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Short communication

Nitric oxide synthase inhibitors do not substitute in rats trained to discriminate phencyclidine from saline

Jenny L. Wiley *, Sarah A. Harvey, Robert L. Balster

Department of Pharmacology and Toxicology, Medical College of Virginia, Virginia Commonwealth University, P.O. Box 980613, Richmond, VA 23298-0613, USA

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Abstract

Release of nitric oxide occurs as a consequence of glutamate stimulation of NMDA receptors and is dependent upon calcium-calmodulin activation of the enzyme nitric oxide synthase. Since nitric oxide may serve as an intracellular messenger for NMDA glutamatergic neurons, it could be hypothesized that blockade of its synthesis may produce pharmacological effects similar to those of NMDA receptor antagonists. The purpose of the present study was to compare the effects of nitric oxide synthase inhibitors to those of the high affinity NMDA open channel blocker phencyclidine in drug discrimination, a pharmacologically selective procedure in which phencyclidine produces distinctive effects. Rats were trained to discriminate 2 mg/kg phencyclidine from saline in a standard two-lever discrimination task with food reward. Whereas phencyclidine dose-dependently substituted for itself, 7-nitroindazole, L-NAME (N^G-nitro-L-arginine methyl ester), and L-NOARG (N^G-nitro-L-arginine) failed to substitute for phencyclidine when administered intraperitoneally. L-NAME and 7-nitroindazole were tested up to doses that disrupted responding, providing evidence that a behaviorally-relevant dosage range was evaluated. Although these results conflict with those of a previous study which found that nitric oxide synthase inhibitors substituted for phencyclidine and produced phencyclidine-like catalepsy in pigeons, they are consistent with research showing that these drugs did not produce phencyclidine-like pharmacological effects in behavioral procedures in rats.

Keywords: Nitric oxide (NO); Discrimination; NMDA receptor; Phencyclidine; (Rat)

1. Introduction

Nitric oxide is a soluble gas that is synthesized in the brain and periphery from the amino acid L-arginine by the enzyme nitric oxide synthase. In the brain, nitric oxide may serve as an intracellular messenger that mediates the effects of the endogenous excitatory amino acid ligand glutamate at a subset of *N*-methyl-D-aspartate (NMDA) neurons that are co-localized with nitric oxide synthase (Garthwaite, 1991). In these neurons, activation of the NMDA receptor by the endogenous excitatory amino acid neurotransmitter glutamate produces an influx of Ca^{2+} which binds to calmodulin. This Ca^{2+}-calmodulin complex activates nitric oxide synthase directly, resulting in a brief 'puff' of nitric oxide that diffuses out of the presynaptic terminal and into astrocytic processes to activate soluble guanylate cyclase (Garthwaite, 1991). In addition, nitric oxide release may result in feedback inhibition of the presynaptic glutamate neuron via action at a modulatory redox site associated with NMDA receptors (Manzoni et al., 1992). Hence, the actions of nitric oxide synthase inhibitors may depend upon their effect upon the balance between excitatory and inhibitory influences in the NMDA-associated nitric oxide system.

Previous animal studies with systemic administration of nitric oxide synthase inhibitors have reported both similarities and differences in the pharmacological profile of these compounds compared to that of NMDA receptor antagonists (Stewart et al., 1994; Nagafusi et al., 1995; Volke et al., 1997; Wiley et al., 1997). Although drug discrimination is more pharmacologically selective than many other behavioral tasks, nitric oxide synthase inhibitors have not been tested extensively in this procedure. There are two exceptions: Jewett et al. (1996) reported that nitric oxide synthase inhibitors substituted for phencyclidine in pigeons trained to discriminate phencyclidine from saline. Green et al. (1997) found that, unlike phencyclidine and other

* Corresponding author. Tel.: +1-804-828-2067; Fax: +1-804-828-2117; E-mail: jwiley@hsc.vcu.edu

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NMDA receptor antagonists (Grant and Colombo, 1992; Sanger, 1993; Shelton and Balster, 1994), the nitric oxide synthase inhibitor L-NAME (N^G-nitro-L-arginine methyl ester) did not substitute for ethanol in rats. Differences in the discriminative stimulus effects of site-selective NMDA receptor antagonists in pigeons vs. rats have been reported previously. Phencyclidine-trained pigeons more readily generalize to NMDA receptor antagonists regardless of their selectivity for other sites within the NMDA receptor complex whereas phencyclidine-trained rats show a greater degree of selectivity for high affinity open channel blockers at this receptor complex (Baron and Woods, 1995; Wiley, 1997). The purpose of the present study was to evaluate the effects of nitric oxide synthase inhibitors in rats trained to discriminate phencyclidine from saline. Test drugs included L-NAME and L-NOARG (N^G-nitro-L-arginine) [nonselective nitric oxide synthase inhibitors] and 7-nitroindazole (a nitric oxide synthase inhibitor that selectively inhibits neuronal nitric oxide synthase) (Moore et al., 1993).

2. Materials and methods

2.1. Subjects

Adult, male Sprague–Dawley rats (310–415 g), obtained from Harlan (Dublin, VA), were individually housed in a temperature-controlled (20–22°C) environment with a 12-h light–dark cycle (lights on at 0700). Rats were maintained within the indicated weight range by restricted post-session feeding and had ad libitum water in their home cages. Rats were drug-naive at the beginning of the study.

2.2. Apparatus

Rats were trained and tested in standard operant conditioning chambers (BRS/LVE, Laurel, MD or Lafayette Instruments, Lafayette, IN) housed in sound-attenuated cubicles. Pellet dispensers delivered 45-mg BIO SERV (Frenchtown, NJ) food pellets to a food cup on the front wall of the chamber between two response levers. Fan motors provided ventilation and masking noise for each chamber. House lights located above the food cup were illuminated during training and testing sessions. A microcomputer with Logic '1' interface (MED Associates, Georgia, VT) and MED-PC software (MED Associates) was used to control schedule contingencies and to record data.

2.3. Drugs

L-NAME HCl (Research Biochemicals International, Natick, MA), L-NOARG (RBI), and phencyclidine (National Institute on Drug Abuse, Rockville, MD) were mixed in sterile water or physiological saline. 7-Nitroindazole (RBI) was dissolved in dimethylsulfoxide (DMSO). L-NAME and 7-nitroindazole were administered i.p. 30 min before the start of the session. Phencyclidine and L-NOARG were injected i.p. 15 min before the start of the session. Phencyclidine, L-NAME, saline, and lower doses of L-NOARG were administered at a volume of 1 ml/kg. Due to solubility problems, the 30 and 100 mg/kg doses of L-NOARG were given as adjusted volumes of a 15 mg/ml solution. 7-Nitroindazole and DMSO were administered at a volume of 0.5 ml/kg.

2.4. Procedure

Rats were trained to press one lever following administration of 2 mg/kg PCP and to press another lever after injection with saline, each according to a fixed-ratio 10 schedule of food reinforcement. Completion of 10 consecutive responses on the injection-appropriate lever resulted in delivery of a food reinforcer. Each response on the incorrect lever reset the ratio requirement on the correct lever. The daily injections for each rat were administered in a double alternation sequence of 2 mg/kg PCP and saline. Rats were injected and returned to their home cages until the start of the experimental session 15 min later. Training occurred during sessions conducted five days a week (Monday–Friday) until the rats had met three criteria during eight of ten consecutive sessions: (1) first completed fixed ratio 10 on the correct lever; (2) percentage of correct-lever responding $\geq 80\%$; and (3) response rate ≥ 0.4 responses/s.

Following successful acquisition of the discrimination, stimulus substitution tests with test compounds were conducted on Tuesdays and Fridays during 15-min test sessions. Training continued on Mondays, Wednesdays, and Thursdays. During test sessions, responses on either lever delivered reinforcement according to a fixed ratio 10 schedule. In order to be tested, rats must have completed the first FR and made at least 80% of all responses on the injection-appropriate lever on the preceding day's training session. In addition, the rat must have met these same criteria during at least one of the training sessions with the alternate training compound (PCP or saline) earlier in the week.

A PCP dose–effect determination was performed first in each rat. Then, rats were tested with some or all of the nitric oxide inhibitors, L-NOARG, L-NAME, and 7-nitroindazole. Doses of each compound were administered in ascending order. Throughout the study, control tests with saline and 2 mg/kg PCP were conducted before each dose–effect curve determination.

2.5. Data analysis

For each test session, percentage of responses on the drug lever and response rate (responses/s) were calculated. When appropriate, ED_{50}'s were calculated separately

for each drug using least-squares linear regression on the linear part of the dose–effect curves (Tallarida and Murray, 1987) for percentage of drug-lever responding, plotted against $\log_{10}$ transformation of the dose. Since rats that responded less than 10 times during a test session did not press either lever a sufficient number of times to earn a reinforcer, their lever selection data only were excluded from data analysis.

3. Results

Phencyclidine dose-dependently substituted for itself ($ED_{50} = 1.0$ mg/kg). In contrast, all of the nitric oxide synthase inhibitors tested produced responding almost exclusively on the saline-associated lever (Fig. 1, top panel). Behaviorally active doses of at least two of the drugs were tested, as indicated by response rate decreases at higher doses of L-NAME and 7-nitroindazole (Fig. 1, bottom panel). In addition, two rats died several hours after receiving 300 mg/kg L-NAME, suggesting significant toxicity at higher doses. L-NOARG had more modest, if any, effects on response rates. Throughout the study, rats responded predominantly (> 80%) on the injection appropriate lever during control tests with saline and phencyclidine.

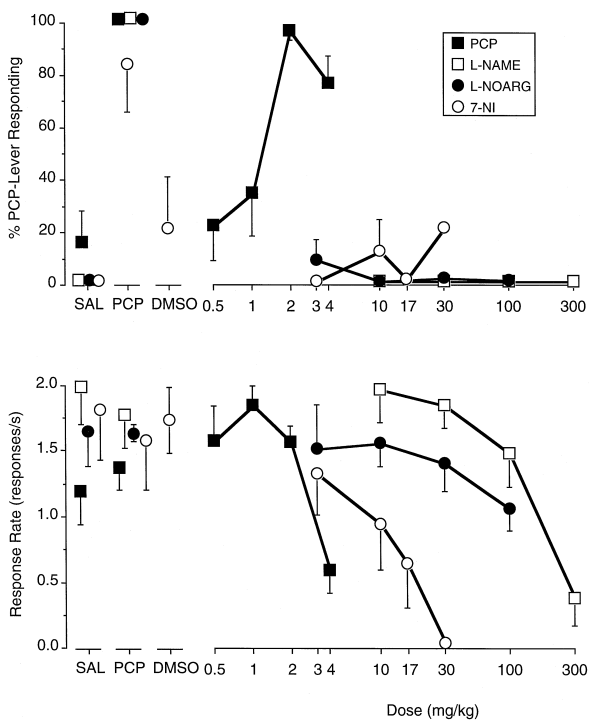

Fig. 1. Effects of phencyclidine and the nitric oxide synthase inhibitors, L-NAME, L-NOARG and 7-nitroindazole, on percentage of phencyclidine-lever responding (top panel) and response rates (bottom panel) in rats trained to discriminate 2 mg/kg phencyclidine from saline. Points above VEH and PCP represent the results of control tests with vehicle and 2 mg/kg PCP conducted before each dose–effect curve determination. A control test was also performed with DMSO prior to beginning tests with 7-nitroindazole. For all dose–effect curve determinations, each value represents the mean (+S.E.M.) of four to eight rats, except for percentage of PCP-lever responding for the 300 mg/kg dose of L-NAME ($n = 2$) and the 17 and 30 mg/kg doses of 7-nitroindazole ($n = 3$ and 1, respectively). In addition, only three rats were tested with saline prior to testing with 7-nitroindazole.

4. Discussion

Jewett et al. (1996) reported that nitric oxide synthase inhibitors produced phencyclidine-like discriminative stimulus effects in pigeons trained to discriminate 1 mg/kg phencyclidine from saline. 7-Nitroindazole fully substituted at a single dose (17.8 mg/kg, i.m.) and systemic injections of L-NAME produced slightly less than full substitution at doses of 560 mg/kg and higher. In addition, these drugs induced catalepsy, an effect that is also produced by phencyclidine in pigeons. In contrast, the results of the present study show that neither of these drugs had phencyclidine-like discriminative stimulus effects in rats, although they were tested up to doses that severely decreased response rates. A third nitric oxide synthase inhibitor, L-NOARG, also failed to have phencyclidine-like effects.

A number of differences between the Jewett et al. study and the present one are evident and may account for the divergent results. In addition to species differences, some crucial differences in experimental parameters between these studies include training dose (1 vs. 2 mg/kg), route of administration (intramuscular vs. intraperitoneal), and pre-session injection interval (variable range from 30 min to 5 h vs. a single set time point for each drug). Previous research has shown that the pharmacological effects of nitric oxide synthase inhibitors are sensitive to these types of alterations. For example, nitric oxide synthase inhibitors produce anticonvulsant or proconvulsant effects in the same seizure model in rats dependent upon dose and time of administration (Rundfeldt et al., 1995). As training dose and pre-session injection interval have been shown to affect the degree to which other drugs produce phencyclidine-like discriminative stimulus effects (Mansbach and Balster, 1991; Baron and Woods, 1995), the possible influence of these variables on the different degrees of substitution of nitric oxide synthase inhibitors for phencyclidine in pigeons and rats should not be neglected. On the other hand, the results of the only other published discrimination study in which a nitric oxide synthase inhibitor was tested in rats suggest that variation in these experimental parameters may not entirely account for the failure of nitric oxide synthase inhibitors to substitute for phencyclidine in rats. In this study, injection interval, route of administration and training dose were manipulated; however, substantial substitution of L-NAME for ethanol under conditions where phencyclidine and the competitive NMDA receptor antagonist, D-CPPene [D-3-(2-carboxypiperazine-4-yl)-1-propenyl-1-phosphonic acid; SDZ EAA

494], produced ethanol-like effects was not achieved (Grant and Colombo, 1992; Green et al., 1997). Nonetheless, this ethanol discrimination study provides only indirect evidence that the pharmacological profile of nitric oxide synthase inhibitors may resemble that of phencyclidine less in rats than in pigeons.

The results of other studies provide more direct evidence that the in vivo effects of nitric oxide synthase inhibitors differ from those of phencyclidine in rodents. Unlike phencyclidine, nitric oxide synthase inhibitors do not disrupt prepulse inhibition of the acoustic startle response in rats nor do they induce popping behavior in mice (Deutsch et al., 1996; Wiley et al., 1997). In fact, attenuation of phencyclidine's effects on these behaviors has been observed following administration of nitric oxide synthase inhibitors (Deutsch et al., 1996; Johansson et al., 1997; Wiley, 1998). The results of the present drug discrimination study are consistent with the failure of nitric oxide synthase inhibitors to produce phencyclidine-like effects in these other rodent paradigms. The exact reasons for the discrepancy between results in rodents and those in pigeons are still unclear.

Although nitric oxide synthase inhibitors lack specific phencyclidine-like effects in rats, they produce pharmacological effects that phencyclidine shares with NMDA receptor antagonists that block other sites within the NMDA receptor complex. Similar to various competitive and glycine-site NMDA receptor antagonists, nitric oxide synthase inhibitors have been shown to produce anxiolytic effects (Volke et al., 1997), anticonvulsant effects (De Sarro et al., 1991), and neuroprotective effects (Nagafusi et al., 1995; Schulz et al., 1995), to impair learning and/or memory (Chapman et al., 1992; Yamada et al., 1995), and to prevent the development of tolerance or sensitization to certain drugs of abuse (Kolesnikov et al., 1992). In the present study, the effects of nitric oxide synthase inhibitors most resembled those of NMDA receptor antagonists acting at non-channel sites, in that none of these classes of drug fully substitutes for phencyclidine in rats (present study; see Wiley, 1997, for a review). Yet, failure to substitute for phencyclidine obviously occurs with drugs that do not affect glutamate neurotransmission. Thus, more research would need to be done to support a conclusion that the pharmacological effects of nitric oxide synthase inhibitors are mediated via alteration of NMDA neurotransmission. In conclusion, although nitric oxide synthase inhibitors and NMDA receptor antagonists have similar pharmacological effects in rats, nitric oxide synthase inhibitors do not appear to have phencyclidine-like discriminative stimulus effects. If this class of drugs proves to have therapeutic value and are developed for clinical use, the results of this and other studies in rodents suggest that they would lack the troublesome behavioral and psychological side effects associated with high affinity NMDA channel blockers, although some contradictory results in pigeons have been reported.

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