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Altered Response to Apomorphine and Haloperidol After Nine Days of Cocaine Injections

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EPSTEIN, P. N. AND H. L. ALTSHULER. *Altered response to apomorphine and haloperidol after nine days of cocaine injections.* PHARMAC. BIOCHEM. BEHAV. 10(2) 189–193, 1979.—Sprague Dawley rats, pretreated with nine daily injections of 20 mg/Kg cocaine or saline, were evaluated for aspects of their behavioral response to apomorphine, haloperidol, or cocaine, twenty-four hours after their last pretreatment injection. Data obtained from saline and cocaine pretreated animals indicated that: cocaine pretreated rats were more sensitive to haloperidol-induced catalepsy, less responsive to some of the stereotypic effects of apomorphine and similar in their responses to the anticataleptic properties of cocaine.

Apomorphine Cocaine Haloperidol Stereotype Catalepsy Tolerance

DURING the past fifty years publications have appeared which describe the effects of chronically administered cocaine [1, 6, 10, 13, 14, 15, 18, 20]. These papers have reported on studies which were principally concerned with the development of tolerance or reverse tolerance. Several have focused on the production of "reverse tolerance" or sensitization to cocaine-induced stereotypy during chronic administration [6, 10, 13, 20].

Since metabolic studies have demonstrated neither decreasing metabolism nor prominent accumulation of cocaine during its chronic administration [10,16], it may be possible that reverse tolerance is caused by neurophysiological or neurochemical changes in the organism. Alteration in central dopaminergic systems has been suggested as a basic component of reverse tolerance [12,13] since dopamine is regarded as a primary mediator of stimulant-induced stereotypy and locomotor activation [2]. Several investigators [13, 17, 19] have suggested that the actions of cocaine are mediated by central dopaminergic systems and further that the reverse tolerance to cocaine might result from the development of supersensitivity of dopaminergic systems to the action of the drug [13].

This study was designed to assess the effects of chronic cocaine administration in rats pretreated with either cocaine or saline by comparing the behavioral potencies of three drugs, apomorphine, haloperidol, and cocaine. Apomorphine is a potent dopamine agonist and psychomotor stimulant which, like cocaine, produces stereotypic behavior in the rat [7]. Haloperidol, on the other hand, is a dopaminergic antagonist that reduces locomotor activity and produces catalepsy in rats [2, 3, 5] at sufficient doses. In addition, haloperidol and cocaine appear to be mutually antagonistic; haloperidol reduces the stereotypic effects of cocaine [8], and cocaine decreases the intensity of haloperidol-induced catalepsy [8,11].

METHOD

The study consisted of three experiments performed with 168 cocaine and 167 saline-pretreated rats. In each experiment a different drug response was examined: stereotypy induced by apomorphine, catalepsy induced by haloperidol, and the antagonism of haloperidol catalepsy by cocaine.

Animals were male Sprague Dawley rats weighing between 300 and 400 g. Cages housed six to eight animals, composed equally of saline- and cocaine-pretreated rats. Food and water were provided ad lib. Lighting was maintained on a regular 12-hour light (7 a.m. to 7 p.m.), dark (7 p.m. to 7 a.m.) cycle.

Pretreatment lasted nine days, during which the animals were weighed every third day. Assignment of rats to saline or cocaine pretreatment regimens was done randomly. During the nine pretreatment days, the animals received daily intraperitoneal (IP) injections of 20 mg/Kg of cocaine or saline. This regimen of cocaine injections was chosen because we found that it produced reverse tolerance in a previous experiment [5], which evaluated several doses of cocaine.

Testing was performed on the tenth day after the start of pretreatment, 24 hours after the last saline or cocaine injection. At that time, the animals were weighed and then placed individually in testing cages for a one-hour habituation period before being injected with the test drugs.

Drugs used were apomorphine HCl (Merck Co.), cocaine HCl (Mallinckrodt Chemical Works), and haloperidol

(McNeil Laboratories); all drugs were dissolved in a 0.9% saline solution. Solutions of apomorphine were prepared immediately before administration. On the basis of preliminary testing, doses of apomorphine, haloperidol, and cocaine were chosen which would elicit a wide range of responses so that either increases or decreases in sensitivity would be obvious.

Testing Apomorphine Stereotypy

Cocaine- and saline-pretreated rats were tested with four doses of apomorphine (0.35, 0.5, 1.0 and 2.0 mg/Kg) administered subcutaneously (SC). Ten animals from each pretreatment group were tested at each dosage of apomorphine, and each animal was used only once.

The animals were rated for the frequency of stereotypic sniffing and the frequency of licking or gnawing during one-minute observations. The observer rated one animal at a time during each one-minute observation, which took place 15, 30, 45 and 60 minutes postdose. The rater was unaware of each animal's pretreatment regimen or apomorphine dosage.

The system of scoring stereotypic licking and gnawing on one scale and stereotypic sniffing on another is a modification of one previously described [4]. Both components of stereotypy were scored on a 0 to 3 scale with half-point increments: 0 if the behavior was not seen, 1 if it occurred only once during the minute, 2 if the behavior occurred more than once, and 3 if the behavior continued during the minute. Half-point increments reflected intensity of the behavior: if low intensity stereotypy was observed, one half-point was subtracted from the scores. The means of the four scores for each one-minute observation were computed and used for further analysis. Data were analyzed for significance with a two-way analysis of variance as the scores of both components were found to be close to normal in distribution.

Testing Haloperidol Catalepsy

The catalepsy induced by SC injections of 0.125, 0.25 and 0.5 mg/Kg of haloperidol was assessed in cocaine- and saline-pretreated animals, using 20 animals from each pretreatment group per dose of haloperidol. Catalepsy test cages were 20 × 25 cm and had a 10-cm high horizontal bar above the middle of the cage. Catalepsy was measured using a procedure described previously [3]. Rats were placed with their forepaws over the horizontal bar, and the time they stayed on the bar was recorded in minutes. The first test was at 60 minutes postdose; thereafter tests were made at 40-minute intervals, and continued through 220 minutes postdose. Means of each animal's catalepsy times were obtained. Analysis of these data were done with nonparametric statistical tests, the Kruskal-Wallis analysis of variance and Newman-Keuls test [22] since the data were not normally distributed.

Measuring Cocaine's Anticataleptic Potency

Cocaine's anticataleptic effect was measured by assessing the reduction it produced in haloperidol-induced catalepsy. Sixty-eight cocaine- and 67 saline-pretreated rats received SC injections of 0.5 mg/Kg of haloperidol. One hundred and ten minutes after the initial injection, a time at which preliminary experiments had shown the catalepsy response to be stable, a second injection of cocaine or saline was given. Three doses of cocaine (10, 20 and 40 mg/Kg) were injected IP. Fifteen animals from each pretreatment group received each dose of cocaine. Injections of saline, also IP, were given to 23 cocaine- and 22 saline-pretreated animals. These animals constituted the two control groups. Catalepsy was tested 20, 40 and 60 minutes after the second injection. Times for the three tests were averaged. Statistical comparisons were made with the Kruskal-Wallis analysis of variance and the Newman-Keuls and Mann-Whitney U tests.

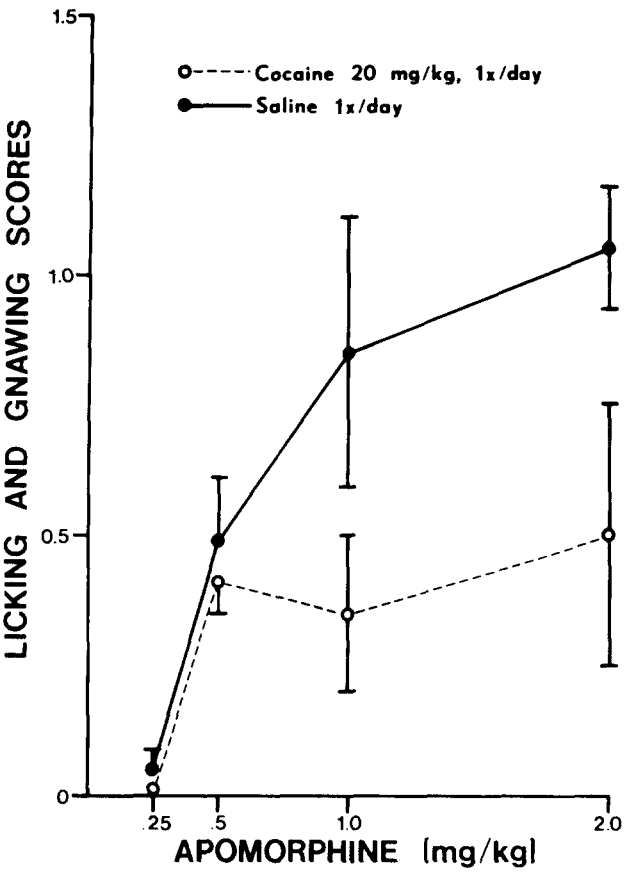

FIG. 1. Dose-response curves for gnawing and licking components of apomorphine stereotype in saline- and cocaine-pretreated rats (n=10 per data point). Mean stereotypy scores (referred to as Licking and Gnawing Scores) are plotted on the ordinate. The dose of apomorphine is displayed along the abscissa. Vertical bars give the standard error of the mean (S.E.M.).

RESULTS

Apomorphine Stereotypy

Cocaine pretreatment reduced the stereotypic licking and gnawing produced by apomorphine. The dose-response curves showing this effect are plotted in Fig. 1. As is evident from the graph, scores of the saline-pretreated animals increased progressively from the lowest to the highest dosages of apomorphine. In contrast, licking and gnawing scores of the cocaine-pretreated animals reached their maximal values at a dose of 0.5 mg/Kg and remained at about the same level for the 1.0 and 2.0 mg/Kg doses. Statistical analysis demonstrated that there was a significant pretreatment effect. The overall test of pretreatment was significant, $F(1,72)=5.02$,

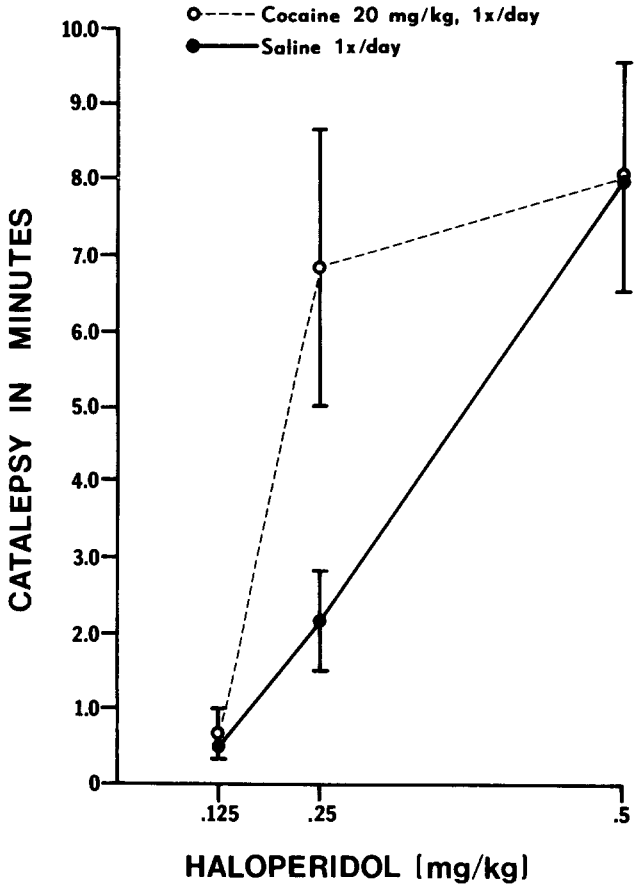

FIG. 2. Dose-response curves for haloperidol-induced catalepsy in saline- and cocaine-pretreated rats (n=20 per data point). Mean catalepsy time in minutes is shown beside the ordinate and the dose of haloperidol is displayed below the abscissa. Vertical bars represent the S.E.M.

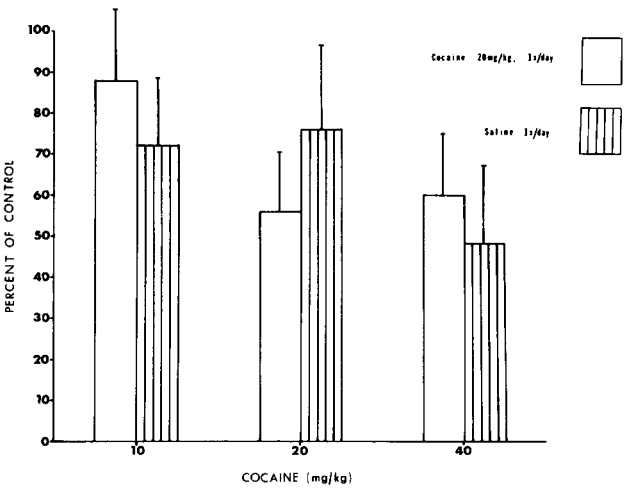

FIG. 3. Anticataleptic effect of three different doses of cocaine in cocaine- and saline-pretreated rats (n=15 per data point). Anticatalepsy scores are given as a percentage of the catalepsy times of control groups (saline control n=22, cocaine control n=23) on the ordinate, dose of cocaine is shown under the abscissa. Vertical bars are S.E.M.

$p<0.05$, as were tests made at the doses of 1.0 and 2.0 mg/Kg of apomorphine, $F(1,72)=4.48$, $p<0.05$, $F(1,72)=4.02$, $p<0.05$ respectively, and the significance was due to the higher scores of the saline-pretreated rats. Sniffing scores (not shown) were not affected by the pretreatment conditions, $F(1,72)>1$, $p>0.2$, at any dose of apomorphine.

Haloperidol Catalepsy

Cocaine pretreatment produced an increase in the cataleptic effect of haloperidol. Figure 2 shows the mean catalepsy time in minutes for both saline- and cocaine-pretreated animals. The mean duration of catalepsy is similar at the highest and lowest doses of haloperidol for both pretreatment groups. However, cocaine-pretreated animals displayed near maximal catalepsy at the dose of 0.25 mg/Kg of haloperidol while the saline-pretreated rats were far below maximum at this dose. Mean catalepsy times of the cocaine-pretreated animals given 0.25 mg/Kg of haloperidol were greater than those for the saline-pretreated animals, $q(\infty,2)=7.7$, $p<0.01$. Differences for the two other haloperidol doses were not significant, $q(\infty,2)=1$, $p>0.25$.

Cocaine's Anticataleptic Potency

Cocaine pretreatment had no clear influence on the acute anticataleptic response to cocaine. The mean catalepsy times, expressed as percentage of mean control catalepsy time, are shown in Fig. 3. Percentages for the three saline-pretreated groups receiving cocaine injections on the test day were computed using the results of the saline-pretreated control group. Similarly, percentages for cocaine-pretreated groups, which were tested with cocaine, were computed from the cocaine-pretreated control group's catalepsy time. Percentage scores were used to eliminate possible confounding due to the effect of pretreatment on the duration of catalepsy, unrelated to cocaine's anticataleptic potency. Scores of the two pretreatment groups did not differ significantly at doses of 10, 20 or 40 mg/Kg of cocaine, $q(\infty,2)\leq 1.2$, $p>0.10$.

DISCUSSION

In this study cocaine pretreatment had no apparent influence on the anticataleptic response to cocaine, reduced stereotypic licking and gnawing responses to apomorphine, and increased the catalepsy induced by haloperidol. Although these results do not explain sensitization, they do imply that cocaine produces important neurophysiological changes in the rat.

The test of cocaine's anticataleptic potency revealed no pretreatment effect. Thus, cocaine's anticataleptic action differs from several of its other properties in demonstrating neither sensitization nor tolerance after chronic treatment. However, this distinction is tentative and will probably remain so since a more sensitive procedure or a longer chronic treatment phase may reveal changes not detected or produced with our procedures. Although we did not compare a variety of dosage regimens or durations of pretreatment, we have previously demonstrated [6] that 9 days of cocaine (20

mg/Kg) produced clear indications of reverse tolerance, and that this effect tended to plateau with more prolonged treatment.

In the test of apomorphine-induced stereotypy, cocaine-pretreated rats demonstrated less stereotypic licking and gnawing behavior but virtually identical stereotypic sniffing behavior relative to control animals. Cocaine's different effects in these two components of stereotypy may be due to conditioning of cocaine-induced patterns of stereotypic behavior during the pretreatment period, since conditioning has been shown to be an important factor in the development of reverse tolerance [21]. Cocaine-induced stereotypies contain similar sniffing behavior as that produced by apomorphine, but lack licking and gnawing components. However, it is improbable that conditioning developed in this study since the animals' pretreatment housing was in large, group cages, and testing was performed in small, individual cages. An alternative explanation is that stereotypic sniffing and stereotypic licking and gnawing may be mediated by different central mechanisms.

The comparatively low licking and gnawing scores reported in this study relative to other reports in the literature which used similar doses of apomorphine may be partly explained by the different scoring system employed in this study, as well as the long period over which these scores were averaged.

Our results for apomorphine-induced stereotypy differ from those of a recent paper [13] which reported enhanced apomorphine-induced stereotypy following cocaine pretreatment. A possible explanation of this discrepency is the differing rating systems used to quantify stereotypy. That study used a global rating system which assigned a single score to complex behavioral patterns while we attempted to rate stereotypy by quantifying the occurrence of discrete behaviors. Since we obtained different results for the two behaviors examined, it seems possible that stereotypy encompasses several different behaviors which may be affected differently by cocaine pretreatment.

Several authors [13,16] have suggested that supersensitive, dopaminergic post-synaptic mechanisms may develop during chronic cocaine administration. However, the reduction found in stereotypic behavior to apomorphine, a dopaminergic agonist [7], and the enhancement of the cataleptic effects of haloperidol, a dopaminergic antagonist [2] following cocaine pretreatment, do not support this possibility. If the catalepsy produced by haloperidol is a result of dopaminergic antagonism, dopaminergic supersensitivity would be expected to reduce the cataleptic response, since treatments which enhance dopamine sensitivity reduce cataleptic effects of haloperidol [1,9].

A mechanism in greater agreement with our results might be that subsensitive dopaminergic mechanisms develop during cocaine treatment rather than the previously suggested supersensitivity. However, both hypotheses are supported only by behavioral evidence obtained during responses to drugs which may have multiple actions. Critical evaluation requires neurophysiological and neurochemical approaches as well as replication with other putative dopamine agonists and antagonists.

In summary, the results of this study demonstrated that cocaine administration alters pharmacological sensitivity of the rat to apomorphine and haloperidol, although they do not suggest an obvious explanation for sensitization to cocaine-induced stereotypy.

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