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BRIEF COMMUNICATION

Chronic Exposure to Lead Attenuates Cocaine-Induced Behavioral Activation

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GROVER, C. A., J. R. NATION AND G. R. BRATTON. *Chronic exposure to lead attenuates cocaine-induced behavioral activation.* PHARMACOL BIOCHEM BEHAV 44(1) 221-225, 1993.—Adult, male rats were exposed to a diet containing 500 ppm (0.05%) lead for 105 days before testing for cocaine-related changes in activity using a Digiscan activity system. Behavioral testing occurred on 6 successive test days. Activity was recorded for 20 min prior to and 40 min after IP injections of either 10, 20, or 40 mg/kg cocaine HCl, with saline injections on the day preceding each drug test day. Cocaine-induced behavioral activation was evident in control diet animals for all three doses (10, 20, and 40 mg/kg). While 10 mg/kg cocaine HCl did not produce behavioral activation in lead-treated animals, both 20 and 40 mg/kg did result in increased activity comparable to that observed in control counterparts.

Activity Cocaine Lead Metal

EXPOSURE to the ubiquitous environmental pollutant lead can result in clearly overt neurotoxic symptoms, as well as numerous more subtle behavioral, cognitive, and neural effects (6,10). Children are in particular at risk of chronic exposure because they consume lead-based paint chips (34) and play in contaminated soil (8). Soil-lead concentrations in several urban areas of the United States, alarmingly, have been reported to be in excess of 2,000 ppm (12).

Early clinical reports of lead-induced hyperactivity in children (13) and experimental findings of increased motor activity in lead-treated animals (29) prompted the seminal investigation on drug-lead interactions (30). Because of its effectiveness in the treatment of some forms of childhood hyperactivity, one of the first drugs to be investigated in lead-treated animals was amphetamine. Lead-treated animals exhibit attenuated amphetamine-induced motor activity (25-27,31) and reduced sensitivity to the stimulus properties of amphetamine in a drug discrimination paradigm (28,35). Apomorphine-induced aggression and stereotypy also are disturbed by lead exposure (4).

Ethanol-related behaviors are included in the list of drug-induced behavioral responses influenced by environmental toxicants. Previous investigations have shown that chronic exposure to low levels of lead, as well as cadmium, result in increased volitional intake of ethanol (14-16,20,21) but decreased lever responding for ethanol reinforcement (7,17). In addition to the effects of these metals on ethanol self-administration, lead and cadmium have been shown to occasion a decreased responsiveness to the pharmacological effects of the drug. For example, chronic treatment with lead has been shown to attenuate the antipunishment effects of ethanol (18), and both lead (32) and cadmium (22) have been shown to compromise the hypnotic properties of ethanol.

More recently, the list of psychoactive drugs affected by inorganometallic compounds has been expanded to include cocaine, that is, cadmium treatment has been shown to alter the effects of acute cocaine challenges (19). When administered 10 mg/kg cocaine HCl IP, adult rats chronically exposed to 100 ppm (0.01%) cadmium in the diet failed to show the typical pattern of cocaine-induced behavioral activation observed in controls. At higher doses, cocaine did produce prominent increases in activity among cadmium-exposed animals, equal to that exhibited by controls. Accordingly, these data suggest a cadmium-related hyposensitivity to the stimulatory effects of cocaine.

As a further demonstration of toxicant-cocaine interac-

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tions, this study was designed to determine whether alterations in cocaine-induced behavioral activation, in a like manner to the attenuation produced by cadmium (19), also would be produced by lead exposure. Specifically, in this study adult, male rats were continuously exposed to either a control diet or a diet containing 500 ppm lead for 105 days before beginning testing for cocaine-induced changes in activity. Previously, in this (15–18) and other laboratories (2,3) it has been shown that this exposure regimen produces moderate lead burdens comparable to those associated with cognitive deficits in children (23). Employing a within-subjects design, animals served as their own controls for IP administration of 10, 20, and 40 mg/kg cocaine HCl (a saline test day occurred prior to each cocaine test day).

METHOD

Animals

Animals used in this study were 11 adult, male Sprague-Dawley rats (Holtzman Company, Madison, WI) approximately 180–200 g at the beginning of the study. In a previous experiment, each of the 11 animals had received operant lever-press training [fixed ratio 1 (FR 1)] for food reinforcement and one IP injection each of saline and 1.0 g ethanol/kg body weight. Throughout the earlier experiment and for the duration of this experiment, six animals were maintained ad lib on a diet that contained no added chemicals (control diet). The remaining five animals received ad lib access to laboratory chow containing 500 ppm lead (lead diet). For all animals, food consumption and body weight was recorded weekly. Throughout the experiment, all animals were individually housed and maintained on a 12 L : 12 D schedule.

Preparation of Food

Pellets of semipurified Teklad Laboratory chow (Harlan Sprague-Dawley, Inc., Madison, WI) were ground in a small food mill and then transferred to a large stainless steel food mixer in 10-kg batches. Two liters of distilled deionized water were added to the mixer for control diet food. For lead diet food, 2 l of distilled deionized water containing the appropriate quantity of lead acetate were added to the mixer. Mixing continued for 20–30 min after the mixture appeared homogenous to ensure complete distribution of lead in the food. The food was then repelleted with a laboratory pelleter (Model CL Laboratory Pellet Mill, California Pellet Mill, Co., San Francisco, CA) and stored at $<0°C$. To avoid cross-contamination between food batches, control diet food was prepared first, followed by lead diet food, and the equipment was cleaned after each mixing.

Apparatus

The behavioral apparatus used in this study involved an automated Digiscan-16 system. The system includes an optical beam activity monitor (Coulbourn Instruments, Lehigh Valley, PA; model no. #E61-32) comprised of 16 vertical and 16 horizontal infrared sensors. Each of four monitors surrounded acrylic activity monitor cages (40 × 40 × 30.5 cm), completely enclosed with 0.5-cm air holes drilled in the top panel. The monitors and cages were located in a sound-proof, radiofrequency shielded room. An E61-58 multiplexer/analyzer, located in an adjacent room, monitored beam breaks from each of the optical beam activity monitors and tracked the simultaneous interruption of beams. The multiplexer/analyzer updated each animal's position in the acrylic cages every 10 ms using a 100% real-time conversion system. Computerized integration of the data obtained from the monitors afforded the recording of updated totals in successive 5-min intervals of 15 different behavioral measures. While several measures are available, previous research in our laboratory has revealed a high degree of intercorrelation across dependent measures (19). Moreover, number of movements (number of times ambulatory activity was initiated for a period greater than 1 s) has consistently been the most reliable index of metal-induced changes in activity. Accordingly, only number of movements data will be reported here. Room lights were on during testing, and to mask extraneous sounds continuous white noise (range 45–55 dB) was present throughout testing.

Procedure

Testing of behavioral activity began on day 105 of exposure to the respective control or lead diets. Animals were allowed to acclimate to the apparatus and saline injections during three 1-h sessions on each of the first 3 days of testing. Alternately, on successive days animals were administered IP injections of saline and increasing doses of cocaine HCl. Each saline test served as the corresponding control for the subsequent drug test. Cocaine HCl was administered serially in doses of 10, 20, and 40 mg/kg. In all cases, the vehicle for cocaine solutions was 1.0 ml/kg volume of saline. The volume of saline administered on saline test days was held constant at 1.0 ml. The 10-, 20-, and 40-mg/kg doses of cocaine HCl were used in an effort to determine possible differential dose-response functions for groups control diet and lead diet.

Animals were run in individual test cages four at a time, counterbalancing for test cage and test time assignment across groups. On all saline and drug test days, animals were tested for 20 min of baseline preinjection activity and 40 min of postinjection activity. Specifically, each animal was placed in a test activity cage and once four successive 5-min baseline data printouts were generated the animal was removed from the apparatus and injected IP with saline or the appropriate drug for that test day. The animal was then returned to the test cage and activity was recorded for an additional eight successive 5-min bins. Thus, each animal was in the test cage for a total of 1 h. Test cages were washed thoroughly with a soap solution following each animal's test.

Chemical Analyses

On day 123 of dietary exposure, 11 days after the final activity test, animals were rendered unconscious in a bell jar with CO_2, then decapitated, and trunk blood was collected. The concentration of lead in blood was then measured via dry ashing and atomic absorption spectrophotometry as previously described (5,33).

RESULTS

Food Consumption

A 2 groups (control diet, lead diet) × 17 weeks repeated-measures analysis of variance (ANOVA) test performed on weekly food consumption revealed that the groups did not differ in food consumption, $F(1, 8) = 0.21$, $p > 0.05$. A weeks main effect, $F(16, 128) = 100.67$, $p < 0.05$, merely reflected that for all animals food intake fluctuated over the weeks. The groups × weeks interaction was not significant, $F(16, 128) = 0.82$, $p > 0.05$. Groups control diet and lead diet mean food consumption for week 1 was 267 and 248 g, respectively, and for week 17 219 and 228 g, respectively.

Body Weight

A repeated-measures ANOVA (2 groups × 18 weeks) on initial and weekly body weights failed to show a significant groups main effect, $F(1, 9) = 0.11, p > 0.05$, or a significant groups × weeks interaction, $F(17, 153) = 0.40, p > 0.05$. A significant main effect for weeks, $F(17, 153) = 212.48, p < 0.05$, indicated that all animals gradually gained weight over the course of the experiment. Initial group mean body weights were 223 and 221 g for groups control diet and lead diet, respectively. The final weekly mean body weights for groups control diet and lead diet were 446 and 444 g, respectively.

Activity Data

Relative to control animals, continuous exposure to lead via the diet resulted in an attenuation of the behavioral activation (number of movements) produced by 10 mg/kg cocaine HCl. There was no evidence of lead-related attenuation of cocaine-induced increases in activity at 20 or 40 mg/kg.

Separate repeated-measures ANOVAs, groups (control diet, lead diet) × injection (saline, cocaine), performed for each pair of the saline and drug test days, on the number of movements during the 5-min interval preceding injection failed to show any group differences (all $p > 0.05$). However, the statistical analysis on the number of movements during the 5-min baseline intervals on the 10-mg/kg cocaine HCl test day and the preceding saline test day showed that the groups main effect approached significance, $F(1, 9) = 3.45, p < 0.10$. See Table 1 for mean number of movements of each group (control diet, lead diet) for the 5-min preinjection interval on all saline and cocaine injection days. To control for individual animal variability in baseline activity, remaining statistical analyses were conducted on postinjection number of movements as a percent of preinjection activity.

A groups (control diet, lead diet) × injections (saline, cocaine) × intervals (5, 10, 15, 20, 25, 30, 35, and 40 min postinjection) repeated-measures ANOVA conducted on the percent of preinjection activity on the 10-mg/kg cocaine HCl test day and preceding saline test day revealed significant main effects for groups, $F(1, 9) = 5.39, p < 0.05$, and injections, $F(1, 9) = 98.35, p < 0.05$ (see Fig. 1). Thus, relative to group lead diet, group control diet showed greater postinjection activity, and postinjection activity was greater on the cocaine test day than on the preceding saline test day. Consistent with other studies from this laboratory (10), on saline test days activity was greater during the preinjection interval than during the postinjection intervals, evident from the postinjection number of movements being approximately 50% of preinjection activity for both groups.

More importantly, the analysis revealed a significant groups × injections interaction, $F(1, 9) = 7.08, p < 0.05$. Post-hoc analyses (Tukey's) indicated that both groups, control diet and lead diet, had greater postinjection activity on the 10-mg/kg cocaine HCl test day than on the saline test day (see Fig. 2). In addition, the postinjection activity was not different for groups control diet (mean = 46%) and lead diet (mean = 35%) on the saline test day, but control diet (mean = 175%) animals did display greater activity than group lead diet (mean = 110%) on the 10-mg/kg cocaine HCl test day.

Separate repeated-measures ANOVAs (2 groups × 2 injections × 8 intervals) on the saline and 20-mg/kg cocaine HCl test days and on the saline and 40-mg/kg cocaine HCl test days did not reveal group differences (all p's > 0.05). However, the analyses did reveal that the main effect of injections differed (all p's < 0.05). In other words, for both diet groups postinjection activity as a percent of preinjection activity was significantly greater on those days that animals received either 20 or 40 mg/kg cocaine HCl than it had been on the respective preceding saline day.

Lead Residues in Blood

Analysis of the lead concentration in blood showed that animals continuously exposed to 500 ppm in the food had greater lead residues in blood [mean = 0.28 ppm ± 0.03 (28 μg/dl ± 3)] than control animals [mean = 0.01 ppm ± .006 (1 μg/dl ± 0.6)] ($p < 0.05$).

DISCUSSION

The results from this study showed that when given 10 mg/kg cocaine HCl IP adult, male rats exposed continuously to 500 ppm lead added to the food did not exhibit cocaine-induced behavioral activation similar to that evinced by their control counterparts. Conversely, when injected with 20 or 40 mg/kg doses of the drug both control diet and lead diet animals demonstrated pronounced increases in movement relative to their baseline activity. Lead treatment did not produce differences in food intake or body weight in this study.

Why continuous dietary exposure to inorganic lead resulted in a reduction of cocaine-induced behavioral activation only at the 10-mg/kg dose is unknown. Nonetheless, this lead-related attenuation of cocaine-induced behavioral activation is similar to earlier findings from this laboratory regarding the effects of cadmium exposure on cocaine-induced behavior

TABLE 1

GROUP MEAN NUMBER OF MOVEMENTS FOR THE 5-min PREINJECTION INTERVAL ON ALL SALINE AND COCAINE DAYS

	Drug					
Group	Saline	Cocaine HCl (10 mg/kg)	Saline	Cocaine HCl (20 mg/kg)	Saline	Cocaine HCl (40 mg/kg)
Control diet						
Mean	36.8	27.8	22.0	21.3	29.0	35.8
SD	9.2	11.2	14.3	18.6	15.3	12.9
Lead diet						
Mean	48.6	36.4	37.3	21.3	27.6	20.5
SD	8.8	7.1	17.6	9.8	14.7	8.5

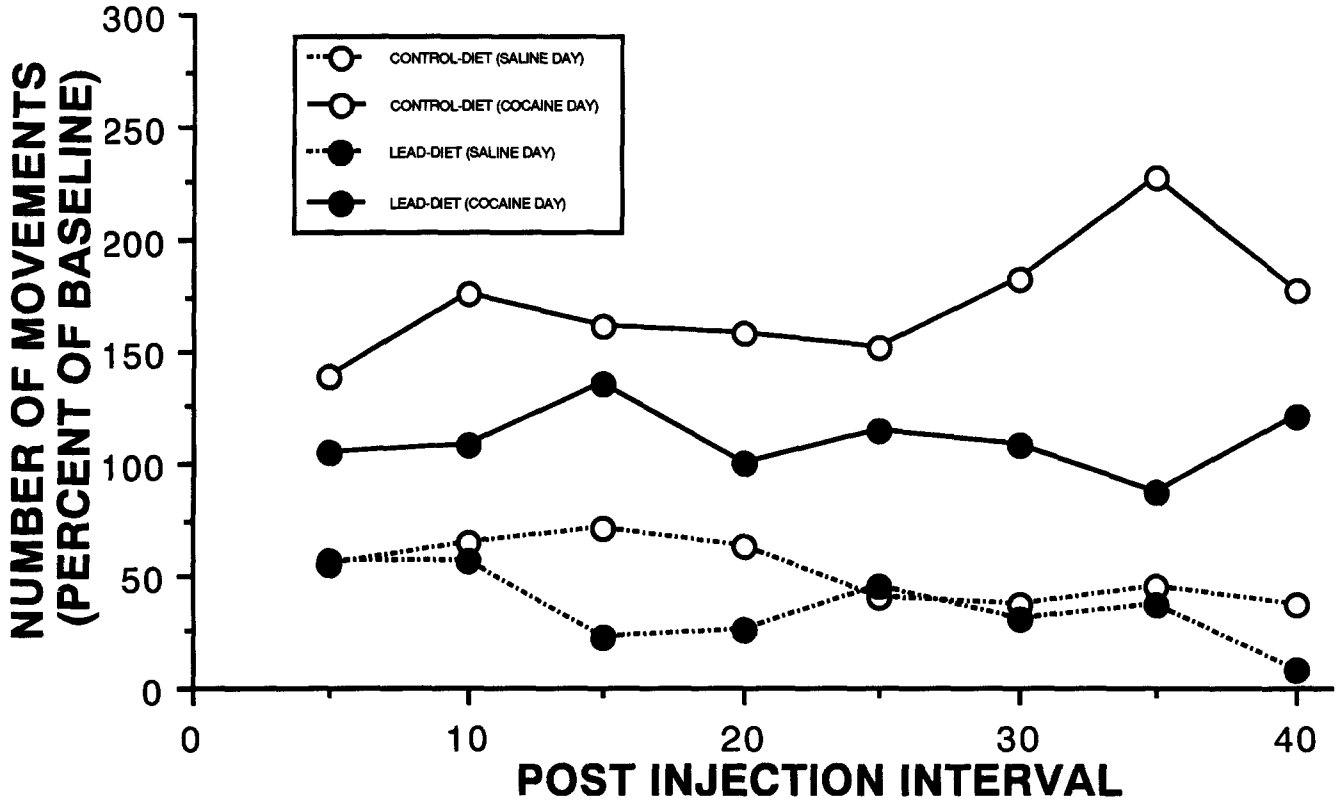

FIG. 1. Number of movements for each group across successive 5-min postinjection (saline, cocaine HCl—10 mg/kg) intervals, as percent of preinjection baseline.

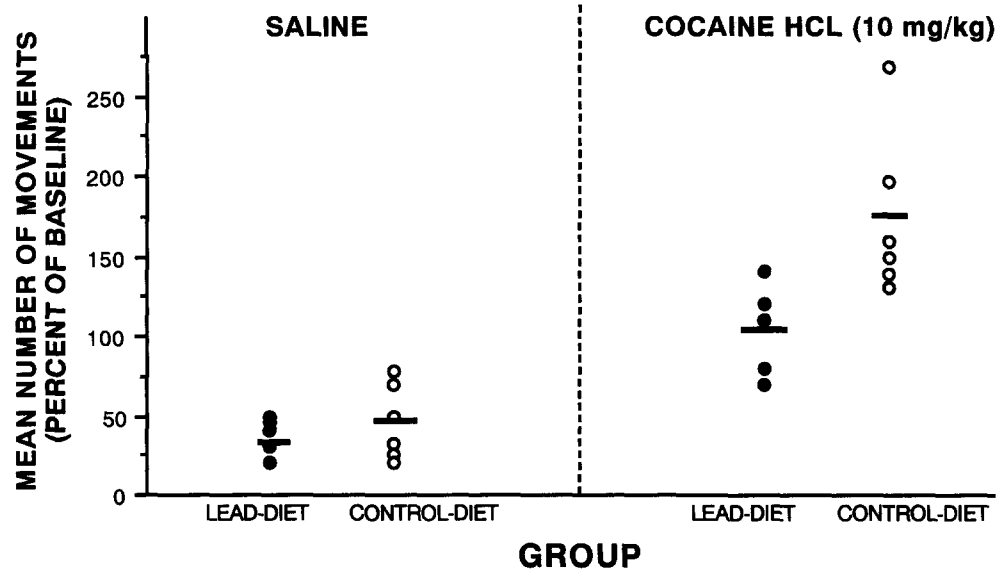

FIG. 2. Mean number of movements as percent of baseline activity (5 min prior to injection) collapsed across the eight postinjection (saline, cocaine HCl) intervals for individual subjects.

(19). While it should be understood that molecular processes may not translate directly into behavioral effects, some consideration for those changes in molecular mechanisms that might contribute to lead-induced behavioral anomalies would seem to be in order. It has been suggested that both inorganolead and inorganocadmium may compete with voltage-dependent calcium influx (1). The behavioral activation produced by cocaine is thought to be due to the drug blocking the reuptake of catecholamines (11) and the calcium-dependent increased release of dopamine by cocaine (9). Calcium antagonists have been found to prevent cocaine-related output of dopamine in the striatum and cocaine-induced motor stimulation (24). One possibility for the attenuation of cocaine behavioral activation produced by these metal toxicants, then, is that lead and cadmium, by competing with calcium at voltage-gated calcium channels, disturb transmitter functions that are inherent in the expression of the neurostimulatory effects of cocaine.

Another possible explanation for the attenuation of cocaine-induced behavioral activation in lead-treated animals is that the pharmacokinetics of cocaine may be differentially affected by the presence of the metal toxicant. In other words, lead may alter the absorption, distribution, and/or metabolism of cocaine. More exhaustive and systematic inquiry into the possible interactive effects between a documented environmental hazard (lead pollution) and a drug with substantial abuse liability (cocaine) is warranted.

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