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Differential modulation by GTPγS of agonist and inverse agonist binding to h5-HT_{1A} receptors revealed by [^{3}H]-WAY100,635

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1 The interaction of serotonergic ligands at human (h) 5-HT_{1A} receptors expressed in Chinese hamster ovary cells was examined with the selective 'neutral' 5-HT_{1A} antagonist [^{3}H]-WAY100,635. Its binding was saturable ($K_D = 0.056$ nM) with a B_{max} (3.65 pmol mg^{-1}) significantly higher than that of two other selective 5-HT_{1A} radioligands: the partial agonist, [^{3}H]-S15535 (2.77 pmol mg^{-1}) and the agonist, [^{3}H]-8-OH-DPAT (2.02 pmol mg^{-1}).

2 The influence of GTPγS (100 μM) on the binding affinity of 15 serotonergic agonists, partial agonists, antagonists and inverse agonists was investigated in competition binding experiments with [^{3}H]-WAY100,635.

3 Agonists, including 5-HT, 8-OH-DPAT and buspirone, displayed biphasic isotherms which shifted to the right in the presence of GTPγS. In contrast, isotherms of the *inverse* agonists, methiothepin, (+)butaclamol and spiperone, were shifted to the left in the presence of GTPγS. Unlabelled WAY100,635 was the only ligand that was unaffected by GTPγS, consistent with 'neutral' antagonist properties.

4 The magnitude of affinity changes induced by GTPγS for 13 ligands was highly correlated ($r = 0.98$) with their efficacy (positive and negative) previously determined by [^{35}S]GTPγS binding.

5 In contrast, the napthylpiperazine derivative and high efficacy agonist, S14506, displayed only a modest GTPγS shift, in accordance with previous indications of 'atypical' binding properties of this ligand. A further full agonist, S14671, which is chemically closely-related to S14506, also displayed a minimal GTPγS shift, underpinning this observation.

6 In conclusion, [^{3}H]-WAY100,635 constitutes a useful neutral antagonist radioligand for the characterization of drug actions at h5-HT_{1A} receptors. GTPγS-induced affinity changes of agonist and inverse agonist competition isotherms generally correlate well with ligand efficacy, with the notable exception of two chemically-similar agents, S14506 and S14671, which are efficacious agonists, yet relatively insensitive to h5-HT_{1A} receptor/G-protein coupling changes.
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Abbreviations: 5-HT, 5-hydroxytryptamine (serotonin); h5-HT_{1A}, recombinant human 5-hydroxytryptamine1A receptor; CHO, Chinese hamster ovary cells; GTPγS, Guanosine-5′-O-(3-thio)-triphosphate

Introduction

Previous studies of G-protein-coupled receptors have shown that agonists display differing affinities in competition binding experiments according to whether the radiolabelled ligand binds preferentially to G-protein-coupled or uncoupled conformations of the receptor (Freedman *et al*., 1988; Sundaram *et al*., 1993). Such differences in affinity may be related to the efficacy of agonists determined in functional tests (e.g. second messenger assays) (Freedman *et al*., 1988; Lahti *et al*., 1992; Egan *et al*., 2000). Comparisons of affinity differences *versus* ligand efficacy were recently undertaken at native, rat 5-HT_{1A} receptors (Assié *et al*., 1999), and at h5-HT_{1A} receptors (Watson *et al*., 2000), using the prototypical agonist [^{3}H]-8-OH-DPAT (Gozlan *et al*., 1983), and the antagonist [^{3}H]-p-MPPF (Kung *et al*., 1996). However, little is known concerning the behaviour of inverse agonists, an issue of particular interest since they should display reversed sensitivity (increased affinity) to guanine nucleotide-induced G-protein-uncoupling (Daeffler & Landry, 2000; Kenakin, 1997). Further, whereas agonists, such as [^{3}H]-8-OH-DPAT, and inverse agonists, such as [^{3}H]-spiperone, label only a fraction of the total receptor population, a selective 'neutral antagonist' would be desirable to address this question by labelling both G-protein-coupled and uncoupled forms of the 5-HT_{1A} receptor (Gozlan *et al*., 1995; Sundaram *et al*., 1993; 1995).

The selective 5-HT_{1A} receptor ligand, WAY100,635, has been extensively employed to characterize 5-HT_{1A} receptor function both *in vitro* and *in vivo*. It acts essentially as a 'neutral' antagonist (Fletcher *et al*., 1996; Newman-Tancredi *et al*., 1997; Routledge, 1996), although it may display inverse agonist properties in certain cell lines and/or buffer conditions (Cosi & Koek, 2000). [^{3}H]-WAY100,635 is of considerable interest as a radioligand for 5-HT_{1A} receptor labelling (Gozlan *et al*., 1995), but has not been commercially available until very recently, and only a single study of [^{3}H]-WAY100,635 binding to recombinant human 5-HT_{1A}

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receptors has been reported (Khawaja *et al*., 1997). Herein, we employed [^{3}H]-WAY100,635 to label h5-HT$_{1A}$ receptors expressed in Chinese hamster ovary (CHO) cells. The binding of [^{3}H]-WAY100,635 was first characterized with respect to its binding kinetics and its saturation binding, in comparison with [^{3}H]-8-OH-DPAT and the selective partial agonist, [^{3}H]-S15535 (Newman-Tancredi *et al*., 1998c). Secondly, we devoted particular attention to the competition binding profile of serotonergic ligands with [^{3}H]-WAY100,635. To determine the affinity of competing ligands for G-protein-coupled and uncoupled conformations of h5-HT$_{1A}$ receptors, affinity constants were determined in the presence or absence of the receptor/G-protein uncoupling agent, GTPγS. The ligands examined consisted of 15 serotonergic agents with diverse chemical structures and varying functional properties (De Vry, 1995). These included high-efficacy agonists, such as [^{3}H]-8-OH-DPAT, (±)flesinoxan and the exceptionally-potent ligands, S14506 and S14671 (Colpaert *et al*., 1992; Millan *et al*., 1992; Schreiber *et al*., 1994), partial agonists, such as buspirone and S15535, antagonists, such as WAY100,635 and (−)UH301 (Cornfield *et al*., 1991; Millan *et al*., 1997a,b; Routledge, 1996), and *inverse* agonists, such as spiperone, methiothepin and (+)butaclamol (Newman-Tancredi *et al*., 1997; 1998b; Stanton & Beer, 1997).

Methods

Drugs and radioligands

S15534 (4-(benzodioxan-5-yl)1-(indan-2-yl)piperazine), eltoprazine and WAY100,635 (N-{2[4-(2-methoxyphenyl)-1-piperazinyl] ethyl}-N-(2-pyridinyl)- cyclo-hexanecarboxamide) were synthesized by J.-L. Peglion, Servier. S14506 (1-[2-(4-fluoroben zoylam ino) ethyl]-4 -(7-metho xynaphtyl)-piperazine), S14671 (1-(7-Methoxynaphth-1-yl)-4-[2-(2-thenoylamino)-ethyl]piperazine) and (±)flesinoxan were synthesized by G. Lavielle, Servier. (+)Butaclamol, spiperone, (±)8-OH-DPAT (8-hydroxy-dipropyl-amino-tetralin), (−)pindolol, (+)UH301 ((+)5-fluoro-8-hydroxy-dipropyl-amino-tetralin), and (−)UH301 ((−)5-fluoro-8-hydroxy-dipropyl-amino-tetralin) were purchased from Research Biochemicals International (Natick, MA, USA). Buspirone and 5-HT were purchased from SIGMA (Saint Quentin Fallavier, France). Methiothepin maleate from Tocris Cookson (Southampton, U.K.). Compounds were dissolved at 10^{-3} M in de-ionised, distilled water or at 10^{-2} M in dimethylsulphoxide (DMSO) and diluted to appropriate concentrations in incubation buffer (see below). DMSO did not itself affect radioligand binding at the concentrations used. [^{3}H]-WAY100,635 (81 Ci/mmol) and [^{3}H]-8-OH-DPAT (225 Ci/mmol) were purchased from Amersham (Les Ulis, France). [^{3}H]-S15535 (43 Ci/mmol) was custom-synthesized by Amersham as described previously (Newman-Tancredi *et al*., 1998c).

[^{3}H]-WAY100,635, [^{3}H]-8-OH-DPAT and [^{3}H]-S15535 ligand binding

Membranes of CHO cells stably expressing recombinant human (h) 5-HT$_{1A}$ receptors were purchased from NEN (Paris, France). Membranes (10 μg protein) were incubated in triplicate with [^{3}H]-WAY100,635, [^{3}H]-8-OH-DPAT or [^{3}H]-S15535 at 22°C for 2.5 h in HEPES 20 mM, pH 7.5, and $MgSO_4$ 5 mM. For saturation binding experiments, radioligand concentrations used were between 0.01 and 10 nM. For competition binding and kinetics experiments, the concentration of [^{3}H]-WAY100,635 was 0.5 nM. Non-specific binding was defined using 5-HT (10 μM). For [^{3}H]-WAY100,635 dissociation binding experiments, reagents were preincubated for 1.5 h before addition of 5-HT (10 μM final concentration). 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).

Data analysis

Binding isotherms were analysed by non-linear regression using the program 'Prism' (GraphPad, S. Diego, CA, U.S.A.). The algorithms used were as described previously (Newman-Tancredi *et al*., 1998), yielding measures of K_{+1obs} (observed association rate constant), K_{-1} (dissociation rate constant), K_{+1} (corrected association rate constant) and t½ (half times of association/dissociation). The kinetic dissociation constant was calculated by $K_D = K_{-1} \div K_{+1}$. Saturation binding experiments yielded B_{max} (maximal binding capacity) and K_D (dissociation constant) values. For competition binding experiments, 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 binding site and a 2 binding sites model. The 'goodness of fit' of the two models was compared using the F-test. In the case of the 2-sites 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.

Results

[^{3}H]-WAY100,635 association/dissociation kinetics

At 22°C, [^{3}H]-WAY100,635 (0.5 nM) associated slowly to CHO-h5-HT$_{1A}$ membranes (t½ = 0.30 ± 0.01; K_{+1obs} = 2.32 ± 0.09), attaining equilibrium after 1½ h incubation. For subsequent saturation and competition binding experiments, an incubation time of 2½ h was adopted to ensure that all components of the reaction mixture (radioligand and competing ligands) had attained equilibrium. [^{3}H]-WAY100,635 also dissociated slowly from CHO-h5-HT$_{1A}$ membranes (t½ = 5.6 ± 0.3 h; K_{-1} = 0.124 ± 0.006). The corrected association constant (K_{+1}) was 4.39 h^{-1} nM^{-1}. From these kinetic data, the calculated dissociation constant (K_D) was 0.03 nM.

[^{3}H]-WAY100,635, [^{3}H]-S15535 and [^{3}H]-8-OH-DPAT saturation binding

[^{3}H]-WAY100,635 binding was saturable with a low non-specific component. Typically, total [^{3}H]-WAY100,635 (0.5 nM) binding amounted to 3000 d.p.m. with 400 d.p.m. non-specific. In saturation binding experiments, [^{3}H]-WAY100,635 exhibited high binding affinity at h5-HT$_{1A}$

receptors with a K_D value (0.056 ± 0.008 nM), lower than [^{3}H]-8-OH-DPAT (0.22 ± 0.03 nM) or [^{3}H]-S15535 (0.54 ± 0.09 nM). [^{3}H]-WAY100,635 also labelled significantly more binding sites (Figure 1): its B_{max} value (3650 ± 80 fmol mg^{-1}) exceeded that of the agonist, [^{3}H]-8-OH-DPAT (2020 ± 60 fmol mg^{-1}; $P<0.05$, 2-tailed t-test), and of the partial agonist, [^{3}H]-S15535 (2770 ± 290 fmol mg^{-1}; $P<0.05$, 2-tailed t-test).

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

The competition binding profile of [^{3}H]-WAY100,635 was consistent with that determined previously with [^{3}H]-8-OH-DPAT: pK_i values determined in previous studies with [^{3}H]-8-OH-DPAT (Newman-Tancredi *et al*., 1998b) correlated well with pK_H values determined with [^{3}H]-WAY100,635 ($r=0.97$, $P<0.0001$). pK_i values determined with [^{3}H]-8-OH-DPAT correlated poorly with pK_i values determined with [^{3}H]-WAY100,635 in the presence of GTPγS (100 μM) ($r=0.50$, $P<0.05$). [^{3}H]-WAY100,635 binding itself was unaffected by GTPγS (100 μM). Under the present conditions, binding in the absence of GTPγS (2870 ± 120 d.p.m.) was not significantly different from in its presence (2820 ± 96 d.p.m.; $P>0.10$, 2-tailed t-test). In competition binding experiments, agonists, such as 5-HT and 8-OH-DPAT, yielded biphasic competition isotherms. In the presence of GTPγS, the isotherms were right-shifted and became monophasic, with an increase in pseudo-Hill coefficients (Table 1, Figure 2). Partial agonists, including S15535, eltoprazine, (−)pindolol and (+)UH301, showed more modest 'GTPγS shifts'. (−)UH301 competition binding curves exhibited small, but significant right-shifts ($P<0.05$, 2-tailed t-test), whereas no change in WAY100,635 affinity was detected. The inverse agonists, methiothepin, spiperone and (+)butaclamol, exhibited less pronounced, but consistent, GTPγS left-ward shifts (pK_i values increased). When the values obtained in parallel experiments – with and without GTPγS – were compared, the left-ward shifts were statistically significant ($P<0.05$, 2-tailed matched pairs t-test; Figure 2).

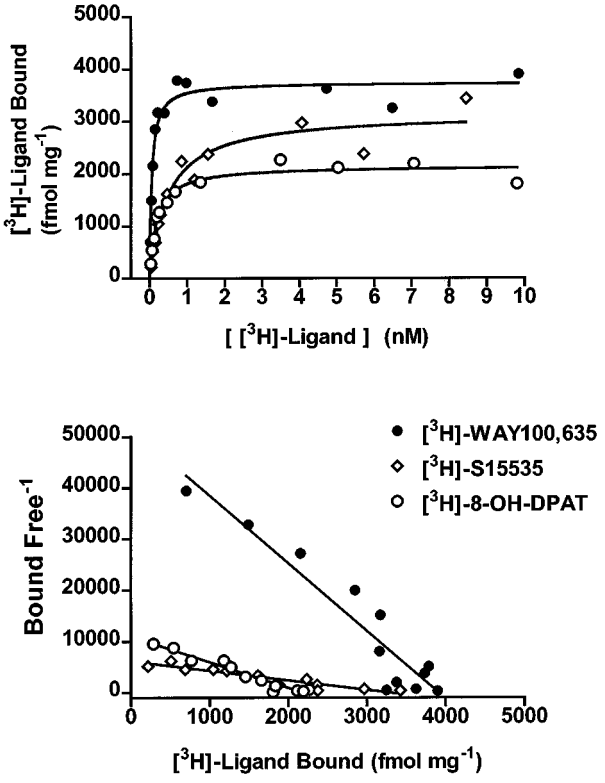

Figure 1 Saturation binding of [^{3}H]-WAY100,635, compared with [^{3}H]-S15535 and [^{3}H]-8-OH-DPAT, at h5-HT$_{1A}$ receptors expressed in CHO cells. (A) Saturation binding of the antagonist, [^{3}H]-WAY100,635, the partial agonist [^{3}H]-S15535, and the agonist, [^{3}H]-8-OH-DPAT, to h5-HT$_{1A}$ receptors. [^{3}H]-WAY100,635 labelled significantly more binding sites that the other radioligands. (B) Scatchard plots of data from (A). Note the steeper slope of [^{3}H]-WAY100,635, reflecting its higher affinity.

Correlation of 'GTPγS shifts' and ligand efficacy

The magnitude of the GTPγS shifts observed in [^{3}H]-WAY100,635 competition binding experiments was calculated for each ligand by subtracting the pK_i determined in the presence of GTPγS from the pK_H (or pK_i if single-site fit) determined in the absence of GTPγS. The resulting affinity changes are listed in Table 1. For 13 of the ligands tested (all except S14506 and S14671), both agonists and inverse agonists showed a high correlation ($r=0.98$; Figure 3) between affinity changes and efficacy of ligands for stimulation of [^{35}S]-GTPγS binding, as determined in previous studies (Newman-Tancredi *et al*., 1997; 1998a,b and unpublished observation). Thus, the full agonist, 5-HT, displayed the greatest affinity change (2.4 log units), partial agonists such as buspirone displayed more modest affinity changes (1.55 log units), and inverse agonists, such as spiperone, yielded negative affinity changes (−0.43 log units). In contrast to the other ligands, the agonists, S14506 and the chemically-similar S14671, behaved in an 'atypical' manner, displaying only modest affinity changes (0.63 and 0.33 log units, respectively), despite their efficacious agonist behaviour in [^{35}S]-GTPγS binding (E_{max} values $\geqslant 90\%$, Table 1; Newman-Tancredi *et al*., 1998b). These values were far removed from the affinity change *vs* efficacy correlation curve derived from the other ligands (Figure 3).

Discussion

The key findings of the present study are that [^{3}H]-WAY100,635 constitutes a useful tool for the characterization of recombinant human 5-HT$_{1A}$ receptors, in agreement with previous reports (Gozlan *et al*., 1995; Khawaja *et al*., 1997). In addition, [^{3}H]-WAY100,635, as a neutral antagonist, is uniquely useful for investigation of ligand efficacy by measuring affinity changes induced by alterations in G-protein-coupling. Further, two naphthylpiperazine agonists, S14506 and S14671, display distinctive binding properties at h5-HT$_{1A}$ receptors. Several features should be highlighted.

First, [^{3}H]-WAY100,635 exhibited significantly higher B_{max} values at h5-HT$_{1A}$ receptors than the other radioligands tested (Figure 1). Thus, [^{3}H]-WAY100,635 labelled about twice as many sites as the agonist, [^{3}H]-8-OH-DPAT (Khawaja *et al*., 1997), which preferentially labels G-protein-coupled receptors (Gozlan *et al*., 1995; Newman-

Table 1 [^{3}H]-WAY100,635 competition binding at h5-HT$_{1A}$ receptors in the presence and absence of GTPγS

	No GTPγS				*With GTPγS (100 μM)*		*Affinity change*	*[^{35}S]-GTPγS*	*[^{3}H]-8-OH-DPAT*[a]
	pK_i/pK_h	*pK_L*	*% High*	*nH*	*pK_i*	*nH*		*E_{max} (%)*	*pK_i*
5-HT	9.84±0.04	7.77±0.07	64±3	0.48±0.02	7.44±0.09	1.05±0.09	2.40	100[a]	9.21
(±)8-OH-DPAT	9.91±0.07	7.98±0.07	67±2	0.67±0.04	7.78±0.05	1.11±0.05	2.13	76[a]	9.24
Eltoprazine	8.74±0.03	7.07±0.15	51±9	0.66±0.08	6.75±0.02	1.18±0.08	1.99	70[b]	8.19
(±)Flesinoxan	9.23±0.14	7.63±0.12	55±4	0.75±0.03	7.39±0.14	1.02±0.19	1.84	75[b]	9.26
S15535	9.66±0.12	8.22±0.13	52±5	0.64±0.02	8.01±0.10	1.35±0.15	1.65	44[b]	9.10
(+)UH301	8.92±0.10	7.19±0.18	45±12	0.72±0.08	7.29±0.03	1.23±0.14	1.63	41[a]	8.56
Buspirone	8.71±0.17	7.07±0.21	60±10	0.76±0.02	7.16±0.16	1.22±0.02	1.55	65[a]	8.05
(−)Pindolol	8.36±0.07			0.95±0.03	7.50±0.06	1.07±0.07	0.86	20[a]	8.19
S14506	10.09±0.22			0.93±0.06	9.46±0.04	1.03±0.08	0.63	90[a]	9.66
S14671	10.27±0.03			1.05±0.02	9.94±0.08	1.04±0.11	0.33	98[a]	10.52
(−)UH301	8.19±0.05			1.18±0.16	8.01±0.03	1.48±0.02	0.18	0[a]	7.87
WAY100,635	9.91±0.04			0.97±0.07	9.92±0.04	0.99±0.03	−0.01	0[a]	9.25
Methiothepin	8.63±0.13			1.22±0.04	8.77±0.10	1.69±0.26	−0.14	−21[a]	8.08
(+)Butaclamol	7.23±0.09			1.19±0.04	7.51±0.03	1.27±0.01	−0.28	−18[a]	6.40
Spiperone	7.82±0.12			0.88±0.03	8.25±0.08	1.30±0.04	−0.43	−28[a]	7.00

[^{3}H]-WAY100,635 (0.5 nM) was competed with serotonergic ligands for binding to h5-HT$_{1A}$ receptors. One-site and two-site fits were compared by F-test. In the presence of GTPγS, all binding isotherms were monophasic. The affinity change was calculated by subtracting the pK_i value determined in the presence of GTPγS by the pK_i/pK_H value determined in its absence. The affinity change values correlated with efficacy values determined by [^{35}S]-GTPγS binding in previous studies ($r=0.98$). nH = Hill coefficient; % High = percentage of high-affinity sites. Data are means±s.e.m. of three or more determinations performed in triplicate. [a]Data from Newman-Tancredi *et al.* 1997; 1998a,b. [b]Unpublished results.

Tancredi *et al.*, 1998c; Sundaram *et al.*, 1995). These data are consistent with studies indicating that the total number of h5-HT$_{1A}$ receptor sites is equivalent to the sum of sites detected with [^{3}H]-8-OH-DPAT and the inverse agonist, [^{3}H]-spiperone (Sundaram *et al.*, 1993; 1995; Watson *et al.*, 2000). Herein, [^{3}H]-S15535 labelled more sites than [^{3}H]-8-OH-DPAT but fewer than [^{3}H]-WAY100,635, reflecting its partial agonist activity at 5-HT$_{1A}$ receptors (Newman-Tancredi *et al.*, 1996; 1998c). In contrast, [^{3}H]-WAY100,635 exhibits neutral antagonist properties at h5-HT$_{1A}$ receptors (Newman-Tancredi *et al.*, 1997), and labels both G-protein-coupled and uncoupled rat h5-HT$_{1A}$ receptors (Gozlan *et al.*, 1995).

Second, the binding kinetics of [^{3}H]-WAY100,635 were slow: 1½ h incubation were necessary to achieve equilibrium at room temperature (22°C), and the dissociation half-life was also long (>5 h). These data indicate that care should be taken to use extended incubation times to allow both [^{3}H]-WAY100,635 and any competing ligands to reach equilibrium conditions (Hulme & Birdsall, 1992). Indeed, this may, at least partly, account for differences between binding affinities in the present study and those of Khawaja *et al.* (1997) who have published the only other study of [^{3}H]-WAY100,635 binding at recombinant h5-HT$_{1A}$ receptors. In their study, using short incubations (~3 times the association half life *vs* >8 in the present study), [^{3}H]-WAY100,635 exhibited 6 fold lower affinity in saturation binding experiments. Further, in competition binding experiments, affinities were ~20–30 fold lower than in the present study (Table 1).

Third, the present study employing [^{3}H]-WAY100,635 demonstrated that correlation between binding affinity changes (induced by modulation of G-protein-coupling) and ligand efficacy (Assié *et al.*, 1999; Freedman *et al.*, 1988; Lahti *et al.*, 1992; Watson *et al.*, 2000) holds true for most ligands at h5-HT$_{1A}$ receptors, whether agonists or inverse agonists. Indeed, competition with agonists, such as 5-HT, 8-OH-DPAT and (+)flesinoxan, yielded biphasic isotherms, consistent with preferential binding to a G-protein-coupled conformation of 5-HT$_{1A}$ receptors. GTPγS, which inhibits receptor-G-protein coupling (Birnbaumer & Birnbaumer, 1995; Daeffler & Landry, 2000), eliminated the high affinity binding components of 5-HT and 8-OH-DPAT leaving only a single, lower affinity binding site, consistent with binding to G-protein-uncoupled h5-HT$_{1A}$ receptors (Figure 2). Buspirone, S15535 and (−)pindolol, exhibited smaller 'GTPγS shifts', consistent with their partial agonist properties (Newman-Tancredi *et al.*, 1996; 1998a,b; Boess & Martin, 1994; De Vry, 1995). Interestingly, (+)UH301 displayed a modest GTPγS shift, as expected from this partial agonist (Cornfield *et al.*, 1991), but its isomer, (−)UH301, also exhibited a small and significant GTPγS shift ($P<0.05$, 2-tailed *t*-test; Table 1), suggesting that it may not be totally devoid of residual agonist properties (Arborelius *et al.*, 1994; Cornfield *et al.*, 1991). It should be noted that unlabelled WAY100,635 was the only ligand which exhibited no sensitivity to GTPγS at all. Indeed, [^{3}H]-WAY100,635 binding was unchanged by GTPγS, consistent with 'neutral' antagonist properties (Fletcher *et al.*, 1996; Newman-Tancredi *et al.*, 1997). In comparison, the competition isotherms of methiothepin, (+)butaclamol and spiperone, were modestly, but significantly displaced to the left by GTPγS (Figure 2; Table 1; $P<0.05$; 2-tailed paired *t*-test), consistent with their inverse agonist properties in [^{35}S]-GTPγS binding studies at h5-HT$_{1A}$ receptors (Daeffler & Landry, 2000; McLoughlin & Strange, 2000; Newman-Tancredi *et al.*, 1997; 1998b; Stanton & Beer, 1997; Watson *et al.*, 2000).

Fourth, the affinity changes induced by GTPγS for most of the ligands tested were quantitatively, and not just qualitatively, related to their efficacy at h5-HT$_{1A}$ receptors. Indeed, the affinity change induced by G-protein uncoupling for 13 of the ligands tested (pK_H−pK_i: positive for agonists, negative for inverse agonists; Table 1), was highly correlated with their efficacy, determined by stimulation or inhibition of [^{35}S]-GTPγS binding (Figure 3; Table 1; Newman-Tancredi *et al.*, 1997; 1998b). This is in agreement with the ternary complex

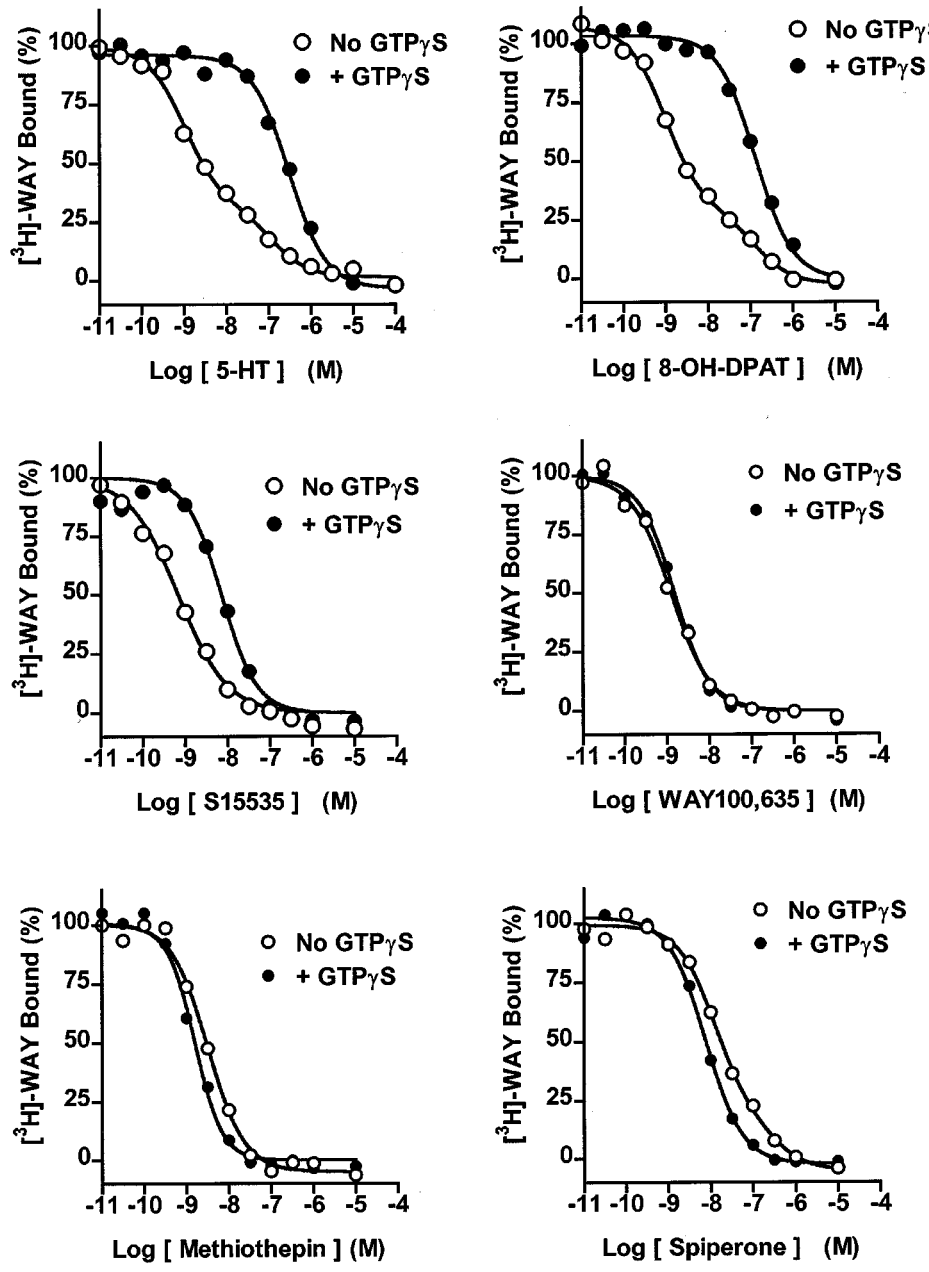

Figure 2 Competition binding profile of [^{3}H]-WAY100,635 at h5-HT$_{1A}$ receptors: influence of GTPγS. [^{3}H]-WAY100,635 (denoted '[^{3}H]-WAY') was competed with the serotonergic ligands indicated, in the presence or absence of the receptor/G-protein uncoupling agent, GTPγS (100 mM). Note that whereas 5-HT shifted to the right in the presence of GTPγS, the inverse agonists, methiothepin and spiperone, shifted to the left.

model of G-protein activation by agonists which predicts that the efficacy of ligands for activation of coupled G-proteins is related to their affinity constants for active (R*) or inactive (R) receptor conformations (Daeffler & Landry, 2000; Kenakin, 1997). Agonists at other G-protein-coupled receptors have, classically, been found to maintain this relationship between efficacy and binding affinity for G-protein-coupled and uncoupled receptor conformations (Freedman *et al.*, 1988; Lahti *et al.*, 1992). In the present study, however, this relationship did not apply to all the ligands tested: S14506 exhibited only modest GTPγS shifts, despite acting as efficacious agonists in *in vitro* and *in vivo* functional models (Colpaert *et al.*, 1992; Newman-Tancredi *et al.*, 1998a; Schreiber *et al.*, 1994). [^{3}H]-S14506 also exhibits 'atypical' binding properties at rat 5-HT$_{1A}$ receptors, being insensitive to G-protein-uncoupling induced by guanine nucleotides and the alkylating agent, N-ethyl-maleimide (NEM; Lima *et al.*, 1997), properties usually associated with antagonists. Further, a recent study found that S14506 displayed little difference in its affinity for rat 5-HT$_{1A}$ receptors labelled by [^{3}H]-8-OH-DPAT *versus* the antagonist [^{3}H]-*p*-MPPF (Assié *et al.*, 1999). Thus, S14506 displays distinctive binding

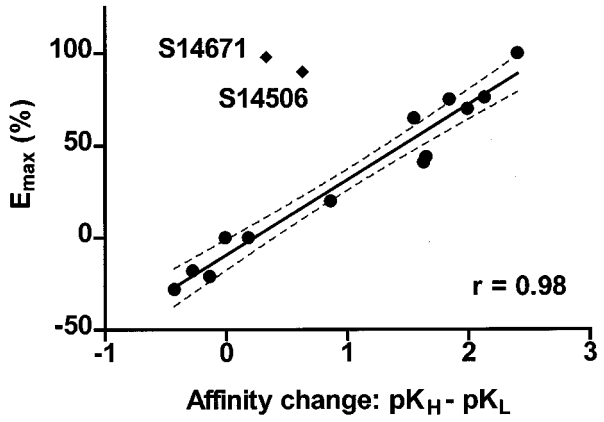

Figure 3 Relationship between affinity changes induced by G-protein uncoupling and ligand efficacy determined by [^{35}S]-GTPγS binding. Correlation of $pK_H - pK_i$ values from competition binding experiments and ligand efficacy determined by stimulation/inhibition of [^{35}S]-GTPγS binding (see Table 1). Dotted lines indicate 95% confidence limits.

properties at 5-HT$_{1A}$ receptors. It is striking that the only other ligand in the present study which displayed little sensitivity to G-protein uncoupling, whilst behaving as an extremely potent and efficacious agonist in functional tests, S14671 (Millan *et al*., 1992), is chemically similar to S14506. This strongly suggests, therefore, that the capacity to activate h5-HT$_{1A}$ receptors in the absence of marked affinity changes is related to the chemical structures of these ligands. A recent modelling study of the binding of S14506 to h5-HT$_{1A}$ receptors (Milligan *et al*., 2000) supports this argument in demonstrating that S14506 may display unusual interaction with h5-HT$_{1A}$ receptors likely involving binding to the 5-HT-site and 'DRY' arginine-switch signalling microdomain (Milligan *et al*., 2000; Jacoby *et al*., 1999). Such interactions may enable S14506 to activate receptor-coupled G-proteins without marked alterations in affinity in response to coupling changes.

Conclusions

The present study shows that [^{3}H]-WAY100,635 constitutes a useful radioligand for the investigation of both agonist and inverse agonist binding at h5-HT$_{1A}$ receptors. [^{3}H]-WAY100,635 binding was saturable and of high affinity without detectable sensitivity to GTPγS, consistent with 'neutral' antagonist properties. Importantly, competition binding assays revealed that most ligands display G-protein uncoupling-induced changes in binding affinity at h5-HT$_{1A}$ receptors that are related to their relative efficacy (positive and negative) determined employing alternative strategies ([^{35}S]-GTPγS binding). However, some ligands (S14506 and S14671) displayed 'atypical' properties suggesting that they are capable of efficacious G-protein activation in the absence of marked affinity change associated with G-protein (un)coupling.

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