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Cocaine Kindling in Mice

Responses to N-Methyl-D,L-Aspartate (NMDLA) and L-Arginine

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Abstract

Previous studies proposed the involvement of the *N*-methyl-D-aspartate (NMDA) type of glutamate receptors in the development of sensitization to the convulsive effect of cocaine (cocaine kindling). The present study was undertaken to determine, first, if cocaine kindling is associated with enhanced sensitivity of the NMDA receptor to the convulsive response of *N*-methyl-D,L-aspartate (NMDLA), and second, whether in vivo modulation of nitric oxide synthase (NOS) function regulates the development of cocaine kindling. The following results were observed:

1. Cocaine-kindled animals were significantly more susceptible to the convulsive effect of the NMDA receptor agonist NMDLA than saline controls;
2. Pretreatment with the NOS inhibitor N^G-nitro-L-arginine methyl ester (L-NAME; 100 mg/kg; ip) blocked the development of cocaine kindling;
3. The protective effect of L-NAME was partially reversed with the coadministration of the NOS substrate, L-arginine (300 mg/kg; ip), but not D-arginine; and
4. L-Arginine (300 mg/kg; ip), but not D-arginine, amplified the development of cocaine kindling. Taken together, these findings suggest that supersensitivity of the NMDA receptor and activation of NOS may underlie the development of cocaine kindling.

Index Entries: Cocaine; convulsions; sensitization, NMDA receptor; nitric oxide (NO).

Introduction

Kindling is considered as a progressive buildup of cellular discharge culminating in full-fledged seizures in response to intermittent low-intensity electrical or chemical stimulation of limbic brain sites (Racine, 1978). Repeated intermittent administration of cocaine results in behavioral sensitization (e.g., increase in locomotor activity and stereotypy) and amplified seizure susceptibility, defined as cocaine kindling (Post, 1977; Shuster et al.,

1977; Post et al., 1988; Johanson and Fischman, 1989). The augmentation in clonic convulsions following repeated administration of cocaine has been compared to the phenomenon of kindling because of their similar electrophysiological characteristics and temporal pattern (Post, 1977; Stripling and Ellinwood, 1977; Post et al., 1988). Cocaine kindling in animal models may relate to kindling induced epilepsy (Dhuna et al., 1991), repeated seizure activity (Alldredge et al., 1989), and panic disorders (Louie et al., 1989) in humans abusing the drug.

The involvement of the *N*-methyl-D-aspartate (NMDA) type of glutamate receptors in electrical-induced kindling (for review, *see* Mody, 1993) and cocaine kindling (Karler et al., 1989; Itzhak and Stein, 1992) had been proposed. Studies from our laboratory demonstrated that cocaine kindling may be associated with the upregulation of cortical NMDA receptors, a phenomenon that is blocked by the noncompetitive NMDA receptor antagonist dizocilpine (MK-801) (Itzhak and Stein, 1992). In addition, evidence suggests a role of NMDA and non-NMDA glutamate receptors, as well as dopamine receptors, in the induction of behavioral sensitization to cocaine (Karler et al., 1989, 1994; Karler and Calder, 1992).

NMDA receptor-mediated neurotoxicity is thought to be associated with overproduction of nitric oxide (NO), produced by activation of brain nitric oxide synthase (NOS), which converts L-arginine to L-citrulline (Garthwaite et al., 1988; Bredt and Snyder, 1989; Garthwaite, 1991; Snyder, 1992). NO may serve as a retrograde messenger affecting the release of various neurotransmitter(s) from presynaptic terminals via increase in cGMP (Gally et al., 1991). Recently, we demonstrated that cocaine-induced kindling and mortality are blocked by pretreatment with NOS inhibitors, such as N^G-nitro-L-arginine methyl ester (L-NAME) and N^G-nitro-L-arginine (Itzhak, 1993, 1994). These findings suggest a role for NMDA/NOS systems in the development of cocaine kindling. In the present study, we investigated first if cocaine kindling is associated with enhanced sensitivity of the NMDA receptor to the convulsive response of *N*-methyl-D,L-aspartate (NMDLA), and second, whether in vivo modulation of NOS function by L-NAME and L-arginine regulates the development of cocaine kindling.

Materials and Methods

Materials

Cocaine-HCl, NMDLA, and L- and D-arginine were purchased from Sigma (St. Louis, MO). L-NAME was purchased from Research Biochemical Incorporated (Natick, MA).

Schedule of Drug Treatment

Male Swiss-Webster mice (28–31 g; Charles River, Willmington, MA) were maintained on a 12-h light/dark lighting schedule and housed in groups of five with free access to food and water. Drug solutions were freshly prepared daily in saline. Animals were divided into several groups, each containing 10 mice. Each group received one of the following treatments by intraperitoneal (ip) injection:

1. Saline (0.1 mL/10 g);
2. Saline 30 min before cocaine (40 mg/kg);
3. L-NAME (100 mg/kg) 60 min before cocaine;
4. L-arginine (300 mg/kg) and L-NAME in one solution 60 min before cocaine;
5. D-arginine (300 mg/kg) and L-NAME in one solution 60 min before cocaine;
6. L-arginine and cocaine in one solution; and
7. D-arginine and cocaine in one solution.

All drug administrations were performed once a day between 11:00 AM and 1:00 PM, for 7 d. The effect of acute ip administration of NMDLA was determined in naive, saline-, and cocaine-treated animals. No significant difference between naive and saline-treated animals in the response to NMDLA was observed.

Assessment of Convulsions

The convulsive response to the drug treatments was assessed as previously described (Itzhak and Stein, 1992). Briefly, immediately

after the drug treatment, animals were examined by an observer unaware of the drug treatment for a 60-min period as either positive or negative for the clear demonstration of tonic-clonic motor convulsions. These were manifested by clonus of the forelimbs and hindlimbs, and flexion of the head or entire body. The results are presented as percent of animals that were marked as positive in such experiments.

Statistical Analysis

Each drug treatment was performed in a group of ten mice, and each experiment was repeated three times. Data presented are the mean ± SEM of three experiments. Analysis of the differences among multiple treatment groups consisted of analysis of variance followed by *post hoc* comparisons (Dunnett's test for control and drug group comparisons and Newmann-Keuls test for comparison between drug groups). Significant differences were defined at $p < 0.05$.

Results

Effect of NMDLA in Saline- and Cocaine-Pretreated Animals

Administration of 200 mg/kg NMDLA to saline-treated animals resulted in running behavior in 60–80% of the animals and tonic-clonic convulsions in only 10% of the animals. The convulsive effect of NMDLA was elevated to 90–100% when the dose of NMDLA was increased to 400 mg/kg. The dose–response effect of NMDLA is described in Fig. 1. At the highest dose tested, 400 mg/kg, convulsions were accompanied with mortality: 60 ± 5% of the animals died within 20–25 min of the drug administration. To determine if cocaine-pretreated animals are more susceptible to the convulsive effect of NMDLA, the ED_{10} (200 mg/kg) was administered to animals that were pretreated for 7 d with cocaine (40 mg/kg). As described in Fig. 2, this schedule of cocaine administration resulted in a progressive increase in convulsions. NMDLA (200 mg/kg; ip) was given 24 h after cocaine administration

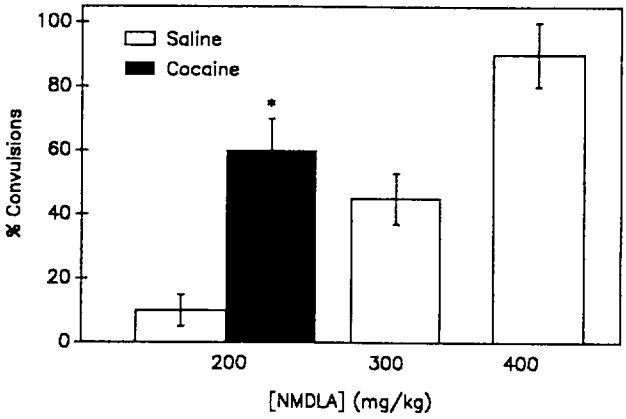

Fig. 1. Augmentation of NMDLA convulsive effect in cocaine-pretreated animals. Swiss Webster mice were pretreated for 7 d with either saline or cocaine (40 mg/kg; ip). Twenty-four hours after the drug administration was stopped, the convulsive response to three different doses of NMDLA given ip was assessed in saline-pretreated animals, and compared to the effect of a single dose of 200 mg/kg given ip to cocaine-pretreated animals. Results represent the mean ± SEM of three experiments. $^*p < 0.05$ for the comparison between the effect of 200 mg/kg given to saline- and cocaine-pretreated animals.

was stopped. The results presented in Fig. 1 indicate a marked increase in the convulsive response to NMDLA in cocaine-pretreated animals, as compared to control. Tonic-clonic convulsions developed in 60 ± 10% of the animals that were pretreated with cocaine, as compared to 10 ± 5% in saline controls. Moreover, the dose of 200 mg/kg NMDLA resulted in mortality in 40% of animals that were pretreated with cocaine, but this dose was not lethal in saline-pretreated animals.

Effect of L-Arginine and L-NAME on the Development of Cocaine Kindling

Daily administration of cocaine (40 mg/kg) resulted in a progressive increase in the convulsive response to cocaine (Fig. 2). Similar results were obtained with the daily administration of 45 mg/kg cocaine, except that the latter schedule also produced augmentation in lethality rate (Itzhak and Stein, 1992). The daily pre-

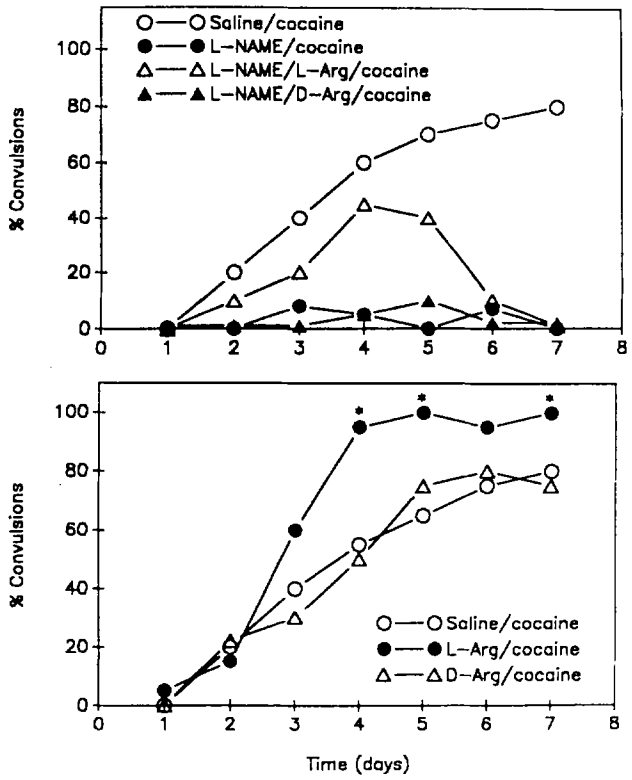

Fig. 2. Modulation of cocaine-induced convulsions by L-NAME, L-arginine, and combinations of drug treatments. Swiss Webster mice were administered ip for 7 d with either cocaine (40 mg/kg) or one of the drug combinations as described in Materials and Methods. The convulsive response to the various treatments was assessed daily for a 60-min period following the drug administration. Results represent the mean of three experiments. SEM was < 5%. $^{*}p < 0.05$.

treatment with L-NAME blocked the development of cocaine kindling (Fig. 2). The protective effect of L-NAME was partially reversed when L-arginine (300 mg/kg), but not D-arginine, was administered along with L-NAME (Fig. 2). These findings suggest that the blockade of NOS by L-NAME and its protective effect are stereospecific. The finding that L-arginine was no longer effective in reversing the protective effect of L-NAME after the sixth day (Fig. 2) suggests that repeated administration of a relatively high dose of L-NAME (100 mg/kg) may cause irreversible inhibition of NOS.

The daily coadministration of L-arginine (300 mg/kg) with cocaine resulted in a significant increase in the convulsive response to cocaine following the third day of drug administration. This phenomenon did not occur with the coadministration of D-arginine (300 mg/kg) and cocaine (Fig. 2). Acute administration of L-arginine (100–1000 mg/kg; ip) to naive animals did not elicit any convulsive response. At a dose above 500 mg/kg, only a slight hyperactivity was observed.

Discussion

Several studies proposed that sensitization to the behavioral and convulsive responses of cocaine is associated with activation of glutamatergic neurotransmission (Karler et al., 1989, 1994; Karler and Calder, 1992). Based on in vitro radioligand binding experiments, we proposed that in the process of cocaine kindling, such activation may be the result of the upregulation of cortical NMDA receptors (Itzhak and Stein, 1992). Result from the present study support the notion that cocaine kindling is associated with upregulation of NMDA receptors, because cocaine-kindled animals were significantly more susceptible to the convulsive effect of NMDLA than control animals. Thus, both in vitro receptor binding assays and the present in vivo experiments suggest that repeated exposure to cocaine that causes the development of kindling is associated with supersensitivity of the NMDA receptors. Further studies are necessary, however, to determine whether supersensitivity of the NMDA receptors is indeed the cause for cocaine kindling or a result of seizure activity.

We have previously reported that NOS inhibitors are capable of blocking the development of cocaine kindling, suggesting that NOS is also involved in the process of increased seizure susceptibility to cocaine (Itzhak, 1993, 1994). In the present study, the finding that L-arginine, but not D-arginine, reversed partially

the protective effect of L-NAME further supports this concept. L-Arginine, however, failed to reverse the protective effect of L-NAME following the sixth day of coadministration of the NOS substrate and inhibitor. This finding implies that prolonged treatment with a high dose of L-NAME (100 mg/kg) may inhibit NOS in an irreversible manner. Thus, it appears that once NOS is inhibited, cocaine kindling is no longer apparent. These results suggest that NOS inhibitors may suppress not only the induction of cocaine kindling, but also the expression of kindled seizures.

Although L-arginine had no convulsive effect in naive animals, this agent amplified the development of convulsions following the third day of its coadministration with cocaine (Fig. 2). These findings, first, suggest that the proconvulsant effect of L-arginine depends on the development of enhanced sensitivity of the NMDA receptor and, second, argue for the involvement of brain NOS in the development of cocaine kindling. These findings are in accord with a previous study demonstrating that icv administration of L-arginine did not evoke seizure activity, but when administered with NMDA (icv), L-arginine markedly lowered the seizure threshold of NMDA, a phenomenon that was blocked by NOS inhibitors (Mollace et al., 1991). These observations, and the present study, support the concept that the proconvulsant effect of L-arginine is contingent to the stimulation of the NMDA receptor and involves the activation of brain NOS.

The hypothesis that NO is involved in cocaine kindling is further supported by several studies that proposed a role for NO in epileptogenic activity. First, NOS inhibitors and methylene blue, which prevents activation of soluble guanylate cyclase by NO, abolish NMDA-evoked seizures from the deep prepiriform cortex (De Sarro et al., 1991). Second, seizures produced by exposure to LiCl and tacrine is associated with increase in brain NOS activity (Bagetta et al., 1993). Third, administration of the NO donor, *S*-nitroso-*N*-acetylpenicillamine (SNAP), to rats produced dose-dependent convulsions similar to those associated with limbic stimulation that were blocked by methylene blue (Gross et al., 1994). Stimulation of brain NOS is not always dependent on the activation of the NMDA receptor, and other neurotransmitter systems may also modulate NOS activity (e.g., Bagetta et al., 1993). The findings that NMDA receptors are activated following repeated administration of cocaine and that the proconvulsant effect of L-arginine emerges in this process suggest that NOS activity is modulated via stimulation of the NMDA receptor in the process of cocaine kindling. Because NO is considered as a retrograde messenger that may modulate the release of various neurotransmitters (Gally et al., 1991; Zhu and Luo, 1992; Lonart et al., 1993; Montague et al., 1994), it is feasible that NMDA-mediated increase in NO production following administration of cocaine contributes to the process of sensitization.

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