Pharmacology, Biochemistry and Behavior 95 (2010) 23–30

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Effects of a positive allosteric modulator of $mGluR_5$ ADX47273 on conditioned avoidance response and PCP-induced hyperlocomotion in the rat as models for schizophrenia

Chantal Schlumberger, Małgorzata Pietraszek, Andreas Gravius, Wojciech Danysz *

Merz Pharmaceuticals GmbH, Dept. In vivo Pharmacology, Alfred-Wegener-Strasse 2, D-60438 Frankfurt am Main, Germany

ARTICLE INFO

Article history:
Received 15 July 2009
Received in revised form 29 November 2009
Accepted 2 December 2009
Available online 5 December 2009

Keywords:
Locomotor activity
Catalepsy
CAR
Phencyclidine
ADX47273
Olanzapine
Aripiprazole
Haloperidol
$mGluR_5$

ABSTRACT

Metabotropic glutamate receptors of the subtype 5 ($mGluR_5$) are located in brain regions implicated in schizophrenia such as the cerebral cortex or the nucleus accumbens. They may therefore provide an interesting target for the treatment of psychoses. Currently available agonists of $mGluR_5$ are not selective, do not penetrate the brain and induce a tonic activation resulting in a rapid desensitization. Therefore, the research focus was shifted to positive allosteric modulators (PAMs). Subsequently several $mGluR_5$ PAMs have been discovered, e.g. ADX47273 (S-(4-fluoro-phenyl)-{3-[3-(4-fluoro-phenyl)-[1,2,4]oxadiazol-5-yl]-piperidin-1-yl}-methanone). In the present study, effects of ADX47273 (1–100 mg/kg) were evaluated in rat models used for detecting antipsychotic-like activity: the conditioned avoidance response (CAR) and the phencyclidine (PCP)-induced hyperlocomotion models. Furthermore, the cataleptogenic potential of ADX47273 was compared to that of haloperidol.

ADX47273 (100 mg/kg) and various clinically used neuroleptics (haloperidol, olanzapine, and aripiprazole) attenuated CAR behaviour in rats. However, ADX47273 and aripiprazole failed to reduce the PCP-induced hyperlocomotion, whereas olanzapine and haloperidol diminished it. In contrast to haloperidol, ADX47273 (100 mg/kg) failed to induce consistent catalepsy in rats. In conclusion, ADX47273 shows promising antipsychotic activity in some tests which require future investigation.

1. Introduction

The glutamate theory of schizophrenia is supported by preclinical evidence and results of post-mortem studies demonstrating alterations in the density of N-methyl-D-aspartate (NMDA) receptors in the cerebral cortices, striata and hippocampi of schizophrenia patients (for review see Meador-Woodruff and Healy, 2000). This theory actually emerged following observation that uncompetitive NMDA receptor antagonists such as, e.g., phencyclidine (PCP) or ketamine induce psychotic symptoms in healthy individuals and exacerbate psychotic symptoms in patients (Krystal et al., 1994; Luby et al., 1959; Malhotra et al., 1997). Both agents have been shown to produce positive and negative symptoms as well as to induce cognitive impairments in humans (Krystal et al., 1994; Luby et al., 1959). Therefore, compounds of this class have been utilized to model schizophrenia symptoms in animals. Indeed, uncompetitive NMDA receptor antagonists increase locomotor activity and induce stereotypy in animals (Danysz et al., 1994; Takahata and Moghaddam, 2003), and these effects are attenuated by both typical and atypical antipsychotic drugs (Maurel-Remy et al., 1995; Nordquist et al., 2008; Ogren and Goldstein, 1994).

Recently, it has also been shown that activation or positive allosteric modulation of $mGluR_5$ can alleviate such behavioural alterations (Chan et al., 2008; Homayoun and Moghaddam, 2008; Liu et al., 2008). Since the stimulation of $mGluR_5$ potentiates the function of NMDA receptors in brain regions relevant for schizophrenia e.g. in the hippocampus, the prefrontal cortex (PFC) and the striatum, this phenomenon has been proposed as a putative mechanism of action of positive allosteric modulators (PAMs) of $mGluR_5$ (Doherty et al., 1997; Homayoun and Moghaddam, 2006; Homayoun et al., 2004; Pisani et al., 2001). Additional support for the $mGluR_5$ being a putative target for schizophrenia therapy comes from the observation of an upregulation of $mGluR_5$ mRNA in the PFC of schizophrenic patients (Ohnuma et al., 1998). Furthermore, intracerebrally administered $mGluR_5$ agonists, e.g., CHPG ((RS)-2-chloro-5-hydroxyphenylglycine), exerted antipsychotic-like effective in some rodent models of schizophrenia (Chan et al., 2008; Kinney et al., 2003). However, administration of $mGluR_5$ agonists desensitises the receptor, precluding application of the substances in *in vivo* studies (Aronica et al., 1993; Gereau and Heinemann, 1998; Lefkowitz, 1993). More recently, $mGluR_5$ receptor PAMs have been discovered (O'Brien

* Corresponding author. Tel.: +49 69 1503 564; fax: +49 69 1503 8869.
E-mail addresses: Chantal.Schlumberger@merz.de (C. Schlumberger), Malgorzata.Pietraszek@merz.de (M. Pietraszek), Andreas.Gravius@merz.de (A. Gravius). Wojciech.Danysz@merz.de (W. Danysz).

0091-3057/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2009.12.002

et al., 2003). Such PAMs do not activate the receptor directly but potentiate the responses to its activation by agonists (Conn et al., 2009). Furthermore, $mGluR_5$ PAMs potentiate enhancement of NMDA receptor currents by $mGluR_5$ agonists (Liu et al., 2006) and display antipsychotic-like activity in animal models of schizophrenia (Epping-Jordan et al., 2005; Lindsley et al., 2004). Homayoun and Moghaddam (2008) demonstrated that the $mGluR_5$ PAM, CDPPB (3-cyano-N-(1,3-diphenyl-1H-pyrazol-5-yl)benzamide), reversed MK-801- or amphetamine-induced stereotypies and excessive firing rates in the orbitofrontal cortex. Similarly, CDPPB reversed hyperlocomotion and prepulse inhibition deficits evoked by amphetamine in rodents (Kinney et al., 2005). Moreover, another $mGluR_5$ PAM, ADX47273 (S-(4-Fluoro-phenyl)-{3-[3-(4-fluoro-phenyl)-[1,2,4]oxadiazol-5-yl]-piperidin-1-yl}-methanone), attenuated PCP-, amphetamine- or apomorphine-induced hyperlocomotion in mice and dose-dependently reduced conditioned avoidance responding (CAR) in rats (Liu et al., 2008). This evidence suggests that $mGluR_5$ PAMs are able to reduce both NMDA antagonist and dopamine agonist induced behaviours (Kinney et al., 2005; Lecourtier et al., 2007).

The aim of the present study was to evaluate the effects of the $mGluR_5$ PAM ADX47273 in animal models relevant for schizophrenia, including CAR and PCP-induced hyperlocomotion, with comparisons made to clinically used neuroleptics. The CAR performance is inhibited by both typical and atypical neuroleptics, and is considered to be highly predictive for antipsychotic activity (Wadenberg and Hicks, 1999). Hyperlocomotion induced by NMDA antagonists represents a common method used to assess possible atypical antipsychotic effects. Finally, ADX47273 was compared to haloperidol in a catalepsy test in order to assess its potential to produce extrapyramidal side effects.

As compared to a previous paper on ADX47273 (Liu et al., 2008), the novelty aspect is enhanced by use of rats instead of mice and also direct comparison with selected neuroleptics (reference agents).

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (Élevage Janvier; Le Genest Saint Isle, France) weighing 200 g at arrival were used in all experiments. The rats were allowed to acclimatise for one week prior to the experiments. The animals were kept in groups of 4–5 individuals, in cages provided with bedding, paper cloth and a red tunnel, under standard conditions (21 ± 1 °C, 60 ± 3%, tap water and chow pellets *ad libitum*), at 12/12 h light–dark cycle (lights on at 7 a.m.). Experiments were carried out between 9 a.m. and 6 p.m. The rats were acclimatised to the experimental rooms for at least 30 min before all experiments. Separate batches of rats were used for each type of experiment, i.e. for CAR, locomotor activity, and catalepsy experiments. For each drug, different sets of animals were used in locomotor activity and catalepsy experiments, and the animals were randomly allotted to the treatment groups. For CAR, animals received more than one type of drug treatment, but were assigned treatments in a pseudorandom order (see below in the CAR section).

All studies were approved by a local Ethical Committee (Regierungspraesidium Darmstadt, Hessen) and were performed in accordance with the recommendations and policies of the U.S. National Institutes of Health Guidelines for the Use of Animals.

2.2. CAR

Training and testing was performed in two GEMINI set-ups (San Diego Instruments, San Diego, CA, USA). Each set-up was subdivided in two connected boxes (21 cm × 24 cm × 17.5 cm) via an aperture (8.5 cm × 7.2 cm) in the middle wall (two-way avoidance paradigm). The animals could move freely from one compartment to the other at any time. The position of the animals was tracked by 8 photocells in each of the boxes. A cue light was situated on the wall opposing the compartment entry. A session started with a 3 minute habituation followed by 20 trials. The cue light was switched on at the start of a trial, and after 10 s, it was combined with a scrambled, 0.3 mA foot shock for 10 further seconds. If the animal changed the compartment in less than 10 s after the start of the cue light, an avoided trial was counted. An escape response was recorded if the rat moved into the opposite compartment during light and foot shock presentation. If a rat did not respond to the foot shock, an escape failure was noted. The intertrial interval was randomly assigned between 20 and 30 s and maximal duration of a daily session was 20 min. Animals were trained 5 days/week until they reached a stable avoidance of above 75%/day on two subsequent days. Before each drug test, a drug-free pre-test was performed. The presented test data are shown as % avoidance values and were compared to the % avoidance values of the pre-test.

Injections were made 30 min before the start of a session on a test day, and testing was done at 30 and 90 min post-injection. Animals were used several times with 1 week drug-free interval between tests, and treatment was assigned to the groups in a pseudorandom order. Before each test, animals' performance was verified on two subsequent days. Additionally, following a publication of Liu et al. (2008), we decided to test ADX47273 at the dose of 100 mg/kg in CAR in a subset of animals.

2.3. Locomotor activity

For the measurement of locomotor activity, Perspex® boxes (ENV-515-16, 43.2 × 43.2 × 30 cm, Med Associates Inc., St Albans, VT, USA) were used. The boxes were placed in noise-proof chambers equipped with a ventilator and a source of white light (5.6 W) 55 cm above a white floor (Med Associates Inc., St Albans, VT, USA). Four arrays of 16 infrared photo beams placed 3 cm above the floor measured horizontal activity. For the measurement of vertical activity, 2 additional sets of 16 photo beams were placed 15 cm above the floor. The output from the counters was integrated and analyzed by a PC computer. Distance travelled (in cm) and vertical movements (counts) were assessed in further analysis as measures of locomotion and rearing, respectively. After the injections with ADX47273 or antipsychotics, animals were placed in the open field for 30 min. Subsequently, PCP was injected and the recording was started and continued for a total of 120 min. For testing ADX47273 alone, it was injected in the home cage. Thirty minutes later, animals were put into the activity boxes and their behaviour was measured for 30 min. A maximum of eight rats from different groups were tested simultaneously in 8 open-field boxes in a pseudorandom order.

2.4. Catalepsy

Before catalepsy experiments, rats were handled for 4 days (5 min/day). Rats were tested on the 5th day at two different placements: placing both front paws on a horizontal bar elevated 9 cm above the floor (bar test) or placing the rat on a vertical wire mesh (grid test). Time to voluntary descending of a paw from the horizontal bar or directed movement on the vertical wire mesh was considered to be the endpoint of cataleptic behaviour (descent latency). Cut-off time for both tests was set to 180 s. The animals were first tested on the horizontal bar immediately followed by the vertical grid. Tests were performed at 30, 60 and 90 min post-injections of ADX47273 or haloperidol.

2.5. Drugs

ADX47273, synthesized by Merz Pharmaceuticals GmbH (Frankfurt am Main, Germany), was dissolved in 70% dimethyl sulfoxide: 30% polyethylene glycol 400. PCP (phencyclidine hydrochloride) was purchased from Sigma-Aldrich, Taufkirchen, Germany, and was

dissolved in saline. Olanzapine (Sequoia Research Products Ltd., Pangbourne, UK) was dissolved in a small amount of 1 N HCl and diluted to the final concentration with water. pH was adjusted to 6 with 1 N NaOH. Aripiprazole (Sequoia Research Products Ltd., Pangbourne, UK) was suspended in water containing Tween 80 (10%). Haloperidol (Haldol-Janssen®, Janssen-Cilag, Beerse, Belgium, ampoules à 5 mg/1 ml solution) was diluted to the final concentration with physiological saline. All substances were injected i.p. in a volume of 1 ml/kg except for aripiprazole which was used in a 2 ml/kg volume. Doses refer to the salt form of the given substance. Injection time point for ADX47273 was chosen based on our previous publication showing high plasma and brain concentrations 30 min after i.p. administration (Schlumberger et al., 2009).

2.6. Data analysis and statistics

2.6.1. CAR

Percent avoidance response was calculated as (number of avoided trials/number of trials)×100. For each rat, the percent avoidances from the test were divided by the percent avoidances from the pre-test and multiplied by 100 and the subsequently obtained values were taken for both analysis and graphic presentation.

Analysis of normalized percent avoidance levels was done by using two-way repeated-measures ANOVA (with treatment and time as variables) followed, if significant, by Duncan's test. Analysis of the percent escape failures was calculated by Kruskal–Wallis ANOVA on ranks followed by Mann–Whitney test for pairwise comparisons for 30 and 90 min time points separately.

2.6.2. Locomotor activity

Locomotion distances and rearing counts were expressed as mean ± SEM. The time course of interactions between ADX47273 and all antipsychotics effect on PCP-induced hyperlocomotion was analyzed via two-way repeated-measures ANOVA followed, if significant, by Duncan's test. The influence of ADX47273 or all antipsychotics on PCP-induced changes of vertical locomotion (rearing), measured by recording the total rearing counts, was analyzed by one-way ANOVA followed, if significant, by Duncan's test (data not shown). The influence of ADX47273 on spontaneous horizontal locomotion (the total distance travelled), or on vertical activity (rearing) was analyzed by one-way ANOVA followed by Duncan's test.

2.6.3. Catalepsy

Results from the catalepsy tests were expressed as medians and quartiles (Q1:Q3) and analyzed by the Kruskal–Wallis ANOVA on ranks followed by Mann–Whitney test for pairwise comparisons at each time point and test quality separately.

3. Results

3.1. CAR

ADX47273 at 100 mg/kg significantly decreased CAR at 30 and 90 min post-injection [3 and 10 mg/kg: $F(2,43)=0.895$; $p=0.067$; for 100 mg/kg: $F(1,15)=21.822$; $p<0.001$; Fig. 1A] having no effect on escape failures at any time point [3 and 10 mg/kg at 30 min: $H=0$; $p>0.1$, $df=2$; 3 and 10 mg/kg at 90 min: $H=0.895$; $p>0.1$, $df=2$; for 100 mg/kg at 30 min: $T=81.00$; $p>0.1$; for 100 mg/kg at 90 min: $T=76.50$; $p>0.1$; Table 1].

At both 30 and 90 min, 0.1 mg/kg haloperidol lowered CAR [$F(2,19)=6.291$; $p=0.008$; Fig. 1B] while it failed to affect escape failures [30 min: $H=0$; $p>0.1$; $df=2$; 90 min: $H=3.397$; $p>0.1$; $df=2$; Table 1]. Both 1.25 and 2.5 mg/kg olanzapine reduced CAR [$F(2,22)=42.883$; $p<0.001$; Fig. 1C]. However, only the effect of the dose of 1.25 mg/kg was specific as at 2.5 mg/kg, olanzapine induced significant escape response failures at both time points [30 min: $H=6.789$; $p=0.034$; $df=2$; 90 min: $H=10.737$; $p=0.005$; $df=2$; Table 1].

Aripiprazole at doses 5 and 10 mg/kg at 30 min and at 2.5–10 mg/kg at 90 min reduced CAR [$F(4,35)=11.389$; $p<0.001$; Fig. 1D] with no increase in response failures at any time [30 min: $H=7.987$; $p=0.092$; $df=4$; 90 min: $H=7.147$; $p>0.1$; $df=4$; Table 1].

3.2. Locomotor activity

PCP dose-dependently induced hyperlocomotion in rats [$F(3,140)=9.899$; $p<0.001$, Fig. 2A]. The effect of PCP was inhibited by haloperidol but only at the highest dose tested (0.4 mg/kg) [$F(4,160)=3.049$; $p=0.031$, Fig. 2B]. Also, olanzapine dose-dependently attenuated PCP-induced hyperlocomotion [$F(3,140)=24.843$; $p<0.001$, Fig. 2C]. In contrast, aripiprazole showed only a tendency to inhibit PCP-hyperlocomotion which failed to reach statistical significance [$F(4,275)=2.392$; $p=0.062$; Fig. 2D]. ADX47273 had no effect on PCP-induced hyperlocomotion at any dose tested (1–100 mg/kg i.p.) [$F(3,130)=1.074$; $p>0.1$, Fig. 2E, $F(2,105)=1.554$; $p>0.1$, Fig. 2F].

PCP had no effect on rearings in habituated rats [$F(3,28)=1.468$; $p>0.1$; data not shown]. Rearings in PCP-treated animals were not changed by aripiprazole [$F(4,55)=1.027$; $p>0.1$] and olanzapine [$F(3,28)=0.380$; $p>0.1$; data not shown]. ADX47273 [$F(3,26)=0.562$; $p>0.1$, for 1–10 mg/kg ADX47273; $F(2,21)=3.213$; $p=0.061$, for 30 and 100 mg/kg ADX47273, data not shown] showed at the highest dose a tendency to increase rearings in PCP-treated animals. In contrast, inhibition was seen following haloperidol at all tested doses [$F(4,32)=4.319$; $p=0.007$; data not shown].

ADX47273 at 30 and 100 mg/kg did not influence the spontaneous locomotion [$F(2,21)=1.331$; $p>0.1$, Fig. 3A], but decreased rearings [$F(2,21)=4.827$; $p=0.019$; Fig. 3B].

3.3. Catalepsy

Haloperidol (0.025–0.6 mg/kg) dose-dependently induced catalepsy with a significant effect observed in rats at doses of 0.4 and 0.6 mg/kg i.p. on the bar test [30 min: $H=27.471$; $p\leq0.001$, $df=4$; 60 min: $H=28.105$; $p\leq0.001$, $df=4$; data not shown] as well as on the grid test [30 min: $H=27.188$; $p\leq0.001$, $df=4$; 60 min: $H=28.228$; $p\leq0.001$, $df=4$; data not shown]. According to this study, the dose of 0.4 mg/kg haloperidol was selected as the positive control for further tests.

In the second experiment, the effect of ADX47273 (30 and 100 mg/kg) was compared to that of haloperidol (0.4 mg/kg) [Fig. 4]. Kruskal–Wallis ANOVA on ranks yielded a significant difference for both endpoints and at all time points [bar descent time: 30 min: $H=15.269$; $p=0.002$, $df=3$; 60 min: $H=14.013$; $p=0.003$, $df=3$; 90 min: $H=13.483$, $p=0.004$, $df=3$; grid descent time: 30 min: $H=13.292$, $p=0.004$, $df=3$; 60 min: $H=13.834$, $p=0.003$, $df=3$; 90 min: $H=18.299$, $p\leq0.001$, $df=3$; Fig. 4]. Post-hoc analysis revealed that haloperidol increased both bar and grid descent latencies at any time point (all time points and descent yielded each $p=0.002$ by Mann–Whitney U-tests), while 100 mg/kg ADX47273 induced only mild prolongation of the grid descent latency 90 min post-injection [$p=0.008$].

4. Discussion

In the present study, the CAR test revealed that ADX47273, haloperidol, olanzapine and aripiprazole decreased avoidance levels being indicative for antipsychotic activity. All these substances had a specific effect in CAR in contrast to the high-dose olanzapine which also induced significant increase in escape failures. In spontaneous locomotor activity test, ADX47273 at high doses of 30 and 100 mg/kg decreased rearings but not horizontal locomotion. ADX47273 failed to attenuate PCP-induced hyperlocomotion. In contrast, typical (haloperidol) and some atypical neuroleptics (olanzapine but not

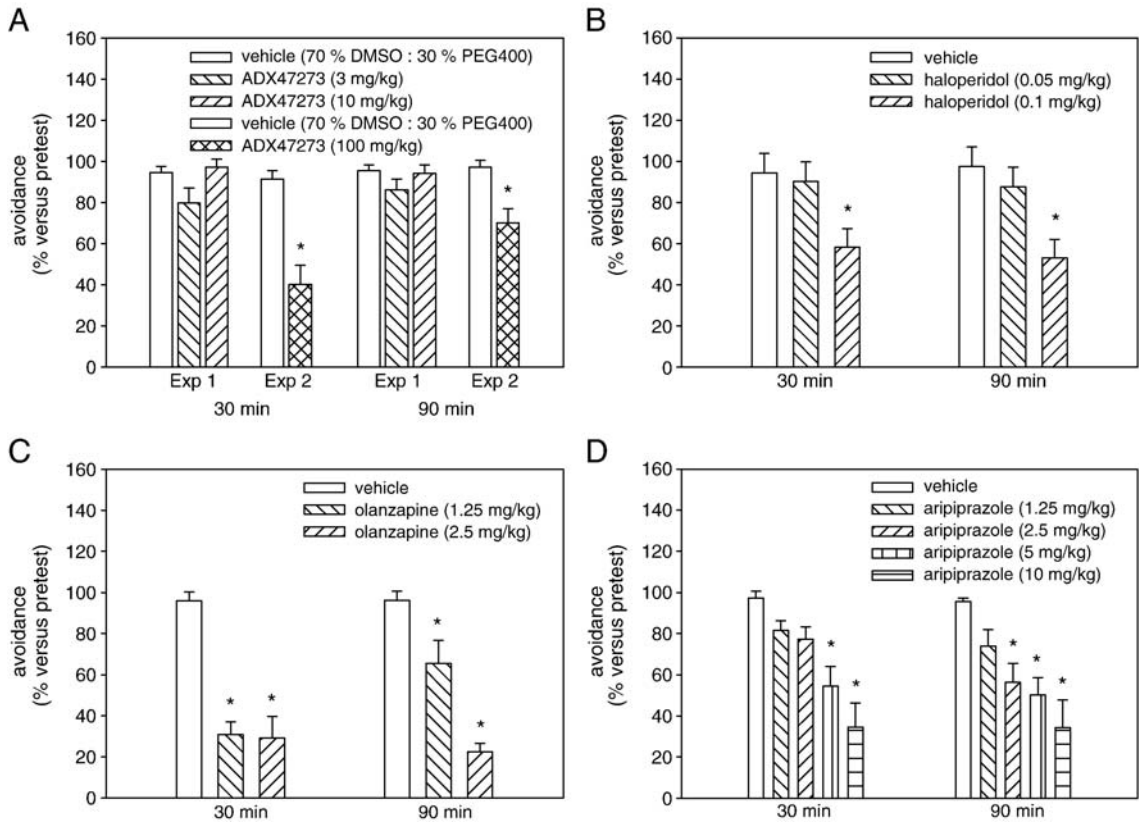

Fig. 1. The effect of ADX47273 (A), haloperidol (B), olanzapine (C) and aripiprazole (D) on CAR levels. Rats were administered 30 min before the test with ADX47273 or any neuroleptic and re-tested 90 min post-injection. Data are shown as % avoidance values and were compared to the % avoidance values of the pre-test. Results are expressed as mean ± SEM. Data were analyzed by two-way ANOVA on repeated measurements followed by Duncan's tests, $^{*}p<0.05$ vs vehicle, $n=6$–10 per group except ADX47273 (3 and 10 mg/kg) with $n=14$–16.

aripiprazole) were able to reverse PCP-induced hyperlocomotion. Additionally, only haloperidol decreased vertical activity (rearings) in PCP-treated animals at all doses tested. Finally, haloperidol and to a milder extent also ADX47273 induced cataleptic behaviour.

Table 1
The effect of ADX47273, haloperidol, aripiprazole and olanzapine on escape failure of CAR. Rats were administered 30 min before the test with ADX47273 or any neuroleptic and re-tested 90 min post-injection. Values are expressed as mean ± SEM, and represent the percentage of escape failures in relation to all trials of one session. Data were analyzed by one-way ANOVA followed by Duncan's test, $^{*}p<0.05$ vs vehicle, $n=6$–10 per group except ADX47273 (3 and 10 mg/kg) with $n=14$–16.

Substance (mg/kg)	% escape failure (mean ± SEM)	
	30 min	90 min
ADX47273		
0	0 ± 0	0.31 ± 0.31
3	0 ± 0	0.31 ± 0.31
10	0 ± 0	0 ± 0
0	0 ± 0	0 ± 0
100	1.25 ± 0.82	1.25 ± 1.25
Haloperidol		
0	0 ± 0	0.71 ± 0.71
0.05	0 ± 0	0.71 ± 0.71
0.1	0 ± 0	7.50 ± 5.43
Olanzapine		
0	0 ± 0	0 ± 0
1.25	6.59 ± 4.47	1.67 ± 0.83
2.5	11.88 ± 4.53 *	9.38 ± 2.74 *
Aripiprazole		
0	0 ± 0	0 ± 0
1.25	0.71 ± 0.71	2.86 ± 1.49
2.5	0 ± 0	2.14 ± 1.49
5	2.00 ± 0.82	3.50 ± 1.30
10	5.00 ± 3.16	5.00 ± 2.86

4.1. CAR

Suppression of CAR is widely used as a model for the detection of potential antipsychotic drugs (Wadenberg and Hicks, 1999). Both typical and atypical neuroleptics inhibit CAR at doses which are not associated with response failures (Courvoisier, 1956; Wadenberg et al., 1990; Wadenberg and Hicks, 1999). This test was shown to mainly detect antipsychotic-like effects of substances with a D_2 receptor antagonistic activity (Seiden and Carlsson, 1963; Ahlenius and Engel, 1971). On the anatomical level, a role of the nucleus accumbens has been implicated in the CAR test due to its motivational drive on locomotion (Salamone et al., 1997). A reduction of CAR was seen after bilateral infusion of the D_2 receptor antagonist (−)-sulpiride in this structure (Wadenberg et al., 1990). In our study, haloperidol decreased CAR at a dose of 0.1 mg/kg without any effect on escape failures, which remains in accordance with literature data (Davidson and Weidley, 1976; Ugale et al., 2004; Wadenberg et al., 2001).

Interestingly, as shown previously, administration of 5-HT_{2A} receptor antagonist MDL 100,907 potentiated the effect of a subthreshold dose of the dopamine D_2 receptor antagonist raclopride in the CAR (Wadenberg et al., 1998) opening the possibility that atypical neuroleptics might modulate the CAR performance also via this receptor. The atypical neuroleptic olanzapine is an antagonist at 5-HT_{2A} and D_2 receptors (Horacek et al., 2006). Accordingly, in the present study olanzapine reduced CAR at both doses tested (1.25 and 2.5 mg/kg) as shown previously (Li et al., 2007; Ugale et al., 2004; Wadenberg et al., 2001). However, the highest dose of olanzapine used in our experiments induced escape failures thus showing that its effect on CAR at this dose was not selective.

Aripiprazole has a different pharmacological profile than olanzapine. Although both the compounds share the antagonism at 5-HT_{2A}

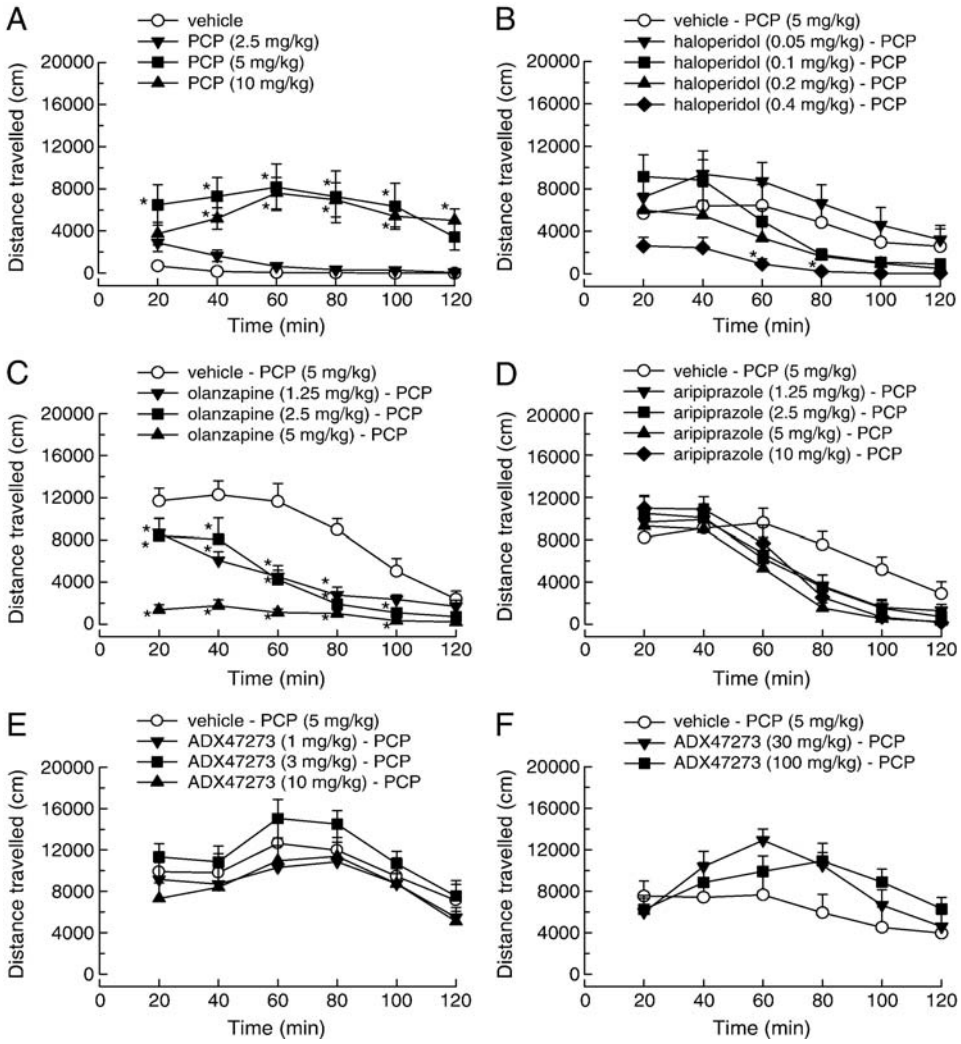

Fig. 2. The effect of PCP on locomotion in rats (A) and the combination with neuroleptics haloperidol (B), olanzapine (C) or aripiprazole (D) and $mGluR_5$ PAM ADX47273 (1–10 mg/kg in (E); 30 and 100 mg/kg in (F)) plotted as a time course over the travelled distance. Rats were administered ADX47273 or antipsychotics and placed in activity chambers for 30 min before starting measurement. After this period, phencyclidine was injected and measurement started. Results are expressed as mean ± SEM. Data were analyzed by two-way ANOVA on repeated measurements followed by Duncan's test, $^*p<0.05$ vs vehicle (A) or $^*p<0.05$ vs vehicle — phencyclidine (B–F), $n=7$–8 per group except aripiprazole with $n=12$.

receptors, aripiprazole is a partial agonist of D_2 receptor (Burris et al., 2002; Horacek et al., 2006). In schizophrenic patients, this compound has an antipsychotic efficacy while inducing no or only very mild extrapyramidal side effects (de Oliveira et al., 2009; Poyurovsky et al., 2008). These clinical observations have been replicated in animal models of schizophrenia. Aripiprazole was effective in models for schizophrenia symptoms such as the CAR (Natesan et al., 2007, 2006 and present study) and amphetamine-induced locomotor activity (Nordquist et al., 2008). Interestingly, even at the doses that produce more that 80% occupancy of dopamine D_2 receptors in the striatum, this compound did not produce catalepsy in animals (Natesan et al., 2006).

The putative neuroleptic ADX47273 reduced the CAR at a dose of 100 mg/kg in rats. Previously Liu et al. (2008) found a CAR impairment starting from 30 mg/kg of ADX47273. As mentioned above, effects of neuroleptics on D_2 transmission have been believed to be a prerequisite for an effect in the CAR. ADX47273 also reduced the amphetamine-induced hyperlocomotion (Liu et al., 2008; Schlumberger et al., 2009) suggesting a potential of ADX47273 to indirectly modulate dopaminergic transmission.

As our animals were trained without the influence of neuroleptics, a contribution of the state-dependent learning cannot be ruled out. However, as shown previously, state dependence does not play a role in the action of pimozide (Beninger et al., 1980). Moreover, ADX47273 (1–10 mg/kg) did not impair context fear conditioning when given either before testing (expression) or before training (acquisition) indicating a lack of state dependency (A. Gravius, unpublished observations).

4.2. Locomotor activity

Administration of NMDA receptor antagonists, e.g. PCP, induces schizophrenia-like symptoms in humans (Luby et al., 1959). In rodents, such antagonists produce an enhancement of locomotor activity and induce stereotypies, believed to be animal behavioural expression of the positive symptoms (Maurel-Remy et al., 1995; Moghaddam and Adams, 1998; Ogren and Goldstein, 1994). The effect of NMDA receptor antagonists can be reversed by both typical and atypical neuroleptics (Maurel-Remy et al., 1995; Nordquist et al., 2008; Ogren and Goldstein, 1994). However, it has been found that atypical neuroleptics, e.g. olanzapine, are more effective in this test than typical ones (e.g. haloperidol), and the present study is in line with that observation (Maurel-Remy et al., 1995).

Changes in the dopaminergic transmission may to some extent contribute to the hyperlocomotion evoked by NMDA receptor antagonists. For example PCP increases dopamine levels in the nucleus accumbens, both after a systemic administration and following direct injections into this structure (McCullough and Salamone, 1992; Moghaddam and Adams,

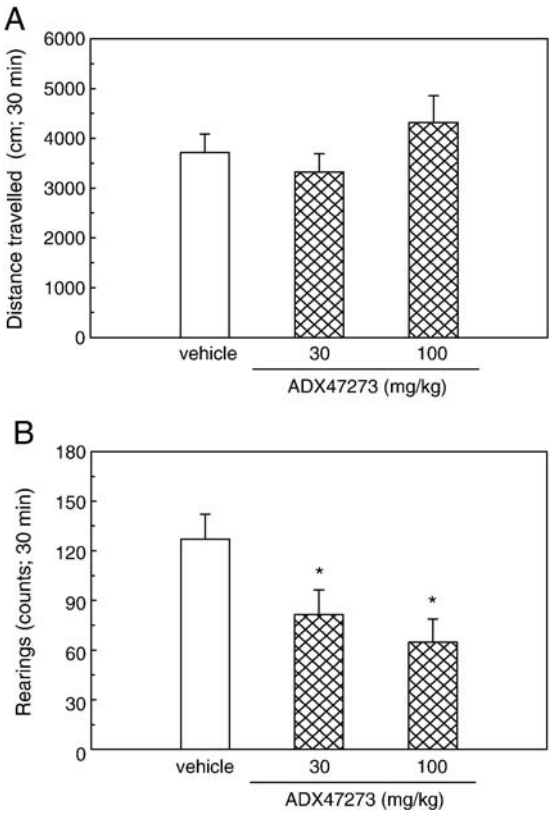

Fig. 3. The effect of ADX47273 (30 and 100 mg/kg) on spontaneous locomotion (A) plotted as total distance travelled and vertical exploration (rearings) (B) in rats plotted as total counts of rearings during time of measurement. Rats were administered with ADX47273, 30 min later they were placed in the activity chambers and the measurement started. Results are expressed as mean ± SEM. Data were analyzed by one-way ANOVA followed by Duncan's test, $^{*}p<0.05$ vs vehicle, $n=8$ per group.

1998). Moreover, PCP-induced hyperlocomotion can be partly antagonized by 6-hydroxydopamine lesions of the nucleus accumbens (French et al., 1985; French and Vantini, 1984; Steinpreis and Salamone, 1993).

The group of Takahata and Moghaddam (2003) suggested that also the PFC may be involved in the enhancing effect of PCP on the locomotor activity because dopamine levels in the PFC corresponds to behavioural activation. The enhancement of a locomotion produced by PCP was attenuated by typical neuroleptics with a high affinity to block dopamine D_2 receptors (e.g. haloperidol) (Maurel-Remy et al., 1995 and present study). In the present study, haloperidol dose-dependently inhibited the PCP-induced hyperlocomotion. It has to be mentioned, however, that a significant effect was observed only at very high doses, which produced catalepsy in rats (see below) suggesting that this effect might be unspecific.

The atypical neuroleptic olanzapine was the most effective neuroleptic against the PCP-induced hyperlocomotion in the present study. Additional to the D_2 antagonism, this effect may be mediated via blockade of 5-HT_{2A} receptors. Indeed, selective 5-HT_{2A} receptor antagonists were shown to be able to reverse the locomotor activity evoked by PCP (Maurel-Remy et al., 1995).

Previous publications showed that aripiprazole attenuated MK-801- or ketamine-induced hyperlocomotion in mice (Leite et al., 2008) or PCP-induced hyperlocomotion in rats (Nordquist et al., 2008). In the present study, aripiprazole only tended to reverse the effect of PCP; the effect, however, did not reach statistical significance (the effect of treatment in 2-way repeated-measures ANOVA: $p=0.062$). In our previous study, this neuroleptic was shown effective at even lower doses against amphetamine-induced hyperlocomotion (Schlumberger et al., 2009).

There are many hints that an interaction between $mGlu_5$ and NMDA receptors may be crucial for the modulation of psychotic-like symptoms (Doherty et al., 1997; Homayoun and Moghaddam, 2006; Homayoun et al., 2004; Pisani et al., 2001). $mGluR_5$ receptor antagonists such as MPEP or MTEP potentiated both the locomotor activity and the deficit of prepulse inhibition evoked by PCP or MK-801 in animals (Henry et al., 2002; Pietraszek et al., 2005). Such effects may be mediated by an indirect inhibition of NMDA receptor function by $mGluR_5$ receptor antagonists. Electrophysiological studies showed that stimulating $mGluR_5$ positively modulates function of NMDA receptors in brain regions relevant for schizophrenia and that such effects are inhibited by $mGluR_5$ antagonists (Doherty et al., 1997; Pisani et al., 2001). Since hypofunction of NMDA receptors may contribute to psychotic symptoms in schizophrenia patients, stimulation of $mGluR_5$ can be expected to produce antipsychotic effects. In animals, $mGluR_5$ PAM CDPPB blocked MK-801-induced excessive firing in neurones of orbitofrontal and prefrontal cortices in rats (Homayoun and Moghaddam, 2008; Lecourtier et al., 2007). Moreover, similar to neuroleptics, CDPPB attenuated the MK-801-induced stereotypy (Homayoun and Moghaddam, 2008). Also,

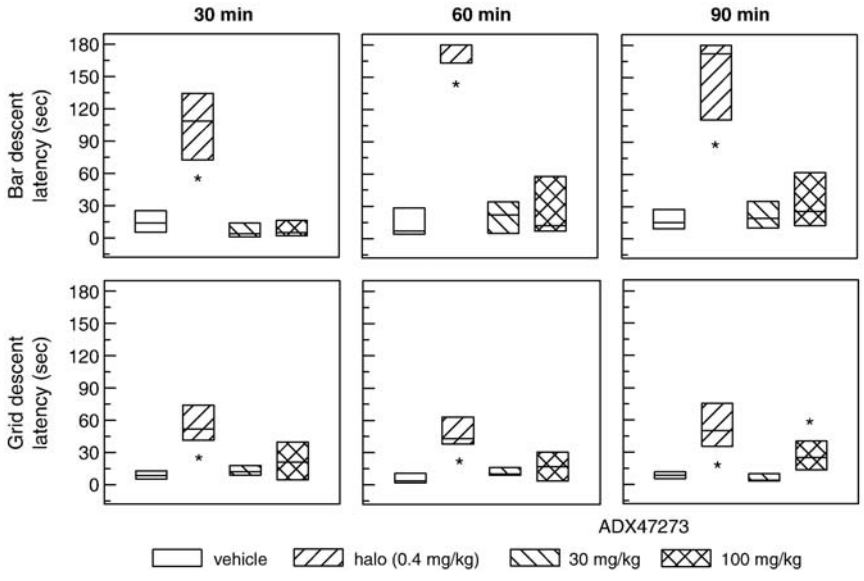

Fig. 4. The effect of ADX47273 and haloperidol on catalepsy in rats. Results are expressed as median levels ± Q1 or Q3, $n=6$–8 rats per group.

intracerebroventricular administration of a $mGluR_5$ agonist CHPG or an $mGluR_5$ PAM DFB reduced ketamine-induced hyperlocomotion and impairment of rotarod performance in mice (Chan et al., 2008). Likewise, Liu et al. (2008) showed recently that the substance used in this study, ADX47273, was able to reverse the PCP (1 mg/kg)-induced hyperlocomotion in mice, however this effect was very weak. In contrast, in our study, ADX47273 (1–100 mg/kg) had no effect on the PCP (5 mg/kg i.p.)-induced hyperlocomotion in rats. In another experiment ADX47273 (30 and 100 mg/kg) also failed to affect the hyperlocomotion produced by lower dose of PCP (3 instead of 5 mg/kg i.p.; data not shown). Thus, it is possible that this apparent discrepancy in the effects of ADX47273 results from different species used (i.e. rats vs mice). It also cannot be excluded that in some conditions, the positive modulation of $mGluR_5$ receptors may enhance NMDA receptor blocked by the channel blockers and in turn also behavioural effects of such. Noteworthy, an enhancement of MK-801 binding to NMDA receptors *in vivo* has previously been shown following treatment with glycine B site agonists (Murray et al., 2000). It should be stressed that ADX47273 alone, at 30 mg/kg failed to affect locomotion up to 140 min after administration (unpublished own data).

4.3. Catalepsy

Catalepsy testing consists of putting a rodent in an unusual, superimposed posture and measuring the time needed for the animal to initiate the movement (Sanberg et al., 1988; Wadenberg, 1996). In rodents, catalepsy is apparent with typical neuroleptics and less obvious with atypical antipsychotics (Arnt and Skarsfeldt, 1998; Natesan et al., 2006). The catalepsy test is often employed to assess a potential of a compound to induce extrapyramidal side effects in humans (Sanberg et al., 1988; Wadenberg, 1996). It has been proposed that catalepsy results from an excessive (>80%) blockade of dopamine D_2 receptors in the striatum (Ezrin-Waters and Seeman, 1977; Meltzer, 1991; Natesan et al., 2006; Ossowska et al., 1990).

In the present study, haloperidol used as a positive control produced catalepsy starting at a dose of 0.4 mg/kg. ADX47273 produced a very mild catalepsy at high dose only which was evidenced by the increased descent time in the grid test at 90 but not 30 or 60 min. In another study, the compound produced mild cataleptic behaviour only at the dose of 300 mg/kg in mice (Liu et al., 2008).

5. Conclusions

Present results confirm that ADX47273 may exert antipsychotic-like effects in some tests for positive symptoms of schizophrenia as published previously, possibly with a low propensity to produce extrapyramidal side effects (Liu et al., 2008; Schlumberger et al., 2009). Further studies are needed to verify the efficacy of ADX47273 in animal models of schizophrenia, in order to fully assess its benefits as compared to currently used therapies in particular where the highest medical need exists i.e. negative symptoms and cognitive impairment.

Acknowledgements

We would like to thank Andrzej Dekundy for his valuable comments and Daniela Schäfer for her excellent technical assistance.

Disclaimer: All authors (C.S., M.P., A.G., and W.D.) are full-time employees of Merz Pharmaceuticals GmbH (Frankfurt am Main, Germany). Before being a full-time employee, C.S. received till June 2009 a stipend from Merz Pharmaceuticals GmbH (Frankfurt am Main, Germany) for her PhD.

References

Ahlenius S, Engel J. Behavioral effects of haloperidol after tyrosine hydroxylase inhibition. Eur J Pharmacol 1971;15:187–92.

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

Aronica E, Dell'Albani P, Condorelli DF, Nicoletti F, Hack N, Balazs R. Mechanisms underlying developmental changes in the expression of metabotropic glutamate receptors in cultured cerebellar granule cells: homologous desensitization and interactive effects involving N-methyl-D-aspartate receptors. Mol Pharmacol 1993;44:981–9.

Beninger RJ, Mason ST, Phillips AG, Fibiger HC. The use of conditioned suppression to evaluate the nature of neuroleptic-induced avoidance deficits. J Pharmacol Exp Ther 1980;213:623–7.

Burris KD, Molski TF, Xu C, Ryan E, Tottori K, Kikuchi T, et al. Aripiprazole, a novel antipsychotic, is a high-affinity partial agonist at human dopamine D2 receptors. J Pharmacol Exp Ther 2002;302:381–9.

Chan MH, Chiu PH, Sou JH, Chen HH. Attenuation of ketamine-evoked behavioral responses by mGluR5 positive modulators in mice. Psychopharmacology (Berl) 2008;198:141–8.

Conn PJ, Lindsley CW, Jones CK. Activation of metabotropic glutamate receptors as a novel approach for the treatment of schizophrenia. Trends Pharmacol Sci 2009;30:25–31.

Courvoisier S. Pharmacodynamic basis for the use of chlorpromazine in psychiatry. J Clin Exp Psychopathol 1956;17:25–37.

Danysz W, Essmann U, Bresink I, Wilke R. Glutamate antagonists have different effects on spontaneous locomotor activity in rats. Pharmacol Biochem Behav 1994;48:111–8.

Davidson AB, Weidley E. Differential effects of neuroleptic and other psychotropic agents on acquisition of avoidance in rats. Life Sci 1976;18:1279–84.

de Oliveira IR, Elkis H, Gattaz WF, Chaves AC, de Sena EP, de Matos E, et al. Aripiprazole for patients with schizophrenia and schizoaffective disorder: an open-label, randomized, study versus haloperidol. CNS Spectr 2009;14:93–102.

Doherty AJ, Palmer MJ, Henley JM, Collingridge GL, Jane DE. (RS)-2-chloro-5-hydroxyphenylglycine (CHPG) activates mGlu5, but no mGlu1, receptors expressed in CHO cells and potentiates NMDA responses in the hippocampus. Neuropharmacology 1997;36:265–7.

Epping-Jordan MP, Nayak S, Derouet F, Dominguez H, Bessis AS, Le Poul E, et al. In vivo characterization of mGluR5 positive allosteric modulators as novel treatments for schizophrenia and cognitive dysfunction. Neuropharmacology 2005;49:243.

Ezrin-Waters C, Seeman P. Tolerance of haloperidol catalepsy. Eur J Pharmacol 1977;41:321–7.

French ED, Vantini G. Phencyclidine-induced locomotor activity in the rat is blocked by 6-hydroxydopamine lesion of the nucleus accumbens: comparisons to other psychomotor stimulants. Psychopharmacology (Berl) 1984;82:83–8.

French ED, Pilapil C, Quirion R. Phencyclidine binding sites in the nucleus accumbens and phencyclidine-induced hyperactivity are decreased following lesions of the mesolimbic dopamine system. Eur J Pharmacol 1985;116:1–9.

Gereau 4th RW, Heinemann SF. Role of protein kinase C phosphorylation in rapid desensitization of metabotropic glutamate receptor 5. Neuron 1998;20:143–51.

Henry SA, Lehmann-Masten V, Gasparini F, Geyer MA, Markou A. The mGluR5 antagonist MPEP, but not the mGluR2/3 agonist LY314582, augments PCP effects on prepulse inhibition and locomotor activity. Neuropharmacology 2002;43:1199–209.

Homayoun H, Moghaddam B. Bursting of prefrontal cortex neurons in awake rats is regulated by metabotropic glutamate 5 (mGlu5) receptors: rate-dependent influence and interaction with NMDA receptors. Cereb Cortex 2006;16:93–105.

Homayoun H, Moghaddam B. Orbitofrontal cortex neurons as a common target for classic and glutamatergic antipsychotic drugs. Proc Natl Acad Sci U S A 2008;105:18041–6.

Homayoun H, Stefani MR, Adams BW, Tamagan GD, Moghaddam B. Functional interaction between NMDA and mGlu5 receptors: effects on working memory, instrumental learning, motor behaviors, and dopamine release. Neuropsychopharmacology 2004;29:1259–69.

Horacek J, Bubenikova-Valesova V, Kopecek M, Palenicek T, Dockery C, Mohr P, et al. Mechanism of action of atypical antipsychotic drugs and the neurobiology of schizophrenia. CNS Drugs 2006;20:389–409.

Kinney GG, Burno M, Campbell UC, Hernandez LM, Rodriguez D, Bristow LJ, et al. Metabotropic glutamate subtype 5 receptors modulate locomotor activity and sensorimotor gating in rodents. J Pharmacol Exp Ther 2003;306:116–23.

Kinney GG, O'Brien JA, Lemaire W, Burno M, Bickel DJ, Clements MK, et al. A novel selective positive allosteric modulator of metabotropic glutamate receptor subtype 5 has in vivo activity and antipsychotic-like effects in rat behavioral models. J Pharmacol Exp Ther 2005;313:199–206.

Krystal JH, Karper LP, Seibyl JP, Freeman GK, Delaney R, Bremner JD, et al. Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine responses. Arch Gen Psychiatry 1994;51:199–214.

Lecourtier L, Homayoun H, Tamagnan G, Moghaddam B. Positive allosteric modulation of metabotropic glutamate 5 (mGlu5) receptors reverses N-methyl-D-aspartate antagonist-induced alteration of neuronal firing in prefrontal cortex. Biol Psychiatry 2007;62:739–46.

Lefkowitz RJ. G protein-coupled receptor kinases. Cell 1993;74:409–12.

Leite JV, Guimaraes FS, Moreira FA. Aripiprazole, an atypical antipsychotic, prevents the motor hyperactivity induced by psychotomimetics and psychostimulants in mice. Eur J Pharmacol 2008;578:222–7.

Li M, Fletcher PJ, Kapur S. Time course of the antipsychotic effect and the underlying behavioral mechanisms. Neuropsychopharmacology 2007;32:263–72.

Lindsley CW, Wisnoski DD, Leister WH, O'Brien JA, Lemaire W, Williams Jr DL, et al. Discovery of positive allosteric modulators for the metabotropic glutamate receptor subtype 5 from a series of N-(1, 3-diphenyl-1H- pyrazol-5-yl)benzamides that potentiate receptor function in vivo. J Med Chem 2004;47:5825–8.

Liu F, Zhang G, Hornby G, Vasylyev D, Bowlby M, Park K, et al. The effect of mGlu5 receptor positive allosteric modulators on signaling molecules in brain slices. Eur J Pharmacol 2006;536:262–8.
Liu F, Grauer S, Kelley C, Navarra R, Graf R, Zhang G, et al. ADX47273 [S-(4-fluoro-phenyl)-{3-[3-(4-fluoro-phenyl)-[1, 2, 4]-oxadiazol-5-yl]-piperidin-1-yl}-methanone]: a novel metabotropic glutamate receptor 5-selective positive allosteric modulator with preclinical antipsychotic-like and procognitive activities. J Pharmacol Exp Ther 2008;327:827–39.
Luby ED, Cohen RC, Rosenbaum B, Gottlieb JS, Kelly R. Study of a new schizophrenomimetic drug: Sernyl. Arch Neurol Psychiatry 1959;81:363–9.
Malhotra AK, Pinals DA, Adler CM, Elman I, Clifton A, Pickar D, et al. Ketamine-induced exacerbation of psychotic symptoms and cognitive impairment in neuroleptic-free schizophrenics. Neuropsychopharmacology 1997;17:141–50.
Maurel-Remy S, Bervoets K, Millan MJ. Blockade of phencyclidine-induced hyperlocomotion by clozapine and MDL 100, 907 in rats reflects antagonism of 5-HT2A receptors. Eur J Pharmacol 1995;280:R9–R11.
McCullough LD, Salamone JD. Increases in extracellular dopamine levels and locomotor activity after direct infusion of phencyclidine into the nucleus accumbens. Brain Res 1992;577:1–9.
Meador-Woodruff JH, Healy DJ. Glutamate receptor expression in schizophrenic brain. Brain Res Brain Res Rev 2000;31:288–94.
Meltzer HY. The mechanism of action of novel antipsychotic drugs. Schizophr Bull 1991;17:263–87.
Moghaddam B, Adams BW. Reversal of phencyclidine effects by a group II metabotropic glutamate receptor agonist in rats. Science 1998;281:1349–52.
Murray F, Kennedy J, Hutson PH, Elliot J, Huscroft I, Mohnen K, et al. Modulation of 3HMK-801 binding to NMDA receptors in vivo and in vitro. Eur J Pharmacol 2000;397:263–370.
Natesan S, Reckless GE, Nobrega JN, Fletcher PJ, Kapur S. Dissociation between in vivo occupancy and functional antagonism of dopamine D2 receptors: comparing aripiprazole to other antipsychotics in animal models. Neuropsychopharmacology 2006;31:1854–63.
Natesan S, Reckless GE, Barlow KB, Nobrega JN, Kapur S. Evaluation of N-desmethylclozapine as a potential antipsychotic—preclinical studies. Neuropsychopharmacology 2007;32:1540–9.
Nordquist RE, Risterucci C, Moreau JL, von Kienlin M, Kunnecke B, Maco M, et al. Effects of aripiprazole/OPC-14597 on motor activity, pharmacological models of psychosis, and brain activity in rats. Neuropharmacology 2008;54:405–16.
O'Brien JA, Lemaire W, Chen TB, Chang RS, Jacobson MA, Ha Jr SN, et al. A family of highly selective allosteric modulators of the metabotropic glutamate receptor subtype 5. Mol Pharmacol 2003;64:731–40.
Ogren SO, Goldstein M. Phencyclidine- and dizocilpine-induced hyperlocomotion are differentially mediated. Neuropsychopharmacology 1994;11:167–77.
Ohnuma T, Augood SJ, Arai H, McKenna PJ, Emson PC. Expression of the human excitatory amino acid transporter 2 and metabotropic glutamate receptors 3 and 5 in the prefrontal cortex from normal individuals and patients with schizophrenia. Brain Res Mol Brain Res 1998;56:207–17.
Ossowska K, Karcz M, Wardas J, Wolfarth S. Striatal and nucleus accumbens D1/D2 dopamine receptors in neuroleptic catalepsy. Eur J Pharmacol 1990;182:327–34.
Pietraszek M, Gravius A, Schafer D, Weil T, Trifanova D, Danysz W. mGluR5, but not mGluR1, antagonist modifies MK-801-induced locomotor activity and deficit of prepulse inhibition. Neuropharmacology 2005;49:73–85.
Pisani A, Gubellini P, Bonsi P, Conquet F, Picconi B, Centonze D, et al. Metabotropic glutamate receptor 5 mediates the potentiation of N-methyl-D-aspartate responses in medium spiny striatal neurons. Neuroscience 2001;106:579–87.
Poyurovsky M, Weizman R, Weizman A. Aripiprazole's receptor pharmacology and extrapyramidal side effects. Am J Psychiatry 2008;165:398 author reply 398–9.
Salamone JD, Cousins MS, Snyder BJ. Behavioral functions of nucleus accumbens dopamine: empirical and conceptual problems with the anhedonia hypothesis. Neurosci Biobehav Rev 1997;21:341–59.
Sanberg PR, Bunsey MD, Giordano M, Norman AB. The catalepsy test: its ups and downs. Behav Neurosci 1988;102:748–59.
Schlumberger C, Pietraszek M, Gravius A, Klein KU, Greco S, More L, et al. Comparison of the mGlu5 receptor positive allosteric modulator ADX47273 and the mGlu2/3 receptor agonist LY354740 in tests for antipsychotic-like activity. Eur J Pharmacol 2009 Nov 25;623(1–3):73–83.
Seiden LS, Carlsson A. Temporary and partial antagonism by L-DOPA of reserpine-induced suppression of a conditioned avoidance response. Psychopharmacologia 1963;4:418–23.
Steinpreis RE, Salamone JD. The role of nucleus accumbens dopamine in the neurochemical and behavioral effects of phencyclidine: a microdialysis and behavioral study. Brain Res 1993;612:263–70.
Takahata R, Moghaddam B. Activation of glutamate neurotransmission in the prefrontal cortex sustains the motoric and dopaminergic effects of phencyclidine. Neuropsychopharmacology 2003;28:1117–24.
Ugale RR, Hirani K, Morelli M, Chopde CT. Role of neuroactive steroid allopregnanolone in antipsychotic-like action of olanzapine in rodents. Neuropsychopharmacology 2004;29:1597–609.
Wadenberg ML. Serotonergic mechanisms in neuroleptic-induced catalepsy in the rat. Neurosci Biobehav Rev 1996;20:325–39.
Wadenberg ML, Hicks PB. The conditioned avoidance response test re-evaluated: is it a sensitive test for the detection of potentially atypical antipsychotics? Neurosci Biobehav Rev 1999;23:851–62.
Wadenberg ML, Ericson E, Magnusson O, Ahlenius S. Suppression of conditioned avoidance behavior by the local application of (−)sulpiride into the ventral, but not the dorsal, striatum of the rat. Biol Psychiatry 1990;28:297–307.
Wadenberg ML, Hicks PB, Richter JT, Young KA. Enhancement of antipsychoticlike properties of raclopride in rats using the selective serotonin2A receptor antagonist MDL 100, 907. Biol Psychiatry 1998;44:508–15.
Wadenberg ML, Soliman A, VanderSpek SC, Kapur S. Dopamine D(2) receptor occupancy is a common mechanism underlying animal models of antipsychotics and their clinical effects. Neuropsychopharmacology 2001;25:633–41.