Available online at www.sciencedirect.com
ScienceDirect
Progress in Neuro-Psychopharmacology & Biological Psychiatry 32 (2008) 336–339
Progress In Neuro-Psychopharmacology & Biological Psychiatry
www.elsevier.com/locate/pnpbp

Phencyclidine-induced cognitive deficits in mice are improved by subsequent subchronic administration of the antibiotic drug minocycline

Yuko Fujita [a], Tamaki Ishima [a], Shinsui Kunitachi [a], Hiroko Hagiwara [a,b], Lin Zhang [a,b], Masaomi Iyo [b], Kenji Hashimoto [a,*]

[a] *Division of Clinical Neuroscience, Chiba University Center for Forensic Mental Health, 1-8-1 Inohana, Chiba 260-8670, Japan*
[b] *Department of Psychiatry, Chiba University Graduate School of Medicine, Chiba, Japan*

Received 14 March 2007; received in revised form 22 August 2007; accepted 22 August 2007
Available online 29 August 2007

Abstract

Background: The *N*-methyl-D-aspartate (NMDA) receptor antagonist phencyclidine (PCP)-induced cognitive deficits have been used as an animal model for schizophrenia. This study was undertaken to determine whether the antibiotic drug minocycline could improve PCP-induced cognitive deficits in mice.
Methods: Saline (10 ml/kg/day, s.c., once daily on day 1–5, 8–12) or PCP (10 mg/kg/day, s.c., once daily on day 1–5, 8–12) were administered to mice for 10 days. Subsequently, vehicle (10 ml/kg/day, i.p.) or minocycline (4.0 or 40 mg/kg/day, i.p.) was injected for 14 consecutive days. One day after the final injection, a novel object recognition test was performed.
Results: PCP-induced cognitive deficits in mice were significantly improved by subsequent subchronic (14 days) administration of minocycline (40 mg/kg), but not minocycline (4.0 mg/kg).
Conclusions: This study suggests that minocycline could be a potential therapeutic drug for cognitive deficits in schizophrenic patients.

Keywords: Cognition; Minocycline; NMDA receptor; Novel object recognition; Schizophrenia

1. Introduction

Cognitive deficits in patients with schizophrenia are a core feature of the illness, which predicts vocational and social disabilities for patients (Green, 1996). Accumulating evidence suggests that *N*-methyl-D-aspartate (NMDA) receptor plays a role in the pathophysiology of schizophrenia (Javitt and Zukin, 1991; Hashimoto et al., 2004, 2005b). The NMDA receptor antagonists such as phencyclidine (PCP) are known to induce schizophrenia-like symptoms including cognitive deficits in healthy subjects (Javitt and Zukin, 1991). In the novel object recognition test (NORT), we found that PCP-induced cognitive deficits in mice could be significantly improved by subsequent subchronic (14 days) administration of clozapine, but not haloperidol (Hashimoto et al., 2005a). These findings suggest that the reversal of PCP-induced cognitive deficits as measured by the NORT may be a potential animal model of atypical antipsychotic activity in relation to the amelioration of cognitive deficits in schizophrenia (Hashimoto et al., 2005a, 2006, 2007a).

Minocycline is a semisynthetic second-generation tetracycline which has anti-inflammatory effects that appear to be completely separate and distinct from its anti-microbial activity. Accumulating evidence suggests that minocycline has potential therapeutic effects in several animal models of neurological diseases (Domercq and Matute, 2004; Yong et al., 2004; Thomas and Le, 2004; Stirling et al., 2005). In addition, we reported that minocycline could ameliorate the behavioral changes (e.g., acute hyperlocomotion and the development of

Abbreviations: ANOVA, One-way analysis of variance; NMDA, *N*-methyl-D-aspartate; NORT, Novel object recognition test; PCP, Phencyclidine.

* Corresponding author. Tel.: +81 423 226 2147; fax: +81 423 226 2150.
E-mail address: hashimoto@faculty.chiba-u.jp (K. Hashimoto).

0278-5846/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.pnpbp.2007.08.031

behavioral sensitization) and neurotoxicity that occur in mice and monkeys due to the administration of methamphetamine or 3,4-methylenedioxymethamphetamine (Zhang et al., 2006a, 2006b; Hashimoto et al., 2007b). Furthermore, we found that the hyperlocomotion and prepulse inhibition deficits in mice that occur after the administration of the NMDA receptor antagonist dizocilpine were significantly attenuated by the administration of minocycline (Zhang et al., 2007). These findings suggest that minocycline may be a potential therapeutic drug for neuropsychiatric disorders including schizophrenia. In the present study, using the NORT, we examined the effects of subsequent subchronic (14 days) treatment with minocycline on cognitive deficits in mice after repeated administration of PCP.

2. Methods

2.1. Animals

Male ICR mice (6 weeks old) weighing 25–30 g were purchased from SLC Japan (Hamamatsu, Shizuoka, Japan). The mice were housed in clear polycarbonate cages (22.5×33.8×14.0 cm) and in groups of 5 or 6 mice under a controlled 12/12-h light–dark cycle (light from 7:00 AM to 7:00 PM) at a room temperature of 23±1 °C and humidity of 55±5%. The mice were given free access to water and to food pellets designed for mice. The experimental procedure was approved by the Animal Care and Use Committee of Chiba University Graduate School of Medicine.

2.2. Drug administration

PCP hydrochloride was synthesized by K.H. in our laboratory. Saline (10 ml/kg/day) or PCP (10 mg/kg/day expressed as a hydrochloride salt) were administered subcutaneously (s.c.) for 10 days (once daily on day 1–5, 8–12), and no treatment was given on days 6, 7, 13 and 14. In the experiment involving subchronic treatment, 3 days (day 15) after the final administration of saline or PCP, vehicle (10 ml/kg/day; physiological saline) or minocycline (4.0 or 40 mg/kg/day, Wako Pure Chemical Ltd., Tokyo, Japan) was administered intraperitoneally (i.p.) for 14 consecutive days (once daily on days 15–28). The dose (40 mg/kg) of minocycline was selected based on the fact that this dose was effective in mitigating the methamphetamine-induced hyperlocomotion (Zhang et al., 2006a) as well as prepulse inhibition deficits in mice after the administration of the NMDA receptor antagonist dizocilpine (Zhang et al., 2007). The dose (4.0 mg/kg) of minocycline was used as a low-dose, negative control dose. The experiments were conducted separately, and the individual dose groups were distributed across the duration of the experiments.

2.3. Novel object recognition test (NORT)

NORT was performed 1 day after a final administration of vehicle (10 ml/kg/day for 14 days) or minocycline (4.0 or 40 mg/kg/day for 14 days). The apparatus for this task consisted of a black open field box (50.8×50.8×25.4 cm). Before the test, mice were habituated in the box for 3 days. During a training session, two objects (various objects differing in shape and color but similar in size) were placed in the box 35.5 cm apart (symmetrically), and each animal was allowed to explore in the box for 10 min (5 min×2). The animals were considered to be exploring the object when the head of the animal was facing the object within an inch of the object or when any part of the body, except for the tail, was touching the object. The time that the mice spent exploring each object was recorded. After the training, the mice were immediately returned to their home-cages, and the box and objects were cleaned with 75% ethanol to avoid any possible instinctive odorant cues. Retention tests were carried out at 1-day intervals following the training. During the retention test, each mouse was placed back into the same box, with one of the objects used during training replaced by a novel object. The mice were then allowed to freely explore for 5 min, and the time spent exploring each object was recorded. Throughout the experiments, the objects were used in a counter-balanced manner in terms of their physical complexity. A preference index, the ratio of the amount of time spent exploring any one of the two objects (training session) or the novel object (retention test session) over the total time spent exploring both objects, was used to measure the memory performance.

2.4. Statistical analysis

Data were expressed as means±S.E.M. Statistical analysis was performed using one-way analysis of variance (ANOVA) and the *post hoc* Bonferroni/Dunn test. *P*-values less than 0.05 were considered statistically significant.

3. Results

During the training session, there were no significant differences (F (4,80)=1.92, p=0.115) among the five groups in the total amount of time spent exploring two objects (Table 1). In the retention session, the exploratory preference (approximately 40%) of the PCP-treated group was significantly lower than that (approximately 50%) of the saline-treated

Table 1
Total amount of time spent exploring both objects during object recognition in the training and retention sessions

Group	Training session (10 min)	Retention session (5 min)
	Time exploring objects (seconds)	
Vehicle+Vehicle	63.97±3.37	48.12±3.81
PCP+Vehicle	65.24±4.64	43.93±3.30
PCP+Minocycline (4.0 mg/kg)	63.47±6.92	42.65±5.99
PCP+Minocycline (40 mg/kg)	82.65±6.74	48.81±3.90
Vehicle+Minocycline (40 mg/kg)	71.08±13.14	32.44±6.67

Data are expressed as the mean±S.E.M (n=9–24). There were no significant differences among five groups.