Pergamon

Pharmacology Biochemistry and Behavior, Vol. 51, Nos. 2/3, pp. 521-523, 1995

0091-3057/95 $9.50 + .00

0091-3057(95)00046-1

Role of Dopamine D_1 Receptors in Cocaine Lethality

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Received 16 July 1994

SCHECHTER, M. D. AND S. M. MEEHAN. *Role of dopamine D_1 receptors in cocaine lethality.* PHARMACOL BIOCHEM BEHAV **51**(2/3) 521-523, 1995. – One group of heterogeneously bred HS mice was assigned to test coadministration of the selective D_1 antagonist SCH 23390 with a dose of cocaine (95 mg/kg) that was observed to produce 80% lethality, whereas a second group was tested by cotreatment with the newly developed full-efficacy D_1 agonist dihydrexidine (DHX) and a dose of (60 mg/kg) cocaine previously shown to be nonlethal. The mice in the former group displayed decreased lethality going from 80% with coadministered vehicle to 15% after pretreatment with the highest dose (0.45 mg/kg) of SCH 23390. In the other group of mice there was no lethality seen when vehicle or 10 mg/kg DHX was coadministered with 60 mg/kg cocaine, but a dose-responsive increase in lethality with increasing DHX doses; the maximal lethality of 80% occurred when 25 mg/kg DHX was coadministered with cocaine. These results confirm the effects of D_1 antagonism decreasing cocaine lethality as reported previously when rats were used, and extend the findings to D_1 agonism; both observations evidence a role for the D_1 receptor in the lethal effects, be they central, cardiopulmonary, or anesthetic, of cocaine.

Cocaine Dopamine SCH 23390 Dihydrexidine HS mice Lethality

THE USE OF cocaine in the United States has reached epidemic proportions and has been reported to be the leading cause of drug-related deaths (9,10,13). Most deaths from cocaine abuse occur shortly after it is used and presumably result from CNS stimulation that causes seizures, hyperthermia, ventricular fibrillation, and/or respiratory arrest (10). Although the mechanism of action by which cocaine induces these effects is unknown, and probably is not the result of cocaine activity upon only one single neurotransmitter system in the brain, the euphorigenic effects of cocaine have generally been ascribed to its ability to increase the concentrations of brain dopamine by blockade of its reuptake (8). Because cocaine acts as a dopamine reuptake inhibitor, it would seem that this increased synaptic dopamine would be able to act upon any and all postsynaptic dopaminergic receptors, two of which have been identified, classified, and named D_1 and D_2 (6). The advent and discovery of a specific antagonist to the D_1 dopamine receptor, SCH 23390, has allowed research to suggest that selective stimulation of this site produces the toxicity reflected in cocaine overdose. Thus, rats administered a lethal dose of cocaine were shown to have a decreased incidence of cocaine-induced death after pretreatment with SCH 23390. This significant reduction in lethality suggested to at least two different laboratories (2,22) that the D_1-type dopamine receptor plays a role in the lethality produced by cocaine and, furthermore, that drugs of this type may prove to be antidotes to cocaine overdose in humans.

This laboratory was the site for recent experiments using the HS strain of mice developed by the Institute of Behavioral Genetics at the University of Colorado at Boulder, where the foundation population was drawn from a genetically heterogeneous stock produced by intercrossing eight inbred mouse lines (13). Employing these mice may help to reduce the reported (11) significant differences in the acute sensitivity to cocaine between mouse strains. These experiments have allowed determination of the LD_{50} and LD_{80} doses of cocaine (17) and suggest that the ability of cocaine to produce seizures may be independent of its lethal effects (18). The recent synthesis and in vitro characterization of a new class of rigid dopamine analogs has allowed for the development of the first potent D_1 agonist that is fully efficacious in stimulating dopamine-sensitive adenylate cyclase (1). This compound, named dihydrexidine (DHX), can allow for the specific activation of D_1 receptors to be tested in behavioral experimentation much as the past use of the selective D_1 antagonist SCH 23390 has allowed for the blockade of these same receptors to be

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investigated. The purpose of the present experimentation was therefore to use the HS line of mice in an effort to determine how an agent with specific activity at D_1 receptors functions when coadministered with a dose of cocaine shown to be too low to produce lethality (17). This work also extends research conducted in rats (2,22) by using the selective D_1 blocker SCH 23390 as pretreatment before a dose of cocaine shown (17) to produce 80% lethality in this line of mice.

METHOD

Subjects and Procedure

A total of 180 (90 male and 90 female) experimentally naive HS mice were used to determine the lethality of cocaine with and without pretreatment with either a D_1 agonist or antagonist. Animals were individually housed in 29 × 18 × 12-cm clear Plexiglas cages containing bedding of aspen shavings and equipped with a wire top. The animals were maintained in a climate-controlled colony room on a 12 L : 12 D cycle with lights on at 0600 h. Food and water were available ad lib, and all mice were 30–32 days of age at the beginning of experimentation.

All procedures were conducted between 1200 and 1800 h, at which time the animals were transported in their home cages from the colony room to an immediately adjacent area. Each mouse was randomly assigned a drug combination and dose level, weighed, and then briefly placed into a clear Plexiglas cage identical to its home cage, but without the bedding. Animals were then injected with either vehicle, SCH 23390, or DHX intraperitoneally (IP) with the total volume injected kept constant and replaced into this test chamber for a 30-min period. During this time, observations were made to determine whether the pretreatment had any effect on their behavior and produced toxicity or lethality. Animals were then injected IP on the contralateral side with an equal volume of either 60 or 95 mg/kg cocaine and replaced into the Plexiglas cage, and a timer was set. Animals were monitored over a 120-min period for indications of toxicity and lethality. Death was operationally defined in this study as the absence of respiration for a period > 30 s. No mouse defined by this criterion recovered. Animals surviving beyond the 120-min observation period remained in the procedure cage and were returned to the holding area with food and water available ad lib. As has been reported in the literature (3), no deaths were observed in mice after 30 min. They were examined again 16 and 24 h and, after this time, all animals were euthanized by CO inhalation.

Determination of Lethality After Cotreatment With SCH 23390 and a Lethal Dose (95 mg/kg) of Cocaine

Animals were randomly assigned to one of two treatment groups: D_1 agonist pretreatment and D_1 antagonist pretreatment. This latter group consisted of 80 mice (40 male and 40 female) randomly assigned to receive either the vehicle used to dissolve SCH 23390 or 0.15, 0.3, or 0.45 mg/kg SCH 23390 injected IP 30 min before the administration of a cocaine dose (95 mg/kg) previously determined (17) to produce lethality in 80% of HS mice. The first administration was IP in a volume of 10 ml/kg, whereas the second, similar volume containing cocaine was administered on the contralateral side of the lower quadrant IP 30 min later, and the animals were immediately placed into the observation chamber.

Determination of Lethality of Coadministered DHX and a Nonlethal Dose (60 mg/kg) of Cocaine

Likewise, 100 mice (50 of each sex) were randomly assigned to be administered one of five treatments: 0, 10, 15, 20, and 25 mg/kg DHX, 30 min before the contralateral IP administration of a dose of cocaine (60 mg/kg) previously shown to be nonlethal (LD_0) (17). The time course between administrations was again 30 min, and the animals were placed into the Plexiglas observation chamber immediately after the second injection.

Statistical Analysis

The ED_{50} value, with 95% confidence limits, of DHX (dose that produced 50% lethality when coadministered with a nonlethal dose of cocaine) and the IC_{50} value of SCH 23390 (dose that decreased lethality when coadministered with a lethal dose of cocaine to 50%) were derived using a computerized version of the Litchfield-Wilcoxon analysis (19).

RESULTS

The results of the experimentation using both male and female mice (n = 10/dose) indicated no significant difference in lethality between the two sexes; therefore, the genders were combined so as to allow for an n = 20/dose. This replicates a previous finding in the same line of mice (Fig. 1) (17). The results of administration of various doses of DHX with the LD0 (60 mg/kg) cocaine, determined previously (17), is represented in Fig. 1 (left side). It may be seen that 10 mg/kg DHX had no effect on lethality but increasing doses of DHX increased the lethality of cocaine administration with the maximum dose of 25 mg/kg DHX plus 60 mg/kg cocaine, allowing for seven of 10 males and eight of 10 females, or 75% of all mice, to die within the 30-min observation period.

Figure 1 (right side) also indicates the results of administration of the vehicle used to dissolve SCH 23390, as well as doses of 0.15, 0.3, and 0.45 mg/kg SCH 23390 preadministered 30 min before 95 mg/kg cocaine. This dose of cocaine was shown to produce mortality in 80% of the mice tested, and increasing doses of SCH 23390 produced decreasing lethality with the

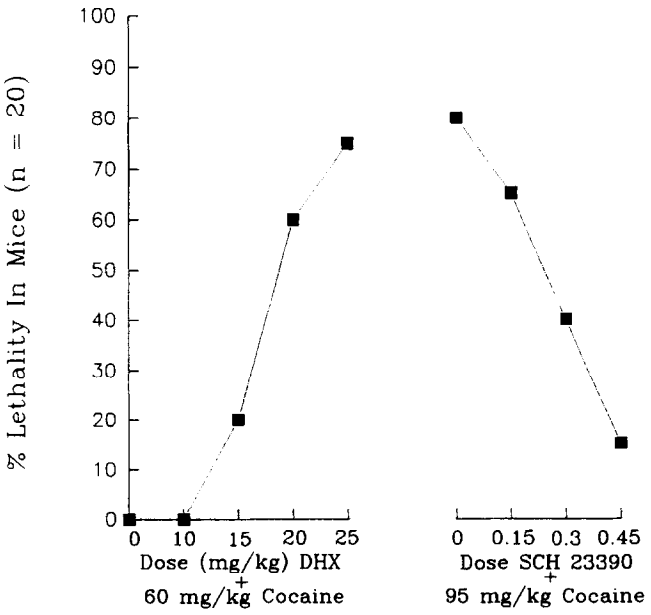

FIG. 1. Lethality of IP coadministered dihydrexidine (DHX) with 60 mg/kg cocaine (left panel) or SCH 23390 with 95 mg/kg cocaine (right panel) in HS mice (n = 20/dose).

highest dose tested (0.45 mg/kg), allowing for two of 10 males and one of 10 females, or 15% of all mice tested, to die within the 30-min observation period.

The ED_{50} value (95% confidence limits) of DHX was calculated (19) to be 19.6 (17.7–21.7) mg/kg, whereas the IC_{50} dose of SCH 23390 (that allowed for a 50% reduction in the lethal effects of 95 mg/kg cocaine) was 0.21 (0.16–0.28) mg/kg.

DISCUSSION

The lethality of cocaine is frequently associated with its effects on heart and brain, and these activities appear to be related to its central and peripheral sympathomimetic properties (5,20). In addition to the stimulation of adrenergic systems by cocaine, its anesthetic properties may contribute to its cardiotoxicity, as manifested by arrhythmias and myocardial infarct (7). Nonetheless, the dopaminergic activation produced by cocaine is the most frequently associated reason for its lethal activity (15). The present work lends evidence to the fact that the D_1 receptor mediates the lethality of cocaine in the HS mouse. Thus, coadministration of the fully effective D_1 receptor-specific agonist dihydrexidine (DHX) with a dose of cocaine (60 mg/kg), both of which produce no deaths, resulted in a dose-related occurrence of lethality. In contrast, pretreatment with the selective D_1 antagonist SCH 23390 before the LD_{80} (95 mg/kg) cocaine was observed to dose-respectively decrease lethality (Fig. 1). This latter effect has been previously observed to occur in rats in which 1.0 mg/kg SCH administered IP 10 min before the LD_{85} dose (70 mg/kg) cocaine reduced lethality to 35%, whereas 0.3 mg/kg SCH 23390 administered 30 min before 100 mg/kg cocaine reduced the lethality of the rats from 80 to 40% (2,22).

Dihydrexidine is approximately 10 times more selective for striatal D_1 over D_2 receptors, and it has been shown to be essentially devoid of receptor affinity to other neurotransmitters (14). The D_1 receptor blocker SCH 23390 was first described in 1983 (4), and the literature is replete with indications that it effectively blocks this receptor [for a review, see (21)]. Evidence of the opposing effect of these agonist-antagonist compounds upon the D_1 receptor site has been evidenced in this laboratory in that rats trained to differentially discriminate the interoceptive cueing effects of DHX were shown to have this capability blocked by pretreatment with SCH 23390 (16). In conclusion, the D_1 receptor antagonist SCH 23390 appears to provide protection against cocaine-induced lethality in the HS mouse. This suggests that not only may the D_1 receptor site mediate the cocaine-induced lethality, but that SCH 23390 may be added to the list of compounds possibly effective in preventing lethality in human abusers. The observation that a fully effective D_1 agonist DHX increases lethality would provide further evidence that the D_1 receptor may be the site of lethal cocaine activity.

ACKNOWLEDGEMENTS

The authors express their thanks to Dr. A. Barnett of Schering Pharmaceuticals for donating the SCH 23390, Dr. Brian F. Thomas of the Research Triangle Institute for supplying the dihydrexidine, the National Institute on Drug Abuse for the cocaine hydrochloride, Dr. Andrew Smolen of the University of Colorado at Boulder for the HS mice, and Sheila Formick and Marty Hilgert for their word-processing expertise.

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