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The putative «silent» 5-HT_{1A} receptor antagonist, WAY 100635, has inverse agonist properties at cloned human 5-HT_{1A} receptors

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Abstract

Agonist binding to G protein-coupled receptors induces the formation of a receptor–G protein complex and subsequent guanosine 5′-diphosphate/guanosine 5′-triphosphate (GDP/GTP) exchange. Some receptors, however, form receptor–G protein complexes and promote GDP/GTP exchange even when not occupied by agonists. Such receptors preferentially activate pertussis toxin-sensitive G proteins (i.e., G_i/G_o), and the interactions of receptors and G proteins are affected by monovalent cations (most notably Na^+), both in the occupied and unoccupied state. We investigated the effects of Na^+ on the intrinsic activity of 5-hydroxytryptamine$_{1A}$ (5-HT_{1A}) receptor ligands, measured as maximal effect (E_{MAX}), using guanosine 5′-0-(3-[^{35}S]thio)-triphosphate ([^{35}S]GTPγS) binding to membranes prepared from human epithelioid carcinoma (HeLa) cells, expressing 500 fmol/mg protein of cloned human 5-HT_{1A} receptor (HA7 cells). A decrease of the NaCl concentration decreased the maximal effect of serotonin, increased basal [^{35}S]GTPγS binding, and increased the negative intrinsic activity of spiperone and *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide (WAY 100635). This ability of WAY 100635 to decrease basal [^{35}S]GTPγS binding was antagonized by (s)-*N*-*tert*-butyl-3-(4-(2-methoxyphenyl)piperazine-1-yl)-2-phenylpropanamide ((s)-WAY 100135) ($pA_2 = 7.77$). Further, WAY 100635 was able to antagonize carboxamidotryptamine (5-CT)-stimulated [^{35}S]GTPγS binding with a pA_2 of 9.9, in standard NaCl conditions, and of 9.7, in the absence of NaCl. Changes in membrane concentration did not affect the ability of WAY 100635 to decrease [^{35}S]GTPγS binding. WAY 100635 did not affect basal [^{35}S]GTPγS binding to membranes from untransfected HeLa cells. Pertussis toxin (200 ng/ml) prevented WAY 100635 and spiperone to decrease [^{35}S]GTPγS binding, showing that their effects were mediated by G proteins of the G_i/G_o family. In conclusion, the constitutive and stimulated activity of human 5-HT_{1A} receptors expressed in HA7 cells is sodium-dependent, which allowed to confirm the 5-HT_{1A} inverse agonist properties of spiperone, and to show that WAY 100635 is an inverse agonist at 5-HT_{1A} receptors that inhibits basal [^{35}S]GTPγS binding to a lesser extent than spiperone. [^{35}S]GTPγS binding to membranes from HA7 cells under low NaCl conditions appears to be especially suitable to evidence and pharmacologically analyze the inverse agonist properties of 5-HT_{1A} receptor ligands. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: [^{35}S]GTPγS binding; 5-HT_{1A} receptor; Inverse agonist; WAY 100635

1. Introduction

A generally accepted interpretation of the cycle of events by which G protein-coupled receptors elicit responses is that agonist binding to G protein-coupled receptors is required to induce the formation of a receptor–G protein complex, and subsequent guanosine 5′-diphosphate/guanosine 5′-triphosphate (GDP/GTP) exchange. In the absence of the agonist, the receptor is thought to exist entirely in the dissociated state, corresponding to the lower affinity component of biphasic agonist displacement in radioligand binding assays. The ternary complex, consisting of the agonist, receptor, and G protein, accounts for the higher affinity component in such assays. It has become apparent in the last few years that while this description is reasonable for receptors coupled primarily or exclusively to G_s (such as the β_2-adrenoceptor), receptors that couple primarily with pertussis toxin-sensitive G proteins (G_i or G_o), and show Na^+-dependent agonist and antago-

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nist binding affinities may behave quite differently. These latter receptors (e.g., δ-opioid receptor, α_2-adrenoceptor, 5-$HT_{1D\alpha/1D\beta}$) appear to form receptor–G protein complexes and promote GDP/GTP exchange even when not occupied by agonists, and do so in a manner that can be affected by monovalent cations (most notably Na^+) (Costa et al., 1990; Jagadeesh et al., 1990; Tian and Deth, 1993; Gray et al., 1997).

Inverse agonism has been described in the in vitro experimental models such as transfected cell lines, in which the receptors are present in sufficiently high densities to cause a significant activation of the signal transduction systems for the inverse agonist to inhibit. For instance, inverse agonist activity of spiperone, but not of *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide (WAY 100635), has been demonstrated using guanosine 5′-0-(3-[^{35}S]thio)-triphosphate binding ([^{35}S]GTPγS binding) to membranes from Chinese hamster ovary (CHO) cells expressing 1.6 pmol/mg protein of human recombinant 5-hydroxytryptamine$_{1A}$ (5-HT_{1A}) receptors (Newman-Tancredi et al., 1997).

Inverse agonism can be affected by variations in [Na^+]. It has been reported that α_2-adrenergic receptor antagonists decreased [^{35}S]GTPγS binding to membranes from PC-12 cells, stably expressing the α_{2D}-adrenoceptors, and that their inverse agonist activity was decreased by increasing the NaCl concentration (Tian et al., 1994). In contrast, agonists' responses were increased at higher NaCl levels (Tian et al., 1994). [^{35}S]GTPγS binding to membranes from human epithelioid carcinoma (HeLa) cells expressing cloned human 5-HT_{1A} receptors (500 fmol/mg protein) (HA7; Fargin et al., 1989), in the presence of 100 mM NaCl, has been used previously to examine the pharmacological properties of 5-HT_{1A} receptor ligands (Stanton and Beer, 1997). Here, we investigated the effects of varying the Na^+ concentrations on the activity of 5-HT_{1A} receptor ligands, such as WAY 100635, to alter [^{35}S]GTPγS binding to membranes prepared from HA7 cells. Part of the present results has been reported previously in abstract form at the Fourth IUPHAR Satellite Meeting on Serotonin in 1998.

2. Methods

2.1. Cell culture

HA7 cells were grown in Dulbecco modified Eagle medium (DMEM) (GIBCO) supplemented with 10% foetal calf serum, gentamicin (100 μg/ml) and geneticin (G418) (400 μg/ml), in 5% CO_2 at 37°C in a water-saturated atmosphere. The cells were plated in 150 cm^2 petri dishes until they reached a 90–100% confluence, after which, they were washed with phosphate buffered saline (PBS) and stored at −80°C. In some experiments, the cells were treated with pertussis toxin (200 ng/ml) for 16 h, then washed with PBS and stored at −80°C.

2.2. [^{35}S]GTPγS binding

The membranes were prepared from frozen cells on the day of the experiment, according to Stanton and Beer (1997), with some modifications. Cells were harvested in ice-cold 20 mM Hepes buffer containing 10 mM ethylenediaminetetraacetic acid (EDTA) (pH 7.4, room temperature (RT)), and were homogenized and centrifuged at 40 000 × *g*, at 4°C for 15 min. The pellet was resuspended in ice-cold 20 mM Hepes containing 0.1 mM EDTA (pH 7.4, RT) and recentrifuged at 40 000 × *g*, at 4°C for 15 min. The final pellet was resuspended in 20 mM Hepes containing 10 mM $MgCl_2$, 10 μM pargyline, 30 μM GDP and different concentrations of NaCl ranging from 100 mM (referred to as the standard NaCl condition) to no NaCl added (referred to as the low NaCl condition). The membranes, 100–50 μg/tube, were incubated in the presence of the test compounds for 1 h at 30°C. In the antagonism studies, different compounds were added at the same time. After 15 min at 0°C, [^{35}S]GTPγS (specific activity is ≈1000 Ci/mmol) was added to a final concentration of 0.1 nM. The membranes were then incubated for an additional 30 min, at 30°C. The reaction was terminated by filtration through Whatman filters using a Brandel harvester, and radioactivity was counted by liquid scintillation spectrometry.

Each concentration–response experiment was performed in triplicate and replicated three to six times. pEC_{50} and E_{MAX} values were estimated from the averaged concentration–response data by means of non-linear regression (sigmoidal model with unit slope; Graphpad Prism). Linear regression and Spearman correlation analyses were performed using Graphpad Prism, and pA_2 values were estimated by means of the Pharm/PCS program by Tallarida and Murray (1987).

2.3. Chemicals

(±)-8-Hydroxy-2-(di-*n*-propylamino)tetralin ((±)-8-OH-DPAT) hydrobromide, (+)-8-hydroxy-2-(di-*n*-propylamino)tetralin ((+)-8-OH-DPAT), 5-carboxamidotryptamine maleate (5-CT), buspirone hydrochloride, (−)-pindolol, 1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl]piperazine (NAN-190) hydrobromide, 8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro-[4,5]decane-7,9-dione-(BMY 7378) dihydrohloride, clozapine, spiperone hydrochloride, and 4-(2′-methoxyphenyl)-1-[2′(*n*-2″-pyridinyl)-*p*-iodobenzamido]ethyl-piperazine (p-MPPI) were purchased from Sigma-RBI; (s)-WAY 100135 hydrochloride, WAY 100635 dihydrochloride, 1[2-(4-fluorobenzoylamino)ethyl]-4-(7-methoxynaphtyl)piperazine (S 14506),

and ipsapirone hydrochloride were synthesized by J.-L. Maurel (Centre de Recherche Pierre Fabre).

3. Results

3.1. Potency and intrinsic activity of serotonergic compounds in standard (100 mM) NaCl conditions

The ability of a variety of compounds with affinity for 5-HT_{1A} receptors to affect basal [^{35}S]GTPγS binding to membranes from HA7 cells was investigated using a reaction buffer that included 100 mM NaCl, as described previously (Stanton and Bear, 1997). Under these conditions, positive intrinsic activity (E_{MAX}) ranged from 419.4 ± 11.70% of basal [^{35}S]GTPγS binding for (+)-8-OH-DPAT to 117.6 ± 4.51% for WAY 100135 (Table 1). Serotonin stimulated basal [^{35}S]GTPγS binding with a pEC_{50} of 7.31 ± 0.036 and an E_{MAX} of 347.3 ± 3.16. The potencies and positive intrinsic activities of these compounds correlated with data reported by Newman-Tancredi et al. (1998) in CHO-h5-HT_{1A} cell membranes (pEC_{50}: $r = 0.85$, slope = 0.99, $P < 0.05$; E_{MAX}: Spearman correlation $r_s = 0.86$, $P < 0.01$).

Negative intrinsic activity was detected for some of the compounds under standard NaCl (100 mM) conditions. WAY 100635, p-MPPI, spiperone and methiotepine inhibited basal [^{35}S]GTPγS binding with pIC_{50} values ranging from 7.70 (spiperone) to 9.54 (WAY 100635), and with E_{MAX} values ranging from 93.1 (WAY 100635) to 72.2 (p-MPPI).

Table 1
Potency and intrinsic activity, in standard NaCl condition, for a series of 5-HT_{1A} receptor ligands using [^{35}S]GTPγS binding to cloned human 5-HT_{1A} receptors stably expressed in HeLa cells

Compounds	pEC_{50} (mean ± SEM)	E_{max} (mean ± SEM)
(+)-8-OH-DPAT	7.70 ± 0.032	419.4 ± 11.70
5-CT	8.40 ± 0.031	394.5 ± 2.56
S14506	9.37 ± 0.052	366.7 ± 31.06
8-OH-DPAT	7.55 ± 0.113	357.6 ± 9.12
5-HT	7.31 ± 0.036	347.3 ± 3.16
Buspirone	6.64 ± 0.267	320.6 ± 6.65
BMY 7378	7.96 ± 0.150	214.2 ± 6.32
Clozapine	6.13 ± 0.065	203.8 ± 19.85
Ipsapirone	7.16 ± 0.070	170.0 ± 12.50
NAN 190	8.59 ± 0.241	152.8 ± 6.79
(−)-Pindolol	7.71 ± 0.120	129.6 ± 17.00
(s)-WAY 100135	7.74 ± 0.262	117.6 ± 4.51
WAY 100635	9.54 ± 0.164	93.1 ± 0.39
Spiperone	7.70 ± 0.088	77.4 ± 0.67
p-MPPI	8.05 ± 0.588	72.2 ± 6.07

Values are expressed as percent of basal [^{35}S]GTPγS binding, and are means ± SEM of three to four independent experiments, each performed in triplicate.

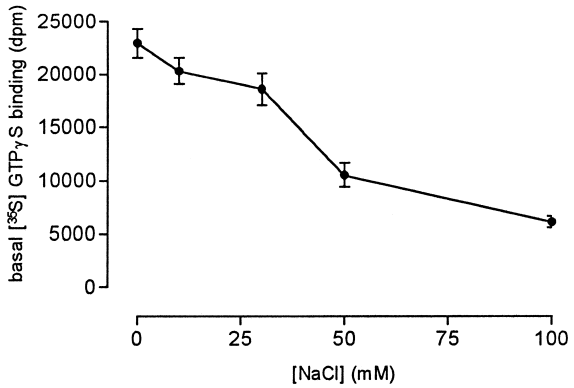

Fig. 1. Effects of different concentrations of NaCl on basal [^{35}S]GTPγS binding in membranes from HeLa cells expressing human 5-HT_{1A} receptors. Values are means ± SEM of 14–16 independent experiments performed in triplicate using different membrane preparations. Data are expressed in dpm.

3.2. Sodium dependency of [^{35}S]GTPγS binding to HA7 cells membranes

NaCl differentially affected basal and drug-induced [^{35}S]GTPγS binding to HA7 cells membranes. NaCl concentration dependently decreased basal [^{35}S]GTPγS binding from 22 943 ± 1357 dpm in the absence of NaCl to 6149 ± 521 dpm in presence of 100 mM NaCl (Fig. 1). In the absence of NaCl, basal [^{35}S]GTPγS binding in untransfected HeLa cells (7216 ± 746.8 dpm) was about 30% of that found in HA7 cells.

NaCl concentration dependently increased the E_{MAX} of serotonin, from 142.7 ± 1.93% at no NaCl added to 347.3 ± 3.16% of basal [^{35}S]GTPγS binding at 100 mM, while the pEC_{50} of 5-HT decreased from 8.43 ± 0.2 to 7.31 ± 0.03, at 0 and 100 mM NaCl, respectively (Fig. 2, right panels). In contrast, decreasing the NaCl concentration increases, in a concentration-dependent manner, the negative intrinsic activity of spiperone and WAY 100635 to a maximum of 39 ± 3.2% and 73 ± 2.5%, respectively, in absence of NaCl, without affecting their pEC_{50} values. As shown by the absolute values expressed in dpm (Fig. 2, left panels), the E_{MAX} of 5-HT was 1.4 times higher than basal in absence of NaCl (basal: 27 730 ± 863 dpm; 5-HT: 37 830 ± 511 dpm) and was 3.3 times higher than basal in the presence of 100 mM NaCl (basal: 7309 ± 206; 5-HT: 24 040 ± 230); the E_{MAX} of spiperone varied from 1.4-fold lower than basal at 100 mM NaCl (basal: 5553 ± 71.2 dpm; spiperone: 3922 ± 62.5 dpm) to 2.8-fold lower than basal in absence of NaCl (29 550 ± 347 dpm; spiperone: 10 570 ± 359 dpm); and the E_{MAX} of WAY 100635 varied from 1.2-fold lower than basal at 100 mM NaCl (basal: 8146 ± 134 dpm; WAY 100635: 6645 ± 35.8 dpm) to 1.6-fold lower than basal in absence of NaCl (29 370 ± 507 dpm; WAY 100635: 18 850 ± 244 dpm).

Changes in membrane concentration did not affect the ability of WAY 100635 to decrease basal [^{35}S]GTPγS

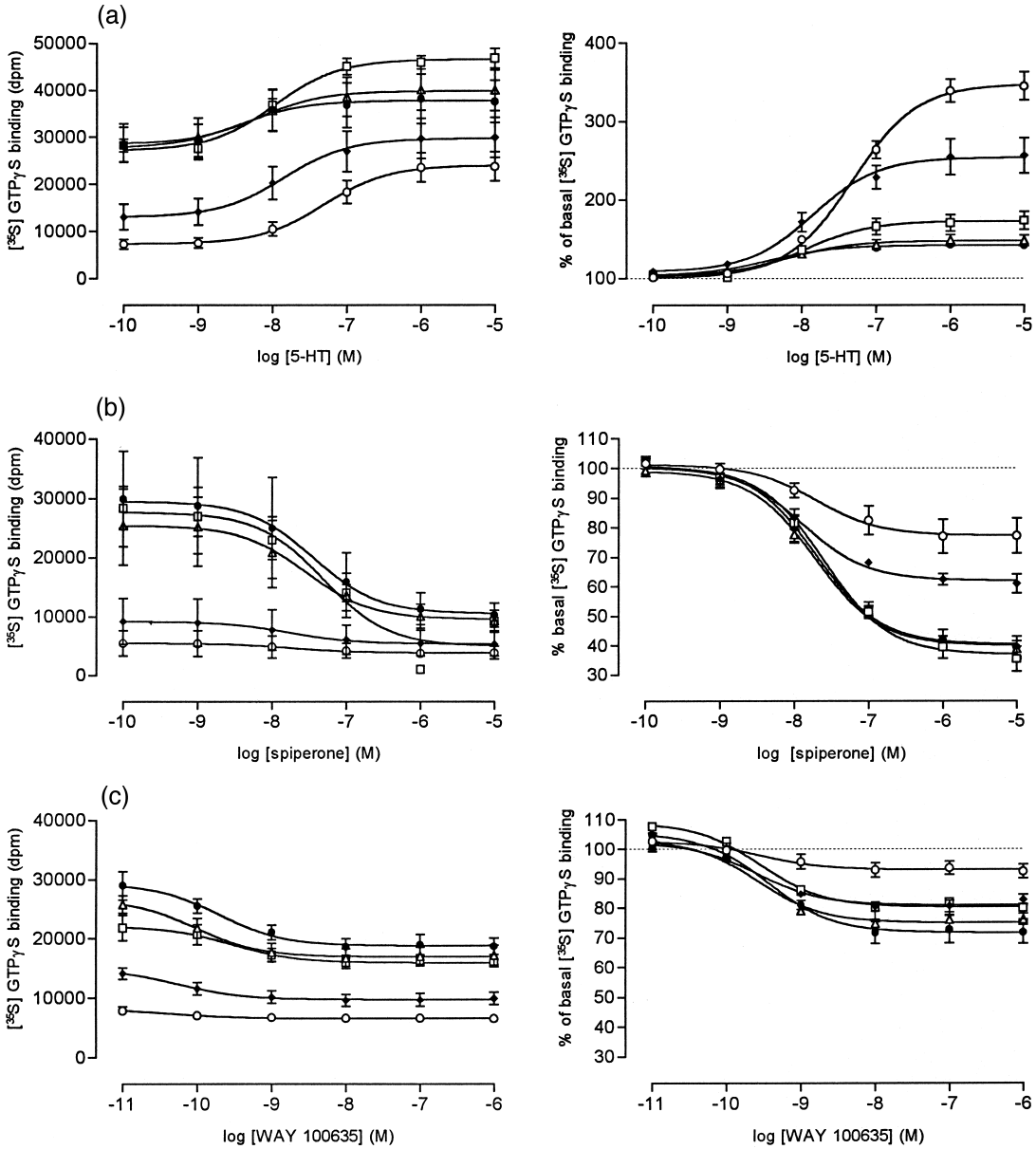

Fig. 2. Effects of different NaCl concentrations on the ability of (a) 5-HT, (b) spiperone and (c) WAY 100635 to affect [^{35}S]GTPγS binding in membranes from HeLa cells, expressing human 5-HT$_{1A}$ receptors. Concentration–response curves were determined in the absence of NaCl (●), and in the presence of the following NaCl concentrations: 10 mM (△), 30 mM (□), 50 mM (◆), 100 mM (○). Values, expressed in dpm in the left panels and as percent of basal [^{35}S]GTPγS binding in the right panels, are means ± SEM of three to seven independent experiments performed in triplicate using different membranes preparations.

binding (data not shown). WAY 100635 did not affect basal [^{35}S]GTPγS binding to membranes from untransfected HeLa cells in the absence of NaCl (data not shown).

3.3. Antagonism studies

In the absence of NaCl, (s)-WAY 100135 (10 nM–1 μM) shifted the concentration–response curve of WAY 100635 to decrease basal [^{35}S]GTPγS binding to the right (Fig. 3), with a pA_2 of 7.77.

The ability of WAY 100635 to antagonize the effects of the full agonist 5-CT was investigated under standard (100 mM) NaCl conditions, and in the absence of NaCl (Fig. 4). In the standard NaCl condition, WAY 100635 (3.2–32 nM) shifted the concentration–response curve of 5-CT to stimulate [^{35}S]GTPγS binding to the right, with a pA_2 value of 9.9. The E_{MAX} of 5-CT was not affected by WAY 100635. At the lowest (0.1, 1 nM) 5-CT concentrations [^{35}S]GTPγS binding was around 80% of basal values. In the absence of NaCl, WAY 100635 shifted the concentration–response curve of 5-CT to stimulate

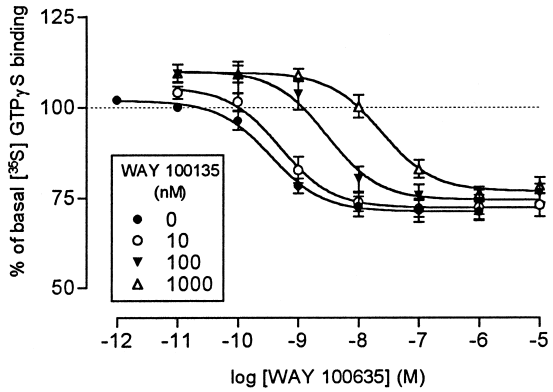

Fig. 3. (s)-WAY 100135 antagonized the ability of WAY 100635 to inhibit [^{35}S]GTPγS binding. Values, expressed as percent of basal [^{35}S]GTPγS binding, are means ± SEM of four independent experiments performed in triplicate using different membrane preparations (low NaCl condition).

[^{35}S]GTPγS binding to the right, with a pA_2 value of 9.7. The E_{MAX} of 5-CT was lower (143.1 ± 0.97%) in the absence of NaCl than in the presence of 100 mM NaCl (394.5 ± 2.56%), and was not affected by the WAY 100635 concentrations used. At the lowest (0.1, 1 nM) concentrations of 5-CT, [^{35}S]GTPγS binding ranged from 64% to 74% of basal values in the presence of WAY 100635.

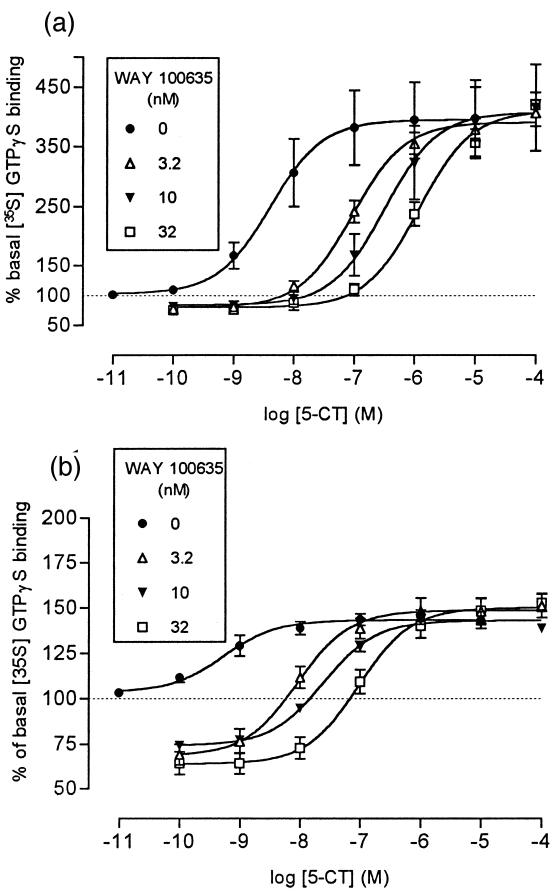

Fig. 4. WAY 100635 antagonized the effects of 5-CT on [^{35}S]GTPγS binding both under standard (a) and under low (b) NaCl conditions. Values, expressed as percent of basal [^{35}S]GTPγS binding, are means ± SEM of three independent experiments performed in triplicate using different membranes preparations.

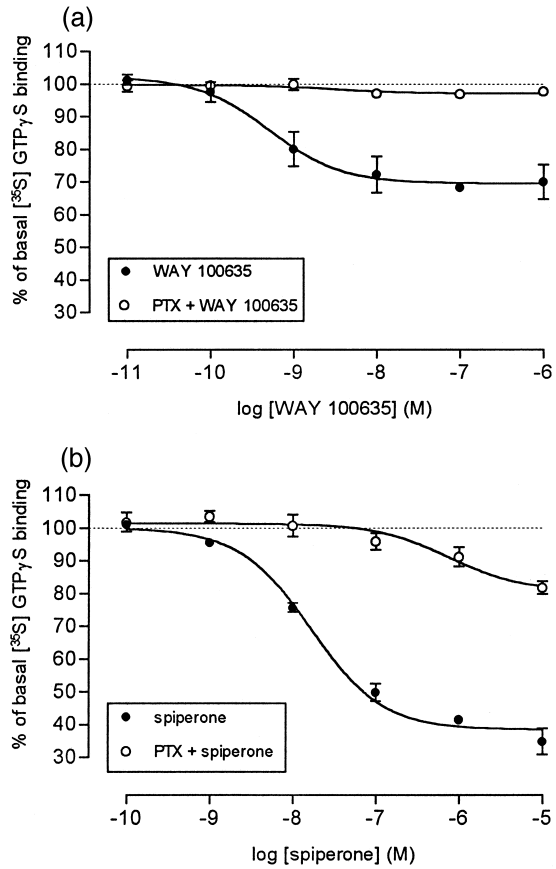

Fig. 5. Pertussis toxin inhibited the ability of WAY 100635 (a) and spiperone (b) to decrease [^{35}S]GTPγS binding. Values, expressed as percent of basal [^{35}S]GTPγS binding, are means ± SEM of three independent experiments performed in triplicate using different membranes preparations, (low NaCl condition).

3.4. Effects of pertussis toxin on the ability of spiperone and WAY 100635 to inhibit basal [^{35}S]GTPγS binding

Pertussis toxin pretreatment completely prevented the inhibition of basal [^{35}S]GTPγS binding produced by WAY 100635, while it reduced the maximal effect of spiperone from 38.4 ± 2.11% to 81 ± 3.08% of basal binding (Fig. 5).

4. Discussion

The effects of a variety of 5-HT$_{1A}$ receptor ligands on basal [^{35}S]GTPγS binding to membranes from HA7 cells were investigated, using the binding conditions previously described by Stanton and Bear (1997) with 100 mM NaCl.

The intrinsic activity and pEC_{50} values obtained here correlated with those reported by Newman-Tancredi et al. (1998), who used [^{35}S]GTPγS binding to membranes from CHO cells expressing human recombinant 5-HT_{1A} receptors.

p-MPPI and spiperone, described as putative inverse agonists at 5-HT_{1A} receptors in transfected cell lines (Barr and Manning, 1997; Newman-Tancredi et al., 1997), decreased basal [^{35}S]GTPγS binding to HA7 membranes. Importantly, WAY 100635, which has been characterized as a «neutral» or «silent» receptor antagonist in CHO-h5-HT_{1A} cells (Newman-Tancredi et al., 1998) and in other in vitro and in vivo models (Fletcher et al., 1996), slightly but consistently inhibited basal [^{35}S]GTPγS binding in HA7 cells under standard NaCl conditions.

Decreasing the concentration of Na^+ has been shown to increase basal [^{35}S]GTPγS binding and, consequently, the maximal effect of putative inverse agonists at α_2-adrenoceptors or 5-$HT_{1D\alpha/1D\beta}$ receptors (Tian et al., 1994; Gray et al., 1997). In the present study, both basal and ligand-induced [^{35}S]GTPγS binding were affected by decreasing the Na^+ concentration. Basal [^{35}S]GTPγS binding was increased by decreasing the concentration of Na^+, suggesting the existence of Na^+-dependent precoupling at 5-HT_{1A} receptors in HA7 cells. Under low Na^+ conditions, basal [^{35}S]GTPγS binding in untransfected HeLa cells was about 30% of that found in HA7 cells, similar to the E_{MAX} of the inverse agonist spiperone (36% of basal binding) and different from the E_{MAX} of WAY 100635 (64% of basal binding). These findings suggest that the human 5-HT_{1A} receptor expressed in HeLa cells may show a level of Na^+-dependent constitutive activity that is sufficiently high to allow discrimination between maximal effects of inverse agonists at this receptor. Decreasing the Na^+ concentration enhanced the maximal inhibitory effect of spiperone and WAY 100635 without affecting their pIC_{50} values, and attenuated the maximal effect of 5-HT and increased its pEC_{50}. These results are in agreement with the effects of Na^+ previously described in PC-12 cells stably expressing α_2-adrenoceptors (Tian et al., 1994), and with the hypothesis that Na^+ might act as a negative modulator of the receptor–G protein precoupling equilibrium in a ternary complex model (Costa et al., 1992). In high Na^+ conditions, 5-HT_{1A} receptors in HA7 cells would behave as non-precoupled receptors, and agonists would contribute to both the formation of ligand–receptor–G protein complex and its augmented rate of nucleotides exchange, measured as [^{35}S]GTPγS exchange. In contrast, low Na^+ conditions would facilitate precoupling of 5-HT_{1A} receptors to such an extent that it can be inhibited by an inverse agonist.

The pharmacological relevance of the WAY 100635 induced decrease of basal [^{35}S]GTPγS binding was demonstrated by the observation that its effect could be antagonized by a selective 5-HT_{1A} receptor antagonist, and by the finding that WAY 100635 was able to maintain its antagonist properties in Na^+-deprived conditions. (s)-WAY 100135 was selected as a tool to antagonize the inverse agonist activity of WAY 100635, because it did not decrease but partially increased basal [^{35}S]GTPγS binding in agreement with its partial agonist activity at 5-HT_{1A} receptors, described previously in vivo (Assié and Koek, 1996). In the absence of Na^+, (s)-WAY 100135 antagonized the effects of WAY 100635 with a pA_2 of 7.77, which is similar to its pA_2 value obtained in rat isolated superior cervical ganglion by extracellular recording (Rhodes et al., 1993). WAY 100635 antagonized 5-CT stimulated [^{35}S]GTPγS binding, both in standard and low Na^+ conditions, with pA_2 values (i.e., 9.7–9.9) similar to those reported previously in isolated guinea pig ileum (Forster et al., 1995). The potency of WAY 100635 to decrease basal [^{35}S]GTPγS binding was similar to its affinity for human 5-HT_{1A} receptors expressed in HA7 cell (unpublished observations), and to its potency to antagonize 5-CT-stimulated [^{35}S]GTPγS binding. This suggests that the same binding site is involved in both effects, that 5-HT_{1A} receptors in HA7 cells are spontaneously active in promoting [^{35}S]GTPγS binding, and that occupation of the ligand recognition site by WAY 100635 reduces the spontaneous receptor activity.

The possibility that an interaction between receptors present in high concentration in the Na^+-deprived reaction buffer could have been responsible for the apparent inverse agonist properties of WAY 100635 appears unlikely, because changes in membrane concentrations did not affect the ability of WAY 100635 to decrease basal [^{35}S]GTPγS binding. Further, the effect of WAY 100635 was due to the presence of 5-HT_{1A} receptors, because WAY 100635 did not affect basal [^{35}S]GTPγS binding to membranes from untransfected HeLa cells. Finally, pertussis toxin prevented both the spiperone-and WAY 100635-induced decrease of basal [^{35}S]GTPγS binding in low Na^+ conditions, showing that these effects are mediated by G proteins of the G_0/G_i family. Pertussis toxin treatment, however, did not completely abolish basal [^{35}S]GTPγS binding in absence of Na^+. The basal dpm detected in HA7 cells after pertussis toxin treatment could reflect an incomplete treatment with pertussis toxin, or more likely, [^{35}S]GTPγS binding to pertussis toxin-insensitive, Na^+-sensitive G protein-linked receptors present in these cells. HA7 cells contain, for example, endogenous histamine H1 receptors linked to the stimulation of phosphoinositide hydrolysis through pertussis toxin-insensitive G proteins (Raymond et al., 1991).

Increasing the sodium concentration increased the maximal effect of 5-HT, in agreement with a recent report that increased sodium enhances the maximal effect of agonist-stimulated [^{35}S]GTPγS to membranes of CHO cells expressing human 5-HT_{1A} receptors (McLoughlin and Strange, 2000). In contrast with the present results, however, McLoughlin and Strange (2000) found that the maximal effect of the inverse agonists, spiperone and methiothepin, were not significantly affected by the concentration

of sodium. Further, they reported that the basal level of [^{35}S]GTPγS binding in untransfected CHO cells was about 35% lower than that in transfected cells, suggesting that the maximum effect a full inverse agonist can exert in this system is about 35%. In the present study, however, basal binding in untransfected HeLa cells was almost 70% lower than in HA7 cells. It is conceivable that differences in G protein pools account in part for these differences between CHO 5HT$_{1A}$ and HA7 cells. Nevertheless, the observation that the difference of basal [^{35}S]GTPγS binding between transfected and untransfected cells is almost twice as large in HA7 cells than in CHO cells, suggests that HA7 cells may afford a more sensitive measure of maximal effect differences of inverse agonists.

In conclusion, the present study indicates that human 5-HT$_{1A}$ receptors expressed in HeLa cells show sodium-dependent constitutive and drug-induced activity, and the results suggest that in HA7 cells, a low NaCl concentration appears to be the most suitable condition to evidence and pharmacologically analyze possible inverse agonist properties of compounds at 5-HT$_{1A}$ receptors. Moreover, the present data confirm the inverse agonist properties of spiperone at human cloned 5-HT$_{1A}$ receptors, and constitute evidence that the putative «silent» 5-HT$_{1A}$ receptor antagonist, WAY 100635, has inverse agonist properties at these receptors.

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