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In vitro and in vivo characterization of F-97013-GD, a partial 5-HT_{1A} agonist with antipsychotic- and antiparkinsonian-like properties

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Abstract

In order to better define the role of 5-HT_{1A} receptors in the modulation of extrapyramidal motor functions, we investigated the effect of 5-HT_{1A} agonists on tacrine-induced tremulous jaw movements (TJM) in rats, a putative model of parkinsonian tremor. Acute injection of 5-HT_{1A} agonists 8-OH-DPAT and buspirone dose-dependently counteracted the tacrine-induced oral movements ($ED_{50} = 0.04$ and 1.0 mg/kg, respectively), an effect reversed by the selective 5-HT_{1A} antagonist WAY 100,635. In contrast to classical antipsychotics, the atypical antipsychotics risperidone ($ED_{50} = 0.3$ mg/kg) and clozapine ($ED_{50} = 1.5$ mg/kg) blocked the oral movements induced by the cholinomimetic agent at or below the doses required for suppression of conditioned avoidance response. The compound F-97013-GD (6-methyl-2-[4-(naphtylpiperazin-1-yl)-butyl]-3-(2H)-pyridazinone), a putative antipsychotic drug that in functional in vitro and in vivo assays behaved as a mixed dopamine D_2-antagonist and 5-HT_{1A}-partial agonist, also displayed a potent antitremorgenic effect in this paradigm ($ED_{50} = 0.5$ mg/kg). Interestingly, pretreatment with WAY 100,635 blocked the inhibitory effect of F-97013-GD but not that of clozapine. The 5-HT depleting agent para-chlorophenylalanine (PCPA) partially attenuated tacrine-induced TJM but did not block the suppressive effect of 5-HT_{1A} agonists. In addition, only high doses of F-97013-GD induced catalepsy in rodents and, like 8-OH-DPAT and clozapine, the compound reversed the haloperidol-induced catalepsy in rats. These results show that 5-HT_{1A} receptors play a role in the regulation of tacrine-induced TJM and suggest that their activation by novel antipsychotics may not only reduce the extrapyramidal side effects EPS liability, but also be effective in the treatment of parkinsonian tremor.

Keywords: Extrapyramidal side effects; Parkinsonism; Tacrine; Tremulous jaw movements; 5-HT_{1A} agonist; Antipsychotic

1. Introduction

Classical antipsychotics improve positive symptoms of schizophrenia but have a high propensity to cause various extrapyramidal side effects (EPS). They also show a poor efficacy against negative symptoms and cognitive disturbances. Despite the beneficial effects of atypical antipsychotics on negative core-symptoms or in neuroleptic-resistant schizophrenia are yet to be conclusively proved, unequivocal evidence supports that they represent a substantial improvement over classical neuroleptics, notably in terms of greatly reduced incidence of EPS. Mechanisms proposed to explain atypicality include a preferential affinity for serotonin 5-HT_{2A} or D_4 receptors versus D_2 receptors, a mixed antagonistic/agonistic action at D_2 and D_1 receptors and more recently, a fast dissociation from D_2 receptors (Brunello et al., 1995; Kapur and Seeman, 2001).

Accumulating evidence suggests that the 5-HT_{1A} receptors may be implicated both in the pathophysiology of schizophrenia and in the atypical profile of certain antipsychotics. Thus,

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increased 5-HT_{1A} receptor density has been found in the prefrontal cortex of schizophrenic patients in post-mortem studies (Bantick et al., 2001) and recent PET imaging studies revealed an increase in cortical 5-HT_{1A} receptor binding in schizophrenia (Kasper et al., 2002). The antipsychotic clozapine, which has been characterized as a partial agonist at human 5-HT_{1A} receptors, occupies this receptor in man at therapeutic doses (Bantick et al., 2001). Another line of evidence supporting a role for 5-HT_{1A} receptors in the management of schizophrenia comes from studies of functional actions of selective 5-HT_{1A} ligands. Thus, 5-HT_{1A} agonists attenuate neuroleptic-induced catalepsy in rodents after acute and repeated treatment and enhance some of the antipsychotic-like effects of neuroleptics (Millan, 2000; Prinssen et al., 2000). Moreover, the 5-HT_{1A} agonist 8-OH-DPAT reduces dyskinesia in monkeys chronically treated with the D_2 antagonist haloperidol (Bantick et al., 2001). Further extrapyramidal behavioral responses to haloperidol in rats, such as vacuous chewing movements and muscle rigidity, are similarly inhibited by 5-HT_{1A} agonists (Naidu and Kulkarni, 2001; Lorenc-Koci et al., 2003). Finally, the 5-HT_{1A} partial agonist tandospirone was shown in clinical studies to improve cognition when added onto haloperidol (Sumiyoshi et al., 2000) and to decrease tardive dyskinesia in patients treated with antipsychotics (Yoshida et al., 1998).

Previous studies have shown that the stimulation of 5-HT_{1A} receptors is associated with an increase in dopamine turnover (Hamon et al., 1988), dopaminergic cell firing (Arborelius et al., 1993) and dopamine release (Ichikawa and Meltzer, 1999), suggesting that 5-HT_{1A} agonists might have potential therapeutic value in the treatment of Parkinson's disease.

Vacuous or tremulous jaw movements in rats are defined as rapid vertical deflections of the lower jaw that resembles chewing but are not directed at any particular stimulus. They can be induced by a number of conditions including neuroleptics, dopamine depletion and cholinomimetics (Salamone et al., 1998). It has been suggested that oral movements after chronic administration of neuroleptics may represent an animal model of tardive dyskinesia (Seiler et al., 1995). However, other studies have reported the appearance of vacuous movements after acute or subchronic treatment with D_2 antagonists, suggesting that they may model acute motor side effects rather than tardive dyskinesia (see Egan et al., 1996). In this line, considerable evidence indicates that the chewing-like movements induced by acute neuroleptics, dopamine depletion and cholinomimetics share many characteristics with human parkinsonian tremor (for review see Salamone et al., 1998). Interestingly, the relative potencies of atypical antipsychotics clozapine and olanzapine to attenuate the tacrine-induced tremulous jaw movements (TJM) markedly differ from that of the typical antipsychotic haloperidol. Thus, whereas the former drugs produce a consistent and robust reduction of tacrine-effect at doses at which they block operant responding, haloperidol failed to attenuate the cholinomimetic effect at doses 10-fold higher than that needed to block the operant behavior (Trevitt et al., 1999).

In this work, we have studied the role of 5-HT_{1A} receptors in the tacrine-induced TJM in rats as well as its contribution to the antiparkinsonian properties and favorable EPS profile of F-97013-GD (Fig. 1), a new potential atypical antipsychotic with D_2 antagonistic and 5-HT_{1A} agonistic activities. A preliminary report was presented at the 32nd Annual Meeting of the Society for Neuroscience (Del Olmo et al., 2002).

2. Methods

2.1. Animals

Unless otherwise specified below, experiments were conducted on male Wistar rats weighing 200–300 g (FAES FARMA S.A., Spain) and male Swiss CD-1 mice weighing 20–35 g (FAES FARMA) kept on standard laboratory conditions. Laboratory temperature was 21 ± 1 °C and humidity was 60 ± 5%. Rats (6 per cage) and mice (10 per cage) were housed in sawdust-lined cages and had a 12-h light/dark cycle (lights on at 08:00 h) with unrestricted access to standard chow and water except in assays using the oral route of administration. In these cases, animals were subjected to food-restriction during 16–18 h before testing to avoid food interference with drug absorption and water was substituted by a solution containing 8% sucrose and 0.2% NaCl (p/v). All animal studies were performed in accordance with the "European Directive for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (European Union Directive 86/606/EEC) and the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health.

2.2. Receptor and transporter binding studies

Competition binding studies were performed at several receptor types (dopaminergic, serotonergic, adrenergic, histaminergic and muscarinic), as well as at dopamine (DA), serotonin (5-HT) and norepinephrine reuptake sites. Animals were killed by guillotine decapitation and the whole brain was quickly removed and the various areas dissected, weighed and immediately frozen at −70 °C until use. Assay conditions are summarized in Table 1. Isotherms were analyzed by nonlinear regression, using the PRISM program (Graphpad Software Inc., USA) to yield inhibitory concentration (IC_{50}) values. K_i values were derived from IC_{50} values 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 is the dissociation constant of the radioligand.

2.3. Stimulation of [^{35}S]GTPγS binding

Agonist-stimulated [^{35}S]GTPγS binding for the 5-HT_{1A} receptor was determined following the method described by Alper and Nelson (1998) with some modifications. Male Wistar rats weighing 200–250 g (Harlan Ibérica, Spain) were killed by guillotine decapitation and the hippocampal tissue was homogenized using a Kinematica GmbH polytron in 10 volumes of cold Tris buffer 50 mM (pH 7.5) twice, 5 s each. The homogenate was centrifuged (40,000 × g, 4 °C for 10 min) and the remaining pellet resuspended in the same buffer. After homogenization, the resuspended fraction was incubated at 37 °C for 15 min and centrifuged again (40,000 × g, 4 °C for 10 min) and the supernatant was decanted. The pellet was resuspended in 10 volumes of Tris buffer (containing 3 mM $MgCl_2$, 100 mM NaCl, 1 mM

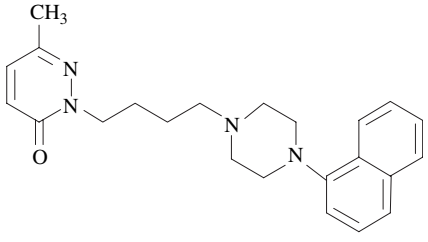

Fig. 1. Structure of F-97013-GD.

Table 1
Standard procedures for receptor and transporter binding studies

Site	Species (tissue)	[^{3}H] ligand (nM)	Non-specific ligand (μM)	References
5-HT$_{1A}$	Rat (TCx)	8-OH-DPAT (0.4)	5-HT (10)	Orjales et al. (2003)
5-HT$_{2A}$	Rat (PFCx)	Ketanserin (0.8)	Methysergide (1)	Orjales et al. (2003)
5-HT$_3$	Rat (ECx)	LY278584 (1.8)	5-HT (10)	Tapia et al. (1999)
5-HT$_4$	Guinea pig (Str)	GR113808 (0.15)	5-HT (100)	Tapia et al. (1999)
D$_1$	Rat (Str)	SCH23390 (0.4)	R(+)SCH23390 (3)	Hess et al. (1986)
D$_2$	Rat (Str)	Raclopride (1.0)	(+)Butaclamol (1)	Tapia et al. (1999)
α_1	Rat (TCx)	Prazosin (0.15)	Phentolamine (10)	Oshita et al. (1991)
α_2	Rat (TCx)	RX821002 (0.75)	Phentolamine (10)	Erdbrügger et al. (1995)
H$_1$	Guinea pig (Cb)	Pyrilamine (1.0)	Astemizole (10)	Benavides et al. (1995)
Mus	Rat (TCx)	QNB (0.2)	Atropine (10)	Watson et al. (1996)
SERT	Rat (TCx)	Paroxetine (0.2)	Fluoxetine (10)	Orjales et al. (2003)
NET	Rat (TCx)	Nisoxetine (0.75)	Mazindol (10)	Orjales et al. (2003)
DAT	Rat (Str)	WIN-35428 (0.6)	Mazindol (10)	Orjales et al. (2003)

TCx, total cortex; PFCx, prefrontal cortex; ECx, entorhinal cortex; Str, striatum; Cb, cerebellum; SERT, serotonin transporter; NET, norepinephrine transporter; DAT, dopamine transporter.

EGTA and 1 mM DL-dithiothreitol), aliquoted in 1 ml microtubes and centrifuged in a microcentrifuge at 20,000 × *g* for 5 min at 4 °C. The supernatants were decanted and the pellets were stored at −70 °C. The incubation buffer contained in a total volume of 250 μl, 3 mM $MgCl_2$, 100 mM NaCl, 1 mM EGTA, 0.2 mM DL-dithiothreitol, 300 μM GDP, 50 mM Tris−HCl, 10 mU/ml adenosine deaminase and 0.1 nM [^{35}S]GTPγS at pH 7.4. Basal binding was determined as that obtained in the absence of the agonist and non-specific binding was defined in the presence of 10 μM unlabelled GTPγS. Stimulated binding was obtained in the presence of increasing concentrations (10^{-9}–10^{-5} M) of 8-OH-DPAT and F-97013-GD, alone or in the presence of 1 μM of WAY 100,635. The incubation for 30 min at 30 °C was started by addition of the membrane suspension (45–60 μg protein/tube). The reaction was terminated by adding twice 5 ml of cold buffer followed by rapid vacuum filtration through Whatman GF/C filters presoaked in the same buffer. The filters were placed into vials containing 4 ml of OptiPhase HiSafe II cocktail (Wallac, UK). After 12 h, the radioactivity present in the vials were determined by liquid scintillation spectrometry (Beckman LS 6000IC). Individual dose–response curves for [^{35}S]GTPγS binding were obtained by non-linear regression analysis. The theoretical maximal effect (E_{max}) and the potency (pEC_{50}) for specific agonist-stimulated [^{35}S]GTPγS binding were calculated using the PRISM program. Data are presented as percent stimulation calculated as: (agonist value-basal value) × 100/basal value, considering the basal value as 100%. Statistical analysis of the results was made using non-paired *t*-test, with a level of significance set at $P < 0.05$.

2.4. [^{3}H]WAY 100,635 competition binding: influence of GTPγS

The method was based on that of Gozlan et al. (1995) with some modifications. Rats were killed by guillotine decapitation and their brains rapidly removed. The hippocampus was dissected and homogenized with a Polytron Ultraturrax T-25 (Janke and Kunkel Staufen, Germany) in 10 volumes of ice-cold 0.32 M sucrose solution for 2 min. The homogenate was centrifuged at 900 × *g* for 10 min at 4 °C, the supernatant collected and centrifuged at 48,000 × *g* for 25 min. The resulting pellet was resuspended in 10 volumes of ice-cold 50 mM Tris−HCl (pH 7.4) buffer and incubated at 37 °C for 15 min to remove endogenous 5-HT. The homogenate was centrifuged at 48,000 × *g* for 25 min and the final pellet was resuspended in 10 volumes of 50 mM Tris−HCl and stored at −70 °C until use. Competition assays with [methoxy-^{3}H]WAY 100,635 (82 Ci/mmol, Amersham Pharmacia, UK) were performed in 96-well plates (Multiscreen HTS, Millipore, USA) in a total volume of 250 μl, consisting of 25 μl of radioligand (0.5 nM), 25 μl of drug, 25 μl of GTPγS (100 μM), 25 μl of hippocampus membranes (1.89 mg/ml) and 150 μl of Hepes buffer 20 mM (pH 7.5). Nonspecific binding was determined in the presence of 5-HT 10 μM. After 60 min of incubation at 22 °C, the plates were filtered and dried at 56 °C for 30 min. Subsequently, 45 μl of scintillation fluid (Microscint-20, Perkin Elmer, USA) was added to each well, the plates sealed on top with an adhesive sheet (TopSeal™, Packard, USA) and the radioactivity counted in a 96-well plate counter (Topcount, Packard, USA). Binding isotherms were analyzed by nonlinear regression using the PRISM program. K_i values were derived from IC_{50} values according to the Cheng–Prusoff equation as mentioned above. All competition isotherms were fitted to both a single and a two-site model. The "goodness of fit" of the two models was compared using the *F*-test. In the case of the two-site model being statistically superior, values of affinity are shown for both high (pK_H) and low (pK_L) affinity sites and for the percentage of sites in the high-affinity binding components.

2.5. Apomorphine climbing test in mice

Animals were orally administered with vehicle or test compounds and 30 min later injected with apomorphine (1 mg/kg, s.c.). Immediately thereafter, mice were individually placed in a wire mesh cylinder (10 cm diameter, 12 cm height). Climbing behavior was scored at 10, 20 and 30 min after the apomorphine injection as follows: (0) four paws on the floor, (1) one or two paws holding the wall and (2) three or four paws holding the wall. The climbing scores of each mouse were summed (maximum score: 6) and the mean value was calculated for each treatment group. ED_{50} values and corresponding 95% confidence limits (CL) were calculated by the method of Litchfield and Wilcoxon (1949).

2.6. Locomotor activity studies in mice and rats

Experiments were performed using a Digiscan System (Omnitech Electronics Inc, USA) equipped with eight activity monitor cages which detect interruptions of 16 photobeams spaced 2.5 cm apart and 2.5 cm above the floor. For spontaneous locomotor activity assays, mice (p.o.) and rats (i.p.) were administered with test compounds and placed singly into activity monitor cages. Distance traveled (cm) was recorded during three consecutive 1-h test periods. To measure the effects on phencyclidine-stimulated motor activity, mice were orally administered and returned to the home-cage. Sixty minutes later, animals were injected with phencyclidine (3 mg/kg s.c.) and placed into the activity cages and monitored for 30 min after a 15-min habituation period. ED_{50} values and 95% CL were calculated by the method of Litchfield and Wilcoxon.

2.7. Conditioned avoidance response (CAR) in rats

A modification of the procedure of Seeger et al. (1995) was used. Four standard, sound-insulated two-way shuttle boxes were used (Coulbourn Instruments, USA). A 4-cm high aluminum hurdle served as a barrier that separated the box into two identical compartments and scrambled foot shocks were

delivered through the grid floor by a constant current shock generator. Rats weighing 150–200 g at the start of the experiments were daily trained for four consecutive days. Training consisted of repeated presentation of a tone-light cue followed 10 s later by a 0.5 mA shock (maximal duration of 5 s). Each animal received 30 trials per session separated by a 15-s intertrial interval. A response was defined as a crossing to the opposite compartment during the tone-light period (avoidance response) or when the shock was on (escape response). The absence of response during the stimulus presentation was considered as an escape failure. Only rats avoiding ≥80% of shocks on day 4 (vehicle-session) were used the next day for drug testing, when drugs were administered i.p. 60 min before the 30-trial session. ED_{50} values and 95% CL were calculated by the method of Litchfield and Wilcoxon.

2.8. Catalepsy in mice and rats

Animals were tested 60, 120 and 180 min after compound administration (p.o. in mice and i.p. in rats) and the catalepsy score for each subject was taken as the average of the three trials. Each trial consisted of placing the animal with its forepaws on a wooden block (9 cm high for rats, 3 cm for mice) and the time spent without a deliberate move to step down was determined (up to maximum of 30 s). ED_{50} values and 95% CL were calculated by the method of Litchfield and Wilcoxon.

2.9. Reversion of haloperidol-induced catalepsy in rats

Thirty minutes after haloperidol injection (1 mg/kg, i.p.), test compounds were administered intraperitoneally (clozapine and F-97013-GD) or subcutaneously (8-OH-DPAT). Catalepsy was measured as described above 30, 60 and 90 min after the second injection. Significant differences from the associated haloperidol-vehicle group were determined by the Mann–Whitney *U*-test.

2.10. Hypothermia and lower lip retraction (LLR) in rats

Animals were transferred to the test room 24 h before the start of the experiments. Body temperature was measured by inserting a rectal probe attached to a digital thermometer (Panlab, Spain) to a depth of 5 cm until a steady read-out was obtained (10 to 15 s). Measurements were taken 30 min before and after drug-injection (i.p., except for 8-OH-DPAT, which was given s.c.). The hypothermic effect of compounds was expressed as temperature change in °C relative to baseline value and corrected for the temperature change observed in the vehicle-treated group. Five minutes after the second temperature measurement, rats were individually placed in transparent observation cages (23 × 23 × 15 cm) and LLR was evaluated for 5 min according to the following scale: (0) lower incisors not visible, (1) lower incisors partly visible, (2) major part of incisors clearly visible and (3) lower incisors completely visible. ED_{50} values and corresponding 95% CL were calculated by the method of Litchfield and Wilcoxon. The ability of WAY 100,635 (1 mg/kg, i.p.) to block the effects of test compounds was evaluated using the same experimental procedure as above, except that the antagonist was injected 30 min before the test compounds. Data of interaction studies were analyzed using either a two-way ANOVA followed by Newman–Keuls test (temperature) or the Mann–Whitney *U*-test (LLR).

2.11. Tacrine-induced TJM in rats

The method was based on that described by Trevitt et al. (1999). Observations of animals were made in transparent cages (23 × 23 × 15 cm) which were elevated 40 cm from the bottom of the table top to allow viewing of the animals from several angles. TJM were defined as rapid vertical deflections of the lower jaw that resembled chewing but were not directed at any stimulus. Rats were placed in the observation cages immediately after tacrine injection (5 mg/kg i.p.) and TJM were counted for 10 min after a 5 min habituation period. Test compounds were i.p. administered 30 min before tacrine, with the exception of 8-OH-DPAT which was injected subcutaneously. Data were analyzed by ANOVA followed by Dunnett's test and ED_{50} values and corresponding 95% CL were calculated by the method of Litchfield and Wilcoxon. In interaction studies, WAY 100,635 (1 mg/kg, i.p.) or vehicle were injected 30 min before the administration of 8-OH-DPAT, F-97013-GD or clozapine. For the studies with the tryptophan hydroxylase inhibitor PCPA, rats received two injections of PCPA (350 mg/kg, i.p.) 48 and 24 h before the tacrine assay. In experiments using WAY 100,635 or PCPA, data were analyzed by two-way ANOVA followed by Newman–Keuls test.

2.12. 5-L-Hydroxytryptophan (5-HTP)-induced head-twitches in rats

The method used was based on that of Rigdon et al. (1996). PCPA (350 mg/kg, i.p.) or saline were injected to rats twice, 48 and 24 h before a challenge of 5-HTP (300 mg/kg, i.p.) and 20 min after 5-HTP injection, rats were placed in transparent observation cages (23 × 23 × 15 cm) and the number of head-twitches were counted for 15 min. Data were analyzed by the Mann–Whitney *U*-test.

2.13. Drugs

Compounds were obtained from the following sources: adenosine deaminase, apomorphine, buspirone, chlorpromazine, DL-dithiothreitol, GDP, GTPγS, haloperidol, 5-HTP, PCPA, phencyclidine, risperidone, ritanserin, scopolamine and tacrine were purchased from Sigma–Aldrich (Madrid, Spain). Clozapine was a gift from Boral Química S.A. (Barcelona, Spain). F-97013-GD and WAY 100,635 were synthesized by FAES FARMA S.A. (Leioa, Spain). In the experiments with 8-hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT) the racemic mixture was used, except in [^{35}S]GTPγS binding assays in which the dextrorotatory enantiomer was utilized (both drugs from Sigma–Aldrich). Unless otherwise indicated, all radioactive ligands were purchased from DuPont NEN (Brussels, Belgium). For binding assays, drugs were dissolved in dimethylsulfoxide (10^{-2} M) and dilutions made in the buffer as appropriate. For behavioral studies, compounds were dissolved or suspended in saline with a drop of Tween-80 and orally given by gavage in a volume of 10 ml/kg or injected in a volume of 5 ml/kg, with the exception of PCPA (10 ml/kg, i.p.). Apomorphine was dissolved in saline containing 1% ascorbic acid (w/v) to prevent oxidation.

3. Results

3.1. Affinities for multiple binding sites

Haloperidol showed high affinity for the dopaminergic D_2 receptor whereas its affinity for serotonergic 5-HT_{2A} and 5-HT_{1A} receptors was low and negligible respectively. Risperidone and clozapine displayed a pronounced preference for 5-HT_{2A} versus D_2 receptors and a modest affinity for the 5-HT_{1A} receptor (Table 2). F-97013-GD showed high affinities for both D_2 and 5-HT_{1A} receptors and moderate to high affinity for the 5-HT_{2A} receptor. Its affinities for adrenergic α_1 and histaminergic H_1 receptors were moderate and it was devoid of significant affinities for D_1 and α_2 receptors. In contrast to clozapine, F-97013-GD showed negligible affinity for muscarinic receptors and like all reference antipsychotics, it did not present any significant affinity for monoamine reuptake sites.

3.2. Stimulation of [^{35}S]GTPγS binding

The reference 5-HT_{1A} agonists 5-HT ($E_{max} = 228.3 \pm 16.1\%$; $pEC_{50} = 6.5 \pm 0.2$) and 8-OH-DPAT ($E_{max} = 150.4 \pm 7.1\%$; $pEC_{50} = 6.9 \pm 0.2$) induced increases in [^{35}S]GTPγS binding in rat hippocampus homogenates (Fig. 2). Incubation with F-

Table 2
Receptor and transporter binding profile of F-97013-GD and reference antipsychotics

Site	F-97013-GD	Haloperidol	Clozapine	Risperidone
5-HT_{1A}	7.1 ± 1.5	>1000	155.3 ± 32.1	393.5 ± 76.9
5-HT_{2A}	23.0 ± 1.9	150.7 ± 24.7	15.9 ± 1.2	0.3 ± 0.07
5-HT_3	>1000	>1000	290.0 ± 51.0	>1000
5-HT_4	>1000	>1000	>1000	>1000
D_1	>1000	134.9 ± 11.0	243.8 ± 21.8	159.6 ± 22.3
D_2	4.9 ± 1.2	4.1 ± 0.9	109.8 ± 18.5	5.7 ± 0.9
α_1	38.9 ± 4.3	21.9 ± 4.4	16.7 ± 1.8	4.0 ± 0.9
α_2	>1000	>1000	373.5 ± 17.7	26.2 ± 3.2
H_1	70.8 ± 11.1	>1000	23.4 ± 7.2	32.3 ± 4.4
Mus	>1000	>1000	107.0 ± 11.2	>1000
SERT	>1000	>1000	>1000	>1000
NET	>1000	>1000	>1000	>1000
DAT	>1000	>1000	>1000	>1000

Data are expressed as mean ± S.E.M. K_i (nM) of at least three separate experiments, each performed in triplicate.

97013-GD also produced a concentration-dependent stimulation of specific [^{35}S]GTPγS binding, with an E_{max} value of 130.8 ± 6.8% and a pEC_{50} value of 6.3 ± 0.1. The selective 5-HT_{1A} antagonist WAY 100,635 (1 μM) completely abolished stimulated [^{35}S]GTPγS binding induced by 5-HT, 8-OH-DPAT and F-97013-GD (data not shown).

3.3. [^{3}H]WAY 100,635 competition binding: influence of GTPγS

5-HT, 8-OH-DPAT and F-97013-GD displayed biphasic competition curves, which were right-shifted and became monophasic in the presence of the receptor/G-protein uncoupling agent GTPγS (Fig. 3). As depicted in Table 3, the differences in pK_i values in the presence or absence of GTPγS were respectively 2.0, 1.74 and 1.18 log units. Risperidone and clozapine showed monophasic isotherms that were also right-shifted in the presence of GTPγS and exhibited modest affinity changes (0.49 and 0.39 log units, respectively). The affinity of WAY 100,635 was unchanged in the presence of GTPγS.

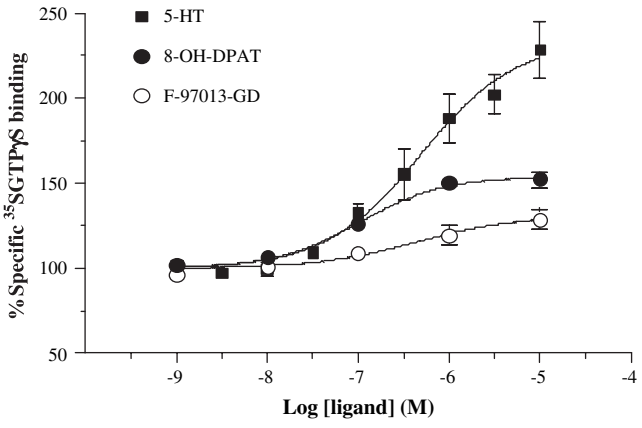

Fig. 2. Dose–response curves for 5-HT-, 8-OH-DPAT- and F-97013-GD-stimulated [^{35}S]GTPγS binding in rat hippocampus. Data points shown are means of triplicate determinations from representative experiments repeated at least four independent times.

3.4. Apomorphine-induced climbing

All compounds antagonized in a dose-related manner the apomorphine-induced climbing in mice (Table 4). Haloperidol and risperidone were the most potent drugs, chlorpromazine and F-97013-GD showed an intermediate potency and clozapine was the less potent compound.

3.5. Phencyclidine-induced hyperactivity

All compounds dose-dependently antagonized the locomotor stimulation elicited by phencyclidine at doses that did not produce motor depression by themselves (Table 4). The selective 5-HT_2 receptor antagonist ritanserin was also evaluated and reversed the PCP-induced hyperlocomotion with an ED_{50} = 0.09 mg/kg (95% CL: 0.05–0.012).

3.6. Conditioned avoidance response

Pretreatment with antipsychotic-drugs and F-97013-GD produced a dose-related disruption of avoidance response in rats trained to avoid signaled shocks by shuttling between the two compartments of the chamber (Table 4). The potency of F-97013-GD (ED_{50} = 4.5 mg/kg) was intermediate between that of chlorpromazine and clozapine. All drugs displayed a higher potency to suppress avoidance response than to induce escape failures [ED_{50} (95% CL)]: haloperidol [0.31 mg/kg (0.23–0.42)], chlorpromazine [7.2 mg/kg (4.5–11.6)], risperidone [2.7 mg/kg (1.7–4.1)], clozapine [21.0 mg/kg (13.9–31.7)] and F-97013-GD [21.2 mg/kg (8.7–51.4)].

3.7. Hypothermia and LLR

8-OH-DPAT, buspirone and F-97013-GD dose-dependently decreased body temperature and produced LLR in rats (Fig. 4). ED_{50} (95% CL) values for the induction of hypothermia were: 8-OH-DPAT 0.10 mg/kg (0.06–0.17), F-97013-GD 1.2 mg/kg (0.7–1.9 mg/kg) and buspirone 2.7 mg/kg (1.5–4.9). Buspirone and F-97013-GD induced the same maximal degree of hypothermia (approximately 1.5 °C), slightly lower than that of 8-OH-DPAT (2.2 °C). Regarding LLR, F-97013-GD [1.3 mg/kg (0.9–2.0)] was more potent than buspirone [4.8 mg/kg (2.7–8.5)] and 10-fold less potent than 8-OH-DPAT [0.12 mg/kg (0.07–0.18)]. In all cases, the pretreatment with WAY 100,635 antagonized both physiological and behavioral effects (Fig. 5).

3.8. Induction of catalepsy

Haloperidol, risperidone and chlorpromazine were, in this order, the most potent compounds to induce catalepsy in

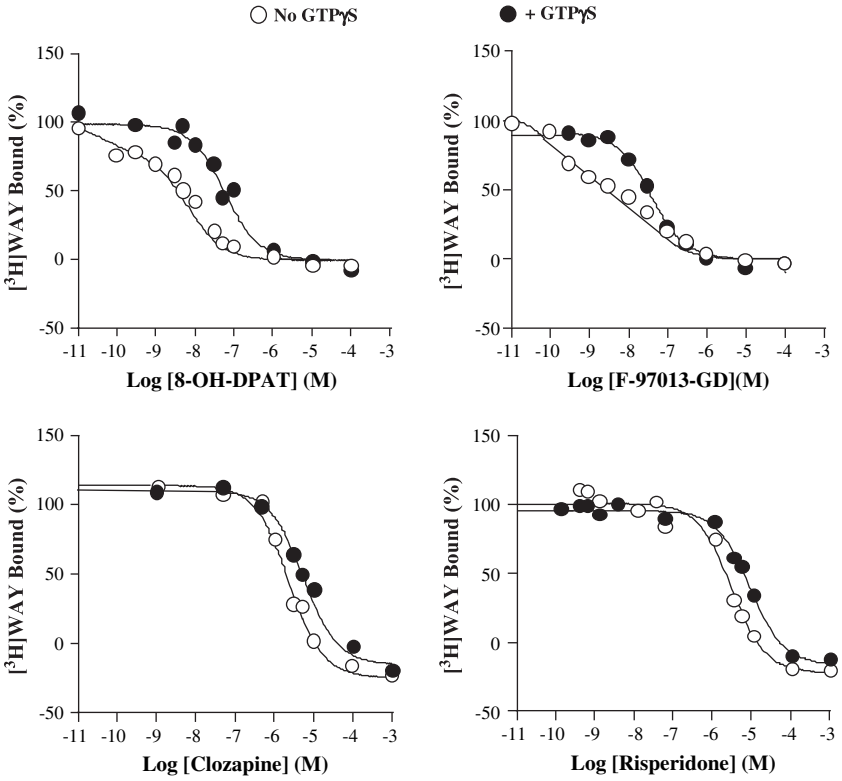

Fig. 3. Influence of GTPγS on affinity of 8-OH-DPAT, F-97013-GD, clozapine and risperidone in competition binding experiments with [^{3}H]WAY 100,635. Radioligand was competed with the ligand indicated, in the presence or absence of the receptor/G-protein uncoupling agent GTPγS. Data points shown are means of triplicate determinations from representative experiments repeated at least two independent times.

mice and rats (Table 4) whereas the cataleptogenic effect of F-97013-GD was markedly lower, close to that of clozapine.

3.9. Reversion of haloperidol-induced catalepsy

Sixty minutes after haloperidol injection rats remained immobile on the wooden block for 23 ± 2 s, reaching the maximal response after 90 min. The ability of 8-OH-DPAT, clozapine and F-97013-GD to reverse haloperidol-induced catalepsy is depicted in Fig. 6. 8-OH-DPAT (1 and 2 mg/kg) and clozapine (10 mg/kg) reduced the haloperidol-induced catalepsy at all time points. The reversion induced by F-97013-GD (5 and 10 mg/kg), was specially marked at 60 min post-haloperidol injection and became weaker at the other tested intervals.

3.10. Tacrine-induced TJM

The cholinesterase inhibitor tacrine produced TJM in the rat in a dose-related manner (1.25–10 mg/kg, data not shown) and the dose of 5 mg/kg was selected for subsequent studies. 8-OH-DPAT and buspirone as well as the muscarinic antagonist scopolamine produced significant dose-dependent reductions in the tacrine-induced effect (Fig. 7A) with ED_{50} (95% CL) values of 0.04 mg/kg (0.02–0.07), 1 mg/kg (0.6–1.7) and 0.09 mg/kg (0.05–0.16), respectively. Risperidone, clozapine and F-97013-GD also blocked the oral movements induced by tacrine ($ED_{50} = 0.3$, 1.5 and 0.5 mg/kg, respectively, Table 4 and Fig. 7B). High doses of haloperidol and chlorpromazine were required to significantly reduce TJM

Table 3
[^{3}H]WAY 100,635 competition binding at rat hippocampal 5-HT_{1A} receptors in the presence and absence of GTPγS

	No GTPγS			With GTPγS	Affinity change
	pK_i or pK_H	pK_L	% High	pK_i	$pK_H - pK_i$
5-HT	9.87 ± 0.43	7.76 ± 0.06	68 ± 1.5	7.87 ± 0.17	2.00
8-OH-DPAT	9.49 ± 0.55	7.97 ± 0.09	63 ± 12	7.75 ± 0.12	1.74
F-97013-GD	9.56 ± 1.10	7.74 ± 0.07	54 ± 10	8.38 ± 0.15	1.18
Clozapine	6.79 ± 0.33			6.40 ± 0.23	0.39
Risperidone	7.18 (7.41; 6.95)			6.69 (6.77; 6.60)	0.49
WAY 100,635	9.39 ± 0.26			9.31 ± 0.13	0.08

[^{3}H]WAY 100,635 (0.5 nM) was competed with serotonergic and antipsychotic ligands for binding to membranes of hippocampus. The affinity change was calculated by subtracting the pK_i value determined in the presence of GTPγS by the pK_i/pK_H value determined in its absence. % High, percentage of high-affinity sites. Data are means ± S.E.M. of at least two determinations performed in triplicate. In the case of two determinations, individual values are given in parentheses.

Table 4
Behavioral effects of F-97013-GD and reference antipsychotics in rodents

Test	Species	F-97013-GD	Haloperidol	Chlorpromazine	Risperidone	Clozapine
Apomorphine-induced climbing	Mice	2.9 (1.8–4.5)	0.11 (0.05–0.25)	2.0 (1.3–3.1)	0.15 (0.07–0.35)	12.3 (9.7–15.7)
Phencyclidine-induced hyperactivity	Mice	0.30 (0.17–0.53)	0.07 (0.03–0.14)	0.58 (0.20–1.72)	0.02 (0.01–0.039)	0.98 (0.49–1.96)
Catalepsy	Mice	67.4 (43.9–103.6)	1.0 (0.3–3.0)	14.1 (9.0–22.0)	7.75 (4.4–13.6)	48.2 (24.8–93.7)
SLA	Mice	12.9 (5.6–29.5)	0.46 (0.21–1.00)	0.66 (0.18–2.48)	0.29 (0.18–0.48)	5.5 (3.5–8.6)
CAR	Rats	4.5 (3.1–6.7)	0.15 (0.12–0.19)	2.4 (1.8–3.1)	0.35 (0.30–0.41)	7.1 (5.2–9.7)
Catalepsy	Rats	31.5 (19.1–52.1)	0.23 (0.11–0.46)	3.3 (1.7–6.4)	1.6 (1.0–2.8)	40.2 (20.4–79.2)
Tacrine-induced TJM	Rats	0.5 (0.3–0.9)	>1	>4	0.3 (0.2–0.5)	1.5 (0.8–2.6)
SLA	Rats	4.2 (1.9–9.7)	0.23 (0.11–0.50)	2.7 (1.4–5.2)	0.20 (0.06–0.68)	10.9 (5.3–22.5)

CAR, conditioned avoidance response; SLA, spontaneous locomotor activity; TJM, tremulous jaw movements. Drugs were administered p.o. (mice) or i.p. (rats). Values represent the ED_{50} (mg/kg) and values in parentheses are 95% CL.

(ED_{50} >1 and >4 mg/kg, respectively). In separate experiments, WAY 100,635 prevented the ability of 8-OH-DPAT and F-97013-GD, but not that of clozapine to block the tacrine-induced TJM (Fig. 7C). Finally, as depicted in Fig. 8, the pretreatment with the 5-HT depleting agent PCPA, which per se reduced the number of tacrine-induced TJM, failed to modify the inhibitory effect of 8-OH-DPAT and F-97013-GD.

3.11. 5-HTP-induced head-twitches

The aim of these experiments was only to assess the efficacy of PCPA to deplete brain 5-HT. PCPA-treated rats (2 × 350 mg/kg) displayed a significant reduction in the number of head-twitches when comparing with control animals (6.2 ± 2.2 and. 25.5 ± 4.9 respectively, $P < 0.01$).

3.12. Inhibition of spontaneous locomotor activity

The potency of compounds to reduce locomotor activity is indicated in Table 4. Mice were generally more sensitive than rats to the sedative properties of drugs. When orally administered to mice, F-97013-GD ($ED_{50} = 12.9$ mg/kg) showed the lowest potency in decreasing locomotor activity. In rats, only clozapine exhibited a lower sedative potency than F-97013-GD ($ED_{50} = 10.9$ and 4.2 mg/kg, i.p., respectively).

4. Discussion

Main symptoms of idiopathic and drug-induced parkinsonism such as akinesia and muscle rigidity can be modeled in rodents after administration of neuroleptic drugs. Regarding tremor, it has been suggested that cholinomimetic-induced jaw movements in rats have many of the temporal, pharmacological and anatomical characteristics of parkinsonian tremor (Salamone et al., 1998). Like other cholinomimetic drugs (pilocarpine, physostigmine) the cholinesterase inhibitor tacrine produced in our hands repetitive bursts of jaw movements characterized by vertical deflections of the lower jaw. Consistent with previous works (Trevitt et al., 1999), we found that the muscarinic antagonist scopolamine and the potent 5-HT_{2A} antagonist risperidone blocked tacrine-induced TJM. Further evidence supporting the role of the serotonergic system in TJM was provided by the 5-$HT_{2A/2C}$ antagonist mianserin and the highly selective 5-HT_{2C} antagonist SB 242084, which also block cholinomimetic-induced jaw movements (Trevitt et al., 1999; Weber et al., 2004). In spite of the potential role of the 5-HT_{1A} receptors in attenuating EPS liability of antipsychotic drugs (Yoshida et al., 1998; Millan, 2000; Bantick et al., 2001), to our knowledge there are no data available in the literature regarding the effect of 5-HT_{1A} agonists on cholinomimetic-enhanced oral movements. The results of

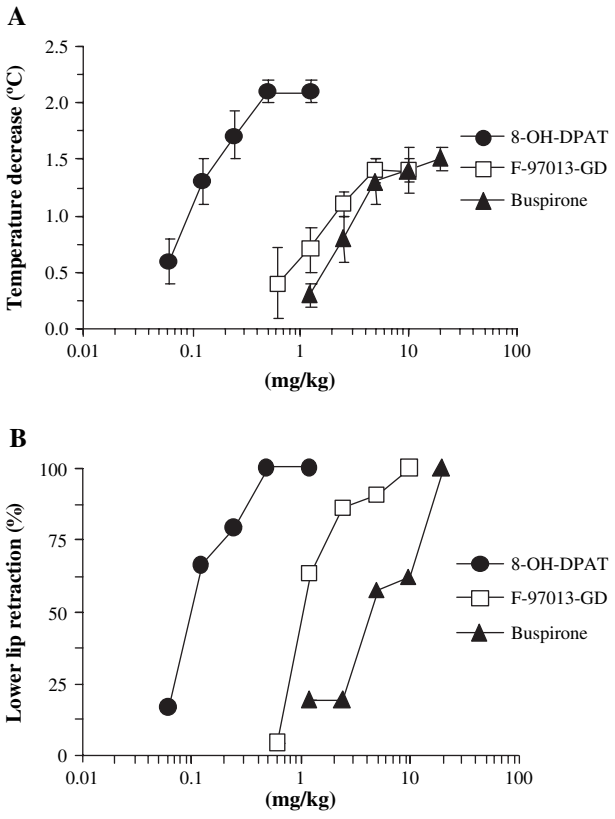

Fig. 4. Dose–response curves of the hypothermia (A) and LLR (B) induced by 8-OH-DPAT, F-97013-GD and buspirone. Temperature was measured 30 min before and after compound administration. LLR was evaluated 5 min after the second temperature measurement. Data are means ± S.E.M. ($n = 10–12$ per group).

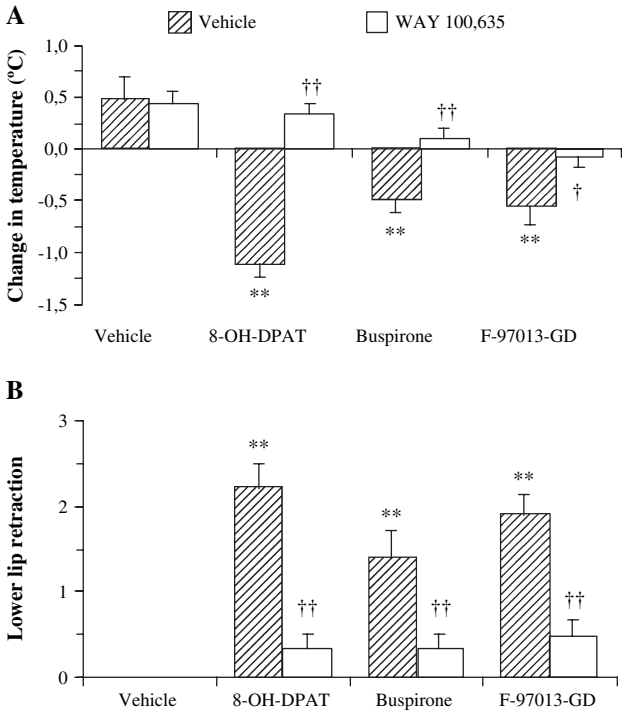

Fig. 5. Effects of WAY 100,635 on hypothermia (A) and LLR (B) induced by 8-OH-DPAT (0.125 mg/kg), buspirone (5 mg/kg) and F-97013-GD (5 mg/kg) in rats. Rectal temperatures were taken 30 min before WAY 100,635 administration (1 mg/kg, i.p.) and 30 min after compound injection. The time between the first and second administration was 30 min. LLR was evaluated 5 min after the second temperature measurement. Data are means ± S.E.M. ($n = 9$–12 per group). Statistical analysis of temperature was performed by a two-way ANOVA followed by Student–Newman–Keuls test and LLR data were analyzed using the Mann–Whitney U-test. ** $P < 0.01$ vs. vehicle-vehicle group; ††$P < 0.01$, †$P < 0.05$ vs. respective vehicle challenged group.

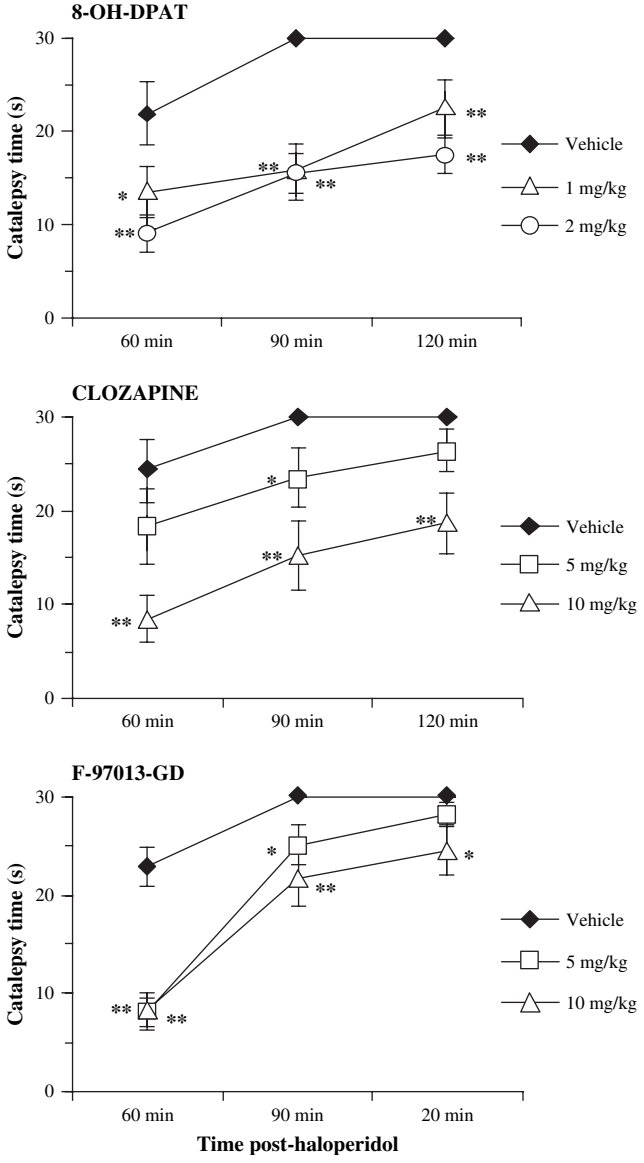

Fig. 6. Effects of 8-OH-DPAT, clozapine and F-97013-GD on haloperidol-induced catalepsy. Rats were injected with the test compounds 30 min after the haloperidol challenge and 30 min before the start of catalepsy testing. Data are means ± S.E.M. ($n = 9$–12 per group). *$P < 0.05$; **$P < 0.01$ vs. haloperidol-vehicle group (Mann–Whitney U-test).

the present study strongly support the involvement of these receptors in the tacrine-induced TJM. Thus, acute administration of the 5-HT_{1A} agonists 8-OH-DPAT and buspirone dose-dependently attenuated the tacrine-induced TJM and the 5-HT_{1A} antagonist WAY 100,635, at a dose which by itself had no effect, reversed significantly the inhibitory effect of 8-OH-DPAT. Interestingly, an association between the level of binding to 5-HT_{1A} receptors in the midbrain raphe and the severity of parkinsonian tremor has been recently found in a PET study (Doder et al., 2003).

The compound F-97013-GD displayed similar high affinities for D_2 and 5-HT_{1A} receptors and moderate to high affinity for the 5-HT_{2A} receptor. In contrast to clozapine, F-97013-GD showed negligible affinity for D_1 and muscarinic receptors. Consistent with its high affinity for the 5-HT_{1A} receptor, F-97013-GD showed a relevant potency in the 5-HT_{1A}-dependent [^{35}S]GTPγS binding assay using rat hippocampal membranes. In comparison to the full agonist 5-HT, 8-OH-DPAT and F-97013-GD stimulated [^{35}S]GTPγS binding with a considerably lower efficacy reflecting their partial agonist activity. The ability of WAY 100,635 to fully inhibit agonist-induced increases in [^{35}S]GTPγS binding demonstrates the 5-HT_{1A} selective nature of the present response. On the other hand, ligand efficacy at the 5-HT_{1A} receptor has been correlated with radiolabelled agonist/antagonist binding affinity differences (Watson et al., 2000) and changes in affinity induced by GTPγS in competition binding studies with [^{3}H]WAY 100,635 (Newman-Tancredi et al., 2001). In the last work, the agonist-preferring high-affinity state of the 5-HT_{1A} receptor is thought to reflect the G protein-coupled conformation of the receptors and this high-affinity component is abolished by the addition of GTPγS. In agreement with these assumptions, we found the greatest affinity changes with the agonists 5-HT and 8-OH-DPAT whereas weak partial agonists such as clozapine and risperidone displayed more modest affinity changes. No change in WAY 100,635 affinity was detected in accordance to its neutral antagonist properties. F-97013-GD showed a clear sensitivity to changes in the receptor-G-

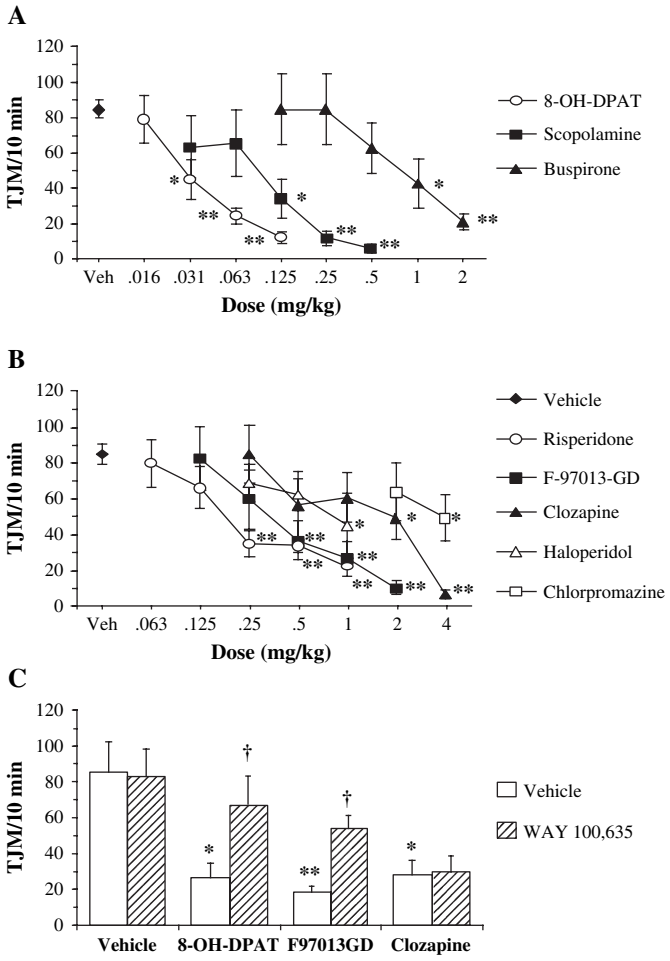

Fig. 7. TJM induced by tacrine (5 mg/kg, i.p.) in rats. Antagonism by 5-HT_{1A} agonists and scopolamine (A), antipsychotics (B) and effect of WAY 100,635 (C) on the inhibitory action of 8-OH-DPAT (0.125 mg/kg), F-97013-GD (2 mg/kg) and clozapine (4 mg/kg). Animals were administered with the test compounds 30 min before the tacrine challenge. In WAY 100,635 experiments, the antagonist was injected 30 min before test compounds. Data are means ± S.E.M. ($n = 9–10$ per group). In (A) and (B), plotted data points for vehicle-tacrine groups were pooled from separate experiments and asterisks denote significance of differences to respective vehicle-tacrine groups (ANOVA followed by Dunnett's test). In (C), symbols (†) denote significance of differences to respective vehicle challenged group and asterisks denote differences to vehicle-vehicle group (two way ANOVA followed by Newman-Keuls test). †,*$P < 0.05$; **$P < 0.01$.

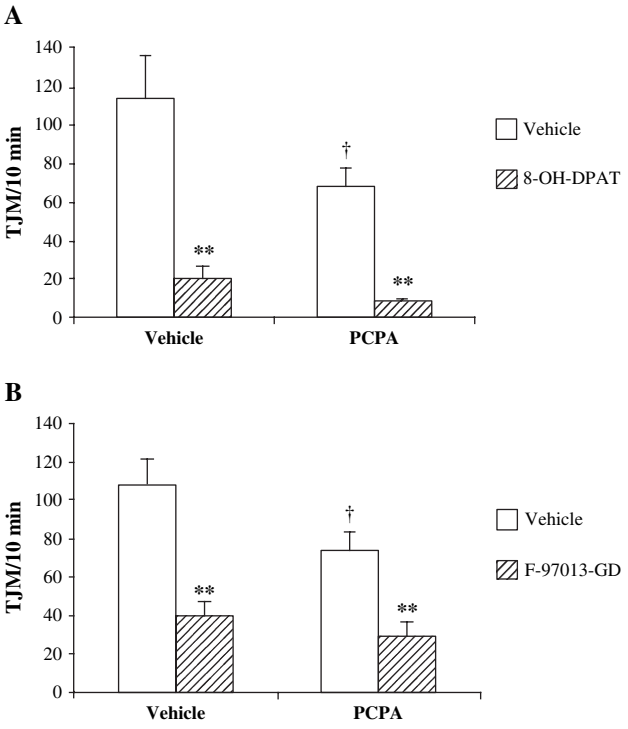

Fig. 8. Effect of the 5-HT-depleting agent PCPA on the suppressive effect of 8-OH-DPAT (0.125 mg/kg, A) and F-97013-GD (2 mg/kg, B) on TJM induced by tacrine (5 mg/kg, i.p.). Rats received two injections of PCPA (350 mg/kg, i.p.) 48 and 24 h before the tacrine-assay. Test compounds were administered 30 min before the tacrine challenge. Data are means ± S.E.M. ($n = 9–10$ per group). Asterisks denote differences to respective vehicle group and symbols (†) denote a significant difference vs. vehicle-vehicle group (two way ANOVA followed by Newman–Keuls). †$P < 0.05$; **$P < 0.01$.

protein-coupling state, being the high-affinity component abolished in the presence of GTPγS. The affinity decrease for F-97013-GD was less pronounced than that for 8-OH-DPAT (1.18 and 1.74 log units, respectively), consistent with its lower efficacy determined in the [^{35}S]GTPγS binding assay.

The biochemical characterization of F-97013-GD as a 5-HT_{1A} partial agonist was supported by in vivo functional assays. The induction of LLR and hypothermia in the rat were chosen as two measures indicative of 5-HT_{1A} activation being LLR the most selective parameter (Berendsen et al., 1989). The fact that F-97013-GD dose-dependently decreased body temperature and induced LLR, and the reversal of these effects by WAY 100,635 confirms the ability of the compound to activate 5-HT_{1A} receptors. When compared with 8-OH-DPAT, the hypothermic responses to F-97013-GD and buspirone were less pronounced whereas the induction of LLR reached the maximal response, in agreement with that previously reported for 5-HT_{1A} partial agonists (Cryan et al., 1999). Inasmuch as 5-HT_{1A} agonist-induced body temperature decrease in rats seems to be postsynaptically mediated (O'Connell et al., 1992), the relative lower efficacy elicited by partial agonists in this model may be due to the lack of receptor reserve at postsynaptic sites (Yocca et al., 1992).

Similar to antipsychotic drugs, F-97013-GD was active in a dose-dependent manner in conventional tests indicative of potential antipsychotic activity such as inhibition of apomorphine-induced climbing and suppression of CAR, probably reflecting the blockade of D_2 receptors. These data support the hypothesis that F-97013-GD should be effective in controlling the positive symptoms of schizophrenia. Moreover, the compound potently antagonized the hyperlocomotion induced by phencyclidine in mice, a model of NMDA receptor hypofunction sensitive to the action of both 5-HT_{2A} and D_2 antagonists (Maurel-Remy et al., 1995).

Substantial differences in the ability of several antipsychotics to inhibit tacrine-induced TJM were found in the present study. Thus, the ED_{50} of clozapine in this procedure was 5-fold lower than the ED_{50} required to block CAR, risperidone exhibited similar potency in both models, while only

cataleptogenic doses of haloperidol and chlorpromazine were able to produce a weak reduction of TJM. In agreement with previous reports (Trevitt et al., 1999), these data support the assumption that a relative high potency to inhibit tacrine-induced TJM could reflect a low propensity to induce EPS. F-97013-GD demonstrated a similar profile to that of clozapine, being markedly more potent against tacrine-induced TJM than against CAR. Although 5-HT_{2A} receptors may also be involved in eliciting this favorable profile, the present study focused on the potential role of 5-HT_{1A} receptors in the control of EPS. Thus, WAY 100,635 significantly reversed the inhibitory effect of F-97013-GD reflecting a receptor-specific action. In contrast, the antagonist did not modify the ability of clozapine to attenuate tacrine-induced TJM, as it has been previously described for other 5-HT_{1A}-like effects of clozapine (Bartoszyk et al., 1996). These results indicate that activation of 5-HT_{1A} receptors contributes to the antitremorgenic activity of F-97013-GD and suggest that antipsychotics with 5-HT_{1A} agonistic properties may ameliorate parkinsonian tremor, in a similar manner to clozapine (Friedman and Lannon, 1990). In support of a favorable EPS profile, F-97013-GD did not only show a low propensity to induce catalepsy but also reversed haloperidol-induced catalepsy in rats, as clozapine and 8-OH-DPAT did.

In our conditions, 8-OH-DPAT and clozapine antagonized haloperidol-induced catalepsy for about a 2-h period, thereafter catalepsy became increasingly severe (not shown). It is noteworthy that F-97013-GD displayed a shorter effective period. The reasons for this may be pharmacokinetic, reflecting a shorter half-life of the compound in brain compared with 8-OH-DPAT and clozapine. Further studies are required to determine the significance of this temporal characteristic.

Other pharmacological properties of F-97013-GD could be also involved in the in vivo effects of this drug. In this respect F-97013-GD displayed an intermediate affinity for α_1 adrenoceptors, blockade of which has been suggested to account for the atypical nature of certain antipsychotics. In general, preclinical data have shown that α_1 antagonists may improve the therapeutic index primarily by increasing antipsychotic efficacy rather than reducing extrapyramidal side effects (Wadenberg et al., 2000). In accordance with this hypothesis, preliminary evidence indicates that α_1 antagonism does not attenuate cholinomimetic-induced jaw movements (Stewart et al., 1988).

The effect of 5-HT_{1A} agonists in the present study mimics previously reported results of 5-$HT_{2A/2C}$ antagonists on cholinomimetic-induced TJM (Salamone et al., 1998), similar to that observed when other related parkinsonian-like symptoms are examined (Neal-Beliveau et al., 1993; Naidu and Kulkarni, 2001). However, the precise role of 5-HT_{2A} and 5-HT_{2C} receptors in these responses needs to be clarified (Stewart et al., 1988; Neal-Beliveau et al., 1993). On the other hand, electrophysiological evidences suggest that 5-HT_{1A} and 5-$HT_{2A/2C}$ receptors exert opposite effects on the excitability and firing activity of pyramidal neurons in the medial prefrontal cortex (Araneda and Andrade, 1991). In the same line, behavioral studies have demonstrated functional interactions between 5-HT_{1A} and 5-$HT_{2A/2C}$ receptors (see Darmani et al., 1990). Further experiments are needed to examine functional interactions between 5-HT_{1A} and 5-$HT_{2A/2C}$ receptors in the mediation of jaw movements.

Cholinomimetic-induced jaw movements in rodents are dependent upon muscarinic receptor stimulation in the ventrolateral striatum, probably of M_4 receptor subtype, and direct and indirect striatonigral pathways can influence neuronal activity in the substantia nigra pars reticulata (SNr), the major output region through which TJM are generated (for review Salamone et al., 1998). Thus, it is possible that muscarinic stimulation in striatum decreases GABA release in SNr through inhibition of the direct pathway, leading to an excessive neuronal activity in SNr, which ultimately is translated into TJM. On the contrary, activation of striatal D_1 receptors could produce excitation of spiny neurons, which in turn would increase GABA release in the SNr. In fact, the full D_1 agonist SKF 82958 suppressed cholinomimetic-induced jaw movements by activation of both striatal and nigral D_1 receptors and this effect was blocked by nigral infusion of the $GABA_A$ antagonist bicuculline (Mayorga et al., 1998). Alternatively, cholinergic stimulation in striatum could also produce an excessive stimulation of nigral neurons through increased glutamate release in SNr because of disinhibition of the subthalamic nucleus (STN). This notion is consistent with data indicating that local injection of glutamate into the central SNr increases jaw movement activity (Salamone et al., 1998).

The mechanism whereby the 5-HT_{1A} agonists exert their potent antitremorgenic effects was studied after inhibiting 5-HT synthesis with PCPA. PCPA-induced effect on brain 5-HT function was measured indirectly by evaluating the number of 5-HTP-induced head-twitches (Darmani et al., 1997). PCPA produced a strong decrease in the 5-HTP-mediated response suggesting a marked reduction of the 5-HT transmission, in line with the reported extensive depletion (>90%) of 5-HT using the same PCPA-treatment regimen (Celada et al., 2001). In agreement with the reduction of pilocarpine-induced jaw movements by PCPA (Stewart et al., 1987), we observed a partial attenuation of tacrine-induced TJM in PCPA-treated rats. These results suggest that cholinomimetic-induced jaw movements are dependent, in part, on intact central stores of 5-HT and that the suppressive effect of 5-HT_{1A} agonists may involve the activation of 5-HT_{1A} autoreceptors. Supporting this notion, stimulation of 5-HT_{1A} localized in the raphe nuclei has been showed to antagonize acute parkinsonian-like effects of neuroleptics in rats (Bantick et al., 2001). It has been hypothesized that the stimulation of 5-HT_{1A} autoreceptors could increase the activity of nigrostriatal DA neurons by releasing the tonic inhibitory influence that 5-HT exerts on midbrain dopamine cell bodies. As mentioned above, increased DA release would then act on D_1 receptors to facilitate GABA transmission in SNr and counteract the tacrine-induced TJM.

On the other hand, the suppressive effect of 8-OH-DPAT and F-97013-GD on tacrine-induced TJM persisted after the 5-HT-depletion. Under the assumption that the high dosage regimen of PCPA used here completely depletes 5-HT system, our results suggest that postsynaptic 5-HT_{1A} receptors play a crucial role in the antitremorgenic action of 5-HT_{1A} agonists.

In support of this notion, the anti-cataleptic action of 8-OH-DPAT has been shown to persist after 5-HT depletion by PCPA (Neal-Beliveau et al., 1993). Modulation of cortical glutamatergic afferents to the basal ganglia may account for postsynaptic 5-HT_{1A} effects. Several studies evidence that activation of 5-HT_{1A} receptors hyperpolarize pyramidal neurons in the medial prefrontal cortex (see Puig et al., 2005), a region that sends direct and indirect excitatory inputs to the STN. This nucleus provides a major excitatory drive on the SNr and thus, depriving the STN of its excitatory glutamatergic input from the cortex following 5-HT_{1A} activation might result in decreased excitation of the SNr and suppression of tacrine-induced TJM. Alternatively, the facilitatory effect that 5-HT_{1A} agonists exert through postsynaptic 5-HT_{1A} receptors on the firing of mesocortical dopamine neurons could modulate glutamatergic projections to the basal ganglia (Espejo and Gil, 1997).

Major symptoms of Parkinson's disease have been associated with an increased activity in the SNr (Vila et al., 1996). Similarly, neurons in the SNr appear to be highly activated during TJM and therefore, activation of 5-HT_{1A} receptors may represent a mechanism to improve parkinsonian symptoms by reducing the excessive activity of this brain area. In support of this notion, recent studies described the antiparkinsonian properties of the 5-HT_{1A} agonist tandospirone in the rat (Ishibashi and Ohno, 2004) as well as the involvement of 5-HT_{1A} receptors in the control of motor behavior in both primates (Bibbiani et al., 2001) and patients with Parkinson's disease (Kannari et al., 2002).

In summary, activation of 5-HT_{1A} receptors by 5-HT_{1A} agonists counteracts the tacrine-induced TJM, a proposed behavioral model of parkinsonian tremor particularly sensitive to atypical antipsychotics. F-97013-GD, a 5-HT_{1A} agonist and D_2 antagonist which shows antipsychotic-like effects and weak cataleptogenic activity in classical paradigms, displayed marked antitremorgenic activity in the tacrine model by an action on 5-HT_{1A} receptors. These results suggest that in addition to a low propensity to induce EPS, activation of 5-HT_{1A} receptors by novel antipsychotics may represent a potential benefit in the treatment of parkinsonian tremor.

Acknowledgments

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