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European Journal of Pharmacology 433 (2001) 55–62

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Agonist, antagonist, and inverse agonist properties of antipsychotics at human recombinant 5-HT_{1A} receptors expressed in HeLa cells

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Received 12 July 2001; received in revised form 25 October 2001; accepted 26 October 2001

Abstract

Agonist and antagonist properties of antipsychotics at human (h) recombinant 5-hydroxytryptamine$_{1A}$ (h5-HT_{1A}) receptor have been examined previously in transfected Chinese hamster ovary (CHO) cells using 5′-*O*-(3-[^{35}S]thio)-triphosphate ([^{35}S] GTPγS) binding. Na^+-dependent [^{35}S] GTPγS binding to membranes from human epithelioid carcinoma (HeLa) cells, expressing 500 fmol/mg protein of h5-HT_{1A} receptor (HA7 cells), appears suitable to characterize not only agonist and antagonist properties of 5-HT_{1A} receptor ligands, but also inverse agonist properties. We therefore examined agonist, antagonist, and inverse agonist activity of antipsychotics at h5-HT_{1A} receptor in HA7 cells. Some antipsychotics had agonist activity and stimulated [^{35}S] GTPγS binding with the following order of efficacy: nemonapride>ziprasidone>clozapine>ocaperidone. Tiospirone and *trans*-5-chloro-2-methyl-2,3,3a,12b-tetrahydro-1*H*-dibenz[2,3:6,7,5]-oxepino-[4,5c]pyrrole (ORG 5222), were more potent h5-HT_{1A} receptor antagonists than raclopride, olanzapine, and risperidone. Haloperidol, chlorpromazine, thioridazine, pimozide, and sertindole showed Na^+-dependent inverse agonist activity at h5-HT_{1A} receptor that could be antagonized by (*s*)-*N*-*tert*-butyl-3-(4-(2-methoxyphenyl)piperazine-1-yl)-2-phenylpropanamide [(*s*)-WAY 100135]. These results are further evidence that interactions with h5-HT_{1A} receptors could play a role in the pharmacological profile of certain antipsychotics, and that Na^+ affects the ability to detect inverse agonist activity at h5-HT_{1A} receptors, likely by influencing receptor precoupling. Also, the manner in which compounds interact with 5-HT_{1A} receptors appears to be related to their K_b/K_i ratio.

Keywords: Inverse agonism; Antipsychotic; 5-HT_{1A} receptor; [^{35}S] GTPγS binding; HA7 cell

1. Introduction

Growing evidence indicates 5-hydroxytryptamine$_{1A}$ (5-HT_{1A}) receptors to be a potential target for novel antipsychotic drugs (for a review, see Bantick et al., 2001). For example, 5-HT_{1A} receptor agonists, such as 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), block haloperidol- and raclopride-induced catalepsy in rats (e.g. McMillen et al., 1988; Wadenberg and Ahlenius, 1991; Andersen and Kilpatrick, 1996), and enhance antipsychotic-like effects of haloperidol and raclopride (Wadenberg and Ahlenius, 1991; Prinssen et al., 1996). 5-HT_{1A} receptor agonists also increase dopamine release in the prefrontal cortex of rodents (Rollema et al., 1997), an effect that has been predicted to improve negative symptoms (Sharma and Shapiro, 1996). Moreover, (putative) atypical antipsychotics such as clozapine, ziprasidone, and tiospirone exert partial agonist activity at h5-HT_{1A} receptors, while conventional antipsychotics such as haloperidol, pimozide, raclopride and chlorpromazine, and some atypical antipsychotics such as risperidone and sertindole, have been characterized as "silent" antagonists at h5-HT_{1A} receptors expressed in Chinese hamster ovary cells (CHO) (Newman-Tancredi et al., 1998).

Drug effects at recombinant human receptors expressed in heterologous systems can be affected by spontaneous receptor precoupling that is influenced by receptor density, G protein pools, and cation concentrations (see De Ligt et al., 2000 for a review). The Na^+ dependency of h5-HT_{1A} receptor precoupling in human epithelioid carcinoma HeLa cells expressing 500 fmol/mg protein of human recombinant 5-HT_{1A} receptor (HA7 cells) allowed us to show that *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl) cyclohexanecarboxamide (WAY 100635), characterized initially as "silent" 5-HT_{1A} antagonist (Newman-Tancredi et al., 1997), has marked inverse agonist properties at h5-HT_{1A} receptors (Cosi and Koek, 2000). This latter finding agrees with previous reports that described a tendency of WAY

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0014-2999/01/$ - see front matter
PII: S0014-2999(01)01493-5

100635 to decrease basal 5*t*-*O*-(3-[^{35}S]thio)-triphosphate ([^{35}S] GTPγS) binding in h5-HT_{1A} CHO cells (Newman-Tancredi et al., 1996), in h5-HT_{1A} C6 glioma cells (Pauwels et al., 1997), and in Cos-7 cells transiently expressing recombinant h5-HT_{1A} receptor fusion proteins (Dupuis et al., 1999). Thus, Na^+-dependent [^{35}S] GTPγS binding to membranes from HA7 cells appears to be especially suitable to characterize inverse agonist properties of 5-HT_{1A} receptor ligands. Here, we therefore examined, in this system, the possible inverse agonist properties of antipsychotics at the h5-HT_{1A} receptor.

2. Methods

2.1. Cell culture

HA7 cells (Fargin et al., 1989) were grown in Dulbecco's modified Eagle medium (DMEM) (GIBCO) supplemented with 10% fetal 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 until used for [^{35}S] GTPγS binding or 5-HT_{1A} radioligand binding assays.

2.2. [^{35}S] GTPγs binding

The membranes were prepared from the 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 [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid] buffer containing 10 mM ethylenediaminetetraacetic acid (EDTA) (pH 7.4, room temperature), and were homogenized and centrifuged at 40 000 × *g*, 4 °C for 15 min. The pellet was suspended in ice-cold 20 mM HEPES containing 0.1 mM EDTA (pH 7.4, room temperature) and centrifuged again at 40 000 × *g*, 4 °C for 15 min. The final pellet was suspended in 20 mM HEPES containing 10 mM $MgCl_2$, 10 μM pargyline, 30 μM GDP, and 100 mM NaCl (called the standard NaCl condition) or no NaCl added (called 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 ≈ 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. For each replication, pEC_{50} and E_{max} values were estimated from concentration–response data by means of non-linear regression (sigmoidal model with unit slope; Graphpad Prism). Linear regression analyses were performed using Graphpad Prism.

pK_b values were calculated from the EC_{50} values of the concentration–response curves of 5-carboxamidotryptamine maleate (5-CT) in the absence (EC_{50} 5-CT) and in the presence of a single concentration of the antagonist (EC_{50} 5-CT + antagonist) as follows: $pK_b = \log(DR - 1) - \log$ ([antagonist]); DR=(EC_{50} 5-CT + antagonist)/(EC_{50} 5-CT). pK_b values for nemonapride and ziprasidone were calculated using the EC_{50} values of the concentration–response curves of 5-CT with the minimum constrained to the minimum value of the 5-CT curve obtained in presence of the antagonist (see Fig. 1).

2.3. 5-HT_{1A} radioligand binding assay

The membranes were prepared from frozen HA7 cells as described below. Cells were harvested in ice-cold Tris (2-amino-2-hydroxymethylpropane-1,3-diol)–HCl pH 7.4, homogenized and centrifuged at 40 000 × *g*, 4 °C for 10

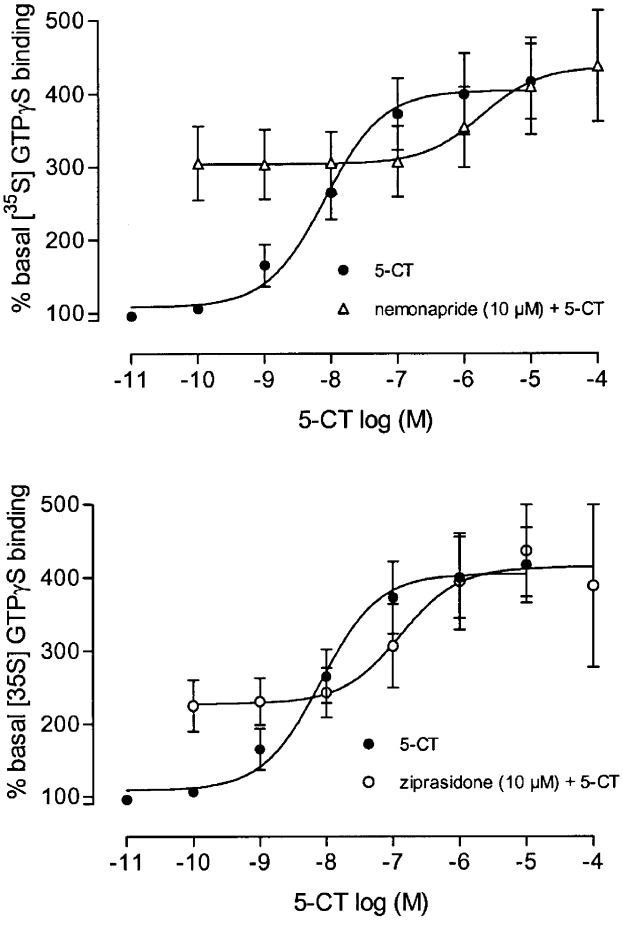

Fig. 1. Antagonist activity of nemonapride (upper panel) and ziprasidone (lower panel). Values are expressed as percentage of basal [^{35}S] GTPγS binding, and are means ± S.E.M. of three to four independent experiments, each performed in triplicate, in the presence of NaCl.

min. The pellet was suspended in the same buffer and centrifuged again. After the second centrifugation, the pellet was suspended in an assay buffer consisting of pargyline (10 μM) and $CaCl_2$ (4 mM) in Tris–HCl (50 mM, pH 7.4). Membrane protein, 0.031–0.084 mg/tube, was incubated with [^{3}H] 8-OH-DPAT (1 nM final concentration) and the test compounds at seven concentrations, for 30 min, room temperature. The reaction was terminated by filtration through Whatman filters using a Brandel harvester, and radioactivity was counted by liquid scintillation spectrometry. The experiments were performed in triplicate. Data were analyzed using the non-linear curve fitting program EBDA/LIGAND (Biosoft). Results expressed as pK_i values are means of three determinations.

2.4. Chemicals

5-CT, clozapine, spiperone hydrochloride, methiothepin mesylate, thioridazine hydrochloride, pimozide, (+)-butaclamol hydrochloride, chlorpromazine hydrochloride, haloperidol, raclopride, and risperidone were purchased from Sigma-RBI. Ocaperidone was a gift from Janssen (Beerse, Belgium), *trans*-5-chloro-2-methyl-2,3,3a,12b-tetrahydro-1*H*-dibenz[2,3:6,7,5]-oxepino-[4,5c]pyrrole (ORG 5222) from Organon (Oss, Netherlands), tiospirone from Bristol Myers (Princeton, NJ, USA), sertindole from Lundbeck (Copenhagen, Denmark), and olanzapine from Eli Lilly (Indianapolis, IN, USA). (*s*)-*N*-*tert*-butyl-3-(4-(2-methoxyphenyl)piperazine-1-yl)-2-phenylpropanamide [(*s*)-WAY 100135] hydrochloride, WAY 100635 dihydrochloride, nemonapride, and ziprasidone hydrochloride were synthesized by J.-L. Maurel (Centre de Recherche Pierre Fabre). [^{3}H] 8-OH-DPAT [8-hydroxy-2-(di-*n*-propylamino)tetralin] (TRK 850; 200–240 Ci/mmol) and guanosine 5′-[γ-^{35}S] triphosphate, triammonium salt, stabilized (SJ 1308; >1000 Ci/mmol) were purchased from Amersham.

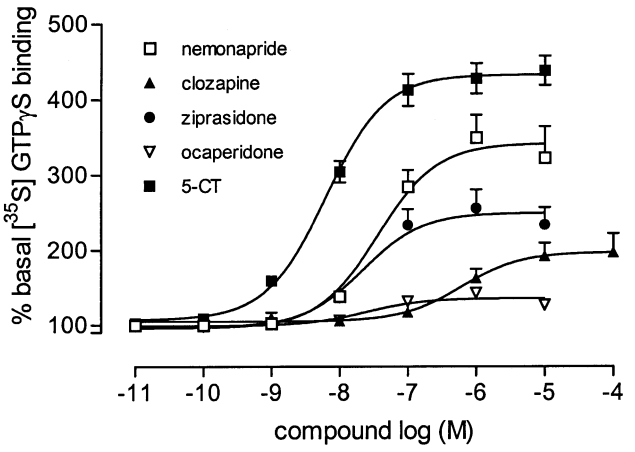

Fig. 2. Agonist activity of antipsychotics at h5-HT$_{1A}$ receptors in comparison with 5-CT. Values are expressed as percentage of basal [^{35}S] GTPγS binding, and are means ± S.E.M. of three to four independent experiments, each performed in triplicate, in the presence of 100 mM NaCl.

3. Results

3.1. Effects of antipsychotics on basal [^{35}S] GTPγS binding under standard (100 mM) Na^+ conditions

Various antipsychotics were tested for their ability to affect basal [^{35}S] GTPγS binding to membranes from HA7

Table 1
Activity of antipsychotics at h5-HT$_{1A}$ receptors

Compounds	h5-HT$_{1A}$		
	E_{max}	pEC$_{50}$	pK_i
nemonapride	342.4 ± 24.35	7.46 ± 0.07	8.42 ± 0.06
ziprasidone	249.8 ± 24.35	7.65 ± 0.03	8.52 ± 0.14
clozapine	203.8 ± 19.85	6.13 ± 0.06	6.99 ± 0.00
raclopride	192.3 ± 14.62	–	5.93 ± 0.34
ocaperidone	135.7 ± 4.70	7.60 ± 0.10	8.08 ± 0.05
olanzapine	107.8 ± 5.76	–	5.84 ± 0.04
ORG 5222	106.5 ± 8.46	–	8.11 ± 0.04
tiospirone	99.48 ± 14.99	–	8.73 ± 0.11
risperidone	95.66 ± 2.37	–	6.56 ± 0.03
pimozide	50.06 ± 3.08	6.41 ± 0.08	6.75 ± 0.07
thioridazine	51.97 ± 10.80	7.38 ± 0.04	6.77 ± 0.05
haloperidol	56.05 ± 10.65	6.81 ± 0.31	5.97 ± 0.21
chlorpromazine	56.58 ± 4.15	6.28 ± 0.13	5.72 ± 0.12
sertindole	48.13 ± 9.53	6.62 ± 0.10	6.41 ± 0.02

Values are expressed as percentage of basal [^{35}S] GTPγS binding, and are means ± S.E.M. of three to four independent experiments, each performed in triplicate, in the presence of NaCl. When the maximal effect was significantly different from basal, a pEC$_{50}$ value was calculated. h5-HT$_{1A}$ affinity values (pK_i) were determined in competition experiments with [^{3}H]8-OH-DPAT and are means ± S.E.M. of three determinations.

Table 2
Na^+-induced changes of the E_{max} value of antipsychotics and 5-HT$_{1A}$ receptor ligands at h5-HT$_{1A}$ receptors

Compounds	100 mM NaCl		No NaCl	
	E_{max}	pEC$_{50}$	E_{max}	pEC$_{50}$
raclopride	192.3 ± 14.62	–	138.0 ± 3.68^b	4.68 ± 0.22
ORG 5222	106.5 ± 8.46	–	106.6 ± 8.53	–
olanzapine	107.8 ± 5.76	–	105.0 ± 1.53	–
tiospirone	99.48 ± 14.99	–	86.41 ± 8.16	–
risperidone	95.66 ± 2.37	–	80.53 ± 3.38^a	6.05 ± 0.15
WAY 100635	93.06 ± 0.39	9.54 ± 0.16	71.79 ± 0.55^c	9.43 ± 0.06
spiperone	77.38 ± 0.67	7.70 ± 0.09	40.62 ± 1.49^a	7.70 ± 0.08
chlorpromazine	56.58 ± 4.15	6.28 ± 0.13	32.67 ± 3.15^b	6.71 ± 0.42
haloperidol	56.05 ± 10.65	6.81 ± 0.31	44.39 ± 5.50	6.97 ± 0.41
thioridazine	51.97 ± 10.80	7.38 ± 0.04	26.08 ± 1.29^a	7.25 ± 0.11
pimozide	50.06 ± 3.08	6.41 ± 0.08	37.92 ± 2.22^a	6.85 ± 0.15
sertindole	48.13 ± 9.53	6.62 ± 0.10	24.02 ± 0.64^a	6.78 ± 0.09
(+)-butaclamol	46.56 ± 12.56	6.74 ± 0.11	34.73 ± 6.26	6.79 ± 0.21
methiothepin	44.21 ± 9.05	8.12 ± 0.04	33.73 ± 2.41	8.17 ± 0.07

Values are the means ± S.E.M. of three to six determinations obtained in the absence and in the presence of 100 mM NaCl. When the maximal effect was significantly different from basal, a pEC$_{50}$ value was calculated. $^aP < 0.05$, $^bP < 0.01$, and $^cP < 0.001$, compared with the E_{max} value obtained in the standard Na^+ condition (*t*-test). Values of WAY 100635 and spiperone are from Cosi and Koek (2000).

cells in standard (100 mM) Na^+ conditions. Basal [^{35}S] GTPγS binding was stimulated by some of the antipsychotics, was inhibited by others, or was not affected (Table 1). The potency with which compounds stimulated or inhibited basal [^{35}S] GTPγS binding correlated with their affinity for h5-HT_{1A} receptors ($r=0.75$, $P<0.025$). The antipsychotics that stimulated basal [^{35}S] GTPγS binding did so to a different extent, and their potencies and maxi-

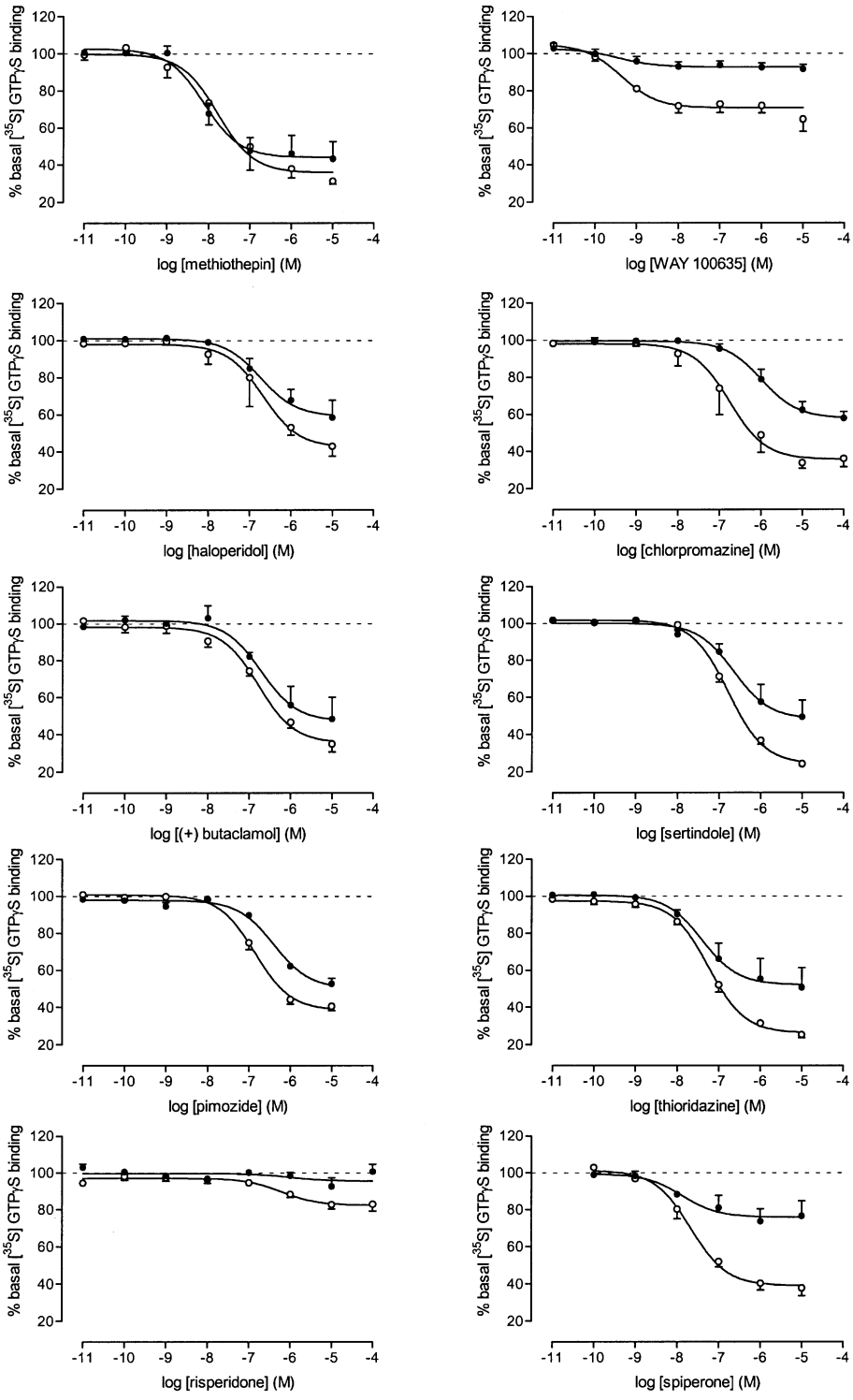

Fig. 3. Inverse agonist activity of antipsychotics and 5-HT_{1A} receptor ligands at h5-HT_{1A} receptors in the presence and in the absence of 100 mM NaCl. Values are expressed as percentage of basal [^{35}S] GTPγS binding, and are means ± S.E.M. of three to four independent experiments, each performed in triplicate. ●: concentration–response curve in the presence of 100 mM NaCl; ○: concentration–response curve in the absence of NaCl.

mal effects were lower than that of the full agonist 5-CT (pEC_{50}= 8.40 ± 0.03; E_{max} = 394.5 ± 2.56%) (Fig. 2). Among the antipsychotics tested, nemonapride was the most efficacious. (Table 1). Raclopride stimulated basal [^{35}S] GTPγS binding only at a single, high (100 μM) concentration, which precluded the estimation of E_{max} and pEC_{50} values by non-linear regression.

Olanzapine, ORG 5222, and tiospirone did not appear to affect basal [^{35}S] GTPγS binding. Risperidone tended to decrease basal [^{35}S] GTPγS binding, but the size of the effect precluded the calculation of a pEC_{50} value.

Pimozide, thioridazine, haloperidol, chlorpromazine, and serindole, decreased basal [^{35}S] GTPγS binding to a similar extent (about 50% of basal [^{35}S] GTPγS binding).

3.2. Na^+ dependency of inverse agonist activity at h5-HT_{1A} receptors

The antipsychotics that either did not affect or decreased basal [^{35}S] GTPγS binding in the standard Na^+ condition were tested also in the low (no NaCl added) Na^+ condition, in comparison with the 5-HT_{1A} inverse agonists, WAY 100635, (+)-butaclamol, methiothepin, and spiperone. The low Na^+ condition did not appear to affect the potency of the compounds to inhibit basal [^{35}S] GTPγS binding, because the pIC_{50} values obtained under both conditions were highly correlated (r = 0.99, slope = 0.86, P < 0.0001).

When NaCl was omitted from the reaction buffer, the E_{max} value varied to an extent that differed among the compounds (Table 2, Fig. 3). Lowering the Na^+ concentration decreased the E_{max} value of raclopride by 28%. It affected significantly the E_{max} of spiperone, chlorpromazine, serindole, and thioridazine by 48–50%, the E_{max} value of WAY 100635, risperidone, and pimozide by16–24%, and appeared to affect the E_{max} value haloperidol, methiotepin, and (+)-butaclamol by 21–26%. ORG 5222 and olanzapine were inactive with or without NaCl. Although the Na^+ concentration affected the E_{max} value of the various compounds in a different manner, it did not markedly affect their rank order (Spearman rank correlation r = 0.87, P < 0.0001).

In the absence of NaCl, (*s*)-WAY 100135 (100 nM) shifted the concentration–response curve of serindole, haloperidol, thioridazine, and chlorpromazine to decrease basal [^{35}S] GTPγS binding to the right with a pK_b varying between 8.37 and 8.91 (Fig. 4). These values are similar to the pK_b of (*s*)-WAY 100135 to shift the concentration–response curve of 5-CT to the right in the presence of 100 mM NaCl [pK_b = 8.33 ± 0.05, similar to the pK_i of (*s*)-WAY 100135 at 5-HT_{1A} receptors, that is, 8.29] (Table 3). (*s*)-WAY 100135 shifted the concentration–response curves of each of the compounds to the right in an apparent parallel manner,

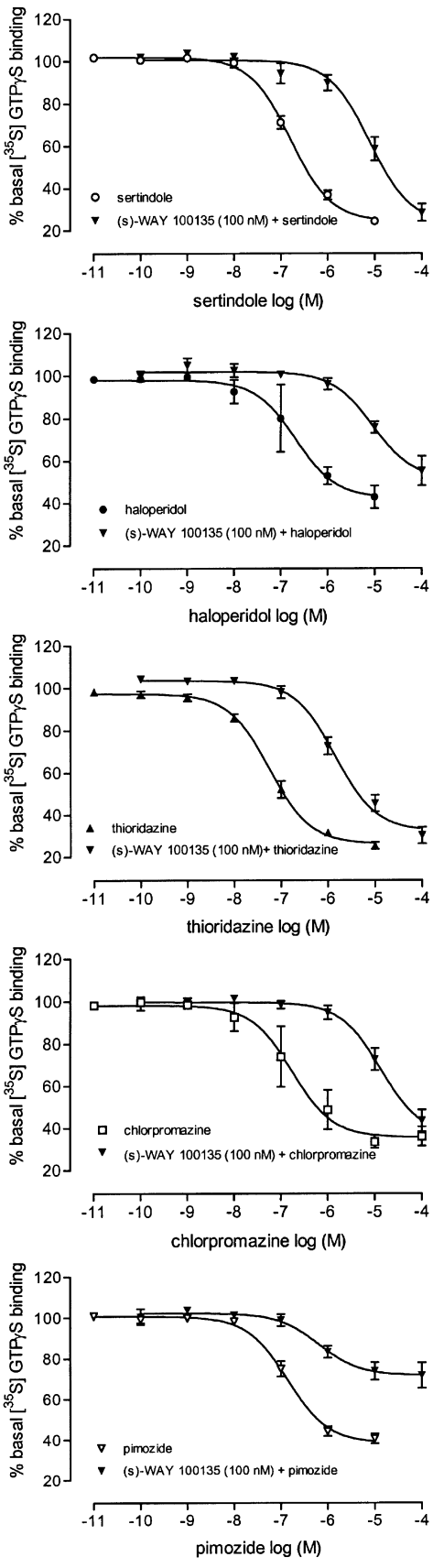

Fig. 4. Antagonism of inverse agonist activity at h5-HT_{1A} receptors by (*s*)-WAY 100135 (100 nM). Values are expressed as percentage of basal [^{35}S] GTPγS binding, and are means ± S.E.M. of three to four independent experiments, each performed in triplicate, in the absence of NaCl.

Table 3
Antagonist properties of 5-HT_{1A} receptor ligands

Compounds (nM)	pK_b	pK_i	antilog(pK_b − pK_i)	E_{max}
(1) spiperone (100)	7.99 ± 0.03	6.57 ± 0.07	26.30	77.38 ± 0.67
(2) (+)-butaclamol (1000)	7.12 ± 0.04	6.04 ± 0.07	12.02	46.56 ± 12.56
(3) methiothepin (10)	8.61 ± 0.04	7.59 ± 0.05	10.47	44.21 ± 9.05
(4) chlorpromazine (1000)	6.59 ± 0.01	5.72 ± 0.12	7.41	56.58 ± 4.15
(5) thioridazine (100)	7.57 ± 0.06	6.77 ± 0.05	6.31	51.97 ± 10.80
(6) WAY 100635 (10)	9.81 ± 0.08	9.20 ± 0.13	4.07	93.06 ± 0.39
(7) sertindole (1000)	6.98 ± 0.05	6.41 ± 0.02	3.72	48.13 ± 9.53
(8) pimozide (1000)	7.26 ± 0.03	6.75 ± 0.07	3.24	50.06 ± 3.08
(9) haloperidol (1000)	6.30 ± 0.06	5.84 ± 0.38	2.88	56.05 ± 10.65
(10) tiospirone (100)	8.90 ± 0.36	8.73 ± 0.11	1.48	99.48 ± 14.99
(11) (*s*)-WAY 100135 (100)	8.33 ± 0.05	8.29 ± 0.02	1.10	117.6 ± 4.51
(12) risperidone (10000)	6.26 ± 0.06	6.56 ± 0.03	0.50	95.66 ± 2.37
(13) ORG 5222 (1000)	7.69 ± 0.10	8.11 ± 0.04	0.38	114.0 ± 3.94
(14) olanzapine (10000)	5.41 ± 0.09	5.84 ± 0.04	0.37	107.8 ± 5.76
(15) ocaperidone (1000)	7.46 ± 0.27	8.08 ± 0.05	0.24	135.7 ± 4.70
(16) clozapine (1000)	6.30 ± 0.15	6.99 ± 0.00	0.20	203.8 ± 19.85
(17) raclopride (10000)	4.80 ± 0.06	5.93 ± 0.34	0.07	192.3 ± 14.62
(18) nemonapride (10000)	6.43 ± 0.28 *	8.42 ± 0.06	0.01	342.4 ± 24.35
(19) ziprasidone (10000)	5.57 ± 0.14 *	8.52 ± 0.14	0.001	249.8 ± 24.35

h5-HT_{1A} receptor antagonist potencies (pK_b) were calculated from the shift of the concentration–response curve of 5-CT by a single concentration of the antagonist, in the presence of NaCl. h5-HT_{1A} affinity values (pK_i) were determined in competition experiments with [^{3}H] 8-OH-DPAT. The effects of the compounds alone are also reported (E_{max}). Values are means ± S.E.M. of three to six determinations.

* Data shown in Fig. 1.

except that of pimozide, which was shifted not only to the right, but also upward (the E_{max} of pimozide changed from 38.46 ± 1.76 to 71.66 ± 2.58) (Fig. 4).

3.3. Antagonist activity at h5-HT_{1A} receptors

Antagonist activity at 5-HT_{1A} receptors was examined, in standard Na^+ conditions, by testing the ability of the compound, at a single concentration, to shift the concentration–response curve of 5-CT to the right. The effects of 5-CT could be antagonized with all the compounds listed in Table 3, which all had affinity for 5-HT_{1A} receptors, but exerted different activity at these receptors, ranging from inverse agonism via neutral antagonism to partial agonism. The pK_b values of the compounds to antagonize 5-CT (Table 3) correlated significantly but weakly with their pK_i values at 5-HT_{1A} receptors ($r = 0.67$, $P < 0.025$). This weak correlation is likely due to the marked variation among the compounds with respect to the ratio of K_b and K_i, which ranged from 26.3 for spiperone to 0.004 for ziprasidone. Note that all compounds with a 5-HT_{1A} antagonist potency at least about 3-fold higher than their 5-HT_{1A} affinity (i.e. having a K_b/K_i ratio greater than ≈ 3) were able to decrease basal [^{35}S] GTPγS binding when tested alone. Such inverse agonism was not observed with compounds that had a smaller K_b/K_i ratio. Compounds with a K_b/K_i ratio lower than 3 but higher than 0.24 were inactive, and those with a K_b/K_i ratio of 0.24 or lower exerted agonist activity. Together, the results obtained with all compounds described in Table 3 showed E_{max} values not to correlate significantly with either K_b or K_i values ($r = -0.28$ and 0.40, respectively), but to correlate strongly ($r = -0.89$, $P < 0.0001$) with the logarithm of the K_b/K_i ratio (Fig. 5). Thus, a pK_b value higher than the pK_i appears to be predictive of inverse agonist activity, similar pK_b and pK_i values suggest neutral antagonism, and pK_b values lower than the pK_i suggest agonist properties.

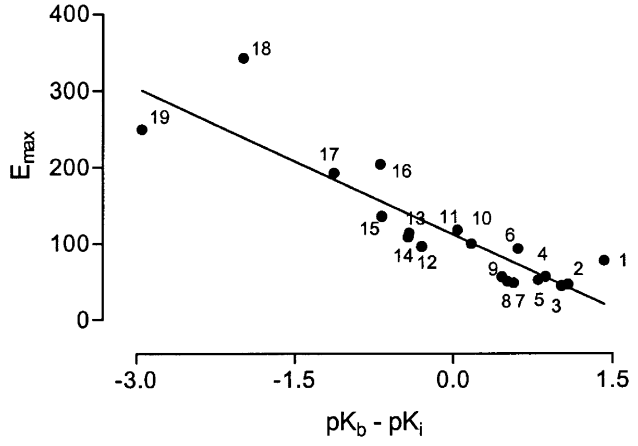

Fig. 5. Correlation between K_b/K_i ratio and efficacy of antipsychotics and 5-HT_{1A} receptor ligands. Compounds are indicated by the numbers listed in Table 3.

4. Discussion

In agreement with previous reports, certain antipsychotics exerted agonist activity at 5-HT_{1A} receptors and others behaved as silent 5-HT_{1A} receptor antagonists. The main finding of the present study is that several antipsychotics (e.g. haloperidol, pimozide, chlorpromazine, thioridazine,

and sertindole) exerted inverse agonist activity at 5-HT_{1A} receptors. This inverse agonist activity, which was Na^+ dependent, was shown by all the compounds whose potency to antagonize the full agonist 5-CT was higher than its affinity for 5-HT_{1A} receptors.

When tested for their ability to affect basal [^{35}S] GTPγS binding to h5-HT_{1A} expressed in HA7 cells, the antipsychotics, nemonapride, ziprasidone, and clozapine were found to have 5-HT_{1A} receptor agonist properties, consistent with previous reports (Assié et al., 1997; Seeger et al., 1995; Zorn et al., 1999). 5-HT_{1A} receptor agonist activity was observed also with ocaperidone. From their maximal stimulation of basal [^{35}S] GTPγS binding, the following efficacy order was apparent: 5-CT>nemonapride>ziprasidone>clozapine>ocaperidone. Because ziprasidone and clozapine had similar affinities for h5-HT_{1A} and hD_2 receptors (Newman-Tancredi et al., 1998) 5-HT_{1A} receptor agonist activity may play a role in their pharmacological profile.

Olanzapine and ORG 5222 behaved as silent antagonists at h5-HT_{1A} receptors: they neither stimulated nor inhibited basal [^{35}S] GTPγS binding, either in the presence or in the absence of added Na^+, and they antagonized the effects of 5-CT with potencies similar to their affinity at 5-HT_{1A} receptors. In the presence of added Na^+, tiospirone (a buspirone analogue reportedly effective in the treatment of schizophrenia while having minimal extrapyramidal side-effects; Jain et al., 1987; Moore et al., 1987) and risperidone behaved the same as olanzapine and ORG 5222, that is, they neither stimulated nor inhibited basal [^{35}S] GTPγS binding, and they antagonized the effects of 5-CT with potencies similar to their affinity at 5-HT_{1A} receptors. Under low Na^+ conditions, however, tiospirone and risperidone tended to decrease basal [^{35}S] GTPγS binding.

In agreement with previous reports, WAY 100635, methiothepin, spiperone, and (+)-butaclamol were inverse agonists at 5-HT_{1A} receptors (Newman-Tancredi et al., 1998; McLoughlin and Strange, 2000; Cosi and Koek, 2000). In contrast with a previous report (Newman-Tancredi et al., 1998) that described the conventional neuroleptics haloperidol, chlorpromazine, and pimozide, as neutral 5-HT_{1A} receptor antagonists, here they showed, together with thioridazine and sertindole, marked inverse agonist activity at h5-HT_{1A} receptors as evidenced by their ability to decrease basal [^{35}S] GTPγS binding, which was enhanced in the absence of added Na^+. They were all able to shift the concentration–response curve of 5-CT to the right, and their pK_b value to antagonize 5-CT was higher than their pK_i at h5-HT_{1A} receptors, and ranging from about 3-fold higher for haloperidol to almost 30-fold higher for spiperone. Neutral antagonists and partial agonists had a pK_b similar to or lower than their pK_i. The results obtained with all compounds showed that the E_{max} values in the [^{35}S] GTPγS binding assay correlate strongly ($r = -0.89$, $P < 0.0001$) with the K_b/K_i ratio. This correlation supports the idea that a high K_b/K_i ratio may be predictive of inverse agonist activity, a K_b/K_i ratio close to one predictive of neutral antagonism, and a ratio lower than one predictive of agonist activity (Newman-Tancredi et al., 1996).

The decrease of basal [^{35}S] GTPγS binding caused by some antipsychotics and 5-HT_{1A} receptor ligands was enhanced by omitting NaCl from the reaction buffer, without affecting their IC_{50}. The extent of the enhancement differed among compounds. Maximal inhibition to about 25% of basal [^{35}S] GTPγS binding was produced by sertindole and thioridazine, in the low Na^+ condition. These results, together with previous findings (see below), suggest that there may be a limit to the extent to which basal [^{35}S] GTPγS binding can be inhibited that depends on the level and efficacy of spontaneous receptor precoupling. For instance, McLoughlin and Strange (2000) recently reported that the maximal effect of the inverse agonist, spiperone, was not significantly affected by the concentration of Na^+ in h5-HT_{1A}–CHO cells, in contrast with the present results. 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%. Basal [^{35}S] GTPγS binding in untransfected HeLa cells, however, has been shown to be almost 70% lower than in HA7 cells (Cosi and Koek, 2000). It is conceivable that differences in G-protein pools account in part for these differences between h5-HT_{1A}–CHO and HA7 cells. Indeed, the affinity of the 5-HT_{1A} receptor for the G protein α-subunit $G_{i\alpha3}$ is higher than that for the $G_{i\alpha2}$ subunit (see references in Raymond et al., 1999). In HeLa cells, $G_{i\alpha3}$ is much more expressed than $G_{i\alpha2}$ (which is almost undetectable by immunoblot), whereas in CHO cells, there is $\approx$9-fold more $G_{i\alpha2}$ than $G_{i\alpha3}$ (Raymond et al., 1993); the former could be a more favorable condition for spontaneous receptor precoupling and consequently more favorable to detect inverse agonist activity. Be that as it may, 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 at 5-HT_{1A} receptors.

Evidence for the involvement of 5-HT_{1A} receptors in the antipsychotics—induced decrease of basal [^{35}S] GTPγS binding, and further evidence for the existence of precoupling of 5-HT_{1A} receptors in HA7 cells (Cosi and Koek, 2000) was provided by the observation that their effects could be antagonized by a selective 5-HT_{1A} receptor antagonist, (*s*)-WAY 100135. In the absence of Na^+, (*s*)-WAY 100135 (100 nM) shifted the concentration–response curves of haloperidol, chlorpromazine, thioridazine, and sertindole, to the right (with a pK_b similar to its pK_b to shift the concentration–response curve of 5-CT in the presence of Na^+), which indicates that the inverse activity of the compounds was mediated by 5-HT_{1A} receptors. The concentration–response curve of pimozide, however, was shifted not only rightward but also upward, suggesting that that the pimozide-induced decrease of basal [^{35}S] GTPγS binding

may involve interactions with sites other than the 5-HT_{1A} receptor.

The therapeutic relevance of the present data for several drugs, including inverse agonists, is unclear since they acted only at very high concentrations at 5-HT_{1A} versus D_2 sites (see affinity values in transfected CHO cells, Newman-Tancredi et al., 1998; Hall and Strange, 1997). Nevertheless, the present finding that antipsychotics interact with 5-HT_{1A} receptors, and do so in a different manner, reinforces the idea that 5-HT_{1A} receptor interactions could play an important role in the pharmacological profile of certain antipsychotic agents. In particular, 5-HT_{1A} receptor agonist properties associated with balanced 5-HT_{1A}/D_2 receptor affinities might be important for antipsychotic activity with less extrapyramidal side effects. Also, the results show that the manner in which compounds interact with the 5-HT_{1A} receptor appears to be related to their K_b/K_i ratio. Finally, the present findings are further evidence that the NaCl concentration can affect the ability to detect inverse agonist activity at cloned human 5-HT_{1A} receptors, likely by influencing receptor precoupling.

Acknowledgements

The authors thank Dr. Marie-Bernardette Assié for helpful discussions and careful reading of the manuscript, and Miss Nathalie Leduc for excellent technical assistance.

References

Andersen, H.L., Kilpatrick, I.C., 1996. Prevention by (±)-8-hydroxy-2-(di-*n*-propylamino)tetralin of both catalepsy and the rises in rat striatal dopamine metabolism caused by haloperidol. Br. J. Pharmacol. 118, 421–427.

Assié, M.-B., Cosi, C., Koek, W., 1997. 5-HT_{1A} receptor agonist properties of the antipsychotic, nemonapride: comparison with bromerguide and clozapine. Eur. J. Pharmacol. 334, 141–147.

Bantick, R.A., Deakin, J.F., Grasby, P.M., 2001. The 5-HT_{1A} receptor in schizophrenia: a promising target for novel atypical neuroleptics? J. Psychopharmacol. 15 (1), 37–46.

Cosi, C., Koek, W., 2000. The putative "silent" 5-HT_{1A} receptor antagonist, WAY 100635, has inverse agonist properties at cloned human 5-HT_{1A} receptors. Eur. J. Pharmacol. 401, 9–15.

De Ligt, R.A.F., Kourounakis, A.P., Ijzerman, A.P., 2000. Inverse agonism at G protein-coupled receptors: (patho)physiological relevance and implication for drug discovery. Br. J. Pharmacol. 130, 1–12.

Dupuis, S.D., Tardif, S., Wurch, T., Colpaert, F.C., Pauwels, P.J., 1999. Modulation of 5-HT_{1A} receptor signaling by point-mutation of cyteine351 in the rat Gαo protein. Neuropharmacology 38, 1035–1041.

Fargin, A., Raymond, J.R., Regan, J.W., Cotecchia, S., Lefkowitz, R.J., Caron, M.G., 1989. Effector coupling mechanisms of cloned 5-HT_{1A} receptor. J. Biol. Chem. 264, 14848–14852.

Hall, D.A., Strange, P.G., 1997. Evidence that antipsychotic drugs are inverse agonist at D2 dopamine receptors. Br. J. Pharmacol. 121, 731–736.

Jain, A.K., Kelwala, S., Moore, N., Gershon, S., 1987. A controlled clinical trial of tiospirone in schizophrenia. Int. Clin. Psychopharmacol. 2, 129–133.

McLoughlin, J.D., Strange, G.P., 2000. Mechanisms of agonism and inverse agonism at serotonin 5-HT_{1A} receptors. J. Neurochem. 74, 347–357.

McMillen, B.A., Scott, S.M., Davanzo, E., 1988. Reversal of neuroleptic-induced catalepsy by novel aryl-piperazine anxiolytic drugs. J. Pharm. Pharmacol. 40, 885–887.

Moore, N.C., Meyendorff, E., Yeragani, V., LeWitt, P.A., Gershon, S., 1987. Tiospirone in schizophrenia. J. Clin. Psychopharmacol. 7, 98–101.

Newman-Tancredi, A., Chaput, C., Verrièle, L., Millan, J.M., 1996. S 15535 and WAY 100635 antagonise 5-HT-stimulated [^{35}S] GTPγS binding at cloned 5-HT_{1A} receptors. Eur. J. Pharmacol. 307, 107–111.

Newman-Tancredi, A., Conte, C., Chaput, C., Spedding, M., Millan, M.J., 1997. Inhibition of the constitutive activity of human 5-HT_{1A} receptors by the inverse agonist, spiperone but not the neutral antagonist, WAY 100635. Br. J. Pharmacol. 120, 737–739.

Newman-Tancredi, A., Gavaudan, S., Conte, C., Chaput, C., Touzard, M., Verrièle, L., Audinot, V., Millan, M.J., 1998. Agonist and antagonist actions of antipsychotic agents at 5-HT_{1A} receptors: a [^{35}S] GTPγS binding study. Eur. J. Pharmacol. 355, 245–256.

Pauwels, P.J., Tardif, S., Wurch, T., Colpaert, F.C., 1997. Stimulated [^{35}S]GTPγS binding by 5-HT_{1A} receptor agonists in recombinant cell lines: modulation of apparent efficacy by G-protein activation state. Naunyn-Schmiedeberg's Arch. Pharmacol. 356, 551–561.

Prinssen, E.P.M., Kleven, M.S., Koek, W., 1996. Effects of dopamine antagonists in a two-way active avoidance procedure in rats: interactions with 8-OH-DPAT, ritanserin, and prazosin. Psychopharmacology 128, 191–197.

Raymond, R.J., Olsen, C.L., Gettys, T.W., 1993. Cell-specific physical and functional coupling of human 5-HT_{1A} receptors to inhibitory G protein α-subunits and lack of coupling to $G_{s\alpha}$. Biochemistry 32, 11064–11073.

Raymond, R.J., Mukhin, V.Y., Gettys, W.T., Garnovskaya, N.M., 1999. The recombinant 5-HT_{1A} receptor: G protein and signaling pathways. Br. J. Pharmacol. 127, 1751–1764.

Rollema, H., Lu, Y., Schmidt, A.W., Zorn, S.H., 1997. Clozapine increases dopamine release in prefrontal cortex by 5-HT_{1A} receptor activation. Eur. J. Pharmacol. 338, R3–R5.

Seeger, T.F., Seymour, P.A., Schmidt, A.W., Zorn, S.H., Schulz, D.W., Lebel, L.A., McLean, S., Guanowsky, V., Howard, H.R., Lowe III, J., Heym, J., 1995. Ziprasidone (CP-88,059): a new antipsychotic with combined dopamine and serotonin receptor antagonist activity. J. Pharmacol. Exp. Ther. 275, 101–113.

Sharma, R.P., Shapiro, L.E., 1996. The 5-HT_{1A} receptor system: possible implications for schizophrenic negative symptomatology. Psychiatry Ann. 26, 88–92.

Stanton, A.J., Beer, S.M., 1997. Characterization of cloned human 5-HT_{1A} receptor cell line using [^{35}S] GTPγS binding. Eur. J. Pharmacol. 320, 267–275.

Wadenberg, M.L., Ahlenius, S., 1991. Antipsychotic-like profile of combined treatment with raclopride and 8-OH-DPAT in the rat: enhancement of antipsychotic-like effects without catalepsy. J. Neural. Transm.: Gen. Sect 83, 495–499.

Zorn, S.H., Lebel, L.A., Schmidt, A.W., Lu, Y., Braselton, J.P., Reynolds, L.S., Sprouse, J.S., Rollema, H., 1999. Pharmacological and neurochemical studies with the new antipsychotic ziprasidone. In: Palomo, T., Benninger, R.J., Archer, T. (Eds.), Interactive Monoaminergic Disorders. Editorial Sintesis, Madrid, Spain, pp. 377–393.