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Dextromethorphan reduces intravenous cocaine self-administration in the rat

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Abstract

Dextromethorphan is a widely used antitussive agent with non-competitive antagonistic effects at the excitatory amino acid receptors of the NMDA type. Since excitatory amino acid neurotransmission has been implicated in cocaine dependence, the aim of the present study was to evaluate the effects of acute systemic administration of dextromethorphan in rats trained to self-administer cocaine intravenously. The experiments were designed to evaluate the effects of dextromethorphan on responding for cocaine and cocaine reward magnitude. The hypothesis was that dextromethorphan could attenuate specific aspects of cocaine-seeking behavior thus providing a preclinical rationale for its clinical use. The results reported reveal that acute pretreatment with dextromethorphan (10–50 mg/kg i.p.) significantly reduced cocaine self-administration in rats self-administering the drug intravenously in a simple continuous reinforcement schedule. In addition, acute pretreatment with an effective dose of dextromethorphan (25 mg/kg) decreased cocaine self-administration in rats tested at various doses of cocaine (0.12–0.5 mg/injection). Finally, dextromethorphan (25 mg/kg) also reduced the absolute reward magnitude of cocaine as measured by responding for cocaine in a progressive ratio schedule. These results encourage further experimental and clinical studies to evaluate the potential use of dextromethorphan during various phases of the natural history of cocaine dependence in humans.

Keywords: Cocaine ; Dextromethorphan ; Self-administration ; NMDA receptor antagonist

1. Introduction

Intravenous cocaine self-administration in rodents has been used as a useful animal analog to study the neural substrates of cocaine dependence. The nucleus accumbens of the basal forebrain has been identified as a critical neural substrate which mediates the acute reinforcing properties of cocaine (Koob, 1992) and, particularly, pharmacological manipulation of dopamine and excitatory amino acid neurotransmission within the nucleus accumbens significantly affects cocaine-seeking behavior (Maldonado et al., 1993; Pulvirenti et al., 1992).

Recent evidence suggests that specific neuroadaptive phenomena which seem to occur during the process of prolonged exposure to drugs of abuse may require excitatory amino acid transmission. These include sensitization to psychostimulants, opiate tolerance and abstinence and ethanol withdrawal (Karler et al., 1990, 1991; Tsai et al., 1995; Herman et al., 1996). These observations open the possibility that pharmacological intervention at the level of excitatory amino acid receptors may effectively modulate various aspects of the natural history of drug dependence. Since antagonism of excitatory amino acid NMDA receptor function within the nucleus accumbens was shown to reduce the expression of the psychostimulant and reinforcing properties of cocaine in the rat (Pulvirenti et al., 1992, 1994), the effects of systemic blockade of NMDA receptors in rats self-administering cocaine deserved experimental attention.

Dextromethorphan is the dextrorotatory isomer of levomethorphan which lacks opioid-like activity and is best known for its antitussive effects (Jaffe and Martin, 1990). Recently, several central nervous system (CNS) actions have been described for dextromethorphan, including high

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affinity binding sites, neuroprotective activity and non-competitive antagonism at the NMDA receptor (see Tortella et al., 1989). For its NMDA antagonistic action and neuroprotective effects, dextromethorphan has been tested in clinical trials of Parkinson's disease, Huntington's chorea, epilepsy and stroke, showing some efficacy (Bonuccelli et al., 1992; Walker and Hunt, 1989; Moses and Choi, 1991).

The aim of the present work was therefore to study the effects of dextromethorphan in rats self-administering cocaine intravenously. One important issue in the evaluation of the effects of drugs in animal models of cocaine dependence, however, is that simple rate measures do not selectively address the problem of the assessment of specific drug effects on cocaine-seeking behavior. Therefore, it is essential to evaluate the relative strength of cocaine-seeking behavior independently of response rate and possible motor impairments. Consequently, in the present study, experiments in which rats were trained to self-administer cocaine at one dose were extended to incorporate training of the animals at various doses of cocaine, in order to generate a complete dose–response function. In this context, the effects of treatment with dextromethorphan on the reinforcing properties of cocaine may be reflected in a shift of the dose–response function. Also, in order to arrive at appropriate interpretations of general increases or decreases in response rate, the absolute reward magnitude of cocaine was assessed in rats self-administering cocaine intravenously in a progressive ratio schedule, an operant measure tailored to establish the motivational strength of the organism to obtain cocaine (Bedford et al., 1978).

2. Materials and methods

2.1. Animals

Male Wistar rats (Charles River, Holester, CA, USA), weighing 200–225 g at the start of the experiment, were housed 3 to a cage and provided with ad lib access to food and water and maintained on a 12-h light-dark cycle (lights on 7:00 a.m.-7:00 p.m.).

2.2. Self-administration

All animals were surgically implanted with a chronic silastic jugular vein catheter under halothane anesthesia, as previously described (Caine et al., 1993). The catheter passed subcutaneously to a piece of marlex mesh secured s.c. on the animal's back. At the time of the self-administration session, the catheter was connected to a swivel system through a metal spring, which was in turn connected to an infusion pump.

Four days following surgery the animals were allowed a 2-h access every day to a metal lever mounted on the side wall of a standard operant-conditioning cage. The cages themselves were housed inside sound attenuating chambers. A lever press resulted in an intravenous injection of 0.1 ml of cocaine hydrochloride (0.25 mg/injection) dissolved in 0.9% physiological saline and delivered over a period of 4 s. A swivel system allowed free movement of the animal in the cage. Coincident with the onset of the injection, a stimulus light was turned on for 20 s during which time the lever became inactive. Lever presses during the period when the signal light was not lit were reinforced on a continuous reinforcement schedule (fixed-ratio 1, FR-1). Once the animals demonstrated stable drug intake for three days (a range of less than 15% of the daily intake over three days), this was taken as baseline and the study was begun. On a test day, the animals were pretreated immediately before the beginning of the session with dextromethorphan (Sigma, St. Louis, MO, USA). There were three different doses of dextromethorphan (0, 10, 25, 50 mg/kg intraperitoneally): each dose was tested only once for each animal using a Latin-square design. The drug was prepared in a vehicle solution of 0.9% physiological saline and injected in a volume of 1.0 ml/kg of body weight. At least two days of baseline self-administration separated the testing days. The number of reinforcers earned at various intervals during the 120-min session (30, 60 and 120 min) was recorded and statistical analysis of the data was computed using a one-way factorial analysis of variance with repeated measures (ANOVA). Individual means comparisons were made using the Newman-Keuls' a posteriori test.

2.3. Between-session cocaine self-administration dose–response

The animals were trained to self-administer cocaine as described above. The criterion for the start of tests of different unit doses of cocaine (0.12, 0.25 and 0.5 mg/injection) or drug pretreatment testing was three consecutive self-administration sessions with less than 15% variation in the total number of reinforcers earned. An effective dose of dextromethorphan as established by the previous experiment (25 mg/kg) was tested. Dose–effect functions were generated using a within-subject Latin-square design to allow analysis of order effects. The total number of reinforcers earned during the 120-min session was recorded and statistical analysis of the data was computed using a two-way factorial analysis of variance with repeated measures (ANOVA). Individual means comparisons were made using the Newman-Keuls' a posteriori test.

2.4. Progressive ratio procedure

Rats were allowed to self-administer cocaine as described above until a stable intake baseline was reached. The following days rats were subjected to a progressive ratio schedule where for the first eight injections the ratio requirement increased by one response per injection. For the next eight injections the ratio was raised by two each

successive ratio, the next eight injections the ratio was increased by four and the next eight injections the ratio was increased by eight responses each successive injection until the ratio requirement reached an FR-120. The breaking point was defined as the last ratio attained by the rat prior to a 1-h period during which a ratio was not completed. Rats were probed with the progressive ratio schedule until a stable response was obtained ($\pm$10% of the average of three successive probes) and then subjected to treatment with dextromethorphan immediately before the beginning of the session. An effective dose of dextromethorphan was used, as established in the first two experiments (25 mg/kg). Data were analyzed using Student's *t*-test.

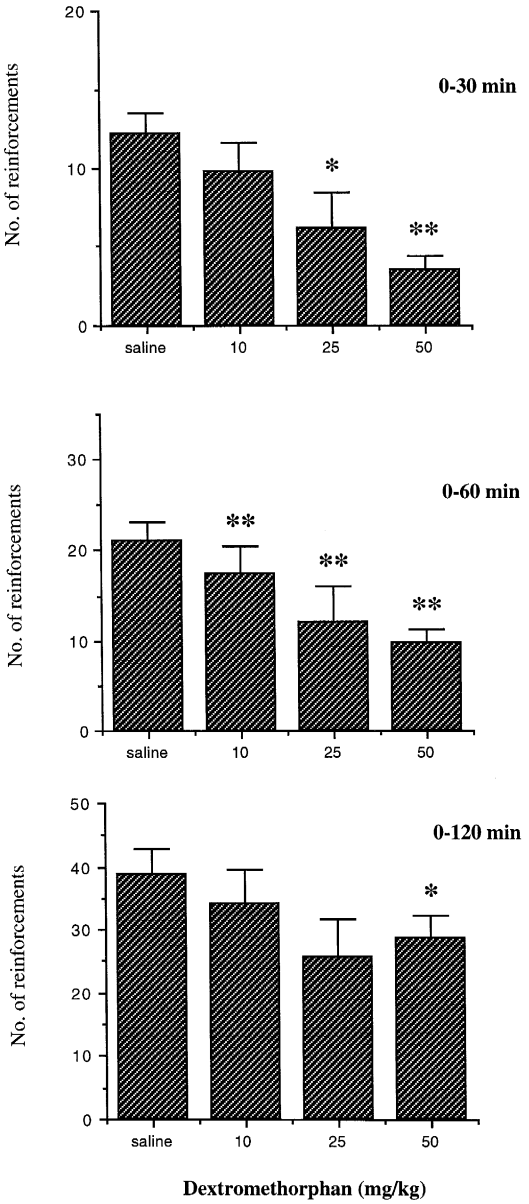

Fig. 1. Effect of treatment with dextromethorphan on i.v. cocaine self-administration. The top, middle and lower panel represent, respectively, the number of self-injection during the first 30, 60 and 120 min of the 2-h session. Values represent means $\pm$ S.E.M. of 7 animals. * $P < 0.05$, ** $P < 0.01$, Newman-Keuls test.

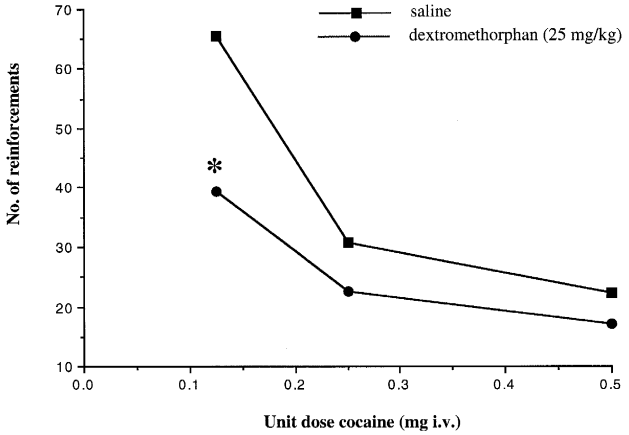

Fig. 2. Effect of pretreatment with dextromethorphan on a between-session dose-effect curve for i.v. cocaine self-administration. Values represent the means of cocaine self-injections of 7 animals.

3. Results

Fig. 1 shows the effects of acute pretreatment with dextromethorphan on responding for cocaine self-administration measured during the first 30 and 60 min and during the entire 120-min session. ANOVA revealed that dextromethorphan significantly reduced cocaine self-administration during all the intervals considered, although more effectively during the first 30 min (0–30 min: $F(3,15) = 8.48$, $P < 0.01$; 0–60 min: $F(3,15) = 7.48$, $P < 0.01$; 0–120 min: $F(3,15) = 3.53$, $P < 0.05$). Newman-Keuls' post-hoc test showed that statistical significance was reached at all the doses of dextromethorphan during the first 60 min of the session (Fig. 1).

Fig. 2 shows the effects of acute pretreatment with dextromethorphan (25 mg/kg) on cocaine self-administra-

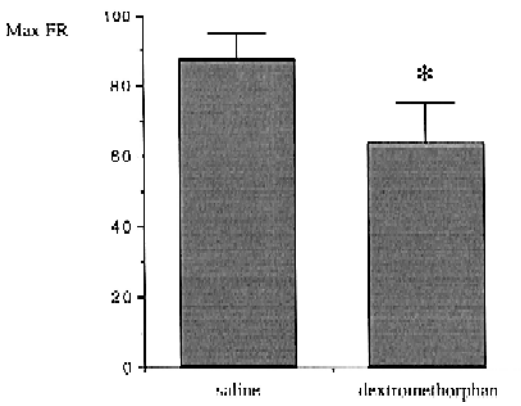

Fig. 3. Effect of pretreatment with dextromethorphan on progressive ratio responding for i.v. cocaine self-administration. Values represent the means $\pm$ S.E.M. of the maximum FR for a single self-injection of cocaine in 9 animals. * $P < 0.05$, Student's *t*-test.

tion using a between-session dose–effect function. ANOVA revealed that dextromethorphan significantly reduced responding for cocaine and there was a significant cocaine × dextromethorphan interaction ($F(2,12) = 10.873$, $P < 0.01$) and Newman-Keuls' post-hoc test showed that statistical significance was reached at the dose of cocaine of 0.12 mg/kg (Fig. 2).

Fig. 3 shows the effect of pretreatment with dextromethorphan on progressive ratio responding for cocaine self-administration. Pretreatment with dextromethorphan at the dose of 25 mg/kg significantly reduced the maximum FR ('breaking point') of responding for cocaine self-administration($df = 8$, $t = 2.602$, $P < 0.05$).

4. Discussion

The results reported here show that dextromethorphan significantly reduces i.v. cocaine self-administration, reduces the between-session dose–effect curve of cocaine self-administration and diminishes the absolute rewarding value of cocaine as measured by the progressive ratio procedure. In rats trained to self-administer a single training dose of cocaine in a simple continuous reinforcement schedule, acute pretreatment with dextromethorphan reduced cocaine intake, thus suggesting a reduction in the motivation to seek cocaine in the presence of dextromethorphan. However, the suppressing effect of dextromethorphan was more fully revealed by the suppression of responding over the entire dose–effect function of cocaine self-administration.

Furthermore, dextromethorphan significantly reduced the maximum FR of responding for cocaine ('breaking point') in the progressive ratio paradigm. The breaking point in a progressive ratio schedule represents a sensitive operant measure designed to assess the organism's motivation to obtain the drug (Roberts et al., 1989). Dextromethorphan decreased baseline cocaine self-administration at each dose tested on the dose–effect function and progressive ratio responding for cocaine. The interpretation of such effects is complicated by the possibility of a non-specific motor effect. This is unlikely for several reasons. First, the animals were capable of responding at higher rates at lower doses in the dose–effect function and the animals are capable of reasonably high FR ratios. In addition, the animals maintained a stable inter-injection interval following dextromethorphan administration. It is interesting to note that competitive NMDA receptor antagonists injected directly into the nucleus accumbens appear to have the opposite effect and actually decrease the inter-injection interval for cocaine self-administration at self-administration doses that are on the descending limb of the dose–effect function. This suggests that systemic non-competitive NMDA receptor antagonists may decrease cocaine self-administration not only by blocking dopamine function within the limbic system, but also by other neuropharmacological circuit interactions involving excitatory amino acid neurotransmission.

Dextromethorphan is a clinically effective antitussive agent which possesses pharmacological activity at the level of the CNS (Musacchio, 1990). Unlike other non-competitive NMDA receptor antagonists known to produce important side effects which greatly limit their clinical use (agitation, hallucination, ataxia and dysphoria), dextromethorphan possesses a favorable therapeutic index and side effects only occur at very high doses in humans (Musacchio, 1990). These observations have been confirmed by several decades of safe use of dextromethorphan as an antitussive agent. Its wide clinical use and approved safety suggests therefore that dextromethorphan may be an ideal candidate for drug treatment in humans.

Neurochemically, dextromethorphan has been shown to act as a non-competitive antagonist at the NMDA receptor complex (Choi, 1987), an effect believed to be responsible for its neuroprotective activity (see Tortella et al., 1989). This is of particular importance since it has been recently suggested that excitatory amino acid neurotransmission may be involved in several aspects of the natural history of cocaine dependence, especially in the nucleus accumbens, a critical structure mediating cocaine self-administration in rodents (Koob, 1992).

Excitatory amino acids represent the main neural afferents to the nucleus accumbens regulating the firing rate of neurons at this level (Kelley and Domesick, 1982; Pennartz and Kitai, 1991). Indeed, functional integrity of glutamate neurotransmission within the nucleus accumbens appears to be essential for the integrated activity of the nucleus accumbens. Behavioral evidence suggests that blockade of nucleus accumbens NMDA receptors reduces cocaine reinforcement (Pulvirenti et al., 1992) and the full expression of the psychoactivating properties of cocaine and other psychostimulant drugs (Pulvirenti et al., 1994), while neurochemical studies indicate that excitatory amino acid within the nucleus accumbens may modulate dopamine tone (Imperato et al., 1990, Youngren et al., 1993) which has been shown to play a key role in cocaine-seeking behavior (Koob, 1992).

It is also noteworthy that an excitatory amino acid-dependent form of long-term enhancement of synaptic efficacy has been shown to occur within the nucleus accumbens (Kombian and Malenka, 1994), suggesting a role for excitatory amino acids in the neuroadaptive phenomena associated with chronic exposure to drugs of abuse in the course of drug dependence.

Support for this hypothesis is experimental evidence that concurrent blockade of excitatory amino acid receptors prevents the development of sensitization to psychostimulant (Karler et al., 1990, 1991). This is an important form of synaptic plasticity occurring within the limbic system, manifested as the augmentation of the locomotor stimulating effects of psychostimulant drugs following repeated exposure to high doses of drugs such as cocaine,

amphetamine and methamphetamine (Robinson and Becker, 1986). Sensitization seems to be a critical component of the addictive process since it seems to represent the neurobiological basis of the paranoid-type psychosis observed in cocaine and amphetamine addicts (Robinson and Becker, 1986) and it has also been hypothesized that drug craving, which is known to be particularly pronounced in psychostimulant addiction, may result from sensitization to the behavioral effects of the abused drug (Robinson and Berridge, 1993).

Therefore, there is growing preclinical evidence suggesting a role of excitatory amino acids in psychostimulant dependence, further supported by the possible involvement of excitatory amino acids in the neuroadaptive changes associated with chronic exposure to other drugs of abuse, including opiates and ethanol (see Tsai et al., 1995 and Herman et al., 1996).

The results of the present study suggest the possibility that dextromethorphan may effectively modulate cocaine dependence by both reducing the acute reinforcing properties of cocaine and suppressing the motivational strength to obtain the drug. Given previous results showing the importance of excitatory amino acid neurotransmission for cocaine reinforcement (Pulvirenti et al., 1992) and the pharmacological profile of dextromethorphan at the NMDA receptor level (Choi, 1987; Musacchio, 1990), it is tempting to speculate that pharmacological intervention at the level of excitatory amino acid neurotransmission may be a novel and effective means of modifying various aspects of the natural history of the addictive process.

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