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Comparison of the $mGlu_5$ receptor positive allosteric modulator ADX47273 and the $mGlu_{2/3}$ receptor agonist LY354740 in tests for antipsychotic-like activity

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ABSTRACT

Recently, it has been proposed that activation of either metabotropic glutamate receptors e.g. $mGlu_5$ by positive allosteric modulators or stimulation of $mGluR_{2/3}$ receptors by agonists may offer new strategy in schizophrenia treatment. The aim of the present study was to compare the effect of $mGlu_5$ receptor positive allosteric modulator, ADX47273 (S-(4-Fluoro-phenyl)-{3-[3-(4-fluoro-phenyl)-[1,2,4]oxadiazol—5-yl]-piperidin-1-yl}-methanone), $mGluR_{2/3}$ agonist, LY354740 ((1S,2S,5R,6S)-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylate monohydrate) and selected neuroleptics in animal models for positive schizophrenia symptoms. ADX47273 (3 and 10 mg/kg i.p.), the typical antipsychotic haloperidol (0.1 and 0.2 mg/kg i.p.), the atypical antipsychotics aripiprazole (1.25–5 mg/kg i.p.) and olanzapine (2.5 and 5 mg/kg i.p.) all reduced amphetamine-induced hyperlocomotion in Sprague–Dawley rats, unlike the $mGlu_{2/3}$ receptor agonist LY354740 (1–10 mg/kg i.p.). Interestingly, haloperidol (0.1 and 0.2 mg/kg i.p.), aripiprazole (1.25–5 mg/kg i.p.) and olanzapine (1.25–5 mg/kg i.p.), but not ADX47273 (1–10 mg/kg i.p.), all reduced spontaneous locomotion and rearings at doses effective against amphetamine-induced hyperlocomotion. This indicates that the effect of ADX47273 in combination with amphetamine may be specific, and also suggests a lack of sedative side effects. Moreover, ADX47273 (30 mg/kg i.p.), haloperidol (0.1 and 0.2 mg/kg i.p.) and aripiprazole (5 and 10 mg/kg i.p.) reversed apomorphine (0.5 mg/kg s.c.)-induced deficits of prepulse inhibition, whereas neither LY354740 (1–10 mg/kg i.p.) nor olanzapine (1.25–5 mg/kg i.p.) produced this effect. Lack of effect of olanzapine was unexpected and at present no convincing explanation can be provided. In conclusion, in selected rodent models for positive schizophrenia symptoms, ADX47273 showed better efficacy than LY354740.

1. Introduction

It is believed that the enhancement of dopaminergic transmission in subcortical structures may lead to psychotic symptoms in schizophrenia (Meltzer and Stahl, 1976; Snyder et al., 1974), a theory which is supported by the efficacy of D_2 receptor blockade by typical neuroleptics (Seeman, 1987; Seeman, 1992).

Glutamatergic transmission may be also involved in the pathomechanism of schizophrenia, and some NMDA receptor antagonists induce psychotic symptoms in healthy volunteers (Adler et al., 1998; Krystal et al., 1994; Luby et al., 1959). Drugs targeting the glutamatergic system, such as the mGlu receptor group II agonist LY404039 ((-)-(1R,4S,5S,6S)-4-amino-2-sulfonylbicyclo[3.1.0]hexane-4,6-dicarboxylic acid), may provide therapeutic benefits for schizophrenic patients (Patil et al., 2007). Furthermore, LY404039 shows fewer side effects e.g. prolactine release and weight gain than olanzapine (Patil et al., 2007). Other mGlu receptor group II agonist, LY354740 ((1S,2S,5R,6S)-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylate monohydrate) was effective against ketamine-induced working memory impairment in healthy volunteers (Krystal et al., 2005) and phencyclidine-induced stereotypy, locomotion, and cortical glutamate efflux in rats (Moghaddam and Adams, 1998; Schlumberger et al., 2009). Based on such animal data, it has been suggested that LY354740's antipsychotic-like mechanism of action involves an inhibition of enhanced glutamate release in the prefrontal cortex. Such enhanced glutamate release resulting from disinhibition in subcortical regions is observed after PCP administration and believed also to take place in schizophrenia (Moghaddam and Adams, 1998; Krystal et al., 2003; Conn et al., 2009). Preclinical experiments suggest that also $mGlu_5$ receptor positive allosteric modulators may have therapeutic potential for this indication (Darrah et al., 2008; Kinney

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et al., 2005; Liu et al., 2008). The use of $mGlu_5$ receptor positive allosteric modulators for the treatment of schizophrenia is supported by the fact that $mGlu_5$ receptor knock-out mice (Kinney et al., 2003; Brody et al., 2004) have deficits in prepulse inhibition of the acoustic startle response which are alleviated by chronic clozapine administration (Gray et al., 2009). Similarly, blockade of the $mGlu_5$ receptors enhances impairment of prepulse inhibition produced by NMDA receptor antagonists (Henry et al., 2002; Pietraszek et al., 2005). Prepulse inhibition impairment is observed both in schizophrenic patients (Braff et al., 1978), and in animals, following dopaminomimetics and NMDA receptor antagonists (Geyer et al., 2001; Swerdlow et al., 2008). Such deficit can be alleviated in animals by antipsychotics (Geyer et al., 2001; Swerdlow et al., 2008). Similarly, the $mGlu_5$ receptor positive modulator CDPPB (3-cyano-N-(1,3-diphenyl-1H-pyrazol-5-yl)benzamide) reduced amphetamine-induced deficits of prepulse inhibition (Kinney et al., 2005; Lindsley et al., 2004). CDPPB as well as another $mGlu_5$ receptor positive modulator ADX47273 (S-(4-Fluoro-phenyl)-{3-[3-(4-fluoro-phenyl)-[1,2,4]oxadiazol—5-yl]-piperidin-1-yl}-methanone) attenuated amphetamine-induced locomotor activity, a standard preclinical test for assessment of antipsychotic-like activity (Kinney et al., 2005; Liu et al., 2008).

The aim of the present study was therefore to compare the effect of the $mGlu_5$ receptor positive allosteric modulator ADX47273 and the $mGlu_{2/3}$ receptor agonist LY354740 (representing a novel group of antipsychotics lacking direct effects on the dopaminergic system) with selected neuroleptics in animal models relevant for the positive symptoms of schizophrenia such as amphetamine-induced hyperlocomotion and apomorphine-induced deficits of prepulse inhibition.

2. Material and methods

2.1. Animals

Male Sprague–Dawley rats obtained from Élevage Janvier (Le Genest Saint Isle, France) were used at a weight of 250–380 g. The rats were allowed to acclimate for one week before the start of the experiments, housed in groups of 4–5 rats per cage, and provided with bedding, paper cloth and a red tunnel. Animals were kept in standard conditions (21 ± 1 °C, 60 ± 3%, water and chow ad libitum) under a 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.

All studies are approved by the 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. Assessment of plasma and brain concentration of ADX47273

Rats were anaesthezised with a ketamine (90 mg/kg)/xylazine (10 mg/kg) mixture, and cannulated with silicone tubing via the right jugular vein. Two days later, prior to the first blood sampling, animals were connected to a counterbalanced system and tubing to perform blood sampling in the freely moving rat. Baseline blood samples (200 µl) were taken immediately before application (predose) followed by the sampling 5, 10, 20, 40, 60, 80, 100 min, 2, 3, 4, 6, 8, 12, 18, 24 and 28 h after application. Blood was collected in heparinised polypropylene tubes stored on ice and subsequently centrifuged at 645 *g* for 10 min at 4 °C. The harvested plasma was kept at −20 °C until being assayed.

To 50 µl of rat plasma sample and calibration standard, 100 µl acetonitrile containing the internal standard (Griseofulvin, 300 ng/ml) was added. Samples and calibration standards were vigorously shaken (10 s) and after 5 min at room temperature centrifuged for 10 min at 6000 × *g* and 20 °C. The particle free supernatant was diluted 1:1 with water and an aliquot of the mixture was transferred to 200 µl sampler vials and subsequently subjected to LC-MS/MS (high performance liquid chromatography/mass spectrometry) with an injection volume of 15 µl.

The HPLC pump flow rate was set to 300 µl/min and the test item was separated on a Gemini C6-Phenyl, 3 µm, 50 × 2.0 mm (Phenomenex, Germany) analytical column with a pre-column (Gemini C6-Phenyl, 3 µm, 4 × 2.0 mm). Gradient elution (is used to accelerate the elution of strongly retained solutes by constantly changing the composition, and hence the polarity, of the mobile phase) with 10 mM ammonium formate/0.1% formic acid as aqueous phase (A) and acetonitrile/0.1% formic acid as organic phase (B) was used for all items: % B (*t* (min)), 5(0–0.2)–97(1.2–4.0)–5(4.2–6.0).

In a separate experiment plasma and brain concentrations were compared. 30 min after i.p administration of ADX47273, rats were given an overdose of pentobarbital (150 mg/kg i.p.) and blood was taken by cardiac puncture and the brains were removed as well. Blood was put in a vial with EDTA to avoid coagulation and was centrifuged after 30 min (5000 × *g* for 10 min). Plasma and brains were stored at −20 °C until analysis. The LC-MS/MS system used for analysis was equipped with an Agilent 1100 HPLC coupled to an API 4000 Q Trap mass spectrometer with an electrospray ionization (ESI) source (Applied Biosystems, Darmstadt, Germany). The analytical column was a Symmetry C18, 5 µm, 50 × 2.1 mm (Waters, Eschborn, Germany). The mobile phase consisted of 60% water and 40% acetonitrile both containing 0.1% formic acid. The chromatographic run was performed over 5 min and consisted of a gradient from 40% acetonitrile in water at the start, to a mobile phase with a composition of 90% acetonitrile in water. For precipitation of proteins from the plasma, 100 µl of the internal standard (2-methyl-6-(phenylethynyl) pyridine hydrochloride, 10.2 ng/ml in acetonitrile) solution were added to a 20 µl sample and then mixed for 15 s using a vortex mixer. After centrifugation (10 min, 20,800 *g* at 2 °C), 2 µl supernatant was transferred to an HPLC vial with an insert and analyzed by LC-MS/MS.

For precipitation of brain protein, 4 ml aCSF (147 mM NaCl, 2.7 mM KCl, 1.2 mM $CaCl_2$, 0.85 mM $MgCl_2$, pH 5.3) were added per 1 g brain and homogenized using an Ultra Turrax. 660 µl of the internal standard solution was then added to 330 µl of the sample and mixed for 15 s using a vortex mixer. After centrifugation (10 min, 20,800 *g* at 2 °C), the supernatant was transferred to an HPLC vial with an insert and analyzed by LC-MS/MS. The lowest limit of detection for ADX47273 was 3 nM.

2.3. Locomotor activity

For the measurement of locomotor activity, Perspex® boxes (ENV-515-16, 43.2 × 43.2 × 30 cm) were placed in a noise-proof chamber equipped with a ventilator and a source of white light (5.6 W) at 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. Measurement of vertical activity was assessed by two additional sets of 16 photo beams placed 15 cm above the floor. The output from the counters was integrated and analyzed on-line by a PC computer. Distance travelled and vertical movements were assessed by further analysis as measures of locomotion and rearing, respectively. For interaction studies of antipsychotics or ADX47273 with amphetamine, animals were injected with ADX47273 or antipsychotics and placed into the locomotor activity chambers for 30 min before amphetamine was administered. For studies involving the interaction of LY354740 and amphetamine, animals were first habituated to the experimental chambers for 20 min and then injected with LY354740. Amphetamine was administered 20 min after LY354740. All measurements started directly after administration of amphetamine and were continued for a total of 120 min. Data are only shown from after the injection of amphetamine. For the measurement of the effect of ADX47273 and neuroleptics on spontaneous locomotion and rearings, all compounds were injected