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Effects of acute phencyclidine administration on arginine metabolism in the hippocampus and prefrontal cortex in rats

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ABSTRACT

Phencyclidine (PCP), a non-competitive N-methyl-D-aspartate glutamate receptor antagonist, induces schizophrenic symptoms in healthy individuals, and altered arginine metabolism has been implicated in schizophrenia. The present study investigated the effects of a single subcutaneous injection of PCP (2, 5 or 10 mg/kg) on arginine metabolism in the sub-regions of the hippocampus and prefrontal cortex in male young adult Sprague-Dawley rats. Animals' general behaviour was assessed in the open field apparatus 30 min after the treatment, and the brain tissues were collected at the time point of 60 min post-treatment. Behaviourally, PCP resulted in reduced exploratory activity in a dose-dependent manner, and severe stereotype behaviour and ataxia at the highest dose. Neurochemically, PCP significantly altered the nitric oxide synthase and arginase activities, the L-arginine, agmatine, spermine, glutamate and GABA levels, and the glutamine/glutamate and glutamate/GABA ratios in a dose-dependent and/or region-specific manner. Cluster analyses showed that L-arginine and its main metabolites L-citrulline, L-ornithine and agmatine formed distinct groups, which changed as a function of PCP mainly in the hippocampus. Multiple regression analysis revealed significant neurochemical-behavioural correlations. These results demonstrate, for the first time, that a single acute administration of PCP affects animals' behaviour and arginine metabolism in the brain.

1. Introduction

Schizophrenia is a debilitating psychiatric disorder characterized by positive symptoms (e.g., hallucinations, delusions and thought disorder), negative symptoms (e.g., deficits in social interaction, emotion and motivation) and cognitive dysfunction (e.g., impairments of attention and working memory). Phencyclidine (PCP), a non-competitive N-methyl-D-aspartate (NMDA) glutamate receptor antagonist, was initially developed as a surgical anaesthetic. A single sub-anaesthetic dose of PCP, however, induced psychotic and negative symptoms, as well as cognitive impairment, in healthy individuals that resemble clinical features of schizophrenia (Luby et al., 1959; Javitt and Zukin, 1991). The symptoms induced by repeated use of PCP could persist for several weeks, even after drug discontinuation (Murray, 2002). These clinical observations suggest that the NMDA glutamate receptor is critically involved in the pathogenesis of schizophrenia. Consistent with this NMDA receptor hypofunction hypothesis, altered NMDA receptor expression and phosphorylation have been found in the post-mortem prefrontal cortex of schizophrenic patients (Akbarian et al., 1996; Dracheva et al., 2001; Emamian et al., 2004). Human genetic studies have shown that the NMDA receptor subunit gene polymorphisms increase susceptibility to schizophrenia (Ohtsuki et al., 2001; Rice et al., 2001; Itokawa et al., 2003).

Given the evidence linking PCP exposure to schizophrenia, PCP has been used in animals to model the disease. Experimental research has demonstrated that PCP induces behavioural changes in laboratory animals that resemble positive symptoms, negative symptoms and cognitive deficits in schizophrenia depending on the treatment regimen. Whereas repeated use of PCP leading to a more persistent schizophrenic symptomatology may better model the chronic symptoms, such as the negative symptoms and cognitive deficits, acute administration of PCP resembles first-episode schizophrenia (for reviews see Jentsch and Roth, 1999; Mouri et al., 2007; Adell et al., 2012). It has been reported that acute PCP treatment results in hyperlocomotion (positive symptoms),

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impaired social behaviour (negative symptoms) and deficits in various learning and memory tests (cognitive dysfunction) (Jentsch and Roth, 1999; Mouri et al., 2007). Acute PCP treatment also disrupts pre-pulse inhibition (PPI) (Mouri et al., 2007; Pålsson et al., 2008), an operational measure of sensorimotor gating that is known as a benchmark test for schizophrenia (Braff, 2010).

L-arginine is a metabolically versatile amino acid widely distributed in mammalian organs, including brain. It can be metabolized by nitric oxide (NO) synthase (NOS) to form NO and L-citrulline, by arginase to generate L-ornithine and urea, and by arginine decarboxylase (ADC) to produce agmatine and carbon dioxide (Wu and Morris, 1998). NO is a gaseous molecule that plays an important role in maintaining normal function of the nervous system at physiological levels (Feil and Kleppisch, 2008; Steinert et al., 2010). It, however, can be neurotoxic when present in excessive amounts due to its nature as a free radical (Calabrese et al., 2007). L-ornithine is the main precursor of polyamines putrescine, spermidine and spermine, but can also be channelled to produce glutamate and γ-aminobutyric acid (GABA) (Wu and Morris, 1998). It has been documented that physiological concentrations of polyamines are essential in maintaining normal cellular function (Williams, 1997; Wallace et al., 2003). Agmatine regulates the production of NO and polyamines, and is considered a novel putative neurotransmitter (Reis and Regunathan, 2000; Halaris and Piletz, 2007). Since agmatine can be metabolized by agmatinase to form putrescine, it is considered a member of the polyamine family (Moinard et al., 2005).

Altered arginine metabolism has been implicated in schizophrenia (Perez-Neri et al., 2006; Fiori and Turecki, 2008). It has been shown, for example, that NO and NOS expression levels are elevated in schizophrenic brains and/or plasma (Baba et al., 2004; Yao et al., 2004; Djordjevic et al., 2010). Human genetic research has identified schizophrenia risk genes encoding neuronal NOS (Shinkai et al., 2002; Reif et al., 2006; Cui et al., 2010). Furthermore, plasma arginase activity has been found to be decreased in schizophrenic patients (Yanik et al., 2003), and the serum levels of L-ornithine positively correlate with the disease duration (Tomiya et al., 2007). Agmatine and polyamines putrescine, spermidine and spermine have also been suggested to play a role in the aetiology and pathology of schizophrenia (Fiori and Turecki, 2008). Recent animal research suggests that NO is critically involved in PCP-induced behavioural impairments (including PPI deficits), and that a NOS inhibitor has an ameliorating effect on PCP treated animals (Wass et al., 2006a,b; 2009; Fejgin et al., 2008). There is, however, no previous research describing how the brain arginine metabolic profile changes following PCP treatment.

It has been well documented that schizophrenia is associated with prefrontal and hippocampal dysfunction (Goldman-Rakic and Selemon, 1997; Harrison, 2004). The present study was therefore designed to investigate the effects of a single administration of PCP at various doses on L-arginine metabolism in these brain regions, by measuring the activities of NOS and arginase and the tissue concentrations of L-arginine and its downstream metabolites. Because there is functional dissociation across the CA1, CA3 and dentate gyrus (DG) sub-region of the hippocampus (for a review see Kesner et al., 2004), neurochemical changes induced by PCP in this region were examined at the sub-regional level. Animals' general behaviour was assessed in the open field apparatus at the time point of 30 min post-treatment.

2. Materials and methods

2.1. Subjects

Thirty-two male Sprague-Dawley rats, weighing between 300 and 370 g, were housed five to six per cage (53 × 33 × 26 cm^3) with free access to water and food, and maintained on a 12-h light/dark cycle (lights on 8 am). Treatments, behavioural assessment and brain tissue collections were conducted during the light period of the light–dark cycle. All experimental procedures were carried out in accordance with the regulations of the University of Otago Committee on Ethics in the Care and Use of Laboratory Animals. Every attempt was made to limit the number of animals used and to minimise their suffering.

2.2. Drug and treatment

The rats were randomly allocated to the saline and three PCP groups ($n = 8$ in each group). PCP was synthesized by the BDG Synthesis Limited (New Zealand) with a purity of 99.7%, and was freshly dissolved in saline. Animals received a subcutaneous (s.c.) injection of saline (2 ml/kg) or PCP at a dose of 2 (PCP-2), 5 (PCP-5) or 10 (PCP-10) mg/kg, and were sacrificed approximately 60 min after the treatment. The variation in animal's body weight was considered and counterbalanced between groups (Saline: 330.9 ± 8.4 g; PCP-2: 315.2 ± 17.8 g; PCP-5: 323.9 ± 7.1 g; PCP-10: 326.8 ± 7.1 g). The experimenters were blind to the grouping information.

2.3. Behavioural procedures

Animals' general behaviour was assessed using an open field chamber. The apparatus consisted of a 60 × 60 cm^2 wooden box with identical walls 20 cm high. All four of the chamber walls and the floor of the box were painted black, and the floor was divided into 36 equal sized grid squares. The apparatus was located in a windowless room with four clear 75 W bulbs mounted on the ceiling, and was elevated approximately 105 cm above the floor. A video camera was mounted at ceiling height in the centre of the room and used for recording animals' behaviour during the testing period. A radio speaker was located adjacent to the video camera at ceiling height to provide background masking noise. The extramaze cues (laboratory furniture, lights and several prominent visual features on the walls, as well as the location of the experimenter) were held constant throughout the entire testing period.

Animals were put into the chamber and allowed to freely explore the apparatus for 5 min, and the order was counterbalanced across the four groups. Animals' behavioural performance was videotaped and analysed offline by a computerised tracking system (HVS 2020; US HVS Image, San Diego, CA). For each animal, the number and duration of wall-supported rearings, the speed and path length animals travelled during the 5-min period, the percentage of maze used, and the percentage of time spent in the outer zone (10 cm from the wall) and the centre (central four 10-cm squares) were analysed (Liu and Bergin, 2009; Liu and Collie, 2009; Bergin and Liu, 2010; Knox et al., 2011).

2.4. Neurochemical procedures

2.4.1. Sample preparation

All rats were sacrificed by decapitation without anaesthesia at the time point of 60 min post-treatment, and the order was counterbalanced across the four groups. The brains were rapidly removed and left in cold saline (4 °C) for at least 45 s. The sub-regions of the hippocampus (CA1, CA2/3 and DG) and the prefrontal cortex (PFC) were dissected freshly on ice (Liu et al., 2004a, 2008a,b,c; 2011; Knox et al., 2011; Gupta et al., 2012). The tissues harvested from one hemisphere were frozen immediately and stored at −80 °C until the NOS and arginase assays. The tissues collected from the other hemisphere were then weighed, homogenized in ice-cold 10% perchloric acid (~50 mg wet weight per millilitre) and centrifuged at 10,000 rpm for 10 min at 4 °C to precipitate protein. The supernatants (the perchloric acid extracts) were frozen immediately and stored at −80 °C until the high performance liquid chromatographic (HPLC) and liquid chromatography/mass spectrometric (LC/MS) assays.

2.4.2. NOS and arginase assays

At the time of the assays, protease-inhibitory buffer containing 50 mM Tris-HCl (pH 7.4), 10 μM phenylmethylsulfonyl fluoride, 15 μM pepstatin A and 2 μM leupeptin (1:10 w/v) was added to the samples on ice. Brain tissues were then homogenised using ultrasonification (Branson Sonifier 150D, Branson Ultrasonics Corporation, Connecticut, USA) and centrifuged at 12,000 *g* for 10 min at 4 °C. Protein concentrations in the supernatant were measured based on the Bradford method (Bradford, 1976) using a Bio-Rad protein assay dye reagent concentrate and Bio-Rad Benchmark Plus microplate spectrophotometer (Bio-Rad Laboratories Inc., California, USA). Each supernatant was then separated and used for the NOS and arginase assays.

We employed a radioenzymatic assay technique to analyse total NOS activity by measuring the ability of tissue homogenates to convert [^{3}H] L-arginine to [^{3}H] L-citrulline in the presence of co-factors, and a spectrophotometric assay method to determine total arginase activity by measuring the amount of newly formed urea from L-arginine, as described previously (Liu et al., 2003a,b; 2004a,b, 2005, 2009b; Knox et al., 2011; Gupta et al., 2012). All assays were performed in duplicate. For each brain region, the tissues from the four groups were processed at the same time and the order was counterbalanced. NOS and arginase activities were expressed as pmol [^{3}H] L-citrulline/30 min/mg protein and μg urea/mg protein, respectively.