Available online at www.sciencedirect.com
SCIENCE DIRECT®
Neuropharmacology 48 (2005) 25–33
NEURO PHARMACOLOGY
www.elsevier.com/locate/neuropharm

Autoradiographic characterisation of [^{35}S]GTPγS binding stimulation mediated by 5-HT$_{1B}$ receptor in postmortem human brain

Ricardo Mostany, Angel Pazos*, M. Elena Castro

Department of Physiology and Pharmacology, School of Medicine, University of Cantabria, Avda/Cardenal Herrera Oria s/n, Santander 39011, Cantabria, Spain

Received 13 May 2004; received in revised form 2 August 2004; accepted 20 August 2004

Abstract

G-protein activation mediated by 5-HT$_{1B}$ receptors was studied in human brain by [^{35}S]GTPγS autoradiographic methods. 5-HT (10 μM) increased [^{35}S]GTPγS binding in caudate–putamen nucleus, globus pallidus, dentate gyrus, CA$_1$, entorhinal cortex and substantia nigra. In basal ganglia and midbrain, this effect was blocked by GR 127935 (5-HT$_{1B/1D}$ antagonist). In contrast, WAY 100635 (selective 5-HT$_{1A}$ antagonist) reversed the effect of 5-HT in hippocampus and entorhinal cortex. Therefore, a detailed pharmacological study was carried out in basal ganglia and substantia nigra using 5-HT and the 5-HT$_{1B/1D}$ agonists GTI and CP 93129. In these areas, these agonists stimulated [^{35}S]GTPγS binding in a concentration-dependent manner, with no significant differences in the potency for a given structure. Furthermore, GTI was more potent in the putamen than in globus pallidus. In caudate–putamen, the three agonists showed the same efficacy, while in globus pallidus and substantia nigra the efficacy of 5-HT was higher than GTI and CP 93129. The selective 5-HT$_{1B}$ antagonist SB-224289 inhibited GTI- and CP 93129-stimulated [^{35}S]GTPγS binding in basal ganglia and substantia nigra, while coincubation with BRL 15572 (selective 5-HT$_{1D}$ antagonist) did not result in any significant change. Here we report the anatomical pattern of distribution of 5-HT$_{1B}$-dependent functionality by using specific pharmacological tools in human brain sections.

Keywords: [^{35}S]GTPγS autoradiography; G-proteins; 5-HT$_{1B}$; Serotonin; Putamen; Substantia nigra; Globus pallidus

1. Introduction

In the human central nervous system, serotonin (5-hydroxytryptamine; 5-HT) exerts diverse physiological responses through multiple receptor subtypes (Barnes and Sharp, 1999; Hoyer et al., 2002). The 5-HT$_1$ receptors group has been subdivided into 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1D}$, 5-ht$_{1E}$ and 5-ht$_{1F}$. Within this group, 5-HT$_{1B}$ and 5-HT$_{1D}$ show a similar pharmacology and anatomical distribution (Waeber and Moskowitz, 1995a; Castro et al., 1997; Domenech et al., 1997; Bonaventure et al., 1998a) despite their moderate (63%) aminoacid sequence homology (Hoyer et al., 2002). 5-HT$_{1B/1D}$ receptors play a role in the regulation of the release of 5-HT and other neurotransmitters (Pauwels, 1997) and are involved in several pathological process, particularly in migraine (Buzzi and Moskowitz, 1991; Moskowitz, 1992), depression (Huang et al., 2003), and degenerative movement disorders (Castro et al., 1998). Therefore, these receptors are interesting pharmacological targets for potential psychotherapeutic drugs (Barnes and Sharp, 1999). Autoradiographic and in situ

* Corresponding author. Tel.: +34 942 201 985; fax: +34 942 201 903.
E-mail address: pazosa@unican.es (A. Pazos).

0028-3908/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuropharm.2004.08.013

hybridisation studies have shown that both 5-HT_{1B} and 5-HT_{1D} receptors are present in basal ganglia and midbrain, among other regions, with a clear predominance of 5-HT_{1B} sites (Bruinvels et al., 1993; Waeber and Moskowitz, 1995b; Bonaventure et al., 1998b). The 5-HT_{1B} receptor subtype belongs to the superfamily of inhibitory G protein-coupled receptors (Hoyer et al., 2002) and its activation mediates the inhibition of adenylyl cyclase and the elevation of intracellular calcium levels (Bouhelal et al., 1988).

During the last decade, methods have been developed to examine the function of G protein-coupled receptors by measuring agonist-stimulated [^{35}S]GTPγS binding (Sim et al., 1995). Thus, for a given receptor, we can obtain a measure of G-protein activation, which is the first step in the transduction signalling converting the receptor activation into an intracellular response. In this regard, the applicability of this technique to measure the functional activation of G-proteins by stimulation of 5-HT_{1B} receptors has previously been reported in different assays using striatal rat membranes (Mize and Alper, 1999) as well as cells transfected with the h5-HT_{1B} receptor (Selkirk et al., 1998; Millan et al., 1999). Regarding the anatomical distribution of this functional response in the central nervous system, few autoradiographic studies have been carried out in rat and guinea-pig tissue, some of them restricted to the substantia nigra (Waeber and Moskowitz, 1997; Millan et al., 1999; Dupuis et al., 1999), and frequently reporting stimulation induced by 5-$HT_{1B/1D}$ agonists in areas known to contain 5-HT_{1A} receptors (Waeber and Moskowitz, 1997). Thus, the degree of pharmacological characterisation of 5-HT_{1B} receptor-stimulated [^{35}S]GTPγS binding throughout the brain is not complete. In addition, available rat brain data cannot be directly extrapolated to human brain due to strong species differences, mainly due to mutations in aminoacid sequence (Oksenberg et al., 1992). These differences are especially relevant regarding the affinity of beta blockers as well as of several agonists (i.e. CP 93129) (see Hoyer and Martin, 1997) between r5-HT_{1B} and h5-HT_{1B} receptors. Finally, no membrane homogenates binding or autoradiographic studies have been performed in human brain about 5-HT_{1B} receptor-mediated [^{35}S]GTPγS binding. Therefore, it is deemed to be of interest to ascertain whether 5-HT_{1B} receptor-mediated G-protein stimulation can be properly detected by autoradiographic techniques in human brain sections, as well as to analyse the regional distribution of their response.

The aim of the present study was to discriminate and characterise, with anatomical resolution, the level of activation of G-proteins mediated by 5-HT_{1B} receptors in human brain sections using both selective and non-selective 5-HT_{1B} agonists by [^{35}S]GTPγS binding methods.

2. Materials and methods

2.1. Postmortem human tissue

Human brain samples (Table 1) were obtained from 12 subjects (6 males/6 females) who died by several causes (neoplasia $n = 7$; heart failure $n = 3$; septicaemia $n = 1$; shock $n = 1$), with an average age of 61.2 ± 2.5 years (mean ± s.e.m.), average postmortem delay of 10.6 ± 1.3 h, and an average freezing storage period of 145.2 ± 25.4 days, without any record of psychiatric or neurological disorder. The brains were removed at autopsy at the Service of Pathology, University Hospital "Marqués de Valdecilla" (Santander, Spain), the tissue collection being approved by the Ethical Committee of the Hospital.

Blocks containing entorhinal cortex, hippocampus, caudate, putamen, globus pallidus and substantia nigra were promptly dissected and immediately stored at −70 °C until assay. Consecutive sections (20 μm thick)

Table 1
Sources of human brain tissue included in this study

Sample	Age (years)	Sex	Postmortem delay (h)	Freezing storage period (days)	Death cause
#1	50	Female	9	110	Breast neoplasia
#2	62	Male	20	108	Lung neoplasia
#3	68	Male	8	95	Stroke
#4	58	Male	14	89	Cardiac arrest
#5	76	Female	6	54	Shock
#6	61	Female	11	47	Gastric neoplasia
#7	73	Female	15	286	Lung neoplasia
#8	65	Female	8	251	Breast neoplasia
#9	58	Male	7	224	Lung neoplasia
#10	48	Male	14	203	Aortic aneurism
#11	58	Female	8	214	Septicaemia
#12	57	Male	7	65	Multiple myeloma

from every tissue block were cut at −25 °C using a microtome-cryostat, mounted on gelatine-coated slides and stored at −25 °C for no longer than two weeks after sectioning.

2.2. Materials

Guanylyl 5′-[gamma-[^{35}S]thio]-triphosphate ([^{35}S]-GTPγS; 1250 Ci/mmol) was purchased from DuPont NEN (Brussels, Belgium). 5-Hydroxytryptamine creatinine sulphate (5-HT), DL-dithiothreitol (DTT), GDP, guanosine-5′-*O*-(3-thiotriphosphate) (GTPγS), adenosine deaminase and *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-2-pyridinyl-cyclohexane-carboxamide-maleate (WAY 100635) were obtained from Sigma. 1,4-Dihydro-3-(1,2,3,6-tetrahydro-4-pyridinyl)-5H-pyrrolo[3,2-*b*]pyridin-5-one dihydrochloride (CP 93129) and 3-[4-(4-chlorophenyl)piperazin-1-yl]l,l-diphenyl-2-propanol hydrochloride (BRL 15572) were purchased from Tocris. 1′-Methyl-5-[[2′-methyl-4-(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-yl]]carbonyl]-2,3,6,7-tetrahydropiro [furo[2,3-f]indole-3,4′-piperidine] oxalate (SB-224289) was kindly donated by SmithKline Beecham Pharmaceuticals (Harlow, U.K.). Serotonin-5-*O*-carboxymethyl-glycil-tyrosamine (GTI) and *N*-[methoxy-3-(4-methyl-1-piperazinyl)phenyl]-(2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)[1,1-biphenyl]-4-carboxamide) (GR 127935) were kindly donated by Dr. J.M. Palacios (Almirall Prodesfarma, S.A., Spain).

2.3. [^{35}S]GTPγS receptor autoradiography

Labelling of brain sections with [^{35}S]GTPγS was carried out as described previously (Sim et al., 1995; Rodriguez-Puertas et al., 2000), with some modifications. Independently of the final purpose of the assay, slide-mounted sections were preincubated for 30 min at room temperature in buffer containing 50 mM Tris–HCl, 0.2 mM EGTA, 3 mM $MgCl_2$, 100 mM NaCl, 1 mM DTT and 2 mM GDP at pH 7.5.

2.3.1. [^{35}S]GTPγS autoradiographic discrimination of 5-HT$_1$ receptors-enriched human brain areas

In order to differentiate between 5-HT$_{1A}$ and 5-HT$_{1B/1D}$ receptors-enriched encephalic areas using [^{35}S]GTPγS receptor autoradiography, tissues were incubated at 25 °C for 2 h in buffer containing adenosine deaminase (3 mU/ml), [^{35}S]GTPγS (0.04 nM) and 10 μM of 5-HT alone or in the presence of 10 μM of WAY 100635 or GR 127935. The concentration of antagonists is that usually assayed in [^{35}S]GTPγS autoradiographic methods in order to assess the pharmacological nature of the G-protein stimulation (Millan et al., 1999; Rodriguez-Puertas et al., 2000).

2.3.2. [^{35}S]GTPγS autoradiographic concentration–response assays in 5-HT$_{1B/1D}$ receptors-enriched human brain areas

Slides containing consecutive sections from caudate nucleus, putamen nucleus, globus pallidus and substantia nigra were incubated at 25 °C for 2 h in buffer containing adenosine deaminase (3 mU/ml), [^{35}S]GTPγS (0.04 nM) and increasing (1 nM–0.1 mM) concentrations of the different agonists used (5-HT, GTI or CP 93129).

2.3.3. [^{35}S]GTPγS autoradiographic characterisation of 5-HT$_{1B}$-mediated response in human brain areas

Sections containing caudate nucleus, putamen nucleus, globus pallidus and substantia nigra were incubated at 25 °C for 2 h in buffer containing adenosine deaminase (3 mU/ml), [^{35}S]GTPγS (0.04 nM) and 10 μM of the agonists GTI or CP 93129, alone or in the presence of 10 μM of the antagonists SB-224289 or BRL 15572. In a complementary experiment, tissues were incubated in the presence of 10 μM of SB-224289 or BRL 15572, to test the effect of these antagonists per se.

In all the assays carried out, non-specific [^{35}S]GTPγS binding was determined in the presence of 10 μM GTPγS, and represented less than 10% of the basal binding. After the incubation, the sections were washed twice for 15 min in cold 50 mM Tris–HCl buffer (pH 7.4), briefly dipped in deionised water at 4 °C and then dried under a cold air stream. Finally, sections were exposed to autoradiographic film (Biomax MR, Kodak) together with ^{14}C microscales (Amersham) at 4 °C for 2 days.

3. Data analysis

Autoradiograms generated were analysed and quantified using a computerised image analysis system (Scion Image, Scion Corporation, Maryland, USA). Optical densities were transformed into nCi/g tissue equivalent based on the microscales radioactivity provided by the supplier. The data are presented as percent stimulation, calculated as: (agonist values − basal values)100/basal values, corresponding 100% value to basal stimulation.

Individual dose–response curves were obtained by non-linear regression analysis. The theoretical maximal effect (E_{max}) and the potency (pEC_{50}) for the agonist-stimulated [^{35}S]GTPγS binding, were calculated using the program GraphPad Prism (GraphPad Software, Inc. California, USA). Statistical comparison between experimental conditions was made using unpaired Student *t*-test (to evaluate the effect of each antagonist on stimulation of [^{35}S]GTPγS binding by the agonists), paired Student *t*-test (to evaluate the effect of the antagonists alone) or ANOVA following

Student–Newman–Keuls test (to compare the E_{max} and pEC_{50} parameters obtained from dose–response curves) using GraphPad Instat (GraphPad Software, Inc. California, USA). The significance level was set at $p < 0.05$.

4. Results

The effects of 5-HT on G protein-coupled receptors in several human brain areas were first analysed. The highest levels of stimulation of [^{35}S]GTPγS induced by 10 μM 5-HT were found in the outer layers of the entorhinal cortex (Table 2 and Fig. 1). High stimulation values were also detected in CA_1 field of hippocampus, globus pallidus and substantia nigra. In caudate and putamen nuclei, globus pallidus and substantia nigra, 5-HT-stimulated [^{35}S]GTPγS binding was significantly inhibited by 10 μM GR 127935 (5-$HT_{1B/1D}$ antagonist), while 10 μM WAY 100635 (selective 5-HT_{1A} antagonist) did not block this effect. On the other hand, in dentate gyrus, CA_1 field of hippocampus and entorhinal cortex, the stimulation of [^{35}S]GTPγS binding induced by 10 μM 5-HT was not affected by GR 127935 but completely abolished in the presence of 10 μM WAY 100635.

According to these results, basal ganglia and substantia nigra, which showed a 5-$HT_{1B/1D}$ receptor pharmacological profile, were selected for a detailed autoradiographic characterisation of [^{35}S]GTPγS binding response with more selective 5-$HT_{1B/1D}$ ligands.

As shown in Table 3 and Fig. 2, the stimulation of [^{35}S]GTPγS binding by the serotonergic agonists used (5-HT, GTI and CP 93129) varied among all the areas analysed. No statistically significant differences were found when the maximal stimulations (E_{max}) of the agonists were compared either in caudate or in putamen nuclei. In globus pallidus, CP 93129, the most selective 5-HT_{1B} agonist used to stimulate [^{35}S]GTPγS binding, showed a significant lower E_{max} value when compared to 5-HT. The maximal efficacy of 5-HT in the substantia nigra was significantly higher than those found for GTI and CP 93129. For each particular brain area examined, no significant differences were found when comparing the potencies of the different agonists to stimulate [^{35}S]GTPγS binding. Furthermore, no regional significant differences were found in potency for 5-HT and CP 93129 to stimulate [^{35}S]GTPγS binding. However, the potency of GTI was slightly lower in globus pallidus and substantia nigra than in putamen nucleus (Table 3).

In order to discriminate 5-HT_{1B}- and 5-HT_{1D} components of [^{35}S]GTPγS binding stimulation induced by the three serotonergic agonists, single concentrations (10 μM) of GTI and CP 93129 were used alone or in the presence of 10 μM of SB-224289 and BRL 15572, selective 5-HT_{1B} or 5-HT_{1D} antagonists, respectively. In this sense, SB-224289 inhibited significantly [^{35}S]GTPγS binding stimulated by GTI and CP 93129 in all the brain areas studied. On the other hand, the selective 5-HT_{1D} antagonist BRL 15572 had no significant effect on GTI- or CP 93129-stimulated [^{35}S]GTPγS binding (Table 4 and Figs. 3 and 4).

The effect of these two selective 5-HT_{1B} and 5-HT_{1D} antagonists (SB-224289 and BRL 15572) on the basal levels of [^{35}S]GTPγS binding was also tested. SB-224289 and BRL 15572 per se did not modify the basal [^{35}S]GTPγS binding (Fig. 5).

Table 2
Effect of 10 μM serotonin, alone or in presence of GR 127935 or WAY 100635, on [^{35}S]GTPγS binding in human brain sections

Brain area	5-HT	5-HT + GR 127935[a]	5-HT + WAY 100635[b]
Striatum			
Caudate	204.4 ± 19.2 (10)	110.2 ± 13.9** (9)	206.3 ± 37.5 (6)
Putamen	192.7 ± 22.4 (10)	97.0 ± 10.6** (9)	187.1 ± 16.6 (6)
Globus pallidus	450.8 ± 60.8 (8)	121.4 ± 15.0** (7)	533.9 ± 137.3 (4)
Hippocampus			
Dentate gyrus	182.5 ± 20.2 (5)	152.0 ± 12.5 (5)	62.5 ± 10.8** (5)
CA_1	415.8 ± 29.5 (5)	362.6 ± 32.2 (5)	70.9 ± 8.1** (5)
Entorhinal cortex			
Outer layers	468.8 ± 67.5 (5)	433.9 ± 60.9 (5)	99.7 ± 15.9** (5)
Intermediate layers	197.8 ± 32.2 (5)	157.3 ± 29.3 (5)	99.3 ± 12.6* (5)
Inner layers	201.7 ± 20.2 (5)	168.1 ± 27.6 (5)	101.8 ± 10.8** (5)
Substantia nigra	422.7 ± 70.4 (8)	149.7 ± 20.2** (7)	487.5 ± 110.8 (5)

The results are expressed as mean ± s.e.m. of the percentage of specific [^{35}S]GTPγS binding with respect to basal value (100%). Statistical comparison between the effect of each antagonist vs. agonist-stimulation alone was made using unpaired Student *t*-test. *$p < 0.05$ and **$p < 0.01$ vs. 5-HT stimulated specific [^{35}S]GTPγS binding. Numbers in brackets indicate number of samples analysed (*n*).

[a] 10 μM.

[b] 10 μM.

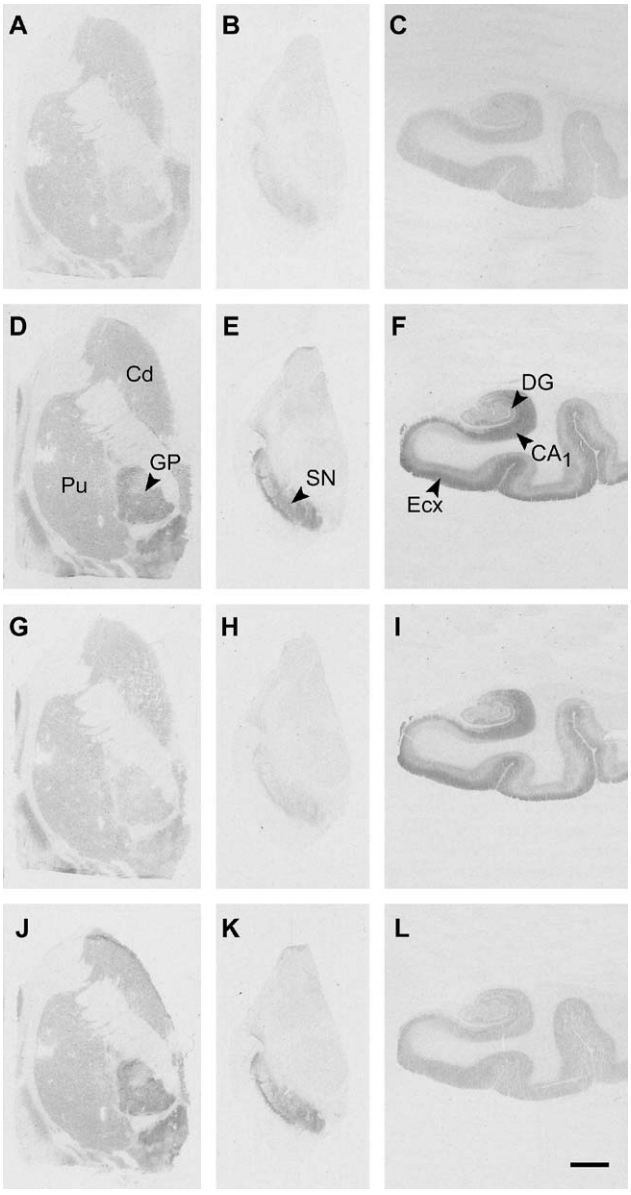

Fig. 1. Representative autoradiograms of 10 μM 5-HT-stimulated [^{35}S]GTPγS binding in basal ganglia (left), midbrain (centre) and hippocampus (right) levels of the human brain. (A, B, C) Basal binding; (D, E, F) 10 μM 5-HT; (G, H, I) 10 μM 5-HT + 10 μM GR 127935; (J, K, L) 10 μM 5-HT + 10 μM WAY 100635. CA_1, CA_1 hippocampal field; Cd, caudate nucleus; DG, dentate gyrus; Ecx, entorhinal cortex; GP, globus pallidus; Pu, putamen nucleus; SN, substantia nigra. Scale bar = 5 mm.

5. Discussion

G-protein activation mediated by 5-HT_{1B} receptors in the human brain was studied assessing agonist-stimulation of [^{35}S]GTPγS binding in tissue sections. We here demonstrate that the stimulation of [^{35}S]GTPγS binding induced by 5-HT, GTI and CP 93129 in caudate and putamen nuclei, globus pallidus and substantia nigra in the human brain, is mainly mediated through the activation of the 5-HT_{1B} receptor subtype and that this response follows a concentration-dependent pattern.

Our results provide novel and interesting information regarding the activation of G-proteins linked to 5-HT_{1B} receptors. First, we have carried out a complete autoradiographic localisation and characterisation of this process in human brain tissue sections. Second, we provide a detailed pharmacological information about 5-HT_{1B}-induced [^{35}S]GTPγS labelling, through the use of selective agonists such as CP 93129 (5-HT_{1B} agonist) and GTI (5-$HT_{1B/1D}$ agonist).

In a preliminary phase of the study, [^{35}S]GTPγS assays carried out in several 5-HT_1 receptors-enriched brain structures (Pazos et al., 1987; Barnes and Sharp, 1999) showed high levels of 5-HT-induced stimulation. In dentate gyrus, CA_1 field of hippocampus and entorhinal cortex, this response was abolished by the selective 5-HT_{1A} antagonist WAY 100635 and little or no effect was detected after incubation with the 5-$HT_{1B/1D}$ antagonist GR 127935. In contrast, in basal ganglia (caudate and putamen, globus pallidus) and substantia nigra the response observed was the opposite; no effect was detected with WAY 100635, but GR 127935 inhibited significantly 5-HT stimulated [^{35}S]GTPγS binding. These results correlate well with the reported presence of 5-HT_1 receptor subtypes in these brain areas, as high densities of the 5-HT_{1A} subtype are present in hippocampus and cerebral cortex (Pazos et al., 1987), while basal ganglia and substantia nigra have been reported to be enriched in 5-$HT_{1B/1D}$ receptors (Bruinvels et al., 1994). This correlation between receptor distribution described in previous studies and functional autoradiography results obtained in the present work, further support the interest of the methodological approach we carried out. The demonstration of a specific 5-HT_{1B}-dependent stimulation of G-proteins in caudate, putamen, globus pallidus and substantia nigra reinforce the proposed role of these receptors in the regulation of motor activity.

When comparing the concentration–response curves obtained in 5-$HT_{1B/1D}$ receptors-enriched areas for the three agonists, the highest stimuli obtained were detected when the non-selective agonist 5-HT was used. No significant differences were observed for the three agonists assayed in caudate and putamen nuclei. However, the maximal efficacy of 5-HT was significantly higher than the one shown by CP 93129 in globus pallidus and than those of the other agonists in the substantia nigra. Several explanations could account for these differences. The different intrinsic activity of the agonists used could be the most obvious explanation to this fact. In addition, the fact that CP 93129 stimulates only 5-HT_{1B} sites could also explain these differential patterns in terms of efficacy. In a similar way, existence in these brain areas of another serotonergic receptor

Table 3
Effect of 5-HT agonists on [^{35}S]GTPγS binding in human brain sections

Brain area	5-HT			GTI			CP 93129		
	E_{max}	pEC_{50}	n	E_{max}	pEC_{50}	n	E_{max}	pEC_{50}	n
Striatum									
Caudate	210.6 ± 26.8	6.5 ± 0.3	6	199.3 ± 28.7	6.7 ± 0.3	6	150.8 ± 8.0	6.6 ± 0.3	6
Putamen	180.4 ± 24.7	6.6 ± 0.3	5	159.9 ± 11.2	7.2 ± 0.3	5	171.8 ± 7.9	6.3 ± 0.5	4
Globus pallidus	543.4 ± 129.4	6.2 ± 0.2	4	353.0 ± 74.5	5.9 ± 0.2	5	194.1 ± 27.4*	6.3 ± 0.3	5
Substantia nigra	472.1 ± 111.9	6.7 ± 0.3	5	247.9 ± 44.2*	6.1 ± 0.2	6	172.7 ± 13.6*	6.2 ± 0.3	5

Values are expressed as mean ± s.e.m. of individual concentration–response curves. E_{max} values are given as percentage of [^{35}S]GTPγS binding with respect to basal value (100%). $pEC_{50} = -\log EC_{50}$. Statistical comparison between experimental conditions was made using ANOVA following Student–Newman–Keuls. *$p < 0.05$ vs. 5-HT stimulated [^{35}S]GTPγS binding.

subtypes with high affinity for 5-HT, such as 5-ht$_{1F}$ site (Castro et al., 1997) should be taken into account. Finally, regional differences in both, distribution and degree of activity of the different subtypes of G-proteins (Young et al., 1993), as well as possible mechanisms of agonist-directed trafficking of receptor signalling, could also be responsible for these findings.

Regarding potency values, our results are in good agreement with those reported for 5-HT and CP 93129 in rat striatal membranes (Mize and Alper, 1999). Furthermore, no regional differences in affinity were found for these agonists. With respect to GTI, EC_{50} values in globus pallidus and substantia nigra were found to be lower than those measured in putamen nucleus. At the present time, we have no clear explanation for this fact. Although the presence of 5-HT$_{1D}$ receptors in these areas could be involved in this response, a regional-dependent coupling to different G-protein subtypes might also account for these differences in affinity.

In our study, the stimulation of [^{35}S]GTPγS binding by GTI and CP 93129 in basal ganglia and substantia nigra was fully antagonised by the selective 5-HT$_{1B}$ antagonist SB-224289, indicating that these responses were mainly mediated by the activation of the 5-HT$_{1B}$ receptor subtype. The selective 5-HT$_{1D}$ antagonist BRL 15572 had no effect on CP 93129-stimulated [^{35}S]GTPγS binding. In contrast, although without

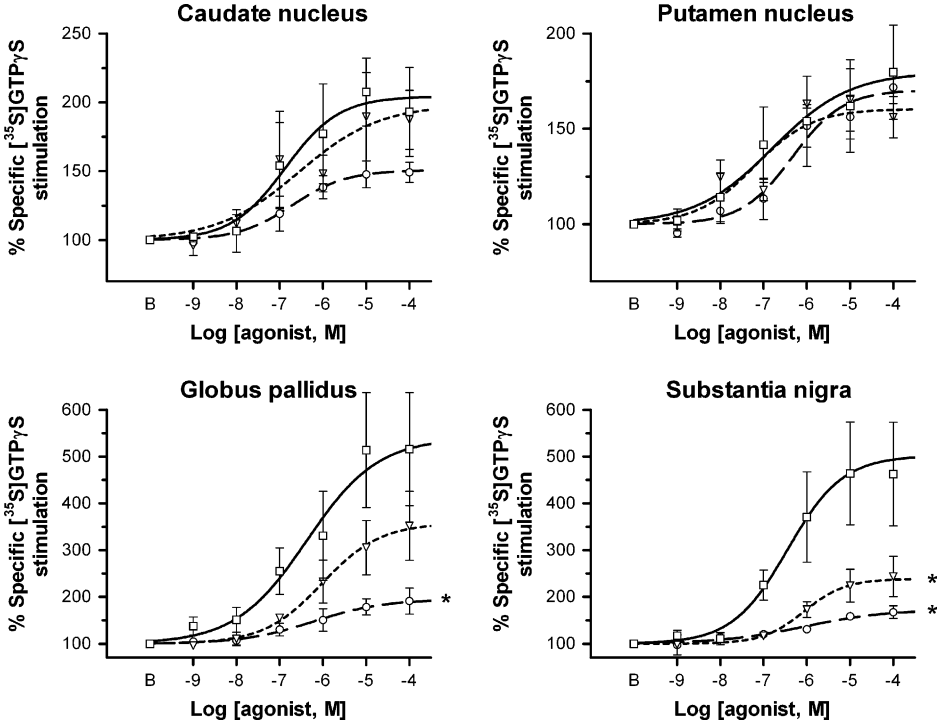

Fig. 2. Autoradiographic concentration–response curves illustrating the effect of 5-HT (*squares*, solid line), GTI (*triangles*, dotted line) and CP 93129 (*circles*, broken line) on [^{35}S]GTPγS binding in human brain areas. Each curve is the mean ± s.e.m. curve of 4–6 individual samples. Statistical comparison between experimental conditions was made using ANOVA following Student–Newman–Keuls. *$p < 0.05$ vs. 5-HT-stimulated [^{35}S]GTPγS binding.

Table 4
Effect of 10 μM GTI and CP 93129, alone or in presence of SB-224289 or BRL 15572, on [^{35}S]GTPγS binding in human brain sections

Brain area	GTI			CP 93129		
	GTI	+SB-224289[a]	+BRL 15572[b]	CP 93129	+SB-224289[a]	+BRL 15572[b]
Striatum						
Caudate	163.1 ± 14.9 (11)	116.6 ± 13.9* (7)	143.7 ± 18.7 (6)	134.9 ± 7.0 (10)	90.9 ± 8.9** (6)	126.6 ± 9.5 (8)
Putamen	179.3 ± 30.2 (10)	106.4 ± 14.0* (7)	153.0 ± 19.4 (5)	136.0 ± 8.2 (9)	98.4 ± 9.6** (6)	138.6 ± 10.7 (7)
Globus pallidus	293.9 ± 46.1 (9)	90.84 ± 13.2** (10)	208.2 ± 39.7 (6)	143.3 ± 9.5 (9)	85.1 ± 5.4** (6)	143.1 ± 26.6 (4)
Substantia nigra	303.4 ± 53.0 (11)	135.0 ± 19.6* (8)	193.9 ± 14.2 (7)	154.6 ± 6.9 (8)	100.5 ± 6.6** (4)	151.1 ± 13.7 (3)

The results are expressed as mean ± s.e.m. of the percentage of [^{35}S]GTPγS binding with respect to basal value (100%). Statistical comparison between the effect of each antagonist vs. agonist-stimulation alone was made using unpaired Student *t*-test. *$p < 0.05$ and **$p < 0.01$ vs. agonist-stimulated [^{35}S]GTPγS binding. Numbers in brackets indicate number of samples analysed (*n*).

[a] 10 μM.

[b] 10 μM.

reaching statistical significance, this 5-HT$_{1D}$ antagonist partially inhibited [^{35}S]GTPγS binding stimulation induced by GTI. Taken together, these results strongly suggest that the functional response elicited by CP 93129 is completely dependent on 5-HT$_{1B}$ receptor stimulation; in line with this, the activation of 5-HT$_{1D}$ receptors would represent only a minor component of the stimulation observed in the assays in which GTI was used.

No inverse agonist properties of SB-224289 could be detected in our study. Controversial data about this issue have been reported. Thus, depending on the receptor source (native receptor or recombinant h5-HT$_{1B}$ receptors expressed in cellular lines), and the methodological approach carried out (binding to membrane homogenates or autoradiographic studies), different authors have reported inverse agonist (Selkirk et al., 1998; Millan et al., 1999) or antagonist (Millan et al., 1999) properties for this compound. It is noteworthy that no inverse agonist effect has been observed in native tissue. It is possible that the appearance of inverse agonist profile (Selkirk et al., 1998; Millan et al., 1999) for SB-224289 in [^{35}S]GTPγS binding might be highly dependent on the concentration of GDP present in the incubation buffer, significantly lower in membrane or cell homogenates binding studies (30–50 μM) than in autoradiographic studies (2 mM). In any case, our results validate the use of this antagonist to assess the selectivity of the agonist-induced stimulation of [^{35}S]GTPγS binding. In a similar way, BRL 15572 did not modify the basal binding, acting as a neutral antagonist, as previously described (Schlicker et al., 1997; Barnes and Sharp, 1999).

In conclusion, the present report demonstrates that [^{35}S]GTPγS binding can be properly carried out in 5-HT$_{1B}$-enriched human brain areas using autoradiographic techniques with selective ligands. Here we report

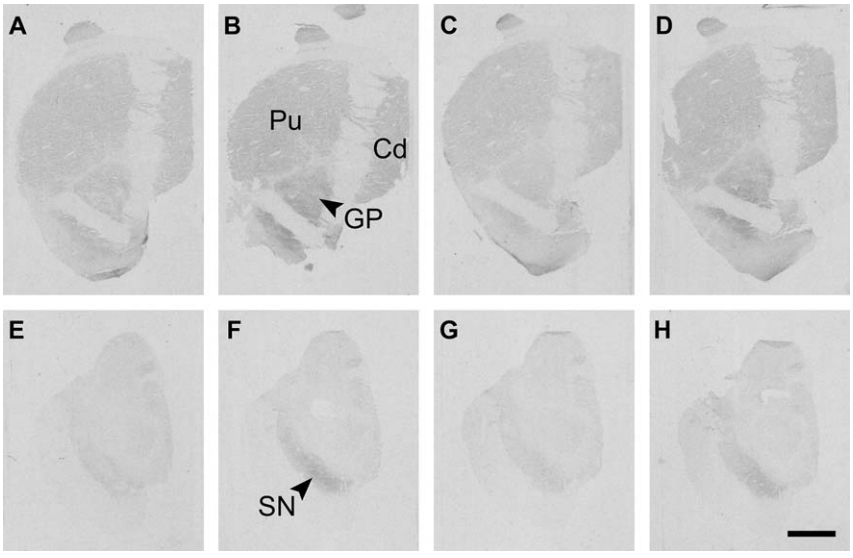

Fig. 3. Representative autoradiograms of 10 μM GTI-stimulated [^{35}S]GTPγS binding coupled to 5-HT$_{1B/1D}$ receptors in human basal ganglia and midbrain. (A, E) Basal binding; (B, F) 10 μM GTI; (C, G) 10 μM GTI + 10 μM SB-224289; (D, H) 10 μM GTI + 10 μM BRL 15572. Cd, caudate nucleus; GP, globus pallidus; Pu, putamen nucleus; SN, substantia nigra. Scale bar = 5 mm.

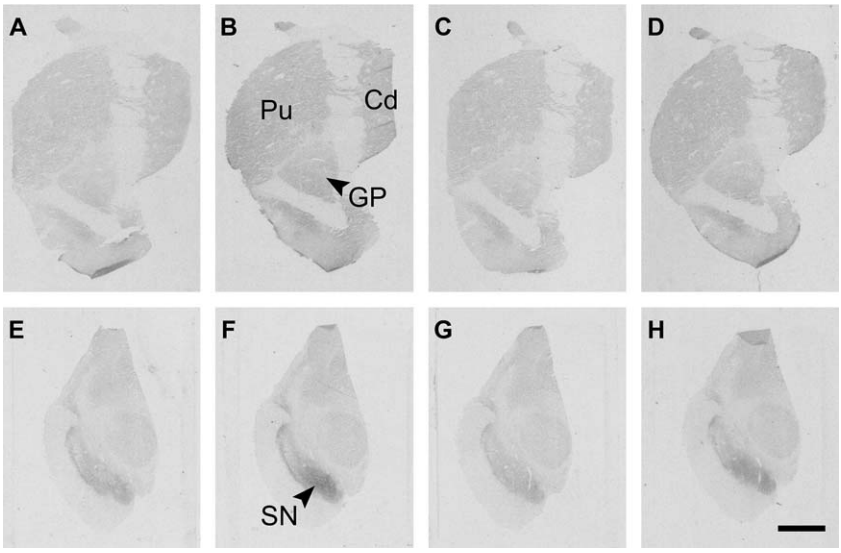

Fig. 4. Representative autoradiograms of 10 μM CP 93129-stimulated [^{35}S]GTPγS binding coupled to 5-HT$_{1B}$ receptors in human basal ganglia and midbrain. (A, E) Basal binding; (B, F) 10 μM CP 93129; (C, G) 10 μM CP 93129 + 10 μM SB-224289; (D, H) 10 μM CP 93129 + 10 μM BRL 15572. Cd, caudate nucleus; GP, globus pallidus; Pu, putamen nucleus; SN, substantia nigra. Scale bar = 5 mm.

a complete visualisation of pharmacologically-assessed 5-HT$_{1B}$ receptor-mediated [^{35}S]GTPγS binding in human brain areas, showing remarkably high levels of G-protein activation in areas regulating motor activity, including basal ganglia structures (caudate, putamen and globus pallidus) as well as substantia nigra, in good agreement with the anatomical distribution of the receptor protein. We also demonstrate that 5-HT, GTI and particularly CP 93129 can be useful pharmacological tools for measuring and studying the regional pattern of stimulated [^{35}S]GTPγS binding through the activation of 5-HT$_{1B}$ receptors. This opens the possibility to explore the functional state of this receptor subtype under diverse pathological conditions as well as after acute and long-term administration of psychotherapeutic drugs.

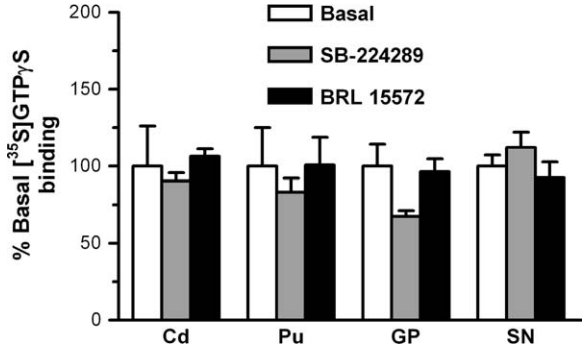

Fig. 5. Effect of 10 μM SB-224289 and BRL 15572 on basal (100%) [^{35}S]GTPγS binding in human basal ganglia and midbrain. Cd, caudate nucleus; GP, globus pallidus; Pu, putamen nucleus; SN, substantia nigra.

Acknowledgements

We are grateful to Almirall-Prodesfarma, S.A. and SmithKline Beecham Pharmaceuticals for the generous gift of drugs. We also wish to thank Ms Josefa Castillo and Ms Lourdes Lanza for their technical assistance. We are deeply in debt to the staff of the Department of Pathology of the University Hospital "Marqués de Valdecilla" for providing us with the human brain samples used in this study. This work was supported by CICYT BFI 2001/0592 and FEDER 1FD97-1597.

References

Barnes, N.M., Sharp, T., 1999. A review of central 5-HT receptors and their function. Neuropharmacology 38, 1083–1152.

Bonaventure, P., Langlois, X., Leysen, J.E., 1998a. Co-localization of 5-HT$_{1B}$- and 5-HT$_{1D}$ receptor mRNA in serotonergic cell bodies in guinea pig dorsal raphé nucleus: a double labeling in situ hybridization histochemistry study. Neuroscience Letters 254, 113–116.

Bonaventure, P., Voorn, P., Luyten, W.H., Jurzak, M., Schotte, A., Leysen, J.E., 1998b. Detailed mapping of serotonin 5-HT$_{1B}$ and 5-HT$_{1D}$ receptor messenger RNA and ligand binding sites in guinea-pig brain and trigeminal ganglion: clues for function. Neuroscience 82, 469–484.

Bouhelal, R., Smounya, L., Bockaert, J., 1988. 5-HT$_{1B}$ Receptors are negatively coupled with adenylate cyclase in rat substantia nigra. European Journal of Pharmacology 151, 189–196.

Bruinvels, A.T., Palacios, J.M., Hoyer, D., 1993. Autoradiographic characterisation and localisation of 5-HT$_{1D}$ compared to 5-HT$_{1B}$ binding sites in rat brain. Naunyn–Schmiedeberg's Archives of Pharmacology 347, 569–582.

Bruinvels, A.T., Landwehrmeyer, B., Probst, A., Palacios, J.M., Hoyer, D., 1994. A comparative autoradiographic study of 5-HT$_{1D}$ binding sites in human and guinea-pig brain using different radioligands. Molecular Brain Research 21, 19–29.

Buzzi, M.G., Moskowitz, M.A., 1991. Evidence for 5-$HT_{1B/1D}$ receptors mediating the antimigraine effect of sumatriptan and dihydroergotamine. Cephalalgia 11, 165–168.

Castro, M.E., Pascual, J., Romon, T., del Arco, C., del Olmo, E., Pazos, A., 1997. Differential distribution of [^{3}H]sumatriptan binding sites (5-HT_{1B}, 5-HT_{1D} and 5-HT_{1F} receptors) in human brain: focus on brainstem and spinal cord. Neuropharmacology 36, 535–542.

Castro, M.E., Pascual, J., Romon, T., Berciano, J., Figols, J., Pazos, A., 1998. 5-HT_{1B} receptor binding in degenerative movement disorders. Brain Research 790, 323–328.

Domenech, T., Beleta, J., Palacios, J.M., 1997. Characterization of human serotonin 1D and 1B receptors using [^{3}H]-GR-125743, a novel radiolabelled serotonin 5-$HT_{1D/1B}$ receptor antagonist. Naunyn–Schmiedeberg's Archives of Pharmacology 356, 328–334.

Dupuis, D.S., Perez, M., Halazy, S., Colpaert, F.C., Pauwels, P.J., 1999. Magnitude of 5-HT_{1B} and 5-HT_{1A} receptor activation in guinea-pig and rat brain: evidence from sumatriptan dimer-mediated [^{35}S]GTPγS binding responses. Molecular Brain Research 67, 107–123.

Hoyer, D., Martin, G., 1997. 5-HT receptor classification and nomenclature: towards a harmonization with the human genome. Neuropharmacology 36, 419–428.

Hoyer, D., Hannon, J.P., Martin, G.R., 2002. Molecular, pharmacological and functional diversity of 5-HT receptors. Pharmacology, Biochemistry and Behavior 71, 533–554.

Huang, Y.Y., Oquendo, M.A., Friedman, J.M., Greenhill, L.L., Brodsky, B., Malone, K.M., Khait, V., Mann, J.J., 2003. Substance abuse disorder and major depression are associated with the human 5-HT_{1B} receptor gene (HTR1B) G861C polymorphism. Neuropsychopharmacology 28, 163–169.

Millan, M.J., Gobert, A., Audinot, V., Dekeyne, A., Newman-Tancredi, A., 1999. Inverse agonists and serotonergic transmission: from recombinant, human serotonin (5-HT)$_{1B}$ receptors to G-protein coupling and function in corticolimbic structures in vivo. Neuropsychopharmacology 21, 61–67.

Mize, A.L., Alper, R.H., 1999. Serotonin 5-$HT_{(1B/1D)}$ agonist-stimulated [^{35}S]GTPγS binding in rat and guinea pig striatal membranes. Brain Research 836, 229–236.

Moskowitz, M.A., 1992. Neurogenic versus vascular mechanisms of sumatriptan and ergot alkaloids in migraine. Trends in Pharmacological Sciences 13, 307–311.

Oksenberg, D., Marsters, S.A., O'Dowd, B.F., Jin, H., Havlik, S., Peroutka, S.J., Ashkenazi, A., 1992. A single amino-acid difference confers major pharmacological variation between human and rodent 5-HT_{1B} receptors. Nature 360, 161–163.

Pauwels, P.J., 1997. 5-$HT_{1B/1D}$ receptor antagonists. General Pharmacology: The Vascular System 29, 293–303.

Pazos, A., Probst, A., Palacios, J.M., 1987. Serotonin receptors in the human brain-III. Autoradiographic mapping of serotonin-1 receptors. Neuroscience 21, 97–122.

Rodriguez-Puertas, R., Gonzalez-Maeso, J., Meana, J.J., Pazos, A., 2000. Autoradiography of receptor-activated G-proteins in post mortem human brain. Neuroscience 96, 169–180.

Schlicker, E., Fink, K., Molderings, G.J., Price, G.W., Duckworth, M., Gaster, L., Middlemiss, D.N., Zentner, J., Likungu, J., Gothert, M., 1997. Effects of selective h5-HT_{1B} (SB-216641) and h5-HT_{1D} (BRL-15572) receptor ligands on guinea-pig and human 5-HT auto- and heteroreceptors. Naunyn–Schmiedeberg's Archives of Pharmacology 356, 321–327.

Selkirk, J.V., Scott, C., Ho, M., Burton, M.J., Watson, J., Gaster, L.M., Collin, L., Jones, B.J., Middlemiss, D.N., Price, G.W., 1998. SB-224289 – a novel selective (human) 5-HT_{1B} receptor antagonist with negative intrinsic activity. British Journal of Pharmacology 125, 202–208.

Sim, L.J., Selley, D.E., Childers, S.R., 1995. In vitro autoradiography of receptor-activated G proteins in rat brain by agonist-stimulated guanylyl 5′-[gamma-[^{35}S]thio]-triphosphate binding. Proceedings of the National Academy of Sciences USA 92, 7242–7246.

Waeber, C., Moskowitz, M.A., 1995a. Autoradiographic visualisation of [^{3}H]5-carboxamidotryptamine binding sites in the guinea pig and rat brain. European Journal of Pharmacology 283, 31–46.

Waeber, C., Moskowitz, M.A., 1995b. [^{3}H]sumatriptan labels both 5-HT_{1D} and 5-HT_{1F} receptor binding sites in the guinea pig brain: an autoradiographic study. Naunyn–Schmiedeberg's Archives of Pharmacology 352, 263–275.

Waeber, C., Moskowitz, M.A., 1997. 5-Hydroxytryptamine$_{1A}$ and 5-hydroxytryptamine$_{1B}$ receptors stimulate [^{35}S]guanosine-5′-*O*-(3-thio)triphosphate binding to rodent brain sections as visualized by in vitro autoradiography. Molecular Pharmacology 52, 623–631.

Young, L.T., Li, P.P., Siu, K.P., Kish, S.J., Warsh, J.J., 1993. Regional distribution of guanine nucleotide binding proteins (G_s and $G_{i\alpha}$) in human brain: correlation with adenylyl cyclase activity. Neurochemistry International 22, 285–291.