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Role of nitric oxide in the development of tolerance and sensitization to behavioural effects of phencyclidine in mice

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1 To determine whether nitric oxide (NO) was involved in tolerance and sensitization to the effects of phencyclidine (PCP), we examined NO synthase activity and the number of NADPH-diaphorase (NADPH-d)-positive cells in discrete brain regions of saline-, acute PCP- and repeated PCP-treated mice. We also investigated the effects of a NO synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME), on the behavioural changes induced by repeated PCP treatment in mice.

2 Acute PCP (1, 3, and 10 mg kg^{-1}, s.c.) treatment induced dose-dependent hyperlocomotion, motor incoordination and stereotyped behaviours, consisting of sniffing, head movement and ataxia in mice.

3 In mice treated repeatedly with PCP (1, 3, and 10 mg kg^{-1} day^{-1}), s.c., once a day for 14 days), the sniffing, head movement, ataxia and motor incoordination induced by PCP were attenuated (indicating the development of tolerance to these behaviours), whereas the hyperlocomotion induced by PCP was potentiated (indicating the development of sensitization to hyperlocomotion). The development of tolerance and sensitization to PCP-induced behaviours in the repeated PCP-treated mice was more marked at the dose of 10 mg kg^{-1} day^{-1}) than at other doses.

4 NO synthase activity in the cerebral cortex and cerebellum, but not in the striatum and hippocampus, was significantly decreased by acute PCP (10 mg kg^{-1}) treatment in comparison with saline treatment, and such changes in activity in the cerebral cortex and cerebellum were reversed by repeated PCP treatment (10 mg kg^{-1} day^{-1}).

5 The number of neurones containing NADPH-d reactivity in the cerebral cortex, nucleus accumbens, and striatum of acute and repeated PCP-treated mice showed no change in comparison with saline-treated mice.

6 Tolerance to PCP (10 mg kg^{-1} day^{-1})-induced ataxia and motor incoordination was significantly attenuated by combined treatment with L-NAME (50 mg kg^{-1} day^{-1} i.p.).

7 Sensitization to PCP-induced hyperlocomotion was further enhanced by combined treatment with L-NAME (50 mg kg^{-1} day^{-1}). However, N^G-nitro-D-arginine methyl ester (D-NAME, 50 mg kg^{-1} day^{-1}, i.p.), a less active enantiomer of L-NAME, had no effect, suggesting a stereospecific mechanism.

8 The PCP-induced behaviours in animals that had exhibited tolerance and sensitization to PCP (10 mg kg^{-1} day^{-1}) were not influenced by acute L-NAME (5 and 50 mg kg^{-1}, i.p.) or D-NAME (50 mg kg^{-1}, i.p.) treatment.

9 These results suggest that NO may play an important role in the development, but not in the maintenance, of tolerance and sensitization to the effects of PCP in mice.

Keywords: Phencyclidine; nitric oxide; nitric oxide synthase; tolerance; sensitization; behavioural changes in mice

Introduction

The repeated administration of phencyclidine (PCP), a psychotomimetic agent, to animals has been reported to induce the development of tolerance to some of its behavioural effects and the development of sensitization to others. Development of tolerance to the behavioural effects of PCP on operant responding (Balster & Chait, 1976; Chait & Balster, 1978; Murray, 1978; Woolverton & Balster, 1979), motor incoordination (Pinchasi *et al.*, 1978a, b; Nabeshima *et al.*, 1982a, b; Hiramatsu *et al.*, 1984; Noda *et al.*, 1995), and stereotyped behaviours (Sturgeon *et al.*, 1982; Nabeshima *et al.*, 1982a, b; 1987; Kitaichi *et al.*, 1995; Noda *et al.*, 1995) has been reported in various animal species. Contrary to these findings, other authors have reported the development of behavioural sensitization or reverse tolerance to other behavioural effects of PCP after repeated PCP treatment: an increase in stereotyped behaviours in stumpai macaques (Schlemmer *et al.*, 1978) and increased locomotor activity, stereotyped sniffing and rearing in rats (Smith *et al.*, 1978; Nabeshima *et al.*, 1987; Xu & Domino, 1994; Kitaichi *et al.*, 1995). However, little is known about the mechanisms involved in the development of tolerance and sensitization to the effects of PCP.

Nitric oxide (NO) may be an important intercellular messenger in nervous and immune systems (Collier & Vallance, 1989) and it may also operate as a neurotransmitter, particularly in the central nervous system (CNS; Garthwaite *et al.*, 1988). NO is produced from L-arginine by NO synthase (Palmer *et al.*, 1988). This enzyme has been found in various neuronal populations, in particular in areas such as the cerebellum, hippocampus, striatum, cortex, hypothalamus, mid brain, and medulla of the rat (Föstermann *et al.*, 1990). NO formation can be blocked by selective enzyme inhibitors, such as N^G-nitro-L-arginine methyl ester (L-NAME; Hecker *et al.*, 1990). The involvement of NO has

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also been demonstrated in the mechanisms of synaptic plasticity, including long-term potentiation (LTP) in the hippocampus (O'Dell *et al.*, 1991; Schuman & Madison, 1991), learning and memory (Chapman *et al.*, 1992; Böhme *et al.*, 1993; Hölscher & Rose, 1993), tolerance to ethanol (Khanna *et al.*, 1993), tolerance to the antinociceptive effect of morphine (Rauhala *et al.*, 1994) and behavioural sensitization to cocaine (Pudiak & Bozath, 1993).

Regarding the involvement of NO in behavioural tolerance and sensitization, the systemic administration of NO synthase inhibitors such as L-NAME has been shown to protect behavioural tolerance and sensitization to the effects of cocaine and morphine or ethanol, respectively (Khanna *et al.*, 1993; Pudiak & Bozath, 1993; Rauhala *et al.*, 1994). Although these findings suggest that NO plays an important role in certain forms of behavioural tolerance and sensitization, it has yet to be elucidated whether NO is involved in the development of tolerance and sensitization to the effects of PCP. In the present study, we investigated the effects of L-NAME, a potent inhibitor of NO synthase (Dwyer *et al.*, 1991), on the behavioural changes induced by repeated PCP treatment in mice, in comparison with the effects of N^G-nitro-D-arginine methyl ester (D-NAME; a less active enantiomer of L-NAME). We also measured NO synthase activity in the brains of mice that had received acute and repeated PCP treatment. Further, we determined NO synthase-containing neurones in the brain by NADPH-diaphorase (NADPH-d) histochemistry, since it has been demonstrated that neuronal NADPH-d is a NO synthase, and that NADPH-d histochemistry provides a specific histochemical marker for neurones producing NO.

Methods

Animals and environment

Male mice of the ddY strain (Japan SLC Inc., Shizuoka, Japan), weighing 27–32 g at the beginning of the experiments, were used. The animals were housed in plastic cages, received food (CE2, Clea Japan Inc., Tokyo, Japan) and tap water *ad lib*, and were kept in a regulated environment (23±1°C, 50±5% humidity), with a 12/12 h light-dark cycle (light on at 09 h00 min).

Schedule for drug treatments

Saline or PCP (1, 3 or 10 mg kg^{-1}, s.c.) were administered once a day for 13 days. On the 14th day, saline-treated animals were challenged with saline (control group) or PCP (1, 3 or 10 mg kg^{-1} s.c.; acute PCP-treated group). PCP-treated animals were challenged with PCP (repeated PCP-treated group). L-NAME (5 or 50 mg kg^{-1}, i.p.) or D-NAME (50 mg kg^{-1}, i.p.) was administered 15 min before PCP treatment.

Behavioural study

On the day of the final treatment, each animal was placed in a transparent acrylic cage (26 × 44 × 40 cm) immediately after PCP or saline-treatment had been given. Thirty min later, the degree of sniffing, head movement, and ataxia was assessed, over a 3-min observation period, in terms of scores ranging from 0 to 3 (0, none; 1, slight; 2, moderate; 3, marked) (Noda *et al.*, 1995). The locomotor activity was simultaneously assessed by counting large movement (movement over 10 cm) to avoid the influence of PCP-induced stereotypies, over a 30-min period using SCANET SV-10 (Toyo Sangyou, Toyama, Japan) (Kitaichi *et al.*, 1995; Noda *et al.*, 1995). Immediately after the measurement of locomotion, the animals were placed on a horizontal bar (30 cm long, 6 mm square and 30 cm above the floor) (Hiramatsu *et al.*, 1984; Noda *et al.*, 1995), and the time that each mouse clung to the bar in 3 trials was measured (cut-off time; 3 min).

NO synthase assay

Immediately after the behavioural study was completed, 6–7 animals were randomly selected and killed by decapitation: the brain was removed rapidly from the skull. The brains were dissected into four regions, the cerebral cortex, striatum, hippocampus, and cerebellum, after which they were rapidly frozen and stored in a deep freezer at −80°C until analyzed.

Brain NO synthase activity was determined as described previously (Bredt & Snyder, 1989), with a minor modification (Komori *et al.*, 1993). The brains were homogenized in 5 vol. (w v^{-1}) of 50 mM Tris-HCl buffer (pH 7.4) containing 0.1 mM EGTA, 0.1 mM EDTA, 1 μM pepstatin, 2 μM leupeptin, 1 mM phenylmethylsulphonyl fluoride, and 0.5 mM dithiothreitol. The homogenates were centrifuged at 20,000 *g* for 45 min, and the supernatants were used in the assay. NO synthase activity was measured by monitoring the conversion of [^{3}H]-arginine to [^{3}H]-citrulline. Briefly, the supernatants were incubated for 12 min at 37°C in a final volumne of 100 μl, containing 100 μl NAPDH, 50 μl L-arginine, 2 mM $CaCl_2$, 0.3 mg calmodulin, 10 μM tetrahydrobiopterin, and 200,000 d.p.m. of L-[^{3}H]-arginine. The assays were terminated by the addition of 2 ml of ice-cold acetate buffer (pH 5.5) containing 1 mM citrulline, 2 mM EDTA, and 0.2 mM EGTA. The samples were applied to 1-ml columns of Dowex AG50W-X8 (Na^+ form) and the eluate was collected. The columns were then further eluted with 2 ml of water. [^{3}H]-citrulline in the combined eluate was quantified by liquid scintillation spectrometer. Protein content was determined according to the method of Lowry *et al.* (1951), with bovine serum albumin used as standard.

NADPH-d histochemistry

Immediately after the end of the behavioural study, 5–7 animals were randomly selected, anaesthetized with sodium pentobarbitone (50 mg kg^{-1}, i.p.), and perfused with physiological saline, followed by perfusion with 4% paraformaldehyde in phosphate-buffered saline (PBS, pH 7.4). The brains were removed, postfixed at 4°C for 2 h in the same fixative, and then cytoprotected in 20% sucrose in PBS. The brains were cut into 20-μm thick coronal slices by a cryostat and were processed by NADPH-d histochemistry.

To demonstrate NADPH-d activity, free-floating sections were incubated in 0.1 M phosphate buffer (pH 7.4) containing 0.3% Triton X-100, 0.1 mg ml^{-1} nitroblue tetrazolium, and 1 mg ml^{-1} β-NADPH at 37°C for 2 h. The sections were washed by PBS, mounted on gelatin-coated glass slides, dried, dehydrated, and coverslipped.

To quantify the number of NADPH-d-positive cells in the cerebral cortex, nucleus accumbens, and striatum, we examined the sections with a computer-assisted image-anaylsis system (C. Imaging System; Compix Inc., Mars, PA, U.S.A.) attached to a light microscope (Olympus BX60-FLB-3; Olympus, Tokyo) fitted with a × 10 objective lens.

Drugs

Phencyclidine HCl (PCP) was synthesized by us. L-[2, 3, 4, 5,-^{3}H]-arginine (37 MBq ml^{-1}) was obtained from Amersham (Arlington Heights, IL, U.S.A.). N^G-nitro-L-arginine methyl ester (L-NAME), N^G-nitro-D-ariginine methyl ester (D-NAME), pepstatin A, leupeptin, phenylmethylsulphonyl fluoride, calmodulin, β-NADPH and nitroblue tetrazolium were purchased from Sigma (St. Louis, MO, U.S.A.). PCP (s.c.), L-NAME (i.p.), and D-NAME (i.p.) were dissolved in a 0.9% saline solution, and were administered in a volume of 0.1 ml 10 g^{-1} body weight.

Statistics

For nonparametric data from stereotypy scores, statistical differences between values for individual two groups and among values for individual over three groups were de-

termined by Mann-Whitney *U*-test and Dunn's multiple comparisons test, respectively. For parametric data from motor coordination, locomotion, and biochemical study, statistical differences between values for individual two groups, and among values for individual over three groups were determined by Student's *t* test and Dunnett multiple comparisons test, respectively.

Results

Effects of acute and repeated PCP treatment

As shown in Figure 1, acute PCP treatment induced sniffing, head movement, ataxia, motor incoordination and hyperlocomotion in mice in a dose-dependent manner. In the repeated PCP-treated mice, sniffing, head movement, ataxia, and motor incoordination induced by PCP challenge were attenuated, whereas the hyperlocomotion induced by the PCP challenge was potentiated in comparison with acute PCP treatment (Figure 1).

Since the development of sensitization and tolerance to the effects of PCP in the repeated PCP-treated mice was more marked at the dose of 10 mg kg^{-1} day^{-1} than at other doses, this dose of PCP was employed in the following experiments.

Effects of acute and repeated PCP treatment on NO synthase activity and NADPH-d reactivity

NO synthase activity in discrete brain regions is shown in Table 1. In the saline-treated mice, the greatest activity was found in the cerebellum, followed by the hippocampus, striatum, and cerebral cortex. NO synthase activity in the cerebral cortex and cerebellum but not in the striatum and hippocampus, of acute PCP-treated mice was significantly less than that of the saline-treated mice. Such effects of acute PCP treatment on the NO synthase activity in the cerebral cortex and cerebellum were significantly reversed by repeated PCP treatment.

NO synthase activity in the cerebral cortex and cerebellum of the acute and repeated L-NAME (50 mg kg^{-1}, i.p.)-treated mice was significantly less than that of saline-treated mice (Table 2).

Table 1 Change in NO synthase activity in saline-, acute PCP-, and repeated PCP-treated mice

Brain region	*Saline*	*Acute PCP (nmol min^{-1} mg^{-1} protein)*	*Repeated PCP*
Cerebral cortex	0.16±0.01 (6)	0.08±0.004 (7)*	0.11±0.01 (7)##
Striatum	0.23±0.01 (6)	0.20±0.006 (7)	0.22±0.02 (7)
Hippocampus	0.30±0.01 (6)	0.28±0.012 (7)	0.31±0.01 (7)
Cerebellum	0.42±0.04 (6)	0.30±0.020 (6)*	0.42±0.03 (7)#

NO synthase activity was measured by monitoring the conversion of [^{3}H]arginine to [^{3}H]citrulline, as described in Methods. Numbers in parentheses show the numbers of animals used. *$P<0.05$ vs saline. #$P<0.05$, ##$P<0.01$ vs acute PCP treatment (Dunnett multiple comparisons test).

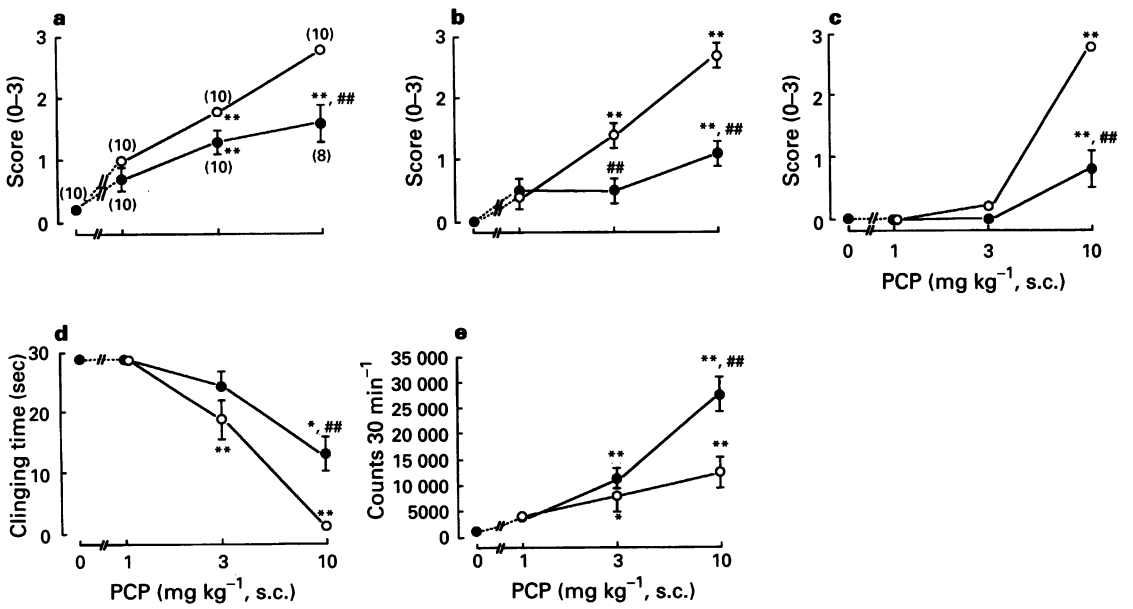

Figure 1 Effects of acute and repeated PCP treatment on (a) sniffing, (b) head movement, (c) ataxia, (d) motor coordination and (e) locomotion in mice. Saline or PCP (1, 3 or 10 mg kg^{-1}, s.c.) was administered once a day for 13 days. On the 14th day, the animals were challenged with saline or PCP (1, 3 or 10 mg kg^{-1}, s.c.) and behavioural studies were then performed, as described in Methods. (○) Acute PCP treatment; (●) repeated PCP treatment. Numbers in parentheses show the numbers of animals used. *$P<0.05$, **$P<0.01$ vs saline alone (stereotypy scores; Dunn's multiple comparisons test, others; Dunnett multiple comparisons test). #$P<0.05$, ##$P<0.01$ vs actue PCP treatment at each dose (stereotypy scores; Mann-Whitney *U*-test; others: Student's *t* test).

The density of NADPH-d positive cells in the cerebral cortex, nucleus accumbens, and striatum of acute and repeated PCP-treated mice was not significantly different from that of the saline-treated mice (data not shown).

Effects of L-NAME and D-NAME on the development of behavioural tolerance and sensitization induced by repeated PCP treatment

The coadministration of L-NAME (50 mg kg^{-1}) during repeated PCP (10 mg kg^{-1} day^{-1}) treatment significantly attenuated the development of tolerance to PCP-induced ataxia and motor incoordination (Figure 2). The development of tolerance to PCP-induced sniffing and head movement was also attenuated by coadministration with L-NAME, but the effect was not significant. In contrast, the development of sensitization in the locomotion induced by repeated PCP treatment was potentiated by coadministration with L-NAME (50 mg kg^{-1}) (Figure 2). However, the same treatment with D-NAME (50 mg kg^{-1}) had no effect (Figure 2).

Effects of acute L-NAME and D-NAME treatment on tolerance to ataxia or motor incoordination and sensitization to the locomotion induced by repeated PCP treatment

The development of tolerance to PCP-induced ataxia or motor incoordination, and the sensitization to hyperlocomotion in mice was again observed in animals repeatedly given PCP (10 mg kg^{-1}) (Figure 3). Neither L-NAME (50 mg kg^{-1}) nor D-NAME (50 mg kg^{-1}), given on the final day of PCP treatment only, had any effect on the sensitization or tolerance to PCP (Figure 3).

Discussion

Repeated PCP treatment resulted in an enhancement of the locomotor-increasing effect seen with acute PCP treatment. In contrast, other behaviours induced by acute PCP were attenuated by repeated PCP treatment, indicating the development of tolerance to the induced sniffing, head movement, ataxia, and motor incoordination. With the exception of the tolerance to sniffing, these findings are consistent with those of other investigators and with our previous reports (Hiramatsu *et al.*, 1984; Nabeshima *et al.*, 1987; Xu & Domino, 1994; Kitaichi *et al.*, 1995). In other words, it has been reported that repeated PCP treatment caused sensitization to sniffing in mice and rats, although here we observed tolerance in mice. In the present study, the tolerance and sensitization to PCP-induced behaviours in the mice receiving repeated PCP-treatment was more marked at the dose of 10 mg kg^{-1} day^{-1} than at other doses. We therefore employed this dose of PCP (10 mg kg^{-1} day^{-1}) in the following experiments, and we examined the involvement of NO in the sensitization and tolerance to PCP-induced behaviours in mice.

In the biochemical study, NO synthase activity in the cer-

Table 2 Change in NO synthase activity in saline-, acute L-NAME- and repeated L-NAME-treated mice

Brain region	*Saline*	*Acute L-NAME (nmol min^{-1} mg^{-1} protein)*	*Repeated L-NAME*
Cerebral cortex	0.119±0.009 (5)	0.040±0.003 (5)**	0.047±0.004 (5)**
Cerebellum	0.300±0.012 (5)	0.099±0.015 (5)**	0.120±0.007 (5)**

NO synthase activity was measured by monitoring the conversion of [^{3}H]-arginine to [^{3}H]-citrulline, as described in Methods. Numbers in parentheses show the numbers of animals used. **$P<0.01$ vs saline (Dunnett multiple comparisons test).

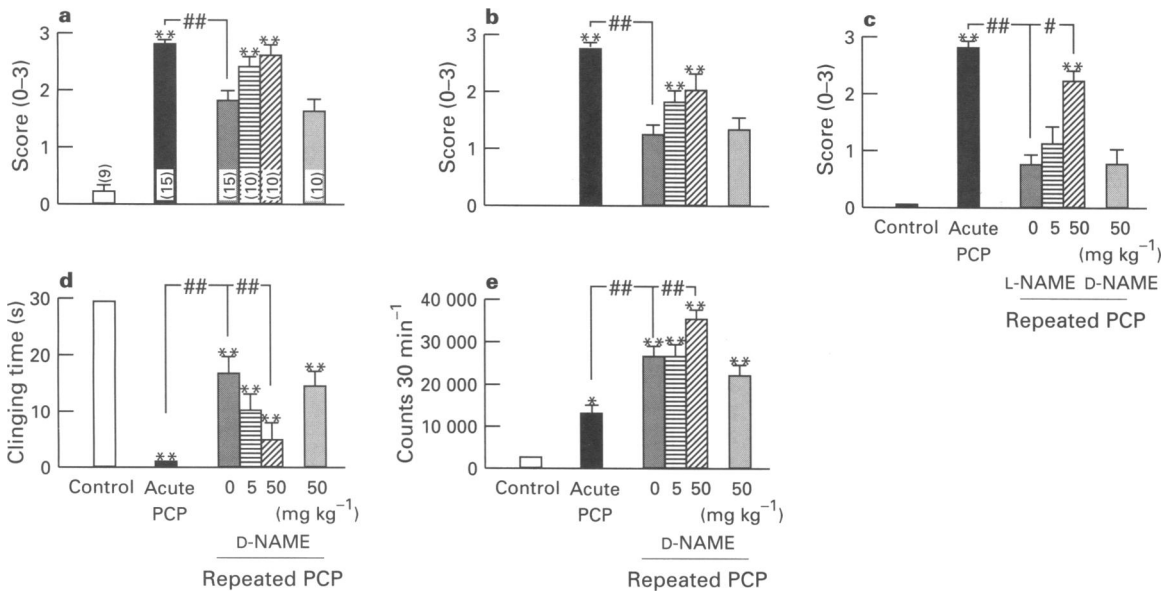

Figure 2 Effects of coadministration of L-NAME and D-NAME on the development of tolerance to (a) sniffing, (b) head movement, (c) ataxia, or (d) motor incoordination and (e) sensitization to hyperlocomotion induced by repeated PCP treatment in mice. L-NAME (5 or 50 mg kg^{-1}, i.p.) or D-NAME (50 mg kg^{-1}, i.p.) was administered 15 min before each PCP treatment (10 mg kg^{-1}, s.c.) for 14 days. Behavioural studies were performed as described in Methods. Numbers in parentheses show the numbers of animals used. *$P<0.05$, **$P<0.01$ vs control. #$P<0.05$, ##$P<0.01$ vs repeated PCP alone treatment (stereotypy scores: Dunn's multiple comparisons test; others: Dunnett multiple comparisons test).

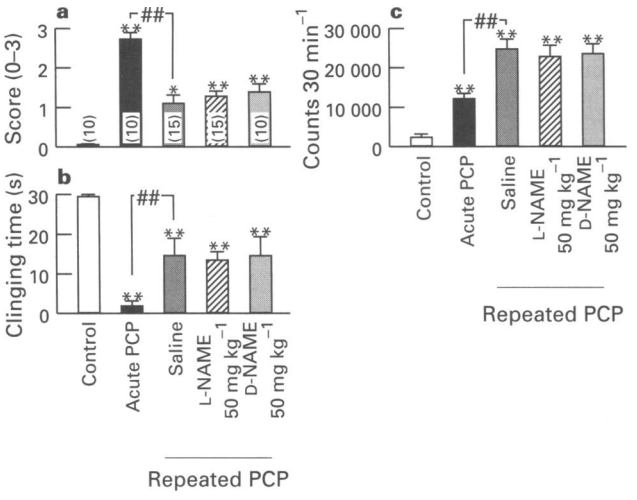

Figure 3 Effects of acute L-NAME treatment on the tolerance to (a) ataxia or (b) motor incoordination and (c) sensitization to hyperlocomotion induced by PCP in mice. PCP (10 mg kg^{-1}, s.c.) was injected once a day for 14 days. On the final day of the treatment with PCP, mice were administered L-NAME (50 mg kg^{-1}, i.p.) 15 min before PCP (10 mg kg^{-1}, s.c.). Behavioural studies were performed as described in Methods. Numbers in parentheses show the numbers of animals used. $*P<0.05$, $**P<0.01$ vs control. $\#\#P<0.01$ vs treatment with repeated PCP alone treatment (stereotypy scores: Dunn's multiple comparisons test; others: Dunnett multiple comparisons test).

ebral cortex and cerebellum was reduced by acute PCP treatment (10 mg kg^{-1}), compared with the effect of saline treatment, and these changes in both brain regions were reversed by repeated PCP treatment (10 mg kg^{-1} day^{-1} for 14 days). Further, in our preliminary *in vitro* experiment, NO synthase activity in each brain region of the saline- and acute PCP-treated mice and in animals receiving repeated PCP treatments, was completely inhibited by L-NAME at 10 μM (data not shown), indicating that the NO synthases in the brains are L-NAME-sensitive forms. In contrast to the NO synthase activity, the reactivity of NADPH-d-containing neurones in the cerebral cortex, nucleus accumbens, striatium, and cerebellum did not differ among saline-, acute PCP-, and repeated PCP-treated mice. A previous report has demonstrated that PCP is a suicide inhibitor of brain NO synthase, suggesting that NO plays an important role in PCP-induced pharmacological and toxicological effects in a variety of physiological processes (Osawa & Davila, 1993). Further, we have found that acute PCP treatment-induced hyperlocomotion, ataxia, and motor incoordination, but not other behavioural changes are modified by L-NAME, suggesting the involvement of central NO production in the mediation of PCP-induced behaviours (Noda *et al.*, 1995). Thus, it is suggested that the mechanisms linked to NO production, without change in NADPH-d-containing neurones are involved in PCP-induced behavioural changes in mice, and it is possible that the repeated PCP treatment-induced behavioural changes in mice may be mediated, at least in part, via the NO pathway.

In the present behavioural study, concomitant treatment of a NO synthase inhibitor, L-NAME, with PCP in mice, blocked the development of tolerance to PCP-induced ataxia and motor incoordination and enhanced the development of sensitization to hyperlocomotion. It has been reported that NO synthase inhibitors, such as L-NAME and N^G nitro-L-arginine, at concentrations up to 100 μM, do not affect either [^{3}H]-MK-801 binding to PCP receptors or [^{3}H]-CGP 39653 binding to NMDA receptors, suggesting that the effects of L-NAME are due to the blockade of NO synthase (Itzhak, 1994). It is unlikely that L-NAME alters the disposition of PCP in the brain: NO synthase inhibitors selectively inhibit the NO synthase, but not cytochrome P-450 reductase (Dudek *et al.*, 1995), although PCP is mainly metabolized by cytochrome P-450 (Holsztynska & Domino, 1983). Further, we found that D-NAME, an inactive isomer, given at the same dose and in the same manner, as L-NAME, had no effect on the repeated effects of PCP, suggesting a stereospecific mechanism. Thus, we conclude that the effects of L-NAME on the development of tolerance and sensitization were a result of its long-term inhibition of NO synthase. Interestingly, in contrast to the enhancing effect of L-NAME on the development of sensitization to hyperlocomotion and its inhibiting effects on the development of tolerance to ataxia or motor incoordination in mice, acute treatment with the same agent, at doses up to 50 mg kg^{-1}, failed to affect the PCP-induced hyperlocomotion, ataxia, and motor incoordination in mice that had previously exhibited sensitization or tolerance to these phenomena. It is unlikely that the lack of effect of L-NAME on the tolerance and sensitization to PCP-induced behaviours is due to insufficient inhibition of NO synthase in the brain, since we confirmed that the remaining NO synthase activity in the brains of the mice used in this experiment was as low as that in the brains of mice used in the experiment of their development (see Table 2). It has been reported that the NO synthase inhibitors prevent the development of tolerance or sensitization to ethanol, morphine, cocaine and methamphetamine, suggesting that NO is involved in the development of tolerance and sensitization to these drugs (Kolesnikov *et al.*, 1992; Khanna *et al.*, 1993; Pudiak & Bozath, 1993; Rauhala *et al.*, 1994; Ohno & Watanabe, 1995). Further, the present phenomenon has been observed in learning and memory experiments, demonstrating that NO plays an important role in the acquisition, but not in the retention, of learning (Chapman *et al.*, 1992; Yamada *et al.*, 1994). The present results suggest that NO may play an important role in the development, but not in the maintenance, of tolerance and sensitization to PCP-induced behaviours.

The mechanisms by which NO modifies the tolerance or sensitization to PCP have yet to be elucidated. When the effects of NO gas on dopamine release in the rat striatum were investigated *in vivo* by use of microdialysis, in animals anaesthetized with urethane, dopamine concentrations were found to have decreased significantly, and such effects were inhibited by the coadministration of haemoglobin (Guevara-Guzman *et al.*, 1994). Thus, it is possible that NO synthase inhibitors could enhance the release of dopamine in the brain. We have found that the concentration of 3,4-dihydroxyphenylacetic acid in the rat brain was significantly increased in L-NAME (60 mg kg^{-1})-treated rats, compared with that in saline-treated rats, suggesting that the long-term inhibition of NO synthase may cause an increase in dopamine turnover in the rat brain (Yamada *et al.*, 1994). We have also reported that dopaminergic systems may be involved in PCP-induced sensitization, since repeated PCP treatment enhances dopamine turnover rates (Nabeshima *et al.*, 1987). Many previous reports have demonstrated that central dopaminergic systems are involved in hyperlocomotion in animals (e.g. Steinpreis & Salamone, 1993). Further, since acute PCP treatment inhibits NO synthase activity (see Table 1), it is possible that PCP and L-NAME release dopamine via the mechanisms linked to NO production. Taken together, we suggest that the enhancing effect of L-NAME on the sensitization to PCP-induced hyperlocomotion may be due to the long-term overstimulation of dopaminergic systems produced by coadministration of PCP and L-NAME, and that PCP releases dopamine via modulation of the NMDA/NO pathway.

The present study demonstrated that the coadministration of L-NAME and PCP blocked the development of tolerance to PCP-induced ataxia and motor incoordination. It should be noted that L-NAME itself did not produce motor incoordination and also that it did not enhance the PCP-induced motor-impairing effect. Further, these effects of L-NAME may not be related to its acute effects, since we have found that pretreatment with L-NAME failed to affect the PCP (10 mg kg^{-1})-induced behavioural deficits in mice (Noda *et al.*, 1995). Thus, inhibition of the development of tolerance to

PCP cannot be attributed to some direct effect of L-NAME itself on motor performance. Khanna *et al.* (1993) have reported that the administration of the NO synthase inhibitor N^G-nitro-L-arginine, blocks the development of rapid tolerance to the motor incoordination induced by ethanol. Further, it has been reported that a NO synthase inhibitor prevents the development of tolerance to morphine (Kolesnikov *et al.*, 1992). These findings demonstrate that NO is involved in the development of tolerance to these drugs. Taken together with these reports, our results suggest that NO is involved in the adaptive processes involved in the development of tolerance to PCP-induced behaviours. This hypothesis is supported by our biochemical data showing that acute PCP treatment inhibited NO synthase activity in the cerebral cortex and cerebellum in mice, and that such effects in both regions were attenuated by repeated PCP treatment, indicating the development of tolerance to the PCP-induced inhibition of NO synthase activity. Both these brain regions are associated with motor function (Gorecki *et al.*, 1991), and PCP/NMDA sites and NO synthase-containing neurones are distributed in the two regions (Sonders *et al.*, 1988; Bredt *et al.*, 1991; Dawson *et al.*, 1991; Vincent & Kimura, 1992). Thus, it is suggested that NO may be involved in the sequence of events associated with PCP-induced motor dysfunction, such as ataxia and motor incoordination.

L-NAME did not exert significant effects on the development of tolerance to PCP-induced sniffing and head movement; the reason for this lack of effect is unknown. One possible explanation may be that L-NAME may exert its effects via modulation of the NMDA/NO pathway. The activation of NMDA receptors has been shown to induce NO synthesis (Garthwaite *et al.*, 1988). PCP-induced motor incoordination and ataxia are indistinguishable from those induced by AP-5, a competitive NMDA receptor antagonist (Schmidt, 1986). Further, we have previously reported that sniffing and head movement induced by acute PCP treatment failed to be modified by the pretreatment with L-NAME (Noda *et al.*, 1995). Thus, it is suggested that the inhibition of NO synthase induced by L-NAME can attenuate the development of tolerance to PCP-induced ataxia and motor incoordination, but not that to PCP-induced sniffing and head movement. Other mechanisms involved in the development of tolerance to the PCP-induced sniffing and head movement remain to be elucidated.

In conclusion, the present results suggest that NO may play an important role in the development of tolerance and sensitization to the behavioural effects of PCP, but that this molecule does not play such a role in the maintenance of these phenomena. Further, the results also suggest that PCP may regulate endogenous NO production in the mouse brain.

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