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Nitric Oxide Synthesis Inhibition Does Not Affect Brain Stimulation Reward

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BOZARTH, M. A., C. M. PUDIAK AND M. MORRIS. *Nitric oxide synthesis inhibition does not affect brain stimulation reward.* PHARMACOL BIOCHEM BEHAV **48**(2) 487–490, 1994.—The effect of nitric oxide synthesis inhibition on brain stimulation reward was examined. A wide range of doses of the nitric oxide synthase inhibitor *N*ω-nitro-L-arginine methyl ester (L-NAME; 30–300 mg/kg IP) failed to affect frequency thresholds for brain stimulation reward. The effect of L-NAME on cocaine's facilitation of brain stimulation reward was also examined. Nitric oxide synthesis inhibition had no effect on cocaine's ability to lower reward thresholds for electrical brain stimulation. Despite reports that nitric oxide may modulate dopamine release, these data suggest that nitric oxide is not involved in the dopamine-dependent rewarding effect of electrical brain stimulation or the reward facilitation produced by cocaine's enhancement of dopaminergic activity. Because L-NAME potently decreases cerebral blood flow, its lack of effect on cocaine-enhanced brain stimulation reward has additional significance. The failure of a moderate dose of L-NAME (30 mg/kg IP) to attenuate cocaine's lowering of reward thresholds argues against pharmacokinetic explanations of L-NAME's effect on other cocaine-induced behaviors.

Nitric oxide Brain stimulation Reward L-NAME Cocaine

BRAIN stimulation reward has a well-established dependence on brain dopamine systems. Manipulations that block dopaminergic function attenuate brain stimulation reward, while manipulations that enhance brain dopamine facilitate reward (3,14). In addition to nitric oxide's proposed role as a retrograde neurotransmitter (4,6,13), it has been reported to modulate dopamine release from striatal slices (5,15). If nitric oxide is involved in regulating dopaminergic neurotransmission, then experimental manipulations affecting nitric oxide should produce corresponding changes in brain stimulation reward.

The following experiments explored the possible involvement of nitric oxide in brain stimulation reward in two ways. First, the effect of blocking nitric oxide synthesis was examined in animals lever-pressing for lateral hypothalamic brain stimulation reward. This experiment assessed the role of nitric oxide in the electrically activated dopamine system. Second, the effect of blocking nitric oxide synthesis on cocaine's facilitation of brain stimulation reward was examined. This assessed the possible role of nitric oxide in drug-augmented dopamine function during electrical brain stimulation. The nitric oxide synthase inhibitor *N*ω-nitro-L-arginine methyl ester (L-NAME) was selected for both studies. L-NAME inhibits nitric oxide formation by competing with the precursor L-arginine for nitric oxide synthase. In addition to possible effects on dopamine release and on cellular neuroadaptation, L-NAME has potent vasopressor effects decreasing cerebral blood flow (9,11). A potential problem with studies using L-NAME to assess the involvement of nitric oxide in various drug-induced effects is that L-NAME might alter the pharmacokinetics of drug action through its cardiovascular effects. This role has received little attention from those studying the behavioral effects of nitric oxide.

METHOD

Subjects

Male Long-Evans rats (Harlan Spraque-Dawley, Indianapolis) weighing 275–350 g at the beginning of the experiment were individually housed with food and water freely available in their home cages. The animal colony was maintained at a constant temperature (23 ± 2°C) and humidity (50 ± 5% relative humidity) with a 14-h light/10-h dark illumination cycle. All behavioral testing was conducted during the light phase of the light/dark cycle.

Rats were anesthetized using sodium pentobarbital (60 mg/kg IP, with 0.4 mg/kg IP atropine sulfate and 100 000 units Penicillin G procaine IM) and stereotaxically implanted with monopolar stimulation electrodes. With the upper incisor bar

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2.4 mm below the interaural line, the stereotaxic coordinates were posterior 3.3 mm from bregma, ±1.5 mm from the midsagittal suture, and 8.4 mm below dura. The stainless steel electrodes were 0.25 mm in diameter and insulated with Formvar, except at the cross-section of the tip. Two to three stainless steel screws mounted in the skull served as the stimulation ground.

After the completion of all behavioral testing, animals were deeply anesthetized with sodium pentobarbital (≈100 mg/kg IP) and transcardially perfused with normal saline followed by 10% phosphate-buffered formalin. The brains were then removed and stored in 10% phosphate-buffered formalin for a minimum of three days before sectioning. Coronal sections (40 μm) were taken using a freezing microtome (−22°C) and mounted on gelatin-coated glass slides. Brain sections were stained with cresyl violet, and electrode placements were histologically verified (8). All electrodes were found to be around the lateral hypothalamic level of the medial forebrain bundle.

Apparatus

Subjects were tested in a 26 × 47-cm operant chamber 38 cm in height with a lever mounted 8 cm above the floor. Stimulation leads were connected to an electrical commutator to permit unrestricted movement of the subjects during testing. Each operant chamber was housed in a ventilated, sound-attenuating chamber with dim illumination.

Each lever press produced a 300-ms train of monophasic cathodal stimulation pulses (300-μ pulse width). The electrode was shunted to ground between stimulation pulses to prevent tissue damage from capacitance buildup (7). All stimulation parameters except frequency were held constant throughout the experiment. Stimulation intensities were individually selected for each rat during initial screening for brain stimulation reward.

Drugs

L-NAME hydrochloride (Sigma Chemical Co., St. Louis) was prepared freshly in sterile physiological saline. Cocaine hydrochloride (Mallinckrodt, St. Louis) was dissolved in physiological saline and sterilized by filtration. All drugs were injected IP, and all drug dosages refer to the drug salts.

Procedure

After recovering from surgery for a minimum of seven days, rats were screened for brain stimulation reward using a 126-Hz frequency at various current intensities (100–400 μA). Rats showing vigorous responding were then tested using a threshold tracking procedure (1). Briefly, the minimum stimulation frequency necessary to maintain lever-pressing was determined throughout 1-h test sessions. The subject was first presented with a series of stimulation frequencies that decreased by 0.1 log units every minute until responding fell below criterion (≥30 presses/min). Each minute the responding fell below criterion (<30 presses/min), the stimulation frequency increased by 0.1 log units until responding met criterion. Alternating descending and ascending stimulation series were presented throughout the test session. The minimum stimulation frequency that maintained the criterion response rate was defined as threshold.

Animals were tested using the threshold tracking procedure until each subject's daily threshold measure varied less than 10% from its running five-day mean (range two to three weeks). After responding for brain stimulation reward had stabilized, rats (n = 14) received L-NAME injections (30–300 mg/kg IP) in a counterbalanced order immediately before testing. Each subject received all doses of L-NAME with a minimum of 72 h between successive drug doses. Frequency thresholds were determined daily during 1-h test sessions throughout the experiment.

A second group of rats (n = 10) was used to determine the effect of L-NAME on cocaine's facilitation of brain stimulation reward. After frequency thresholds had stabilized, these subjects received saline (1 ml/kg IP), cocaine (10 mg/kg IP), and L-NAME (30 mg/kg IP) plus cocaine (10 mg/kg IP) in a counterbalanced order. Saline and cocaine injections were given immediately before testing, and L-NAME was administered 30 min prior to the cocaine injection. All subjects were tested under each treatment condition with a minimum of 72 h between drug tests. Frequency thresholds were measured daily using 1-h test sessions.

RESULTS

The effect of each treatment was compared to the two- to three-day baseline period prior to each injection. Stimulation thresholds are expressed as a percentage of these baseline thresholds computed for each subject. An analysis of variance (ANOVA) was used to determine if these percentage measures differed across treatments. Response rates were measured at threshold stimulation frequencies, and an ANOVA was used to determine if significant changes in lever-pressing rates occurred across treatments.

Figure 1 summarizes the effect of L-NAME on brain stimulation reward. Nitric oxide synthesis inhibition failed to affect thresholds for brain stimulation reward, $F(3, 39) = 1.80$, $p > .10$. Similarly, there was no decrease in response rates (measured at threshold) even at the highest dose of L-NAME tested, $F(3, 39) = 2.14$, $p > .10$. Decreases in response rates would be indicative of gross behavioral toxicity, and it is interesting that the strong hypertensive effect of L-NAME did not slow lever-pressing rates for brain stimulation reward.

Figure 2 shows that cocaine injections produced a marked decrease in thresholds for rewarding brain stimulation, $F(2, 18) = 34.54$, $p < .001$. Pretreatment with L-NAME did not

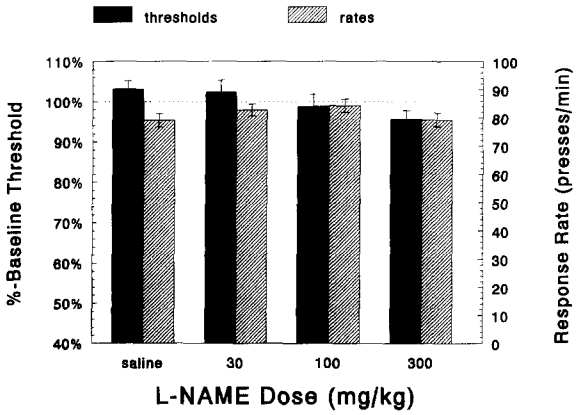

FIG. 1. Effect of L-NAME on frequency thresholds and response rates for brain stimulation reward. The figure shows the mean ± SEM percent of baseline frequency thresholds and the mean ± SEM lever-presses/min. Nitric oxide synthesis inhibition was ineffective in modifying stimulation thresholds and failed to affect response rates at threshold frequencies.

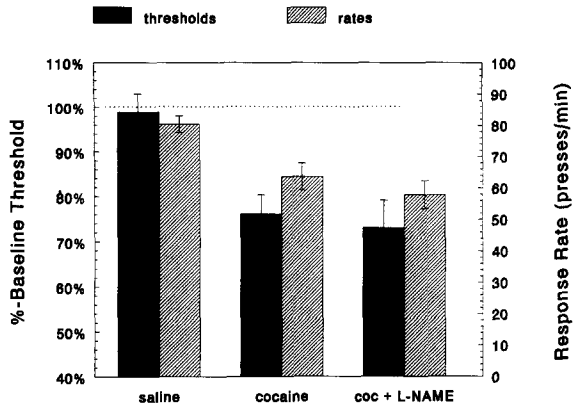

FIG. 2. Effect of L-NAME pretreatment on cocaine's facilitation of brain stimulation reward. The figure shows the mean ± SEM percent of baseline frequency thresholds and the mean ± SEM lever-presses/min. Cocaine effectively lowered stimulation thresholds, while nitric oxide synthesis inhibition failed to modify cocaine's effect. Note that the decreased response rates during the cocaine treatment are obtained at the lower stimulation-frequency thresholds.

affect cocaine's overall lowering of reward thresholds during the 60-min test session. This finding indicates that L-NAME does not significantly inhibit total cocaine distribution to brain sites involved in modifying brain stimulation reward. However, an examination of threshold changes within the 60-min session revealed that L-NAME pretreatment produced a slight alteration in the time course of cocaine's threshold-lowering effect as shown in Fig. 3: treatment effect, $F(2, 18) = 32.04$, $p < .001$, and Treatment × Time interaction, $F(6, 54) = 4.48$, $p < .005$. Pretreatment with L-NAME delayed the time to cocaine's peak effect, Tukey's (a) test for unconfounded means, $p < .05$, and also slightly prolonged cocaine's threshold-lowering effect. The entire time course of cocaine's effect on brain stimulation appeared shifted 15 min to the right by L-NAME pretreatment.

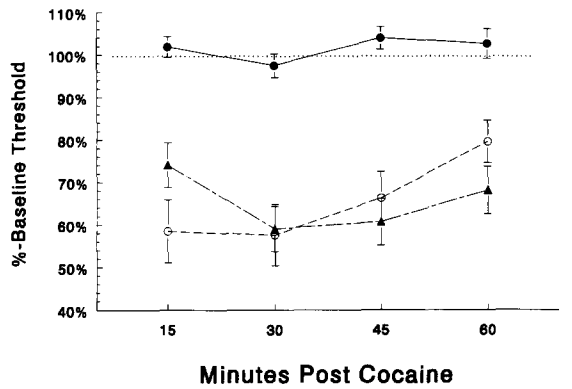

FIG. 3. Time course of cocaine's facilitation of brain stimulation reward thresholds with and without L-NAME pretreatment. The figure shows the mean ± SEM percent of baseline frequency thresholds. Nitric oxide synthesis inhibition caused a slight shift in the time course of cocaine's effect on brain stimulation reward without altering the overall threshold-lowering effect measured during the 60-min period (cf. Fig. 2). ●, saline; ○, cocaine only; ▲, cocaine pretreated with L-NAME.

Figure 2 also shows decreased lever-pressing rates at threshold frequencies following cocaine treatment, $F(2, 18) = 12.72$, $p < .001$. This is somewhat misleading because the response rates are measured at threshold stimulation frequencies and the rate measures obtained during the cocaine treatment are at much lower stimulation frequencies than those obtained during saline treatment. Cocaine would be expected to increase lever-press rates for constant stimulation parameters (2,12). By definition of threshold (i.e., minimum stimulation frequency maintaining ≥30 presses/min), these subthreshold stimulation frequencies would maintain <30 presses/min during saline treatment. Thus response rates increased from <<30 presses/min to >58 presses/min following cocaine treatment (viz., >100% increase in response rates).

DISCUSSION

The failure of L-NAME to affect brain stimulation reward suggests that nitric oxide is not involved in the electrically stimulated release of dopamine, at least not in the brain dopamine system mediating the rewarding effect of lateral hypothalamic stimulation. If dopamine release were significantly modified, then corresponding changes in brain stimulation thresholds should have been evident. L-NAME was also ineffective in modifying cocaine-facilitated brain stimulation reward, further suggesting that nitric oxide is not involved in modulating the dopamine-enhancing effect of cocaine. In an earlier study (10), L-NAME pretreatment appeared to block cocaine's stimulation of locomotor activity. This effect was probably due to a nonspecific sedative effect of L-NAME because the same dose of L-NAME was ineffective in attenuating cocaine's facilitation of brain stimulation reward. The earlier study also reported that L-NAME alone produced an almost 50% reduction in locomotor activity, although this sedative action was insufficient to decrease lever-pressing rates in the present study, even at 10 times the dose previously reported to decrease locomotor activity. The threshold tracking method of measuring brain stimulation reward appears relatively insensitive to sedative drug action.

Although the overall effect of cocaine on brain stimulation reward during the 60-min test session was not affected by L-NAME pretreatment, nitric oxide synthesis inhibition did appear to alter the time course of this effect. L-NAME inhibits cerebral blood flow (9,11), and this action may have delayed the time-to-peak-effect in the present study. Also, the same decrease in cerebral blood flow may have slightly prolonged the duration of cocaine's effect, since elimination of cocaine from the biophase may have been similarly retarded. The net threshold lowering over the course of the 60-min session, however, remains unchanged by the L-NAME pretreatment. The fact that decreased cerebral blood flow following L-NAME does not significantly diminish the overall effect of cocaine is important for ruling out pharmacokinetic explanations of an earlier study using these drug doses (10). The behavioral data suggest that the pharmacokinetic area-under-the-curve (i.e., cocaine brain levels over time) is not affected by L-NAME pretreatment despite the slight shift in cocaine's time course. Even if L-NAME pretreatment significantly inhibited brain clearance of cocaine at times not measured in the present study (i.e., >60 min postcocaine), this would only prolong slightly cocaine's duration of action.

Pudiak and Bozarth (10) reported that L-NAME pretreatment blocked sensitization to the locomotor-stimulating effect of repeated cocaine administration. That effect was interpre-

ted as supporting the proposed role of nitric oxide in learning and neuroadaptation. Two alternative explanations of that effect remained, however. First, L-NAME could have decreased total cocaine distribution to the brain, thus decreasing the effective dose of cocaine. Second, L-NAME could have inhibited cocaine's enhancement of dopamine, thus diminishing the impact of repeated cocaine treatment. Both potential effects would be expected to attenuate behavioral sensitization from repeated cocaine administration, but both alternative explanations are ruled out by the present study. The failure of nitric oxide synthesis inhibition to affect cocaine-facilitated brain stimulation reward, considered with the earlier report that L-NAME inhibits cocaine sensitization, supports the proposed role of nitric oxide in neuroadaptation and suggests nitric oxide is not involved in the short-term regulation of dopamine in the brain system mediating this rewarding effect.

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