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Comparison between intraperitoneal and subcutaneous phencyclidine administration in Sprague–Dawley rats: A locomotor activity and gene induction study

Mikhail Kalinichev [a,*], Melanie J. Robbins [a], Elizabeth M. Hartfield [a], Peter R. Maycox [a], Susan H. Moore [b], Kevin M. Savage [a], Nigel E. Austin [b], Declan N.C. Jones [a]

[a] *Biology Department, Psychiatry Centre of Excellence for Drug Discovery, GlaxoSmithKline plc, New Frontiers Science Park, Third Avenue, Harlow, Essex, CM19 5AW, UK*
[b] *Drug Metabolism and Pharmacokinetics, Psychiatry Centre of Excellence for Drug Discovery, GlaxoSmithKline plc, New Frontiers Science Park, Third Avenue, Harlow, Essex CM19 5AW, UK*

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Abstract

In a putative model of acute phencyclidine (PCP)-induced psychosis we evaluated effects of the drug on locomotor activity (LMA) and immediate early gene (IEG) induction in the rat using two routes of drug administration, intraperitoneal (i.p.) and subcutaneous (s.c.). Adult male rats received saline or PCP (1.0–5.0 mg/kg) either i.p or s.c. and were assessed for LMA for 60 min. At the end of the LMA testing animals were culled and blood and brain samples were collected for PCP concentration analysis. Separate cohorts of animals received 5.0 mg/kg PCP (i.p. or s.c.) and were used to investigate (1) the pharmacokinetics of PCP or (2) induction of IEG (Arc, c-*fos*, BDNF, junB, Krox-20, sgk-1, NURR1, fra-2, Krox-24, and egr-3) mRNA expression in the prefrontal cortex (PFC). Administration of PCP resulted in locomotor hyperactivity which was more robust and longer-lasting in animals dosed s.c. compared to i.p.-treated-animals. Differences in hyperlocomotion were paralleled by higher concentrations of PCP in the blood and in the brain of s.c.-treated animals compared to i.p.-treated animals. The differences in the concentration of PCP between the two routes of administration were detected 30 min after dosing and persisted for up to 4 h. Administration of PCP via the s.c. route resulted in induction of more IEGs and consistently larger magnitudes of induction than that via the i.p. route. Therefore, we have outlined the dosing conditions to induce rapid and robust effect of acute PCP on behaviour, gene induction, and pharmacokinetic profile, to allow investigation of this as a potential animal model of acute psychosis.

Keywords: Animal model; Hyperactivity; Pharmacokinetics; Psychosis; Route of administration; Schizophrenia

1. Introduction

Phencyclidine (PCP), a non-competitive antagonist of the NMDA glutamate receptor, was introduced into clinical trials in late 1950s as an anaesthetic but was soon discontinued due to high incidents of symptoms typically associated with schizophrenia (Jacob et al., 1981; Jentsch and Roth, 1999). Remarkable similarity between the symptoms of schizophrenia and PCP-induced toxic psychosis combined with the evidence of marked exacerbation of those symptoms in schizophrenic patients by PCP (Allen and Young, 1978; Itil et al., 1967) brought forward involvement of NMDA receptors in pathophysiology of schizophrenia (Javitt and Zukin, 1991; Tamminga, 1998).

Over the last decade, pre-clinical research in schizophrenia has been characterized by marked increases in animal models based on administration of PCP or its congeners (ketamine, MK-801). In these studies PCP-treated animals are evaluated for signs and behaviours that mimic or are relevant to those observed in schizophrenic patients. These include locomotor hyperactivity and stereotypy (Martin et al., 1979; Murray and Horita, 1979; Phillips et al., 2001), memory- and learning-related cognitive deficits (Abdul-Monim et al., 2006; Fletcher et al., 2005; Handelmann et al., 1987; Laurent and Podhorna, 2004; Stefani

Abbreviations: PCP, phencyclidine; LMA, locomotor activity.
* Corresponding author. Tel.: +44 1279 627122; fax: +44 1279 875389.
E-mail address: mikhail.m.kalinichev@gsk.com (M. Kalinichev).

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and Moghaddam, 2002; Verma and Moghaddam, 1996), social withdrawal (Lee et al., 2005; Sams-Dodd, 1996), reduced motivation/reward (Pallares et al., 1995; Spielewoy and Marcou, 2003; Stevens et al., 1997) and deficit in sensorimotor gating (Geyer et al., 1984). Furthermore, PCP-treated animals have been evaluated for anatomical (Olney and Farber, 1995; Wang and Johnson, 2005), neurochemical (Carboni et al., 1989; Cochran et al., 2003; Jentsch et al., 1997; Steinpreis and Salamone, 1993; Steinpreis, 1996), and transcriptional (Ito, 2002; Kaiser et al., 2004; Näkki et al., 1996; Ouchi et al., 2005; Toyooka et al., 2002) changes that might play a role in aetiology of schizophrenia.

Despite the wide use of PCP in pre-clinical studies, marked inconsistencies in behavioural and neurochemical effects of the drug have been seen in the scientific literature. For example, in male rats the minimum effective dose (MED) of PCP stimulating locomotor activity was 1.0 mg/kg following subcutaneous (s.c.) dosing (Greenberg and Segal, 1985, 1986; Van Ree and Leys, 1985), compared to 4.0–5.0 mg/kg following intraperitoneal (i.p.) administration (Kesner et al., 1981; Pryor et al., 1977; Yang et al., 1991). Furthermore, acute i.p. administration of PCP (5.0 or 10 mg/kg) to male Wistar rats resulted in dose-dependent increases in DA and DOPAC concentrations in the medial prefrontal cortex (PFC) and anterior cingulate cortex (CC), but not in the nucleus accumbens (NAc) or striatum (Yang et al., 1991). In contrast, s.c. administration of PCP at similar dose-range (2.5–10 mg/kg) to male Sprague–Dawley rats stimulated DA release in NAc and dorsal caudate (Carboni et al., 1989). These behavioural and neurochemical discrepancies can, in part, be explained by variations in experimental variables and specifically by interchangeable use of i.p. and s.c. routes of PCP administration in these studies. Thus, in order to achieve comprehensive understanding of neurobiological substrate altered by PCP, its behavioural expression and its relevance to schizophrenia, the route of PCP administration requires systematic investigation.

In a putative animal model of acute PCP-induced psychosis we evaluated effects of the drug on locomotor activity and immediate early gene (IEG) induction in the rat using two, most-commonly used routes of drug administration, i.p. and s.c. The purpose of using i.p. and s.c. routes was to establish a mode of PCP administration in the rat, which is simple to perform, while providing robust behavioural and gene induction effects paralleled by rapid increases and high peak brain concentrations of the drug. We chose s.c. route based on previous evidence that in the rat pharmacokinetic values determined from the infusion of PCP via i.v. and s.c. routes were similar, with the latter route having much smaller coefficient of variation (Wessinger, 1991). Based on this evidence we hypothesized that the s.c. route of administration would result in larger magnitudes of PCP-stimulated locomotion compared to the i.p. route, paralleled by higher concentrations of PCP in both blood and brain. Furthermore, for the first time we investigated the impact of route on the induction of several IEGs in the prefrontal cortex (PFC). There is a large body of converging evidence from pre-clinical and clinical studies implicating PFC in the expression of cognitive and negative symptoms of schizophrenia (Jentsch and Roth, 1999; Yang and Chen, 2005). We used TaqMan RT-PCR (Bond et al., 2002; Harrison and Bond, 2005; Medhurst et al., 2000) to assess expression of activity-regulated cytoskeleton-associated protein (Arc), JunB, brain-derived neurotrophic factor (BDNF), c-*fos*, Krox-20, Krox-24, fra-2, NURR1, serum- and glucocorticoid-regulated kinase 1 (sgk-1), early growth response 3 (Erg-3) in response to 5.0 mg/kg PCP administered either i.p. or s.c.

2. Methods

2.1. Animals

The total of 78 adult male Sprague–Dawley rats (200–300 g; Charles River, UK) was used in this study. Animals were housed in groups of 5 and maintained on a 12-h light/dark cycle (lights on from 0600 to 1800 h) under constant temperature (21 + 1 °C) and humidity (50–58%) conditions. Food (Harlan Tekland 2014, Harlan UK Ltd., Bicester, UK) and water was available ad libitum. Animals were acclimated for at least 5 days before the experiment. Studies were conducted in full compliance with the Home Office Guidance on the operation of the UK Animals (Scientific Procedures) Act 1986, and were approved by the GlaxoSmithKline Procedures Review Panel.

2.2. Drugs

PCP hydrochloride (Sigma–Aldrich, UK) was dissolved in saline and administered i.p. and s.c.; control animals received saline. Animals administered PCP or saline via s.c. route were injected in the flank. We chose a lower dose range of PCP concentrations (1.0–5.0 mg/kg) in order to minimize the impact of stereotypy and ataxia on locomotor activity (Castellani and Adams, 1981).

2.3. Locomotor activity and PCP distribution study

Locomotor activity (horizontal counts) was monitored using twenty-four Perspex boxes (42 × 21 × 21 cm) in conjunction with "AM Logger" AM1052 activity monitors as described previously (Thorn et al., 1997). The system is equipped with 2 sets (lower and upper) of 16 × 8 infrared photobeam interruption sensors (positioned 25 mm apart) and a computerized analysis system (Linton Instruments, Diss, UK). Horizontal activity counts were generated as a result of interruption of any beam on the lower level. Stereotypic behaviours induced by PCP, such as oral stereotypy (repetitive movements of mouth and tongue) and horizontal head movements, were left largely undetected by the system. Therefore, horizontal activity counts represent primarily, if not exclusively, forward locomotion.

Animals were individually placed in those boxes, habituated for 30 min, dosed (i.p. or s.c.) with either saline or PCP (1.0, 3.2, 5.0 mg/kg) and activity recorded for further 1 h (6/route/dose). A random subset of these animals (n = 3/route/dose) was used for analysis of PCP concentrations in the blood and brain. Immediately after the locomotor activity testing (i.e. 1 h after dosing) these animals were killed via decapitation and trunk blood was collected into EDTA-coated tubes. Brains were removed and stored at −80 °C.