British Journal of Pharmacology (1997) 120, 737–739

SPECIAL REPORT

Inhibition of the constitutive activity of human 5-HT_{1A} receptors by the inverse agonist, spiperone but not the neutral antagonist, WAY 100,635

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At recombinant human 5-hydroxytryptamine (5-HT)5-HT_{1A} receptors expressed in Chinese hamster ovary cells (CHO-5-HT_{1A}), 5-carboxamidotryptamine (5-CT), acted as a full agonist (relative to 5-HT = 100%) for stimulation of receptor-mediated [^{35}S]-GTPγS (guanylyl 5′-[γ-thio]-tryphosphate) binding. In contrast, spiperone inhibited basal [^{35}S]-GTPγS binding by 30.2% (IC_{50} = 55.5 nM) in CHO-5-HT_{1A} membranes but not in control untransfected membranes. The antagonist, N-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-N-(2-pyridinyl)-cyclohexane-carboxamide (WAY 100,635), blocked both 5-CT-induced stimulation and spiperone-induced inhibition of [^{35}S]-GTPγS binding without itself modifying [^{35}S]-GTPγS binding. It is concluded that, in this heterologous expression system, 5-HT_{1A} receptors display 'constitutive' activation of G-proteins and that spiperone displays inverse agonist activity whereas WAY 100,635 acts as a 'neutral' antagonist at this site.

Keywords: 5-HT_{1A} receptors; constitutive activity; inverse agonism; [^{35}S]-GTPγS binding; G-proteins; WAY 100,635

Introduction The study of G-protein-coupled receptors in heterologous expression systems has revealed that some ligands, referred to as 'inverse agonists', inhibit the basal activity observed in second messenger or G-protein activation assays (Barker *et al*., 1994; Thomas *et al*., 1995). On the assumption that endogenous agonist(s) are not present at the receptor, this action may be attributed to inhibition of endogenous or 'constitutive' coupling/activation of the receptors themselves. This action has not, as yet, been investigated for the 5-HT_{1A} receptor, which is important as an autoreceptor in the control of 5-hydroxytryptaminergic transmission. The present study therefore addressed this issue by use of a Chinese hamster ovary cell line stably expressing human cloned 5-HT_{1A} receptors (CHO-5-HT_{1A}, Newman-Tancredi *et al*., 1992). We examined the modulation of G-protein activation (in [^{35}S]-GTPγS (guanylyl 5′-[γ-thio]-tryphosphate) binding assays by 5-carboxamidotryptamine (5-CT), a prototypical 5-HT_1 receptor agonist, and spiperone, a classical 5-HT_{1A} receptor 'antagonist'. In addition, we tested the novel, 5-HT_{1A}-selective, 'silent' phenylpiperazine, N-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-N-(2-pyridinyl)-cyclohexane-carboxamide (WAY 100,635; Fletcher *et al*., 1996; Newman-Tancredi *et al*., 1996).

Methods CHO-5-HT_{1A} cells expressing approximately 1.6 pmol of human recombinant 5-HT_{1A} receptor mg^{-1} protein (Newman-Tancredi *et al*., 1992) were washed by resuspending in ice-cold Earle's Buffered Saline Solution before centrifugation (450 *g*, 10 min, 4°C). Cells were resuspended in buffer A (HEPES (pH 7.4) 20 mM; $MgSo_4$ 3 mM) and membranes were prepared by Polytron homogenization followed by 2 centrifugations at 48,000 *g* for 25 min at 4°C. Membranes were resuspended in buffer A and stored at −80°C. Efficacy was determined by measuring [^{35}S]-GTPγS binding as described previously (Newman-Tancredi *et al*., 1996). Briefly, ligands and CHO-5-HT_{1A} membranes (30 μg protein) were incubated (20 min, 37°C) in buffer B (HEPES 20 mM, ph 7.4, GDP 3 μM, $MgSO_4$ 3 mM, [^{35}S]-GTPγS 0.2 nM). Final volume was 0.5 ml. For antagonist tests, the reagents were preincubated for 30 min at 22°C before addition of [^{35}S]-GTPγS. Incubations were terminated by rapid filtration. Binding isotherms were analysed by non-linear regression and results are expressed as arithmetic means ± s.e.mean of *n* (number in parentheses) determinations. pK_b values were calculated according to Lazareno & Birdsall (1993).

Results 5-HT increased [^{35}S]-GTPγS binding 2.1 fold from basal levels of 104 ± 10 (4) fmol mg^{-1} to 219 ± 17 (4) fmol mg^{-1}. Whereas 5-CT concentration-dependently increased [^{35}S]-GTPγS binding to CHO-5-HT_{1A} cell membranes, spiperone inhibited it by about 30% (Table 1). The 5-CT and spiperone binding isotherms shifted to the right in the presence of WAY 100,635 (10 nM) without altering their maximal effects (Table 1; Figure 1a). WAY 100,635 did not, by itself, alter basal binding (*n* = 3) but concentration-dependently reversed 5-CT (100 nM)-induced stimulation of [^{35}S]-GTPγS

Table 1 Antagonism by WAY 100,635 of 5-CT-induced stimulation and spiperone-induced inhibition of [^{35}S]-GTPγS binding to CHO-5-HT_{1A} cell membranes

	pEC_{50} or pIC_{50}	*E_{max} (%)*	n
5-CT	8.79 ± 0.07	100.5 ± 4.6	3
5-CT + WAY 100,635	6.99 ± 0.03	104.3 ± 5.4	3
Spiperone	7.30 ± 0.13	−30.2 ± 4.4	4
Spiperone + WAY 100,635	6.18 ± 0.06	−32.4 ± 1.6	3

[^{35}S]-GTPγS binding was carried out in membranes of CHO cells stably expressing the human 5-HT_{1A} receptor. pEC_{50} values for 5-CT and pIC_{50} values for spiperone are shown in the absence or presence of 10 nM WAY 100,635. pK_b values for WAY 100,635 calculated from these data were 9.77 and 9.02 respectively. For E_{max} values, 0% is defined as basal [^{35}S]-GTPγS binding determined in the absence of ligand(s), and 100% is defined as [^{35}S]-GTPγS binding determined in the presence of a maximally effective concentration (10 μM) of 5-HT. Results are presented as means ± s.e.mean.

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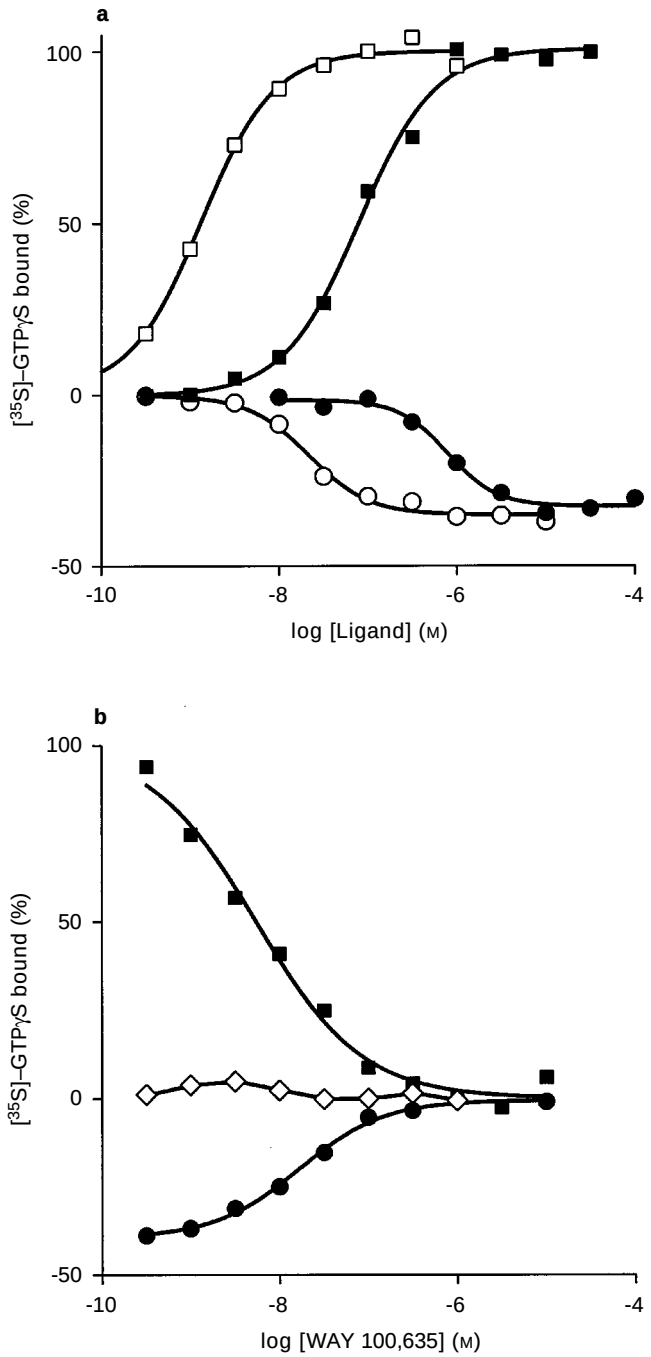

Figure 1 (a) Modulation of [^{35}S]-GTPγS binding to CHO-5-HT$_{1A}$ membranes by 5-CT alone (□) and in the presence of 10 nM WAY 100,635 (■); and by spiperone alone (○) and in the presence of 10 nM WAY 100,635 (●). (b) Effect of WAY 100,635 alone (◇) and antagonism by WAY 100,635 of the modulation of [^{35}S]-GTPγS binding to CHO-5-HT$_{1A}$ membranes induced by 100 nM 5-CT (■) and 1000 nM spiperone (●). Points are means of triplicate determinations from representative experiments. Similar results were obtained on three or more occasions; 0% is defined as basal [^{35}S]-GTPγS binding determined in the absence of ligand(s) and 100% is defined as [^{35}S]-GTPγS binding induced by 10 μM 5-HT.

binding (pIC$_{50}$ = 8.29 ± 0.12, n = 3; pK_b = 10.05). WAY 100,635 also reversed spiperone (1000 nM)-induced inhibition of [^{35}S]-GTPγS binding (pEC$_{50}$ = 8.11 ± 0.12, n = 5; pK_b = 9.32; Figure 1b). In control experiments, neither spiperone, 5-CT nor WAY 100,635 altered [^{35}S]-GTPγS binding to membranes of control, untransfected CHO cells (n = 3), which was significantly lower (29 ± 7 fmol mg^{-1} (3); P < 0.01, Student's 2-tailed t test) than basal binding to CHO-5-HT$_{1A}$ membranes.

Discussion In the present study the modulation of G-protein activation at human recombinant 5-HT$_{1A}$ receptors by [^{35}S]-GTPγS binding was characterized (Newman-Tancredi *et al*., 1996). Whereas 5-CT acted as a potent and 'full' agonist at 5-HT$_{1A}$ receptors, relative to 5-HT, spiperone inhibited basal [^{35}S]-GTPγS binding. The suggestion that spiperone may be antagonizing the action of residual agonist(s) can essentially be discounted. Firstly, the cell membranes were extensively washed (see Methods). Indeed, in control experiments in which they underwent three additional washes, similar results were obtained (unpublished results). Secondly, inhibition of basal activity was not observed in untransfected CHO cell membranes, showing that this effect is specifically mediated by 5-HT$_{1A}$ receptors. Thirdly, if basal activity in CHO-5-HT$_{1A}$ membranes was due to agonist activation of 5-HT$_{1A}$ receptors, the antagonist, WAY 100,635, would also block it, which it does not. Instead, WAY 100,635 induced no change in [^{35}S]-GTPγS binding, when tested alone, but shifted the activation/inhibition isotherms of 5-CT and spiperone to the right in a parallel manner, suggesting that WAY 100,635 competitively antagonizes both agonist and inverse agonist activity (Figure 1a). The pK_bs calculated for WAY 100,635 were slightly higher for antagonism of 5-CT (9.77 and 10.05; see Results and legend to Table 1) than for antagonism of spiperone (9.02 and 9.32 nM). Whilst these values are in the same range as the pK_i for WAY 100,635 at 5-HT$_{1A}$ receptors (9.25, Newman-Tancredi *et al*., 1996) it is possible that 5-CT and spiperone may stimulate/inhibit activation of different populations of intracellular G-proteins. Nevertheless, taken together, these data suggest that 5-HT$_{1A}$ receptors constitutively activate G-proteins in CHO cells, an interpretation supported by the 3.6 fold increase in basal [^{35}S]-GTPγS binding between CHO-5-HT$_{1A}$ membranes and control CHO membranes. The negative efficacy of spiperone shows that it has inverse agonist activity at CHO-5-HT$_{1A}$ receptors, which is probably due to an induction or stabilization of an inactive conformation of 5-HT$_{1A}$ receptors. This agrees with the observation that [^{3}H]-spiperone binds preferentially to G-protein-uncoupled 5-HT$_{1A}$ receptors with an affinity (pK_i = 7.76; Sundaram *et al*., 1993) similar to its potency (pIC$_{50}$ = 7.30, Table 1) for inhibition of [^{35}S]-GTPγS binding. Indeed, spiperone also exhibits inverse agonist properties at 5-HT$_{2A}$ and dopamine D$_2$ receptors (De Lean *et al*., 1982; Barker *et al*., 1994). In contrast, the present data confirm previous findings that WAY 100,635, which is highly selective for 5-HT$_{1A}$ receptors, exhibits no intrinsic agonist activity (Fletcher *et al*., 1996; Newman-Tancredi *et al*., 1996) and, in addition, demonstrate that WAY 100,635 is also devoid of inverse agonist activity at recombinant 5-HT$_{1A}$ receptors.

In conclusion, the present data demonstrate the constitutive activity of recombinant human 5-HT$_{1A}$ receptors and show that whilst spiperone is an inverse agonist at these sites, WAY 100,635 acts as a neutral antagonist, blocking both agonist and inverse agonist activity.

References

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.

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.

FLETCHER, A., FORSTER, E.A., BILL, D.J., BROWN, G., CLIFFE, I.A., HARTLEY, J.E., JONES, D.E., MCLENACHAN, A., STANHOPE, K., CRITCHLEY, D.J.P., CHILDS, K.J., MIDDLEFELL, V.C., LANFUMEY, L., CORRADETTI, R., LAPORTE, A.-M., GOZLAN, H., HAMON, M. & DOURISH, C.T. (1996). Electrophysiological, biochemical, neurohormonal and behavioural studies with WAY 100,635, a potent, selective and silent 5-HT$_{1A}$ receptor antagonist. *Behav. Brain Res.* **73,** 337–353.

LAZARENO, S. & BIRDSALL, N.J.M. (1993). Estimation of competitive antagonist affinity from functional inhibition curves using the Gaddum, Schild and Cheng-Prusoff equations. *Br. J. Pharmacol.*, **109,** 1110–1119.

NEWMAN-TANCREDI, A., CHAPUT, C., VERRIELE, L. & MILLAN, M.J. (1996). 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., 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.

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. Receptor Signal Transduct. Res.*, **15,** 199–211.

(Received October 11, 1996
Accepted December 12, 1996)