Naunyn-Schmiedeberg's Arch Pharmacol (1997) 356:328–334

ORIGINAL ARTICLE

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Characterization of human serotonin 1D and 1B receptors using [^{3}H]-GR-125743, a novel radiolabelled serotonin 5HT1D/1B receptor antagonist

Received: 13 February 1997 / Accepted: 18 May 1997

Abstract The study of serotonin (5-HT) receptors from the points of view of their anatomical localization and pharmacological characterization has been linked to the availability of highly selective radioligands exhibiting high affinity for their targets. This is particularly so in the case of serotonin receptors, since many different subtypes with overlapping pharmacological profiles have been described. Of these, the serotonin 5-HT$_1$ receptor family appears to be the most complex in terms of molecular diversity and pharmacological properties. The lack of appropriate tools to characterize the different receptor subtypes included in this family has hampered progress in the understanding of biological function. In the case of serotonin 5-HT$_{1D}$ receptors all the radioligands used so far in their characterization behave as agonists from the functional point of view. This agonistic character is regarded as a disadvantage for radioligands since their interaction with the receptors under study depends on factors other than the abundance of the receptor, such as the coupling of the receptors with G-proteins.

We describe here the binding properties of [^{3}H]-GR-125743, a new radiolabelled derivative of a compound that exhibits selective antagonistic properties with respect to the serotonin human (h5-HT$_{1D}$) and human (h5-HT$_{1B}$) receptors. The compound has been characterized for its ability to label the cloned h5-HT$_{1D}$ and h5-HT$_{1B}$ receptors. The binding obtained in both cases was specific, saturable and reversible, whereas the percentage of specific binding depended on the level of expression of the receptors. Using saturation analysis we have found that, on the specific clones used in this study, the compound labels a receptor population 5 to 10–fold higher that the one revealed using [^{3}H]-5-carboxamidotryptamine, a compound with agonist properties for these receptors in functional assays.

Using [^{3}H]-GR-125743 as a radioligand we have characterized the pharmacological profile of the same cloned h5-HT$_{1D}$ and h5-HT$_{1B}$ receptor preparations for a range of serotonin reference compounds by means of displacement assays.The affinities found have been compared, using regression analysis, with those obtained for the same radioligand and compounds in membranes obtained from human substantia nigra, a tissue known to be rich in h5-HT$_{1B/1D}$ receptors. We have found a better correlation, both in terms of correlation coefficient and of slope, between the substantia nigra data and the h5-HT$_{1B}$ data compared with the h5-HT$_{1D}$ data (0.94 and 1.05 vs. 0.86 and 0.64 respectively). Finally, the addition of 100 μM GTP reduced the binding of [^{3}H]-GR-125743 to h5-HT$_{1D}$ and h5-HT$_{1B}$ receptor subtypes by approximately 20% without affecting the affinities obtained for different displacers. Therefore, [^{3}H]-GR-125743 appears to be a suitable radioligand for the characterization of h5-HT$_{1D}$ and h5-HT$_{1B}$ receptor subtypes, being potentially more useful than previously existing compounds.

Key words Human · h5-HT$_{1B}$ receptor · h5-HT$_{1D}$ receptor · Radioligand binding · Substantia nigra

Introduction

Serotonin receptors have been classified, on the basis of operational and molecular biological criteria into seven major types: 5-HT$_1$ to 5-HT$_7$. Among the 5-HT receptor classes, the 5-HT$_1$ class is by far the most heterogeneous, comprising at least five different subtypes, 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1D}$, 5-HT$_{1E}$ and 5-HT$_{1F}$ (Hoyer et al. 1994).

The 5-HT$_{1D}$ receptor, a member of the seven transmembrane G protein-coupled receptor family, was originally identified in bovine brain as being distinct from 5-HT$_{1A}$ and 5-HT$_{1B}$ subtypes and was operationally defined as a population of binding sites that binds [^{3}H]-5-HT in the presence of 100 nM 8-OH-DPAT and 100 nM

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mesulergine (Heuring and Peroutka 1987). 5-HT_{1D} sites in human brain are enriched in the substantia nigra, the basal ganglia, and the dorsal subiculum and show a distribution very similar to that of the 5-HT_{1B} sites in rat brain (Waeber et al. 1990).

Molecular cloning revealed the existence of two distinct receptor populations closely related to the human 5-HT_{1D} receptor. These were named 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ subtypes, each of which was shown to be negatively coupled to adenylate cyclase (Hartig et al. 1992; Weinshank et al. 1992; Maenhaut et al. 1993).

It has been shown that the 5-$HT_{1D\beta}$ human receptor is the species homologue of the rat 5-HT_{1B} receptor, even though their pharmacological properties are quite different (Weinshank et al. 1992; Adham et al. 1992; Voigt et al. 1991; Hamblin et al. 1992), and a single aminoacid mutation (human threonine^{355}/rat asparagine^{351}) yields a receptor with a pharmacological profile superimposable on that of the rat brain 5-HT_{1B} receptor (Metcalf et al. 1992). On the other hand, 5-HT_{1D}-like receptors have been described in rat brain exhibiting a pharmacological profile similar to their human counterpart (Adham et al. 1992).

More recently it has been proposed that predominance should be given to the human sequence of genes and, in accordance with that, human 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptors have been renamed as 5-HT_{1D} and 5-HT_{1B} receptors, respectively (Hartig et al. 1996). This nomenclature has been adopted in the present paper.

Selective receptor antagonists are important tools in the understanding and characterization of the physiological functions of receptors. Direct radiolabelling of receptors with agonist ligands is limited, in the case of G-protein coupled receptors, to a subpopulation of high affinity binding sites (Hoyer and Boddeke 1993).The identification of "silent" antagonists for 5-HT_1 receptors is still limited. In the case of h5$HT_{1D/1B}$ receptors, no selective antagonist has been available until recently, and compounds like metergoline and methiothepine have been used, in spite of their known lack of selectivity. For these receptors progress in the characterization of agonists has been made more rapidly due to their known therapeutic value in the treatment of pathologies such as migraine (Doenicke et al. 1988).

GR-127935 (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) is a highly selective and potent 5-$HT_{1B/1D}$ receptor antagonist (Humphrey et al. 1988; Skingle et al. 1993). GR-127935 displaces, with high affinity, [^{3}H]-5HT binding to HeLa cells transfected with h5-HT_{1D} and h5-HT_{1B} receptors (pKi values of 9.9 and 8.9, respectively) and displays a good degree of selectivity over other 5-HT receptors (pKi<6) (Skingle et al. 1993; Clitherow et al. 1995).

Antagonist activity for GR-127935 has been demonstrated in the following functional 5-$HT_{1B/1D}$ receptor-mediated responses: sumatriptan-induced contractions of dog basilar artery and saphenous vein isolated rings (each of which contains vascular 5-HT_1 receptors equivalent to the 5-HT_{1B} receptor subtype from bovine and human cerebral arteries); contralateral turning elicited by the unilateral intranigral infusion of the 5-HT_1 receptor agonist GR-56764 in the guinea-pig substantia nigra; sumatriptan-evoked 5-HT release in guinea-pig dorsal raphe nucleus slices and stimulation of central 5-$HT_{1B/1D}$ receptors causing hypothermia in the guinea-pig (Hamel et al. 1993; Skingle et al. 1993; Starkey and Skingle 1993; Skingle et al. 1994; Starkey and Skingle 1994).

Recently, GR-127935 and metergoline, another 5HT receptor antagonist, have been defined in different models as either "silent" antagonists (Pauwels and Palmier 1995), partial agonists (Watson et al. 1996) or even full agonists (Walsh et al. 1995) at the level of adenylate cyclase modulation.

GR-125743 (N-[4-Methoxy-3-(4-methylpiperazin-1-yl)phenyl]-3-methyl-4-(4-pyridyl)benzamide) is another selective 5-$HT_{1B/1D}$ antagonist that shows 100-fold selectivity for 5-$HT_{1B/1D}$ receptors with respect to other serotonin receptors. That compound has a pKi value of 8.5 for the 5-$HT_{1B/1D}$ receptors and shows CNS and oral activity (Hatcher et al. 1995).

In the present paper we describe the characterization of the binding of tritiated GR-125743 to cloned h5-HT_{1D} and h5-HT_{1B} receptors separately expressed in HeLa cell lines.

Methods

Cell culture. The cloned HeLa cell lines 5AET14 and MA6A permanently expressing the h5-HT_{1B} and h5-HT_{1D} receptor genes (Doménech et al. 1994) were grown in Eagle minimal essential medium with Earle salts supplemented with 2 mM glutamine, 1% nonessential amino acids, 10% heat inactivated FCS and 600 µg G418/ml. Two days prior to pharmacological experiments, the medium was changed, being substituted with medium containing dialysed FCS and without geneticin.

Membrane preparation and binding assays. Human brains were obtained from the University of Barcelona Neurological Tissue Bank. Tissues were dissected and kept frozen at –80°C until used. The donors were two females and a man with ages ranging from 58 to 72 years and with immediate cause of death and post mortem delay as follows: cardiogenic shock (8 h), bronchopneumonia (3 h) and myocardial infarct (3 h). Tissue samples from the three donors were pooled prior to membrane preparation.

The cloned HeLa cell lines 5AET14 and MA6A were grown to approximately 90% confluence, scraped into 1× cold PBS and recovered by centrifugation (1,100 g, 10 min. at 4°C). Cell pellets were stored at –80°C until used.

Partially thawed cell pellets or brain samples were resuspended in 50 mM Tris-HCl, pH 7.7 buffer at room temperature and homogenized with a Polytron (2×15 s at full speed). The membranes were pelleted by centrifugation (20,000 g at 4°C for 45 min) and washed with the same buffer and re-centrifugated (20,000 g, 45 min at 4°C). The final membrane pellet was resuspended in binding buffer (50 mM Tris-HCl, pH 7.7, containing 10 µM pargyline, 0.1% ascorbic acid and 4 mM $CaCl^2$). Membranes were immediately frozen in aliquots by immersion in liquid nitrogen and stored at –80°C until used. Protein content was measured using the assay described by Bradford (1976), using bovine serum albumin as standard.

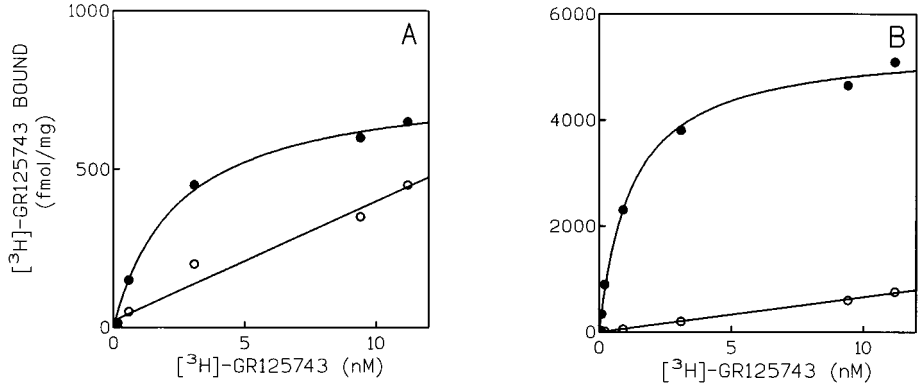

Fig. 1 Representative saturation analysis assays of [^{3}H]-GR 125743 binding to cloned human 5-HT$_{1D}$ (**A**) and 5-HT$_{1B}$ receptors (**B**). Non-specific binding was defined by 100 μM GR-127935. Data points were assayed in triplicate. (● specific binding, ○ non-specific binding)

Binding assays were performed in triplicate in 1 ml total volume containing 180 or 90 μg protein membranes/ml when MA6A or 5AET14 cell membranes were used respectively. [^{3}H]-GR-125743 was diluted in binding buffer at room temperature to a final concentration, for competition experiments, of 0.5 nM. Tubes were incubated at 37°C for 30 min and then rapidly filtered under reduced pressure using a Brandel cell harvester (model MB-48R), fitted with Whatman GF/B filters that had been presoaked in 0.3% polyethylenimine in ice-cold wash buffer (50 mM TRIS-HCl pH 7.7). The filters were then washed rapidly four times with 4 ml of ice-cold buffer and dried at 60°C for 30 min. The amount of radioactivity bound to the filters was measured by liquid scintillation counting, using 5 ml of Optiphase Hisafe II (EG&G) per filter. 1 μM GR-127935 was used to define nonspecific binding.

Saturation experiments were performed in triplicate using a final radioligand concentration ranging from 0.01 to 10 nM. Kinetic-association experiments were performed at the stated temperatures and samples were taken at 1, 2, 4, 6, 10, 20, 30 and 60 min. For the dissociation assays, the tubes containing standard reaction mixtures were initially allowed to equilibrate for 60 min. At that time 10 μM cold GR-127935 was added, and samples were taken at the same time intervals used for the association assays. For competition experiments and experiments involving incubation in the presence of 100 μM GTP, at least seven concentrations of displacing drugs ranging from 10^{-4} to 10^{-10} M, were assayed in triplicate.

Reagents and drugs. [^{3}H]-GR-125743 (70 Ci/mmol) was kindly provided by Amersham (UK). Serotonin-5-O-carboxymethyl-glycyl[^{125}I]tyrosinamide ([^{125}I]GTI; 2200 Ci/mmol) and [^{3}H]-5-carboxamidotryptamine ([^{3}H]-5CT; 50.4 Ci/mmol) were obtained from ANAWA (Switzerland) and Amersham (UK), respectively. 5-HT (5-hydroxytryptamine, serotonin) and 5-methoxytryptamine were obtained from Sigma (Madrid, Spain). Ketanserin, ritanserin, (±)-8-hydroxy-2(N-dipropylamino)tetralin (8-OH-DPAT) and methiothepin were obtained from Research Biochemicals Inc. (Natick,USA). Metergoline was from Farmitalia Carlo Erba (Italy). Sumatriptan, naratriptan, GR-127935, CP-122288 and 5-carboxamidotryptamine (5-CT) were prepared at the Medicinal Chemistry Department, Laboratorios Almirall (Barcelona,Spain). Geneticin was from GIBCO (Grand Island, N.Y., USA).

Data analysis. Linear and non linear regression calculations were performed using the programme InPlot (GraphPad Software, San Diego, Calif., USA). For displacement assays the values are given as the mean of the IC_{50} or slope values obtained in three individual experiments run in triplicate ± SEM. For the saturation and linear regression analysis the 95% confidence intervals of values or regression lines are given respectively.

Kd values derived from kinetic assays were calculated according to Bennett and Yamamura (1985).

Results

Saturation experiments

Saturable and high affinity [^{3}H]-GR-125743 binding was observed in membranes from cells expressing either h5HT$_{1D}$ or h5HT$_{1B}$ receptor subtypes (Fig. 1). At the radioligand concentration used in displacement assays, non specific binding was less than 50% for the h5-HT$_{1D}$ membranes, whereas it was less than 5% for the h5-HT$_{1B}$ membranes. Similar percentajes of specific signal were obtained when other antagonists, such as methiothepin, were used to define non-specific binding. The B_{max} and K_d values obtained for h5-HT$_{1D}$ receptors were 639 fmol/mg (584–695, n = 3) and 6.2 nM (4.9–7.4, n = 3), respectively; whereas for h5-HT$_{1B}$ receptors, values of 4732 fmol/mg (4607–4857) and 1.02 nM (0.92–1.11) were obtained.

In order to compare these results, the agonist [^{3}H]-5CT was used as radioligand with the same membrane preparations. The B_{max} and K_d values obtained in this case in saturation experiments were: 64 fmol/mg (52–82, n = 6) and 0.6 nM (0.5–0.7, n = 6) respectively for h5-HT$_{1D}$ receptors and 1057 fmol/mg (1086–1330, n = 6) and 0.6 nM (0.4–0.8, n = 6) respectively for h5-HT$_{1B}$ receptors.

Kinetic characterization

The kinetic characterization of the binding of [^{3}H]-GR-125743 to h5-HT$_{1B}$ cloned receptors was performed at 4°, 25° and 37°C. At 4°C equilibrium was not reached after the 60 min incubation period. At the two higher temperatures equilibrium was complete after 30 min. Association and dissociation rate constants were determined for specific [^{3}H]-GR-125743 binding at 37°C and the rate constants obtained were used to calculate a K_d value of 1.9 nM as described under methods.

Similar assays performed using the h5-HT$_{1\,D}$ receptor membranes showed that at 25°C and 37°C equilibrium was again reached in less than 30 min, but the low signal

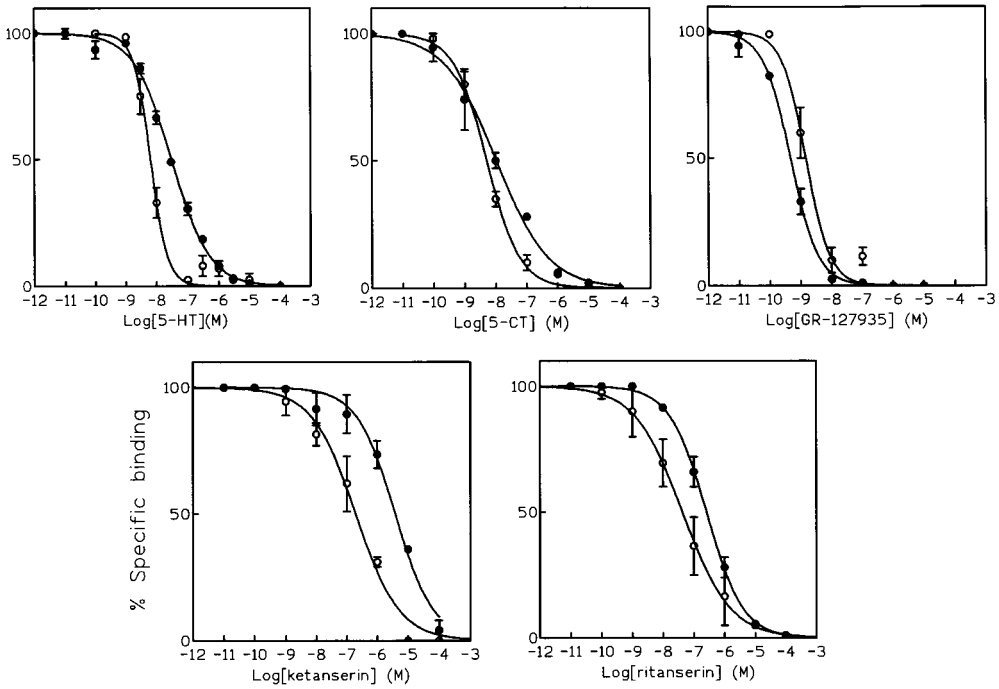

Fig. 2 Displacement curves of [^{3}H]-GR 125743 specific binding to the human cloned 5-HT_{1D} (○) and 5-HT_{1B} (●) receptors by selected serotonergic compounds

to-noise ratio obtained hampered the accurate measurement of the corresponding association and dissociation rate constants.

Pharmacological characterization

The affinities of different drugs for the transfected h5-HT_{1D} and h5-HT_{1B} receptors were characterized in competition experiments.

Displacement curves of [^{3}H]-GR-125743 binding to the two receptor subtypes using selected drugs, are shown in Fig. 2. For h5-HT_{1B} receptors, GR-129735 showed a slope of 1.0±0.1, whereas known agonists at this receptor such as 5-HT, sumatriptan, CP-122288, 5-CT, 8-OH-DPAT, 5-MeOT and naratriptan showed slopes ranging from 0.73 to 0.53. These compounds showed affinity values ranging from 0.43 to 4350 nM, with the following order of potency: GR-127935 > metergoline > methiothepin > naratriptan > 5-CT > 5-HT > 5-MeOT > sumatriptan > ritanserin > 8-OH-DPAT > ketanserin (Table 1).

For the h5-HT_{1D} receptors, the high non specific binding found, due to the low levels of receptor expressed by the MA6A cell clone, precluded a reliable analysis of the slopes obtained. The compounds tested as displacers of [^{3}H]-GR-125743 from this receptor, showed affinity values ranging from 1.08 to 111 nM with the following order of potency: metergoline > naratriptan = 5-MeOT = 5-CT > GR-127935 > methiothepin > 5-HT > sumatriptan > CP-122288 > 8-OH-DPAT > ritanserin > ketanserin (Table 1).

Table 1 Binding affinities (IC_{50}, nM) and slopes of displacement curves (nH) of a series of serotonergic standard compounds for cloned h5-HT_{1D} and h5-HT_{1B} receptor subtypes and for human substantia nigrausing [3H]-GR-125743 as radioligand

	h5-HT_{1D}		h5-HT_{1B}		Human S. Nigra	
Compound	IC_{50}	n_H	IC_{50}	n_H	IC_{50}	n_H
5-HT	3.4±1.2	0.81±0.28	38±9.0	0.69±0.04	9.0±2.0	0.77±0.10
Sumatriptan	8.4±1.7	0.80±0.10	60±6.0	0.69±0.04	28±10	0.84±0.06
5-CT	2.4±0.6	0.85±0.06	12±1.9	0.65±0.05	6.0±0.9	0.74±0.02
5-MeOT	1.7±0.4	0.77±0.16	38±1.4	0.53±0.08	35±3.6	0.74±0.10
Naratriptan	1.5±0.4	0.85±0.08	9.4±0.7	0.69±0.03	5.1±1.1	0.80±0.11
CP-122288	22±4.0	1.55±0.25	26±6.0	0.73±0.04	24±4.0	0.85±0.04
8-OH DPAT	47±33	0.90±0.08	710±430	0.58±0.10	700±130	0.91±0.04
Metergoline	1.1±0.2	0.74±0.14	5.5±2.0	0.89±0.10	9.4±0.3	0.94±0.01
GR127935	2.1±0.7	1.2±0.35	0.43±0.1	1.0±0.10	1.3±0.5	1.0±0.20
Methiothepin	5.9±1.2	0.61±0.06	5.7±0.9	0.68±0.04	31.6±12	0.80±0.02
Ketanserin	111±43	0.68±0.18	4350±460	0.78±0.10	2515±465	0.62±0.04
Ritanserin	72±0.5	0.56±0.07	289±63	0.75±0.01	266±40	0.65±0.15

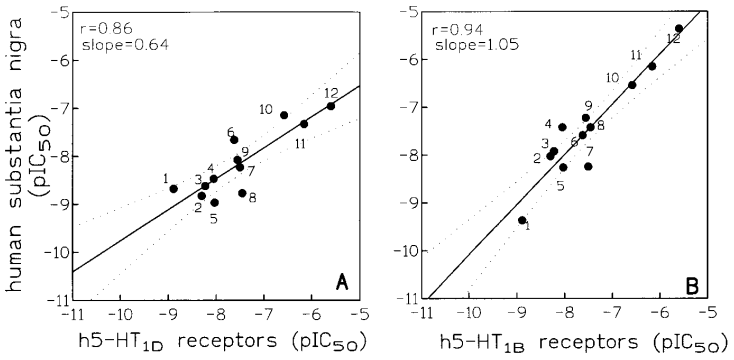

Fig. 3 Correlation of binding affinities of serotonergic reference compounds for the 5-HT_{1D}/5-HT_{1B} receptors in human substantia nigra vs. the coloned human 5-HT_{1D} (panel **A**) and 5-HT_{1B} (panel **B**) receptors (1. GR-127935, 2. naratriptan, 3. 5-CT, 4. 5-HT, 5. metergoline, 6. CP-122288, 7. methiothepin, 8. 5-MeOT, 9. sumatriptan, 10. ritanserin, 11. 8-OH DPAT, 12. ketanserin)

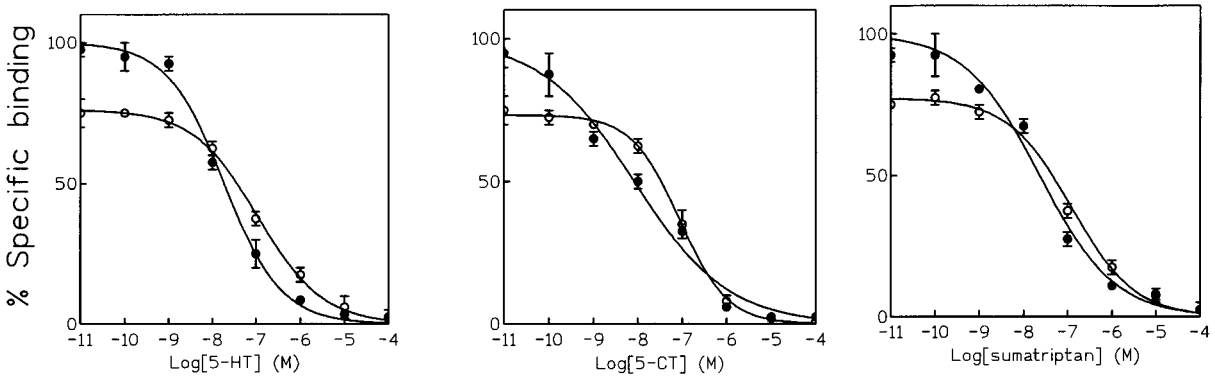

Fig. 4 Displacement curves of [^{3}H]-GR 125743 specific binding to the cloned human 5-HT_{1B} receptor membranes by selected serotonergic agonists in the absence (●) and in the presence (○) of 100 μM GTP

When the same compounds were tested in membrane preparations from human substantia nigra labelled with [^{3}H]-GR-125743, their affinities varied between 1.3 to 2515 nM, presenting the following order of affinity: GR-125743 > naratriptan > 5-CT > 5-HT > metergolin > CP-122288 > sumatriptan > methiothepin > 5-MeOT > ritanserin > 8-OH-DPAT > ketanserin (Table 1).

The affinity values obtained for the same drugs as displacers of [^{3}H]-GR-125743 from both the human cloned receptors were compared by linear regression with those observed using human substantia nigra membranes (Fig. 3). A good correlation was found in both cases, but the correlation coefficient obtained with the h5-HT_{1B} receptors (0.94) and the slope of the regression line (1.05) were significantly better that those obtained with the h5-HT_{1D} receptor membranes (0.86 and 0.64, respectively).

The addition of 100 μM GTP to standard incubation mixtures containing either h5-HT_{1D} or h5-HT_{1B} membranes resulted in a 20% reduction of the radioligand bound in the absence of displacers. No effect on either the affinity or the slope of the displacement curves was found for agonists or antagonists. The results obtained with representative displacers are shown in Fig. 4.

Discussion

The use of silent antagonists as radioligands for G-protein coupled receptors, instead of agonists, is generally encoureged because they recognize every receptor available with the same affinity, independently of their coupling to G-proteins, whereas agonists have higher affinity for the receptor population that is coupled to G-proteins. This implies that the proportion of receptors labelled by agonists in membranes obtained from different tissues or cell types will be influenced by the G-protein abundance in each particular tissue and on the extent of their coupling to the receptors, whereas this appears not to be the case for antagonists. Therefore, radiolabelled antagonists will give better estimations of the receptor abundance in a particular preparation (De Lean et al. 1980).

Very few selective antagonists have been described for serotonin h5HT_{1B} and h5HT_{1D} receptors and, until now, no radiolabelled antagonists were commercially available, with the exception of ^{125}I-cyanopindolol, the use of which is limited to the study of rodent 5-HT_{1B} receptors (Hoyer et al. 1985, Adham et al. 1992).

We have characterized the binding of [^{3}H]-GR-125743, a putative 5-$HT_{1B/1D}$ receptor antagonist, using membranes prepared from HeLa cell lines permanently transfected with either the h5HT_{1B} or h5HT_{1D} receptors. In saturation experiments the radioligand showed low non-specific binding for the h5HT_{1B} receptor preparation, whereas this value increased substantially when the h5HT_{1D} membrane preparation was used. This is likely

to be due to the low expression level of receptor in the latter preparation, since the absolute values for non-specific binding obtained in both cases were very similar. Under these conditions [^{3}H]-GR-125743 showed a 5-fold selectivity for the h5HT$_{1B}$ receptor with respect to the h5HT$_{1D}$ receptor according to the K_d values obtained.

The receptor density obtained for both the h5HT$_{1B}$ and h5HT$_{1D}$ transfected cell lines using [^{3}H]-GR-125743 was 10–fold and 5–fold higher respectively than the density obtained using the agonist [^{3}H]-5CT as radioligand. This illustrates that the antagonist radioligand can recognize a larger population of receptors than the agonist in our membrane preparations.

The kinetic studies performed indicate that the radioligand equilibrates rapidly with the receptors at 25°C and 37°C. In the case of the h5HT$_{1B}$ membranes a K_d value of 1.9 nM could be calculated, similar to the one obtained in saturation assays. A similar calculation for the h5HT$_{1D}$ membranes was precluded by the low specific signal obtained.

The pharmacological characterization of both receptor membrane preparations using several serotonin related drugs as displacers showed an order of potency typical of the h5HT$_{1D}$/5HT$_{1B}$ receptors. In the case of the h5-HT$_{1B}$ enriched membranes the displacement curves allowed the discrimination between the agonists used, which exhibited a shallow slope, and known antagonists such as GR-129735, that displayed a slope close to 1.

In order to establish if [^{3}H]-GR-125743 could be used as a diagnostic tool to characterize the relative h5-HT$_{1D}$ and h5-HT$_{1B}$ receptor populations in other membrane systems we tested the same compounds as displacers of this radioligand from human substantia nigra membranes. The affinity values obtained (expressed as pIC$_{50's}$) were compared by linear regression with the pIC$_{50}$ values obtained for the two cell membrane preparations (Table 1). It was found that the h5HT$_{1B}$ data fitted better with the substantia nigra values than the h5HT$_{1D}$ data, particularly in terms of slope of the regression line (1.05 for the former vs. 0.64 for the latter). Predominance of 5-HT$_{1B}$ over the 5-HT$_{1D}$ receptors has already been described in human cortex using a similar approach but using the agonist [^{125}I]-GTI as radioligand (Beer and Middlemis 1993).

The differences in affinity found for some of the displacers used have previously been described for the same receptor systems cloned from human and rabbit sources using the endogenous agonist [^{3}H]-5-HT as radioligand. Zgombick et al. (1995) reported a higher affinity for the recombinant human 5HT$_{1D}$ subtype, relative to the 5HT$_{1B}$ receptor, using the 5-HT$_{2A}$ receptor antagonists ketanserin and ritanserin (71 and 22-fold, respectively). Slightly lower ratios have been found in our system (39 and 4-fold, respectively). Bard et al. (1996) have extended these observations to the cloned rabbit 5-HT$_{1D}$ and 5-HT$_{1B}$ receptors. In this case, ketanserin was also found to be partially 5-HT$_{1D}$ selective (22-fold), whereas the non selective 5-HT$_{1D}$ antagonist methiothepin was described as having the opposite selectivity (16-fold). This latter observation does not seem to hold for the human receptor counterparts (Bard et al. 1996, this study).

Finally, the effect of the G-protein uncoupler GTP was determined in competition assays using known agonists as displacers. No significant effect on either the slope of the displacement curves or in the affinity of the different displacers was found for the two receptor preparations, but a small but consistent reduction in the [^{3}H]-GR-125743 binding was found in all cases. This indicates a direct effect of GTP on the affinity of the radioligand for both receptors, and suggests that GR-125743 is not a silent antagonist but may have some degree of intrinsic activity which will result in this compound acting as a full or a partial agonist in systems that have high degree of receptor reserve. A similar situation has already been described for other compounds defined as antagonists at these receptors, such as GR-129735 (Walsh et al. 1995; Pauwels and Palmier 1995). A reduction in the population of receptors labelled by the radiolabelled agonist [^{125}I]-GTI in the presence of GTP has been described for the 5-HT$_{1D}$ receptors (Palacios et al. 1992). The effect of GTP on [^{3}H]-GR-125743 binding is lower than that obtained using [^{125}I]-GTI, since GTP is able to reduce the binding of this agonist to the h5-HT$_{1B}$ an h5-HT$_{1D}$ receptor membranes used in this study by more than 50% (Doménech et al., manuscript in preparation).

In conclusion, [^{3}H]-GR-125743 appears to be a suitable radioligand for the characterization of h5-HT$_{1D}$ and h5-HT$_{1B}$ receptors. Being an antagonist it appears to be particularly suited for the study of receptor abundance in tissues. Preliminary results indicate that this radioligand also recognizes 5-HT$_{1B}$ receptors in rat striatum membrane preparations as well as h5-HT$_{1D}$ and h5-HT$_{1B}$ receptors in autoradiographic studies (Mengod et al. 1996)

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