite their low affinity for rat 5-HT$_{1B}$ sites, manifest up to 1000-fold higher affinity at h5-HT$_{1B}$ sites (Audinot et al., 2001a). Further, with the exception of ziprasidone, all behave as inverse agonists. This suggests

that a role of 5-HT_{1B} receptors in their clinical actions may have been neglected.

4.3. General discussion

As discussed above, in line with previous studies of cloned, human, native guinea pig and native mouse 5-HT_{1B} receptors, [^{3}H]GR125,743 provides an excellent ligand for the radiolabelling of native, rat 5-HT_{1B} receptors. The present study also prompts several general remarks.

First, although characterization of [^{3}H]GR125,743 binding throughout the rat CNS would obviously be of interest, this study was restricted to the FCX in view of its key role in the control of mood, motor behaviour and cognition, and since the inhibitory influence of 5-HT_{1B} receptors upon the release of 5-HT (and other transmitters) is particularly well-characterized in this region (see Millan et al., 2000). Further, in focussing on one structure, it was possible to determine the affinities of a very extensive (28) range of agonists and antagonists, as well as diverse (10) antipsychotic agents (Audinot et al., 2001a).

Second, it remains controversial as to whether 5-HT_{1B} receptors display constitutive activity in vivo (Gaster et al., 1998; see Millan et al., 1999). The availability of [^{3}H]-GR125,743 offers a novel approach to examine this question for native, cerebral 5-HT_{1B} receptors. Thus, we recently showed that for cloned h5-HT_{1A} receptors the displacement by inverse agonists as compared to neutral antagonists of the radiolabelled antagonist [^{3}H]WAY100,635 is differentially modified by guanine nucleotides (Newman-Tancredi et al., 2001). Similar studies of rat 5-HT_{1B} sites with [^{3}H]-GR125,743 may be of interest.

Third, functional studies of the actions of GR125,743 at pre- and postsynaptic 5-HT_{1B} receptors in rodents (Hatcher et al., 1995; Millan et al., 1999; Skingle et al., 1996) suggest that it possesses low, if any, intrinsic activity in native 5-HT_{1B} receptors. Nevertheless, it should be noted that in certain studies of cloned, human 5-HT_{1B} receptors, weak, partial agonist properties of GR125,743 were seen (Doménech et al., 1997; Gränäs and Larhammar, 1999), analogous to the chemically related GR125,793 (Watson et al., 1996, Millan et al., 1999)].

4.4. Conclusions

In conclusion, the present results demonstrate that [^{3}H]GR125,743 specifically labels a homogeneous population of 5-HT_{1B} receptors in rat FCX. They extend previous studies of the utility of [^{3}H]GR125,743 for the characterization of 5-HT_{1B} sites to the rat, a key species used for exploration of their functional significance. In light of this, the determination of affinities for a substantial number of ligands in rat 5-HT_{1B} receptors should prove instructive for the future functional studies in this species. Inasmuch as [^{3}H]GR125,743 behaves as an antagonist in 5-HT_{1B} sites, it will be of particular use for their further characterization.

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