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Dextro-Naloxone Counteracts Amphetamine-Induced Hyperactivity

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CHATTERJIE, N., J. A. SECHZER, K. W. LIEBERMAN AND G. J. ALEXANDER. *Dextro-naloxone counteracts amphetamine-induced hyperactivity*. PHARMACOL BIOCHEM BEHAV **59**(2) 271–274, 1998.—The locomotor stimulating effect of *d*-amphetamine in mice was counteracted by the administration of *l*-naloxone [(−)-naloxone], a known opiate receptor antagonist. Mice injected with amphetamine reached a peak locomotor activity within 30 min. When treated simultaneously with amphetamine and *l*-naloxone, these subjects showed low motility. Furthermore, when mice were treated not with *l*-naloxone but with its mirror image, *d*-naloxone [(+)-naloxone], a compound that by itself does not antagonize opiates and does not affect spontaneous motility, they showed no amphetamine-induced hyperactivity. The finding that an enantiomer of naloxone, with no opiate antagonist activity, is able to block the excitatory action of amphetamine, suggests the existence of a hitherto unknown mechanism of counteracting some of the effects of stimulants and euphoriants like amphetamine and cocaine. © 1998 Elsevier Science Inc.

(+)-Naloxone *d*-Naloxone *dextro*-Naloxone *l*-Naloxone Amphetamine Hyperactivity Locomotor activity

DEXTRO-AMPHETAMINE has been used routinely as a stimulant and appetite suppressant. It enhances motor activity and produces restlessness and agitation. Intake of amphetamine produces a temporary feeling of euphoria followed by a feeling of depression and concomitant craving for more of the drug. In laboratory animals, intake of amphetamine led to increased locomotor activity.

Amphetamine does not seem to have a strong affinity for any of the opiate receptor sites in the brain, and its relationship with endogenous opiates is uncertain. Yet, systemic administration of opiate receptor antagonists, *l*-naloxone and *l*-naltrexone, as well as of the partial antagonist buprenorphine, counteracts amphetamine-induced agitation. It has been shown in laboratory animals that *l*-naloxone prevented or moderated the enhancement of motility due to amphetamine intake (3,12,14–16). We now report that not only the opiate antagonist, *l*-naloxone, but also its mirror image isomer, *d*-naloxone, in a somewhat higher dose, was able to block the hyperactivity caused by amphetamine (4,7). The stereospecificity of the *dextro*-isomer differed significantly from that of the active opiates (Fig. 1); it showed no opioid antagonist activity (11,13). We present here the results of our studies of amphetamine-induced enhancement of spontaneous motility and the effects of both *l*- and *d*-naloxone in counteracting that enhancement.

METHOD

Animals

A total of 110 Swiss–Webster albino male mice, 25–35 g, purchased from Charles River Laboratories (Wilmington, MA), was used in these experiments. The mice were housed four to six per cage and given tap water and Purina Chow ad lib. The animal colony was on a 12 h on–12 h off light cycle. All injections were administered intraperitoneally. Animals were treated in accord with the NIH guidelines. The only invasive treatment that they received consisted of drug injections.

Chemicals

In this study *d*-amphetamine sulfate (Sigma Chemical Co., St. Louis, MO) was used exclusively. Although excitatory in

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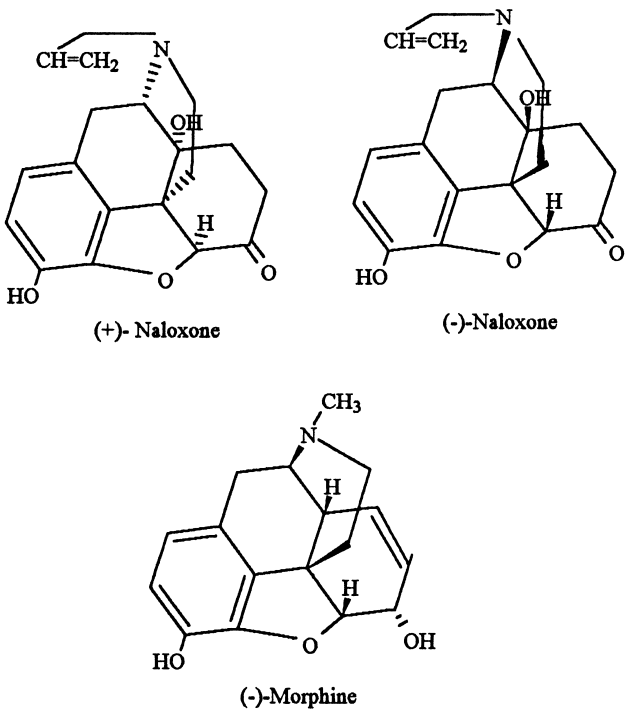

FIG. 1. Structures of *d*-naloxone [(+)-naloxone], *l*-naloxone [(−)-naloxone] and morphine.

moderate doses, amphetamine can become a depressant in very high doses; thus, it was important for us to determine that the dose we use produces hyperactivity in our animals. We tested the effect of doses from 0.5 to 4.0 mg/kg. We did not test the effects of *l*-amphetamine. However, we did test the effects of two isomers of naloxone, *l*-naloxone—received from the Mallinckrodt Corp., St. Louis, MO, and *d*-naloxone—received from the National Institute of Drug Abuse, Bethesda, MD. Both isomers were injected as hydrochlorides.

Treatment Groups

The first experiment was designed to study the changes in locomotor activity and behavior caused by amphetamine 30 min after intake and to study modifications in these changes, when *l*-naloxone was injected along with amphetamine. A total of 20 animals was divided by weight into five groups of four. At 0 time they were given injections of amphetamine alone or a mixture of *l*-naloxone and amphetamine. The dose of amphetamine was 2.0 mg/kg in all cases. The dose of *l*-naloxone was varied from 0.5 to 2.0 mg/kg. At 30 min the locomotor activity was recorded for 10 min. The experiment was repeated with 20 additional animals and the results combined.

The second experiment was designed to investigate the effects of *d*-naloxone, the isomer that does not affect morphine analgesia. A total of 24 mice in six groups was treated with saline, amphetamine, *d*-naloxone (2.0 or 4.0 mg/kg), or with mixtures of amphetamine (2.0 mg/kg), and 2.0 or 4.0 mg/kg *d*-naloxone. This experiment, too, was repeated with additional 24 mice.

The last experiment was designed to study the effect of the *d*-isomer of naloxone on amphetamine-induced hyperactivity, when the compound was not injected simultaneously with amphetamine as in Experiment 2, but at a time when hyperactivity induced by amphetamine was already near its peak, i.e., 30 min after injection of amphetamine. A total of 16 mice was given amphetamine, and 30 min later one-half received saline, the other half *d*-naloxone.

Observations of Behavior

Animals were habituated to test chambers and then observed by individuals who did not know their treatment history. Locomotor activity and behavioral patterns were recorded. Among the observed traits were spontaneous motility, rotary movements, vocalization, displacement motions (i.e., grooming, etc.), resistance to handling, frequent sniffing, and unusual excretory or urinary frequency.

Quantitative Assay of Spontaneous Motor Activity

Activity was measured in an Opto-Varimex Apparatus (Columbus Instruments International Corp., Columbus, OH) equipped to measure motility of four subjects simultaneously. In the first two experiments, subjects were placed individually in motility chambers 30 min after treatment. The number of times a subject interrupted ("crossed") a beam of light was recorded for 10 min. In the last experiment subjects received saline or *d*-naloxone 30 min after amphetamine; motility was recorded at 10-min intervals.

Statistics

Data from animals treated the same way were combined, averaged, and their standard deviations computed. Data were treated with one-way ANOVA, using commercial statistics software.

RESULTS

Animals given amphetamine increased their locomotor activity significantly. They moved continuously, and displayed repeated grooming and circling motions and some vocaliza-

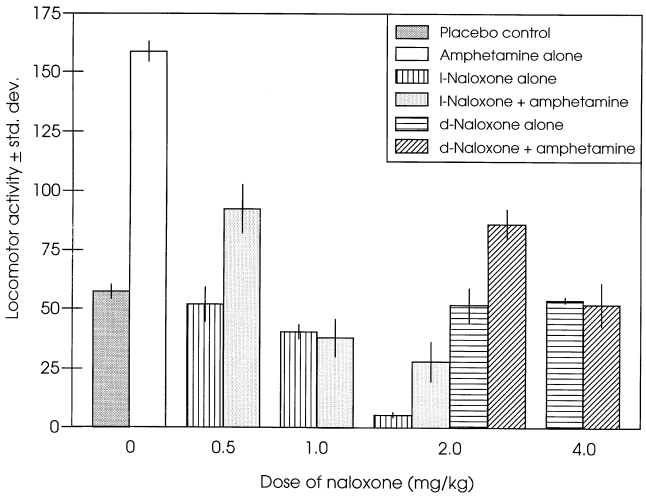

FIG. 2. Amphetamine-induced locomotor activity in mice. Number of crossings/min (i.e., breaking a light beam) in the presence of *l*-naloxone [(−)-naloxone], (0.5, 1.0, or 2.0 mg/kg), or *d*-naloxone [(+)-naloxone], (2.0 or 4.0 mg/kg). Dose of amphetamine: 2 mg/kg.

tion. They resisted handling more than controls. The hyperactivity was reflected in the quantitative data. Peak motor activity was reached between 30 and 50 min following injection and waned thereafter. Control subjects showed a motility of 57.3 ± 3.0 crossings per min. Treatment with 0.5 mg/kg amphetamine led to a minimal increase in motility; treatment with 1.0 mg/kg increased motility 50%. Motility tripled after 2.0 mg/kg amphetamine to 162.2 ± 5.5 (Fig. 2), and rose to 225.5 ± 12.0 after 4.0 mg/kg. In subsequent experiments, 2.0 mg/kg was used.

Administration of *l*-naloxone, in doses above 0.5 mg/kg, resulted in a drop in activity. At 30 min after injection, animals treated with 1.0 mg/kg showed a 30% decrease in motility; animals treated with 2 mg/kg crossed the light beams only a few times per min (Fig. 2). However, mice treated with the *d*-naloxone isomer showed no change in motility even after administration of 4.0 mg/kg. At 30 min after injection of 2.0 mg/kg of *d*-naloxone, the number of crossings was 51.8 ± 7.1 and after 4.0 mg/kg 54.8 ± 1.2, comparable to the number exhibited by saline controls. Thus, *d*-naloxone by itself did not exert any observable effect on locomotor activity.

When amphetamine and *l*-naloxone were injected simultaneously, locomotor activity was altered significantly. After treatment with 0.5 mg/kg of *l*-naloxone amphetamine-induced motility was 92.5 ± 10.3. A dose of 1 mg/kg canceled the effect of 2 mg/kg amphetamine (Fig. 2). A higher dose, 2 mg/kg, along with 2 mg/kg amphetamine, caused a drop in motility to a level below that of control subjects. When *d*-naloxone, 2 mg/kg, was injected along with amphetamine, the animals showed an average of 86.8 ± 6.0 crossings/min, lower than amphetamine-treated subjects but higher than controls. A dose of 4 mg/kg of *d*-naloxone, along with amphetamine, brought the locomotor activity to control levels, an effect comparable to that of 1.0 mg/kg of the *l*-isomer.

In the last experiment, a group of mice treated with 2.0 mg/kg amphetamine, which tripled their motility, received a second treatment in 30 min. One-half received 4 mg/kg of *d*-naloxone and the other half received saline. Unlike the saline-treated animals, *d*-naloxone–treated mice rapidly decreased their locomotor activity (Fig. 3). Within 20 min after the second treatment the number of crossings/min of the amphetamine subjects treated with *d*-naloxone decreased to control levels, while the motility of the amphetamine subjects given placebo remained elevated. Thus, *d*-naloxone was effective in counteracting amphetamine excitation not only when injected simultaneously with the stimulant but also when injected at a time when the stimulant effect was near its peak.

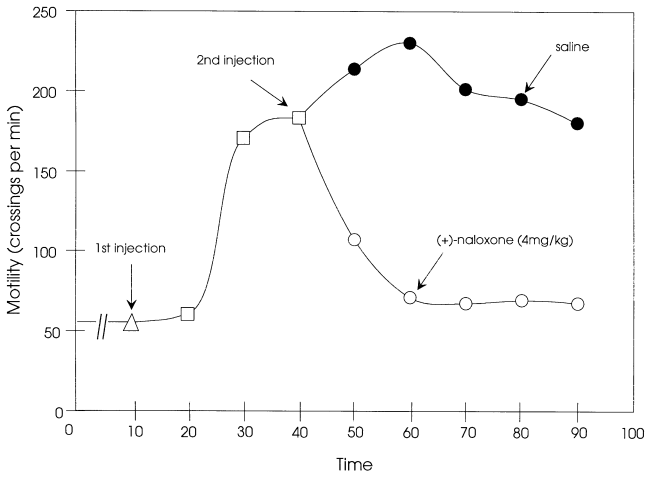

FIG. 3. Motility after treatment with amphetamine (2 mg/kg) (first injection), followed 30 min later by either saline or *d*-naloxone (4.0 mg/kg) (second injection).

DISCUSSION

Among the known effects of amphetamine is its interference with dopamine and norepinephrine pathways in the CNS (5,11,12,17). We have used amphetamine to lower the levels of brain norepinephrine and thereby decrease susceptibility to convulsive seizures in rodents (1,2). The relationship between amphetamine and opiate antagonists has been explored in a large number of studies over the years, with a consensus that the two compounds counteract each other (3,5,12,14–17).

The primary mode of action of *l*-naloxone is to block the μ-opiate receptor sites in the CNS (13,19). However, naloxone, with its five chiral carbons, can exist in 32 isomeric forms, only one of which, *l*-naloxone, has been found to act as an opiate antagonist. The mirror image isomer, *d*-naloxone, shows almost no opiate antagonist activity: it is 1,000–10,000 times less effective in binding opiate sites than is *l*-naloxone (11,13). However, *d*-naloxone seems to affect peripheral muscular events, and it is useful in dealing with cardiac arrhythmias (6,18).

The properties of *d*-naloxone have not been extensively investigated in part because of the difficulties in obtaining it in sufficient amounts. We have a limited supply and have tested it *in vivo* in mice against cocaine-induced excitation (10). Preliminary results of our studies of the interaction between *d*-naloxone and amphetamine have been reported (4,7). We now show that, while less effective than *l*-naloxone, it can prevent amphetamine from enhancing locomotor activity.

Many researchers have reported that opiate antagonists affect amphetamine activity (5,12,14–16). It was tacitly assumed, however, that this effect of naloxone or naltrexone is due to their opiate antagonist activity. In view of the data presented here, this assumption needs to be modified. An isomer of naloxone that does not affect opiate receptors is also capable of interfering with amphetamine-induced hyperactivity.

Heretofore, the use of opiate antagonists as a treatment modality in cases of amphetamine intoxication was somewhat limited by the fact that these compounds can enhance pain perception and can prevent endogenous opiates from alleviating pain and trauma. The finding that an enantiomer of naloxone, with no opiate antagonist properties and no effect on locomotor activity per se, is also able to block agitation caused by amphetamine, may lead to a discovery of an entirely new class of chemicals able to counteract drug-induced excitation. Other compounds in this class and their spirohydantoin and benzoylhydrazone derivatives (8,9) merit a careful investigation.

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