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Calcium Antagonists Isradipine and Nimodipine Suppress Cocaine and Morphine Intravenous Self-Administration in Drug-Naive Mice

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KUZMIN, A., E. ZVARTAU, G. L. GESSA, M. C. MARTELLOTTA AND W. FRATTA. *Calcium antagonists isradipine and nimodipine suppress cocaine and morphine intravenous self-administration in drug-naive mice.* PHARMACOL BIOCHEM BEHAV **41**(3) 497–500, 1992.—The effect of isradipine and nimodipine, two dihydropyridine calcium antagonists, on intravenous self-administration of cocaine and morphine in naive mice has been investigated. When morphine or cocaine injections were made contingent upon nose-poke response by naive mice, they increased their rate of nose-poking with respect to animals receiving contingent saline injections or yoked control animals, receiving noncontingent cocaine or morphine injections. Pretreatment of mice with isradipine (1.0–3.0 mg/kg, SC) or nimodipine (5–20 mg/kg, SC) inhibited in a dose-related manner self-administration both of cocaine and morphine contingent upon a nose-poke response. The ED_{50} of isradipine against cocaine and morphine self-administration was 1.7 and 2.1 mg/kg, respectively. The relative values for nimodipine were 14.5 and 11.4 mg/kg, respectively. These data suggest that nimodipine and, especially, isradipine suppress the reinforcing properties of morphine and cocaine and may be an effective pharmacotherapy for treatment of cocaine and heroin abuse.

Cocaine Morphine Isradipine Nimodipine Self-administration by naive mice

CONSIDERABLE evidence indicates that dopaminergic neurons, particularly of the mesolimbic system, are critically involved in the rewarding action of cocaine and morphine (1,7,15,18). Accordingly, recent studies showed that morphine and cocaine increase the output of dopamine (DA) in the ventral striatum and nucleus accumbens, as measured by microdialysis (5). Whereas morphine-induced DA release seems to be secondary to the activation of DA neurons in the ventrotegmental area (10), cocaine effect is attributed to the blockade of the reuptake of DA released by nerve impluse (3,7). As expected from a nerve-impulse-dependent mechanism, both morphine- and cocaine-induced DA output is calcium dependent, as indicated by the fact that removal of Ca^{2+} from perfusion medium in microdialysis experiments has been shown to abolish the effect both of cocaine and morphine on DA output (2). In agreement with the foregoing premises, we recently showed that two dihydropyridine calcium channel antagonists, isradipine and nimodipine, are able to inhibit not only cocaine-induced DA release in the rat ventral striatum, as measured by in vivo microdialysis (12), but also cocaine-induced motor stimulation (13) and cocaine-induced place preference in rats (14). Moreover, results in preparation from our laboratory have shown that isradipine also abolishes morphine-induced DA output in the rat ventral striatum and nucleus accumbens.

The aim of the present study was to determine the ability of isradipine and nimodipine to inhibit the reinforcing properties of cocaine and morphine as measured by the intravenous self-administration technique in drug-naive mice.

METHOD

Animals

Swiss albino mice aged about 2 weeks and weighing 20–24 g were used. Upon arrival, animals were housed six per cage and acclimated to the laboratory conditions (12L : 12D cycle,

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lights on at 0800 h, 21 ± 1°C room temperature, and 60% relative humidity) for at least 1 week prior to use. Food and water were available ad lib until the time of testing.

Drugs

Cocaine HCl and morphine HCl were obtained from Sigma (USA), naloxone HCl was obtained from Endo Lab. (USA), isradipine was a gift from Sandoz (Italy) and nimodipine from Bayer (Italy), and diazepam was obtained from Hoffman-La Roche (Basel, Switzerland). Cocaine, morphine, and naloxone were freshly dissolved in saline, isradipine, nimodipine, and diazepam in 9% Tween-80 solution in distilled water. On the basis of previous experiments (12-14), the calcium antagonists were injected SC 80 min prior to the experiment at doses that did not modify spontaneous motor activity. Naloxone was injected SC 3 min prior to the experiment. Dosages are referred to as the base substances.

Technique

Experiments were performed between 1300 and 1600. The technique described by Criswell and Ridings (4) was modified as follows. Mice were tested in pairs of identical test cages (8 × 8 × 8 cm); the "active" mouse was placed in one cage and the "yoked passive" mouse was confined to the other cage. Each test cage presented a frontal hole for nose-poking and a vertical chink, in the back wall, through which the mouse's tail was extended outside the box and taped to a horizontal surface allowing access to the lateral tail veins for intravenous injections, as well as ensuring a firm hold on the animal. The frontal hole was provided with an infrared detector for nose-poke response (NPR) recording. Each nose-poke of the active mouse resulted in a contingent injection of 1.4 μl of either saline or of the drug dissolved in saline both to the active mouse and to the yoked passive mouse. Therefore, the passive yoked mouse received the same amount of a drug at the same time intervals as the active mouse. Nose-pokes of the yoked control were counted but had no programmed consequences. Responses of the yoked control served as a measure of the effect of the drug treatment on motor activity and operant behavioral output.

After the first 10 min of habituation in the test cage, an IV injection was made contingent upon each nose-poke of the active animal. As a measure of the reinforcing effect of a drug the ratio, R, between the cumulative number of NPR's of the active and passive mouse during a 30-min period was used. The effect of the drug was considered reinforcing, neutral, and negative reinforcing when R was higher, equal, and smaller than 1, respectively.

Statistic

The significance of values with respect to the level of saline self-administration has been calculated with the aid of the Mann-Whitney U-test. The ED_{50} of drugs has been calculated with the aid of the Litchfield-Wilcoxon method.

RESULTS

There was no statistically significant difference in the mean number of NPR's of the active and passive mice when saline injections were contingent upon NPR's. Therefore, R in these conditions was not different from unity. Cocaine influenced R in a concentration-dependent manner (Table 1). Thus, the lowest concentration tested, 1 mg/ml, failed to modify R because it did not modify NPR's in either the active or the passive mouse. The concentrations of 2 and 3 mg/ml markedly increased NPR's of the active mice, but had no significant effect in the nose-poking of the passive ones; therefore, these concentration increased R and are considered reinforcing. The highest cocaine concentration tested, 4 mg/ml, reduced NPR's in the active mouse to a greater extent than in the passive one; therefore, it reduced R and may be considered a negative reinforcing effect.

TABLE 1

CONCENTRATION-DEPENDENT REINFORCING EFFECT OF COCAINE IN DRUG-NAIVE MICE

Cocaine Concentration (mg/ml)	Cumulative NPR in 30 min: Active Mouse (A)	Cumulative NPR in 30 min: Passive Mouse (P)	(No. of Pairs)	R (A/P)
(saline) 0	21.6 ± 5.3	26.0 ± 9.3	(25)	0.8
1.0	24.2 ± 3.5	26.4 ± 6.5	(20)	0.9
2.0	49.7 ± 2.7*†	18.3 ± 1.5	(18)	2.7
3.0	72.7 ± 15.3*†	25.3 ± 7.7	(24)	2.9
4.0	5.7 ± 1.1*†	18.5 ± 4.6	(16)	0.3

An IV administration of 1.4 μl cocaine at the indicated concentration was made contingent upon each NPR of the active mouse.

Each value is the mean ± SEM of the number of mice indicated in parentheses.

*$p < 0.01$ with respect to mouse self-administering saline.

†$p < 0.01$ with respect to the yoked passive mouse.

Morphine concentrations of 0.5 and 1 mg/kg decreased NPR's in the passive mouse, but did not modify and increased, respectively, NPR's in the active mouse (Table 2). Therefore, these concentrations, by increasing R, produced a positive reinforcement. The concentration of 2 mg/kg reduced nose-poking both in the passive and to a greater extent in the active mouse. Therefore, this concentration may be considered negative reinforcing.

The calcium antagonists, isradipine and nimodipine, were tested against the concentrations of cocaine and morphine that produced the maximal reinforcement, that is, 2 and 1 mg/ml, respectively. On the basis of previous observations (12,14), isradipine and nimodipine were given at doses ranging

TABLE 2

CONCENTRATION-DEPENDENT REINFORCING EFFECT OF MORPHINE IN DRUG-NAIVE MICE

Morphine Concentration (mg/ml)	Cumulative NPR in 30 min: Active Mouse (A)	Cumulative NPR in 30 min: Passive Mouse (P)	(No. of Pairs)	R (A/P)
0 (saline)	21.6 ± 5.3	26.2 ± 9.2	(25)	0.8
0.5	22.7 ± 4.3†	14.1 ± 3.4	(20)	1.6
1	31.6 ± 3.7*†	12.4 ± 2.6	(18)	2.5
2	8.4 ± 5.1*†	16.0 ± 6.4	(16)	0.5

An IV administration of 1.4 μl morphine at the indicated concentration was made contingent upon each NPR of the active mouse.

Each value is the mean ± SEM of the number of mice indicated in parentheses.

*$p < 0.01$ with respect to mouse self-administering saline.

†$p < 0.01$ with respect to the yoked passive mouse.

between 1-3 and 5-20 mg/kg, respectively, 80 min prior to the test. As shown in Table 3, isradipine and nimodipine inhibited the reinforcing effect of cocaine in a dose-dependent manner. A dose of isradipine of 1 mg/kg selectively decreased the number of NPR's of the active mouse contingent upon cocaine injections, but failed to modify nose-poking in the passive one. Higher isradipine doses decreased NPR's in both animals. Nimodipine, 10 mg/kg, selectively inhibited NPR's in the active mouse, while the dose of 20 mg/kg inhibited nose-poking in both animals. Naloxone at the dose of 1 mg/kg failed to influence the reinforcing effect of cocaine. As shown in Table 4, isradipine and nimodipine inhibited in a dose-related manner the reinforcing effect of morphine. In fact, doses of 2 and 10 mg/kg, respectively, decreased the number of NPR's of the active mouse, but did not modify it in the passive mouse. The higher doses tested reduced the responses both in the active and passive mice. Finally, naloxone antagonized, as expected, the reinforcing effect of morphine, and also reduced the suppressant effect of morphine on NPR in the passive mouse.

To evaluate if possible stress effects could interfere with the self-administration pattern, mice were pretreated with diazepam 0.5 mg/kg IP 30 min before starting the self-administration session. This dose of diazepam was chosen as the highest dose that did not modify spontaneous motor activity. As shown in Table 5, diazepam pretreatment failed to significantly modify either cocaine or morphine self-administration patterns with respect to vehicle-pretreated mice.

DISCUSSION

The method of self-administration by naive mice has a great advantage over other methods in which trained animals are used because it allows a rapid screening of drugs in a large number of animals. The yoked passive animal receiving the same amount of drug as the active one may provide an index of the unspecific effects of the drug on motor activity and coordination. Validity of the method is confirmed by the fact that, in spite of the large individual variabilities in naive animals, the mean number of NPR's of active and passive animals was not statistically different when saline injection was made contingent upon the response, whereas NPR's of the active mouse selectively increased when injection of a proper concentration of cocaine and morphine was contingent upon nose-poking. The finding that naloxone suppressed morphine but not cocaine self-administration further supports the validity of the method for demonstrating the reinforcing properties of drugs. Our results have shown that the reinforcing effect of morphine and cocaine in drug-naive mice is concentration dependent according to a bell-shaped curve. As expected from previous studies (9), high doses of these drugs become negative reinforcing. The mechanism by which isradipine and nimodi-

TABLE 3

ANTAGONISM BY ISRADIPINE AND NIMODIPINE OF THE REINFORCING EFFECT OF COCAINE (2 mg/ml) IN DRUG-NAIVE MICE

Pretreatment (mg/kg)	Cumulative NPR in 30 min: Active Mouse (A)	Cumulative NPR in 30 min: Passive Mouse (P)	(No. of Pairs)	R (A/P)
Vehicle	49.7 ± 2.7†	18.3 ± 1.5	(18)	2.7
Isradipine				
1.0	23.3 ± 3.6*	21.2 ± 7.1	(20)	1.1
2.0	5.5 ± 1.1*	5.7 ± 1.3	(15)	0.9
3.0	3.3 ± 0.4*	4.5 ± 0.8	(15)	0.7
Nimodipine				
5.0	57.4 ± 11.2†	26.3 ± 9.1	(10)	2.2
10.0	29.2 ± 6.5*	22.4 ± 7.1	(20)	1.3
20.0	8.1 ± 0.8*	8.9 ± 2.2	(10)	0.9
Naloxone				
1.0	55.1 ± 16†	22.2 ± 8.1	(11)	2.5

An IV administration of 1.4 μl cocaine at the indicated concentration was made contingent upon each NPR of the active mouse.

Each value is the mean ± SEM of the number of mice indicated in parentheses.

*$p < 0.01$ with respect to mouse pretreated with vehicle.

†$p < 0.01$ with respect to yoked passive mouse.

TABLE 4

ANTAGONISM BY ISRADIPINE, NIMOPIDINE, AND NALOXONE OF THE REINFORCING EFFECT OF MORPHINE (1 mg/ml) IN DRUG-NAIVE MICE

Pretreatment (mg/kg)	Cumulative NPR in 30 min: Active Mouse (A)	Cumulative NPR in 30 min: Passive Mouse (P)	(No. of Pairs)	R (A/P)
Vehicle	31.6 ± 3.7†	12.4 ± 2.6	(18)	2.5
Isradipine				
1.0	25.1 ± 6.1†	13.5 ± 3.1	(11)	1.8
2.0	16.3 ± 1.9*	11.3 ± 4.1	(20)	1.4
3.0	5.1 ± 0.8*	6.1 ± 3.5	(15)	0.8
Nimodipine				
5.0	35.6 ± 3.8†	18.4 ± 2.6	(25)	1.9
10.0	18.3 ± 4.1†	9.1 ± 1.6	(16)	2.0
20.0	9.6 ± 3.1*	8.6 ± 2.1	(10)	1.1
Naloxone				
1.0	18.0 ± 2.1*	24.2 ± 3.1	(10)	0.7

An IV administration of 1.4 μl morphine at the indicated concentration was made contingent upon each NPR of the active mouse.

Each value is the mean ± SEM of the number of mice indicated in parentheses.

*$p < 0.01$ with respect to mouse pretreated with vehicle.

†$p < 0.01$ with respect to the yoked passive mouse.

TABLE 5

FAILURE OF DIAZEPAM TO MODIFY THE REINFORCING EFFECT OF COCAINE (2 mg/ml) AND MORPHINE (1 mg/ml) IN DRUG-NAIVE MICE

Pretreatment	Drug Infused	Cumulative NPR in 30 min: Active Mouse (A)	Cumulative NPR in 30 min: Passive Mouse (P)	(No. of Pairs)	R (A/P)
Vehicle	Cocaine	44.8 ± 6.3	19.6 ± 4.7	(14)	2.4
Diazepam	Cocaine	41.2 ± 5.8	21.4 ± 5.2	(16)	1.9
Vehicle	Morphine	35.3 ± 4.9	16.4 ± 3.5	(13)	2.2
Diazepam	Morphine	33.9 ± 3.8	17.6 ± 4.2	(15)	1.9

An IV administration of 1.4 μl cocaine or morphine at the indicated concentration was made contingent upon each NPR of the active mouse.

Each value is the mean ± SEM of the number of mice indicated in parentheses.

pine suppress cocaine and morphine self-administration is not clear. It is possible that the inhibition of the reinforcing effect of cocaine and morphine is due to the ability of these calcium antagonists to inhibit cocaine- and morphine-induced DA output in the ventral caudate and nucleus accumbens (13). This effect may, in turn, be dependent on the ability of the calcium antagonists to block L-type calcium channels (8,11). Accordingly, the relative potency of isradipine and nimodipine in suppressing cocaine and morphine self-administration is similar to their relative potency in inhibiting cocaine-induced DA release in the ventral striatum (12,13). The different potency between isradipine and nimodipine might be due, at least in part, to differences in pharmacokinetic and capability to cross the blood–brain barrier.

The site of action of calcium channel antagonists in inhibiting cocaine- and morphine-induced DA release is not known. In vitro experiments seem to exclude the possibility that L-type calcium channel blockers may act on DA nerve terminals (6,16).

Irrespective of the mechanism involved, the finding that nimodipine and especially isradipine are able to potently suppress cocaine- and morphine-reinforcing effects is of great practical interest because it suggests that these calcium antagonists may be useful for treatment of cocaine and heroin abuse, as well as the simultaneous abuse of the two, a rather common clinical condition.

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