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Apomorphine Fails to Inhibit Cocaine-Induced Behavioral Hypersensitivity

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RIFFEE, W. H., E. WANEK AND R. E. WILCOX. *Apomorphine fails to inhibit cocaine-induced behavioral hypersensitivity*. PHARMACOL BIOCHEM BEHAV **29**(2) 239–242, 1988.—The subchronic administration of cocaine will induce a behavioral sensitization to challenge doses of the drug administered several days after cessation of treatment. This sensitization is similar behaviorally to that observed for other stimulants such as amphetamine. Similarities and differences in the sensitization induced by cocaine and amphetamine (which are thought to have different mechanisms of actions although common behavioral outcomes) have not been thoroughly studied. The purpose of the present experiment was to examine the effects of these two drugs on basic horizontal locomotion and changes occurring subsequent to their subchronic administration in mice. Cocaine and amphetamine were administered acutely in various doses to compare time and dose responses in the behavioral paradigm used (infrared detection of horizontal locomotion). Subsequently, cocaine (10 mg/kg) or amphetamine (2.5 mg/kg) were administered twice a day for 5 days and the animals challenged 3 days after the last treatment with the same doses received subchronically. Two other groups of mice received the same subchronic treatment and in addition were administered 80 μg/kg apomorphine (5 to 15 min after each dose of the stimulant) and then tested for their response to challenge doses of the stimulants 72 hours after the last pretreatment dose. Acutely, cocaine produced a maximum locomotor activity that was significantly lower than that of amphetamine and the former had a much shorter duration of action than the latter. After subchronic administration, both stimulants induced sensitization, however, apomorphine inhibited the sensitization induced by amphetamine but failed to do so in the cocaine-treated animals. Possible mechanisms for these differences are discussed.

Cocaine Amphetamine Sensitization Locomotor activity Apomorphine

THE subchronic administration of cocaine induces a sensitization to the locomotor [10,16] and stereotypic [8] responses to challenge doses of the drug following the cessation of the treatment. This sensitization has been suggested to be essentially the same as that observed for amphetamine [9] which has been well described [1, 5, 11]. Hypotheses regarding the biological basis of sensitization to amphetamine include: (1) an increase in postsynaptic dopamine (DA) receptors; (2) an increase in DA synthesis; (3) an increase in DA utilization and/or release; and (4) a decrease in DA autoreceptors [14]. Robinson and Becker [14] further state that there is not convincing evidence for an increase in postsynaptic DA receptors or in DA synthesis in animals sensitized to amphetamine. In contrast, they suggest that there is convincing evidence to support the notion that behavioral sensitization is due to enhanced mesotelencephalic DA release, especially upon re-exposure to the drug. Other studies by Robinson and Becker [13] have shown that subchronic treatment of rats with amphetamine enhances the release of dopamine from striatal slices while others [6] have shown that similar treatment with methylphenidate enhances the release of dopamine from slices of nucleus accumbens. These results suggest that changes in the release processes and in the concentration of dopamine maintained in the synaptic cleft may play a role in the development of amphetamine-induced behavioral hypersensitivity.

The research surrounding the hypothesis that this increase in synaptic DA causes autoreceptor subsensitivity and that this phenomenon may underlie the changes in release, has been equivocal and thus other mechanisms must be explored. In addition, it has not been established that sensitization induced by amphetamine and uptake inhibitors such as cocaine have similar neurological bases.

We [11] and others [3] have shown that acute administration of apomorphine in low microgram doses blocks amphetamine-stimulated locomotor activity. We have also observed that apomorphine inhibits the locomotor activity induced by indirect-acting stimulants such as cocaine, amfonelic acid and methylphenidate (data unpublished). More recently, we have shown that concomitant administration of

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apomorphine with amphetamine subchronically, eliminated the amphetamine-induced sensitization. Our objective in this study was to observe possible sensitization induced by cocaine and to determine the effects of a "presynaptic dose" of apomorphine administered subchronically in combination with the cocaine on cocaine-induced behavioral sensitization in mice.

METHOD

General

Naive male albino CD-1 mice weighing 20–30 g were used throughout the study. They were maintained in the Animals Resources Center of the University of Texas. The mice had continual access to food and water but were food-deprived 24 hr prior to testing. A 12 hr light/dark cycle (lights on at 7 a.m.) was maintained and all testing was done between the hr of 9 a.m. and 5 p.m. Drugs used in the experimentation were R-(−)-apomorphine (MacFarland Smith, Edinburgh, Scotland), (+)-amphetamine and cocaine sulfate (Sigma, St. Louis, MO). Drugs were prepared without preservatives immediately prior to use.

Locomotor Activity

Locomotor activity was measured as described earlier [11] using Digiscan infrared activity monitors (Omnitech Electronics, Columbus, OH). All animals were pretreated with saline and given a one hour habituation to the test chambers. The mice were then administered cocaine or amphetamine and returned to the test environment. Locomotor activity was recorded for 60 min. Data from the detectors represented actual distance traveled (in inches) per 5-min period. A microprocessor measures the actual distance traveled by the animal and not merely the number of light beams interrupted. Continuous interruption of one beam by a behavior such as head-bobbing would not be recorded as total distance traveled but as stereotypic activity. Preliminary studies showed that a second saline injection to control for the concomitant apomorphine injection, produced results identical to those mice receiving only the single saline injection twice a day for five days. Therefore, in subsequent experiments, one control group was used for both the experiments where the stimulant drug was administered alone subchronically and for the experiments in which the stimulant and apomorphine were administered concomitantly for the subchronic treatment period. Data analysis was done using analysis of variance and post-hoc Newman-Keuls tests for significance.

Acute administration of cocaine was done using 2.5–10 mg/kg of the drug to compare dose and time response with those of acute administration of amphetamine 1.25–5 mg/kg. Mice treated subchronically were administered saline, cocaine (10 mg/kg) IP, amphetamine (2.5 mg/kg) IP, or vehicle (isotonic saline) twice a day (12 hr apart) for 5 days. Each of these pretreatments was followed in 5 min (for cocaine) or 15 min (for amphetamine) by the administration of apomorphine 80 μg/kg subcutaneously. The control groups were treated in an identical manner substituting isotonic saline for drug injections. The animals were tested for locomotor activity with challenge doses (10 and 2.5 mg/kg, respectively) of cocaine and amphetamine 3 days after the cessation of the subchronic treatment.

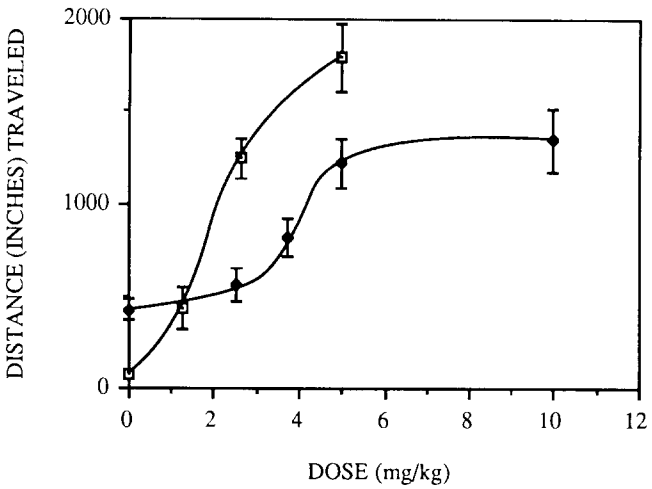

FIG. 1. Dose-response relationship for amphetamine and cocaine-induced locomotor activity. Mice were treated with saline and placed into the infrared detection cages and permitted to acclimate for 60 min. Each animal was then removed from the cage, administered the test dose of the drug and replaced into the cage. The values represent the mean distance (inches) traveled (±S.E.M.) during five minutes at the peak time of activity for each drug at each dose. Ten to 12 mice were used at each dose level. □—AMP; ◆—cocaine.

RESULTS

Acute Administration

The acute dose-response curves for cocaine and amphetamine (Fig. 1) show some basic differences that are important when describing the effect of subchronic treatments. In this animal model, amphetamine had a relatively steeper dose-response relationship than cocaine, reaching its maximum at about the same dose as cocaine but with the intermediate doses of cocaine producing a more gradual increase in locomotor activity compared to amphetamine. An interesting result was the apparent difference in the maximum locomotor activity produced by the two compounds. Cocaine's maximum locomotor activity was only 72% of that observed for amphetamine. Higher doses of either compound (e.g., 10 mg/kg of amphetamine or >30 mg/kg cocaine in this strain of mice) became confounded due to the appearance of competing, non-locomotor behaviors (e.g., stereotypic movements) which interfered with the expression of drug-induced horizontal locomotion. Time-response data showed that the peak activity of cocaine was at 5 min followed by an immediate decline. In contrast, amphetamine induced changes that peaked at 15 to 25 minutes and declined gradually over the remaining 60 min. This difference in peak activity accounts for the baseline differences seen in Fig. 1. This is to say that when comparing cocaine to matched controls, one must consider that the administration of saline alone produces an initial burst of locomotion following the injection. Therefore, when constructing a dose-response curve, the inclusion of the saline data as the zero dose results in a relatively higher baseline activity in cocaine-challenged mice when measured at five min after drug injection. The zero-dose locomotor values in the amphetamine dose-response curve are those of saline-treated mice 15 min after they received the saline injection and therefore the value for that data point was derived at a

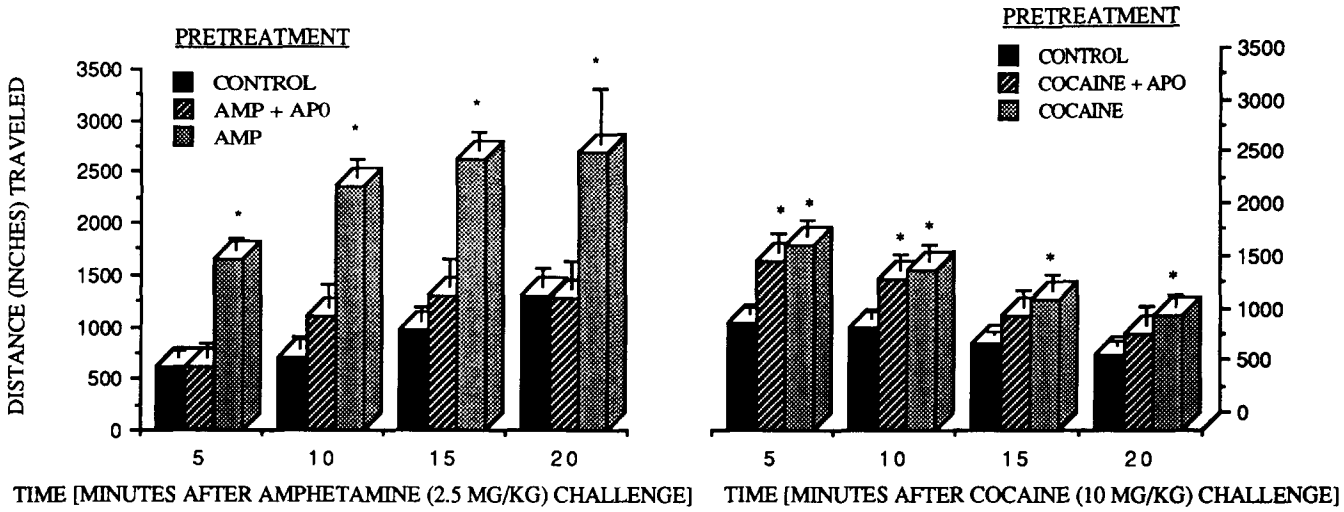

FIG. 2. Time-response for amphetamine (2.5 mg/kg) and cocaine (10 mg/kg) administered IP in mice for time periods of 5 to 20 minutes after drug injection. These challenge doses of the drugs were administered 3 days after the last subchronic injection of the drugs of interest. The solid black bar represents the mean response (inches traveled±S.E.M.) in mice which had been treated with saline twice a day for 5 days. The striped bar represents the response in mice which had been treated with either amphetamine or cocaine followed (in 15 min or 5 min, respectively) by an injection of apomorphine (80 μg/kg, SC) twice a day for 5 days. The stippled bar represents the response of mice which had been treated with amphetamine or cocaine alone twice a day for 5 days. The * indicates statistically significant differences ($p<0.05$) from control.

time point past that initial burst of activity in response to the injection. Thus the amphetamine baseline (zero dose) was lower when compared to that of cocaine.

Subchronic Administration

The results of the subchronic administration show that those mice treated with cocaine and amphetamine alone and challenged with cocaine or amphetamine three days after the last pretreatment dose (Fig. 2), responded in a typically hypersensitive manner to the challenge doses of the two drugs when compared to those treated subchronically with saline and challenged with the stimulants. It should also be noted that the amphetamine response in sensitized animals was much more robust than that for cocaine at the doses used in the subchronic study. Therefore, since the doses used for both of these drugs were those which produced the maximum locomotor response (without confounding behaviors such as stereotypy interfering), it would appear that the two compounds differ in their ability to alter the processes involved in that sensitization.

Concomitant Administration of Apomorphine

As expected, those mice treated subchronically with a combination of amphetamine plus apomorphine (80 μg/kg given 15 min after the amphetamine, twice a day for five days), showed no statistically significant differences in their response to amphetamine when compared to that of the saline-pretreated mice [12]. However, the animals which received concomitant administration of cocaine and apomorphine were statistically no different from those which had received cocaine alone. Thus concomitant administration of apomorphine prevented amphetamine-induced sensitization but was not effective in preventing that produced by cocaine.

DISCUSSION

We have previously shown that subchronic treatment of mice with amphetamine induces a sensitization to subsequent challenge doses of amphetamine [11]. Co-administration of low doses of apomorphine with the amphetamine subchronically prevented the development of the sensitization response to amphetamine [12]. We suggested in that paper that the prevention by apomorphine of the sensitization normally induced by subchronic amphetamine was not due simply to the prevention of the pharmacological effect of amphetamine but to some interference with processes occurring subsequent to the stimulation of neurotransmitter release by the stimulant.

This effect of apomorphine has led us to speculate that the site at which apomorphine is interacting in our model to inhibit the sensitization normally observed after subchronic amphetamine administration may be even more directly linked to dopamine synthesis or dopamine release than is an autoreceptor. This view is consistent with the recent studies which have shown that the rate of dopamine synthesis (as reflected in the activity of tyrosine hydroxylase) is not linked to dopamine autoreceptor control [3,4] as was formerly believed. In addition, such a suggestion is consistent with the reports which indicate that amphetamine is dependent for its effects on newly synthesized dopamine located in an easily releasable pool within the nerve ending and with the studies demonstrating apomorphine's ability to depress tyrosine hydroxylase activity in the rat striatum in a manner which appears to be autoreceptor independent [15]. Further, reports have shown differential adaptations of striatal D_2 DA receptors, release, synthesis and metabolism following subchronic apomorphine administration [17,18].

The inability of apomorphine to prevent the sensitization induced by subchronic cocaine administration suggests that sensitization can be induced by more than one mechanism. Apomorphine was apparently unable to alter the process in-

volved in cocaine sensitization whereas the former was extremely effective in altering the process mediating amphetamine sensitization. McMillan [7] has suggested that stimulants such as cocaine act *via* dopamine uptake inhibition and through a process involving increased DA exchange from less mobile "storage pools" to more labile pools lead to "neurogenic overflow" resulting in more DA being made available for release upon neuronal stimulation. He further states that neurogenic overflow is far less dependent on dopamine synthesis than is the more readily releasable pool which responds to amphetamine. The lack of effect of apomorphine in cocaine-treated animals observed in this study, supports the neurogenic overflow model since apomorphine is known not to interact with processes associated with the exchange between storage and labile pools. The absence of an effect of apomorphine in the cocaine-induced sensitization may also reflect a possible subsensitization of autoreceptors. Thus sensitization induced by this central nervous system stimulant, known to inhibit DA uptake, may be due to either an increase in the exchange of DA from storage to labile pools or subsensitive autoreceptors or both. The data showing the relatively large difference in the magnitude of sensitization induced by amphetamine and cocaine when administered alone subchronically also suggest that there may be differences in the processes involved in the development of sensitization. It may also be hypothesized that drugs which induce sensitization by altering synthetic processes as has been suggested for amphetamine [14], may induce a sensitization of greater magnitude than compounds which may alter processes not involving transmitter synthesis. Only further research in this area will elucidate the relative contribution of these processes to the amphetamine and cocaine-induced sensitization phenomenon.

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