ELSEVIER

European Journal of Pharmacology 307 (1996) 107–111

ejp

Short communication

S 15535 and WAY 100,635 antagonise 5-HT-stimulated [^{35}S]GTPγS binding at cloned human 5-HT$_{1A}$ receptors

Adrian Newman-Tancredi *, Christine Chaput, Laurence Verrièle, Mark J. Millan

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

Received 11 March 1996; accepted 12 March 1996

Abstract

In Chinese hamster ovary (CHO) cells expressing cloned human 5-HT$_{1A}$ receptors, S 15535 (4-(benzodioxan-5-yl)1-(indan-2-yl)piperazine) exhibited high affinity (K_i = 0.79 nM), similar to that of 5-HT (0.61 nM), (±)-8-hydroxy-2-(di-*n*-propylamino)tetralin ((±)-8-OH-DPAT; 0.58 nM) and *N*-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl)cyclo-hexanecarboxamide (WAY 100,635; 0.56 nM). In these cells, 5-HT stimulated [^{35}S]GTPγS binding 3-fold (EC_{50} = 15 nM) whereas (±)-8-OH-DPAT exhibited 73% efficacy relative to 5-HT (EC_{50} = 6.0 nM). WAY 100,635 completely blocked 5-HT- and (±)-8-OH-DPAT-stimulated [^{35}S]GTPγS binding. Likewise, S 15535 antagonised 5-HT-stimulated [^{35}S]GTPγS binding, reducing it to 30.1% of control values. S 15535 (100 nM) also shifted the 5-HT and (±)-8-OH-DPAT stimulation curves to the right, to EC_{50} values of 870 and 313 nM, respectively. However, unlike WAY 100,635, which by itself did not stimulate [^{35}S]GTPγS binding, S 15535 alone increased it by 34.7% relative to 5-HT (EC_{50} = 5.8 nM). In conclusion, S 15535 antagonises the stimulation of 5-HT$_{1A}$ receptors by 5-HT, whilst itself exerting weak partial agonist activity at these sites.

Keywords: S 15535; WAY 100,635; (±)-8-OH-DPAT ((±)-hydroxy-2-(di-*n*-propylamino)tetralin); 5-HT$_{1A}$; [^{35}S]GTPγS binding

1. Introduction

S 15535 (4-(benzodioxan-5-yl)1-(indan-2-yl)piperazine) is a highly selective and potent ligand at rat hippocampal 5-HT$_{1A}$ sites (K_i = 1.8 nM versus > 200 nM at other 5-HT receptors; Millan et al., 1994b). In vivo, S 15535 shows agonist and antagonist properties at pre- and post-synaptic 5-HT$_{1A}$ receptors, respectively. Hence, in electrophysiological tests, S 15535 behaves as an agonist in completely inhibiting the firing of raphe-localised serotonergic neurons. In contrast, in animal tests which reflect post-synaptic receptor activation, S 15535 antagonises 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT)-induced hypothermia, spontaneous tail-flicks and flat-body posture (Millan et al., 1994b). The mixed 'agonist/antagonist' behaviour of S 15535 raises the question of the degree of its intrinsic activity at 5-HT$_{1A}$ receptors. This issue, which has not been previously addressed in in vitro models of receptor activation, was studied by use of a [^{35}S]GTPγS binding protocol. [^{35}S]GTPγS, a non-hydrolysable analogue of GTP, binds to agonist-activated G-proteins, providing a measure of agonist efficacy at diverse receptor types, including muscarinic m_1–m_4, dopamine D_4, 5-HT$_{1D}$ and 5-HT$_{1A}$ (Lazareno et al., 1993; Chabert et al., 1994; Thomas et al., 1995; Newman-Tancredi et al., 1996). The present study examined the ability of S 15535 to both stimulate basal [^{35}S]GTPγS binding and antagonise the stimulation induced by 5-HT and the 5-HT$_{1A}$-selective agonist (±)-8-OH-DPAT. The behaviour of S 15535 in these tests was compared to that of the novel and selective 5-HT$_{1A}$ 'silent' antagonist *N*-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl)cyclo-hexanecarboxamide (WAY 100,635), which has antagonist properties at both pre- and post-synaptic 5-HT$_{1A}$ receptors (Fletcher et al., 1994; Forster et al., 1995).

2. Materials and methods

[^{35}S]GTPγS (1300 Ci/mmol) was obtained from NEN and [^{3}H](±)-8-OH-DPAT (225 Ci/mmol) from Amersham. S 15535 and WAY 100,635 were synthesised by Servier chemists. 5-HT was purchased from Sigma and (±)-8-OH-DPAT from RBI.

* Corresponding author. Tel.: +33 1 41.18.24.46; fax: +33 1 41.18.24.70.

0014-2999/96/$15.00 Published by Elsevier Science B.V.
PII S0014-2999(96)00303-2

Membranes were prepared from recombinant Chinese hamster ovary (CHO) cells stably expressing the human 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. Membranes (10–20 μg protein) were incubated with [^{3}H](±)-8-OH-DPAT at 22°C for 2.5 h. Efficacy was determined by measuring agonist stimulation of [^{35}S]GTPγS binding, as described previously (Newman-Tancredi et al., 1996). CHO-5-HT_{1A} membranes (50 μg protein) were incubated (20 min, 22°C) in triplicate in a buffer containing Hepes 20 mM (pH 7.4), GDP 3 μM, $MgSO_4$ 3 mM, [^{35}S]GTPγS (1300 Ci/mmol, NEN) 0.1 nM. 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. In antagonist tests, the antagonist was pre-incubated with cell membranes (30 min, 22°C) prior to addition of the [^{35}S]GTPγS. Experiments were terminated by rapid filtration through Whatman GF/B filters using a Brandel cell harvester. Radioactivity retained on the filters was determined by liquid scintillation counting and binding isotherms were analysed by non-linear regression using the program PRISM (Graphpad). K_b values were calculated by the equation: $K_b = [\text{antagonist}]/\{(EC'_{50}/EC_{50}) - 1\}$; where [antagonist] is the concentration of antagonist, EC'_{50} is the effective concentration(50) in the presence of antagonist and EC_{50} is the effective concentration(50) in the absence of antagonist. Results are expressed as the mean ± S.E.M. of (*n*) independent determinations.

3. Results

[^{3}H](±)-8-OH-DPAT competition binding experiments yielded the following K_i values: S 15535: 0.79 ± 0.10 nM (4); 5-HT: 0.61 ± 0.15 nM (3); (±)-8-OH-DPAT: 0.58 ± 0.03 nM (3) and WAY 100,635: 0.56 ± 0.09 nM (3).

In efficacy measurements, 5-HT concentration dependently increased specific [^{35}S]GTPγS binding from basal levels of 4170 ± 1060 dpm (3) to a maximum of 12 620 ± 2030 dpm (3). (±)-8-OH-DPAT increased [^{35}S]GTPγS binding with an efficacy of 72.6 ± 3.6% (3) relative to 5-HT (Table 1) whereas WAY 100,635 did not stimulate [^{35}S]GTPγS binding but instead showed a tendency to

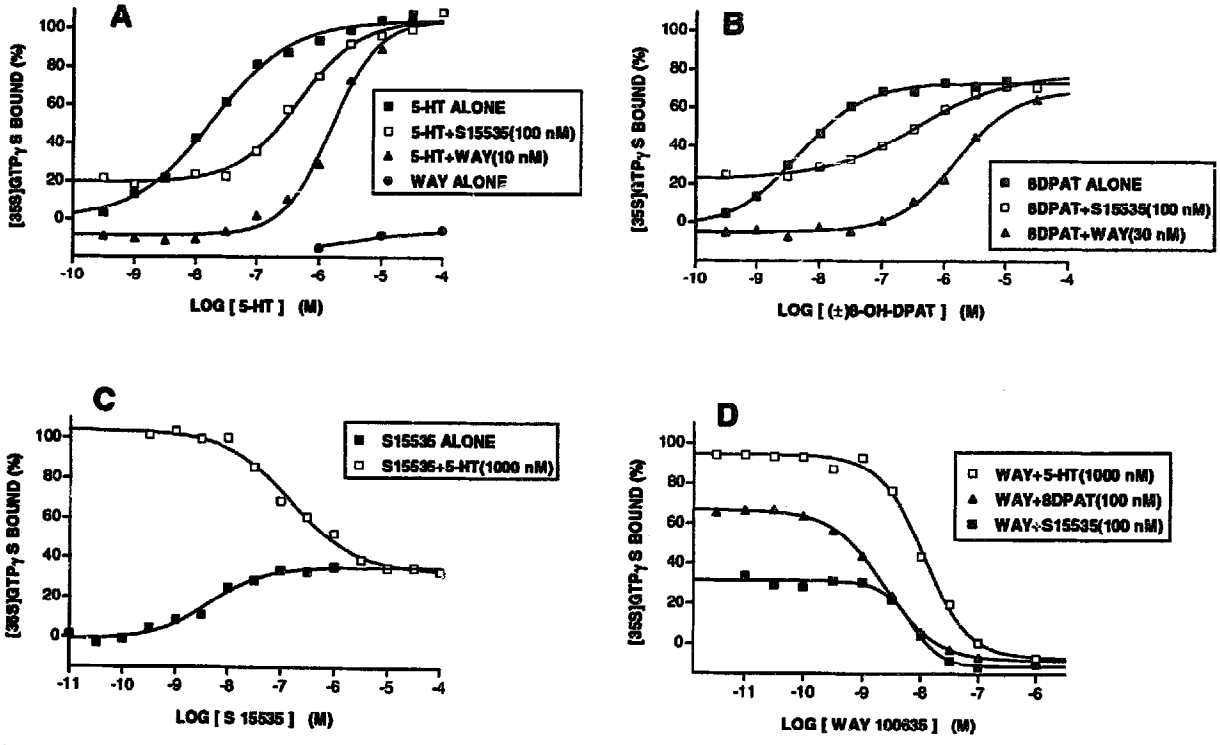

Fig. 1. Agonism and antagonism at cloned human 5-HT_{1A} receptors defined by [^{35}S]GTPγS binding. Membranes of CHO cells stably expressing the human 5-HT_{1A} receptor were incubated with different ligands in a buffer containing Hepes (20 mM, pH 7.4), GDP (3 μM), $MgSO_4$ (3 mM) and [^{35}S]GTPγS (0.1 nM) for 20 min at 22°C. Non-specific binding was defined with 10 μM GTPγS. Experiments were terminated by rapid filtration and binding isotherms were analysed by non-linear regression. Points shown are means of triplicate determinations from representative experiments. [^{35}S]GTPγS binding is expressed as a percentage of the maximal stimulation given by 5-HT. Abbreviations: WAY, WAY 100,635; 8DPAT, (±)-8-OH-DPAT. A: Stimulation of [^{35}S]GTPγS binding by 5-HT alone, by WAY 100,635 alone, or by 5-HT in the presence of S 15535 (100 nM) or WAY 100,635 (10 nM). B: Stimulation of [^{35}S]GTPγS binding by (±)-8-OH-DPAT alone or in the presence of S 15535 (100 nM) or WAY 100,635 (30 nM). C: Stimulation of [^{35}S]GTPγS binding by S 15535 alone and inhibition by S 15535 of [^{35}S]GTPγS binding induced by 5-HT (1000 nM). D: Inhibition by WAY 100,635 of [^{35}S]GTPγS binding induced by 5-HT (1000 nM), (±)-8-OH-DPAT (100 nM) or S 15535 (100 nM).

Table 1
Agonism by 5-HT and (±)-8-OH-DPAT and antagonism by S 15535 and WAY 100,635 at cloned human 5-HT_{1A} receptors

	Agonist alone		Agonist + S 15535	Agonist + WAY 100,635
	Efficacy (%)	EC_{50} (*n*)	EC_{50} (*n*) K_b	EC_{50} (*n*) K_b
5-HT	100	15.0 ± 2.2 (3)	870 ± 300 (4)	1450 ± 280 (3)
			2.37 ± 0.61	*0.11 ± 0.02*
(±)-8-OH-DPAT	72.6 ± 3.6 (3)	6.0 ± 0.7 (3)	313 ± 77 (4)	2320 ± 250 (3)
			2.60 ± 0.92	*0.08 ± 0.01*

[^{35}S]GTPγS binding was carried out using membranes of CHO cells stably expressing the human 5-HT_{1A} receptor. Agonist efficacy is expressed relative to that of 5-HT (= 100%) which stimulated [^{35}S]GTPγS binding from basal values of 4170 ± 1060 dpm (3) to a maximum of 12 620 ± 2030 dpm (3). EC_{50} values (nM) are shown for 5-HT and (±)-8-OH-DPAT either alone or in the presence of a fixed concentration of either S 15535 or WAY 100,635. Corresponding K_b values (nM) are shown in italics. Results are expressed as means ± S.E.M. of (*n*) independent determinations. The antagonist concentration of S 15535 was 100 nM, that of WAY 100,635 was 10 nM against 5-HT and 30 nM against (±)-8-OH-DPAT.

decrease [^{35}S]GTPγS binding below basal levels (Fig. 1A (3)). The stimulation of [^{35}S]GTPγS binding induced by 5-HT (1000 nM) and (±)-8-OH-DPAT (100 nM) was completely antagonised by WAY 100,635 (Fig. 1D) yielding IC_{50} values of 9.89 ± 1.73 nM (4) and 2.87 ± 0.47 nM (3), respectively. S 15535 also antagonised the stimulation of [^{35}S]GTPγS binding induced by 5-HT (1000 nM) with an IC_{50} of 198 ± 57 nM (4), reducing it to 30.1 ± 2.2% (3) of control values. S 15535 alone increased [^{35}S]GTPγS binding by a maximum of 34.7 ± 2.2% (10) relative to 5-HT and with an EC_{50} of 5.8 ± 1.4 nM (3) (Fig. 1C). The stimulation of [^{35}S]GTPγS binding induced by S 15535 (100 nM) was completely antagonised by WAY 100,635 (Fig. 1D) with an IC_{50} of 4.93 ± 0.71 nM (4). S 15535 (100 nM), like WAY 100,635 (10 or 30 nM), shifted the 5-HT and (±)-8-OH-DPAT stimulation curves to the right with EC_{50} and K_b values as shown in Table 1 (Fig. 1A,B).

4. Discussion

The present data indicate that the novel benzodioxopiperazine, S 15535, has high affinity at cloned human 5-HT_{1A} receptors. Its K_i value at 5-HT_{1A} receptors (0.79 nM) is similar to that of (±)-8-OH-DPAT, 5-HT and WAY 100,635 and compares well with a value of 1.8 nM at native, rat hippocampal receptors (Millan et al., 1994b). Although they display similar affinity, these ligands differ in their ability to stimulate h5-HT_{1A} receptor-mediated G-protein activation, as measured by [^{35}S]GTPγS binding. The endogenous agonist, 5-HT, induced a 3-fold increase in specific [^{35}S]GTPγS binding and, although absolute experiment-to-experiment variation in 5-HT-stimulated [^{35}S]GTPγS binding was about 16% (12 620 ± 2030 dpm), the efficacies of the other ligands, *relative to 5-HT* (which was tested in each experiment), exhibited standard errors of under 4%. Hence, the prototypic 5-HT_{1A} receptor agonist (±)-8-OH-DPAT behaved as an efficacious agonist, stimulating [^{35}S]GTPγS binding by 73% relative to 5-HT. This value for (±)-8-OH-DPAT is similar to efficacies determined in rat hippocampus of 79% for stimulation of GTPase activity (Odagaki and Fuxe, 1995) and 77% for inhibition of forskolin-stimulated adenylyl cyclase activity (De Vivo and Maayani, 1986). The selective 5-HT_{1A} receptor antagonist WAY 100,635 did not stimulate [^{35}S]GTPγS binding (Fig. 1A), consistent with its absence of agonist activity in a variety of in vivo tests (Forster et al., 1995). In contrast, WAY 100,635 completely antagonised the activation of [^{35}S]GTPγS binding induced by 5-HT or (±)-8-OH-DPAT (Fig. 1D), indicating that this is mediated specifically by 5-HT_{1A} receptors.

S 15535 (100 nM), like WAY 100,635, exhibited antagonist properties in shifting the concentration-response curves of 5-HT and (±)-8-OH-DPAT to the right (Fig. 1A,B) with an associated increase in EC_{50} values of 58- and 52-fold, respectively (Table 1). In addition, S 15535 concentration dependently antagonised the stimulation of [^{35}S]GTPγS binding induced by 5-HT. However, unlike WAY 100,635, S 15535 did not reduce binding to basal levels (Fig. 1C). Indeed, S 15535 (alone) stimulated [^{35}S]GTPγS binding by 34.7%, indicating that it is a weak partial agonist at 5-HT_{1A} receptors. These results agree with rat hippocampal binding data obtained using the radiolabeled form of the compound ([^{3}H]S 15535). Unlike [^{3}H]8-OH-DPAT, [^{3}H]S 15535 binding is only slightly inhibited (−10%) by 100 μM GTP (Peglion et al., 1995).

An important factor, when considering agonist efficacies, is the expression level of the receptor. The CHO-5-HT_{1A} membrane preparation used here exhibits an expression level, measured by [^{3}H]8-OH-DPAT saturation binding, of 1.0 pmol of receptor/mg. This is at least 4-fold lower than the amount of agonist-activated G-protein determined in [^{35}S]GTPγS saturation binding experiments (unpublished observations). Therefore, the agonist stimulation levels observed in the present study represent the intrinsic efficacies of the compounds in this system, without 'exaggeration' due to receptor reserve. In contrast, in heterologous expression systems which express much higher levels of receptor, even partial agonists can behave like full agonists (cf. pindolol at 5-HT_{1B} receptors; Adham et al., 1993). It is concluded that the CHO-5-HT_{1A} cell line used here constitutes a model of *post*-synaptic receptors in

vivo, since the efficacy of serotonergic agonists in this cell line agrees well with those observed in rat hippocampus (Newman-Tancredi et al., 1992), a region which also lacks 5-HT_{1A} receptor reserve (Yocca et al., 1992). It appears, therefore, that the ≈35% efficacy of S 15535 is low enough to produce marked antagonist effects post-synaptically in vivo (see Introduction). However, at serotonergic cell bodies, the weak partial agonist activity of S 15535 may be sufficient to fully activate 5-HT_{1A} autoreceptors, since they exhibit high receptor reserve and low tonic activity (Meller et al., 1990). This profile of activity likely underlies the effectiveness of S 15535 in animal models of potential anxiolytic and antidepressant activity (Millan et al., 1994a).

The 'agonist/antagonist' properties of S 15535 raise several further issues which deserve comment. First, whereas the K_b value (≈2.5 nM) of S 15535, which shows weak partial agonist activity, is about 3-fold higher than its K_i value (0.79 nM), WAY 100,635 exhibited a K_b value (≈0.1 nM) which was over 5-fold *lower* than its K_i value (0.56 nM). This suggests that WAY 100,635 may be an inverse agonist, a hypothesis which would be consistent with (a) its tendency to reduce [^{35}S]GTPγS binding below basal levels (Fig. 1A), (b) the increase in [^{3}H]WAY 100,635 binding provoked by GTP (Gozlan et al., 1995) and (c) the slight stimulation by WAY 100,635 of raphe firing rates (Forster et al., 1995). The idea (which requires further investigation) that a high K_i/K_b ratio may be predictive of inverse agonism, is also valid for spiperone, which exhibits a K_i/K_b ratio of 25 (Newman-Tancredi et al., 1992) and which shows inverse agonist binding properties at h5-HT_{1A} receptors (Sundaram et al., 1993). Second, the EC_{50} values for activation of [^{35}S]GTPγS binding by S 15535 and (±)-8-OH-DPAT (5.8 and 6.0 nM) were about 10-fold higher than their K_i values determined in competition against [^{3}H](±)-8-OH-DPAT. Differences in K_i and EC_{50} values have been observed for 5-HT (37-fold) and clozapine (15-fold; Newman-Tancredi et al., 1996). Since [^{3}H](±)-8-OH-DPAT selectively labels receptors which are coupled to G-protein (Sundaram et al., 1993), these differences suggest that the EC_{50} values measured by [^{35}S]GTPγS binding may correlate better with the affinity (K_i) at receptors *not* coupled to G-protein. This interpretation supports the findings of Chamberlain et al. (1993) who showed that agonist IC_{50} values for inhibition of forskolin-stimulated adenylyl cyclase activity at rat hippocampal 5-HT_{1A} receptors correlate better with K_i values at low-affinity [^{3}H](±)-8-OH-DPAT binding sites.

In conclusion, using [^{35}S]GTPγS binding as a measure of efficacy at cloned human 5-HT_{1A} receptors, these data demonstrate the ability of S 15535 to antagonise the stimulation of these sites by 5-HT and (±)-8-OH-DPAT, whilst itself exhibiting modest intrinsic activity. The activation induced by S 15535 was completely blocked by WAY 100,635, a selective 5-HT_{1A} receptor antagonist.

References

Adham, N., B. Ellerbrock, P. Hartig, R.L. Weinshank and T. Branchek, 1993, Receptor reserve masks partial agonist activity of drugs in a cloned rat 5-hydroxytryptamine$_{1B}$ receptor expression system, Mol. Pharmacol. 43, 427.

Chabert, C., C. Cavegn, A. Bernard and A. Mills, 1994, Characterization of the functional activity of dopamine ligands at human recombinant dopamine D_4 receptors, J. Neurochem. 63, 62.

Chamberlain, J., S.J. Offord, B.B. Wolfe, L.S. Tyau, H.-L. Wang and A. Frazer, 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.

De Vivo, M. and S. Maayani, 1986, Characterisation 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.

Fletcher, A., D.J. Bill, I.A. Cliffe, E.A. Forster, D. Jones and Y. Reilly, 1994, A pharmacological profile of WAY 100,635, a potent and selective 5-HT_{1A} receptor antagonist, Br. J. Pharmacol. 112 (Proceedings Supplement), 91P.

Forster, E.A., I.A. Cliffe, D.J. Bill, G.M. Dover, D. Jones, Y. Reilly and A. Fletcher, 1995, A pharmacological profile of the selective silent 5-HT_{1A} receptor antagonist, WAY 100,635, Eur. J. Pharmacol. 281, 81.

Gozlan, H., S. Thibault, A.-M. Laporte, L. Lima and M. Hamon, 1995, The selective 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.

Lazareno, S., T. Farries and N.J.M. Birdsall, 1993, Pharmacological characterisation of guanine nucleotide exchange reactions in membranes from CHO cells stably transfected with human muscarinic receptors m1–m4, Life Sci. 52, 449.

Meller, E., M. Goldstein and K. Bohmaker, 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.

Millan, M.J., M. Brocco, R. Schreiber, J.-M. Rivet, P. Hjorth, R. Lacroix, R. Samanin, R. Jaffard, S. File, M. Spedding and J.-L. Peglion, 1994a, S 15535, a novel and selective antagonist at postsynaptic 5-HT_{1A} receptors and agonist at 5-HT_{1A} autoreceptors: actions in models of antidepressive, anxiolytic and promnesic activity, Soc. Neurosci. 20, 1544.

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

Newman-Tancredi, A., R. Wootton and P.G. Strange, 1992, High-level stable expression of recombinant 5-HT_{1A} 5-hydroxytryptamine receptors in Chinese hamster ovary cells, Biochem. J. 285, 933.

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

Odagaki, Y. and K. Fuxe, 1995, 5-HT_{1A} receptor-mediated activation of high-affinity GTPase in rat hippocampal membranes, Eur. J. Pharmacol. 288, 385.

Peglion, J.-L., A. Newman-Tancredi, L. Verrièle and M.J. Millan, 1995, [^{3}H]S 15535, a novel selective radioligand at serotonin (5-HT)$_{1A}$ receptors: characterisation of binding to cloned human and rat hippocampal 5-HT_{1A} receptors, Soc. Neurosci. 21, 1855.

Sundaram, H., A. Newman-Tancredi and P.G. Strange, 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.

Thomas, D.R., S.A. Faruq, J.M. Balcarek and A.M. Brown, 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.

Yocca, F.D., L. Iben and E. Meller, 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.