Rapid cortico-limbic alterations in AMPA receptor densities after administration of PCP: Implications for schizophrenia

Katerina Zavitsanou [a,b,*], Vu Nguyen [a], Kelly Newell [b,c], Patrice Ballantyne [a], Xu Feng Huang [b,c]

[a] Australian Nuclear Science and Technology Organisation (ANSTO), Sydney, NSW, Australia
[b] Schizophrenia Research Institute (SRI), Sydney, NSW, Australia
[c] School of Health Sciences, University of Wollongong, Wollongong, NSW, Australia

ARTICLE INFO

ABSTRACT

Phencyclidine (PCP), a non-competitive NMDA/glutamate receptor antagonist, is a psychotomimetic drug that produces a syndrome in normal humans that resembles schizophrenia. The present study investigated the mechanisms of PCP actions by examining the density of glutamate and muscarinic receptors in the rat brain 4 h after a single injection of PCP. We used receptor autoradiography and [^{3}H]MK801, [^{3}H]AMPA, [^{3}H]pirenzepine and [^{3}H]AFDX384 to target glutamate NMDA, glutamate AMPA and muscarinic M1 and M2 receptors, respectively. The major outcome from the present study was an overall decrease in levels of the glutamate AMPA receptor density (F = 14.5, d.f. = 1, $p < 0.001$) in the PCP treated rats. More specifically, PCP-treated animals displayed decreased AMPA receptor density in hippocampus CA1 (−16%), hippocampus CA2 (−25%), dentate gyrus (−27%), parietal cortex layers III–VI (−19%), central nucleus of the amygdala (−40%), and basolateral amygdala (−19%). Other brain regions examined were unaffected. PCP administration did not significantly affect glutamate NMDA, muscarinic M1 and M2 receptor density. The present study demonstrates the limbic system as the anatomical locus of alterations in AMPA receptor density after acute administration of PCP and may have implications for models of schizophrenia that focus on glutamatergic dysfunction in limbic cortical regions.

© 2008 Elsevier B.V. All rights reserved.

1. Introduction:

Phencyclidine (PCP), an antagonist of the glutamate NMDA receptor, is a potent psychotomimetic drug that produces a syndrome in normal humans that resembles schizophrenia (Morris et al., 2005). Reported effects of acute and long-term exposure to PCP or its analogue ketamine in humans include psychosis, thought disorder, delusions, flattened affect and withdrawal (Jentsch and Roth, 1999). Furthermore, long lasting cognitive impairments in episodic memory and attention have been reported in recreational users of ketamine (Morgan et al., 2004). PCP-induced psychosis provides the best evidence to date in support of the glutamate hypofunction hypothesis of schizophrenia (Jentsch and Roth, 1999). This pharmacological model of schizophrenia suggests that the neural substrates affected by PCP are vulnerable in subjects with schizophrenia (Javitt and Zukin, 1991; Hori et al., 2000; Abe

et al., 2001). Numerous experimental investigations also suggest that PCP administration mimics the behavioural features of schizophrenia in animal models (Jentsch and Roth, 1999).

Acute administration of PCP or other NMDA receptor antagonists such as ketamine and MK801, have been shown to stimulate locomotor behaviour, reduce social interaction in rats and monkeys and to disrupt prepulse inhibition, a measure of sensorimotor gating that is impaired in schizophrenia. Administration of PCP, ketamine or MK801 to rats impairs performance on tasks that seem to depend upon hippocampal or amygdalar function (reviewed in Jentsch and Roth, 1999). Persistent neurotoxicity and spatial learning deficits have been shown in mice treated with a single high dose of MK801 (Wozniak et al., 1996), whilst acute PCP administration to monkeys impairs performance of working memory tasks and tasks that measure motivation (Frederick et al., 1995).

Several neurochemical and neuroanatomical substrates for altered behaviour in PCP-treated animals have been proposed. Acute administration of ketamine or PCP dramatically increases cortical glutamate and dopamine efflux (Moghaddam et al., 1997). Laboratory animals given PCP, ketamine or MK801 show metabolic activation that is especially prominent in the limbic cortex. Both hippocampus and anterior cingulate areas of the limbic cortex

were affected early after administration (Tamminga et al., 2003). More specifically, acute administration of PCP results in biphasic changes in both brain glucose metabolism and glutamate receptor density. Studies of the uptake of 2-deoxyglucose in the brains of PCP-treated rats have revealed a short-lived (in the order of several hours) increase in forebrain metabolic activity, followed by a longer lasting reduction in metabolism, which lasts about 2 days (Gao et al., 1993). In addition NMDA receptor binding in the hippocampus is transiently decreased (at 3 h) and subsequently increased (for approximately 2 days) after single dose PCP administration (Gao and Tamminga, 1996).

PCP has also been shown to interact strongly with the cholinergic system (Weinstein et al., 1973; Maayani et al., 1974; Paster et al., 1974). Acute administration of PCP to rats increased cortical acetylcholine turnover (Murray and Cheney, 1981) and release (Giovannini et al., 1994; Hutson and Hogg, 1996). Furthermore, high dose acute PCP administration has been shown to induce a pattern of neurotoxicity in limbic brain regions (Olney et al., 1989; Corso et al., 1997) that can be prevented by antipsychotic drugs and cholinergic antagonists (Olney et al., 1999).

We have recently shown that chronic treatment (14 days) with PCP increased [^{3}H]MK801 binding to NMDA receptors in the hippocampus in mice 1 and 24 h after the last PCP treatment, whereas binding was decreased 14 days after the last PCP injection (Newell et al., 2007b). The same treatment regimen increased [^{3}H]pirenzepine binding to muscarinic M1 receptors at 1 and 24 h and decreased binding at 14 days after last PCP administration (Newell et al., 2007a). These studies were consistent with the notion that adaptations to both NMDA and muscarinic receptors may be involved in psychotomimetic actions of long-term PCP treatment. However, it was not clear how rapidly these receptor systems adapt to PCP treatment. As a corollary, increasing evidence suggests impairment of both glutamatergic and cholinergic neurotransmission in schizophrenia (Zavitsanou et al., 2002, 2004). PCP has been shown to affect functions that are controlled by the glutamatergic and cholinergic system in both humans and experimental animals. A better understanding of glutamatergic and cholinergic adaptations produced by PCP might, therefore, clarify the mechanisms underlying psychosis.

The present study investigated the mechanisms of PCP actions by examining the density of NMDA, AMPA and muscarinic M1 and M2 receptors in the rat brain 4 h after a single injection of PCP. We used quantitative autoradiography and [^{3}H]MK801, [^{3}H]AMPA, [^{3}H]pirenzepine, and [^{3}H]AFDX384; radioligands that target glutamate NMDA, glutamate AMPA and muscarinic M1 and M2 receptors respectively, and found that PCP rapidly induced changes in AMPA receptor density in limbic cortical areas that are reminiscent of changes observed in schizophrenia.

2. Materials and methods

2.1. Animals

All experiments were carried out in accordance with the University of Wollongong Animal Care Ethics Committee guidelines, which follow the National Health and Medical Research Council guidelines for animal experimentation. All efforts were made to minimize animal suffering. Female Sprague–Dawley rats (228–270 g) were obtained from the Animal Resources Center (Perth, WA, Australia). They were housed two per cage in environmentally controlled conditions (temperature 22 °C, light cycle from 0600 to 1800 h and dark cycle from 1800 to 0600 h), and allowed ad libitum access to standard laboratory chow and water throughout the study. Rats were allowed 1 week to acclimatize to their new environment before the study began. The day of the PCP treatment rats were randomised and divided into one control and one treated group. The control group (*n* = 8) was injected with saline vehicle while the treatment group (*n* = 8) received

PCP (40 mg/kg, i.p.). Both groups were decapitated under carbon dioxide anaesthesia 4 h after the injection. The brains were quickly removed, frozen in chilled isopentane (−45 °C) and stored at −80 °C. Twenty micrometers coronal sections were cut from all brains in a cryostat and mounted immediately on gelatinized microscope slides. Consecutive sections (six per slide) were taken from two brain compartments. These compartments correspond to Plates 12–15 and 28–32 from a standard rat brain atlas (Paxinos and Watson, 1997).

2.2. Autoradiography

For all autoradiographic experiments, sections were processed simultaneously to minimize experimental variance. [^{3}H]MK801 binding was performed based on the method of Subramanian and McGonigle (1991). Sections were incubated at room temperature for 2.5 h in 30 mM HEPES buffer, pH 7.5, containing 100 μM glycine, 100 μM glutamate, 1 mM EDTA and 20 nM [^{3}H]MK801 (specific activity 17.1 Ci/mmol, PerkinElmer, USA). Non-specific binding was determined by incubating adjacent sections with [^{3}H]MK801 in the presence of 20 μM MK801. Following the incubation, sections were washed twice for 20 min each at 0 °C in 30 mM HEPES containing 1 mM EDTA (pH 7.5).

[^{3}H]AMPA binding was performed based on the method of Nielsen et al. (1990): Sections underwent a pre-wash for 30 min at 4 °C in 50 mM Tris–HCl buffer (pH 7.2) containing 2.5 mm CaCl$_2$ and then incubated at 4 °C for 60 min in 50 mn Tris–HCl buffer (pH 7.2) containing 46 nM [^{3}H]AMPA (specific activity 48.7 Ci/mmol, PerkinElmer, USA in the presence of 2.5 mM CaCl$_2$ and 100 mM potassium thiocyanate (KSCN). Non-specific binding was determined by incubating adjacent sections with [^{3}H]AMPA in the presence of 1 mM glutamic acid. After the incubation the sections were rinsed three times in cold 50 mM Tris–HCl buffer containing 2.5 mM CaCl$_2$ for 60 s and dried with warm air.

[^{3}H]Pirenzepine binding was performed based on the method used previously (Piggott et al., 2002). The sections were pre-incubated for 15 min at room temperature in 22 mM HEPES (pH 7.4), and then incubated for 90 min at room temperature in the same buffer containing 10 nM [^{3}H]pirenzepine (specific activity 79 Ci/mmol, PerkinElmer, USA). Adjacent sections were incubated with [^{3}H]pirenzepine plus 10 μM of atropine to determine non-specific binding. After the incubation the sections were rinsed three times (4 min each) in ice-cold buffer with a final dipping in ice-cold distilled water and air-dried.

[^{3}H]AFDX384 binding was performed based on the method used previously (Piggott et al., 2002). Briefly, all sections were pre-incubated for 15 min at room temperature in 10 mM KH$_2$PO$_4$, 10 mM Na$_2$HPO$_4$ (pH 7.4). Sections were then incubated for 1 h in the same buffer containing 4 nM [^{3}H]AFDX384 (specific activity 120 Ci/mmol, PerkinElmer, USA). Non-specific binding was determined by incubating adjacent sections in [^{3}H]AFDX384 plus 10 μM atropine. Following incubation, sections were washed twice for 2 min each in room temperature buffer, and dipped once in distilled water and air dried.

For all ligands, the concentration of each was measured in 10 μl aliquots taken from the incubation mixture. After the assays, dried sections were exposed to Kodak MR film for 96 days for [^{3}H]MK801, 97 days for [^{3}H]AMPA, 37 days for [^{3}H]pirenzepine and 40 days for [^{3}H]AFDX384. [^{3}H]Microscales from Amersham were used as standards.

2.3. Quantitative analysis of autoradiographic images

Autoradiographs were developed at room temperature in Kodak X-ray developer (4 min) and then exposed to cold tap water (15 s), Kodak X-ray fixer (4 min), cold tap water (10 min) and room temperature air to dry. For the confirmation of anatomical structures, sections were stained with cresyl violet after the development of the autoradiographs. Section images on the film were then superimposed on the stained sections and observed under an Olympus BX-50 microscope to determine the distribution of each ligand among each brain region. Quantification analysis of the resulting autoradiographic images was performed by using an MCID image analyzer (MCID Elite, Imaging Research, Canada). A total of eight animals per group were analyzed. However, due to sectioning problems some of the sections were torn and unsuitable for processing. Therefore, for some of the brain regions the final value represents average from seven animals.

For each brain region, at least six measurements were randomly taken. Optical density measurements were converted into fmoles [^{3}H]ligand per mg tissue equivalent, according to the calibration curve obtained from the tritium standards. Non-specific binding was subtracted from the total binding for the determination of specific binding.

2.4. Statistical analyses

Statistically significant variation in radioligand binding was identified by two-way analysis of variance (ANOVA) with treatment status (PCP treated versus controls), and brain regions as factors. Further analysis using unpaired Student's *t*-tests were then used to identify the source of significant variation between the treatment groups. Variance associated with brain regions was further analyzed using Bonferroni post hoc tests. Differences were regarded as statistically significant if *p* < 0.05. All data was analyzed using the SPSS statistical package.