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Pharmacology, Biochemistry and Behavior 74 (2003) 811–825
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

The glycine transporter-1 inhibitors NFPS and Org 24461: a pharmacological study

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Received 14 August 2002; received in revised form 15 November 2002; accepted 25 November 2002

Abstract

The in vitro and in vivo pharmacology of two glycine transporter-1 (GlyT1) inhibitors, *N*[3-(4′-fluorophenyl)-3-(4′-phenylphenoxy)-propyl]sarcosine (NFPS) and *R*,*S*-(+/−)*N*-methyl-*N*-[(4-trifluoromethyl)phenoxy]-3-phenyl-propylglycine (Org 24461), was studied. NFPS and Org 24461 inhibited the uptake of [^{3}H]glycine in hippocampal synaptosomal preparation with IC_{50} values of 0.022 and 2.5 μM. Neither NFPS nor Org 24461 (0.1 μM) showed significant binding to α-1, α-2, and β-adrenoceptors, D_1 and D_2 dopamine receptors, and 5-HT_{1A} and 5-HT_{2A} serotonin receptors in membranes prepared from rat brain or to cloned 5-HT_6 and 5-HT_7 receptors. At 10 μM concentrations, binding affinity was measured for NFPS to 5-HT_{2A} and 5-HT_{2C} serotonin receptors and α-2 adrenoceptors and for NFPS and Org 24461 to 5-HT_7 serotonin receptors. Glycine (0.1 mM) and sarcosine (5 mM) increased [^{3}H]glycine efflux from superfused rat hippocampal slices preloaded with [^{3}H]glycine. NFPS and Org 24461 (0.1 mM) did not influence [^{3}H]glycine efflux, however, they inhibited glycine-induced [^{3}H]glycine release. These findings indicate that NFPS and Org 24461 selectively inhibit glycine uptake without being substrates of the transporter protein. Several antipsychotic tests were used to characterize antipsychotic effects of NFPS and Org 24461 in vivo. These compounds did not alter apomorphine-induced climbing and stereotypy in a dose of 10 mg/kg po in mice and did not induce catalepsy in a dose of 10 mg/kg ip in rats. The ID_{50} values of NFPS were 21.4 mg/kg and higher than 30 mg/kg ip for inhibition of phencyclidine (PCP)- and D-amphetamine-induced hypermotility in mice and these values were 2.5 and 8.6 mg/kg ip for Org 24461. NFPS and Org 24461 did not exhibit anxiolytic effects in light–dark test in mice, in the *meta*-chlorophenylpiperazine (mCPP)-induced anxiety test (minimal effective dose or MED was higher than 3 mg/kg ip) and in the Vogel conflict drinking test in rats (MED was higher than 10 mg/kg ip). Both NFPS and Org 24461 (1–10 mg/kg ip) reversed PCP-induced changes in EEG power spectra in conscious rats. These data support the view that GlyT1 inhibitors may have potential importance in treatment of negative symptoms of schizophrenia.

Keywords: NFPS; Org 24461; [^{3}H]Glycine uptake; Glycine transporter-1 inhibitors; [^{3}H]Glycine efflux; Antipsychotic agents; Antipsychotic tests; Negative symptoms of schizophrenia; Anxiolytic tests; Electroencephalogram

1. Introduction

Novel antipsychotic agents that block D_2 dopamine and 5-HT_2 receptors exhibit favorable side effect profile although their clinical efficacy in blocking some symptoms of schizophrenia has certain limits (Zimbroff et al., 1997). Despite improved antipsychotic effect of clozapine, there remains a population of patients irresponsive to clozapine as its impact upon primary negative symptoms may be limited (Kane and Freeman, 1994; Meltzer, 1995). In addition, atypical antipsychotic drugs that act on 5-HT_{2A} receptors are often delayed and not fully restorative (Tamminga, 1998a). The lack of full efficacy of antipsychotics may be explained by the fact that not only dopaminergic and

Abbreviations: NFPS, *N*[3-(4′-fluorophenyl)-3-(4′-phenylphenoxy)-propyl]sarcosine; Org 24461, *R*,*S*-(+/−)*N*-methyl-*N*-[(4-trifluoromethyl)phenoxy]-3-phenyl-propylglycine.

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doi:10.1016/S0091-3057(02)01078-X

serotonergic but other neurotransmission mechanisms are also involved in the pathology in schizophrenia. Hypoactivity of *N*-methyl-D-aspartate (NMDA) receptor-mediated glutamatergic neurotransmission has been implicated in induction of negative symptoms of schizophrenia (Javitt and Zukin, 1991; Coyle, 1996). Although the mechanisms by which reduced activity of NMDA receptor-mediated glutamatergic tone occurs are not known, dysregulation of glutamate receptor expression or altered metabolism in glutamatergic transmission has been suggested (Tsai et al., 1998).

Competitive NMDA antagonists that were developed primarily to treat brain ischemia exhibit psychotomimetic effects (Kristensen et al., 1992; Grotta et al., 1995). Moreover, phencyclidine (PCP) and other noncompetitive NMDA antagonists also induce schizophrenia-like psychotic symptoms in humans as they induce hallucinations and delirium, thought disturbance and cognitive dysfunction and also negative symptoms (Heresco-Levy et al., 1996; Lahti et al., 2001). Thus, PCP, which blocks the action of glutamate at NMDA receptors, gives further support to the hypoglutamatergic theory of schizophrenia (Tamminga, 1998b). PCP has been proposed to be a pharmacological model of schizophrenia because it can mimic the full spectrum of schizophrenic disorders (Moghaddam and Adams, 1998).

An important breakthrough in understanding NMDA receptor-mediated glutamatergic neurotransmission was the recognition of glycine as a coagonist of glutamate at this ion channel-coupled receptor (Johnson and Ascher, 1987; Berger et al., 1998). Glycine and glutamate act as cotransmitters for opening NMDA-sensitive ionotropic glutamate receptors in a strychnine-insensitive manner (Foster and Kemp, 1989) influencing the permeability of the receptor-coupled ion channel for mono- and bivalent cations (Reynolds and Miller, 1988). NMDA receptors consist of NR1 and NR2 subunits, the former possesses binding site for glycine ($glycine_B$ binding site) and the latter binds glutamate to the agonist binding site (Parsons et al., 1998). Certain D-amino acids like D-cycloserine or D-serine exert partial agonist effects at $glycine_B$ site (Tsai et al., 1998; Mothet et al., 2000; Snyder and Kim, 2000) whereas kynurenic acid and some other compounds (2-carboxyindoles, 2-carboxy-tetrahydroquinolines, 4-hydroxy-2-quinolones, quinoxaline-2,3-diones) act as antagonists at the glycine coagonist site of NMDA receptors (Danysz and Parson, 1998). Activation of NMDA receptors requires occupancy of $glycine_B$ binding sites by endogenous glycine released from neighboring cells into the vicinity of NMDA receptors. A potent glycine transport mechanism assures to set glycine concentrations below the level required to saturate glycine sites at NMDA receptors (Sato et al., 1995). The high-affinity glycine transport system, which primarily inactivates glycine in glutamatergic synapses, is located in membranes of neurons and glial cells (Zafra et al., 1995).

Glycine transporters are members of the Na^+- and Cl^--dependent neurotransmitter transporter family (Uhl and Hartig, 1992; Amara et al., 1995). Two glycine transporter genes (GlyT1 and GlyT2) have been identified and cloned (Adams and Kuhar, 1993; Kim et al., 1994). In addition, three isoforms for GlyT1 (GlyT1a, GlyT1b, and GlyT1c) and two isoforms for GlyT2 (GlyT2a and GlyT2b) have been identified, cloned, and characterized (Borowsky and Hoffman, 1998; Pronce et al., 1998). Glycine transporters, which regulate glycine concentrations in excitatory synapses, belong to GlyT1 transporters and transport proteins have been shown to be colocalized with NMDA receptors (Smith et al., 1992; Fedele et al., 1993).

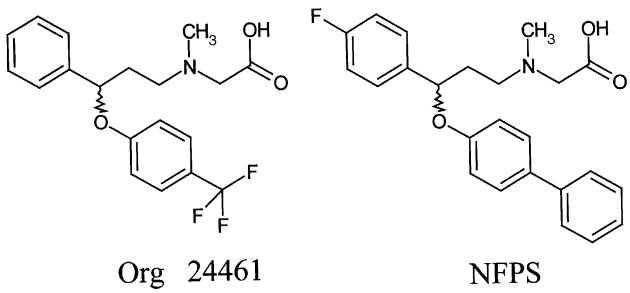

Fig. 1. The chemical structures of Org 24461 and NFPS.

A number of compounds have been reported to have selective and high affinity to glycine transporters. Toth and Lajtha (1986) and Toth et al. (1986) have demonstrated that glycine inhibits PCP-induced hyperactivity in mice and of the glycine derivatives, glycyldodecylamide (GDA) was found particularly active in this respect. Later, the glycine reuptake inhibitory effect of GDA has been demonstrated (Javitt and Frusciante, 1997; Javitt et al., 1999). In the glycine transporter inhibitor *N*[3-(4′-fluorophenyl)-3-(4′-phenylphenoxy)-propyl]sarcosine (NFPS) and *R*,*S*-(+/−)*N*-methyl-*N*-[(4-trifluoromethyl)phenoxy]-3-phenyl-propylglycine (Org 24461), the amino group of sarcosine was substituted with lipophilic heterocycles (Bergeron et al., 1998; Ge et al., 1999; Atkinson et al., 2000). The aim of the present investigation was to further characterize the pharmacology of the GlyT1 inhibitor Org 24461 and NFPS (Fig. 1) in in vitro and in vivo models. It was found that these GlyT1 inhibitors reverse PCP-induced alterations suggesting that they may have a therapeutic role in influencing negative symptoms of schizophrenia. A preliminary report of these findings was presented at the 35th Winter Conference of Brain Research (Harsing et al., 2002).

2. Methods

2.1. Animals

The experimental protocol used in this study was approved by the Ethic Committee, Division of Preclinical Research at EGIS Pharmaceuticals and all procedures are in compliance with the National Institute of Health Guide for

Care and Use of Laboratory Animals. Male Wistar rats weighing 260–300 g were used in the experiments. All rats were obtained from Charles River, Hungary except those tested for electroencephalogram (EEG), which were raised in the laboratory. In some experiments, male NRMI mice weighing 25–33 g were used and purchased from Charles River, Hungary. Animals were housed five to a cage in a temperature- and humidity-controlled animal facility on a 12-h light–dark cycle (6.00 a.m. on; 6.00 p.m. off) with food and water available ad libitum. The animals were allowed at least 1 week of habituation to their housing prior to experimentation.

2.2. Drugs used in this study

NFPS (ALX-5407) and Org 24461 were synthesized by Dr. P. Matyus. D-Amphetamine sulfate, apomorphine HCl, butaclamol HCl, 1-(3-chlorophenyl)piperidine (mCPP) 2HCl, clozapine, cyproheptadine HCl, diazepam, glycine HCl, haloperidol, methiotepine mesylate, mianserin HCl, MK-801, phentolamine mesylate, prazosin HCl, ritanserin, sarcosine HCl, *R*(+)SCH-23390 HCl, and serotonin creatinine sulfate were purchased from Sigma (St. Louis, MO, USA). Buspirone, PCP, and risperidone were synthesized at EGIS Pharmaceuticals, Budapest, Hungary. [^{3}H]Glycine, [^{3}H]MK-801, [^{3}H]ketanserin, and [^{3}H]LSD were purchased from New England Nuclear Life Science Products (Boston, MA, USA). [^{3}H]8-OH-DPAT, [^{3}H]mesulergine, [^{3}H]prazosin, [^{3}H]idazoxan, [^{3}H]SCH-23390, and [^{3}H]spiperone were obtained from Amersham Life Sciences, UK. All other chemicals were of analytical grade.

2.3. Neurochemical studies

2.3.1. Receptor binding assays

Competition binding studies for the GlyT1 inhibitors and some antipsychotic drugs were performed at NMDA receptors, multiple dopaminergic and serotonergic receptors, and adrenoceptors. The protocols employed for examination of drug affinities at specific receptor types are summarized in Table 1.

2.3.2. Glycine uptake in hippocampal synaptosome

Synaptosomal P_2 fraction was prepared as described by Gray and Whittaker (1962). Rats were decapitated, the brains were removed from the skull and the hippocampi were dissected and placed into ice-cold saline. The tissue was homogenized in 40 volumes (w/v) of 0.32 M sucrose. The homogenate was centrifuged at $1000 \times g$ for 5 min to remove nuclei and debris, then the supernatant was centrifuged again at $12{,}000 \times g$ for 20 min. The P_2 pellet was resuspended in Krebs solution with the following composition (in mM): NaCl 125, KCl 3, $CaCl_2$ 1.2, NaH_2PO_4 1, $MgSO_4$ 1.2, $NaHCO_3$ 22, glucose 10, pH 7.4, and gassed with 95% O_2/5% CO_2. [^{3}H]Glycine (specific activity 41.1 Ci/mmol) uptake in synaptosomal

Table 1
Conditions for competition binding at monoaminergic receptors (summarized experimental conditions)

Receptor	Rat NMDA	Rat 5-HT$_{1A}$	Rat 5-HT$_{2A}$	Pig 5-HT$_{2C}$	Human 5-HT$_6$	Human 5-HT$_7$	Rat α_1	Rat α_2	Rat D_1	Rat D_2
Tissue	forebrain	frontal cortex	frontal cortex	choroid plexus	HEK293 cells	CHO cells	frontal cortex	frontal cortex	striatum	striatum
Radioligand (nM)	[^{3}H]MK-801 (5)	[^{3}H]8-OH-DPAT (0.8)	[^{3}H]ketanserin (1)	[^{3}H]mesulergine (1)	[^{3}H]LSD (3)	[^{3}H]LSD (5.5)	[^{3}H]prazosin (0.3)	[^{3}H]idazoxan (8.9)	[^{3}H]SCH-23390 (2.8)	[^{3}H]spiperone (0.6)
Nonspecific ligand (μM)	MK-801 (10)	5-HT (10)	cyproheptadine (10)	mianserin (1)	5-HT (100)	clozapine (25)	prazosin (1)	phentolamine (10)	(+)SCH-23390 (1)	(+)butaclamol (1)
Buffer components and concentrations (mM)	HEPES (5)–NaOH glutamate (0.01), glycine (0.01)	Tris (50), $CaCl_2$ (6.66), pargyline (0.01666), ascorbic acid (0.166% w/v)	Tris (50)	Tris (50), $CaCl_2$ (4), pargyline (0.01), ascorbic acid (0.1% w/v)	Tris (50), $MgCl_2$ (10), EDTA (0.5)	Tris (50), $MgSO_4$ (10), EDTA (0.5)	Tris (50)	Tris (50)	Tris (50), $MgSO_4$ (5), EDTA (1)	Tris (50), NaCl (120), KCl (5), $CaCl_2$ (2), $MgCl_2$ (1), ascorbic acid (0.1% w/v)
pH	7.4	7.7	7.7	7.7	7.4	7.4	7.7	7.7	7.4	7.4
Incubation	120 min, 25 °C	30 min, 25 °C	15 min, 37 °C	30 min, 37 °C	60 min, 37 °C	120 min, 27 °C	45 min, 25 °C	30 min, 25 °C	30 min, 37 °C	15 min, 37 °C
Reference ligand (K_i, nM)	MK-801 (9)	buspirone (19)	ritanserin (0.9)	mianserin (2.3)	methiothepin (0.62)	methiothepin (0.14)	prazosin (0.15), WB4101 (3.8)	phentolamine (6.7), clonidine (8.6)	(+)SCH-23390 (1.8)	haloperidol (4.8)
Literature reference	Foster and Wong (1987)	Peroutka (1986)	Leysen et al. (1982)	Pazos et al. (1985)	according to information sheet	according to information sheet	Reader et al. (1987) and Greengrass and Brenner (1979)	Mallard et al. (1992) and Reader et al. (1987)	Wallace et al. (1989) and Billard et al. (1984)	Creese et al. (1979)

P_2 fraction was carried out as described by Fedele and Foster (1992). Aliquots of the P_2 fraction (0.25 mg protein) were preincubated in 2 ml of Krebs solution for 5 min at 37 °C. [^{3}H]Glycine was then added and the incubation continued for 3 min. The assay mixture was filtered under vacuum through Whatman GF/B filters that were rapidly washed three times with 5 ml of ice-cold Krebs solution. Blanks were determined by incubation at 0 °C. Filters were placed into scintillation vials with 3 ml of scintillation liquid and radioactivity was measured by liquid scintillation spectrometry. To achieve the desired ligand concentrations, [^{3}H]glycine was diluted by adding unlabeled glycine. In the experiments, 0.25 μM [^{3}H]glycine (Herdon et al., 2001) or 30 μM [^{3}H]glycine (Fedele and Foster, 1992) was used and the drugs tested were present from the beginning of the experiments at concentrations of 1–100 μM. Protein concentrations were determined by the Bradford assay using bovine serum albumin as a standard (Bradford, 1976).

2.3.3. [^{3}H]Glycine efflux from rat hippocampal slices

Rats were killed by decapitation and the brains were removed from the skulls. Slices approximately 350 μm thick from the hippocampus were prepared using a McIlwain tissue chopper (The Mickie Laboratory Engineering, Gomshall, UK). The slices were collected into ice-cold Krebs–bicarbonate buffer, pH 7.4 with the following composition (in mM): NaCl 118, KCl 4.7, $CaCl_2$ 1.25, NaH_2PO_4 1.2, $MgCl_2$ 1.2, $NaHCO_3$ 25, glucose 11.5. The Krebs–bicarbonate buffer used throughout the experiments was continuously gassed with 5% CO_2 in O_2. The brain slices were incubated with [^{3}H]glycine (2.5 μCi/ml) in Krebs–bicarbonate buffer for 30 min at 36 °C (Harsing et al., 2001). The tissues were then transferred into low volume (0.3 ml) superfusion chambers (Experimetria, Budapest, Hungary) and superfused with aerated and preheated (37 °C) Krebs–bicarbonate buffer. The flow rate was kept at 1 ml/min by a Gilson multichannel peristaltic pump (type M312, Villiers-Le-Bel, France). The superfusate was discarded for the first 60-min period of the experiments then 22 three-minute fractions were collected by a Gilson multichannel fraction collector (type FC-2038, Middleton, WI, USA). The GlyT1 inhibitors were added to the buffer from fraction 5 and maintained throughout the experiments. At the end of superfusion, the tissue was collected from the superfusion chambers, homogenized, and an aliquot was processed for determination of tissue content of radioactivity. To determine the radioactivity released from the tissue, a sample of the superfusate was mixed with liquid scintillation reagent (Optifluor, Packard, Groningen, The Netherlands) and subjected to liquid scintillation spectrometry. The efflux of [^{3}H]glycine was expressed as a fractional rate, i.e., as a percentage of the amount of radioactivity in the tissue at the time the release was determined (Harsing et al., 1992). A computer program (Quattro Pro) was used to estimate the fractional rate of [^{3}H]glycine efflux.

2.4. Behavioral studies

2.4.1. Apomorphine-induced climbing and stereotypy in mice

Male NMRI mice of 20–26 g were used. The test compound or the vehicle was administered orally in a volume of 20 ml/kg at the beginning of the test. Thirty minutes after the administration of the vehicle or test compound, the animals were placed individually into the experimental chamber (a wire mesh cage of 12 × 12 × 12 cm) for habituation. At the end of the 60-min pretreatment period, 1 mg/kg apomorphine HCl was administered subcutaneously to the animals. The measurement of stereotypy started immediately after the apomorphine treatment and

Table 2
Radioligand binding affinities of Org 24461 and NFPS as compared to antipsychotic drugs at multiple receptors

Receptors	Percent inhibition at different concentrations				K_i (nM)		
	Org 24461		NFPS		Haloperidol	Clozapine	Risperidone
	10^{-7} M	10^{-5} M	10^{-7} M	10^{-5} M			
NMDA	–	2	–	2	–	–	–
5-HT_{1A}	2	2	3	4	31,000	207	610
5-HT_{2A}	3	0	1	33	122	19.2	0.5
5-HT_{2C}	15	19	25	58	7900	5.8	36
5-HT_6	0	6	0	0	12,000	6	4053
5-HT_7	12	24	5	21	398	47	0.7
α-1	3	0	2	9	21	21	1
α-2	5	0	0	25	9800	230	6
β	1	0	3	9	–	–	–
D_1	4	1	2	6	131	435	148
D_2	0	1	0	3	5	630	5

Radioligand binding affinities were expressed as percent inhibition at 10^{-7} or 10^{-5} M concentrations for Org 24461 and NFPS or as K_i (inhibition constant, nM) for the antipsychotic agents. Data are means of at least two determinations performed in triplicate. For receptor binding assay conditions, see the Methods section and Table 1.

lasted 25 min. Stereotypy was scored from 0 to 4 according to the following criteria:

0 = absence of stereotyped behavior, similar to control;
1 = presence of continuous exploration, intermittent sniffing, and movements of head;
2 = intense stereotyped movements of head and/or sniffing, periodic exploration;
3 = intermittent licking, biting or gnawing with intense sniffing, or head weaving;
4 = intense licking or gnawing confined to a small area without exploration and locomotion.

Climbing behaviour was scored in an "all or none" manner 15 min after apomorphine administration for 10 min. The reaction was considered positive if the mouse clung with at least three paws on the grid wall.

Median of stereotypy scores for each group was calculated from the score maximums. The Mann–Whitney *U* test was used for statistical evaluations. ID_{50} values were calculated by linear regression analysis using percent inhibitions. Climbing frequency was calculated for each group. The result of the control group was considered as 100%. ED_{50} value was computed from the data of dose–effect relationship by the method of Litchfield and Wilcoxon (1949).

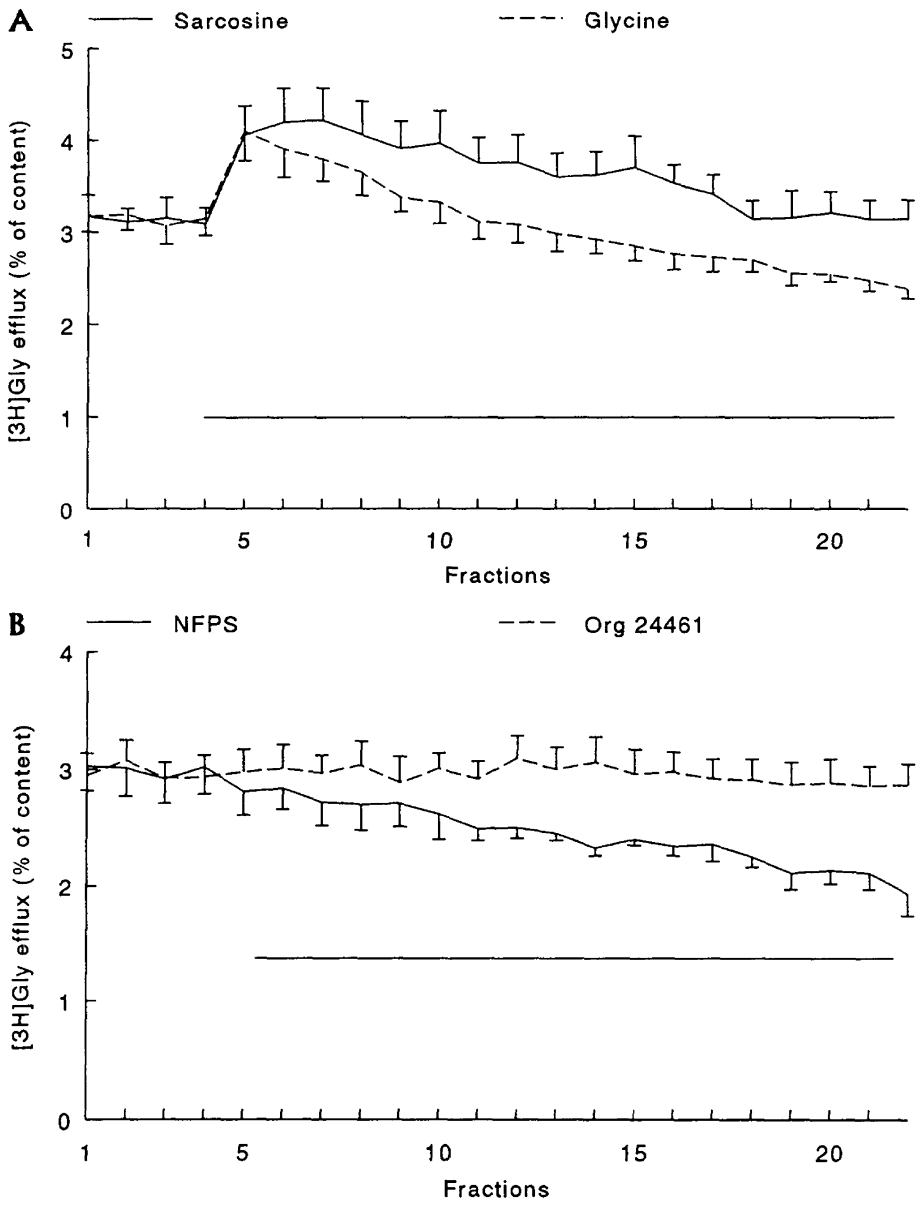

Fig. 2. Effects of GlyT1 inhibitors on [^{3}H]glycine efflux from hippocampal slices of the rat. (A) Glycine and sarcosine added in concentrations of 0.1 and 5 mM, respectively, increased [^{3}H]glycine efflux. (B) Org 24461 and NFPS added in a concentration of 0.1 mM did not influence [^{3}H]glycine efflux. Horizontal line indicates drug administration. Slices from rat hippocampus were prepared, loaded with [^{3}H]glycine, and superfused with Krebs–bicarbonate buffer. Drugs were added to hippocampal slices from fraction 5 and maintained throughout the experiments. Values shown are the means ± S.E.M. of four experiments.

2.4.2. PCP- and D-amphetamine-induced hypermotility in mice

Increasing doses of the test compound were applied intraperitoneally in a volume of 10 ml/kg to 10 mice per group. Thirty minutes later they received injections of PCP (3 mg/kg, ip). Fifteen minutes after the PCP treatment, the mice were placed in a 10-channel Dews apparatus for 45 min. The number of infrared light beam interruptions was recorded for each channel. Data were analyzed by analysis of variance (ANOVA) followed by the Duncan's test and ID_{50} values were calculated by linear regression analysis using percent inhibitions.

When effects on D-amphetamine-induced hypermotility were investigated, increasing doses of the test compound were applied intraperitoneally in a volume of 10 ml/kg to 10 mice per group. Thirty minutes later, they received injections of D-amphetamine (4 mg/kg, sc). Fifteen minutes after the D-amphetamine treatment, the mice were placed in a 10-channel Dews apparatus for 30 min. The number of infrared light beam interruptions was recorded. Data were analyzed by ANOVA followed by the Duncan's test (STATISTICA) and ID_{50} values were calculated by linear regression analysis using percent inhibitions.

2.4.3. Catalepsy in rats

The cataleptogenic effect was determined in rats by the method of Morpurgo (1962). The forepaws of rats were placed on 3- or 6-cm-high columns and an immobilization period of 10 s was scored as 0.5 or 1. A total score of 3 was considered as a 100% effect. Cataleptic activity was determined in every 30 min for a 4-h period. The Mann–Whitney *U* test was used for determination of statistical significance after the calculation of maximum score values. The compounds tested were administered intraperitoneally or orally to rats.

2.4.4. Light–dark test in mice

Test was performed in a room illuminated with a 2-lx light source. An animal activity monitor equipped with six 2-compartment automated test chambers (Omnitech, Digiscan, Model RXYZCM16) was used for all experiments. Each box consisted of one dark and one lit compartment. Both areas measured 39 × 20 × 29 cm. Access between the two compartments was provided by an 8 × 8 cm passageway. A 60-W white tungsten light bulb was used to illuminate the lit area. Interruptions of the 32 infrared beams (16 at 2 cm and 16 at 8 cm high above the box floor) in both compartments were automatically recorded by the Digiscan analyzer and transmitted to a computer. Male NMRI mice weighing 25–33 g were used for the test. Mice were kept in a dark room, treated intraperitoneally 30 min prior to test and were placed individually in the center of the lit area. Behavioral activity was detected for 5 min. Time spent in each area, number of transitions, and horizontal and vertical activities were recorded (Costall et al., 1989). Means ± S.E.M. values were calculated and the statistical analysis of data was performed by one-way ANOVA followed by the Duncan's test.

2.4.5. mCPP-induced anxiety in rats

Parameters of the experimental equipment were same as described above, except that a 40-W red tungsten light bulb was used to illuminate the lit area of the box. Male Wistar rats weighing 160–200 g were used for the test. The animals were treated intraperitoneally with vehicle or test compound. Twenty minutes later, the animals were treated subcutaneously with saline or mCPP (0.5 mg/kg). They were kept in the next 10 min in dim red light in the room where the experiment was carried out. The rats were then placed individually at the center of the dark compartment and behavioral activity was detected for 5 min. Time spent in each area, number of transitions, and horizontal and vertical activity were recorded (Bilkei-Gorzo et al., 1998). Means ± S.E.M. values were calculated and the statistical analysis of data was performed by one-way ANOVA followed by the Duncan's test.

2.4.6. Conflict drinking test in rats

Experiments were performed in a computer-operated LIIKOSYS (Experimetria) consisting of eight test chambers (20 × 20 × 20-cm Plexiglas boxes), each of which was equipped with a water fountain system mounted at an appropriate height on the wall of the chamber and metal grid floor for delivering electric shocks. Male Wistar rats weighing 160–180 g were deprived of drinking water for 48 h and fasted for 24 h prior to test. Test and reference compounds or vehicle were administered intraperitoneally 30 min prior to test. All procedures were carried out in a quiet, air-conditioned room between 0730 and 1300 h at an ambient temperature of 232 °C. At the beginning of the experiment, the animals were placed in the test chamber where they had free access to drinking water for a 30-s grace period. After that, electric shocks (600 μA, 0.6 s) were applied through the drinking spout following every 20 licks during a 5-min test period (Vogel et al., 1971). Number of

Table 3
Lack of effects of Org 24461 and NFPS on apomorphine-induced climbing and stereotypy in mice and on catalepsy in rats

Compounds	Apomorphine-induced		Catalepsy
	Climbing	Stereotypy	MED (mg/kg)
	ED_{50} (mg/kg)	ID_{50} (mg/kg)	
Org 24461	>10	>10	>10
NFPS	>10	>10	>10
Haloperidol	0.3	0.25	1
Clozapine	11	35	>40
Risperidone	0.02	0.08	1

Apomorphine-induced climbing and stereotypy were determined in mice after oral administration of the drugs as described in the Methods section. Catalepsy was determined in rats according to Morpurgo (1962). GlyT1 inhibitors were administered intraperitoneally; the antipsychotic agents were given orally. For details, see Methods section.

punished licks was recorded and stored by a computer. Means ± S.E.M. of numbers of tolerated shocks were calculated in each group, statistical analysis of data was performed by one-way ANOVA followed by the Duncan's test.

2.5. EEG testing

Male Wistar rats weighing 500 g were anesthetized by chloral hydrate (350 mg/kg ip) and mounted in a stereotaxic frame (Sebban et al., 1999). Two holes were drilled in the left and right prefrontal regions and two others in the right and left sensorimotor cortex regions, and four transcortical bipolar electrodes were inserted. The animals were earthed via a stainless steel screw fixed in the frontal cranial bone. Ten days after the surgery the animals were habituated for EEG recording for an additional 10–14 days. Two EEG recordings were performed for each dose of a drug tested in the same rat. The first recording lasted

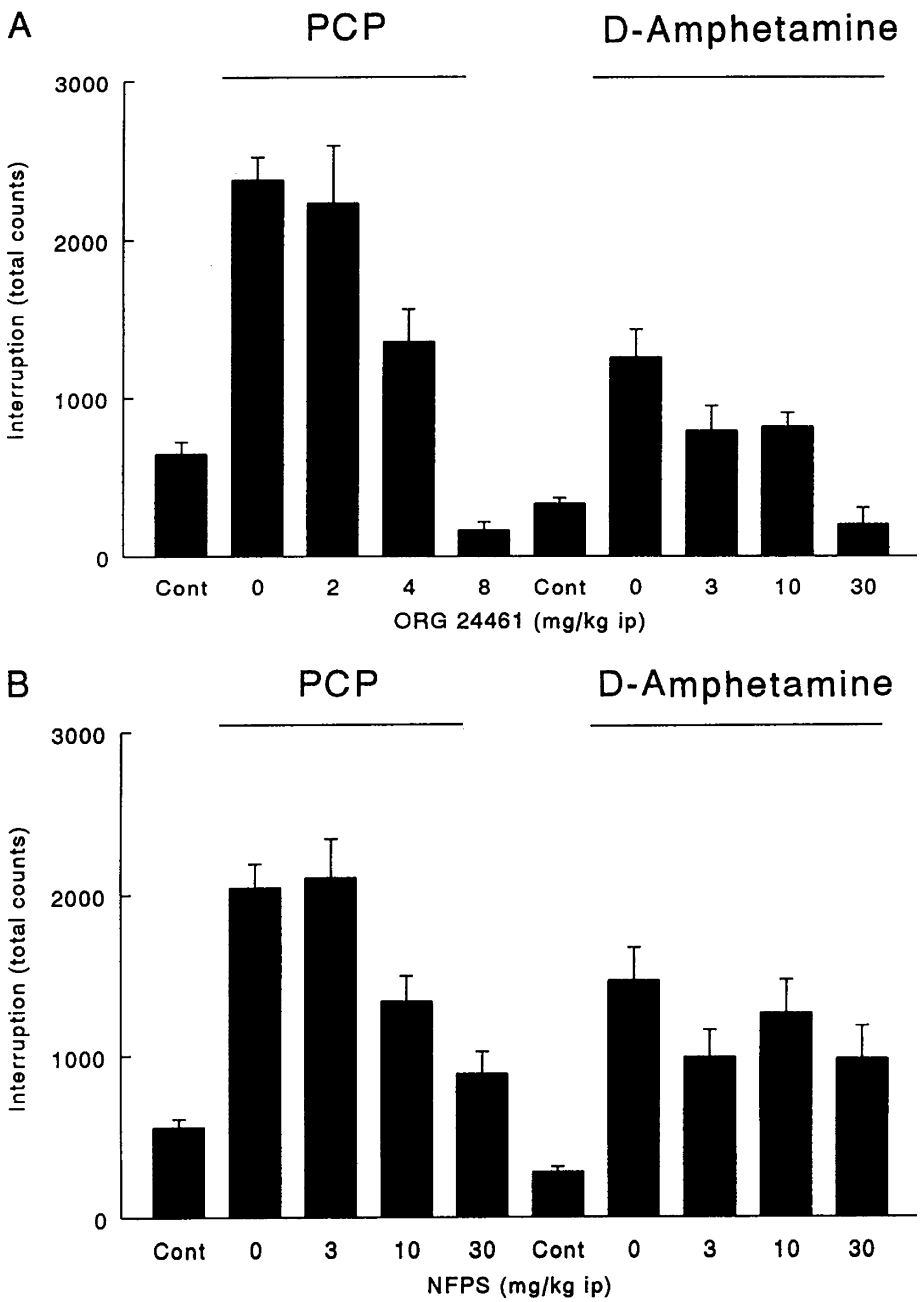

Fig. 3. Effect of Org 24461 (A) and NFPS (B) on PCP-induced and D-amphetamine-evoked hypermotility in mice. The ID_{50} values to inhibit PCP- and D-amphetamine-induced hypermotility were 3.8 and 13.5 mg/kg ip for Org 24461 and these values for NFPS were 21.4 and higher than 30 mg/kg ip. Hyperlocomotion was evoked by PCP (3 mg/kg ip) or D-amphetamine (4 mg/kg sc) as described in the Methods section. The total counts indicate the number of beams that have been interrupted by the animal during the ambulatory activity. Values shown are the means ± S.E.M. ($n = 10$).

for 165 min after vehicle administration. The second recording was done 24 h later for the same duration after drug administration. The two records were used to evaluate the treatment effect relative to the vehicle effect. For recording, EEG signals were amplified and filtered for Fourier transformation, which allowed calculation of the power variable (μV^2). Absolute power spectra of EEG signals were computed every 30 s from 1 to 30 Hz in steps of 1 Hz. In each rat, hertz by hertz drug-induced power changes were evaluated by determining spectral power obtained following the injection of PCP and drug tested versus the spectral power obtained following administration of PCP (1 mg/kg sc). The EEG spectral power from the prefrontal and sensorimotor cortices of the two hemispheres was averaged for every 5-min period for each 165-min recording session.

2.6. Statistical analyses

One-way ANOVA followed by the Dunnett's or Duncan's multiple comparison test, the Student *t* statistics for two means, and the paired *t* test were used for statistical analysis of the data. The Mann–Whitney *U* test was used for nonparametric analysis. The mean ± S.E.M. was calculated and the number of independent determinations (*n*) is indicated. A level of probability (*P*) less than 5% was considered significant.

3. Results

3.1. Neurochemical testing

3.1.1. Receptor binding assays

Using receptor binding assays, we determined the binding properties of the Org 24461 and NFPS in comparison with other antipsychotic agents. At 0.1 μM concentration, NFPS and Org 24461 did not show significant binding to α-1, α-2, and β-adrenoceptors, D_1 and D_2 dopamine receptors, and 5-HT_{1A} and 5-HT_{2A} serotonin receptors in membranes prepared from rat brain or in membranes from cells expressing recombinant h5-HT_6 and h5-HT_7 receptors (Table 2). At higher concentrations (10 μM), binding affinity was measured for NFPS at 5-HT_{2A} serotonin receptors and α-2 adrenoceptors and for NFPS and Org 24461 at 5-HT_7 serotonin receptors were measured; no receptor affinity was measured to NMDAs receptors. NFPS also showed inhibition of 5-HT_{2C} serotonin receptor binding at 0.1 and 10 μM concentrations in membranes prepared from choroid plexus, this effect of Org 24461 was less pronounced (Table 2). For comparison, the receptor binding profiles of haloperidol, clozapine, and risperidone are also shown in Table 2. These antipsychotic agents showed major affinity to a number of receptors tested. Thus, haloperidol showed affinity to α-1 and D_2 receptors, clozapine exhibited strong affinity to α-1, 5-HT_{2A}, 5-HT_{2C}, and 5-HT_6 receptors, and risperidone showed preferential affinity to 5-$HT_{2A,}$ and α-1 and α-2 adrenoceptors and also to D_2 dopamine receptors (Table 2).

3.1.2. Effects on [^{3}H]glycine uptake in hippocampal synaptosome preparation

The uptake of [^{3}H]glycine in rat hippocampal P_2 synaptosomal preparation was found to be linear for at least 4 min. Exposition of synaptosomal P_2 fraction to hyposomotic shock led to a complete inhibition of [^{3}H]glycine uptake. The uptake of [^{3}H]glycine was Na^+-dependent as reduction of Na^+ from 148 to 23 mM in the buffer resulted in a 75% reduction of [^{3}H]glycine accumulation. Competition studies with increasing concentrations of unlabeled glycine (40–10,000 μM) revealed the presence of a high- and low-affinity glycine uptake in hippocampal synaptosomes. The high- and low-affinity glycine uptake were characterized with K_m values of 0.06 and 3 mM and maximal velocity (V_{max}) of 1.1 and 6.3 nmol/mg protein/min, respectively. These data correspond to those published in the literature (Fedele and Foster, 1992; Herdon et al., 2001). Using 0.25 μM [^{3}H]glycine concentration in the assay, we found that NFPS and Org 24461 inhibited high-affinity [^{3}H]glycine uptake with IC_{50} values of 0.022 and 2.5 μM in rat hippocampal P_2 synaptosomal preparations. These values were 2 and 28 μM for NFPS and Org 24461, respectively, when 30 μM [^{3}H]glycine was used for the determination of [^{3}H]glycine uptake.

3.1.3. Effects on [^{3}H]glycine efflux in hippocampal slices

After a 60-min preperfusion period, the spontaneous [^{3}H]glycine outflow from hippocampal slices occurred at a rate of 3.08 ± 0.15 kBq/g in 3 min (n = 4) and it decreased to 2.67 ± 0.22 kBq/g in 3 min during a 66-min period of superfusion. The content of radioactivity in hippocampal tissue was found to be 294 ± 10 kBq/g (n = 4). Efflux rate of [^{3}H]glycine from superfused hippocampal slices was increased by the addition of glycine (0.1 mM) or sarcosine

Table 4
Lack of anxiolytic effect of Org 24461 and NFPS in anxiolytic tests

Compounds	MED (mg/kg ip)		
	Light–dark test	mCPP light–dark test	Conflict drinking test
Org 24461	>3	>3	>10
NFPS	>3	>3	>10
Diazepam	0.1	0.1	5

Testing of drugs in light–dark test was carried out in mice as described by Costall et al. (1989) after intraperitoneal administration of Org 24461, NFPS, and diazepam. For determination of mCPP-induced anxiety (Bilkei-Gorzo et al., 1998), rats were treated with the test compounds intraperitoneally and 20 min later 0.5 mg/kg mCPP was injected subcutaneously. A modification of the Vogel et al.'s (1971) methods was used for the conflict drinking test in rats, Org 24461, NFPS, and diazepam were administered intraperitoneally. For details, see the Methods section.

(5 mM) to the superfusion buffer (Fig. 2). The effects of 0.1 mM Org 24461 or NFPS on [^{3}H]glycine outflow were determined in hippocampal slices in concentrations 3.5 and 50 times higher than those in inhibited [^{3}H]glycine uptake by 50%. Addition of 0.1 mM of Org 24461 or NFPS to the superfusion buffer did not affect [^{3}H]glycine outflow (Fig. 2). In addition, Org 24461 as well as NFPS (0.1 mM) inhibited the stimulatory effect of 0.1 mM glycine on

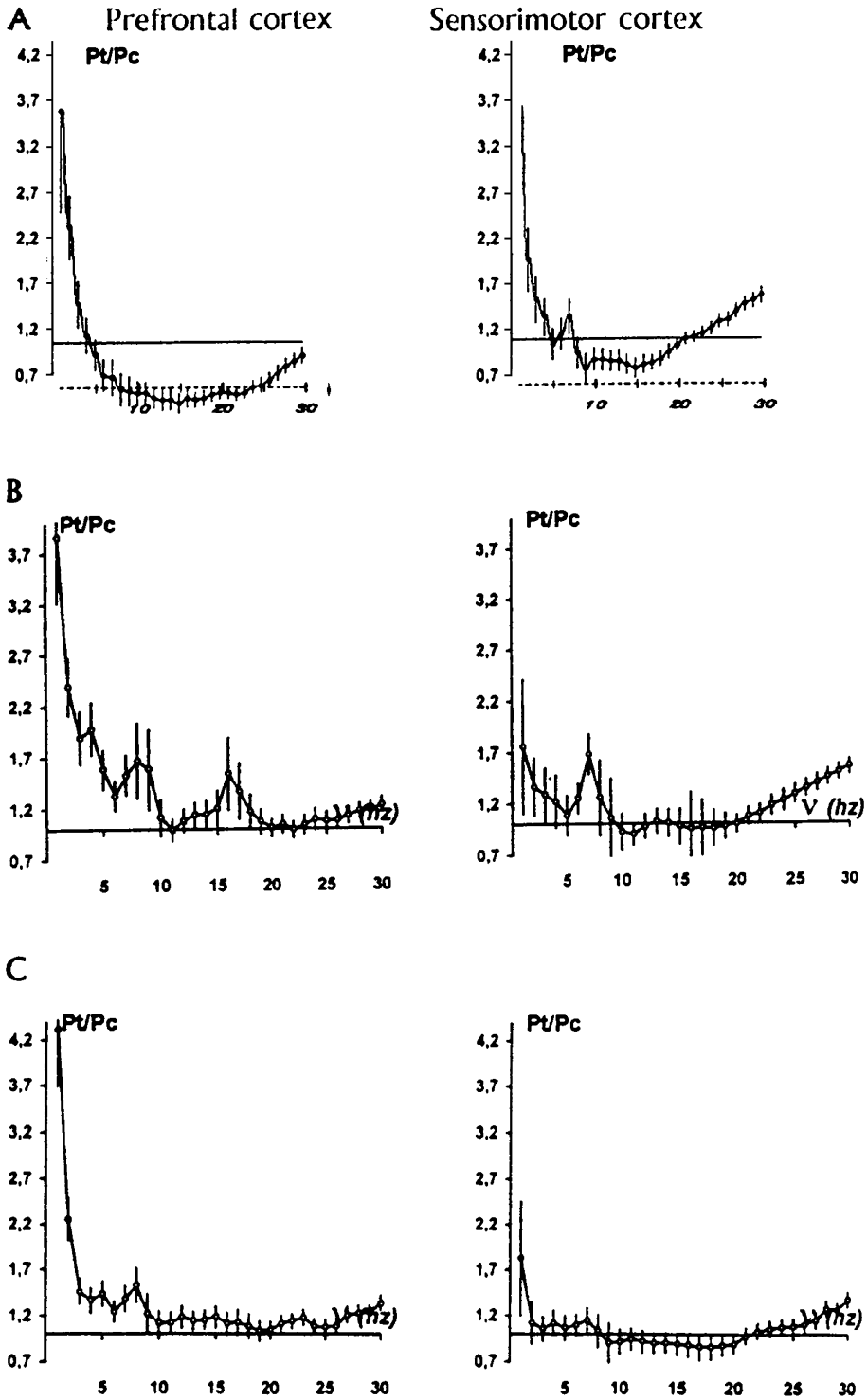

Fig. 4. Effect of Org 24461 and NFPS on PCP-induced EEG power spectral changes in prefrontal cortex (on the left) and sensorimotor cortex (on the right) of conscious rats. (A) Administration of PCP alone (1 mg/kg sc). Coadministration of PCP with Org 24461 (B) and with NFPS (C). The GlyT1 antagonists were administered in a dose of 10 mg/kg ip 30 min after PCP injection. Abscissa represents the EEG spectral component at each frequency between 1 and 30 Hz, the ordinate shows changes in the EEG power spectrum produced by drug administration. Vertical bars indicate 95% confidence intervals ($n=6$).

[^{3}H]glycine efflux. The glycine-induced [^{3}H]glycine efflux was 3.89 ± 0.49% in the absence, and 1.67 ± 0.16% and 2.08 ± 0.21% of content in the presence of Org 24461 and NFPS, respectively [$F(2,9) = 13.499$, $P < .01$, $n = 4$].

3.2. Behavioral testing

3.2.1. Apomorphine-induced stereotypy and climbing in mice

Org 24461 and NFPS had no effect on the induction of stereotypy or climbing behavior in mice by the dopaminergic agonist apomorphine even when they were administered in an oral dose as high as 10 mg/kg (Table 3). Haloperidol and risperidone effectively inhibited apomorphine-induced stereotypy and climbing, whereas clozapine exerted only weak antagonistic effects (Table 3).

3.2.2. PCP- and D-amphetamine-induced hypermotility in mice

Org 24461 antagonized the action of PCP on locomotion with a greater potency than that of D-amphetamine; the calculated ID_{50} values were 3.8 and 13.5 mg/kg ip against PCP and D-amphetamine (Fig. 3). NFPS was less effective than Org 24461 on PCP-induced hypermotility (ID_{50} 21.4 mg/kg ip) and it was ineffective on D-amphetamine-induced hyperlocomotion in mice (ID_{50}>30 mg/kg ip, Fig. 3). The locomotion elicited by D-amphetamine was blocked by haloperidol with a far greater potency than that required to inhibit the PCP-evoked hypermotility (ID_{50} 0.05 and 1.2 mg/kg sc). The hyperlocomotion elicited by PCP or D-amphetamine was blocked by risperidone with equal potency as the ID_{50} values were 0.07 mg/kg sc in both tests. Clozapine displayed markedly greater potency against PCP- than D-amphetamine-induced hypermotility, the ID_{50} values were 2.9 and higher than 10 mg/kg sc in mice, respectively.

3.2.3. Effect of Org 24461 and NFPS on catalepsy compared with antipsychotic agents

Org 24461 and NFPS were found inactive in inducing catalepsy in rats in doses as high as 10 mg/kg (Table 3). Clozapine was also found ineffective in inducing catalepsy whereas haloperidol and risperidone elicited catalepsy in rats (Table 3).

3.2.4. Anxiolytic tests

As shown in Table 4, Org 24461 and NFPS did not induce major anxiolytic effects in light–dark test in mice or in mCPP-induced anxiety test in rats (minimal effective dose or MED was higher than 3 mg/kg ip). In conflict drinking test, Org 24461 and NFPS were also ineffective at doses of 10 mg/kg ip. Diazepam exerted anxiolytic effects in all three anxiolytic tests employed. The MED of diazepam was 0.1 mg/kg ip in light–dark test and mCPP-induced anxiety test and it was 5 mg/kg in the conflict drinking test (Table 4).

3.3. EEG power spectral analysis in prefrontal and sensorimotor cortex of conscious rats

PCP when injected in a dose of 1 mg/kg sc induced complex EEG changes according to the frequency and cortical brain region (Fig. 4). The most characteristic changes in EEG power were an increase at very low frequencies (<4 Hz) in the prefrontal and sensorimotor cortices and a power decrement at higher frequencies. EEG changes in sensorimotor cortex were mainly characterized by an increase in 7- to 8-Hz frequency band and by a further increase in power for the 20- to 30-Hz band.

Org 24461 and NFPS were injected in doses of 1, 3, and 10 mg/kg ip in order to study their effects on PCP-induced changes in EEG power spectra in conscious rats. Org 24461 and NFPS dose-dependently attenuated the power decrement evoked by PCP at higher frequencies in the prefrontal and sensorimotor cortices (Fig. 4). In addition, Org 24461, in interaction with PCP, also induced synchronization peaks at 3–5 and 8–20 Hz frequency bands in the prefrontal cortex. NFPS nearly completely inhibited the decrease in power between 5 and 30 Hz evoked by PCP and only a small synchronization at 7 Hz was observed in the prefrontal cortex (Fig. 4).

4. Discussion

The sarcosine derivative NFPS and Org 24461 inhibited [^{3}H]glycine uptake in hippocampal synaptosomal preparations and this inhibition depended on the concentration of [^{3}H]glycine used in the assay. NFPS was found to be equipotent in inhibition of glycine uptake in synaptosomes prepared from hippocampus (this study) or cerebral cortex (Herdon et al., 2001). Org 24461 inhibited glycine uptake in CHO cells expressing hGlyT1b with an IC_{50} value of 0.3 μM (Brown et al., 2001), a potency one magnitude higher than what we found in hippocampal synaptosomes. Although the reason of this discrepancy is not clear, differences in inhibition of glycine uptake determined in cell line expressing GlyT1 and synaptosomes were also demonstrated by Herdon et al. (2001). It has been reported that glycine transporter proteins, which regulate glycine concentrations in glutamatergic excitatory synapses, belong to GlyT1 transporters (Smith et al., 1992; Fedele et al., 1993). NFPS and Org 24461 inhibited glycine uptake in HEK292 cells and CHO cells expressing GlyT1c and hGlyT1b, respectively (Herdon et al., 2001; Brown et al., 2001). We therefore believe that NFPS and Org 24461 preferably inhibited GlyT1 transporter in our experimental models using hippocampal synaptosomes or slice preparations.

Brown et al. (2001) reported that Org 24598, the *R*-(−) isomer of Org 24461, showed no appreciable affinity at dopamine, serotonin, and noradrenaline receptors in radioligand binding experiments. We may confirm that the glycine uptake inhibitory effects of Org 24461 and NFPS

were associated with no major affinity to a series of neurotransmitter receptors although some receptor binding affinity of these compounds was observed at 5-HT_{2A}, 5-HT_{2C}, and 5-HT_7 serotonin receptors and α-2 adrenoceptors at the higher concentrations (10 μM) tested. Clozapine and risperidone, on the other hand, interact with multiple dopaminergic, serotonergic, and adrenergic receptors (Gunasekara et al., 2002). The interactions of these two antipsychotic agents with neurotransmitter receptor systems markedly differ from that of haloperidol, which preferentially binds to dopamine D_2 receptors. These distinctive receptor binding profiles may explain a number of differences observed in the neuropharmacological actions of GlyT1 inhibitors and antipsychotic agents tested.

To further characterize the inhibition by Org 24461 and NFPS of GlyT1 proteins, their effects were also determined on [^{3}H]glycine efflux in hippocampal slice preparations. We found that neither Org 24461 nor NFPS influenced the efflux rate of [^{3}H]glycine albeit the two GlyT1 inhibitors were added to superfused hippocampal slices in concentrations several times higher than those in inhibited [^{3}H]glycine uptake. In contrast, sarcosine and even glycine enhanced [^{3}H]glycine efflux rate in hippocampal slices. This latter finding may correlate with the observation of Herdon et al. (2001) who showed that addition of sarcosine or glycine to HEK293 cells containing hGlyT1c transporters and preloaded with [^{3}H]glycine resulted in a decrease in content of radioactivity. The fact that sarcosine and glycine enhanced, whereas Org 24461 and NFPS did not influence, [^{3}H]glycine efflux from hippocampal slices indicates different mechanisms of these compounds in glycine transporter inhibition (Aubrey and Vanderberg, 2001).

The glycine- or sarcosine-induced [^{3}H]glycine release may be consequent to the transport of these molecules into the glycine-containing cells through the glycine carriers. Glycine-releasing cells probably express homocarriers for the recapture of the released glycine and homo- or heterocarriers through which sarcosine can penetrate and evoke glycine release. The effect of sarcosine or glycine is presumably due to exchange with preloaded [^{3}H]glycine via normal operation of the transporter resulting in an increase in [^{3}H]glycine efflux. On the other hand, Org 24461 and NFPS may be true inhibitors of GlyT1 and their inhibitory effect resembles that of GDA reported earlier (Harsing et al., 2001). It is worthwhile to point out that Org 24461 and NFPS, which were without effects on [^{3}H]glycine outflow by themselves, were able to reduce the glycine-induced [^{3}H]glycine efflux from hippocampal slices. These results may suggest that both Org 24461 and NFPS are nontransportable inhibitors of GlyT1 proteins whereas sarcosine may inhibit the uptake of glycine by competing with glycine at the binding site of its transporters.

Hippocampal tissue was chosen to study the effects of GlyT1 inhibitors on glycine uptake and release as the density of $glycine_B$ binding sites associated with NMDA receptors is greatest in this brain area (Pullan and Powell, 1992). Hippocampus is also an area that has been proposed to be the primary locus of action for the dissociative action of PCP (Corssen and Domino, 1966; Miyasaka and Domino, 1968), a noncompetitive antagonist of NMDA receptors (Anis et al., 1983). In experimental animals, PCP induces complex behavior syndrome and the ability of a compound to antagonize these behavioral alterations would be predictive for its antipsychotic properties in humans (Bujas-Bobanovic et al., 2000). PCP mimics symptoms of schizophrenia by at least two mechanisms. First, PCP induces increased glutamate release in intracortical circuitry by increasing impulse flow in thalamocortical inputs (Aghajanian and Marek, 2000). Second, PCP reduces NMDA receptor-mediated functions by blocking the receptor, which then leads to disinhibition of the thalamic filter (Carlsson, 1988). Both mechanisms will result in an overload of information for the cerebral cortex leading to disruption of integrative cortical functions.

Accordingly, PCP evokes dose-dependent characteristic changes in EEG power spectra both in the prefrontal and sensorimotor cortices of conscious rats (Sebban et al., 2001). Thus, PCP increased EEG power at low frequency (1 Hz), an effect proved to be resistant to the influence of a number of drugs. Over a frequency range of 5–20 Hz, PCP decreased EEG power and the desynchronization observed at this power band may be associated with hypervigilance, which is believed to be part of schizophrenia. It was also shown that clozapine and haloperidol inhibited the PCP-induced decrease in EEG power between 5 and 30 Hz in the prefrontal cortex although differences between the actions of the two antipsychotic agents were also observed (Millan et al., 1998; Sebban et al., 2001). Thus, both clozapine and haloperidol added with PCP resulted in a synchronization at 7 Hz in the sensorimotor cortex and haloperidol evoked similar effect in the prefrontal cortex.

The GlyT1 inhibitors markedly influenced the effects of PCP on EEG spectra in the cerebral cortex and their effects resemble those of antipsychotics. NFPS homogenously attenuated the effect of PCP and these changes were characteristic for both prefrontal and sensorimotor cortices. The effect of Org 24461 was found to be more complex as this compound induced synchronization with the appearance of peaks in low (3–5 Hz) and higher (5–20 Hz) frequency bands in the prefrontal cortex. These findings suggest that NMDA receptors associated with $glycine_B$ binding sites may also be involved in the cortical desynchronization of EEG power at higher frequencies. As the PCP-induced EEG desynchronization observed at higher frequencies is likely related to an increase in cortical signal processing that corresponds to hallucination, GlyT1 inhibitors that alter this frequency range may also attenuate hallucination in schizophrenia.

The behavioral changes induced by PCP in animals consist of stereotyped behavior, ataxia, and also hyperlocomotion (Bujas-Bobanovic et al., 2000). PCP-evoked hyperlocomotion may involve serotonergic as well as dop-

aminergic mechanisms and activation of 5-HT_{2A} receptors (Martin et al., 1997; Millan et al., 1998) or increase in dopamine release in the limbic or extrapyramidal systems after PCP administration have been demonstrated (Phillips et al., 2001; Balla et al., 2001). Clozapine, an atypical antipsychotic drug with multireceptor action (Lieberman., 1993), has been reported to block hyperlocomotion induced by acute PCP administration (Maurel-Remy et al., 1995) and this effect is believed to be mediated by the blockade of 5-HT_{2A} receptors in the nucleus accumbens (Millan et al., 1998). The classic antipsychotic agent haloperidol also antagonizes increases in locomotor activity after PCP administration and this effect of haloperidol is probably due to its blockade of D_2 dopamine receptors (Kitaichi et al., 1994; Maurel-Remy et al., 1995). Another antipsychotic agent, risperidone, which shows a pharmacology that partially overlaps both haloperidol and clozapine, also blocks the development of PCP-induced locomotor hyperactivity probably by acting on D_2 and 5-HT_{2A} receptors (Kitaichi et al., 1994). Similar to the antipsychotic drugs, the GlyT1 inhibitor Org 24461 and NFPS also inhibited PCP-induced hyperlocomotion in our experiments. The fact, however, that neither Org 24461 nor NFPS expressed affinity to dopamine or 5-HT receptors in binding assays suggests that GlyT1 inhibitors influence PCP-induced locomotion by other mechanisms, probably by increasing glycine concentrations at $glycine_B$ binding sites on NMDA receptors. It may be important to call attention to the preferential effects of GlyT1 inhibitors on PCP- versus D-amphetamine-induced hyperlocomotion (see below) that may suggest their potency to control negative symptoms.

Whereas PCP elicits negative symptoms or both negative and positive symptoms of schizophrenia (Cosgrove and Newell, 1991; Javitt and Zukin, 1991), the use of direct and indirect dopamine agonists has been proposed to be the principal model for positive symptoms of schizophrenia (Arnt and Skarsfeldt, 1998). Therefore, in the first series of experiments, the indirect dopamine agonist D-amphetamine was employed to test the effects of GlyT1 inhibitors on dopamine receptor-mediated effects. D-Amphetamine induces locomotion, an effect mediated mainly by D_2 dopamine receptor stimulation in the nucleus accumbens (Arnt, 1995). We found that Org 24461 inhibited the D-amphetamine-induced hypermotility whereas NFPS was found inactive in this test. This finding suggests there might be some differences in the mechanism of action of the two GlyT1 inhibitors as Org 24461, which contains fluoxetine moiety, that may alter some other neurotransmitter system(s) at receptor or transporter levels.

In another series of experiments, we have determined the effects of Org 24461 and NFPS on apomorphine-induced climbing and stereotypy in mice. Inhibition of apomorphine-induced climbing is an effect related to D_2 dopamine receptor stimulation mainly in the limbic system and that may be predictive for antipsychotic activity of a drug (Kahn and Davis, 1995). On the contrary, inhibition of apomorphine-induced stereotypy is an effect related to D_2 dopamine receptor stimulation particularly in the extrapyramidal system, and positive data obtained in this test may predict potential extrapyramidal side effects of an antipsychotic drug candidate (Costall and Naylor, 1977; Ninan and Kulkarni, 1999). The fact that the GlyT1 inhibitor Org 24461 and NFPS did not influence apomorphine-induced climbing and stereotypy indicates that these compounds may have limited potential in mediation of limbic and extrapyramidal dopaminergic neurotransmission. Clozapine, haloperidol, and risperidone used as reference compounds in this study, however, inhibited the apomorphine-induced climbing and stereotyped behavior according to their binding potency to dopamine receptors.

Antipsychotic agents elicit an extrapyramidal motor syndrome by the interruption of activity at striatal D_2 or D_1 dopamine receptors and catalepsy induced in rats may be predictive for an extrapyramidal side effect in humans (Hoffman and Donovan, 1995). Haloperidol potently elicited catalepsy in rats, a response reflecting inhibition of activity at D_2 dopamine receptors in the striatum. Risperidone, which shows D_2 and 5-HT_{2A} affinities but no agonist effect on 5-HT_{1A} receptors, also induced catalepsy in rats further confirming the extrapyramidal side effect-inducing ability of this compound observed in humans (Gunasekara et al., 2002). Clozapine, however, was found to be less potent in inducing catalepsy and its reduced extrapyramidal side effects may be explained by marked affinity to muscarinic receptors (Brunello et al., 1995). Similarly to the apomorphine-induced stereotypy, the GlyT1 inhibitor Org 24461 and NFPS did not induce catalepsy in rats presumably suggesting the lack of extrapyramidal side effects of these compounds in patients. The lack of effect of GlyT1 inhibitors on D_2 receptors speaks for improving negative symptoms of schizophrenia with a benign extrapyramidal potential without eliciting tardive dyskinesia after a long-term treatment.

Anxious symptoms are frequently encountered in many psychiatric disorders and it was proposed that anxiolytic properties would be useful for an antipsychotic agent (Millan et al., 1999). In contrast to haloperidol, clozapine possesses some anxiolytic properties in certain experimental models (Wiley et al., 1993). Since GlyT1 inhibitors also shared antipsychotic activity in some but not all tests employed, we determined whether these compounds possess anxiolytic activity. The GlyT1 inhibitor Org 24461 and NFPS, which indirectly activate $glycine_B$ binding site by increasing glycine levels at the vicinity of NMDA receptors, failed to demonstrate anxiolytic effects in a number of tests suitable to detect anxiolytic activity. It is interesting to mention that glycine, a full agonist at $glycine_B$ binding site, was also inactive in animal models of anxiety (Winslow et al., 1990; Chojnacka-Wojcik et al., 1996). On the other hand, D-cycloserine, a partial agonist at $glycine_B$ site, showed an anxiolytic-like activity in the Vogel conflict drinking test in rats (Klodzinska and Chojnacka-Wojcik,

2000) and a similar effect has also been reported in the elevated plus maze paradigm (Karcz-Kubicha et al., 1997). In the Vogel conflict drinking test, however, the anxiolytic effect of D-cycloserine and that of ACPC, another partial agonist at glycine$_B$ binding site, was not affected by pretreatment with glycine (Chojnacka-Wojcik et al., 1996; Klodzinska and Chojnacka-Wojcik, 2000). These findings, in addition to our results obtained with GlyT1 inhibitors, may indicate that glycine$_B$ binding site at NMDA receptors is probably not directly involved in anxiogenic/anxiolytic mechanisms in the central nervous system (Klodzinska and Chojnacka-Wojcik, 2000).

In summary, the GlyT1 inhibitors Org 24461 and NFPS display marked differences in mode of action to haloperidol and the atypical antipsychotic clozapine and risperidone. Their receptor, glycine transporter inhibitory, and functional profiles suggest that they may be able to control negative symptoms of schizophrenia in the relative absence of effects on positive symptoms and extrapyramidal motor functions. Thus, GlyT1 inhibitors are likely to be of use in the treatment of schizophrenia with a narrower margin of influencing symptoms in comparison of currently used antipsychotic agents. In schizophrenia, the reduced thalamic filter may result in a higher glutamatergic tone in the cerebral cortex, which is associated with hypofunctional NMDA receptors located on postsynaptic neural substrates. GlyT1 blockers may reverse NMDA receptor hypofunction by the blockade of glycine reuptake, which then leads to increase of glycine levels in the vicinity of glycine$_B$ binding sites at NMDA receptors. The hypothesis that schizophrenia may be associated with decreased central nervous system glycine levels was further shown by the observation that high oral doses of glycine improved negative symptoms of schizophrenic subjects (Heresco-Levy et al., 1996). Thus, GlyT1 inhibitors may influence beneficially symptoms of schizophrenia by the inhibition of glycine uptake process.

Acknowledgements

This research was supported in part by the Hungarian Science Research Fund (OTKA) Grant No. T-32983 and the Research Council for Health Sciences, Hungarian Ministry of Health and Welfare (ETT-107/2000).

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