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Short communication

The neuronal selective nitric oxide synthase inhibitor, N^{ω}-propyl-L-arginine, blocks the effects of phencyclidine on prepulse inhibition and locomotor activity in mice

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Abstract

Phencyclidine has frequently been used to model schizophrenia in animals. In the present study, the ability of the neuronal selective nitric oxide synthase (NOS) inhibitor, N^{ω}-propyl-L-arginine, to block the behavioural effects of phencyclidine in mice was investigated. N^{ω}-propyl-L-arginine (20 mg/kg) was found to block both phencyclidine (4 mg/kg)-induced disruption of prepulse inhibition and phencyclidine-induced stimulation of locomotor activity in the mice tested. It is concluded that the NOS-sensitive behavioural effects of phencyclidine in rodents is dependent on neuronal NOS and that NO may play a role in the psychotomimetic effects of phencyclidine.

Keywords: Prepulse inhibition; Phencyclidine; Locomotor activity; N^{ω}-Propyl-L-arginine; Nitric oxide; (Mouse)

1. Introduction

Due to its unique psychotomimetic properties in humans, phencyclidine has frequently been used to model various aspects of schizophrenia in animals. Thus, phencyclidine has been shown to alter several behavioural and biochemical measures in animals, some of which are sensitive to antipsychotic treatment (Jentsch and Roth, 1999). Recent studies also show that some of the behavioural and biochemical effects of phencyclidine can be blocked by the nitric oxide synthase (NOS) inhibitor, N^{ω}-nitro-L-arginine methyl ester, suggesting that NOS inhibition may be a useful pharmacological approach for the treatment of schizophrenia (Johansson et al., 1997, 1998; Klamer et al., 2001). N^{ω}-nitro-L-arginine methyl ester acts as a non-selective NOS inhibitor, however, affecting both endothelial NOS (eNOS) and neuronal NOS (nNOS) with about equal affinity. Thus, it does not only affect neuronal activity but also several other biological functions in the organism. Importantly, it affects cerebral blood flow, which may both invalidate its use as an antipsychotic agent (as may it also question the use of other eNOS inhibitors in this setting), but also explain some of the results obtained. Furthermore, eNOS has recently been identified in neurons (O'Dell et al., 1994), and eNOS may consequently be important also in neuronal transmission. Accordingly, long-term potentiation has been shown significantly reduced in mutant mice lacking both nNOS and eNOS but not in mice lacking only nNOS (Son et al., 1996). Recent development of L-arginine derivatives, which has provided new NOS inhibitors with increased selectivity for the nNOS isoenzyme, may help to elucidate this issue. In the present study, N^{ω}-propyl-L-arginine, a reversible NOS inhibitor with a 149-fold selectivity for nNOS over eNOS (Zhang et al., 1997), was used to further investigate the involvement of nNOS in the effects of phencyclidine on behaviour. To this end, the effect of N^{ω}-propyl-L-arginine on phencyclidine-induced disruption of prepulse inhibition of the acoustic startle response and phencyclidine-induced stimulation of locomotor activity in an open field was tested.

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2. Materials and methods

2.1. Animals

Male NMRI mice (Charles River, Sulzfeld, Germany), 30–40 g, were used. The mice arrived at the animal facilities one week prior to the start of the experiments. They were housed maximum 10 per cage (37×21×15 cm) in a colony room under constant temperature (20±1 °C) and humidity (55±5%). Food (B&K Feeds) and tap water were available ad libitum. The daylight cycle was maintained artificially (dark 1800–0600 h). Experiments were performed during the light hours and each mouse was used in one experiment only. The Ethics Committee for Animal Experiments, Göteborg, Sweden, approved the experimental procedures used in this study.

2.2. Drugs

Phencyclidine (1-(1-phenylcyclohexyl) piperidine HCl) (RBI, Natick, USA) and N^{ω}-propyl-L-arginine (Tocris, London, UK) were used. The drugs were dissolved in saline (0.9% NaCl) and administrated intraperitoneally (i.p.) in a volume of 10 ml/kg. The dose of phencyclidine (4 mg/kg) used to disrupt prepulse inhibition and to stimulate locomotor activity in the mice tested were chosen from previous findings (Bird et al., 2001; Klamer et al., 2001, 2004). The dose used of N^{ω}-propyl-L-arginine was based on unpublished data from the present laboratory suggesting it to be the highest dose not producing effects on these behaviours by itself.

2.3. Prepulse inhibition experiments

2.3.1. Apparatus

Acoustic startle was recorded by a MOPS 2b startle response recording system (Metod och Produkt, Svenska, Göteborg, Sweden). Each mouse was placed in a small wire-mesh cage (5.5×10×5.5 cm) made of stainless steel, which was suspended at one point at the top to a piston in such way that it could freely move under the piston. A sudden movement of the mouse inside the cage caused a displacement of the piston, the acceleration of which was converted to an analogue signal by a moving coil transducer. The signal was sampled and digitalised with a 12-bit digital resolution by a microcomputer, which also served to control the delivery of acoustic stimuli. Startle amplitude was defined as the maximum signal amplitude (digital units) that occurred during the first 40 ms after the delivery of the startle-eliciting stimulus. This time period was considered to cover the response latency in mice sufficiently, which was always shorter than 15 ms. The cage was housed in dimly lit and sound-attenuated enclosure (52×42×38 cm). Three cages were used simultaneously and a mouse tested in one cage was always tested in the same cage at subsequent tests. The acoustic signal consisted of white noise delivered to the mouse by two high frequency loudspeakers built into the ceiling of the enclosure. A continuous signal provided a white background noise level of 62 dB inside the enclosure. This signal was interrupted at stimulus presentation by a burst of white noise with a rise/decay time of less than 1 ms.

2.3.2. Testing procedure

The animals were first placed in the startle cages for a 10-min adaptation period. After this period, they were presented with a series of five startle pulse-alone trials followed by a series of five prepulse-alone trials. The pulse-alone trials served only to accommodate the animals to the sudden change in stimulus conditions and were omitted from the data analysis and the prepulse-alone trials were analysed only to ensure that these stimuli did not evoke any startle responses on their own. Thereafter, the animals were presented, three times repeatedly, with a series of five prepulse–pulse trials followed by a series of five pulse-alone trials, i.e., a total of 30 trials. The time between trials was always 10 s and the time between any series of trials was 70 s. Startle pulse intensity was set to 105 dB and prepulse intensity to 70 dB. The prepulse was 60 ms in duration and presented immediately before the startle pulse, which was 20 ms in duration. The startle pulse was set to 105 dB, since this intensity was found to evoke a robust startle amplitude that showed a minimum of habituation and at the same time did not cause a ceiling effect. Similarly, prepulse intensity was set to 70 dB (8 dB above background noise) to produce a robust prepulse inhibition.

2.3.3. Drug treatment

Testing started 1 week after the arrival of the animals from the breeder the first test being a pretest with no drug treatment. The mice (n=11) were then tested every third day using a semirandomised crossover design, each animal receiving all treatments of an experiment. N^{ω}-propyl-L-arginine (20 mg/kg) or saline was administrated 10 min prior to a second injection of either phencyclidine (4 mg/kg) or saline, which was administered 15 min prior to the presentation of the first startle stimulus.

2.3.4. Data analysis

The mean response amplitude for pulse-alone trials (P) was calculated for each rat and treatment condition. This measure was used in the statistical analysis to assess drug-induced changes in startle reactivity. The mean response amplitude for prepulse–pulse trials (PP) was also calculated and used to express the percent prepulse inhibition according to the following formula:

$$\text{Prepulse inhibition } (\%) = 100 - [(PP/P) \times 100]$$

Using this formula, a 0% value denotes no difference between pulse-alone and prepulse–pulse response amplitudes and consequently no prepulse inhibition. Statistical analysis was performed by one-way analysis of variance (ANOVA) with treatment as within-subjects factor followed

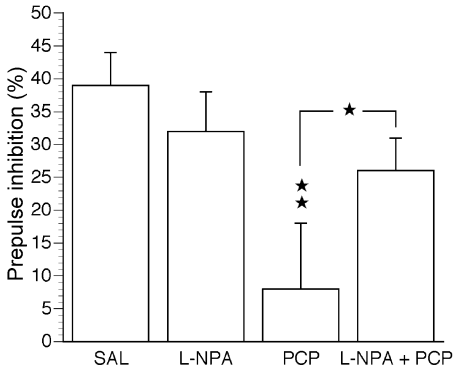

Fig. 1. The effect of N^{ω}-propyl-L-arginine (L-NPA, 20 mg/kg, i.p.) on phencyclidine-induced (PCP, 4 mg/kg, i.p.) disruption of prepulse inhibition in mice. The results are represented by the mean values±S.E.M. of 11 mice tested every third day receiving all treatments of the experiment in a semirandomised order. ★P<0.05, ★★P<0.01 compared to saline (SAL) treatment unless otherwise indicated (statistically significant ANOVA followed by Fisher's PLSD for comparisons between treatments).

by Fisher's protected least significant difference test (PLSD) for difference between treatments. Two-tailed levels of significance were used and P<0.05 was considered statistically significant.

2.4. Locomotor activity experiments

2.4.1. Apparatus

Locomotor activity was recorded by means of eight box-shaped Plexiglas® activity measuring devices with a floor area of 40×40 cm (Kungsbacka mät-och reglerteknik, Fjärås, Sweden). The activity boxes were housed in ventilated, dimly lit and sound-attenuated cabinets (100×100×45 cm). Five times five rows of photocell beams at the floor level enabled a computer-based system to determine the horizontal location of the animal at any time.

2.4.2. Testing procedure

After an initial 50-min adaptation period in the activity boxes, the mice were removed and treated with either saline+saline (n=11), saline+phencyclidine (n=10), N^{ω}-propyl-L-arginine+saline (n=5), or N^{ω}-propyl-L-arginine+-phencyclidine (n=6). Immediately after their removal, N^{ω}-propyl-L-arginine (20 mg/kg) or saline were administrated to the animals and 10 min thereafter a second injection of either saline or phencyclidine (4 mg/kg). After the drug treatments, the mice were put back into the activity boxes and locomotor activity was recorded for three consecutive 20-min time periods.

2.4.3. Data analysis

The locomotor activity data achieved were analysed by a two-way ANOVA with treatment as between-subjects factor and time period as within-subjects factor followed by Fisher's PLSD test for difference between treatments. P<0.05 was considered statistically significant.

3. Results

3.1. Effect of N^{ω}-propyl-L-arginine on phencyclidine-induced disruption of prepulse inhibition

Statistical analysis showed a significant effect of treatment on prepulse inhibition in the mice tested (ANOVA, effect of treatment: F(3,30)=4.37, P=0.01, Fig. 1). Phencyclidine caused a significant decrease in prepulse inhibition (P<0.01, Fisher's PLSD test) while N^{ω}-propyl-L-arginine had no effect on prepulse inhibition compared to saline treatment. Pretreatment with the selective nNOS inhibitor, N^{ω}-propyl-L-arginine, markedly reduced the phencyclidine-induced disruption of prepulse inhibition. The effect of phencyclidine treatment in combination with N^{ω}-propyl-L-arginine was significantly different from that of phencyclidine alone (P<0.05, Fisher's PLSD test), and did not significantly differ from that of saline treatment.

Startle reactivity (pulse-alone response), on the other hand, was not significantly affected by N^{ω}-propyl-L-arginine and/or phencyclidine treatment (ANOVA, effect of treatment: F(3,30)=1.12, P=0.36, Table 1).

3.2. Effect of N^{ω}-propyl-L-arginine on phencyclidine-induced stimulation of locomotor activity

Statistical analysis of the first 20-min time period showed a significant effect of treatment on locomotor activity (ANOVA, effect of treatment: F(3,28)=9.02, P<0.001, Fig. 2). Phencyclidine caused a significant increase in locomotor activity (P<0.001, Fisher's PLSD test), whereas that of N^{ω}-propyl-L-arginine did not significantly differ from that of saline treatment. Furthermore, pretreatment with N^{ω}-propyl-L-arginine significantly reduced the phencyclidine-induced stimulation of locomotor activity. Thus, the effect of phencyclidine treatment in combination with N^{ω}-propyl-L-arginine was significantly decreased compared to that of phencyclidine alone (P<0.01, Fisher's PLSD test), and did not significantly differ from that of saline treatment. Statistical analysis of the second and third 20-min time periods also showed significant effects of treatment, but at these time periods no difference in locomotor activity

Table 1
The effect of N^{ω}-propyl-L-arginine (L-NPA, 20 mg/kg, i.p.) and phencyclidine (PCP, 4 mg/kg, i.p.) on acoustic startle response amplitude (pulse-alone response) in mice

Treatment	Acoustic startle response
Saline	341±43
L-NPA	345±41
PCP	306±43
L-NPA+PCP	324±47

The results are represented by means (digital units)±S.E.M. of 11 mice tested every third day receiving all treatments of the experiment in a semirandomised order. No statistically significant differences between treatments were obtained.

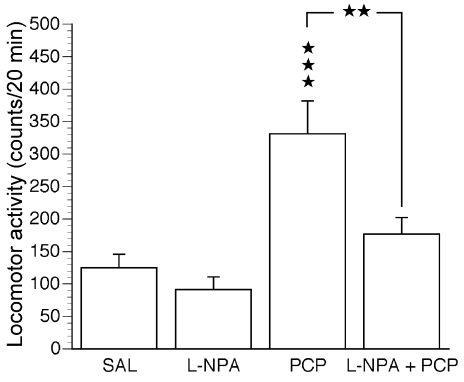

Fig. 2. The effect of N^{ω}-propyl-L-arginine (L-NPA, 20 mg/kg, i.p.) on phencyclidine-induced (PCP, 4 mg/kg, i.p.) stimulation of locomotor activity in mice. The results are represented by the mean values±S.E.M. of mice treated with either saline (n=11), phencyclidine (n=10), N^{ω}-propyl-L-arginine (n=5), or N^{ω}-propyl-L-arginine+phencyclidine (n=6). ★★P<0.01, ★★★P<0.001 compared to saline (SAL) treatment unless otherwise indicated (statistically significant ANOVA followed by Fisher's PLSD for comparisons between treatments).

stimulation was obtained between phencyclidine-treated and N^{ω}-propyl-L-arginine+phencyclidine-treated mice (data not shown).

4. Discussion

In the present study, N^{ω}-propyl-L-arginine was shown to block both phencyclidine-induced disruption of prepulse inhibition and phencyclidine-induced stimulation of locomotor activity. N^{ω}-propyl-L-arginine exerted these effects at a dose which by itself did not significantly alter either prepulse inhibition or locomotor activity. These observations corroborate previous studies suggesting the involvement of nNOS in the behavioural effects of phencyclidine (Johansson et al., 1999; Klamer et al., 2001; Wiley, 1998), and also suggest that the results obtained with the non-selective agent, N^{ω}-nitro-L-arginine methyl ester, may depend on its inhibitory action on nNOS.

N^{ω}-propyl-L-arginine was able to block the effect of phencyclidine on prepulse inhibition in the mice tested at a dose comparable to that of N^{ω}-nitro-L-arginine methyl ester in a previous study, see Klamer et al. (2001). This common property is also paralleled by a comparable potency in inhibiting nNOS. An IC_{50}=57 nM has been shown for N^{ω}-propyl-L-arginine (Zhang et al., 1997) compared to 22 nM for N^{ω}-nitro-L-arginine, the probable active metabolite of N^{ω}-nitro-L-arginine methyl ester, with a 50-fold higher potency compared to the native compound (Cooper et al., 2000; Pfeiffer et al., 1996). A selectivity of N^{ω}-propyl-L-arginine for nNOS has also been confirmed in vivo (Wang et al., 2004). Thus, the ability of N^{ω}-propyl-L-arginine and N^{ω}-nitro-L-arginine methyl ester to block the behavioural effects of phencyclidine corresponds to their ability to inhibit nNOS, which further suggests this isoenzyme as the primary site of action.

The present results also argue against the possibility that the NOS-sensitive effects of phencyclidine are dependent on vascular effects. N^{ω}-propyl-L-arginine and N^{ω}-nitro-L-arginine methyl ester have both been shown to change blood pressure but in opposite directions. N^{ω}-propyl-L-arginine was recently shown to cause hypotension with no effect on blood flow in renal tissue, whereas N^{ω}-nitro-L-arginine methyl ester caused hypertension and a decrease in renal blood flow (Kakoki et al., 2001). Thus, it is unlikely that a common effect of N^{ω}-propyl-L-arginine and N^{ω}-nitro-L-arginine methyl ester on mechanisms related to blood flow explains their ability to block the behavioural effects of phencyclidine.

The effects of N^{ω}-propyl-L-arginine obtained in the present study were short in duration, however. N^{ω}-propyl-L-arginine effectively blocked the stimulatory effect of phencyclidine on locomotor activity only during the first 20-min recording period. This short duration may be explained by its reversible action as a NOS inhibitor (Cooper et al., 2000). A reversible NOS inhibitor should affect nitric oxide formation only as long as its plasma concentration is sufficiently high to maintain inhibition. N^{ω}-nitro-L-arginine methyl ester, on the other hand, seems to exert longer inhibitory action (Johansson et al., 1997) possibly due to the fact that its active metabolite, N^{ω}-nitro-L-arginine, acts as an irreversible NOS inhibitor (Dwyer et al., 1991).

The present study further suggests that nNOS may be involved in the psychotomimetic effects of phencyclidine and that this isoenzyme could possibly be a target for the development of new antipsychotic treatments.

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