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European Journal of Pharmacology 438 (2002) 75–78

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Short communication

Phencyclidine increases vesicular dopamine uptake

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Received 16 January 2002; accepted 22 January 2002

Abstract

Phencyclidine (PCP) rapidly (within 1 h) increased vesicular dopamine uptake and binding of the vesicular monoamine transporter-2 (VMAT-2) ligand, dihydrotetrabenazine. Uptake returned to basal values 3 h in the striatum after a high-dose administration of this drug (15 mg/kg i.p.). In contrast, a similar pretreatment with another non-competitive NMDA receptor antagonist, dizocilpine;([5R,10S]-[+]-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine; MK-801; 1 mg/kg, i.p.), was without effect on vesicular dopamine uptake. Pretreatment with the dopamine D2 receptor antagonist, eticlopride, blocked the increase in vesicular dopamine uptake caused by PCP administration. These data demonstrate a heretofore unreported mechanism that may contribute to the ability of PCP to influence dopamine neuronal function and exert its pharmacological effects. © 2002 Published by Elsevier Science B.V.

Keywords: Vesicular monoamine transporter; Dopamine D2 receptor; NMDA receptor

1. Introduction

Phencyclidine (PCP) is a psychotomimetic that acts as both a non-competitive NMDA receptor antagonist (Ohmori et al., 1992; Balster et al., 1995) and a dopamine transporter (DAT) inhibitor (Nishijima et al., 1996; Dersch et al., 1994). This latter effect contributes to the ability of this agent to increase extracellular dopamine concentrations (Nishijima et al., 1996).

Several recent studies have demonstrated agents that alter extracellular dopamine levels, rapidly affect the function of the vesicular monoamine transporter-2 (VMAT-2). For instance, Brown et al. (2001a) reported that DAT inhibitors including cocaine, 1-[2-(diphenylmethoxy)ethyl]-4-(3-phenylpropyl)piperazine) (GBR 12935) and amfonelic acid increase vesicular dopamine uptake, as assessed in vesicles prepared from the striata of treated rats. In contrast, methamphetamine administration rapidly decreases vesicular dopamine uptake in such preparations (Brown et al., 2000; Hogan et al., 2000). The impact of other transmitters systems, such as glutamate on VMAT-2 function, has not been described. The purpose of the present study was to determine the effects of PCP, an NMDA receptor antagonist and a unique psychotomimetic, on vesicular dopamine uptake. Results reveal that PCP administration increased vesicular uptake by increasing dopamine D2 receptor activity.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (280–340 g; Simonsen Laboratories, Gilroy, CA) were group-housed (8 animals/cage) with an alternating light/dark cycle (lights on 14 h/day) and treated in an ambient temperature of 24 °C. Food and water were provided ad libitum. Rats were sacrificed by decapitation. All experiments were conducted in accordance with National Institute of Health guidelines for the care and use of laboratory animals.

2.2. Drugs and radioligands

Phencyclidine was supplied by the National Institute on Drug Abuse (Bethesda, MD). MK-801 was purchased from Research Biochemicals International (Natick, MA). Eticlopride was purchased from Sigma (St. Louis, MO). 7,8-[^{3}H]dopamine (49 Ci/mmol) was purchased from Amersham Life Sciences (Arlington Heights, IL) and α-[2-^{3}H]dihydrotetrabenazine (20 Ci/mmol) was purchased from American Radioligand Chemicals (St. Louis, MO). Tetrabenazine was kindly donated by Drs. Jeffrey Erickson, Helene Varoqui

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0014-2999/02/$ - see front matter © 2002 Published by Elsevier Science B.V.
PII: S0014-2999(02)01298-0

(Louisiana State University Health Science Center, New Orleans, LA), and Erik Floor (University of Kansas, Lawrence, KS). Drugs were administered as indicated in figure legends; doses were calculated as the respective free base.

2.3. Vesicular [^{3}H]dopamine uptake and [^{3}H]dihydrotetrabenazine binding

Vesicular [^{3}H]dopamine uptake was accomplished as described by Brown et al. (2000). Briefly, striatal synaptic vesicles were obtained from synaptosomes prepared from rat striatum as described previously (Fleckenstein et al., 1997). Synaptosomes were resuspended and homogenized in cold distilled deionized water. Osmolarity was restored by the addition of HEPES and potassium tartrate (final concentrations in mM: 25 and 100, respectively; pH 7.5 at 4 °C). Samples were centrifuged for 20 min at 20,000 × *g* (4 °C) to remove lysed synaptosomal membranes. $MgSO_4$ (final concentration: 1 mM) was added to the supernatant, which was then centrifuged for 45 min at 100,000 × *g* (4 °C). The resulting vesicular pellet was resuspended in cold wash buffer (see below) at a concentration of 50 mg/ml (original tissue weight). Vesicular [^{3}H] dopamine uptake was determined by incubating 100 μl of synaptic vesicle samples (~ 2.5 μg of protein) at 30 °C for 3 min in assay buffer (final concentration in mM: 25 HEPES, 100 potassium tartrate, 1.7 ascorbic acid, 0.05 EGTA, 0.1 EDTA, 2 ATP-Mg^{2+}, pH 7.5 at 30 °C) in the presence of [^{3}H]dopamine (final concentration: 30 nM). The reaction was terminated by the addition of 1 ml of cold wash buffer (assay buffer containing 2 mM $MgSO_4$ substituted for the ATP-Mg^{2+}, pH 7.5 at 4 °C) and rapid filtration through Whatman GF/F filters soaked previously in 0.5% polyethylenimine. Filters were washed three times with cold wash buffer using a Brandel filtering manifold (Brandel, Gaithersburg, MD). Radioactivity trapped in filters was counted using a liquid scintillation counter. Nonspecific values were determined by measuring vesicular [^{3}H]dopamine uptake in wash buffer (i.e., no ATP present) at 4 °C.

Binding of [^{3}H]dihydrotetrabenazine was performed as described by Teng et al. (1998). Briefly, 200 μl of the synaptic vesicle preparation (~ 6 μg of protein) was incubated in wash buffer in the presence of [^{3}H]dihydrotetrabenazine (final concentration: 2 nM) for 10 min at 25 °C. The reaction was terminated by the addition of 1 ml of cold wash buffer and rapid filtration through Whatman GF/F filters soaked in 0.5% polyethylenimine. Filters were washed three times with cold wash buffer. Radioactivity trapped in filters was counted using a liquid scintillation counter. Nonspecific binding was determined by coincubation with 20 μM tetrabenazine. All protein concentrations were determined by a Bio-Rad protein assay (Bio-Rad, Richmond, CA).

2.4. Statistical analyses

Statistical analyses were performed using an Analysis of Variance followed by a Fisher's protected least-significant

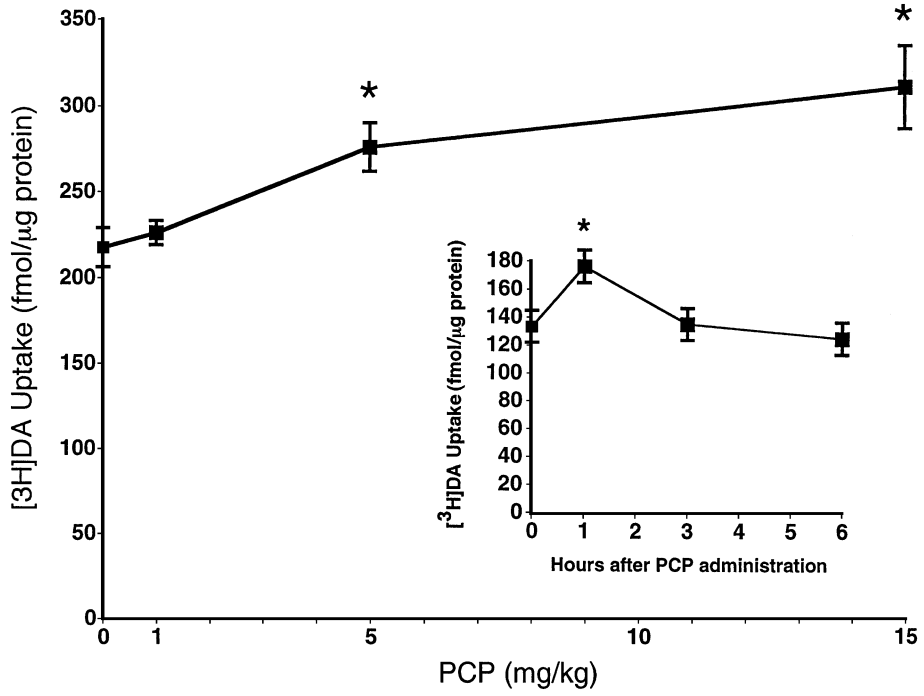

Fig. 1. Rats received a single injection of PCP (1–15 mg/kg, i.p.) or saline vehicle (1 ml/kg, i.p.) and were decapitated 1 h later. Inset: Rats received a single injection of PCP (15 mg/kg, i.p.) or saline vehicle (1 ml/kg, i.p.) and were decapitated 1–6 h later. Symbols represent the means and vertical lines ± 1 S.E.M. of determinations in six rats. * Values for PCP-treated rats that are significantly different from saline-treated controls ($P \leq 0.05$).

difference post hoc comparison or Student's *t*-test as indicated. Differences were considered significant if probability error was $\leq 5\%$.

3. Results

Results presented in Fig. 1 demonstrate that PCP administration increased vesicular dopamine uptake. Specifically, a single intraperitoneal injection of 5 or 15 mg/kg significantly elevated uptake by 27% and 43%, respectively, as assessed 1 h after drug treatment in vesicles prepared from the striatum of rats. In a separate experiment, a single 15 mg/kg intraperitoneal injection also increased dihydrotetrabenazine binding by 30% (i.e., from 8.6 ± 0.47 to 11.2 ± 0.86 fmol/μg protein; $n = 6$ rats/group) 1 h after drug treatment. The increase in vesicular uptake induced by 15 mg/kg of PCP returned to basal value 3 h after drug treatment (Fig. 1, inset). In contrast, administration of another non-competitive NMDA receptor antagonist, dizocilpine([5R,10S]-[+]-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine; MK-801; 0.1-1.0 mg/kg, i.p.; $n = 6$ rats/group) was without effect on vesicular dopamine uptake 1 h after treatment (data not shown).

It has been reported that PCP, in addition to acting as an NMDA receptor antagonist, is a DAT inhibitor (Nishijima et al., 1996; Dersch et al., 1994). Because dopamine D2 receptor antagonists have been shown to prevent the increase in vesicular dopamine reuptake caused by administration of the DAT inhibitor, cocaine (Brown et al., 2001b), the ability of eticlopride to prevent the PCP-induced increase in vesicular dopamine uptake was examined. Results presented in Fig. 2 reveal that the pretreatment with the dopamine D2 receptor antagonist prevented the increase in vesicular dopamine uptake caused by PCP administration. As reported previously, (Brown et al., 2001a) eticlopride, per se, did not affect vesicular dopamine uptake.

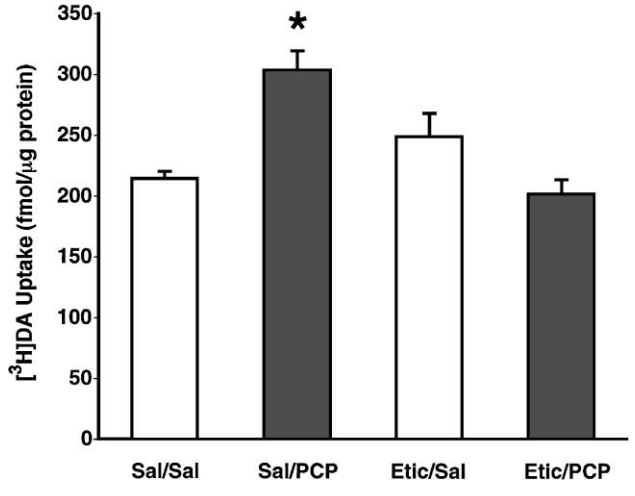

Fig. 2. Fifteen minutes prior to receiving a single administration of PCP (solid columns; 15 mg/kg i.p.) or saline (Sal) vehicle (open columns; 1 ml/kg i.p.), rats were pretreated with eticlopride (Etic; 1 mg/kg i.p.) or saline vehicle (1 ml/kg i.p.). Rats were decapitated 1 h after PCP or saline administration. Columns represent the means and vertical lines ± 1 S.E.M. of determinations in five or six rats. * Value for Sal/PCP-treated rats that is significantly different from saline or Etic/PCP-treated rats ($P \leq 0.05$).

4. Discussion

The present report presents the novel finding that PCP treatment increases vesicular dopamine uptake, as assessed in purified striatal vesicles. These vesicles are associated predominantly with dopamine neurons, as it has been demonstrated previously that destruction of serotonergic terminals projecting to the striatum does not affect vesicular dopamine uptake in the resulting vesicular preparation (Brown et al., 2000; Darchen et al., 1989).

To determine if the NMDA receptor-blocking properties of PCP per se caused the increase in vesicular dopamine uptake caused by the agent, effects of another NMDA receptor antagonist, MK-801, were assessed. MK-801 was administered at a doses ranging from 0.1 to 1.0 mg/kg, i.p. In contrast to the effects of PCP, MK-801 administration was without effect on vesicular uptake suggesting that the PCP effect was not due to its NMDA receptor-blocking action.

In addition to its effect on glutamatergic systems, PCP is an inhibitor of the DAT and thereby increases extracellular dopamine levels (Nishijima et al., 1996; Dersch et al., 1994). This effect may contribute to the ability of PCP to increase vesicular uptake, as it has been demonstrated that DAT inhibitors such as cocaine, GBR12935 and amfonelic acid increase vesicular dopamine uptake (Brown et al., 2001a). Consonant with the possibility that a PCP-induced increase in vesicular uptake is mediated by an increase in extracellular dopamine concentrations is the observation that the effect was blocked by pretreatment with a dopamine D2 receptor antagonist, eticlopride. A role for dopamine D2 receptors in increasing vesicular dopamine uptake has been demonstrated previously: specifically, eticlopride pretreatment attenuates the increase in vesicular dopamine uptake caused by cocaine administration (Brown et al., 2001b). Moreover, administration of the dopamine D2 receptor agonist, quinpirole, also increases vesicular dopamine uptake (Brown et al., 2001b). Because the increase in vesicular uptake after both PCP and cocaine treatment (Brown et al., 2001a) is associated with an increase in binding of the VMAT-2 ligand dihydrotetrabenazine, this change may reflect a redistribution of vesicles within dopamine nerve terminals (i.e., into the pool purified in the present vesicular preparation). The nature of this putative redistribution requires further study.

In conclusion, the present data demonstrate that PCP administration increases vesicular dopamine uptake in a manner resembling the effects of DAT inhibitors; an effect mediated by dopamine D2 receptors. We speculate that this effect may represent a redistribution of vesicles within the

synapse after drug treatment, and subsequently contributes to the ability of PCP to influence dopamine neuronal function.

Acknowledgements

These studies were supported by NIH-NIDA Grants DA04222, DA11389 and DA00869.

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