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Efficacy of antipsychotic agents at human 5-HT_{1A} receptors determined by [^{3}H]WAY100,635 binding affinity ratios: relationship to efficacy for G-protein activation

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Abstract

5-HT_{1A} receptors are implicated in the aetiology of schizophrenia. Herein, the influence of 15 antipsychotics on the binding of the selective 'neutral' antagonist, [^{3}H]WAY100,635 ([^{3}H]*N*-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl)-cyclo-hexanecarboxamide), was examined at human 5-HT_{1A} receptors expressed in Chinese Hamster Ovary cells. In competition binding experiments, 5-HT displayed biphasic isotherms which were shifted to the right in the presence of the G-protein uncoupling agent, GTPγS (100 μM). In analogy, the isotherms of ziprasidone, quetiapine and S16924 (((*R*-2-{1-[2-(2,3-dihydro-benzo[1,4]dioxin-5-yloxy)-ethyl]-pyrrolidin-3yl}-1-(4-fluoro-phenyl)-ethanone), were displaced to the right by GTPγS, consistent with agonist actions. Binding of several other antipsychotics, such as ocaperidone, olanzapine and risperidone, was little influenced by GTPγS. Isotherms of the neuroleptics, haloperidol, chlorpromazine and thioridazine were shifted to the left in the presence of GTPγS, suggesting inverse agonist properties. For most ligands, the magnitude of affinity changes induced by GTPγS (alteration in pK_i values) correlated well with their previously determined efficacies in [^{35}S]GTPγS binding studies [Eur. J. Pharmacol. 355 (1998) 245]. In contrast, the affinity of the 'atypical' antipsychotic agent, clozapine, which is a known partial agonist at 5-HT_{1A} receptors, was less influenced by GTPγS. When the ratio of high-/low-affinity values was plotted against efficacy, hyperbolic isotherms were obtained, consistent with a modified ternary complex model which assumes that receptors can adopt active conformations in the absence of agonist. In conclusion, modulation of [^{3}H]-WAY100,635 binding by GTPγS differentiated agonist vs. inverse agonist properties of antipsychotics at 5-HT_{1A} receptors. These may contribute to differing profiles of antipsychotic activity.

Keywords: 5-HT_{1A} receptor; WAY100,635; Inverse agonist; Clozapine; S16924; Antipsychotic

1. Introduction

5-HT_{1A} receptors, located post-synaptically in cortical and hippocampal structures and as inhibitory autoreceptors on serotonergic cell bodies in the raphe nuclei, play an important role in the control of mood, cognition and memory (De Vry, 1995; Kroeze and Roth, 1998; Barnes and Sharp, 1999). There is evidence that their function is perturbed in psychosis (Harvey and Balon, 1995; Sharma and Shapiro, 1996) and the density of 5-HT_{1A} receptors in hippocampus and cortex is altered in schizophrenic patients (Burnet et al., 1997; Gurevich and Joyce, 1997; Bantick et al., 2000), although the molecular basis for this effect is currently unclear (Millan, 2000). Further, 5-HT_{1A} (auto)receptor stimulation may improve cognitive and negative symptoms, stabilise mood and attenuate undesirable extrapyramidal symptoms elicited by striatal dopamine D_2 receptor blockade. Thus, 5-HT_{1A} receptor agonists reverse the catalepsy (a model of extrapyramidal symptoms) induced in rats by dopamine D_2 receptor antagonists, such as the neuroleptic agent, haloperidol (Invernizzi et al., 1988; McMillen et al., 1988; Andersen and Kilpatrick, 1995; Millan et al., 1998b). This observation suggests that antipsychotic agents displaying 5-HT_{1A} receptor agonist properties may exhibit a lowered propensity to induce EPS. This hypothesis is supported by the observation that the 'atypical' antipsychotic agent, clozapine, which is es-

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sentially free of EPS, behaves as a partial agonist at 5-HT_{1A} receptors both in vitro and in vivo (Newman-Tancredi et al., 1996; Millan et al., 1998b; Rollema et al., 1997; see Millan, 2000 for review) and occupies 5-HT_{1A} receptors in humans at therapeutic doses (Bantick et al., 2000).

We have previously investigated the agonist efficacy of antipsychotic agents at recombinant human 5-HT_{1A} receptors expressed in Chinese hamster ovary (CHO) cells by the technique of [^{35}S]GTPγS binding and shown that several 'atypical' antipsychotic agents show partial agonist activity (Newman-Tancredi et al., 1998a). Ligand efficacy may also be investigated by changes in ligand affinity in response to G-protein uncoupling induced by guanine nucleotides such as GTPγS, a hydrolysis-resistant analogue of GTP. Indeed, whereas agonists stabilise receptors in G-protein-coupled conformation(s), inverse agonists stabilise the receptor in non-G-receptor-coupled state(s) (Daeffler and Landry, 2000; Samama et al., 1994; Leff, 1995; Kenakin, 1997). 'Neutral antagonists' do not distinguish, by definition, between coupled and uncoupled receptors.

The present study employed the neutral antagonist radioligand, [^{3}H]WAY100,635 (Fletcher et al., 1996; Gozlan et al., 1995; Newman-Tancredi et al., 1997; Khawaja et al., 1997), to label human 5-HT_{1A} receptors expressed in Chinese hamster ovary (CHO) cells. We carried out [^{3}H]WAY100,635 competition binding experiments in the presence and absence of GTPγS, to derive measures of affinity at both G-protein-coupled and uncoupled forms of the 5-HT_{1A} receptor (Sundaram et al., 1995; Watson et al., 2000; Assié et al., 1999). As we have recently shown (Newman-Tancredi et al., 2001), the magnitude of the affinity changes induced by GTPγS in competition binding experiments with [^{3}H]WAY100,635 is quantitatively related to agonist and inverse agonist efficacy at human 5-HT_{1A} receptors for most ligands. However, certain ligands exhibit little sensitivity to GTPγS whilst behaving as efficacious agonists, suggesting unusual relationships between binding affinity and functional properties. We, therefore, applied this methodology to the characterisation of antipsychotic agent interaction at 5-HT_{1A} receptors.

2. Materials and methods

2.1. Drugs and radioligands

S18327 base (1-[2-[4-(6-fluoro-1,2-benzisoxazol-3-yl) piperidin-1-yl]ethyl]-3-phenyl-imidazolidin-2-one), quietapine hemifumarate, olanzapine base and risperidone base were synthesised by J.-L. Peglion, Servier. S16924 ((*R*-2-{1-[2-(2,3-dihydro-benzo[1,4]dioxin-5-yloxy)-ethyl]-pyr-

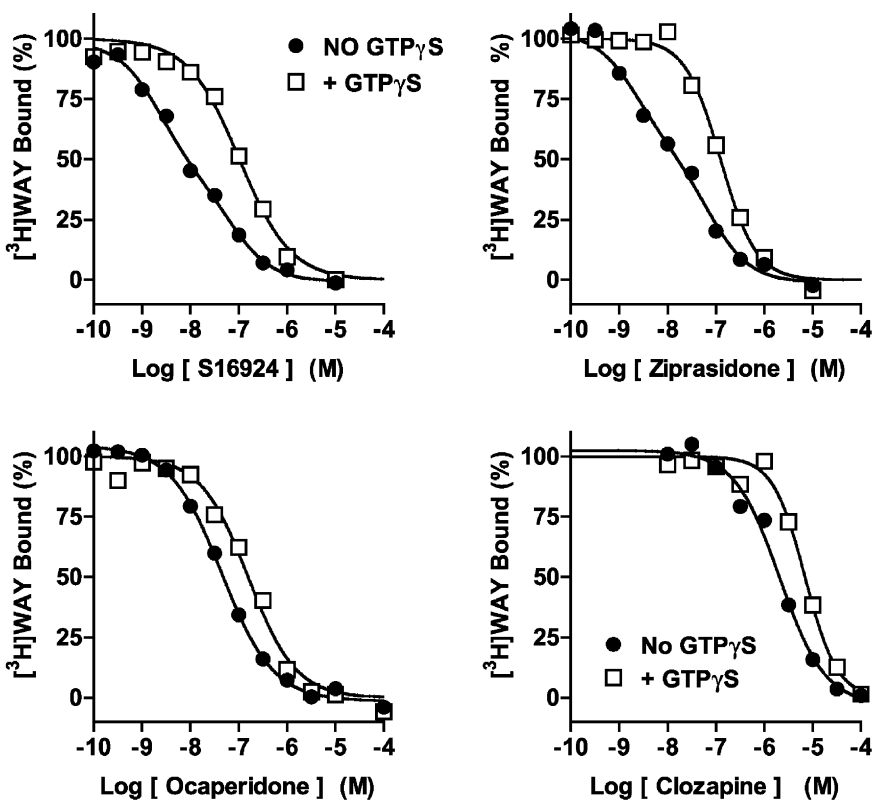

Fig. 1. Competition binding profile of [^{3}H]-WAY100,635: influence of GTPγS on antipsychotic agents with agonist properties at recombinant human 5-HT_{1A} receptors. [^{3}H]-WAY100,635 was competed with the antipsychotic agents indicated, in the presence or absence of the receptor/G-protein uncoupling agent, GTPγS (100 μM). Marked shifts to the right were observed with GTPγS, consistent with agonist properties.

rolidin-3yl}-1-(4-fluoro-phenyl)-ethanone) HCl and ziprasidone HCl were synthesised by G. Lavielle, Servier. Clozapine base, (+)-butaclamol HCl and spiperone HCl were purchased from Research Biochemicals International (Natick, MA). 5-HT creatinine sulphate, chlorpromazine HCl, thioridazine HCl and haloperidol base were purchased from Sigma (Saint Quentin Fallavier, France). Tiospirone HCl and BMY14802 (1-[4-(4-fluorophenyl)-4-hydroxybutyl]-4-(5-fluoropyrimidin-2-yl)piperazine) HCl were donated by Bristol Myers Squibb (Wallingford, CT). Ocaperidone base was donated by Janssen (Beerse, Belgium). Loxapine succinate was donated by Lederle (Wayne, NJ).

Compounds were dissolved in dimethylsulphoxide (DMSO) at 10^{-2} M or in deionised, distilled water at 10^{-3} M and diluted to appropriate concentrations in incubation buffer (see below). [^{3}H]WAY100,635 (81 Ci/mmol) was purchased from Amersham (Les Ulis, France).

2.2. [^{3}H]WAY100,635 competition binding and data analysis

Membranes of CHO cells stably expressing recombinant human 5-HT$_{1A}$ receptors were purchased from NEN (Paris, France). For competition binding experiments, membranes (10 μg protein) were incubated in triplicate with [^{3}H]WAY100,635 (0.5 nM) at 22 °C for 2.5 h in HEPES 20 mM, pH 7.5, and $MgSO_4$ 5 mM. Nonspecific binding was defined using 5-HT (10μM). All experiments were terminated by rapid filtration through Whatman GF/B filters (pretreated with 0.1% polyethyleneimine) and radioactivity was determined by liquid scintillation counting. Protein concentration was determined using a bicinchonic acid assay kit (Sigma).

Binding isotherms were analysed by nonlinear regression using the program 'Prism' (GraphPad, San Diego, CA). The algorithms used were as described previously (Newman-Tancredi et al., 1998b). K_i values were calculated by $K_i = IC_{50} \div (L + K_d)$, where L is the concentration of radioligand. All competition isotherms were fitted to both a single- and a two-binding site models. The 'goodness of fit' of the two models was compared using the *F*-test. In the case of the two-site model being statistically superior, values of affinity are shown for both high (pK_H)- and low (pK_L)-affinity sites and for the percentage of sites in the high-affinity binding components.

3. Results

3.1. [^{3}H]WAY100,635 competition binding: influence of GTPγS

5-HT exhibited biphasic competition isotherms with [^{3}H]WAY100,635. Similarly, BMY14802, ziprasidone, S16924 and quietapine also exhibited biphasic isotherms, which became steeper and right-shifted in the presence of GTPγS (Fig. 1). Clozapine, ocaperidone, loxapine, olanzapine, tiospirone and risperidone displayed monophasic isotherms, with pK_i values which were significantly reduced by GTPγS. In contrast, the neuroleptics, thioridazine, haloperidol and chlorpromazine exhibited 'reversed' sensitivity to GTPγS. Their competition binding curves shifted to the left, in the presence of GTPγS, i.e. their binding affinity increased (Fig. 2).

The change in affinity induced by receptor uncoupling was calculated by subtracting the pK_i value determined

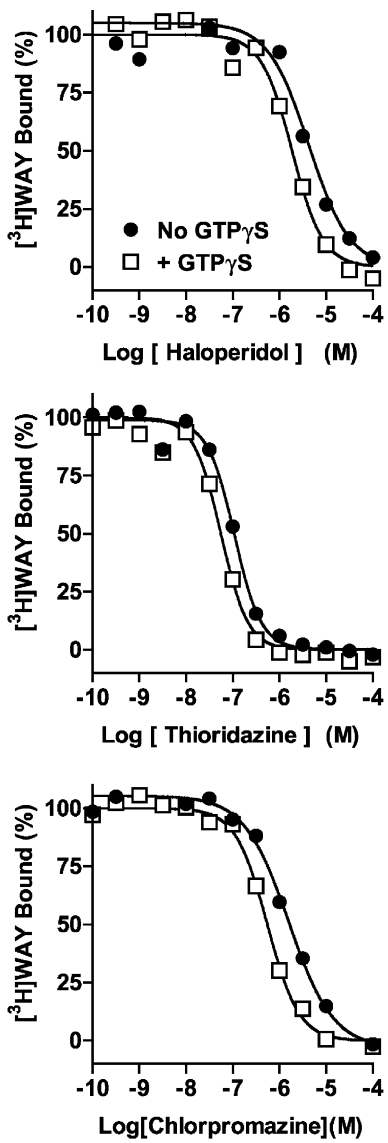

Fig. 2. Competition binding profile of [^{3}H]-WAY100,635: influence of GTPγS on antipsychotic agents with inverse agonist properties at recombinant human 5-HT$_{1A}$ receptors. [^{3}H]-WAY100,635 was competed with the antipsychotic agents indicated, in the presence or absence of the receptor/G-protein uncoupling agent, GTPγS (100 μM). Isotherms were shifted to the left in the presence of GTPγS, consistent with inverse agonist properties.

with GTPγS from that determined in its absence (Newman-Tancredi et al., 2001). The greatest affinity changes were for 5-HT and BMY14802 (> 2.2 log units) (Table 1). S16924, ziprasidone and quietapine exhibited intermediate values (1.73–1.28), whereas clozapine, olanzapine and risperidone, exhibited more modest affinity changes (0.65–0.25). Thioridazine, haloperidol and chlorpromazine exhibited negative affinity changes (−0.24–−0.36). Changes in affinity were statistically significant ($P < 0.05$, two-tailed *t*-test) for all compounds except thioridazine.

Changes in affinity were also expressed as K_i/K_H ratios, where K_i is the binding affinity (in nM) determined in the presence of GTPγS and K_H is the binding affinity (in nM) for the high-affinity binding component of the isotherms in the absence of GTPγS. K_i/K_H ratios exceeded 1 for agonists and were inferior to 1 for inverse agonists (see Table 1).

3.2. GTPγS-induced affinity changes: correlation with efficacy for G-protein stimulation

The affinity change values of 16 ligands in Table 1 (all except clozapine) were highly correlated ($r^2 = 0.94$, $P < 0.001$) with their efficacies for G-protein activation determined by stimulation of [^{35}S]GTPγS binding (see Newman-Tancredi et al., 1998a). In contrast, clozapine, which behaves as a partial agonist for G-protein activation, with an efficacy ($E_{max} = 53\%$) similar to that of ziprasidone and S16924, was less sensitive to GTPγS-induced receptor-G-protein uncoupling. Its affinity change values were close to those of its two chemical congeners, olanzapine and loxapine, which display markedly lower efficacy ($E_{max} \sim 25\%$; Table 1). Thus, the clozapine data point fell outside the affinity change vs. efficacy linear regression established for the other ligands (Fig. 3A).

The values of affinity change expressed as K_i/K_H ratios were plotted against efficacy (Fig. 3B). A similar type of plot has been used to model the simple vs. modified ternary complex models of receptor/G-protein coupling (Egan et al., 2000). Herein, the K_i/K_H ratio vs. E_{max} plot yielded a rectangular hyperbola tending towards a maximum of 100%, consistent with a modified rather than the simple ternary complex model. It is interesting that clozapine, once again, is markedly different to its congeners, loxapine and olanzapine. The data points belonging to clozapine, S16924 and ziprasidone fall outside the hyperbola but little can be inferred from this observation. This is because the K_i/K_H plot is not well suited to detecting ligands for which affinity changes correlate poorly with efficacy, due to the 'compression' of the

Table 1
[^{3}H]-WAY100,635 competition binding at recombinant human 5-HT$_{1A}$ receptors in the presence and absence of GTPγS

	No GTPγS				With GTPγS		Affinity change		[^{35}S]-GTPγS
	pK_i or pK_H	pK_L	% High	n_H	pK_i	n_H	$pK_H - pK_i$	K_i/K_H	E_{max} (%)[a]
5-HT	9.84 ± 0.06	7.85 ± 0.06	70 ± 2	0.58 ± 0.05	7.54 ± 0.02	1.00 ± 0.04	2.30	200	100
BMY14802	8.18 ± 0.24	6.59 ± 0.05	53 ± 6	0.68 ± 0.03	5.90 ± 0.04	1.00 ± 0.17	2.28	191	82
Ziprasidone	9.61 ± 0.22	8.16 ± 0.20	47 ± 12	0.74 ± 0.02	7.88 ± 0.01	1.12 ± 0.04	1.73	54	55
S16924	9.52 ± 0.25	8.02 ± 0.23	51 ± 12	0.74 ± 0.06	7.80 ± 0.07	1.13 ± 0.13	1.72	52	54
Quietapine	6.67 ± 0.07	5.15 ± 0.20	45 ± 10	0.75 ± 0.04	5.48 ± 0.17	0.96 ± 0.14	1.28	19	60
Ocaperidone	8.33 ± 0.09			0.89 ± 0.02	7.68 ± 0.06	1.12 ± 0.12	0.65	4.5	29
Loxapine	6.16 ± 0.26			0.91 ± 0.08	5.51 ± 0.01	1.18 ± 0.01	0.65	4.5	25[b]
S18327	7.49 ± 0.01			0.83 ± 0.08	6.89 ± 0.03	1.23 ± 0.07	0.60	4.0	29[b]
Clozapine	6.74 ± 0.06			0.90 ± 0.05	6.18 ± 0.04	1.27 ± 0.07	0.56	3.6	53
Olanzapine	6.00 ± 0.07			1.05 ± 0.20	5.60 ± 0.03	1.02 ± 0.02	0.40	2.5	24
Tiospirone	8.83 ± 0.07			1.11 ± 0.14	8.48 ± 0.10	1.29 ± 0.21	0.35	2.2	17
Risperidone	6.92 ± 0.05			1.13 ± 0.08	6.67 ± 0.05	1.25 ± 0.06	0.25	1.8	9[b]
Thioridazine	7.60 ± 0.27			1.49 ± 0.16	7.84 ± 0.33	1.47 ± 0.02	−0.24	0.6	−16[b]
(+)-Butaclamol[a]	7.23 ± 0.09			1.19 ± 0.04	7.51 ± 0.03	1.27 ± 0.01	−0.28	0.5	−18
Haloperidol	6.52 ± 0.08			1.09 ± 0.22	6.87 ± 0.12	1.12 ± 0.17	−0.35	0.4	0
Chlorpromazine	6.87 ± 0.12			0.92 ± 0.08	7.23 ± 0.01	1.42 ± 0.14	−0.36	0.4	0
Spiperone[a]	7.82 ± 0.12			0.88 ± 0.03	8.25 ± 0.08	1.30 ± 0.04	−0.43	0.37	−28

[^{3}H]-WAY100,635 (0.5 nM) was competed with antipsychotic agents for binding to h5-HT$_{1A}$ receptors. One- and two-site fits were compared by *F*-tests. In the presence of GTPγS (100μM), all isotherms were monophasic. Affinity changes were calculated (i) by subtracting the pK_i value determined with GTPγS from the pK_i/pK_H value determined without it; and (ii) by dividing the K_i value (with GTPγS) by the K_i/K_H value (no GTPγS). Affinity changes ($pK_H - pK_i$) were statistically significant ($P < 0.05$, two-tailed *t*-test) for all compounds except thioridazine. Efficacy (E_{max}) values were determined by [^{35}S]-GTPγS binding. n_H = Hill coefficient; %High = percentage of high-affinity sites. Data are means ± S.E.M. of ≥ 3 determinations performed in triplicate.

[a] Data from Newman-Tancredi et al. (1998a, 2001) and Millan et al. (1998a, 2000).

[b] Unpublished results.

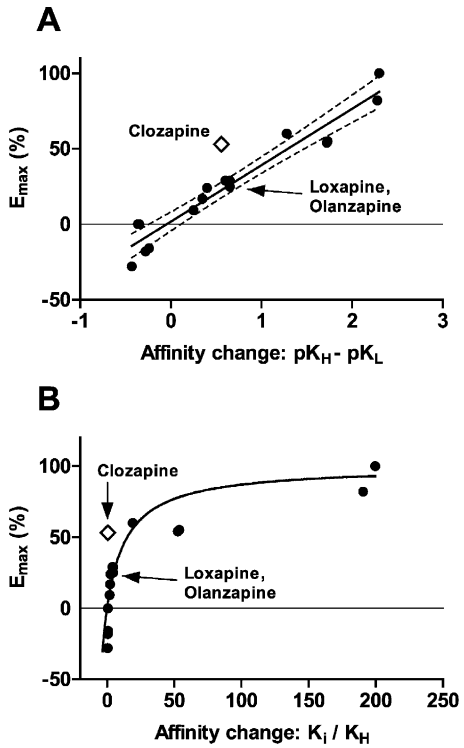

Fig. 3. Relationship between affinity changes induced by G-protein uncoupling and ligand efficacy determined by [^{35}S]-GTPγS binding at recombinant human 5-HT$_{1A}$ receptors. Panel A: Correlation of affinity change ($pK_i - pK_H$) values from competition binding experiments and ligand efficacy determined by stimulation/inhibition of [^{35}S]GTPγS binding (see Table 1). Dotted lines indicate the 95% confidence interval of the regression isotherm ($r^2 = 0.94$). Panel B: Correlation of affinity change (K_i / K_H) values from competition binding experiments and ligand efficacy. The hyperbolic isotherm is consistent with fitting to the revised ternary complex model (Egan et al., 2000).

distribution of the majority of points which are located between K_i/K_H ratios of 0 and 20.

4. Discussion

The present study investigated the actions of antipsychotic agents at recombinant human 5-HT$_{1A}$ receptors in competition binding experiments with the selective antagonist, [^{3}H]WAY100,635, in the presence and absence of the receptor/G-protein uncoupling agent, GTPγS. Thus, competition with 5-HT yielded biphasic isotherms, indicative of preferential binding to a G-protein-coupled conformation of 5-HT$_{1A}$ receptors. The high-affinity component was abolished by addition of GTPγS. Such an effect is typical of agonists, which display lower affinity for receptors in a non-G-protein-coupled state (Assié et al., 1999; Egan et al., 2000; Freedman et al., 1988; Lahti et al., 1992; Watson et al., 2000). The binding of [^{3}H]WAY100,635 itself is not influenced by GTPγS (Newman-Tancredi et al., 2001).

In analogy to 5-HT, both ziprasidone and S16924, which display 'atypical' profiles of antipsychotic activity in preclinical models (Millan et al., 1998a,b; Seeger et al., 1995), displayed biphasic isotherms in the absence of GTPγS and shifts to the right in its presence, reflecting agonist actions at 5-HT$_{1A}$ receptors. These data are consistent with our previous study of G-protein activation determined by [^{35}S]GTPγS binding (Newman-Tancredi et al., 1998a) and, therefore, further underpin the 5-HT$_{1A}$ receptor agonist properties of these 'atypical' antipsychotic agents (Millan et al., 1998b; Millan, 2000; Ichikawa and Meltzer, 1999; Ichikawa et al., 2001). Interestingly, quietapine also showed marked GTPγS shifts, suggesting that 5-HT$_{1A}$ receptor agonist properties might be involved in its actions, an issue which deserves further investigation. Indeed, the affinities of ziprasidone, quietapine and S16924, as well as clozapine, at human 5-HT$_{1A}$ receptors were similar to, or higher than, their affinity at hD$_2$ receptors (Newman-Tancredi et al., 1998a; Richelson and Souder, 2000). This supports the assertion that agonism at 5-HT$_{1A}$ sites may be involved in their reduced incidence of extrapyramidal symptoms (Millan et al., 1998b; Millan, 2000; Ichikawa and Meltzer, 1999).

Other antipsychotic ligands, including ocaperidone, olanzapine and tiospirone, exhibited markedly lower agonist properties in competition binding assays with [^{3}H]WAY100,635, with much more modest 'shifts' induced by GTPγS, consistent with lower efficacy for activation of coupled G-proteins (Newman-Tancredi et al., 1998a). Interestingly, risperidone, which may interact with 5-HT$_{1A}$ receptors at high doses in vivo (Ichikawa and Meltzer, 2000), also exhibited a slight, but significant, GTPγS shift (Table 1), suggesting that it possesses mild agonist properties, albeit at concentrations which are $>$30-fold higher that its affinity at dopamine D$_2$ receptors (Newman-Tancredi et al., 1998a).

In contrast to the above ligands, the neuroleptics, haloperidol and chlorpromazine, which are effective in the treatment of positive schizophrenic symptoms, but induce marked extrapyramidal symptoms, displayed shifts to the left in the presence of GTPγS, suggesting inverse agonist properties at 5-HT$_{1A}$ receptors, similar to those of the neuroleptics, (+)-butaclamol and spiperone (Table 1; Newman-Tancredi et al., 1998b, 2001). However, consistent inhibition of basal [^{35}S]GTPγS binding by haloperidol and chlorpromazine was not observed in our previous study of G-protein activation (Newman-Tancredi et al., 1998a; Table 1), so the precise nature of their inverse agonist properties remains to be clarified. Further, their affinities at human 5-HT$_{1A}$ receptors were at least an order of magnitude lower than at human D$_2$ receptors, emphasising the predominant role of the latter in their functional profiles (Ichikawa and Meltzer, 1999; Millan, 2000). Indeed, there is currently no evidence that inverse agonist properties at 5-HT$_{1A}$ receptors are involved in the physiological actions of these ligands. The precise extent of the

(modest) leftward shift is, no doubt, dependent on the degree of constitutive in the system, receptor/G-protein stoichiometry and membrane environment. Nevertheless, the present data suggest that inverse agonist activity may be predicted on the basis of equilibrium competition binding experiments with a neutral radiolabelled antagonist.

It was previously shown at human 5-HT_{1A} receptors using [^{3}H]WAY100,635 (Newman-Tancredi et al., 2001), and at rat 5-HT_{1A} and human 5-HT_{1A} receptors employing other radioligands (Watson et al., 2000; Assié et al., 1999), that the magnitude of the affinity differences for G-protein coupled/uncoupled receptors is correlated with their efficacy for G-protein activation. This was indeed the case for most of the antipsychotic ligands characterised here. An excellent correlation ($r^2 = 0.94$; Fig. 3A) was obtained between changes in pK_i values induced by G-protein uncoupling ($pK_H - pK_i$: positive for agonists, negative for inverse agonists), and E_{max} values determined by [^{35}S]GTPγS binding (Newman-Tancredi et al., 1998a; Fig. 3). However, clozapine (Fig. 3) exhibited only a modest GTPγS shift, whilst acting as a partial agonist both in vitro and in vivo (Newman-Tancredi et al., 1996, 1998a; Millan, 2000; Rollema et al., 1997). In fact, the efficacy of clozapine for G-protein activation ($E_{max} = 53\%$) is markedly greater than that of its chemical analogues, olanzapine and loxapine ($E_{max} \sim 25\%$), and similar to that of ziprasidone and S16924 ($E_{max} \sim 55\%$; Table 1; Millan et al., 1998a). In contrast, whereas the competition binding isotherms of the latter ligands were markedly biphasic, clozapine binding curves were steep ($n_H = 0.90$) and could not be resolved into high- and low-affinity components (Table 1). It is interesting to note recent data for 5-HT_{1A} receptor-mediated mitogen activated protein kinase (MAPK) activation in a CHO-5-HT_{1A} cell line: unlike ziprasidone and S16924, clozapine only poorly induced MAPK phosphorylation (Cussac et al., 2001), underlining its unusual properties for activation of this receptor. The actions of clozapine at 5-HT_{1A} receptors are reminiscent of two potent 5-HT_{1A} ligands, S14506 (1-[2-(4-fluorobenzoylamino)ethyl]-4-(7-methoxynaphtyl)-piperazine) and S14671 (1-(7-methoxynaphth-1-yl)-4-[2-(2-thenoylamino)-ethyl]piperazine). Their affinity is weakly influenced by GTPγS, despite their full agonist properties (Newman-Tancredi et al., 2001), observations which may be related to interaction with conserved amino acid residues in the 5-HT_{1A} receptor binding pocket (Jacoby et al., 1999; Bennet et al., 2000; Milligan et al., 2001).

A different kind of analysis of affinity changes can be carried out, whereby K_i/K_H ratios were plotted against E_{max} values (Fig. 3B; Table 1). Such an analysis yielded a hyperbolic curve which, in previous studies at 5-HT_{2A} and 5-HT_{2C} receptors, was shown to be more closely mimicked by a modified (or 'revised') ternary complex model of receptor/G-protein coupling rather than the classical (or 'simple') ternary complex model (Egan et al., 2000). The revised ternary complex model postulates that receptors can exist in a conformation that can activate coupled G-proteins in the absence of agonists. Such 'constitutive activity' may be detected in functional assays using inverse agonists (Daeffler and Landry, 2000; Newman-Tancredi et al., 1997). Thus, the present K_i/K_H ratios vs. E_{max} data at human 5-HT_{1A} receptors are consistent with detection of marked constitutive activity in this system (Newman-Tancredi et al., 1997, 1998a,b). It may be noted that hyperbolic K_i/K_H ratios vs. E_{max} plots are not best suited to detect ligands, such as clozapine, which do not fall on the isotherms. Nevertheless, the clozapine data point falls some distance from those of its chemical congeners, loxapine and olanzapine, reinforcing the differences noted above between these ligands.

In conclusion, the present study of [^{3}H]WAY100,635 binding examined the sensitivity of antipsychotic agents to GTPγS-induced 5-HT_{1A} receptor/G-protein uncoupling. The results show that GTPγS-induced affinity changes correlate, in most cases, with their efficacy determined by [^{35}S]GTPγS binding. Caution should be exercised in extrapolating from the present in vitro data to physiological situations, because the therapeutic properties of antipsychotic agents reflect actions at diverse other targets, including 5-HT_{2A} and dopamine D_2, D_3 and D_4 receptors. Nevertheless, taken together, the present data suggest that the in vivo relevance of 5-HT_{1A} receptor inverse agonist properties of neuroleptics is likely minor, in view of the latter's much greater affinity at dopaminergic receptors, but support the hypothesis of a role of partial agonism at 5-HT_{1A} receptors in the actions of certain 'atypical' antipsychotics.

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