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Inverse Agonist Properties of Antipsychotic Agents at Cloned, Human (h) Serotonin (5-HT)$_{1B}$ and h5-HT$_{1D}$ Receptors

Valérie Audinot, Ph.D., Adrian Newman-Tancredi, Ph.D., Didier Cussac, Ph.D., and Mark J. Millan, Ph.D.

The actions of diverse antipsychotics at cloned h5-HT$_{1B}$ and h5-HT$_{1D}$ receptors were examined employing [^{3}H]-GR125,743 and [^{35}S]-GTPγS for determination of affinities and efficacies, respectively. Compared with hD$_2$ receptors, haloperidol, chlorpromazine and olanzapine showed markedly (>100-fold) lower affinity for h5-HT$_{1D}$ and h5-HT$_{1B}$ receptors at which they expressed inverse agonist properties. Clozapine, risperidone and ocaperidone likewise behaved as inverse agonists at h5-HT$_{1B}$ and h5-HT$_{1D}$ receptors but their affinities were only ~10-fold lower than at hD$_2$ receptors. Moreover, ziprasidone, S16924 and ORG5222 interacted at h5-HT$_{1B}$ and h5-HT$_{1D}$ receptors with affinities similar to hD$_2$ sites. While S16924 and ORG5222 were inverse agonists at h5-HT$_{1B}$ and h5-HT$_{1D}$ sites, ziprasidone was an inverse agonist at h5-HT$_{1D}$ receptors yet a partial agonist at h5-HT$_{1B}$ receptors. These actions of antipsychotics were abolished by the selective, neutral antagonist, S18127. In conclusion, with the exception of ziprasidone, all antipsychotics were inverse agonists at h5-HT$_{1B}$ and h5-HT$_{1D}$ receptors, although they differed markedly in their potency at these sites as compared to hD$_2$ receptors.

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KEY WORDS: *5-HT$_{1B}$; 5-HT$_{1D}$; Schizophrenia; 5-HT$_{1B}$; 5-5-Schizophrenia; Antipsychotic; Clozapine; Inverse agonist*

While neuroleptics, such as haloperidol and chlorpromazine, interact preferentially with D_2 receptors, serotonergic mechanisms may participate in the superior clinical profile of clozapine (Brunello et al. 1995; Meltzer 1995; Roth and Meltzer 1995; Cunningham-Owens 1996; Lieberman et al. 1998). In line with this hypothesis, the recently-characterized antipsychotics, risperidone, olanzapine and ORG5222, display more pronounced activity at 5-HT$_{2A}$ vs. D_2 receptors and control positive symptoms in the relative absence of extrapyramidal side-effects (Meltzer 1995; Arnt and Skarsfeldt 1998; Ichikawa and Meltzer 1999; Remington and Kapur 2000). A further, clinically-effective antipsychotic, ziprasidone, as well as the novel benzopyrrolidine, S16924, share this preference for 5-HT$_{2A}$ vs. D_2 sites and likewise reveal "atypical" profiles in functional studies (Seeger et al. 1995; Millan et al. 1998; see Millan 2000). In addition—in analogy to clozapine—they show agonist actions at 5-HT$_{1A}$ autoreceptors, activation of which is implicated in their functional actions: notably an improvement of mood, cognitive and negative symptoms and an attenuation of extrapyramidal, motor side-effects (Ichikawa and Meltzer 1999 and Millan 2000).

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Although they have received comparatively little attention, 5-HT_{1B} receptors (Moret and Briley 1993; Boess and Martin 1994; Gerhardt and van Heerikhuizen 1997; Barnes and Sharp 1999) are also of potential interest in the pathogenesis and management of schizophrenia.

First, postsynaptic populations are concentrated in regions implicated in psychotic disorders and their treatment: notably, the hippocampus, frontal cortex, amygdala and striatum. These populations principally comprise heteroceptors on the terminals of non-serotonergic neurones (Bruinvels et al. 1993; Saudou and Hen 1994; Bonaventure et al. 1997, 1998; Barnes and Sharp 1999; Sari et al. 1999). Further, inhibitory 5-HT_{1B} receptors are situated presynaptically on serotoninergic neurones themselves (Saudou and Hen 1994; Barnes and Sharp 1999). Of these, a minority may be co-localized with 5-HT_{1A} sites on serotoninergic cell bodies, but they are predominantly found on their terminals (Trillat et al. 1997; Gaster et al. 1998; Evrard et al. 1999; Sarhan and Fillion 1999; Millan et al. 2000a). In this regard, bearing in mind the broad implication of serotonergic mechanisms in schizophrenia (Brunello et al. 1995; Leiberman et al. 1998; Millan 2000), it is of interest to note that terminal-localized 5-HT_{1B} receptors interact with uptake sites for 5-HT (Daws et al. 2000). Indeed, there are indications that polymorphisms of the 5-HT transporter may be associated with alterations in psychosis scores (Malhotra et al. 1998) and that 5-HT transporters are altered in schizophrenics (Joyce et al. 1993; Dean et al. 1995). Further, 5-HT reuptake inhibitors may modify the subjective effects of hallucinogenic agents and antipsychotics in schizophrenic patients (Goff et al. 1990; Bonson et al. 1996; Evins and Goff 1996; Silver et al. 2000; Sills et al. 2000).

Second, 5-HT_{1B} receptors modulate the activity of ascending dopaminergic pathways via actions integrated both at terminals and cell bodies in the ventrotegmental area (mesolimbic and frontocortical pathways) and substantia nigra (nigrostriatal projection) (Johnson et al. 1992; Iyer and Bradberry 1996; Gobert et al. 1997; Barnes and Sharp 1999; Millan et al. 2000a). Activation of 5-HT_{1B} receptors enhances DA release in the nucleus accumbens (Hållbus et al. 1997; Boulenguez et al. 1998) and frontal cortex (Iyer and Bradberry 1996; Matsumoto et al. 1999), yet suppresses DA release in the striatum (Sarhan et al. 1999). Although the relationship of such local actions to the overall influence of systemically-administered selective 5-HT_{1B} ligands remains to be clarified (Galloway et al. 1993; Gobert et al. 1997; Fletcher and Korth 1999a,b; Millan et al. 1999), stimulation of 5-HT_{1B} receptors facilitates cocaine-induced release of mesolimbic DA in rats (Parsons et al. 1999). This action probably reflects disinhibition of mesolimbic dopaminergic pathways via inhibition of GABAergic interneurones (Johnson et al. 1992) and likely underlies potentiation of cocaine-induced reinforcement by engagement of 5-HT_{1B} receptors (Rocha et al. 1998; Parsons et al. 1998; Belzung et al. 2000;—though see Fletcher and Korth 1999a, b). It is also related to the positive influence of 5-HT_{1B} receptors upon motor behavior (Rempel et al. 1993; Saudou et al. 1994; Skingle et al. 1996; Chaouloff et al. 1999; O'Neill et al. 2000). Moreover, 5-HT_{1B} receptors reduce hippocampal release of acetylcholine suggesting an inhibitory influence upon cognitive-attentional processes (Maura et al. 1989; Buhot 1997; Barnes and Sharp 1999; Malleret et al. 1999; Meneses 1999; Sarhan and Fillion 1999). Notably, 5-HT_{1B} receptor activation disrupts "pre-pulse inhibition" and "latent inhibition" (Cassaday et al. 1993; Boulenguez et al. 1998; Dulawa et al. 2000a,b)—sensory processes defective in schizophrenics.

Third, activation of 5-HT_{1B} receptors inhibits aggressive behavior and facilitates anxious states, both of which are prominent in psychotic patients (Saudou et al. 1994; Buhot 1997). Fourth, inasmuch as clozapine and other antipsychotic agents provoke weight gain, the inhibitory and facilitory influence of 5-HT_{1B} receptor agonists and antagonists, respectively, upon food intake deserves mention (Trail et al. 1996; Lucas et al. 1998; De Vry and Schreiber 1999).

One reason underlying the dearth of information concerning actions of antipsychotics at 5-HT_{1B} receptors is the marked difference of rodent vs. homologous, human 5-HT_{1B} receptors (Price et al. 1996; Bonaventure et al. 1999; Barnes and Sharp 1999). Several drugs, despite high affinities at h5-HT_{1B} sites, fail to recognize their rodent counterparts. While guinea pig 5-HT_{1B} sites resemble those of man, this species is difficult to exploit for functional studies of antipsychotics (Sipes and Geyer 1996; Bonaventure et al. 1999; Barnes and Sharp 1999).

In addition to 5-HT_{1B} sites, closely-related 5-HT_{1D} receptors also operate as inhibitory autoreceptors upon serotoninergic perikarya (Davidson and Stamford 1995a,b; Pineyro et al. 1995; Bonaventure et al. 1998; Millan et al. 1999; Xie et al. 1999) and may, in theory, likewise be implicated in psychotic states and their treatment. However, owing to a lack of selective antagonists and knock-out mice, their functional significance remains unclear (Bruinvels et al. 1993; Barnes and Sharp 1999).

In light of the above, the present study examined the actions of haloperidol, clozapine and several other antipsychotics at native, rat and guinea pig 5-HT_{1B} sites and, in particular, at cloned h5-HT_{1B} and h5-HT_{1D} receptors. For determination of affinities, the novel ligand, [^{3}H]-GR 125,743 (Audinot et al. 1997; Bonaventure et al. 1999) was employed and, for evaluation of efficacies, [^{35}S]-GTPγS binding was exploited. This technique quantifies the initial step of h5-HT_{1B} and h5-HT_{1D} receptor coupling to G-proteins (Thomas et al. 1995; Selkirk et al. 1998; Millan et al. 1999; Mize and Alper 1999; Newman-Tancredi et al. 2000; Audinot et al. 2001). Inasmuch

as 5-HT_{1B} and 5-HT_{1D} receptors show constitutive activity (Thomas et al. 1995; Pauwels et al. 1996; Selkirk et al. 1998; Middlemiss et al. 1999; Millan et al. 1999; Audinot et al. 2001), actions of antipsychotics were compared both to those of 5-HT and to those of the prototypical inverse agonist, methiothepin. In addition, interaction studies with the selective, neutral 5-$HT_{1B/1D}$ antagonist, S18127 (Millan et al. 1999) were performed in order to confirm specificity of drug actions.

METHODS

Binding studies at native and recombinant 5-HT_{1B} and 5-HT_{1D} receptors

The procedures employed were as described in Audinot et al. (1997). Briefly, for rat cortex and guinea pig caudate, [^{3}H]-GR125,743 (0.8nM; 70Ci/mmol, Amersham Pharmacia Biotech, Orsay, France) binding assays were carried out for 60 min at 22°C in a buffer containing 50mM Tris-HCl (pH 7.7 at 22°C), 4mM $CaCl_2$, 0.1% ascorbic acid and 10μM pargyline. Cell membranes expressing the human 5-HT_{1B} and 5-HT_{1D} receptors respectively denominated CHO-h5-HT_{1B} and CHO-h5-HT_{1D}, were incubated for 60 min at 22°C in the same buffer without pargyline, with [^{3}H]-GR125,743 (1nM; Audinot et al. 1997; Doménech et al. 1997). 5-HT (10μM) was used to define non- specific binding. Incubations were terminated by rapid filtration using a cell harvester through Whatman GF/B filters pretreated with polyethylenimine (0.1% v/v). Data were analyzed by non-linear regression using the program PRISM (GraphPad Software Inc., San Diego, CA), to yield IC_{50} (Inhibitory Concentration$_{50}$) values. Inhibition constants (K_i) were calculated according to the Cheng-Prusoff equation: $K_i = IC_{50}/[1+(L/K_D)]$, where L is the concentration of the radioligand and K_D the dissociation constant.

Binding studies at native rat and recombinant human D_2 receptors

The procedures employed were as described in Millan et al. (1998). Briefly, for [^{3}H]-raclopride (2 nM, NEN, Les Ulis, France) binding to rat striatal membranes, incubations lasted 30 min at 22°C in a buffer containing 50mM Tris-HCl (pH 7.7 at 22°C), 4mM $CaCl_2$, 0.1% ascorbic acid, 5mM KCl, 1mM $MgCl_2$, 120mM NaCl and 10μM pargyline. Non-specific binding was defined with spiperone (10μM). For [^{125}I]-iodosulpride (0.1nM, Amersham Pharmacia Biotech, Orsay, France) binding to recombinant human D_2 receptors expressed in CHO cells, incubations lasted 30 min at 30°C in the same buffer without pargyline. Non-specific binding was defined with raclopride (10μM).

Effects of receptor ligands on [^{35}S]-GTPγS binding at CHO-h5-HT_{1B} and CHO-h5-HT_{1D} membranes

Receptor-linked G protein activation at h5-HT_{1B} and h5-HT_{1D} receptors was determined by measuring stimulation of [^{35}S]-GTPγS (1000 Ci/mmol, Amersham Pharmacia Biotech, Orsay, France) binding. Briefly, as previously (Audinot et al. 2001) membranes (15–25μg) were incubated (30 min at 22°C) with ligand in a final volume of 250μl of buffer B (20 mM HEPES, pH 7.4 at 22°C, 3 μM GDP, 3 mM $MgCl_2$, 100 mM NaCl and 0.1 nM [^{35}S]-GTPγS). Non-specific binding was defined with GTPγS (10μM). Incubations were terminated by rapid filtration through Whatman GF/B filters. Data were analyzed by non-linear regression using the program PRISM, to yield EC_{50} (Effective Concentration$_{50}$) or IC_{50} values (Inhibitory Concentration$_{50}$). For antagonism experiments, K_B values of S18127 were calculated according to Lazareno and Birdsall (1993).

Membranes and compounds

CHO-h5-HT_{1B} and CHO-h5-HT_{1D} cell membranes expressing 8 and 1.6 pmol/mg receptors respectively were purchased from Euroscreen (Brussels, Belgium). 5-HT sulphate, haloperidol, clozapine and chlorpromazine were purchased from SIGMA (Saint Quentin Fallavier, France) and, methiothepin maleate from Tocris-Fisher Bioblock (Illkirch, France). Olanzapine was obtained from Eli Lilly (Indianapolis, USA), ORG5222 from Organon (Oss, the Netherlands) and ocaperidone from Janssen (Beerse, Belgium). S16924 HCl and ziprasidone HCl were synthesized by G. Lavielle, Servier and S18127 HCl and risperidone by J.L. Peglion, Servier. GR125,5743 is (*N*-[4-methoxy-3-(4-methylpiperazin-1-yl)phenyl}-3-methyl-4-(4-pyridyl)benzamide); S16924 is (*R*)-2-1-{1-[2-(2,3-dihydro-benzo[1,4] dioxin-5-yloxy)-ethyl]-pyrrolodin-3yl}-1-(4-fluorophenyl) ethanone; ORG-5222 is {trans-5-chloro-2-methyl-2,3,3a,12b-tetrahydro-1*H* dibenz[2,3:6,7]oxepino[4,5-c]pyrrole} and S18127 is N-[1-(1,4-benzodioxan-5yl)piperidin 4-yl] N-(indan 2-yl) amine.

RESULTS

Influence upon [^{3}H]-GR125,743 binding at native 5-HT_{1B} receptors

As shown in Table 1, each antipsychotic displayed low affinity at native, rat 5-HT_{1B} receptors relative to native rat D_2 receptors (Millan et al. 1998, 2000b). The affinity of haloperidol at native, guinea pig 5-HT_{1B} receptors was similarly low as compared to native, rat D_2 receptors. Chlorpromazine and olanzapine shared the relatively low affinity of haloperidol at guinea pig 5-HT_{1B} vs. rat D_2 receptors. On the other hand, clozapine, ris-

Table 1. Affinities of Drugs at Native Rat 5-HT_{1B}, Native Guinea Pig 5-HT_{1B}, Cloned h5-HT_{1B} and Cloned h5-HT_{1D} Receptors

	[^{3}H]-GR 125,743				[^{3}H]-raclopride	[^{125}I]-iodosulpride
	r5-HT_{1B}	gp5-HT_{1B}	h5-HT_{1B}	h5-HT_{1D}	rD_2	hD_2
5-HT	9.07 ± 0.04	8.66 ± 0.03	7.79 ± 0.06	9.07 ± 0.04	5.36 ± 0.09	<6
Methiothepin	7.04 ± 0.09	7.98 ± 0.04	8.32 ± 0.04	7.69 ± 0.15	9.10 ± 0.08	9.31 ± 0.03
Haloperidol	<6	<6	5.29 ± 0.05	6.26 ± 0.09	8.94 ± 0.05	9.37 ± 0.05
Chlorpromazine	<6	5.94 ± 0.13	6.06 ± 0.04	6.24 ± 0.17	8.56 ± 0.03	8.55 ± 0.18
Olanzapine	<6	6.57 ± 0.15	6.00 ± 0.13	5.76 ± 0.10	8.04 ± 0.03	8.23 ± 0.07
Clozapine	<6	6.02 ± 0.06	6.01 ± 0.13	6.36 ± 0.04	6.66 ± 0.07	7.12 ± 0.12
Risperidone	<6	7.49 ± 0.15	7.43 ± 0.10	7.69 ± 0.12	8.58 ± 0.11	8.48 ± 0.11
Ocaperidone	6.35 ± 0.11	7.86 ± 0.12	7.55 ± 0.20	8.43 ± 0.20	9.03 ± 0.09	9.52 ± 0.10
S16924	6.04 ± 0.09	7.17 ± 0.11	7.11 ± 0.08	7.69 ± 0.07	7.67 ± 0.09	7.45 ± 0.14
ORG5222	6.91 ± 0.05	8.53 ± 0.16	8.01 ± 0.11	8.21 ± 0.26	9.27 ± 0.05	8.86 ± 0.11
Ziprasidone	5.97 ± 0.16	8.35 ± 0.17	8.79 ± 0.36	8.62 ± 0.08	8.12 ± 0.05	8.40 ± 0.04
S18127	7.88 ± 0.03	8.13 ± 0.07	7.43 ± 0.09	7.40 ± 0.06	5.88 ± 0.01	<6

Affinities are expressed as pK_i and are compared to drug affinities at rat D_2 and cloned hD_2 receptors (Millan et al. 1998, 2000b). Data are Means ± SEMs of at least three independent experiments.

peridone and ocaperidone showed affinities at guinea pig 5-HT_{1B} sites which were only 10 to 20-fold lower than at native rat D_2 receptors. Further, for S16924, ORG5222 and ziprasidone, affinities at guinea pig 5-HT_{1B} sites were similar to rat D_2 receptors.

Influence upon [^{3}H]-GR125,743 binding at h5-HT_{1B} and h5-HT_{1D} receptors

Drug affinities at h5-HT_{1B} receptors were similar to those determined at native, guinea pig 5-HT_{1B} receptors and markedly higher than affinities at native, rat 5-HT_{1B} receptors (Table 1). The affinities of haloperidol, olanzapine and chlorpromazine at h5-HT_{1B} receptors were substantially inferior to their affinities at cloned hD_2 receptors. Clozapine, risperidone and ocaperidone displayed affinities some 10-fold lower than those at hD_2 sites, while S16924, zisprasidone and ORG5222 revealed affinities at h5-HT_{1B} sites similar to their affinities at hD_2 receptors. With the exception of haloperidol, which displayed (like 5-HT) a 10-fold preference for h5-HT_{1D} vs. h5-HT_{1B} receptors, antipsychotics manifested similar affinity for h5-HT_{1D} as compared to h5-HT_{1B} sites.

Influence upon [^{35}S]-GTPγS binding at h5-HT_{1B} and h5-HT_{1D} receptors

5-HT dose-dependently stimulated [^{35}S]-GTPγS binding with a maximal effect (relative to basal values = 100 %) of +252% and +133% at h5-HT_{1B} and h5-HT_{1D} receptors, respectively, (Figure 1, Table 2). These data are in line with our previous studies of these sites (Audinot et al. 2001, Newman-Tancredi et al. 2000). In contrast, methiothepin dose-dependently inhibited basal [^{35}S]-GTPγS binding at h5-HT_{1B} and h5-HT_{1D} receptors with a similar magnitude at both subtypes (Figure 1). S18127 did not significantly modify [^{35}S]-GTPγS binding at either h5-HT_{1B} or h5-HT_{1D} receptors over a substantial concentration range (Figure 1, Table 2). At h5-HT_{1B} receptors, haloperidol, chlorpromazine, olanzapine, clozapine, S16924, risperidone, ocaperidone and ORG 5222, all suppressed basal [^{35}S]-GTPγS binding in analogy to methiothepin and suggestive of inverse agonist properties (Figure 2, Table 2). S16924 and olanzapine showed the least and most pronounced "negative" efficacy in this respect. In contrast to these agents, ziprasidone behave as a weak partial agonist relative to 5-HT (Table 2). Antipsychotic potencies (pIC_{50}s) in exerting inverse agonist actions correlated significantly ($r = 0.74$, $p < .05$) with their affinities (pK_is) at these sites. At h5-HT_{1D} receptors, all antipsychotics manifested inverse agonist actions. Risperidone, zipradone and haloperidol were the most "efficacious" in this regard, while clozapine and S16924 showed relatively modest negative efficacy (Figure 2, Table 2). Again, antipsychotic potencies in exerting inverse agonist actions correlated well ($r = 0.90$, $p < .005$) with their affinities at these sites. Negative efficacies were significantly ($p < .05$) more pronounced at h5-HT_{1D} vs. h5-HT_{1B} sites for haloperidol, ziprasidone and risperidone—as well as ziprasidone—whereas they were similar for other drugs.

Blockade of drug actions with S18127

For antagonist studies with S18127, six key drugs were selected, two from each group of antipsychotics; those with relatively low h5-$HT_{1B/1D}$ vs. hD_2 affinity (haloperidol and olanzapine); those with modest affinity (clozapine and risperidone) and those with relatively high h5-$HT_{1B/1D}$ activity (S16924 and ziprasidone). S18127, which did not itself markedly affect [^{35}S]-GTPγS binding at h5-$HT_{1B/1D}$ receptors (Figure 3) concentration-dependently and completely reversed the inverse agonist effects of haloperidol, olanzapine, clozapine, ris-

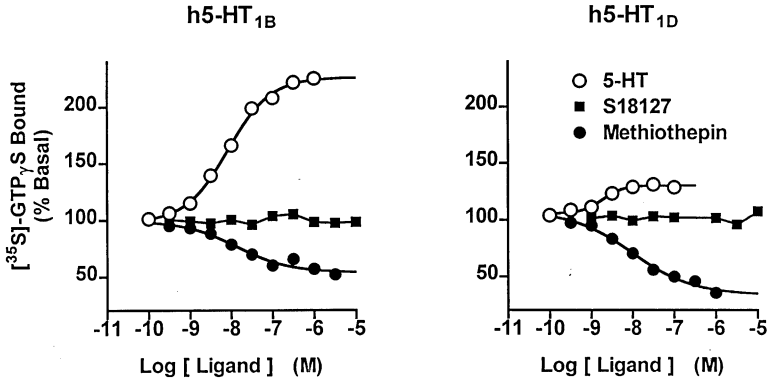

Figure 1. Concentration-response isotherms for influence of the agonist, 5-HT (○), the antagonist, S18127 (■), and the inverse agonist, methiothepin (●), upon [^{35}S]-GTPγS binding to CHO-h5-HT$_{1B}$ (panel A) and CHO-h5-HT$_{1D}$ (panel B) membranes. Results are expressed as a percentage of effect vs. basal level (absence of receptor ligand). Points shown are from representative experiments performed in triplicate and repeated on at least three independent occasions.

peridone and S16924 at both h5-HT$_{1B}$ and 5-HT$_{1D}$ receptors (Figures 3 and 4). This was also the case for the inverse agonist effect of ziprasidone at h5-HT$_{1D}$ receptors (Figure 4). While its partial agonist effect at h5-HT$_{1B}$ receptors was also reversed, this effect was too weak to obtain dose-dependent inhibition (not shown). S18127 exerted its antagonistic action with similar potencies in all cases, although pK_B values were somewhat lower for S16924 and methiothepin at h5-HT$_{1B}$ receptors, and ziprasidone and methiothepin at h5-HT$_{1D}$ receptors (Table 3).

DISCUSSION

[^{35}S]-GTPγS binding at h5-HT$_{1B}$ and h5-HT$_{1D}$ receptors: agonist, antagonist and inverse agonist actions

In corroboration of prior investigations both by this laboratory (Millan et al. 1999; Newman-Tancredi et al. 2000; Audinot et al. 2001) and by others (Thomas et al. 1995; Pauwels et al. 1996; Gaster et al. 1998; Selkirk et al. 1998), [^{35}S]-GTPγS binding revealed agonist properties of 5-HT at both h5-HT$_{1B}$ and h5-HT$_{1D}$ receptors at concentrations

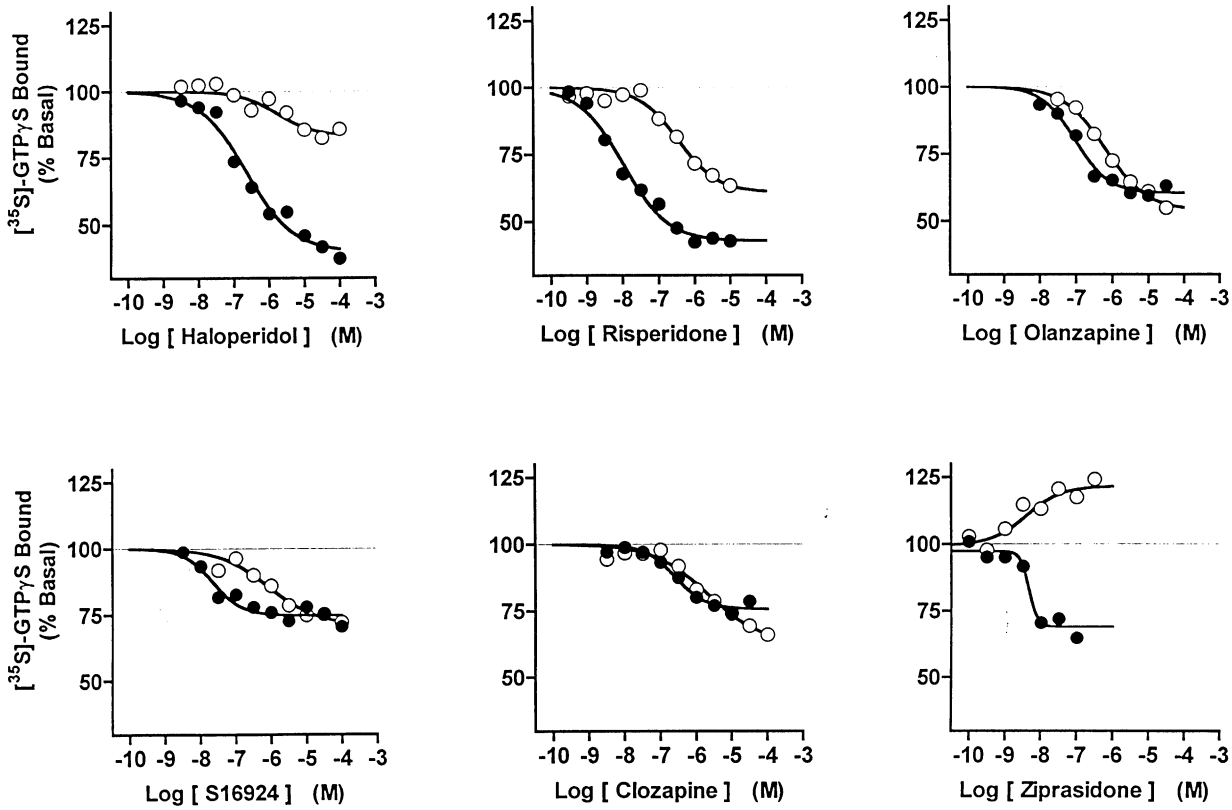

Figure 2. Concentration-response isotherms for influence of the antipsychotics upon [^{35}S]-GTPγS binding to CHO-h5-HT$_{1B}$ (○) and CHO-h5-HT$_{1D}$ (●) membranes. Results are expressed as a percentage of effect vs. basal level (absence of receptor ligand). Points shown are from representative experiments performed in triplicate and repeated on at least three independent occasions.

Table 2. Influence of Drugs upon [^{35}S]-GTPγS Binding at h5-HT_{1B} and h5-HT_{1D} Receptors

	h5-HT_{1B}		h5-HT_{1D}	
	pIC_{50}/pEC_{50}[a]	E_{max}	pIC_{50}/pEC_{50}[a]	E_{max}
5-HT	8.07 ± 0.05[a]	252 ± 13	8.89 ± 0.05[a]	133 ± 5
Methiothepin	7.83 ± 0.14	58 ± 4	8.18 ± 0.17	47 ± 6
Haloperidol	5.66 ± 0.39	76 ± 5	6.96 ± 0.08	34 ± 3
Chlorpromazine	6.15 ± 0.09	68 ± 6	7.29 ± 0.12	50 ± 4
Olanzapine	6.16 ± 0.05	54 ± 2	6.45 ± 0.22	56 ± 3
Clozapine	5.77 ± 0.10	67 ± 1	6.62 ± 0.16	76 ± 1
Risperidone	6.59 ± 0.17	65 ± 4	8.45 ± 0.27	35 ± 9
Ocaperidone	5.93 ± 0.09	59 ± 5	7.98 ± 0.16	30 ± 8
S16924	5.75 ± 0.08	80 ± 1	7.41 ± 0.12	72 ± 6
ORG 5222	8.45 ± 0.15	65 ± 3	8.32 ± 0.19	50 ± 8
Ziprasidone	8.21 ± 0.16[a]	115 ± 1	8.57 ± 0.25	59 ± 5

pEC_{50} ([a]) values are shown for agonist effects, whereas pIC_{50} values are shown for inverse agonist effects. E_{max} is [^{35}S]-GTPγS binding expressed as the percentage of that observed in the absence of receptor ligand (100 %). Data are Means ± SEMs of at least three independent experiments.

corresponding to its affinity for these sites. The stimulation by 5-HT of [^{35}S]-GTPγS binding was less pronounced at h5-HT_{1D} than h5-HT_{1B} sites. This difference is possibly due to the lower density of h5-HT_{1D} vs. h5-HT_{1B} receptor expression inasmuch as receptor reserve, together with factors such as receptor-G protein stoichiometry, may influence agonist efficacy at these sites (Adham et al. 1992; Thomas et al. 1995; Zgombick et al. 1996; Selkirk et al. 1998; Milligan 2000; Audinot et al. 2001; Newman-Tancredi et al. 2000). Both h5-HT_{1B} and h5-HT_{1D} receptors show constitutive activity (Gaster et al. 1998; Millan et al. 1999; Newman-Tancredi et al. 2000; Audinot et al. 2001) and, in confirmation of previous work, a robust inhibition of basal [^{35}S]-GTPγS binding

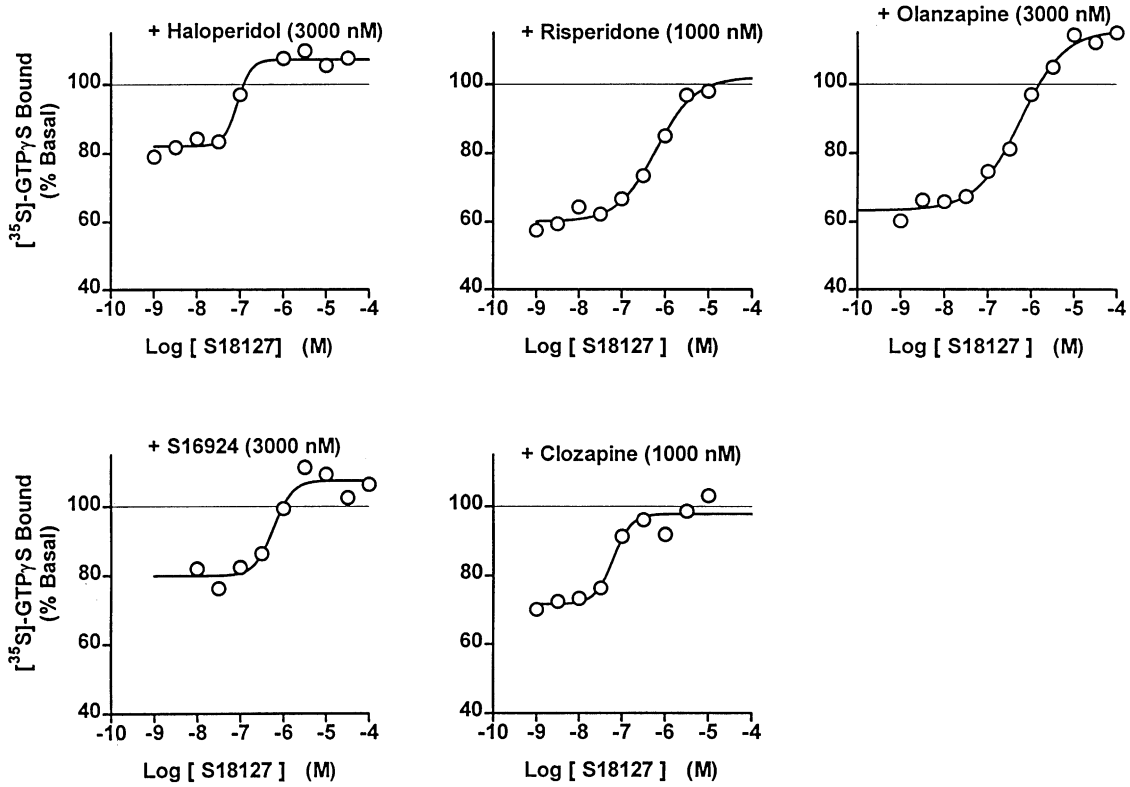

Figure 3. Concentration-response isotherms for influence of S18127 on the inhibition of [^{35}S]-GTPγS binding by haloperidol (3000 nM), clozapine (1000 nM), olanzapine (3000 nM), S16924 (3000 nM), risperidone (1000 nM) at CHO-h5-HT_{1B} membranes. Results are expressed as a percentage of effect vs. basal level (absence of receptor ligand). Points shown are from representative experiments performed in triplicate and repeated on at least three independent occasions.

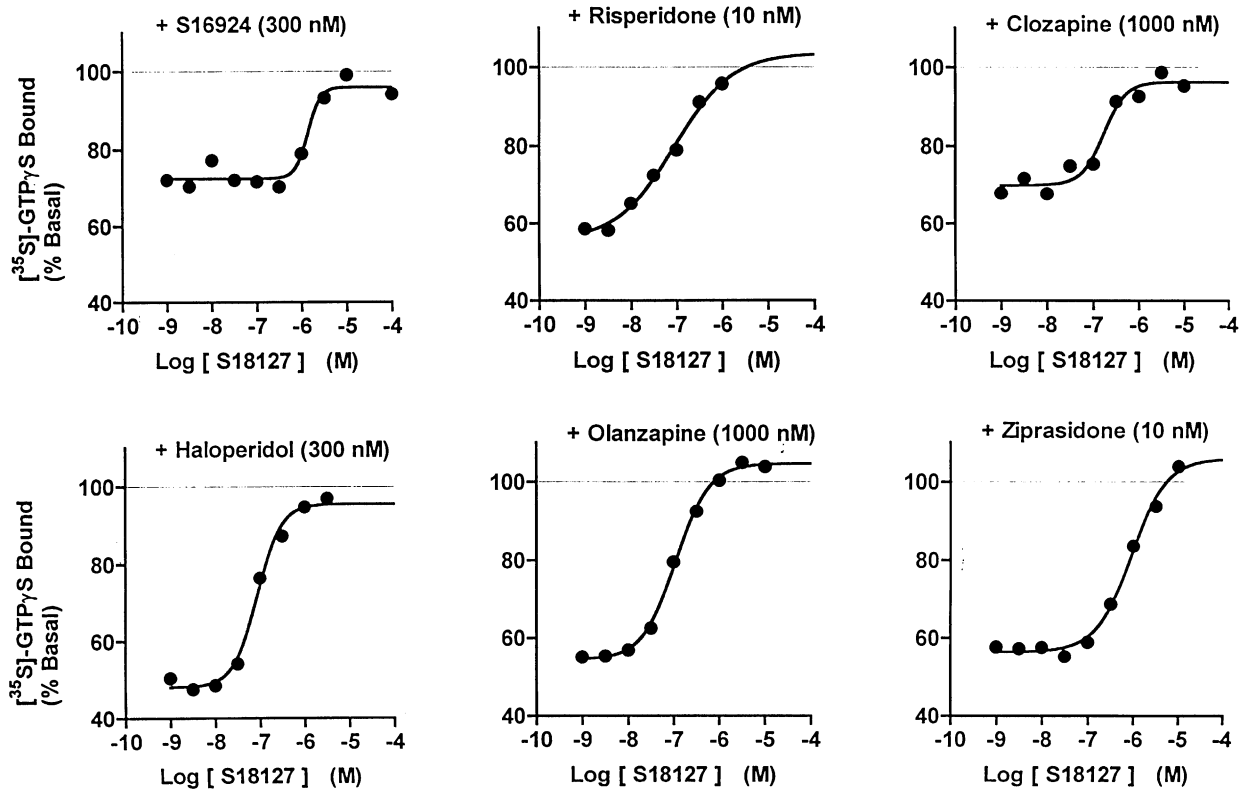

Figure 4. Concentration-response isotherms for influence of S18127 on the inhibition of [^{35}S]-GTPγS binding induced by haloperidol (300 nM), clozapine (1000 nM), olanzapine (1000 nM), S16924 (300 nM), risperidone (10 nM) and ziprasidone (10 nM) at CHO-h5-HT$_{1D}$ membranes. Results are expressed as a percentage of effect vs. basal level (absence of receptor ligand). Points shown are from representative experiments performed in triplicate and repeated on at least three independent occasions.

was elicited by the inverse agonist, methiothepin (Thomas et al. 1995; Pauwels et al. 1996; Zgombick et al. 1996). In distinction, the novel, selective h5-HT$_{1B}$/h5-HT$_{1D}$ ligand, S18127 (Millan et al. 1999), behaved as a "neutral" antagonist. The maximal reduction of [^{35}S]-GTPγS binding was more marked at h5-HT$_{1D}$ than at h5-HT$_{1B}$ receptors and, in analogy to agonists, such differences likely reflect diverse factors including receptor-G-protein stoichiometry and the proportion of spontaneously G-protein-coupled vs. non-coupled receptors: this issue is discussed in detail elsewhere (Audinot et al. 2001; Adham et al. 1992; Thomas et al. 1995; Dickenson and Hill 1998; Newman-Tancredi et al. 1998; Millan et al. 1999; Milligan 2000). Irrespective of such considerations, the present conditions are, evidently, appropriate for the characterization and differentiation of potential agonist vs. inverse agonist actions of antipsychotics at h5-HT$_{1B}$ and h5-HT$_{1D}$ receptors.

Antipsychotic binding affinities at h5-HT$_{1B}$ and h5-HT$_{1D}$ receptors

In corroboration of previous investigations, antipsychotic agents displayed low to negligible affinity for native, rat 5-HT$_{1B}$ receptors (Leysen et al. 1988; Moore et al. 1993; Seeger et al. 1995) suggesting that interactions at these sites are unlikely to play a major role in functional actions in rodents. On the other hand, at both guinea pig 5-HT$_{1B}$ receptors and homologous h5-HT$_{1B}$ sites, they showed a broad range of affinities. In this regard, ziprasidone was a potent agent, extending previ-

Table 3. Influence of the Neutral Antagonist, S18127, upon Agonist (5-HT) and Inverse Agonist Modulation of [^{35}S]-GTPγS Binding at h5-HT$_{1B}$ and h5-HT$_{1D}$ Receptors

	pK$_B$ (S18127)	
Drug	**h5-HT$_{1B}$**	**h5-HT$_{1D}$**
5-HT	7.53 ± 0.10	7.45 ± 0.11
Methiothepin	6.53 ± 0.09	6.77 ± 0.04
Haloperidol	7.49 ± 0.09	7.81 ± 0.10
Olanzapine	7.39 ± 0.13	7.26 ± 0.12
Clozapine	7.73 ± 0.03	7.39 ± 0.03
Risperidone	7.08 ± 0.08	7.90 ± 0.06
S16924	6.52 ± 0.06	7.04 ± 0.08
Ziprasidone	—	6.60 ± 0.14

Antagonist potency (pK$_B$) of S18127 was calculated from IC$_{50}$ values for inhibition of the [^{35}S]-GTPγS binding stimulation induced by 5-HT, or for reversal of the inhibition of [^{35}S]-GTPγS binding induced by methiothepin and antipsychotics. Results are Means ± SEMs of at least three independent experiments.

ous report of its high affinity at bovine, caudate 5-HT_{1B} receptors labeled with the non-selective agent, [^{3}H]5-HT, in the presence of mesulergine and 8-OH-DPAT to mask binding to other sites (Seeger et al. 1995). Further, likewise employing an agonist, [^{3}H]-alniditan, ziprasidone displayed high affinity at h5-HT_{1B} receptors expressed in a L929 cell line (Schotte et al. 1996). Indeed, the latter study, while employing experimental conditions different from the present investigation, observed affinities for various antipsychotics similar to those acquired herein, underpinning the coherence of these findings. In addition, employing a C6 glioma cell line, Schotte et al. 1996 reported affinities for several antipsychotics at h5-HT_{1D} receptors remarkably close to those obtained in the present study and, as herein, haloperidol revealed a (modest) preference for h5-HT_{1D} vs. h5-HT_{1B} receptors. S16924 (Millan et al. 1998), which has not been previously evaluated, mimicked the low affinity of other antipsychotics at rat 5-HT_{1B} sites yet manifested marked affinity for both h5-HT_{1B} and h5-HT_{1D} as well as guinea pig 5-HT_{1B} receptors.

For all known antipsychotic agents, blockade of (mesolimbic) dopamine D_2 receptors is a crucial element in the control of core, positive symptoms (Seeman and Tallerico 1999; Remington and Kapur 2000). It is, thus, important to express and interpret antipsychotic affinities at h5-HT_{1B} and h5-HT_{1D} receptors relative to those at D_2 receptors, an approach previously adopted for other 5-HT receptor types (Roth and Meltzer 1995; Lieberman et al. 1998; Newman-Tancredi et al. 1998; Millan et al. 2000b). Within this perspective, haloperidol, chlorpromazine and olanzapine interacted with h5-HT_{1B} and h5-HT_{1D} sites only at concentrations far higher than hD_2 sites, whereas the degree of separation was modest for clozapine, risperidone and ocaperidone. For the latter agents, h5-HT_{1B} and h5-HT_{1D} sites may, therefore, be of functional pertinence *in vivo*. Moreover, this assertion is clearly valid for S16924, ziprasidone and ORG5222, which display comparable affinities for h5-HT_{1B}, h5-HT_{1D} and hD_2 receptors.

Inverse agonist properties of antipsychotics at h5-HT_{1B} and h5-HT_{1D} receptors

With the exception of a very recent report showing inverse agonist action of ocaperidone at 5-HT_{1D} receptors expressed in a bacillovirus expression system (Brys et al. 2000), this is the first study of functional actions of antipsychotic agents at cloned h5-HT_{1B} and h5-HT_{1D} receptors. Importantly, we used a mammalian expression system, and the key finding was that antipsychotics all behaved as inverse agonists at both h5-HT_{1D} receptors and, with the exception of ziprasidone, at h5-HT_{1B} receptors. In fact, the magnitude of h5-HT_{1B} receptor stimulation elicited by ziprasidone was low. In the presence of 5-HT, then, ziprasidone will exert principally antagonist properties at 5-HT_{1B} receptors. This contention is consistent with the report of Seeger et al. 1995 that ziprasidone antagonizes the influence of 5-HT upon adenylyl cyclase in guinea pig substantia nigra, a structure enriched in 5-HT_{1B} receptors. Nevertheless, under conditions of low serotonergic tone, partial agonist properties of ziprasidone at 5-HT_{1B} receptors may be revealed.

In terms of inverse agonist efficacy, haloperidol, oraperidone and ziprasidone revealed a more marked influence at h5-HT_{1D} vs. h5-HT_{1B} sites, although the significance of differential degrees of inverse agonist efficacy remains unclear (see Millan et al. 1999 and below). Notably, pIC_{50} values for inhibition of basal [^{35}S]-GTPγS binding at h5-HT_{1B} or h5-HT_{1D} receptors were significantly correlated with drug affinities at these sites. This observation supports the specificity of drug actions at h5-HT_{1B} and h5-HT_{1D} receptors in the mediation of their inverse agonist properties. This contention is powerfully supported by studies with the selective, neutral antagonist, S18127 (Millan et al. 1999), which abolished the influence of antipsychotics upon [^{35}S]-GTPγS binding—without itself exerting activity.

As indicated in Table 3, pK_b values for blockade of the actions of methiothepin at both h5-HT_{1B} and h5-HT_{1D} sites by S18127 were lower than those for 5-HT. This difference might reflect blockade of inverse agonist as compared to agonist actions. However, this appears improbable inasmuch as pK_b values for blockade of the actions of several other inverse agonists (such as haloperidol) by S18127 were comparable to those of 5-HT. In fact, it has previously been suggested that a low pK_b for S18127 against methiothepin at h5-HT_{1B} sites reflects a distinctive influence of methiothepin upon receptor-G protein coupling or stability (Audinot et al. 2001). Similar arguments have been advanced as concerns the unusual interaction of methiothepin at h5-HT_{1A} receptors (McLoughlin and Strange 2000). Such differences may similarly underlie contrasting pK_b values for S18127 against other ligands, and notably low pK_bs vs. S16924. Information concerning pK_b values of selective neutral antagonists in blocking the actions of inverse agonists are virtually non-existent. Indeed, while K_b values of neutral antagonists should be constant against various agonists, it remains unclear as to whether this likewise applies to inverse agonists. This question would justify, thus, additional investigation.

Functional significance of inverse agonist and/or antagonist properties of antipsychotics at h5-HT_{1B} and h5-HT_{1D} receptors

The above observations demonstrate that—relative to 5-HT—all antipsychotics behaved as antagonists at h5-HT_{1B} and h5-HT_{1D} receptors and, with the exception of ziprasidone at the former, all showed inverse agonist

properties. Based on observations outlined at the beginning of this article, antagonism (or inverse agonism) at h5-HT_{1B} receptors in schizophrenic patients may have important functional consequences for ziprasidone, ORG5222 and S16924 as well as, possibly, clozapine, ocaperidone and risperidone—although it is improbable that haloperidol, chlorpromazine and olanzapine attain concentrations sufficient to occupy these sites. In particular, actions at 5-HT_{1B} sites may modulate mesolimbic, frontocortical or nigrostriatal DA release, cognitive function and sensory gating and mood.

In fact, it has been suggested that methiothepin elevates resting 5-HT release in the hypothalamus by inverse agonist actions at terminal 5-HT_{1B} receptors (Moret and Briley 1993). However, subsequent studies of methiothepin and selective 5-HT_{1B} antagonists, employing diverse measures of receptor-coupling, 5-HT release and other functional models, have not provided additional evidence for such inverse agonist actions in corticolimbic structures: indeed, it is difficult to differentiate potential inverse agonist actions from antagonism of the inhibitory effects of endogenous 5-HT upon serotonergic neurons (Roberts et al. 1997; Alper and Nelson 1998; Selkirk et al. 1998; Mize and Alper 1999; Stenfors et al. 2000). Moreover, although an increase in 5-HT release by antipsychotics could, theoretically, alleviate depressive states co-morbid with psychotic symptoms, agonist properties of clozapine, S16924 and ziprasidone at inhibitory 5-HT_{1A} autoreceptors would mask any potential increase in 5-HT release due to their inverse agonist/ antagonist actions at 5-HT_{1B} receptors (Sprouse et al. 1997; Millan et al. 1998; Millan 2000). Interestingly, actions at 5-HT_{1B} sites may be involved in the elevation by risperidone of frontocortical 5-HT release in rats (Hertel et al. 1998, 1999), although this action also involves blockade of α_2-adrenoceptors (Hertel et al. 1997). Finally, though studies in guinea pig could, in principle, reveal inverse agonist actions of antipsychotic agents, at 5-$HT_{1B/1D}$ receptors, their numerous interactions at other receptors (Brunello et al. 1995) would render identification of such effects challenging. Further, it remains under discussion as to whether inverse agonist properties are genuinely expressed at 5-HT_{1B}, 5-HT_{1D} or other 5-HT receptor types *in vivo* (see Millan et al. 1999).

SUMMARY AND CONCLUSIONS

In conclusion, antipsychotic agents examined herein show low affinity for rat 5-HT_{1B} receptors questioning the significance of actions at these sites to their functional properties in rodents. However, at both guinea pig and homologous, human 5-HT_{1B} (and 5-HT_{1D}) receptors, they show a spectrum of affinities relative to hD_2 receptors ranging from weak (haloperidol, chlorpromazine and olanzapine) through intermediate (clozapine, olanzapine and risperidone) to pronounced (S16924, ORG5222 and ziprasidone). All antipsychotics manifested low efficacy at h5-HT_{1B} and h5-HT_{1D} sites indicating essentially antagonist properties with respect to 5-HT. Indeed, with the exception of ziprasidone at h5-HT_{1B} sites, all behaved as inverse agonists. The potential significance of inverse agonist properties at cerebral populations of h5-HT_{1B} and h5-HT_{1D} receptors remains unclear. However, as outlined at the beginning of this article, the broad implication of h5-HT_{1B} receptors in the modulation of functions pertinent to schizophrenia and its treatment (see Introduction) suggests that, for certain antipsychotic agents actions at h5-HT_{1B} and/or h5-HT_{1D} receptors may be of functional significance.

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REFERENCES

Adham N, Ellerbrock B, Hartig P, Weinshank RL, Branchek T (1992): Receptor reserve masks partial agonist activity of drugs in a cloned rat 5-hydroxytryptamine$_{1B}$ receptor expression system. Mol Pharmacol 43:427–433

Alper RH, Nelson DL (1998): Characterization of serotonin$_{1A}$ receptor-mediated [^{35}S]GTPγS binding in rat hippocampal membranes. Eur J Pharmacol 343:303–312

Arnt J, Skarsfeldt T (1998): Do novel antipsychotics have similar pharmacological characteristics? A review of the evidence. Neuropsychopharmacol 18:63–101

Audinot V, Lochon S, Newman-Tancredi A, Lavielle G, Millan MJ (1997): Binding profile of the novel 5-$HT_{1B/1D}$ receptor antagonist, [^{3}H]GR 125,743, in guinea-pig brain: a comparison with [^{3}H]5-carboxamidotryptamine. Eur J Pharmacol 327:247–256

Audinot V, Newman-Tancredi A, Millan MJ (2001): Constitutive activity at serotonin 5-HT_{1D} receptors: detection and quantification by homologous GTPγS versus [^{35}S]-GTPγS binding isotherms. Neuropharmacology 40:57–64

Barnes NM, Sharp T (1999): A review of central 5-HT receptors and their function. Neuropharmacology 38:1083–1152

Belzung C, Scearce-Levie K, Barreau S, Hen R (2000): Absence of cocaine-induced place conditioning in serotonin 5-HT_{1B} receptor knock-out mice. Pharmacol Biochem Behav 66:221–225

Boess FG, Martin IL (1994): Molecular biology of 5-HT receptors. Neuropharmacology 33:275–317

Bonaventure P, Schotte A, Cras P, Leysen JE (1997): Autoradiographic mapping of 5-HT_{1B} and 5-HT_{1D} receptors in human brain using [^{3}H]alniditan, a new radioligand. Receptors and channels 5:225–230

Bonaventure P, Langlois X, Leysen JE (1998): Co-localization of 5-HT_{1B} and 5HT_{1D} receptor mRNA in serotonergic

cell bodies in guinea pig dorsal raphé nucleus : a double labeling in situ hybridization histochemistry study. Neurosci Let 254:113–116

Bonaventure P, Umans L, Bakker MHM, Cras P, Langlois X, Luyten WHML, Megens AAHP, Serneels L, Van Leuven F, Leysen JE (1999): Humanization of mouse 5-hydroxytryptamine$_{1B}$ receptor gene by homologous recombination: *In vitro* and *in vivo* characterization. Mol Pharmacol 56:54–67

Bonson KR, Buckholtz JW, Murphy DL (1996): Chronic administration of serotonergic antidepressants attenuates the subjective effects of LSD in humans. Neuropsychopharmacology 14:425–436

Boulenguez P, Peters SL, Mitchell SN, Chauveau J, Gray JA, Joseph MH (1998): Dopamine release in the nucleus accumbens and latent inhibition in the rat following microinjections of a 5-HT_{1B} agonist into the dorsal subiculum : implications for schizophrenia. J Psychopharmacol 12:258–267

Bruinvels AT, Palacios JM, Hoyer D (1993): Autoradiographic characterization and localization of 5-HT_{1D} compared with 5-HT_{1B} binding sites in rat brain. Naunyn Schmeidelberg's Arch Pharmacol 347:569–582

Brunello N, Masotto C, Steardo L, Markstein R, Racagni G (1995): New insights into the biology of schizophrenia through the mechanism of action of clozapine. Neuropsychopharmacology 13:177–213

Brys R, Josson K, Castelli MP, Jurzak M, Lijnen P, Gommeren W, Leysen JE (2000): Reconstitution of the human 5-HT_{1D} receptor-G-protein coupling: evidence for constitutive activity and multiple receptor conformations. Mol Pharmacol 57:1132–1141

Buhot MC (1997): Serotonin receptors in cognitive behaviors. Curr Opinion Neurobiolol 7:243–254

Cassaday HJ, Hodges H, Gray JA (1993): The effect of ritanserin, RU24969 and 8-OH-DPAT on latent inhibition in the rat. J Psychopharmacol 7:63–71

Chaouloff F, Courvoisier H, Moisan MP, Mormède P (1999): GR 127935 reduces basal locomotor activity and prevents RU 24969 but not D-amphetamine-induced hyperlocomotion, in the Wistar-Kyoto hyperactive (WKHA) rat. Psychopharmacology 141:326–331

Cunningham-Owens DC (1996): Adverse effects of antipsychotic agents: Do newer agents offer advantages? Drugs 51:895–930

Davidson C, Stamford JA (1995a): The effect of paroxetine on 5-HT efflux in the rat dorsal raphe nucleus is potentiated by both 5-HT_{1A} and 5-$HT_{1B/D}$ receptor antagonists. Neurosci Let 188: 41–44

Davidson C, Stamford JA (1995b): Evidence that 5-hydroxytryptamine release in rat dorsal raphe nucleus is controlled by 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} autoreceptors. Br J Pharmacol 114:1107–1109

Daws LC, Gould GG, Teicher SD, Gerhardt GA, Frazer A (2000): 5-HT_{1B} receptor-mediated regulation of serotonin clearance in rat hippocampus *in vivo.* J Neurochem 75:2113–2122

Dean B, Opeskin K, Pavey G, Naylor L, Hill C, Keks N, Copolov DL (1995): [^{3}H]paroxetine binding is altered in the hippocampus but not the frontal cortex or caudate nucleus from subjects with schizophrenia. J Neurochem 64:1197–1202

De Vry J, Schreiber R (1999): Effects of selected serotonin 5-HT_1 and 5HT_2 receptor agonists on feeding behavior: possible mechanisms of action. Neurosci and Biobehav Rev 24:341–353

Dickenson JM, Hill SJ (1998): Human 5-HT_{1B} receptor stimulated inositol phospholipid hydrolysis in CHO cells: synergy with Gq-coupled receptors. Eur J Pharmacol 348:279–285

Doménech T, Beleta J, Palacios JM (1997): Characterization of the human serotonin$_{1D}$ receptors using [^{3}H]-GR-125743, a novel radiolabeled serotonin 5$HT_{1D/B}$ receptor antagonist. Naunym Schmiedebergs Arch Pharmacol 356:328–334

Dulawa SC, Gross C, Stale KL, Hen R, Geyer MA (2000a): Knock-out mice reveal opposite roles for 5-HT_{1A} and 5-HT_{1B} receptors in prepulse inhibition. Neuropsychopharmacology 22:650–659

Dulawa SC, Scearce-Levie KA, Hen R, Geyer MA (2000b): Serotonin releasers increase prepulse inhibition in serotonin$_{1B}$ knockout mice. Psychopharmacology 149:306–312

Evins AE, Goff DC (1996): Adjunctive antidepressant drug therapies in the treatment of negative symptoms of schizophrenia. CNS Drugs 6:130–144.

Evrard A, Laporte AM, Chastanet M, Hen R, Hamon M, Adrien J (1999): 5-HT_{1A} and 5-HT_{1B} receptors control the firing of serotoninergic neurons in the dorsal raphe nucleus of the mouse: studies in 5-HT_{1B} knock-out mice. Eur J Neurosci 11:3823–3831

Fletcher PJ, Korth KM (1999a): Activation of 5-HT_{1B} receptors in the nucleus accumbens reduces amphetamine-induced enhancement of responding for conditioned reward. Psychopharmacology 142:165–174

Fletcher PJ, Korth KM (1999b): RU-24969 disrupts d-amphetamine self-administration and responding for conditioned reward via stimulation of 5-HT_{1B} receptors. Behav Pharmacol 10:183–193

Galloway MP, Suchowski CS, Keegan MJ, Hjorth S (1993): Local infusion of the selective 5HT_{1B} agonist CP-93,129 facilitates striatal dopamine release *in vivo.* Synapse 15:90–92

Gaster LM, Blaney FE, Davies S, Duckworth M, Ham P, Jenkins S, Jennings AJ, Joiner GF, King FD, Mulholland KR, Wyman PA, Hagan JJ, Hatcher J, Jones BJ, Middlemiss DN, Price GW, Riley G, Roberts C, Routledge C, Selkirk J, Slade PD (1998): The selective 5-HT_{1B} receptor inverse agonist 1′-methyl-5-[[2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-yl]-2,3,6,7-tetrahydro-spiro[furo[2,3-f]indole-3,4′-piperidine] (SB-224289) potently blocks terminal 5-HT autoreceptor function both *in vitro* and *in vivo.* J Med Chem 41:1218–1235

Gerhardt CC, van Heerikhuizen H (1997): Functional characteristics of heterologously expressed 5-HT receptors. Eur J Pharmacol 334:1–23

Gobert A, Rivet JM, Cistarelli L, Millan MJ (1997): Potentiation of the fluoxetine-induced increase in dialysate levels of serotonin (5-HT) in the frontal cortex of freely moving rats by combined blockade of 5-HT_{1A} and 5-HT_{1B} receptors with WAY 100,635 and GR 127,935. J Neurochem 68:1159–1163

Goff DC, Brotman AW, Waites M, McCormick S (1990): Trial of fluoxetine added to neuroleptics for treatment-resistant schizophrenic patients. Am J Psychiatry 147:492–494

Hållbus M, Magnusson T, Magnusson O (1997): Influence of 5-$HT_{1B/1D}$ receptors on dopamine release in the guinea pig nucleus accumbens: a microdialysis study. Neurosci Let 225:57–60

Hertel P, Lindblom N, Nomikos GG, Svensson TH (1998): Modulation of central serotonergic neurotransmission by risperidone: underlying mechanisms and significance of action. Prog Neuro-Psychopharmacol & Biol Psychiat 22:815–834

Hertel P, Nomikos GG, Schilstrom B, Arborelius L, Svensson TH (1997): Risperidone dose-dependently increases extracellular concentrations of serotonin in the rat frontal cortex : role of α_2 adrenoceptor antagonism. Neuropsychopharmacology 17:44–55

Hertel P, Nomikos GG, Svensson TH (1999): The antipsychotic drug risperidone interacts with auto- and heteroreceptors regulating serotonin output in the rat frontal cortex. Neuropharmacology 38:1175–1184

Ichikawa J, Meltzer HY (1999): Relationship between dopaminergic and serotonergic neuronal activity in the frontal cortex and the action of typical and atypical antipsychotic drugs. Eur Arch Psychiatry Clin Neurosci 249:4 IV/90–IV/98

Iyer RN, Bradberry CW (1996): Serotonin-mediated increase in prefrontal cortex dopamine release: pharmacological characterization. J Pharmacol Exper Ther 377:40–47

Johnson SW, Mercuri NB, North RA (1992): 5-hydroxytryptamine$_{1B}$ receptors block the $GABA_B$ synaptic potential in rat dopamine neurons. J Neurosci 12:2000–2006

Joyce JN, Shane A, Lexow N, Winokur A, Casanova MF, Kleinman JE (1993): Serotonin uptake sites and serotonin receptors are altered in the limbic system of schizophrenics. Neuropsychopharmacology 8:315–336

Lazareno S, Birdsall NJM (1993): Estimation of antagonist K_B from inhibition curves in functional experiments: alternatives to the Cheng-Prusoff equation. Trends Pharmacol Sci 14:237–239

Leysen JE, Gommeren W, Eens A, De Chaffoy De Courcelles D, Stoof JC, Janssen PAJ (1988): Biochemical profile of risperidone, a new antipsychotic. J Pharmacol Exper Ther 247:661–665

Lieberman JA, Mailman RB, Duncan G, Sikich L, Charos M, Nichols DE, Kraus JE (1998): A decade of serotonin research : role of serotonin in treatment of psychosis. Biol Psychiatry 44:1099–1117

Lucas JJ, Yamamoto A, Scearce-Levie K, Saudou F, Hen R (1998): Absence of fenfluramine-induced anorexia and reduced c-fos induction in the hypothalamus and central amygdaloid complex of serotonin 1B receptor knock-out mice. J Neurosci 18:5537–5544

Malhotra AK, Goldman D, Mazzanti C, Clifton A, Breier A, Pickar D (1998): A functional serotonin transporter (5-HTT) polymorphism is associated with psychosis in neuroleptic-free schizophrenics. Mol Psychiatry 3:328–332

Malleret G, Hen R, Guillou JL, Segu L, Buhot MC (1999): 5-HT_{1B} receptor knock-out mice exhibit increased exploratory activity and enhanced spatial memory performance in the morris water maze. J Neurosci 19:6157–6168

Matsumoto M, Togashi H, Mori K, Ueno KI, Miyamoto A, Yoshioka M (1999): Characterization of endogenous serotonin-mediated regulation of dopamine release in the rat prefrontal cortex. Eur J Pharmacol 383:39–48

Maura G, Fedele E, Raiteri M (1989): Acetylcholine release from rat hippocampal slices is modulated by 5-hydroxytryptamine. E J Pharmacol 165:173–179

McLoughlin DJ, Strange PG (2000): Mechanisms of agonism and inverse agonism at serotonin 5-HT_{1A} receptors. J Neurochem 74:347–357

Meltzer HY (1995): Atypical antipsychotic drugs. In Bloom FE, Kupfer DJ (eds), Psychopharmacology: The Fourth Generation in Progress. New York, Raven Press, Ltd, pp 1277–1286

Meneses A (1999): 5-HT system and cognition. Neurosci Biobehav Rev 23:1111–1125

Middlemiss DN, Göthert M, Schlicker E, Scott CM, Selkirk JV, Watson J, Gaster LM, Wyman P, Riley G, Price GW (1999): SB-236057, a selective 5-HT_{1B} receptor inverse agonist, blocks the 5-HT human terminal autoreceptor. Eur J Pharmacol 375:359–365

Millan MJ (2000): Improving the treatment of schizophrenia: focus on 5-HT_{1A} receptors. J Pharmacol Exp Ther 295: 853–861

Millan MJ, Gobert A, Newman-Tancredi A, Audinot V, Lejeune F, Rivet JM, Cussac D, Nicolas JP, Muller O, Lavielle G (1998): S16924 ((R)-2-{1-[2-(2,3-dihydrobenzo[1,4] dioxin-5-yloxy)-ethyl]-pyrrolidin-3yl}-1-(4-fluoro-phenyl)-ethanone), a novel, potential antipsychotic with marked serotonin (5-HT)$_{1A}$ agonist properties: I. Receptorial and neurochemical profile in comparison with clozapine and haloperidol. J Pharmacol Exper Ther 286: 1341–1355

Millan MJ, Gobert A, Audinot V, Dekeyne A, Newman-Tancredi A (1999): Inverse agonists and serotonergic transmission: from recombinant, human serotonin (5-HT)$_{1B}$ receptors to G-protein coupling and function in corticolimbic structures *in vivo.* Neuropsychopharmacology 21:615–675

Millan MJ, Lejeune F, Gobert A (2000a): Reciprocal autoreceptor and heteroreceptor control of serotonergic, dopaminergic and noradrenergic transmission in the frontal cortex: relevance to the actions of antidepressant agents. J Psychopharmacol 14:114–138

Millan MJ, Brocco M, Rivet JM, Audinot V, Newman-Tancredi A, Maiofiss L, Queriaux S, Despaux N, Peglion JL, Dekeyne A (2000b): S18327 (1{2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)piperid-1-yl]ethyl}3-phenyl imidazolin-2-one), a novel, potential antipsychotic displaying marked antagonist properties at α_1- and α_2-adrenergic receptors : II. Functional profile and a multiparametric comparison with haloperidol, clozapine and 11 other antipsychotic agents. J Pharmacol Exp Ther 292:54–66

Milligan G (2000): Insights into ligand pharmacology using receptor-G-protein fusion proteins. Trends Pharmacol Sci 21:24–29

Mize AL, Alper RH (1999): Serotonin 5-$HT_{1B/1D}$ agonist-stimulated [^{35}S]GTPγS binding in rat and guinea pig striatal membranes. Brain Res 836:229–236

Moore NA, Calligaro DO, Wong DT, Bymaster F, Tye NC (1993): The pharmacology of olanzapine and other new antipsychotic agents. Curr Opin Invest Drugs 2(4):281–293

Moret C, Briley M (1993): The unique effect of methiothepin

on the terminal serotonin autoreceptor in the rat hypothalamus could be an example of inverse agonism. J Psychophamacol 7:331–337

Newman-Tancredi A, Audinot V, Moreira C, Verrièle L, Millan MJ (2000): Inverse agonism and constitutive activity as functional correlates of serotonin h5-HT_{1B} receptor/G-protein stoichiometry. Mol Pharmacol 50: 1042–1049

Newman-Tancredi A, Gavaudan S, Conte C, Chaput C, Touzard M, Verrièle L, Audinot V, Millan MJ (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

O'Neill MF, Dobson DR, Sanger GJ (2000): 5-$HT_{1B/1D}$ receptor agonist, SKF99101H, induces locomotor hyperactivity in the guinea pig. Eur J Pharmacol 399:49–55

Parsons LH, Weiss F, Koob GF (1998): Serotonin$_{1B}$ receptor stimulation enhances cocaine reinforcement. J Neurosci 18:10078–10089

Parsons LH, Koob GF, Weiss F (1999): RU 24969, a 5-$HT_{1B/1A}$ receptor agonist, potentiates cocaine-induced increases in nucleus accumbens dopamine. Synapse 32:132–135

Pauwels PJ, Palmier C, Wurch T, Colpaert FC (1996): Pharmacology of cloned human 5-HT_{1D} receptor-mediated functional response in stably transfected rat C6-glial cell lines: further evidence differentiating human 5-HT_{1D} and 5-HT_{1B} receptors. Naunyn Schmeidelberg's Arch Pharmacol 353:144–156

Pineyro G, De Montigny C, Blier P (1995): 5-HT_{1D} receptors regulate 5-HT release in the rat raphe nuclei: *in vivo* voltametry and *in vitro* superfusion studies. Neuropsychopharmacology 13:249–260

Price GW, Roberts C, Watson J, Burton MJ, Mulholland K, Middlemiss DN, Jones B (1996): Species differences in 5-HT autoreceptors. Behav Brain Res 73:79–82

Remington G, Kapur S (2000): Atypical antipsychotics: are some more atypical than others? Psychopharmacology 148:3–15

Rempel NL, Callaway CW, Geyer MA (1993): Serotonin$_{1B}$ receptor activation mimics behavioral effects of presynaptic serotonin release. Neuropsychopharmacology 8: 201–211

Roberts C, Price GW, Gaster L, Jones BJ, Middlemiss DN, Routledge C (1997): Importance of h-5HT_{1B} receptor selectivity for 5-HT terminal autoreceptor activity: an *in vivo* microdialysis study in the freely moving guinea-pig. Neuropharmacology 36:549–557

Rocha BA, Scearce-Levie K, Lucas JJ, Hiroi N, Castanon N, Crabbe JC, Nestler EJ, Hen R (1998): Increased vulnerability to cocaine in mice lacking the serotonin-$_{1B}$ receptor. Nature 393:175–178

Roth BL, Meltzer HY (1995): The role of serotonin in schizophrenia. In Bloom FE, Kupfler DJ (eds), Psychopharmacology: Fourth Generation of Progress. Raven Press Ltd, New York

Sarhan H, Clöez-Tayarani I, Massot O, Fillion MP, Fillion G (1999): 5-HT_{1B} receptors modulate release of [^{3}H]dopamine from rat striatal synaptosomes. Naunyn Schmeidelberg's Arch Pharmacol 359:40–47

Sarhan H, Fillion G (1999): Differential sensitivity of 5-HT_{1B} auto and heteroreceptors. Naunyn Schmeidelberg's Arch Pharmacol 360:382–390

Sari Y, Miquel M-C, Brisorgueil M-J, Ruiz G, Doucet E, Hamon M, Vergé D (1999): Cellular and subcellular localization of 5-hydroxytryptamine$_{1B}$ receptors in the rat central nervous system: immunocytochemical, autoradiographic and lesion studies. Neuroscience 88: 899–915

Saudou F, Hen R (1994): 5-hydroxytryptamine receptor subtypes in vertebrates and invertebrates. Neurochem Int 25:503–532

Saudou F, Amaral DA, Diérich A, LeMeur M, Ramboz S, Segu L, Buhot MC, Hen R (1994): Enhanced aggressive behavior in mice lacking 5-HT_{1B} receptor. Science 265: 1875–1878

Schotte A, Janssen PFM, Gommeren W, Luyten WHML, Van Gompel P, Lesage AS, De Loore K, Leysen JE (1996): Risperidone compared with new and reference antipsychotic drugs: *in vitro* and *in vivo* receptor binding. Psychopharmacolgy 124: 57–73

Seeger TF, Seymour PA, Schmidt AW, Zorn SH, Schulz DW, Lebel LA, McLean S, Guanowsky V, Howard HR, Lowe JA III, 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

Seeman P, Tallerico T (1999): Rapid release of antipsychotic drugs from dopamine D_2 receptors: an explanation for low receptor occupancy and early clinical release upon withdrawal of clozapine or quietapine. Am J Psychiatry 156:876–884

Selkirk JV, Scott C, Ho M, Burton MJ, Watson J, Gaster LM, Collin L, Jones BJ, Middlemiss DN, Price GW (1998): SB224,289—a novel selective (human) 5-HT_{1B} receptor antagonist with negative intrinsic activity. Br J Pharmacol 125:202–208

Sills TL, Greenshaw AJ, Baker GB, Fletcher PJ (2000): Subchronic fluoxetine treatment induces a transient potentiation of amphetamine-induced hyperlocomotion: possible pharmacokinetic interaction. Behav Pharmacol 11:109–116

Silver H, Barash I, Aharon N, Kaplan A, Poyurovsky M (2000): Fluvoxamine augmentation of antipsychotics improves negative symptoms in psychotic chronic schizophrenic patients: a placebo-controlled study. Int Clin Psychopharmacol 15:257–261

Sipes TE, Geyer MA (1996): Functional behavioral homology between rat 5-HT_{1B} and guinea pig 5-HT_{1D} receptors in the modulation of prepulse inhibition of startle. Psychopharmacology 125:231–237

Skingle M, Beattie DT, Scopes DIC, Starkey SJ, Connor HE, Feniuk W, Tyers MB (1996): GR127935, a potent and selective 5-HT_{1D} receptor antagonist. Behav Brain Res 73: 157–161

Sprouse JS, Reynolds L, Rollema H (1997): Do 5-$HT_{1B/1D}$ autoreceptors modulate raphe cell firing? *In vivo* electrophysiological studies in guinea pigs with GR127935. Neuropharmacology 36: 559–567

Stenfors C, Yu H, Ross SB (2000): Enhanced 5-HT metabolism and synthesis rate by the new selective r5-HT_{1B} receptor antagonist, NAS-181, in the rat brain. Neuropharmacology 39:553–560

Thomas DR, Faruq SA, Balcarek JM, Brown AM (1995): Pharmacological characterisation of [^{35}S]-GTPγS bind-

ing to Chinese Hamster Ovary cell membranes stably expressing cloned human 5-HT_{1D} receptor subtypes. J Recep Sig Trans Res 15:199–211

Trail B, Bright F, Lightowler SL, Kennett GA (1996): Effects of selective 5-HT_{1B} receptor ligands on indices of appetite control in the rat. Br J Pharmacol 98:238 pages

Trillat AC, Malagié I, Scearce K, Pons D, Anmella MC, Jacquot C, Hen R, Gardier AM (1997): Regulation of serotonin release in the frontal cortex and ventral hippocampus of homozygous mice lacking 5-HT_{1B} receptors: *In vivo* microdialysis studies. J Neurochem 69:2019–2025

Xie Z, Lee SP, O'Dowd BF, George SR (1999): Serotonin 5-HT_{1B} and 5-HT_{1D} receptors form homodimers when expressed alone and heterodimers when co-expressed. FEBS Let 456:63–67

Zgombick JM, Schechter LE, Adham N, Kucharewicz SA, Weinshank RL, Branchek TA (1996): Pharmacological characterizations of recombinant human 5-HT_{1D} alpha and 5-HT_{1D} beta receptor subtypes coupled to adenylate cyclase inhibition in cell lines: apparent differences in drug intrinsic efficacies between human 5-HT_{1D} subtypes. Naunyn Schmiedebergs Arch Pharmacol 354: 226–236