Nitric Oxide Synthesis Inhibition Attenuates Haloperidol-Induced Supersensitivity

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Increased responsiveness to psychomotor stimulants can be produced by either chronic stimulant administration or by chronic dopamine receptor blockade. This study examined the role of nitric oxide in the development of neuroleptic-induced supersensitivity. Nω-nitro-L-arginine methyl ester hydrochloride (L-NAME) administered during chronic intraperitoneal (ip) haloperidol treatment (0.2 mg/kg/d for 14 d) was used to inhibit nitric oxide synthesis. Locomotor activity following a cocaine$_{ip}$ challenge injection (10 mg/kg) administered at 3 and 10 d after cessation of haloperidol treatment demonstrated the development of supersensitivity. Haloperidol animals pretreated with L-NAME$_{ip}$ (30 mg/kg) showed significantly less cocaine-stimulated locomotor activity on both tests than saline-pretreated animals. This finding suggests that nitric oxide is involved in haloperidol-induced supersensitivity and that a common neural mechanism may underlie the development of supersensitivity and stimulant-induced sensitization. This study also suggests that treatment with a nitric oxide synthesis inhibitor may decrease the side effects accompanying long-term treatment with antipsychotic medications.

Key Words: antipsychotic medication, cocaine, haloperidol, L-NAME, locomotor activity, neuroleptics, nitric oxide, supersensitivity

INTRODUCTION

Antipsychotic drugs (for example, haloperidol) block dopamine receptors and are used in the treatment of schizophrenia. However, neuroleptic medications frequently produce side effects such as tremor and rigidity (Seeman 1995), and chronic neuroleptic treatment has been associated with the development of a condition called supersensitivity (Snyder and others 1974; Muller and Seeman 1978). Supersensitivity is caused by dopamine receptors that respond to amounts of dopamine previously unable to elicit any biological effects. This enhanced responsiveness can produce tardive dyskinesia (Muller and Seeman 1978; Tarsy 1983), which is characterized by unorganized and uncontrollable movements.

The molecular mechanisms that mediate the development of supersensitivity remain unclear. However, a similar neuroadaptive process that may help to identify the molecular mechanisms that mediate the development of supersensitivity is stimulant-induced sensitization. Sensitization is produced by repeated psychomotor stimulant administration (for example, cocaine, amphetamine) and results in the progressive enhancement of a stimulant's locomotor-activating effects (Robinson 1988; Robinson and Berridge 1993). Both sensitization and supersensitivity produce increased responsiveness to the stimulatory action of psychomotor stimulants

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(VonVoigtlander and others 1975; Robinson 1988), but they are produced by 2 distinctively different experimental treatments (that is, chronic dopamine agonist administration and chronic dopamine receptor antagonist administration, respectively).

Several studies have shown administration of the noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist MK-801 blocks behavioral sensitization to repeated stimulant administration (Karler and others 1989; Kuribara and others 1992; Pudiak and Bozarth 1993). In addition, NMDA receptor activation has been implicated in the conversion of L-arginine to nitric oxide by means of nitric oxide synthase (Garthwaite 1993). Pudiak and Bozarth (1993) have recently reported that L-NAME, a nitric oxide synthase inhibitor, is also effective in inhibiting behavioral sensitization produced by chronic cocaine treatment. These data suggest that both NMDA receptor activation and nitric oxide formation are involved in the development of sensitization to stimulants, and they support the proposed role of nitric oxide in cellular learning and adaptation (Garthwaite 1991; Snyder and Bredt 1992). The present study used the nitric oxide synthase inhibitor L-NAME to determine if nitric oxide was involved in the development of supersensitivity following chronic neuroleptic treatment.

METHODS

Subjects and apparatus

Experimentally naive, male Long-Evans rats (Harlan Sprague-Dawley, Indianapolis, USA) weighing 260 to 324 g at the beginning of the experiment were used. The animals were individually housed with free access to food and water, except during behavioral testing. The animal colony was maintained at 22 ± 2 °C during a 14-h light/10-h dark illumination cycle with lights on from 08:00 to 22:00. All behavioral testing occurred during the light phase between 13:00 and 18:00.

Locomotor activity was measured using a 31× 70 × 38 cm high chamber constructed of aluminum and Plexiglas walls with a tubular stainless steel floor. Infrared photocells were placed 3.5 cm above the floor every 10 cm along the longitudinal axis. Photocell arrays consisted of an infrared-emitting diode (900 nm typical, 650 μW) with a photodarlington transistor positioned 31 cm across the test chamber. Photodetectors were connected to an IBM-compatible computer (8088-12 MHz) using a specially designed interface. Each photocell beam interruption constituted one activity count. Total counts per 5-min period were automatically collected during 30-min test sessions. Each activity chamber was placed in a dimly illuminated, sound-attenuating chamber with an exhaust fan providing ventilation and noise masking.

Drugs

L-NAME (Sigma Chemical, St Louis, USA) was dissolved daily in sterile physiological saline prior to injecting. Cocaine hydrochloride (National Institute on Drug Abuse, Rockville, USA) was prepared in physiological saline, sterilized by filtration (0.22 μm filter), and stored at room temperature. Haloperidol (Sigma Chemical, St Louis, USA) was dissolved in saline and glacial acetic acid and stored at 4 °C; its pH was adjusted (pH = 6.4) by adding sodium hydroxide. All the drugs were injected intraperitoneally, and all drug dosages refer to the drug salts except for haloperidol, which was used in the free base form.

Procedure

Animals were tested in activity chambers for 30 min following an injection of physiological saline (1 mL/kg). The next day, all animals were given an injection of cocaine hydrochloride (10 mg/kg) immediately before being placed in activity chambers, and locomotor activity was measured for 30 min. All animals received 2 daily injections and were otherwise left undisturbed in their home cages for 14 consecutive days. One group of animals (n = 12) was pretreated with physiological saline (1 mL/kg) and another group (n = 12) with L-NAME (30 mg/kg) 30 min prior to receiving a daily injection of haloperidol (0.2 mg/kg). The animals were not injected on days 15 and 16 of the study. Seventy-two hours after their last haloperidol injection, all animals received a challenge injection of cocaine hydrochloride (10 mg/kg) immediately before being placed into activity chambers for 30 min. Seven days later (that is, 10 d after their last haloperidol injection), all animals were tested again in activity chambers for 30 min immediately following an injection of cocaine hydrochloride (10 mg/kg). The day 10 test ruled out any sedative or motor-impairing effects from the daily L-NAME injections and also assessed the stability of any changes in sensitivity to cocaine.

RESULTS

To assure that differences between the groups were due to the experimental treatments and could not be attributed to preexisting group differences in activity levels or responsiveness to a cocaine challenge, saline and cocaine pretest activity scores were measured for both groups of animals before starting haloperidol treatment. Figure 1 compares locomotor-activity scores for the 2 experimental groups after a saline challenge and after a cocaine challenge before beginning the chronic treatments. Independent t tests revealed that no differences in activity levels ($t[22] = 0.62$, $P > 0.05$) or responsiveness to a cocaine challenge ($t[22] = 0.92$, $P > 0.05$) existed between the 2 groups prior to the introduction of the 14-d injection sequence.

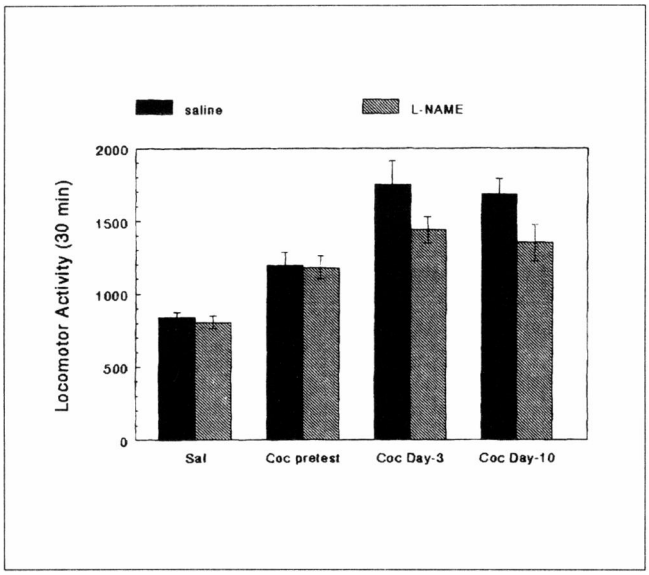

Figure 1. Supersensitivity from chronic haloperidol treatment. The figure shows the mean ± SEM number of activity counts for the 4 locomotor activity tests. The solid bars illustrate the group pretreated daily with saline; the shaded bars illustrate the group pretreated daily with L-NAME. Sal = saline test; Coc pretest = 1st test with cocaine; Coc Day-3 = cocaine test 72 h after termination of the haloperidol treatment; Coc Day-10 = cocaine test 10 d after termination of the haloperidol treatment.

To avoid residual drug effects during the sensitization tests, subjects received a challenge dose of cocaine hydrochloride 72 h and 10 d after their last drug treatments. The day 10 test assured that any motor-impairing or sedative effects that may have resulted from cumulative L-NAME injections were absent. Dependent *t* tests were used to determine the effect of treatment on locomotor activity for the 2 treatment conditions. Chronic haloperidol treatment produced increased locomotor stimulation from cocaine. The saline-pretreated group showed significantly more locomotor activity on the day 3 test ($t[11] = 3.66$, $P < 0.01$) and on the day 10 test ($t[11] = 3.29$, $P < 0.01$) than on the cocaine pretest. The L-NAME–pretreated group showed slightly more locomotor activity during the day 3 test ($t[11] = 2.15$, $P < 0.05$), but this effect was absent on the day 10 test ($t[11] = 1.22$, $P > 0.05$). A 2 × 2 analysis of variance (ANOVA) with repeated measures on 1 factor showed that L-NAME pretreatment significantly inhibited the development of supersensitivity (treatment effect: $[F(1,22) = 4.91, P < 0.05]$); the days effect and the days × treatment interaction were not significant. Planned comparisons showed significant differences between the saline and L-NAME pretreated groups on both day 3 ($t[22] = 1.96$, $P < 0.05$) and day 10 ($t[22] = 1.89$, $P < 0.05$) tests.

DISCUSSION

Changes in neural sensitivity may contribute to the development of mental illness and drug addiction. Chronic neuroleptic treatment may not only result in motoric side effects (Seeman 1995) and tardive dyskinesia (Kane and Smith 1982; Meltzer and others 1986; Baldessarini 1990) but may also be responsible for the increased incidence of stimulant abuse among individuals with schizophrenia (LeDuc and Mittleman 1995). The incidence of stimulant abuse (for example, cocaine) in people with schizophrenia is 2 to 5 times higher than in the general population (LeDuc and Mittleman 1995). Thus supersensitivity following long-term neuroleptic treatment may produce alterations in dopamine receptors that result in an increased sensitivity both to the neurotransmitter dopamine and to cocaine's reinforcing effects.

An understanding of the molecular mechanism underlying supersensitivity could lead to more effective treatments for schizophrenia, with an increased efficacy of long-term antipsychotic medication. Although supersensitivity and sensitization are produced by opposite experimental manipulations (that is, chronic dopamine receptor antagonist administration and chronic dopamine agonist administration, respectively), the molecular mechanisms underlying the development of neuroleptic-induced supersensitivity and stimulant-induced sensitization may in fact be very similar. Data obtained from this study show that inhibition of nitric oxide synthesis attenuates haloperidol-induced supersensitivity to cocaine, suggesting that nitric oxide formation is involved in the development of neuroleptic-induced supersensitivity.

These data suggest that drugs used to inhibit nitric oxide synthesis may be useful for minimizing neuroleptic-induced supersensitivity during the treatment of schizophrenia. The prevention of neuroleptic-induced supersensitivity may reduce motoric side effects associated with long-term neuroleptic administration and may decrease the incidence of stimulant abuse among patients with schizophrenia.

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REFERENCES

Baldessarini RJ. 1990. Drugs and the treatment of psychiatric disorders. In: Gilman AG, Rall TW, Nies AS, Taylor P,

editors. The pharmacological basis of therapeutics. New York: Pergamon Press. p 383–435.

Garthwaite J. 1991. Glutamate, nitric oxide and cell-cell signalling in the nervous system. Trends Neurosci 14:60–7.

Garthwaite J. 1993. Nitric oxide signalling in the nervous system. Semin Neurosci 5:171–80.

Kane JM, Smith JM. 1982. Tardive dyskinesia: prevalence and risk factors, 1959 to 1979. Arch Gen Psychiatry 39:473–81.

Karler R, Calder LD, Chaundhry IA, Turkanis SA. 1989. Blockade of "reverse tolerance" to cocaine and amphetamine by MK-801. Life Sci 45:599–606.

Kuribara H, Asami T, Ida I, Iijima Y, Tadokora S. 1992. Effect of repeated MK-801 on ambulation in mice and in sensitization following methamphetamine. Psychopharmacology 108:271–5.

LeDuc PA, Mittleman G. 1995. Schizophrenia and psychostimulant abuse: a review and re-analysis of clinical evidence. Psychopharmacology 121:407–27.

Meltzer HY, Schyve PM, Moore KE. 1986. Drugs for therapy of psychosis. In: Pradhan SN, Maickel RP, Dutta SN, editors. Pharmacology in medicine: principles and practice. Bethesda (MD): SP Press International. p 325–37.

Muller P, Seeman P. 1978. Dopaminergic supersensitivity after neuroleptics: time-course specificity. Psychopharmacology 60:1–11.

Pudiak CM, Bozarth MA. 1993. L-NAME and MK-801 attenuate sensitization to the locomotor-stimulating effect of cocaine. Life Sci 53:1517–24.

Robinson TE. 1988. Stimulant drugs and stress: factors influencing individual differences in the susceptibility to sensitization. In: Kalivas PW, Barnes CW, editors. Sensitization of the nervous system. Caldwell (NJ): Telford Press. p 145–73.

Robinson TE, Berridge KC. 1993. The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Res Brain Res Rev 18:247–91.

Seeman P. 1995. Dopamine receptors and psychosis. Scientific American 2:28–37.

Snyder SH, Banerjee SP, Yamamura HI, Greenberg D. 1974. Drugs, neurotransmitters, and schizophrenia. Science 184:1243–52.

Snyder SH, Bredt DS. 1992. Biological roles of nitric oxide. Scientific American 266:68–77.

Tarsy D. 1983. Neuroleptic-induced extrapyramidal reactions: classification, description, and diagnosis. Clin Neuropharmacol 6(1 Suppl):9S–26S.

VonVoigtlander PF, Losey EG, Triezenberg HG. 1975. Increased sensitivity to dopaminergic agents after chronic neuroleptic treatment. J Pharmacol Exp Ther 193:88–94.