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Research report

Positive modulation of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors reverses sub-chronic PCP-induced deficits in the novel object recognition task in rats

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ABSTRACT

Cognitive deficits are a major clinical unmet need in schizophrenia. The psychotomimetic drug phencyclidine (PCP) is widely applied in rodents to mimic symptoms of schizophrenia, including cognitive deficits. Previous studies have shown that sub-chronic PCP induces an enduring episodic memory deficit in female Lister Hooded rats in the novel object recognition (NOR) task. Here we show that positive modulation of AMPA receptor (AMPAR) mediated glutamate transmission alleviates cognitive deficits induced by sub-chronic PCP treatment. Female Lister hooded rats were treated sub-chronically with either vehicle (0.9% saline) or PCP (2 mg/kg two doses per day for 7 days), followed by a 7 days washout period. 30 min prior to the acquisition trial of the NOR task animals were dosed with either vehicle, CX546 (10, 40 or 80 mg/kg) or CX516 (0.5, 2.5, 10, 40 or 80 mg/kg). Our results show that sub-chronic PCP treatment induced a significant decrease in the discrimination index (DI) and both ampakines CX546 and CX516 were able to reverse this disruption of object memory in rats in the novel object recognition task. These data suggest that positive AMPAR modulation may represent a mechanism for treatment of cognitive deficits in schizophrenia.

1. Introduction

Schizophrenia is a complex psychiatric disorder comprised of different symptom classifications including positive, negative, and cognitive symptoms. Studies have shown that the rehabilitation of a patient into society strongly correlates with the degree of cognitive symptoms [25]. To date, there is no approved treatment for this symptom class resulting in a large unmet need for novel therapies. The National Institute of Mental Health (NIMH) has funded two initiatives focusing on the cognitive symptoms of schizophrenia, namely MATRICS (Measurement And Treatment Research to Improve Cognition in Schizophrenia) and TURNS (Treatment Units for Research on Neurocognition and Schizophrenia). One of the main goals of these initiatives is to define key aspects of cognition in schizophrenia and identify potential treatment targets to assist the development of novel compounds addressing cognitive deficit in schizophrenia [[40], http://www.turns.ucla.edu/].

The non-competitive N-methyl D-aspartate receptor (NMDAR) antagonist PCP has been shown to exacerbate psychotic symptoms in schizophrenic patients and to induce schizophrenia-like symptoms in healthy individuals [36]. These observations have led to the NMDAR hypofunction hypothesis of schizophrenia. Subsequently, animal models involving treatment with PCP have proven to be useful tools in mimicking several symptom classes of schizophrenia, including cognitive symptoms [29,30]. Among others, sub-chronic PCP dosing of female Lister hooded rats induces deficits in reversal learning [2], attentional set shifting [41] and novel object recognition (NOR) [23,42]. A reversal of these deficits in the respective tests was reported by different atypical, but not typical antipsychotic drugs [2,23,41]. Sub-chronic PCP treatment thus provides a valuable disease model for the assessment of cognitive symptoms in schizophrenia.

The NMDA receptor hypofunction hypothesis has drawn the attention of the schizophrenia research field to glutamatergic signalling pathways. NMDA as well as AMPA receptors belong to the ionotropic glutamate receptor channels, which have been shown to be essential for the induction and maintenance of long-term potentiation (LTP), a model believed to be a cellular correlate of learning and memory [10,13]. NMDA receptors are volt-

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age dependent receptors comprised of four subunits that form a membrane-spanning pore. This pore is, at resting potential, blocked by a magnesium ion. Upon depolarization, the magnesium block is removed and the NMDA receptor can be activated. AMPA receptors on the other hand are fast acting, ligand-gated glutamate receptors. Activation of AMPA receptors leads to cell depolarization, which in turn removes the magnesium block from the NMDA receptors and enables ion flux through the NMDA receptor channel [7,10]. It is generally accepted that AMPAR activity and subsequent NMDAR activation mediate changes in synaptic plasticity, underlying the induction and maintenance of LTP, and learning and memory [7,13,39].

The increased interest in glutamatergic signalling accelerated the development of drugs acting directly on the glutamatergic system, with positive AMPAR modulators, so-called ampakines, being just one example. Ampakines modulate AMPARs by slowing down both deactivation and desensitization and thereby facilitating the receptor's activity. The balance between effects on deactivation versus desensitization can vary between different ampakines leading to individual drug profiles. The two ampakines employed in the present study, CX516 and CX546, have different effects on receptor kinetics. CX516 induces a four-fold decrease in the rate of deactivation of the AMPAR and a smaller decrease in the rate of desensitization [5]. CX546 has an even greater effect on deactivation, i.e. a 10-fold decrease in the rate of deactivation, and also some effect on desensitization [6]. Both, CX516 and CX546 affect LTP in hippocampal slices with CX516 reducing the threshold for LTP induction and CX546 increasing the magnitude of potentiation [7].

Since ampakines do not possess either agonistic or antagonistic properties, they only affect receptors in their activated state. Consequently, facilitation of AMPAR signalling is achieved only in currently active neuronal networks minimising the risk of unwanted effects [37]. Interestingly, some studies have shown a reduction of AMPAR gene expression in the temporal lobe of schizophrenia patients [15]. The rationale for investigating ampakines as possible treatment in schizophrenia is therefore highly valid, although results with ampakines tested for procognitive potential in the clinic vary [20,21,28].

The NOR task assesses episodic memory and has previously been shown to be a useful paradigm for examining cognitive deficits of relevance to schizophrenia and efficacy of potential procognitive drugs when used in conjunction with sub-chronic PCP treatment [23]. The aim of the present study was to examine the potential efficacy of CX516 and CX546 to alleviate deficits of an animal model of schizophrenia in NOR.

2. Materials and methods

Adult female Lister Hooded rats (Charles River Ltd, UK) were used for the present studies. Animals were dosed intraperitoneally (i.p.) with either vehicle (0.9% saline) or PCP (2 mg/kg) in a volume of 1 ml/kg, two doses/day for 7 days, followed by a 7-day washout period.

The animals were housed 5 per cage and kept on a 12-h light/dark cycle (lights on at 07:00 h) in a temperature controlled room (21 ± 2 °C) with a humidity of 45–55%. Animals had free access to food and water ad libitum except during testing. All experimental procedures were carried out in accordance with the Animals (Scientific Procedures) Act, UK (1986) and were approved by the University of Bradford ethical review process.

Rats sub-chronically treated with PCP were assigned to receive a subcutaneous (s.c.) dose of either the relevant vehicle, CX546 (10, 40, or 80 mg/kg) or CX516 (0.5, 2.5, 10, 40, or 80 mg/kg), respectively, in a volume of 5 ml/kg, 30 min prior to the acquisition trial of the NOR task. CX546 was dissolved in 10% HP-β cyclodextrin and CX516 in 0.9% saline. Doses were based on previous studies using CX516 between 10 and 50 mg/kg [11,26,33,46]. Doses higher than 80 mg/kg were not applied due to increased risk of adverse events, such as seizures.

The NOR paradigm was carried out as previously described [23,42]. The experimental arena consisted of an open box (52 cm wide × 52 cm long × 40 cm high) with black Plexiglas walls and a white floor. The floor is divided by equidistant black lines, 2 vertical and 2 horizontal, into 9 equal sized squares; these lines are used for assessment of locomotor activity. The two sets of objects used for the experiments were Coca Cola® cans and white plastic bottles (17.5 cm high, diameter 7.5 cm). The novel object and its placement in the box were determined in a pseudo randomised fashion.

Rats were habituated to the experimental arena for 20 min per day, 3 days prior to the experiments. During this initial habituation rats were placed in the empty arena, 5 rats at a time. On the day of the experiment each rat received an additional, 7 min habituation. Following the habituation period, animals were singly placed in the arena for the acquisition trial during which each rat was allowed to freely explore two identical objects placed in diagonally opposite corners of the arena for 3 min. The rat was removed from the arena and placed in its home cage for a 1 min intertrial interval (ITI). During this time objects and arena were cleaned with 10% alcohol to eliminate odour cues as explained in [23]. Subsequently, a triplicate of the familiar object and a novel object were placed in the arena. The rat was then re-introduced to the arena and allowed to explore the objects for a 3 min retention trial.

Exploration was defined as sniffing, licking or touching the object while facing it. Experiments were recorded on videotape and exploration time manually scored. The discrimination index (DI) was calculated as [(time spent exploring novel object – time spent exploring familiar object)/total exploration time].

For acquisition trial data, the exploration of the left and the right object were compared using a paired *t*-test. Likewise, exploration of the novel and the familiar object in retention trials were compared by a paired *t*-test.

The locomotor activity of animals was scored as the total number of line crossings in the acquisition and the retention trial. Both the locomotor activity data and the DIs were compared using a one-way ANOVA and Dunnett's post hoc test with the sub-chronic PCP group as the control group.

The exposure study was performed at H. Lundbeck A/S using 48 female Lister hooded rats (Charles River Ltd, Germany). These rats were housed two per cage under controlled conditions (12 h of light starting at 06:00 h; 20 ± 2 °C; 30–70% humidity). All animal procedures were carried out in compliance with European Commission Directive 6/609/EEC and with Danish law regulating experiments on animals. Rats were dosed according to the same sub-chronic PCP regimen (excluding vehicle group). Following washout rats were treated acutely with either CX516 or CX546 at doses indicated above ($n = 3$). Thirty or 60 min after receiving the compound rats were euthanized by CO_2 followed by decapitation and preparation of blood and brain samples. Blood samples were subsequently centrifuged for 15 min at 3600 rpm, 4 °C and the plasma supernatant extracted. Plasma and brain samples were kept at −80° C until analysis. Brains were homogenized with four volumes of 70% MeCN followed by centrifugation and the supernatant was isolated for analysis. Plasma and brain concentrations were determined by liquid chromatography/tandem mass spectrometry on a Sciex API 3000 (Applied Biosystems).

3. Results

3.1. The effect of CX546 on object recognition in sub-chronic PCP rats

In the NOR task, vehicle-, but not sub-chronic PCP- treated animals significantly discriminated between the novel and familiar object during the retention trial. CX546 treatment at all three doses significantly reversed this deficit (paired *t*-test, $p < 0.01$–$p < 0.001$, Fig. 1b). Statistical analysis of the DIs revealed an overall difference between groups (one-way ANOVA, $F(4,45) = 3.03$, $p = 0.028$), with the DI being significantly decreased in the sub-chronic PCP group compared to the vehicle group (Dunnett's post hoc test, $p = 0.031$). This decrease was fully reversed by treatment with 80 mg/kg CX546 (Dunnett's post hoc test, $p = 0.012$), but not by 10 or 40 mg/kg (Fig. 1c).

No significant differences were observed during acquisition on exploration of the left versus right object in any of the groups (paired *t*-test, Fig. 1a). To assess the effect of CX546 treatment on locomotor activity, the total number of line crossings during acquisition and retention was scored (Fig. 1d). Here, the group receiving 80 mg/kg CX546 showed a significant decrease in the number of line crossings (one-way ANOVA, $F(4,45) = 11.31$, $p < 0.001$, Fig. 2d), indicating reduced levels of activity in the NOR arena during acquisition and retention.

Taken together, in this experiment the sub-chronic PCP-induced episodic memory deficit was fully restored by acute treatment with 80 mg/kg CX546.