ELSEVIER

European Journal of Pharmacology 355 (1998) 245–256

ejp

Agonist and antagonist actions of antipsychotic agents at 5-HT_{1A} receptors: a [^{35}S]GTPγS binding study

Adrian Newman-Tancredi *, Samantha Gavaudan, Caroline Conte, Christine Chaput, Manuelle Touzard, Laurence Verrièle, Valerie Audinot, Mark J. Millan

Department of Psychopharmacology, Institut de Recherches Servier, 125 Chemin de Ronde, 78290 Croissy-sur-Seine, Paris, France

Received 2 April 1998; revised 19 June 1998; accepted 26 June 1998

Abstract

Recombinant human (h) 5-HT_{1A} receptor-mediated G-protein activation was characterised in membranes of transfected Chinese hamster ovary (CHO) cells by use of guanosine-5′-*O*-(3-[^{35}S]thio)-triphosphate ([^{35}S]GTPγS binding). The potency and efficacy of 21 5-HT receptor agonists and antagonists was determined. The agonists, 5-CT (carboxamidotryptamine) and flesinoxan displayed high affinity (subnanomolar K_i values) and high efficacy ($E_{max} > 90\%$, relative to 5-HT = 100%). In contrast, ipsapirone, zalospirone and buspirone displayed partial agonist activity. EC_{50}s for agonist stimulation of [^{35}S]GTPγS binding correlated well with K_i values from competition binding ($r = +0.99$). Among the compounds tested for antagonist activity, methiothepin and (+)butaclamol exhibited 'inverse agonist' behaviour, inhibiting basal [^{35}S]GTPγS binding. The actions of 17 antipsychotic agents were investigated. Clozapine and several putatively 'atypical' antipsychotic agents, including ziprasidone, quetiapine and tiospirone, exhibited partial agonist activity and marked affinity at h5-HT_{1A} receptors, similar to their affinity at hD_2 dopamine receptors. In contrast, risperidone and sertindole displayed low affinity at h5-HT_{1A} receptors and behaved as 'neutral' antagonists, inhibiting 5-HT-stimulated [^{35}S]GTPγS binding. Likewise the 'typical' neuroleptics, haloperidol, pimozide, raclopride and chlorpromazine exhibited relatively low affinity and 'neutral' antagonist activity at h5-HT_{1A} receptors with K_i values which correlated with their respective K_b values. The present data show that (i) [^{35}S]GTPγS binding is an effective method to evaluate the efficacy and potency of agonists and antagonists at recombinant human 5-HT_{1A} receptors. (ii) Like clozapine, several putatively 'atypical' antipsychotic drugs display balanced serotonin h5-HT_{1A}/dopamine hD_2 receptor affinity and partial agonist activity at h5-HT_{1A} receptors. (iii) Several 'typical' and some putatively 'atypical' antipsychotic agents displayed antagonist properties at h5-HT_{1A} sites with generally much lower affinity than at hD_2 dopamine receptors. It is suggested that agonist activity at 5-HT_{1A} receptors may be of utility for certain antipsychotic agents.

Keywords: 5-HT_{1A} receptor; [^{35}S]GTPγS binding; Schizophrenia; Clozapine; Ziprasidone; Risperidone

1. Introduction

The 5-HT_{1A} receptor is a member of the superfamily of monomeric transmembrane G-protein-coupled receptors and has been heterologously expressed in COS7 (african green monkey kidney), HeLa, CHO (Chinese hamster ovary), and other cell lines, enabling the study of its coupling to G-proteins and second messenger systems (Raymond et al., 1992; Newman-Tancredi et al., 1992; Boess and Martin, 1994; Kenakin, 1996). 5-HT_{1A} receptors are localised as somatodendritic autoreceptors in raphe nuclei and postsynaptically in corticolimbic structures such as hippocampus and cortex, reflecting a key role in the modulation of affective disorders, including anxiety disorders and depression (De Vry, 1995; Maes and Meltzer, 1995; Millan et al., 1997b).

5-HT_{1A} receptors have also attracted interest as potential targets for novel antipsychotic agents. First, clinical studies have reported that the 5-HT_{1A} receptor partial agonist, buspirone, markedly ameliorates the lowered mood and social withdrawal of schizophrenics, through its antianxiety effects (Sovner and Parnell-Sovner, 1989; Goff et al., 1991; Harvey and Balon, 1995). Second, post-mortem studies have shown that frontal cortex 5-HT_{1A} receptor

* Corresponding author. Tel.: +33-1-4118-2446; Fax: +33-1-4118-2470; E-mail: newman_tancredi@hotmail.com

0014-2999/98/$19.00
PII: S0014-2999(98)00483-X

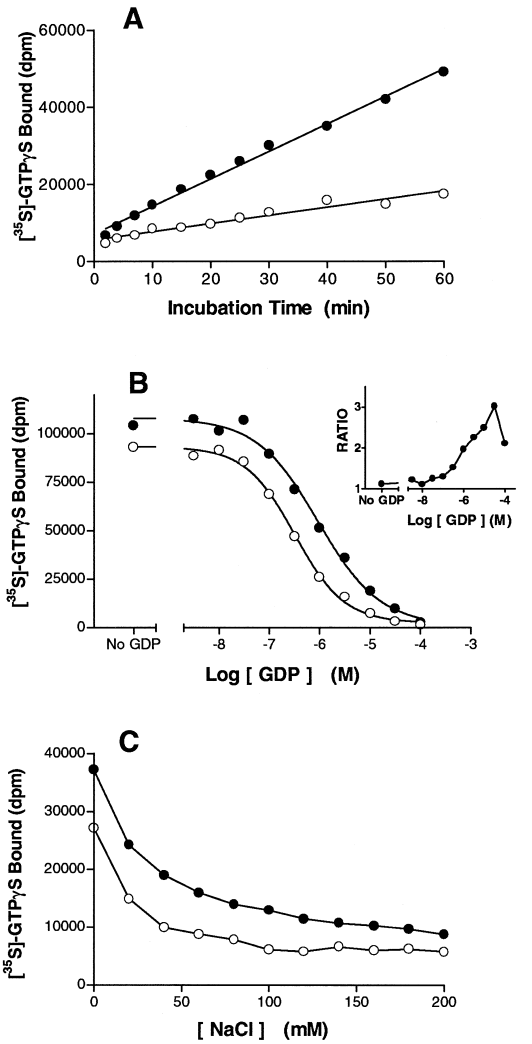

Fig. 1. Effect of (A) time, (B) GDP and (C) NaCl on [^{35}S]GTPγS binding to membranes of CHO cells stably expressing cloned human 5-HT$_{1A}$ receptors. Panel A: Basal and 5-HT-stimulated [^{35}S]GTPγS binding determined over time points ranging from 1 to 60 min. Panel B: Basal and 5-HT-stimulated [^{35}S]GTPγS binding determined in the presence of GDP concentrations between 0 and 100 μM. Inset: Effect of GDP concentration on agonist stimulation ratio. The stimulation ratio was calculated as 5-HT-stimulated [^{35}S]GTPγS binding divided by basal [^{35}S]GTPγS binding. Panel C: Basal and 5-HT-stimulated [^{35}S]GTPγS binding determined in the presence of concentrations of NaCl between 0 and 200 mM. Points shown are means of triplicate determinations from representative experiments repeated on at least three independent occasions.

density is increased in schizophrenic patients (Hashimoto et al., 1991; Burnet et al., 1997). Third, 5-HT$_{1A}$ receptor agonists, such as 8-OH-DPAT (8-hydroxy-dipropylamino-tetralin), block the catalepsy induced in rats by neuroleptics such as haloperidol or raclopride (McMillen et al., 1988; Andersen and Kilpatrick, 1995), suggesting that an antipsychotic agent which displays 5-HT$_{1A}$ receptor agonist activity may show a lower incidence of extrapyramidal symptoms in humans. Fourth, schizophrenic patients suffer from a functional deficiency in the frontal cortex, and in rat dialysis studies, 5-HT$_{1A}$ receptor agonists selectively reinforce dopamine and noradrenaline release in this region, suggesting that 5-HT$_{1A}$ receptor agonism may alleviate 'hypofrontality' (Millan et al., 1997b). Fifth, the 'atypical' antipsychotic, clozapine, which is clinically effective against both positive and negative symptoms in the absence of extrapyramidal symptoms, displays marked affinity at human brain 5-HT$_{1A}$ receptors (Mason and Reynolds, 1992) and partial agonism at recombinant human 5-HT$_{1A}$ receptors (Newman-Tancredi et al., 1996a). Indeed, Corbett et al. (1993) suggested that the capacity of antipsychotic agents to treat psychotic symptoms in the absence of extrapyramidal symptoms is due to agonist actions at 5-HT$_{1A}$ receptors. However, no direct evidence was provided to support this hypothesis and the present study therefore undertook a systematic analysis of this issue by investigating the intrinsic activity of antipsychotic agents for modulation of 5-HT$_{1A}$ receptor-mediated signal transduction.

5-HT$_{1A}$ receptors modulate the activity of diverse second messenger pathways, including potassium and calcium channels, inositol phosphate metabolism, arachidonic acid production and adenylyl cyclase activity (Schoeffter and Hoyer, 1988; Pauwels et al., 1993; Boess and Martin, 1994). Whilst these approaches yield valuable information about 5-HT$_{1A}$ receptor activation, they measure responses which are several steps 'downstream' of the receptor per

Table 1
Agonist action of 5-HT receptor ligands at recombinant h5-HT$_{1A}$ receptors determined by [^{35}S]GTPγS binding

Ligand	K_i (nM) (n)	E_{max} (%)	EC_{50} (nM)	E_{max}[a] (%)	EC_{50}[a] (nM)
S 14671	0.03±0.01	98.1±2.5	0.44±0.12		
5-CT	0.08±0.01	96.3±3.7	2.2±0.4	106	1.1
S 14506	0.22±0.06	90.1±2.2	1.0±0.3		
(+)Flesinoxan	0.54±0.11	94.3±10.4	26.0±8.0		
(±)8-OH-DPAT	0.58±0.03	75.7±3.2	7.4±0.8	79	11
5-HT	0.61±0.15	100	18.5±1.6	100	18
RU 24969	1.13±0.18	95.4±4.8	60.8±8.4	111	20
LY 165,163	1.2±0.2	82.9±3.8	14.1±4.9		
Spiroxatrine	1.45±0.57	45.1±1.9	13.2±1.7		
Ipsapirone	2.5±0.5	49.0±3.6	21.8±6.7	71	18
(+)UH 301	2.74±0.02	34.8±6.3	40.5±18.5		
Tandospirone	5.85±1.12	100.5±0.7	268±31.8		
Zalospirone	7.2±2.2	47.1±4.5	33.6±11.8		
Buspirone	8.9±1.2	65.4±4.4	114±12	62	150

[^{35}S]GTPγS binding was carried out on membranes of CHO-h5-HT$_{1A}$ cells. Agonist efficacy is expressed relative to that of 5-HT (=100%). Affinity (K_i values) at h5-HT$_{1A}$ receptors were determined in competition experiments with [^{3}H]8-OH-DPAT. Results are expressed as means ±S.E.M. of at least three determinations.

[a] Data from Odagaki and Fuxe (1995) for stimulation of GTPase activity in rat hippocampal membranes. Their E_{max} values are expressed as a percentage of the effect of 5-HT. Their data correlated with the values from the present study (EC_{50} values: $r=+0.91$, slope = 1.2, $P<0.01$; E_{max} values: $r=+0.89$, slope = 0.87, $P<0.01$).

se and depend on the cell types investigated (Liu and Albert, 1991; Raymond et al., 1993). In addition, 5-HT_{1A} receptors can modulate different second messenger systems with different potencies (Fargin et al., 1989; Gudermann et al., 1996) or the same second messenger systems in a different manner depending on brain region (Clarke et al., 1996; Johnson et al., 1997). These differing responses complicate the interpretation of second messenger effects. In contrast, both adenylyl cyclase inhibition and phosphoinositide hydrolysis are mediated by the same G-protein, at least in HeLa cells (Fargin et al., 1991), suggesting that investigation of agonist activity at the G-protein level may overcome some of these difficulties. Odagaki and Fuxe (1995) measured G-protein activation by determining GTPase activity in rat hippocampal membranes, whilst guanosine-5′-*O*-(3-[^{35}S]thio)-triphosphate ([^{35}S]GTPγS) binding methodology can be applied to 5-HT_{1A} receptors in recombinant systems (Newman-Tancredi et al., 1996b, 1997a; Stanton and Beer, 1997).

The present study had several aims. First, to characterise the optimal experimental conditions for determination of agonist stimulation of 5-HT_{1A}-receptor-mediated [^{35}S]GTPγS binding in a CHO cell line stably expressing recombinant human 5-HT_{1A} receptors. Second, to evaluate the agonist/antagonist activity (by stimulation of [^{35}S]GTPγS binding) and binding affinity (by competition with [^{3}H]8-OH-DPAT) of serotonergic reference compounds. These include serotonergic agonists, antagonists, and agents proposed for the treatment of affective disorders, such as buspirone and (+)flesinoxan. Third, to investigate the 5-HT_{1A} receptor activity of a range of antipsychotic drugs. These included older antipsychotic agents, such as chlorpromazine, haloperidol, pimozide and raclopride, which are known to provoke marked extrapyramidal symptoms (Meltzer, 1996; Baldessarini, 1996). Several recent drugs, intended to display clozapine-like clinical efficacy without extrapyramidal symptoms induction, were also evaluated, including risperidone, ziprasidone, quetiapine, olanzapine and sertindole (Schwartz and Brotman, 1992; Fleischhacker and Hummer, 1997).

2. Materials and methods

2.1. Determination of affinity (K_i) at CHO-h5-HT_{1A} receptors

Membranes were prepared from CHO-h5-HT_{1A} cells stably expressing the human 5-hydroxytryptamine 5-HT_{1A} receptor (Newman-Tancredi et al., 1992). Cells grown in suspension culture were harvested by centrifugation and homogenised in buffer A (HEPES 20 mM, pH 7.5 and $MgSO_4$ 5 mM) using a Kinematica Polytron. The homogenate was centrifuged at 50,000 × *g* for 30 min and the membrane pellet resuspended in buffer A. For competition binding experiments, membranes (10–20 μg protein)

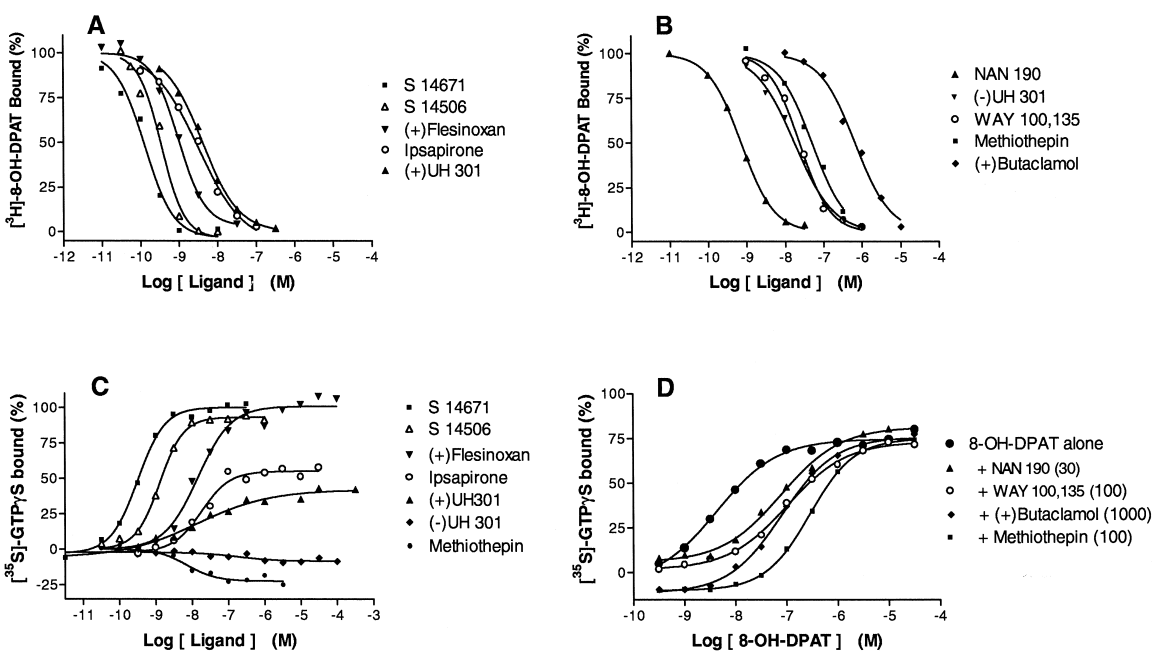

Fig. 2. Action of serotonergic agonists and antagonists at h5-HT_{1A} receptors. Panel A: Competition by serotonergic agonists for [^{3}H]8-OH-DPAT binding to h5-HT_{1A} receptors. Panel B: Competition by antagonists for [^{3}H]8-OH-DPAT binding to h5-HT_{1A} receptors. Panel C: Stimulation/inhibition of [^{35}S]GTPγS binding by serotonergic ligands. 100% stimulation is defined as that observed with 10 μM 5-HT. Methiothepin inhibited basal binding. Panel D: Stimulation of [^{35}S]GTPγS binding by 8-OH-DPAT alone or in the presence of fixed concentrations (nM) of antagonists. Points shown are means of triplicate determinations from representative experiments repeated on at least three independent occasions.

Table 2
Action of antagonist ligands at cloned human 5-HT_{1A} receptors determined by shift of 8-OH-DPAT concentration–response isotherm of [^{35}S]GTPγS binding

	K_b (nM)	K_i (nM)
NAN 190 (30)	0.97 ± 0.09	0.45 ± 0.06
(−)Tertatolol (100)	11.1 ± 0.8	6.7 ± 1.9
WAY 100,135 (100)	17.0 ± 6.9	11.5 ± 2.3
(−)UH 301 (100)	10.5 ± 5.1	13.5 ± 1.3
Methiothepin (100)	1.71 ± 0.19	8.3 ± 4.1
Spiperone (100)	9.97 ± 1.85	105 ± 24
(+)Butaclamol (1000)	86.3 ± 19.3	369 ± 65

[^{35}S]GTPγS binding was carried out using membranes of CHO cells stably expressing human 5-HT_{1A} receptors. Compounds are listed in order of affinity (K_i) at 5-HT_{1A} receptors, determined in competition experiments with [^{3}H]8-OH-DPAT. Antagonist potencies (K_b values) were determined by the shift in the 8-OH-DPAT stimulation curve of [^{35}S]GTPγS binding to a higher concentration in the presence of a fixed concentration of antagonist (nM). Results are expressed as mean ± S.E.M. of at least three independent determinations.

were incubated with [^{3}H]8-OH-DPAT (225 Ci/mmol; Amersham) at 22°C for 2.5 h. Non-specific binding was defined with 5-HT (10 μM). Inhibition constants (K_i values) were calculated from IC_{50} values by the Cheng-Prusoff equation: $K_i = IC_{50}/\{(L/K_d) + 1\}$; where L is the concentration of radioligand and K_d is the dissociation constant of [^{3}H]8-OH-DPAT at CHO-h5-HT_{1A} receptors (0.65 nM). The receptor expression level in CHO-h5-HT_{1A} cells is 1.6 pmol/mg (Newman-Tancredi et al., 1997a).

2.2. Determination of agonist efficacy at CHO-h5-HT_{1A} receptors

Efficacy was determined by measuring agonist stimulation of [^{35}S]GTPγS binding, as described previously (Newman-Tancredi et al., 1996a). Briefly, CHO-h5-HT_{1A} membranes (50 μg protein) were incubated (20 min, 22°C) in triplicate in a buffer containing 20 mM HEPES (pH 7.4), 3 μM GDP, 3 mM $MgSO_4$, NaCl 100 mM, 0.1 nM [^{35}S]GTPγS (1300 Ci/mmol, NEN). Non-specific binding was defined with 10 μM GTPγS. Agonist efficacy is expressed relative to that of 5-HT (= 100%) which was tested at a maximally effective concentration (10 μM) in each experiment. The efficacy of the antipsychotic agent, tiospirone was determined using membranes of CHO-h5-HT_{1A} cells purchased from NEN (Les Ulis, France). In control experiments, CHO-h5-HT_{1A} membranes from NEN (expression level = 1.3 pmol/mg) displayed the same competition and [^{35}S]GTPγS binding characteristics as those from Newman-Tancredi et al. (1992). For antagonist experiments, K_b values were calculated as described previously (Newman-Tancredi et al., 1996b, 1997b).

Experiments were terminated by rapid filtration through Whatman GF/B filters using a Brandel cell harvester and radioactivity determined by liquid scintillation counting. Binding isotherms were analysed by non-linear regression using the program PRISM (Graphpad Software, San Diego, CA). Results are expressed as the mean ± S.E.M. of three or more independent determinations. Protein concentration was determined by use of a bichinconic acid kit (Sigma, S. Quentin Fallavier, France).

2.3. Compounds

Compounds were obtained from the following sources: S 14671 (1-[2-(2-thenoylamino)ethyl]-4-[1-(7-methoxynaphtyl)]piperazine), S 14506 (1-[2-(4-fluorobenzoylamino)ethyl]-4-(7-methoxynaphtyl)piperazine), FG 5893 ({2-[4-[4,4-bis(4-fluorophenyl)butyl]-1-piperazinyl]-

Table 3
Agonist action of antipsychotic agents at cloned human 5-HT_{1A} receptors determined by stimulation of [^{35}S]GTPγS binding

Ligand	h5-HT_{1A}			hD$_2$	K_i ratio
	Efficacy (%)	EC_{50} (nM)	K_i (nM)	K_i (nM)	(h5-HT_{1A}/hD$_2$)
Ziprasidone	54.9 ± 4.2	12.6 ± 5.4	1.24 ± 0.06	4.7[a]	0.26
FG 5893	61.4 ± 5.1	5.57 ± 1.09	1.97 ± 0.41	15.6[b]	0.13
Tiospirone	17.0 ± 1.9	77.3 ± 9.1	4.65 ± 1.30	2.16[b]	2.2
ORG 5222	14.3 ± 1.4	91.1 ± 37.8	4.67 ± 0.68	1.47[b]	3.2
Ocaperidone	29.4 ± 3.4	62.8 ± 17.5	10.1 ± 2.1	0.32[c]	32
Nafadotride	68.0 ± 5.6	1401 ± 210	45.6 ± 7.9	4.84[b]	9.4
BMY 14802	82 ± 1.2	3670 ± 1290	62.8 ± 12.2	645[b]	0.10
Clozapine	53.3 ± 4.0	3390 ± 670	132 ± 30	76[a]	1.7
Quetiapine	60.4 ± 10.9	11 100 ± 2900	250 ± 36	186[a]	1.3
Olanzapine	24.2 ± 4.7	6220 ± 3500	1637 ± 23	6.1[c]	270

[^{35}S]GTPγS binding experiments were carried out on membranes of CHO cells stably expressing recombinant human 5-HT_{1A} receptors. Agonist efficacy is expressed relative to that of 5-HT (= 100%). Compounds are listed in order of affinity (K_i) at 5-HT_{1A} receptors, determined in competition experiments with [^{3}H]8-OH-DPAT. Results are expressed as means ± S.E.M. of three or more independent determinations. K_i values at recombinant human D_2 receptors are shown for comparison. The K_i ratio is calculated by dividing the K_i value at h5-HT_{1A} receptors by that at hD$_2$ receptors.
[a]Audinot et al., 1995.
[b]Unpublished observations.
[c]Millan et al., 1995b.

pyridine-3-carboxylic acid}), ziprasidone, nafadotride, LY 165,163 (1-(2-4-aminophenyl)ethyl-4-(3-trifluoromethylphenyl)-piperazine) and 5-CT (5-carboxamidotryptamine), were synthesised by G. Lavielle, Servier; risperidone and WAY 100,135 (*N*-tertiobutyl-3-[4-(2-methoxyphenyl) piperazinyl]-2-phenylpropanamide) was synthesised by J.-L. Peglion, Servier, (−)tertatolol from Servier, (+)flesinoxan from Duphar (Weesp, Netherlands), RU 24969 (5-methoxy-3-(1,2,3,6-tetrahydropyridin-′-yl)-1*H*-indole) from Roussel-Uclaf (Romainville, France), Ipsapirone from Troponwerke (Cologne, Germany), Zalospirone from Wyeth-Ayerst (Princeton, NJ), Methiothepin from Hoffmann-La Roche (Basel, Switzerland), ocaperidone from Janssen (Beerse, Belgium); ORG 5222 ({*trans*-5-chloro-2-methyl-2,3,3*a*,12*b*-tetrahydro-1*H*-dibenz[2,3:6,7]-oxepino-[4,5*c*]pyrrole) from Organon (Oss, Netherlands); olanzapine from Eli Lilly (Indianapolis, USA); raclopride from Astra (Sodertalje, Sweden); quetiapine (ICI 204,636) from Zeneca (Macclesfield, UK); sertindole from Lundbeck (Copenhagen, Denmark); tiaspirone and BMY 14802 (1-[4-(4-fluorophenyl)-4-hydroxybutyl]-4-(5-fluoropyrimidin-2-yl)piperazine) from Bristol-Myers (Wallingford, USA), thioridazine, tandospirone and NAN 190 (1-(2-methoxyphenyl)-4-[4-(2-phthalimino)butyl]piperazine) were purchased from Tocris-Cookson (Bristol, UK), (±)8-OH-DPAT (8-hydroxy-dipropylaminotetralin), spiroxatrine, pimozide, clozapine, (+)butaclamol, (+)UH 301 and (−)UH 301 from Research Biochemicals International (Illkirch, France), chlorpromazine, haloperidol, 5-HT (5-hydroxytryptamine) and buspirone from Sigma (S. Quentin Fallavier, France).

3. Results

3.1. Definition of [^{35}S]GTPγS binding conditions

5-HT (10 μM) stimulated [^{35}S]GTPγS binding to 5-HT_{1A} membranes in a linear manner over the first 20 min (3) of time course experiments, and a standard incubation time of 20 min was therefore used. In contrast, no stimulation of [^{35}S]GTPγS binding was observed in membranes of untransfected CHO cells (results not shown). Basal (non-agonist-stimulated) binding of [^{35}S]GTPγS to CHO-h5-HT_{1A} membranes was dependent on the concentration of GDP present in the buffer (Fig. 1B) and was reduced from about 90,000 dpm in the absence of GDP to about 15,000 dpm at a GDP concentration of 3 μM. In contrast, agonist-dependent [^{35}S]GTPγS binding (i.e., the difference between agonist-stimulated and basal binding) amounted to about 20,000 dpm (Legend to Table 1) and was not markedly decreased by GDP concentrations up to 3 μM. The decrease in basal binding augmented the ratio of agonist-stimulated to basal [^{35}S]GTPγS binding to 2.3 fold at GDP concentrations of 3 μM (Fig. 1B, inset). Like GDP, NaCl reduced basal [^{35}S]GTPγS binding, from 27,000 dpm in the absence of NaCl, to 7000 dpm at a concentration of 100 mM (Fig. 1C). A set of standard experimental conditions was defined (3 μM GDP, 3 mM $MgCl_2$, 100 mM NaCl, 20 min incubation) which yielded the highest agonist stimulation of [^{35}S]GTPγS binding and which was used in all subsequent experiments.

3.2. Agonist activity of serotonergic ligands at CHO-h5-HT_{1A} receptors

A series of serotonergic agonists were tested for their binding affinity and ability to stimulate [^{35}S]GTPγS binding (Table 1; Fig. 2A,C). K_i values were closely corre-

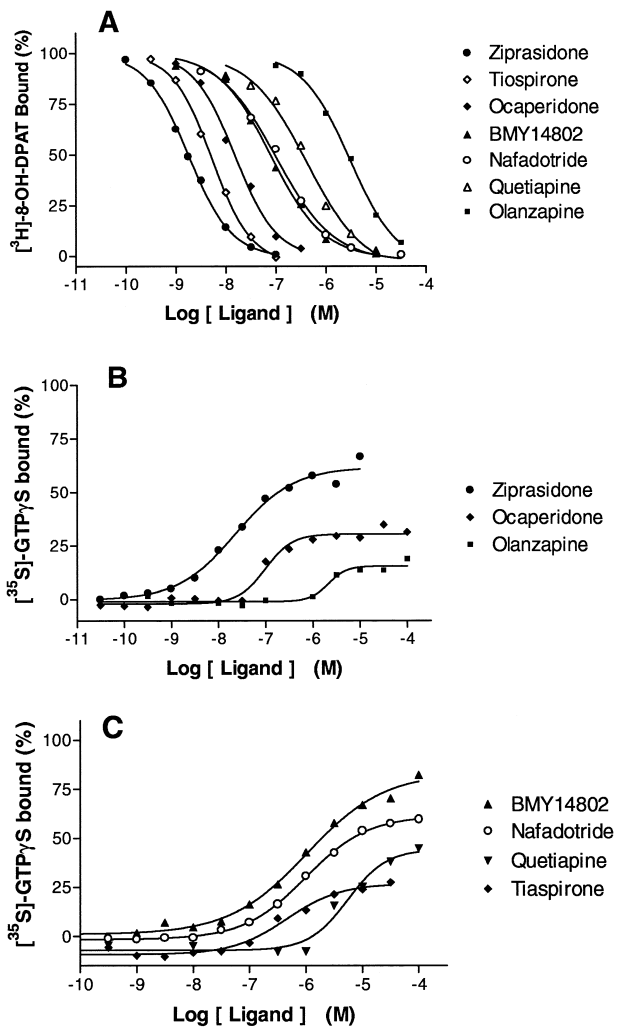

Fig. 3. Agonist actions of antipsychotic agents at h5-HT_{1A} receptors. Panel A: Competition by antipsychotic agents for [^{3}H]8-OH-DPAT binding to h5-HT_{1A} receptors. Panels B and C: Stimulation of [^{35}S]GTPγS binding by antipsychotic agents. One hundred percent stimulation is defined as that observed with 10 μM 5-HT. Points shown are means of triplicate determinations from representative experiments repeated on at least three independent occasions.

Table 4
Antagonist action of antipsychotic agents at cloned human 5-HT_{1A} receptors determined by inhibition of 5-HT-stimulated [^{35}S]GTPγS binding

Ligand	h5-HT_{1A}			hD_2	K_i ratio
	IC_{50} (nM)	K_b (nM)	K_i (nM)	K_i (nM)	(h5-HT_{1A}/hD_2)
Thioridazine	477 ± 93	72.8 ± 14	83.6 ± 22.2	6.95[a]	12
Pimozide	1224 ± 274	193 ± 43	155 ± 17	1.31[a]	120
Risperidone	6039 ± 378	950 ± 60	292 ± 7	3.63[b]	80
Sertindole	1874 ± 246	294 ± 39	433 ± 134	2.06[c]	210
Chlorpromazine	4402 ± 1603	692 ± 167	642 ± 92	1.23[a]	520
Haloperidol	7674 ± 1687	1205 ± 264	1910 ± 250[d]	0.42[b]	4550
Raclopride	n.d.	n.d.	6960 ± 640	1.07[b]	6510

Antagonist potency (K_b) was calculated from IC_{50} values for the inhibition of 5-HT (100 nM)-stimulated [^{35}S]GTPγS binding to CHO-h5-HT_{1A} cell membranes. Compounds are listed in order of affinity at h5-HT_{1A} receptors (K_i), determined in competition experiments with [^{3}H]8-OH-DPAT. Results are expressed as means ± S.E. of the mean of at least three independent experiments.
n.d. = not determined.
K_i values at recombinant human D_2 receptors are shown for comparison. The K_i ratio is calculated by dividing the K_i value at h5-HT_{1A} receptors by that at hD_2 receptors.
[a]Unpublished observations.
[b]Millan et al., 1995b.
[c]Audinot et al., 1995.
[d]Newman-Tancredi et al., 1996b.

lated with EC_{50} values ($r = +0.94$, slope = 1.01, $P < 0.001$). EC_{50} values were, however, an average of 21.6 ± 4.1 ($n = 17$) fold higher than their respective K_i values. The observed agonist efficacies (E_{max} values) ranged from 34.8% for (+)UH 301 to essentially full agonist efficacy for S 14671, 5-CT, (+)flesinoxan, RU 24969 and tandospirone whilst the azapirone derivatives, buspirone and ipsapirone, displayed partial agonist efficacy.

3.3. Antagonist activity of serotonergic ligands at CHO-h5-HT_{1A} receptors

Serotonergic antagonists were tested in competition binding with [^{3}H]8-OH-DPAT and for their ability to 'shift' the 8-OH-DPAT stimulation curve of [^{35}S]GTPγS binding (Table 2; Fig. 2B,D). NAN 190, which exhibited only very slight agonism when tested alone ($E_{max} = 9.3 \pm 3.0\%$, $EC_{50} = 2.8 \pm 1.8$ nM), potently 'shifted' the 8-OH-DPAT stimulation curve with a K_b value which corresponded closely to its K_i value. (−)Tertatolol, WAY 100,135 and (−)UH 301 did not alter [^{35}S]GTPγS binding from basal levels when tested alone, and displayed K_b values which resembled their K_i values. In contrast, methiothepin inhibited [^{35}S]GTPγS binding decreasing it below basal levels ($E_{max} = -21.6 \pm 1.5\%$, $EC_{50} = 18.4 \pm 7.6$ nM). (+)Butaclamol also displayed negative efficacy: $E_{max} = -17.7 \pm 2.5\%$ (mean ± range, $n = 2$) and $EC_{50} = 852 \pm 367$ nM (mean ± range, $n = 2$). The K_b values calculated for methiothepin and (+)butaclamol were lower than their respective K_i values.

3.4. Agonist activity of antipsychotic agents at CHO-h5-HT_{1A} receptors

Several compounds proposed as 'atypical' antipsychotic agents were found to exhibit agonist activity at 5-HT_{1A} receptors (Table 3; Fig. 3), including clozapine, ziprasidone, FG 5893, nafadotride, tiospirone, ocaperidone and quetiapine. As in the case of the serotonergic agonists,

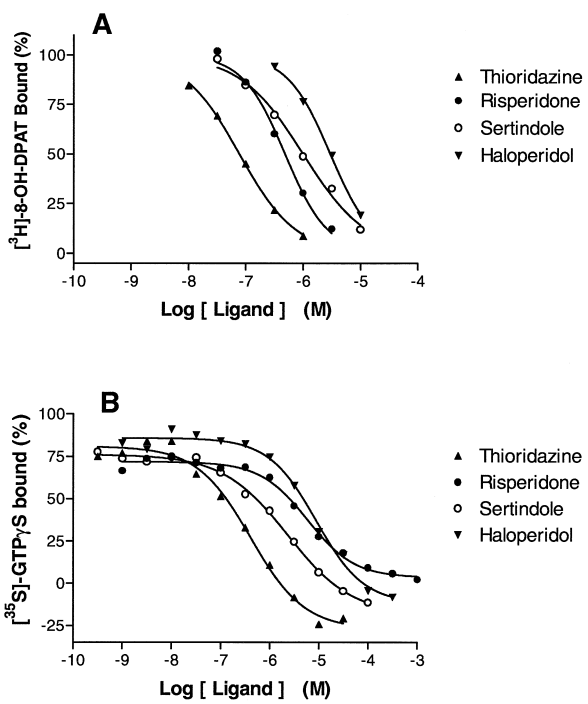

Fig. 4. Antagonist actions of antipsychotic agents at h5-HT_{1A} receptors. Panel A: Competition by antipsychotic agents for [^{3}H]8-OH-DPAT binding to h5-HT_{1A} receptors. Panel B: Inhibition of 5-HT (100 nM)-stimulated [^{35}S]GTPγS binding by antipsychotic agents. One hundred percent stimulation is defined as that observed with 10 μM 5-HT. Points shown are means of triplicate determinations from representative experiments repeated on at least three independent occasions.

EC_{50} values were greater than the respective K_i values (21.8 ± 5.8-fold difference, $n = 10$) and a high degree of correlation was observed ($r = +0.93$, slope = 1.10, $P < 0.001$). All the compounds displayed high or marked binding affinity at CHO-h5-HT1A receptors except for olanzapine, which exhibited a micromolar K_i value at h5-HT_{1A} receptors (Table 3).

3.5. Antagonist activity of antipsychotic agents at CHO-h5-HT_{1A} receptors

Some antipsychotic agents were found to exhibit antagonist activity at CHO-h5-HT_{1A} receptors (antagonism of 5-HT (100 nM)-stimulated [^{35}S]GTPγS binding; Table 4; Fig. 4) including the 'typical' neuroleptics haloperidol, chlorpromazine and pimozide, but also risperidone and sertindole. None of the antagonists altered [^{35}S]GTPγS binding from basal levels when tested alone and K_b values correlated closely with respective K_i values ($r = +0.95$, slope = 0.84, $P < 0.001$). The affinities of the antagonist antipsychotics at 5-HT_{1A} receptors were generally lower than those of the agonist group. The h5-HT_{1A}/hD_2 receptor K_i ratios were ≥ 80 for six of the seven compounds tested (Table 4).

4. Discussion

4.1. Affinity / efficacy of 5-HT agonists at 5-HT_{1A} receptors

A range of serotonergic agonists and partial agonists were tested for their capacity to stimulate 5-HT_{1A} receptor mediated [^{35}S]GTPγS binding in CHO-h5-HT_{1A} membranes. The methoxynaphtylpiperazine ligand, S 14671, was the most potent agonist tested, with virtually full agonist activity, relative to 5-HT (Table 1; Fig. 2C) consistent with its exceptionally potent and efficacious actions in in vivo functional paradigms (Millan et al., 1992; Schreiber et al., 1994). Its analogue, S 14506 was also a highly potent and efficacious ligand (E_{max} = 90%) in agreement with previous in vivo studies (Schreiber et al., 1994). (+)UH 301 exhibited partial agonist activity at 5-HT_{1A} receptors (E_{max} = 35%) similar to that reported for inhibition of forskolin-stimulated adenylyl cyclase activity in rat hippocampal membranes (47%, Cornfield et al., 1991). The roughly 20-fold difference between EC_{50} values for stimulation of [^{35}S]GTPγS binding and the respective K_i values for inhibition of [^{3}H]8-OH-DPAT binding, probably reflects the labelling by [^{3}H]8-OH-DPAT of 5-HT_{1A} receptors which are in a G-protein-coupled state (Mongeau et al., 1992; Sundaram et al., 1993; Gozlan et al., 1995). In contrast, EC_{50} values probably correlate better with the affinities of the ligands at uncoupled receptors (Cornfield et al., 1991; Chamberlain et al., 1993). The overall order of potency of the agonists for activation of [^{35}S]GTPγS binding corresponds closely to that for inhibition of [^3H]8-OH-DPAT binding ($r = 0.94$) and agrees with the pharmacological profile of the classically characterised 5-HT_{1A} receptor. Further, the efficacies reported here are positively correlated with those reported using GTPase activity as a measure of G-protein activation by native rat hippocampal 5-HT_{1A} receptors (Odagaki and Fuxe, 1995; Table 1 legend). Agonist efficacies determined at CHO-h5-HT_{1A} receptors are also broadly comparable with those for inhibition of adenylyl cyclase activity in native brain hippocampal membranes (De Vivo and Maayani, 1986; Schoeffter and Hoyer, 1988). Thus, whilst caution should be exercised in extrapolating data from recombinant to physiological systems (which are likely to express differing levels of receptors and/or G-proteins), the marked correspondence between the agonist efficacies determined by [^{35}S]GTPγS binding in CHO-h5-HT_{1A} membranes and those determined by GTPase or adenylyl cyclase determinations in hippocampal membranes is striking. Thus, it may be concluded that agonist-mediated stimulation of [^{35}S]GTPγS binding to CHO-h5-HT_{1A} membranes, constitutes a model of activation of native hippocampal 5-HT_{1A} receptors.

In contrast, it is likely that agonist efficacies at presynaptic receptors would be 'amplified' by the presence of 'spare receptors'. Indeed, in cortical neurons in primary culture, ipsapirone and buspirone act as antagonists whereas in hippocampal neurons they acted as partial agonists (Dumuis et al., 1988). Further, ipsapirone acts as a full agonist at presynaptic 5-HT_{1A} autoreceptors (Cox et al., 1993). These differences in efficacy have been attributed to the presence of significant receptor reserve at presynaptic but not at postsynaptic 5-HT_{1A} receptors (Meller et al., 1990; Yocca et al., 1992), a situation which may be modelled by cell lines which express high densities of recombinant 5-HT_{1A} receptors (Varrault et al., 1992). Indeed, when CHO-h5-HT_{1A} cells were manipulated to express 3-fold more receptors, the partial agonist, eltoprazine, behaved virtually as a full agonist (Newman-Tancredi et al., 1997a).

4.2. Affinity / potency of serotonergic antagonists at 5-HT_{1A} receptors

Marked differences were observed between the actions of 'antagonists' (Table 2; Fig. 2D). The α_1/5-HT_{1A} receptor ligand, NAN 190 (Glennon et al., 1988), demonstrated its antagonist properties by a parallel shift of the 8-OH-DPAT stimulation curve, consistent with competitive antagonism. This suggests that its antagonist activity is predominant, at least in the present model of post-synaptic 5-HT_{1A} receptors, although previous reports have shown that it may exhibit some agonist properties at somatoden-

dritic 5-HT_{1A} autoreceptors (Greuel and Glaser, 1992; Lejeune et al., 1994).

In contrast, methiothepin and (+)butaclamol, as well as spiperone, exhibited negative efficacy by concentration-dependently inhibiting [^{35}S]GTPγS binding below basal levels, indicating that they act as inverse agonists in this system (Results, Fig. 2C; Newman-Tancredi et al., 1997a). It is interesting to note that methiothepin appears to exhibit inverse agonist activity at a range of serotonergic receptors including 5-HT_{1D} and 5-HT_{2C} receptors (Barker et al., 1994; Thomas et al., 1995; Stanton and Beer, 1997). Further, (+)butaclamol exhibits negative efficacy at dopamine D_2 receptors (Hall and Strange, 1997), whilst spiperone discriminates differential affinity states of native porcine dopamine D_2 (De Lean et al., 1982) as well as 5-HT_{1A} receptors (Sundaram et al., 1993).

WAY 100,135, (−)UH 301 and the 5-HT_{1A} receptor (and β-adrenergic) receptor antagonist, (−)tertatolol, acted as 'neutral' antagonists, exhibiting antagonist activity without any detectable agonist or inverse agonist effects. This is in accordance with previous 'in vitro' reports of the actions of these compounds at post-synaptic 5-HT_{1A} receptors (Bjork et al., 1991; Escandon et al., 1994), although studies of raphe-localised serotonergic neuron firing suggest that WAY 100135, but not (−)UH 301 or (−)tertatolol, may have some weak partial agonist action (Arborelius et al., 1994; Escandon et al., 1994; Lejeune et al., 1994).

4.3. Agonist activity of antipsychotic agents at 5-HT_{1A} receptors

We have previously shown that the atypical antipsychotic, clozapine, exhibits partial agonist properties for stimulation of [^{35}S]GTPγS binding at CHO-h5-HT_{1A} cell membranes (Newman-Tancredi et al., 1996a). The present study confirms our previous report on clozapine and demonstrates that several other putatively 'atypical' antipsychotic drugs also exhibit some agonist activity at 5-HT_{1A} receptors (Table 3; Fig. 4). Tiospirone, a buspirone analogue which is effective in the treatment of psychoses with minimal extrapyramidal symptoms (Jain et al., 1987; Moore et al., 1987), exhibits partial agonist activity and has balanced hD_2/h5-HT_{1A} receptor affinities. Quetiapine and ziprasidone displayed marked 5-HT_{1A} receptor agonist activity, similar to that of clozapine ($E_{max} \sim 50\%$) and also share clozapine's balanced 5-HT_{1A}/hD_2 receptor affinity (Table 3). In vivo ziprasidone has a high threshold for induction of catalepsy in rats, which Seeger et al. (1995) tentatively attributed to its 5-HT_{1A} receptor agonist actions. Clozapine and, probably, quetiapine and ziprasidone, display a distinctive clinical profile, compared to the 'typical' antipsychotic, haloperidol, with a lower incidence of extrapyramidal symptoms and, possibly, greater effectiveness against negative symptoms of schizophrenia (Fleischhacker and Hummer, 1997; Tollefson and Sanger, 1997). In contrast, although the preferential D_3 receptor antagonist, and potential antipsychotic agent, nafadotride, showed marked efficacy at h5-HT_{1A} receptors ($E_{max} = 68\%$), its affinity at this site was about ten-fold lower than at dopamine hD_2 receptors (Table 3) and about 100-fold lower than at hD_3 dopamine receptors (Sautel et al., 1995). Thus, nafadotride's preferential dopaminergic actions may be responsible for its induction of catalepsy in vivo (Sautel et al., 1995).

An important issue is the level of efficacy at 5-HT_{1A} receptors which is necessary for in vivo physiological and behavioural effects to be expressed. Bartoszyk et al. (1996) have shown that clozapine mediates some of its antianxiety actions by a 5-HT_{1A} receptor mediated mechanism. Further, the increase in frontal cortex dopamine levels induced by clozapine is (at least partially) due to 5-HT_{1A} receptor activation (Rollema et al., 1997). The selective 5-HT_{1A} receptor ligand (and anxiolytic drug) S 15535, ($E_{max} = 35\%$ in the present system, Newman-Tancredi et al., 1996b), displays full agonist activity at presynaptic somatodendritic 5-HT_{1A} receptors although acting as an antagonist in in vivo models of post-synaptic 5-HT_{1A} receptor activation (Millan et al., 1993, 1994). These observations suggest that efficacies equivalent or greater than that of buspirone, as displayed by BMY 14802 and, possibly, FG 5893 and quetiapine, may be necessary to observe post-synaptic 5-HT_{1A} receptor effects (eg. hypothermia, serotonin syndrome) but lower efficacies would likely be sufficient to provoke activation of 5-HT_{1A} autoreceptors on serotonergic neurons in the raphe nuclei. Thus the partial agonist properties of clozapine, quetiapine and tiospirone, as well as the more efficacious agonists, may be expressed physiologically at somatodendritic 5-HT_{1A} sites. In contrast, although the tricyclic drug olanzapine is a structural analogue of clozapine (Bymaster et al., 1996), it displayed lower affinity at 5-HT_{1A} receptors, with a 270-fold preference for dopamine hD_2 vs. serotonin h5-HT_{1A} receptors, compared with clozapine's equilibrated 5-HT_{1A}/hD_2 receptor affinities (Table 3). Further, olanzapine exhibited only about half the efficacy of clozapine at CHO-h5-HT_{1A} receptors (24% vs. 53%). Thus, it may be hypothesised that 5-HT_{1A} receptor-mediated actions of clozapine are not a feature of olanzapine's therapeutic profile. Nevertheless, in clinical trials, olanzapine has significant effects in negative symptom improvement with a low incidence of extrapyramidal symptoms (Tran et al., 1997) indicating that activity at other receptor subtypes is also important. Indeed, Bartoszyk et al. (1996) showed that the anticataleptic action of clozapine was not reversed by the selective 5-HT_{1A} receptor antagonist, WAY 100,635. Further, BMY 14802, which in the present study displayed 10-fold selectivity for serotonin h5-HT_{1A} vs. dopamine hD_2 receptors with efficacious agonist activity ($E_{max} = 82\%$, Table 3) is ineffective against schizophrenia in humans, whilst not inducing extrapyramidal symptoms (Gewirtz et al., 1994).

The relative importance of 5-HT_{1A} vs. other receptor subtypes may be evaluated using novel antipsychotic agents which display high affinity for h5-HT_{1A} receptors. One such putatively 'atypical' antipsychotic in clinical trials, S 16924, exhibits 25-fold h5-HT_{1A} vs. hD_2 selectivity (K_i = 1.8 vs. 46 nM; Millan et al., 1995a) and has clozapine-like partial agonist properties at CHO-h5-HT_{1A} receptors (E_{max} = 66%; Newman-Tancredi et al., 1995). In vivo, S 16924, like clozapine, blocks both amphetamine-induced locomotion and haloperidol-induced catalepsy in rats.

4.4. Antagonist activity of antipsychotic agents at 5-HT_{1A} receptors

Spiperone is often used as a serotonergic antagonist (Escandon et al., 1994) and (+)butaclamol is used as a stereoselective receptorial ligand for the in vitro study of dopaminergic and serotonergic receptors (Sundaram et al., 1993; Hall and Strange, 1997). However, these compounds were originally intended as neuroleptic agents for the treatment of psychosis and although spiperone has not been tested as an antipsychotic agent in humans, (+)butaclamol, an inverse agonist at CHO-h5-HT_{1A} receptors, induces marked extrapyramidal symptoms in man (Clark et al., 1977). Other 'typical' antipsychotic drugs which are known to induce extrapyramidal symptoms, including chlorpromazine, pimozide and haloperidol, displayed antagonist behaviour at CHO-h5-HT_{1A} receptors (Table 2). This group of ligands, including haloperidol, raclopride, risperidone and pimozide, exhibited much higher affinity at dopamine D_2 than at serotonin 5-HT_{1A} receptors (Table 3) with selectivity ratios of at least 80-fold. This is also true of spiperone and (+)butaclamol, which display K_i values at hD_2 dopamine receptors of 0.06 and 1.3 nM (unpublished observation), i.e., at least two orders of magnitude higher affinity that at h5-HT_{1A} receptors (Table 2).

It is tempting to speculate that equilibrated 5-HT_{1A}/hD_2 receptor affinity and agonist activity at 5-HT_{1A} receptors would impart an 'atypical' profile to antipsychotic agents. However, as stated above, the profile of action of antipsychotic drugs at other receptor subtypes plays an important role in reduced induction of extrapyramidal symptoms. Indeed, both sertindole and risperidone, which are widely considered to exhibit certain 'atypical' features, displayed marked selectivity for dopamine hD_2 vs. serotonin h5-HT_{1A} receptors (K_i ratios of 80 and 210, respectively; Table 4, Fig. 4) and antagonist activity at 5-HT_{1A} receptors. Although antagonism at 5-HT_{1A} receptors may be associated with an amelioration of cognitive deficits and have promnesic proprieties (Harder et al., 1996; Buhot, 1997), it is unlikely to be involved in reducing extrapyramidal symptoms. 'Atypicality' may also be related to higher affinity at serotonin 5-HT_{2A} vs. dopamine D_2 receptors and actions at D_4 or D_3 receptors, as well as other 5-HT receptor subtypes such as 5-HT_6 and 5-HT_7 (Schotte et al., 1996; Seeman et al., 1997; Millan et al., 1997a; Roth et al., 1998). However, unlike clozapine, which is a 5-HT_{1A} receptor partial agonist, risperidone does dose-dependently induce extrapyramidal symptoms, although at a lower incidence than haloperidol (Schwartz and Brotman, 1992; Fleischhacker and Hummer, 1997), suggesting that an additional agonist activity at 5-HT_{1A} receptors may help to mimic a clozapine-like 'atypical' therapeutic profile.

4.5. Conclusions

The present study demonstrates that [^{35}S]GTPγS binding can be applied to CHO-h5-HT_{1A} receptors to characterise a wide range of agonist, antagonist and inverse agonist ligands. The ligand binding and functional profile of CHO-h5-HT_{1A} receptors resembles that of native hippocampal 5-HT_{1A} receptors. The characterization of the affinity and efficacy at h5-HT_{1A} receptors of a range of antipsychotic agents, indicates that, although the hypothesis that 5-HT_{1A} receptor agonism contributes to an 'atypical' profile is attractive, substantial further investigation is required. Nevertheless, clozapine and several other putatively atypical drugs displayed agonist activity at 5-HT_{1A} receptors whereas several 'typical' antipsychotic agents did not. These observations suggest that, although high intrinsic efficacy at 5-HT_{1A} receptor is insufficient, in itself, for antipsychotic action, agonist activity at this site may impart beneficial properties in the treatment of psychotic disorders.

References

Andersen, H.L., Kilpatrick, I.C., 1995. Prevention by (±)-8-hydroxy-2-(di-*n*-propylamino)tetralin of both catalepsy and the rises in rat striatal dopamine metabolism caused by haloperidol. Br. J. Pharmacol. 118, 421–427.

Arborelius, L., Hook, B.B., Hacksell, U., Svensson, T.H., 1994. The 5-HT_{1A} receptor antagonist (S)UH 301 blocks (*R*)-8-OH-DPAT-induced inhibition of serotonergic dorsal raphe cell firing in the rat. J. Neural Transm. Gen. Sect. 96, 179–186.

Audinot, V., Newman-Tancredi, A., Jacques, V., Chaput, C., Millan, M.J., 1995. Comparative profiles of interaction of atypical antipsychotics at cloned human dopamine hD_2, hD_3 and hD_4 receptors: S 16924 shows a marked preference for hD_4 sites. Eur. Neuropsychopharmacol. 5, 4–13.

Baldessarini, R.J., 1996. Drugs and the treatment of psychiatric disorders. In: Hardman, J.G., Limbird, L.E. (Eds.), Goodman and Gilman's The Pharmacological Basis of Therapeutics. pp. 399–430.

Barker, E.L., Westphal, R.S., Schmidt, D., Sanders-Bush, E., 1994. Constitutively active 5-hydroxytryptamine$_{2C}$ receptors reveal novel inverse agonist activity of receptor ligands. J. Biol. Chem. 269, 11687–11690.

Bartoszyk, G.D., Roos, C., Ziegler, H., 1996. 5-HT_{1A} receptors are not involved in clozapine's lack of cataleptogenic potential. Neuropharmacology 35, 1645–1646.

Bjork, L., Cornfield, L.J., Nelson, D.L., Hillver, S.E., Anden, N.E., Lewander, T., Hacksell, U., 1991. Pharmacology of the novel 5-hy-

droxytryptamine$_{1A}$ receptor antagonist (*S*)-5-fluoro-8-hydroxy-2-(dipropylamino)-tetralin: inhibition of (*R*)-8-hydroxy-2-(dipropyl amino)-tetralin-induced effects. J. Pharmacol. Exp. Ther. 258, 58–65.

Boess, F.G., Martin, I.L., 1994. Molecular biology of 5-HT receptors. Neuropharmacology 33, 275–317.

Buhot, M.-C., 1997. Serotonin receptors in cognitive behaviors. Current Opinion in Neurobiology 7, 243–254.

Burnet, P.W.J., Eastwood, S.L., Harrison, P.J., 1997. [^{3}H]WAY 100,635 for 5-HT$_{1A}$ receptor autoradiography in human brain: a comparison with [^{3}H]8-OH-DPAT and demonstration of increased binding in the frontal cortex in schizophrenia. Neurochem. Int. 30, 565–574.

Bymaster, F.P., Calligaro, D.O., Falcone, J.F., Marsh, R.D., Moore, N.A., Tye, N.C., Seeman, P., Wong, D.T., 1996. Radioreceptor binding profile of the atypical antipsychotic olanzapine. Neuropsychopharmacology 14, 87–96.

Chamberlain, J., Offord, S.J., Wolfe, B.B., Tyau, L.S., Wang, H.-L., Frazer, A., 1993. Potency of 5-hydroxytryptamine$_{1A}$ agonists to inhibit adenylyl cyclase activity is a function of affinity for the 'low-affinity' state of [^{3}H]8-hydroxy-*N,N*-dipropylaminotetralin ([^{3}H]8-OH-DPAT) binding. J. Pharmacol. Exp. Ther. 266, 618–625.

Clark, M.L., Paredes, A., Costiloe, J.P., Wood, F., 1977. Evaluation of butaclamol in chronic schizophrenic patients. J. Clin. Pharmacol. 17, 529–536.

Clarke, W.P., Yocca, F.D., Maayani, S., 1996. Lack of 5-hydroxytryptamine$_{1A}$-mediated inhibition of adenylyl cyclase in dorsal raphe of male and female rats. J. Pharmacol. Exp. Ther. 277, 1259–1266.

Corbett, R., Hartman, H., Kerman, L.L., Woods, A.T., Strupczewski, J.T., Helsey, G.C., Conway, P.C., Dunn, R.W., 1993. Effects of atypical antipsychotic agents on social behavior in rodents. Pharmacol. Biochem. Behav. 45, 9–17.

Cornfield, L.J., Lambert, G., Arvidsson, L.-E., Mellin, C., Vallgarda, J., Hacksell, U., Nelson, D.L., 1991. Intrinsic activity of enantiomers of 8-hydroxy-2-(di-*n*-propylamino)tetralin and its analogs at 5-hydroxytryptamine$_{1A}$ receptors that are negatively coupled to adenylate cyclase. Mol. Pharmacol. 39, 780–787.

Cox, R.F., Meller, E., Waszczak, B.L., 1993. Electrophysiological evidence for a large receptor reserve for inhibition of dorsal raphe neuronal firing by 5-HT$_{1A}$ agonists. Synapse 14, 297–304.

De Lean, A., Kilpatrick, B.F., Caron, M.G., 1982. Dopamine receptor of the porcine anterior pituitary gland: evidence for two affinity states discriminated by both agonists and antagonists. Mol. Pharmacol. 22, 290–297.

De Vivo, M., Maayani, S., 1986. Characterization of the 5-hydroxytryptamine$_{1A}$ receptor-mediated inhibition of forskolin-stimulated adenylate cyclase activity in guinea pig and rat hippocampal membranes. J. Pharmacol. Exp. Ther. 238, 248–253.

De Vry, J., 1995. 5-HT$_{1A}$ receptor agonists: recent developments and controversial issues. Psychopharmacology 121, 1–26.

Dumuis, A., Sebben, M., Bockaert, J., 1988. Pharmacology of 5-hydroxytryptamine$_{1A}$ receptors which inhibit cAMP production in hippocampal and cortical neurons in primary culture. Mol. Pharmacol. 33, 178–186.

Escandon, N.A., Zimmermann, D.C., McCall, R.B., 1994. Characterization of the serotonin$_{1A}$ receptor antagonist activity of WAY-100135 and spiperone. J. Pharmacol. Exp. Ther. 268, 441–447.

Fargin, A., Raymond, J.R., Regan, J.W., Cotecchia, S., Lefkowitz, R.J., Caron, M.G., 1989. Effector coupling mechanisms of the cloned 5-HT$_{1A}$ receptor. J. Biol. Chem. 264, 14848–14852.

Fargin, A., Yamamoto, K., Cotecchia, S., Goldsmith, P.K., Spiegel, A.M., Lapetina, E.G., Caron, M.G., Lefkowitz, R.J., 1991. Dual coupling of the cloned 5-HT$_{1A}$ receptor to both adenylyl cyclase and phospholipase C is mediated via the same G$_i$ protein. Cell. Signal. 3, 547–557.

Fleischhacker, W.W., Hummer, M., 1997. Drug treatment of schizophrenia in the 1990s: achievements and future possibilities in optimising outcomes. Drugs 53, 915–929.

Gewirtz, G.R., Gorman, J.M., Volavka, J., Macaluso, J., Gribkoff, G., Taylor, D.P., Borison, R., 1994. Neuropsychopharmacology 10, 37–40.

Glennon, R.A., Naiman, N.A., Pierson, M.E., Titeler, M., Lyon, R.A., Weisberg, E., 1988. NAN-190: an arylpiperazine analog that antagonizes the stimulus effects of the 5-HT$_{1A}$ agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT). Eur. J. Pharmacol. 154, 339–341.

Goff, D.C., Midha, K.K., Brotman, A.W., McCormick, S., Waites, M., Amico, E.T., 1991. An open trial of buspirone added to neuroleptics in schizophrenic patients. J. Clin. Psychopharmacol. 11, 193–197.

Gozlan, H., Thibault, S., Laporte, A.-M., Lima, L., Hamon, M., 1995. The selective 5-HT$_{1A}$ antagonist radioligand [^{3}H]WAY 100635 labels both G-protein-coupled and free 5-HT$_{1A}$ receptors in rat brain membranes. Eur. J. Pharmacol. 288, 173–186.

Greuel, J.M., Glaser, T., 1992. The putative 5-HT$_{1A}$ receptor antagonists NAN-190 and BMY 7378 are partial agonists in the rat dorsal raphe nucleus in vitro. Eur. J. Pharmacol. 211, 211–219.

Gudermann, T., Kalkbrenner, P., Schultz, G., 1996. Diversity and selectivity of receptor–G-protein interaction. Annu. Rev. Pharmacol. Toxicol. 36, 429–459.

Hall, D.A., Strange, P.G., 1997. Evidence that antipsychotic drugs are inverse agonists at D$_2$ dopamine receptors. Br. J. Pharmacol. 121, 731–736.

Harder, J.A., Maclean, C.J., Alder, J.T., Francis, P.T., Ridley, R.M., 1996. The 5-HT$_{1A}$ antagonist, WAY 100,635, ameliorates the cognitive impairment induced by fornix transection in the marmoset. Psychopharmacology 127, 245–254.

Harvey, K.V., Balon, R., 1995. Augmentation with buspirone: a review. Ann. Clin. Psychiatry 7, 143–147.

Hashimoto, T., Nishino, N., Nakai, H., Tanaka, C., 1991. Increase in serotonin 5-HT$_{1A}$ receptors in prefrontal cortex and temporal cortices of brains from patients with chronic schizophrenia. Life Sci. 48, 355–363.

Jain, A.K., Kelwala, S., Moore, N., Gershon, S., 1987. A controlled clinical trial of tiaspirone in schizophrenia. Int. Clin. Psychopharmacol. 2, 129–133.

Johnson, R.G., Fiorella, D., Winter, J.C., Rabin, R.A., 1997. [^{3}H]8-OH-DPAT labels a 5-HT site coupled to inhibition of phosphoinositide hydrolysis in the dorsal raphe. Eur. J. Pharmacol. 329, 99–106.

Kenakin, T., 1996. The classification of seven transmembrane receptors in recombinant expression systems. Pharmacol. Rev. 48, 413–463.

Lejeune, F., Audinot, V., Gobert, A., Rivet, J.-M., Spedding, M., Millan, M.J., 1994. Clozapine inhibits serotonergic transmission by an action at α_1-adrenoceptors not at 5-HT$_{1A}$ receptors. Eur. J. Pharmacol. 260, 79–83.

Liu, J.F., Albert, P.R., 1991. Cell-specific signaling of the 5-HT$_{1A}$ receptor. J. Biol. Chem. 266, 23689–23697.

Maes, M., Meltzer, H.Y., 1995. The serotonin hypothesis of major depression. In: Bloom, F.E., Kupfer, D.J., (Eds.), Psychopharmacology: The Fourth Generation of Progress. Raven Press, New York, NY, pp. 933–944.

Mason, S.L., Reynolds, G.P., 1992. Clozapine has sub-micromolar affinity for 5-HT$_{1A}$ receptors in human brain tissue. Eur. J. Pharmacol. 221, 397–398.

McMillen, B.A., Scott, S.M., Davanzo, E., 1988. Reversal of neuroleptic-induced catalepsy by novel aryl-piperazine anxiolytic drugs. J. Pharm. Pharmacol. 40, 885–887.

Meller, E., Goldstein, M., Bohmaker, K., 1990. Receptor reserve for 5-hydroxytryptamine$_{1A}$-mediated inhibition of serotonin synthesis: possible relationship to anxiolytic properties of 5-hydroxytryptamine$_{1A}$ agonists. Mol. Pharmacol. 37, 231–237.

Meltzer, H.Y., 1996. Pre-clinical pharmacology of atypical antipsychotic drugs: a selective review. Br. J. Psychiatry 168, 23–31.

Millan, M.J., Rivet, J.-M., Canton, H., Lejeune, F., Bervoets, K., Brocco, M., Gobert, A., Lefebvre de Ladonchamps, B., LeMarouille-Girardon, S., Verriele, L., Laubie, M., Lavielle, G., 1992. S 14671: a

naphtylpiperazine 5-hydroxytryptamine$_{1A}$ agonist of exceptional potency and high efficacy possessing antagonist activity at 5-hydroxytryptamine 1C/2 receptors. J. Pharmacol. Exp. Ther. 262, 451–463.

Millan, M.J., Rivet, J.-M., Canton, H., Lejeune, F., Gobert, A., Widdowson, P., Bervoets, K., Brocco, M., Peglion, J.-L., 1993. S 15535: a highly selective benzodioxopiperazine 5-HT$_{1A}$ receptor ligand which acts as an agonist and an antagonist at presynaptic and postsynaptic sites respectively. Eur. J. Pharmacol. 230, 99–102.

Millan, M.J., Canton, H., Gobert, A., Lejeune, F., Rivet, J.-M., Bervoets, K., Brocco, M., Widdowson, P., Mennini, T., Audinot, V., Honoré, P., Renouard, A., LeMarouille-Girardon, S., Verrièle, L., Gressier, H., Peglion, J.-L., 1994. Novel benzodioxopiperazines acting as antagonists at post-synaptic 5-HT$_{1A}$ receptors and as agonists at 5-HT$_{1A}$ autoreceptors: a comparative pharmacological characterisation with proposed 5-HT$_{1A}$ antagonists. J. Pharmacol. Exp. Ther. 268, 337–351.

Millan, M.J., Audinot, V., Brocco, M., Maurel-Remy, S., Joly, F., Newman-Tancredi, A., Canton, H., Hautefaye, P., Spedding, M., Muller, O., Lavielle, G., 1995a. S 16924: a pyrrolidine-substituted benzodioxane with higher affinity at 5-HT$_{1A/2A/2C}$ and D$_4$ than D$_2$ receptors, and an atypical antipsychotic profile in the rat. Br. J. Pharmacol. 114, 156P.

Millan, M.J., Peglion, J.-L., Vian, J., Rivet, J.-M., Brocco, M., Gobert, A., Newman-Tancredi, A., Dacquet, C., Bervoets, K., Girardon, S., Jacques, V., Chaput, C., Audinot, V., 1995b. Functional correlates of dopamine D$_3$ receptor activation in the rat in vivo and their modulation by the selective antagonist, (+)S 14297: 1. Activation of postsynaptic D$_3$ receptors mediates hypothermia while blockade of D$_2$ receptors elicits prolactin secretion and catalepsy. J. Pharmacol. Exp. Ther. 275, 885–898.

Millan, M.J., Gressier, H., Brocco, M., 1997a. The dopamine D$_3$ receptor antagonist, (+)S 14297, blocks the cataleptic properties of haloperidol in rats. Eur. J. Pharmacol. 321, R7–R9.

Millan, M.J., Newman-Tancredi, A., Rivet, J.-M., Brocco, M., Audinot, V., Cistarelli, L., Gobert, A., 1997b. S 15535, a novel benzodioxopiperazine ligand of serotonin 5-HT$_{1A}$ receptors: interaction with cloned human (h)5-HT$_{1A}$, dopamine hD$_2$/hD$_3$ and hα_{2A}-adrenergic receptors in relation to modulation of cortical monoamine release and potential antidepressant properties. J. Pharmacol. Exp. Ther. 282, 132–147.

Mongeau, R., Welner, S.A., Quirion, R., Suranyi-Cadotte, B.E., 1992. Further evidence for differential affinity states of the serotonin$_{1A}$ receptor in rat hippocampus. Brain Res. 590, 229–238.

Moore, N.C., Meyendorff, E., Yeraggani, V., LeWitt, P.A., Gershon, S., 1987. Tiaspirone in schizophrenia. J. Clin. Psychopharmacol. 7, 98–101.

Newman-Tancredi, A., Wootton, R., Strange, P.G., 1992. High level stable expression of recombinant 5-HT$_{1A}$ 5-hydroxytryptamine receptors in Chinese Hamster Ovary cells. Biochem. J. 285, 933–938.

Newman-Tancredi, A., Audinot, V., Spedding, M., Chaput, C., Verrièle, L., Millan, M.J., 1995. Efficacy of antipsychotic drugs at cloned human serotonin 5-HT$_{1A}$ and dopamine D$_4$ receptors determined by stimulation of [^{35}S]-GTPγS binding. Am. Soc. Neurosci. 21, 727.20.

Newman-Tancredi, A., Chaput, C., Verrièle, L., Millan, M.J., 1996a. Clozapine is a partial agonist at cloned, human serotonin 5-HT$_{1A}$ receptors. Neuropharmacology 35, 119–121.

Newman-Tancredi, A., Chaput, C., Verrièle, L., Millan, M.J., 1996b. S 15535 and WAY 100,635 antagonise 5-HT-stimulated [^{35}S]GTPγS binding at cloned human 5-HT$_{1A}$ receptors. Eur. J. Pharmacol. 307, 107–111.

Newman-Tancredi, A., Conte, C., Chaput, C., Millan, M.J., 1997a. Inhibition of the constitutive activity of human 5-HT$_{1A}$ receptors by the inverse agonist, spiperone but not the neutral antagonist, WAY 100,635. Br. J. Pharmacol. 120, 737–740.

Newman-Tancredi, A., Audinot, V., Chaput, C., Verrièle, L., Millan, M.J., 1997b. [^{35}S]Guanosine-5′-*O*-(3-thio)triphosphate binding as a measure of efficacy at human recombinant dopamine D$_{4.4}$ receptors: actions of antiparkinsonian and antipsychotic agents. J. Pharmacol. Exp. Ther. 282, 181–191.

Odagaki, Y., Fuxe, K., 1995. Pharmacological characterisation of the 5-hydroxytryptamine$_{1A}$ receptor-mediated activation of high-affinity GTP hydrolysis in rat hippocampal membranes. J. Pharmacol. Exp. Ther. 274, 337–344.

Pauwels, P.J., Van Gompel, P., Leysen, J.E., 1993. Activity of serotonin (5-HT) receptor agonists, partial agonists and antagonists at cloned human 5-HT$_{1A}$ receptors that are negatively coupled to adenylate cyclase in permanently transfected HeLa cells. Biochem. Pharmacol. 45, 375–383.

Raymond, J.R., Albers, F.J., Middleton, J.P., 1992. Functional expression of human 5-HT$_{1A}$ receptors and differential coupling to second messengers in CHO cells. Naunyn-Schmiedeberg's Arch. Pharmacol. 346, 127–137.

Raymond, J.R., Olsen, C.L., Gettys, T.W., 1993. Cell-specific and functional coupling of the human 5-HT$_{1A}$ receptors to inhibitory G protein α-subunits and lack of coupling to G$_{s\alpha}$. Biochemistry 32, 11064–11073.

Rollema, H., Lu, Y., Schmidt, A.W., Zorn, S.H., 1997. Clozapine increases dopamine release in prefrontal cortex by 5-HT$_{1A}$ receptor activation. Eur. J. Pharmacol. 338, R3–R5.

Roth, B.L., Meltzer, H.Y., Khan, N., 1998. Binding of typical and atypical antipsychotic drugs to multiple neurotransmitter receptors. Adv. Pharmacol. 42, 482–485.

Sautel, F., Griffon, N., Sokoloff, P., Schwartz, J.-C., Launay, C., Simon, P., Costentin, J., Schoenfelder, A., Garrido, F., Mann, A., Wermuth, C.G., 1995. Nafadotride, a potent preferential dopamine D$_3$ receptor antagonist, activates locomotion in rodents. J. Pharmacol. Exp. Ther. 275, 1239–1246.

Shoeffter, P., Hoyer, D., 1988. Centrally acting hypotensive agents with affinity for 5-HT$_{1A}$ binding sites inhibit forskolin-stimulated adenylate cyclase activity in calf hippocampus. Br. J. Pharmacol. 95, 975–985.

Schotte, A., Janssen, P.F.M., Gommeren, W., Luyten, W.H.M.L., Van Gompel, P., Lesage, A.S., De Loore, K., Leysen, J.E., 1996. Risperidone compared with new and reference antipsychotic drugs: in vitro and in vivo receptor binding. Psychopharmacology 124, 57–73.

Schreiber, R., Brocco, M., Gobert, A., Veiga, S., Millan, M.J., 1994. The potent activity of the 5-HT$_{1A}$ receptor agonists S 14506 and S 14671, in the rat forced swim test is blocked by novel 5-HT$_{1A}$ receptor antagonists. Eur. J. Pharmacol. 271, 537–541.

Schwartz, J.T., Brotman, A.W., 1992. A clinical guide to antipsychotic drugs. Drugs 44, 981–992.

Seeger, T.F., Seymour, P.A., Schmidt, A.W., Zorn, S.H., Schultz, D.W., Lebel, L.A., McLean, S., Guanowsky, V., Howard, H.R., Lowe, J.A., Heym, J., 1995. Ziprasidone (CP-88,059): a new antipsychotic with combined dopamine and serotonin receptor antagonist activity. J. Pharmacol. Exp. Ther. 275, 101–113.

Seeman, P., Corbett, R., Van Tol, H.H.M., 1997. Atypical neuroleptics have low affinity for dopamine D$_2$ receptors or are selective for D$_4$ receptors. Neuropsychopharmacology 16, 93–110.

Sovner, R., Parnell-Sovner, N., 1989. Use of buspirone in the treatment of schizophrenia. J. Clin. Psychopharmacol. 9, 61–62.

Stanton, J.A., Beer, M., 1997. Characterisation of a cloned human 5-HT$_{1A}$ receptor cell line using [^{35}S]GTPγS binding. Eur. J. Pharmacol. 320, 267–275.

Sundaram, H., Newman-Tancredi, A., Strange, P.G., 1993. Characterisation of recombinant human serotonin 5-HT$_{1A}$ receptors expressed in Chinese hamster ovary cells: [^{3}H]spiperone discriminates between the G-protein-coupled and uncoupled forms. Biochem. Pharmacol. 45, 1003–1009.

Thomas, D.R., Faruq, S.A., Balcarek, J.M., Brown, A.M., 1995. Pharmacological characterisation of [^{35}S]GTPγS binding to chinese hamster ovary cell membranes stably expressing cloned human 5-HT$_{1D}$ receptor subtypes. J. Recept. Signal Transduct. Res. 15, 199–211.

Tollefson, G.D., Sanger, T.M., 1997. Negative symptoms: a path analytic approach to a double-blind, placebo- and haloperidol-controlled clinical trial with olanzapine. Am. J. Psychiatry 154, 466–474.

Tran, P.V., Dellva, M.A., Tollefson, G.D., Beasley Jr., C.M., Potvin, J.H., Kiesler, G.M., 1997. Extrapyramidal symptoms and tolerability of olanzapine versus haloperidol in the acute treatment of schizophrenia. J. Clin. Psychiatry 58, 205–211.

Varrault, A., Journot, L., Audigier, Y., Bockaert, J., 1992. Transfection of human 5-hydroxytryptamine$_{1A}$ receptors in NIH-3T$_3$ fibroblasts: effects of increasing receptor density on the coupling of 5-hydroxytryptamine$_{1A}$ receptors to adenylyl cyclase. Mol. Pharmacol. 41, 999–1007.

Yocca, F.D., Iben, L., Meller, E., 1992. Lack of apparent receptor reserve at postsynaptic 5-hydroxytryptamine$_{1A}$ receptors negatively coupled to adenylyl cyclase activity in rat hippocampal membranes. Mol. Pharmacol. 41, 1066–1072.