Pharmacology Biochemistry and Behavior, Vol. 41, pp. 415–423, 1992
Printed in the U.S.A.

0091-3057/92 $5.00 + .00

Acute and Multiple Injection Effects of Magnesium on Responding Maintained by Cocaine, Extinction From Cocaine, Glucose + Saccharin, and Food

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Received 22 July 1991

KANTAK, K. M., S. J. WASSERMAN, S. I. LAWLEY AND T. O'CONNOR. *Acute and multiple injection effects of magnesium on responding maintained by cocaine, extinction from cocaine, glucose + saccharin, and food.* PHARMACOL BIOCHEM BEHAV **41**(2) 415–423, 1992. – In a variety of behavioral experiments, magnesium has effects that are similar to cocaine and other psychomotor stimulants. Of particular relevance to the present experiments is the recent finding that magnesium maintains responding in cocaine-trained rats. It would be expected, therefore, that injections of magnesium would alter the rate of responding maintained by self-administered cocaine in rats. Five experiments examined the specificity and selectivity of this interaction. Acute and multiple injections of $MgCl_2$ (15–250 mg/kg) produced dose-dependent reductions in responding maintained by cocaine (0.1–2 mg/kg/infusion). Testing for acute injection effects occurred following injections, while testing for multiple injection effects occurred prior to daily injections. Doses of 30 and 125 mg/kg $MgCl_2$ reduced responding maintained by doses of cocaine that were below the training dose of 0.75 mg/kg/infusion. $MgCl_2$ in a dose of 250 mg/kg markedly suppressed responding maintained by each dose of cocaine. A magnesium-deficient diet produced a dose-dependent increase in responding maintained by 0.1 mg/kg/infusion cocaine. In order to determine the specificity and selectively of these effects, acute and multiple injections of $MgCl_2$ were examined on glucose + saccharin- and food-maintained responding. The acute effects of $MgCl_2$ injections were specific because food-maintained responding was not affected, except by the highest dose of 250 mg/kg. This demonstrates that lever pressing was not nonspecifically reduced by 30 and 125 mg/kg $MgCl_2$ during cocaine availability. However, the effects on cocaine-maintained responding were not selective for cocaine because glucose + saccharin-maintained responding and responding during extinction from cocaine were affected by $MgCl_2$ in a manner similar to cocaine-maintained responding. The multiple injection effects of $MgCl_2$ were both specific and selective for cocaine-maintained responding because multiple injections did not influence glucose + saccharin- or food-maintained responding.

Cocaine Cocaine-maintained responding Cocaine extinction responding Food-maintained responding
Glucose + saccharin-maintained responding Magnesium chloride Magnesium-deficient diet Rats
Self-administration

LOW doses of magnesium chloride ($MgCl_2$) have many behavioral effects like those of psychomotor stimulants. Studies on mouse aggression (13–15), drug-induced stereotypy and locomotion (17), and conditioned place preference (23,24) support this assertion. Other recent studies suggest that $MgCl_2$ substitutes for self-administered cocaine. Under a FR 1 reinforcement schedule, $MgCl_2$-maintained responding occurred in cocaine-trained rats in a dose-dependent manner to maintain a constant level of $MgCl_2$ intake (19). Responding was maintained by $MgCl_2$ over a 10–20-day period of time both with and without access to cocaine on test days (19). Responding was also maintained by $MgCl_2$ under FR 5 and progressive ratio schedules, further indicating that in cocaine-trained rats, $MgCl_2$ maintains responding (19). From these data it would be expected that Mg^{2+} treatments might alter the rate of responding maintained by cocaine. Previous studies have shown that the rate of responding maintained by cocaine is decreased following injections of a variety of substances from different drug classes. These include *d*-amphetamine (36), bromocriptine (12,21), chlordiazepoxide (10) and buprenorphine or naloxone (26). Other drugs which are primarily dopamine antagonists, increase the rate of responding maintained by cocaine (8,22) or shift the cocaine dose-effect curve to the right (3). In the present experiments, injections of various doses of $MgCl_2$ and a Mg^{2+}-deficient diet were examined for their effects on the rate of self-administration maintained by various doses of cocaine. Furthermore, to determine the specificity and selectivity of changes, the effects of injections of $MgCl_2$ on food-maintained responding and glucose + saccharin-maintained responding were examined (5), as well as responding during extinction from cocaine self-administration.

METHOD

Animals

Male Wistar rats (Charles River Breeding Labs, Wilmington, MA), weighing approximately 250 ± 20 g upon arrival, were individually housed in hanging stainless steel 10 × 7 × 7 inch cages and had free access to tap water and Purina Lab Chow until they were used in experiments. In Experiments 1–4 Purina Lab Chow was restricted to 16 g per day. This ration of food initially maintained body weight at approximately 80% of the free-feeding weight and then weight slowly increased over time. Rats were maintained at weights ranging from 200 to 450 g during the various experiments. In Experiment 5, rats were fed either a magnesium-deficient diet containing 15% of the daily requirement of Mg^{2+} (78 mg/kg of food) or a control diet containing 100% of the daily requirement (500 mg/kg of food). These diets were purchased from Nutritional Biochemicals (Cleveland, OH) in a solid pellet form and were restricted to 16 g per day. Light (0800 h on, 2000 h off) and temperature (74 ± 4°F) were automatically controlled.

Apparatus

For procedures used to study food-maintained responding and cocaine-maintained responding, 4 Gerbrands Model A operant conditioning units were each outfitted with a response lever, feeder, food cup and stimulus light. The units were each enclosed in a lighted and ventilated sound-attenuated cubicle. In self-administration studies, the designs of the leash, swivel and balance arm were identical to that described by Smith et al. (32). The line from the swivel was connected to a Sage Instruments Single-Channel Syringe Pump which delivered precise volumes of drug. The pump was set at a rate of 0.57 ml/min and was outfitted with a 20 ml plastic syringe and 0.2 μm syringe filter. Experimental events were maintained by an AT compatible computer and a Med Associates Interface (East Fairfield, VT).

For procedures used to study glucose + saccharin-maintained responding, a hanging-style 10 × 7 × 7 in stainless steel rat cage was used as the testing chamber. A 3 × 3 in. Plexiglas sheet covered the grid mesh on the front of the cage, and a 1 cm diameter hole was drilled through the Plexiglas, 2.5 in. from the floor, in order to access the drinking spout. The spout was recessed 0.5 cm from the Plexiglas barrier, requiring that the animal orient itself at the Plexiglas opening in order to lick at the spout. The spout was connected to a capacitance-sensitive touch detector (25). This triggered the release of 0.006 ml of fluid per lick through a solenoid valve. The calibration of the flow through the solenoid valve and spout was maintained by an adjustable capillary valve. A 50 ml plastic syringe served as a fluid reservoir. The spout, touch detector, solenoid valve and capillary valve were mounted onto a 15 × 15 in. Plexiglas board. An AT compatible computer with a MetraByte Interface was used to schedule and record events from 8 experimental chambers.

Surgery

Each rat used in self-administration studies was surgically implanted with a jugular catheter (35) under general pentobarbital anesthesia with Halothane or Brevital anesthesia as needed. The catheter was passed subcutaneously to the dorsal surface and attached to a stainless steel anchor button which was sutured to the back muscles of the rat. Stainless steel tubing, which emerged 1 cm from the teflon acorn nut top of the anchor button, was closed off with a crimped piece of teflon tubing. The outside skin was sutured over the anchor button, finishing with a purse string stitch around the center post of the anchor button. All rats were infused daily with 0.5 ml of 8.5 units/ml heparinized saline followed by 0.1 ml of 1000 units/ml heparinized saline.

Drugs

Cocaine hydrochloride (NIDA, Rockville, MD) was dissolved in 8.5 units per ml heparinized saline and was in a concentration of 4 mg/ml. During drug-maintained responding, each lever press activated the infusion pump for the number of seconds needed to deliver a precise volume of cocaine that was required for the specified mg/kg dose (8). Infusion rate was 0.57 ml/min. Average infusion length was approximately 6 s for the 0.75 mg/kg/infusion training dose of cocaine. $MgCl_2$ (Fisher Scientific, Medford, MA) was dissolved in distilled water and was injected subcutaneously in a volume of 3 ml/kg. Doses are expressed as the anhydrous salt of $MgCl_2 \cdot 6H_2O$. Physiological saline was used for the 0 mg/kg control dose. Solutions of 3% D-(+)-glucose + 0.125% saccharin (Sigma, St. Louis, MO) were made with tap water and kept at room temperature for 3 days (4,5).

Cocaine Self-Administration Training

To initiate self-administration of cocaine, rats were trained to lever press under a FR 1 schedule of food delivery (45 mg pellets) and drug delivery as previously described (19). Briefly, rats were exposed daily to a 2-component mixed schedule of reinforcement: FR 1: food (10-min period), FR 1: cocaine (3-h period). A training dose of 0.75 mg/kg/infusion was used and a stimulus light was activated for the duration of drug infusion. Any additional lever presses during an infusion had no consequences.

Procedures

Experiment 1. Acute and multiple injection effects of $MgCl_2$ on cocaine-maintained responding. All rats received 5 days of baseline access to the 0.75 mg/kg/infusion training dose of cocaine in 3-h sessions. Following this cocaine baseline period, the dose-response functions of self-administered cocaine (2, 1, 0.5, 0.25 and 0.1 mg/kg/infusion) were examined in 5 groups of randomly assigned rats. Each group was also injected with its own daily dose of $MgCl_2$ 1 h into the cocaine session. Each group received either 0 (n = 8), 15 (n = 4), 30 (n = 4), 125 (n = 4) or 250 (n = 4) mg/kg $MgCl_2$. Rats were tested Monday–Friday with the dose of cocaine changed daily in a descending order to determine the dose-effect curve. The time of occurrence for each response for individual animals was recorded by the computer. From this information, the number of responses per h and mg cocaine intakes per h were calculated and individual event records were generated. Cocaine-maintained responding was compared during baseline, during the first h of cocaine access on magnesium injection days (preinjection period), and during the first and second hour of cocaine access following injections of 0, 15, 30, 125 or 250 mg/kg $MgCl_2$ (postinjection period). Changes in cocaine-maintained responding during the daily preinjection period would indicate if there were any effects of multiple injections of $MgCl_2$, while changes in cocaine-maintained responding during the daily postinjection period would indicate if there were any acute effects of $MgCl_2$ injections.

Experiment 2. Effects of $MgCl_2$ injections on cocaine-maintained responding and responding during extinction from cocaine. Following a cocaine baseline, the cocaine dose-response functions (2, 1, 0.5, 0.25 and 0.1 mg/kg/infusion) were determined twice in 3 groups of rats. Each group was injected with either 0 (n = 10), 30 (n = 7) or 125 (n = 6) mg/kg $MgCl_2$ 1 h into the 3-h cocaine session. Rats were tested Monday–Friday

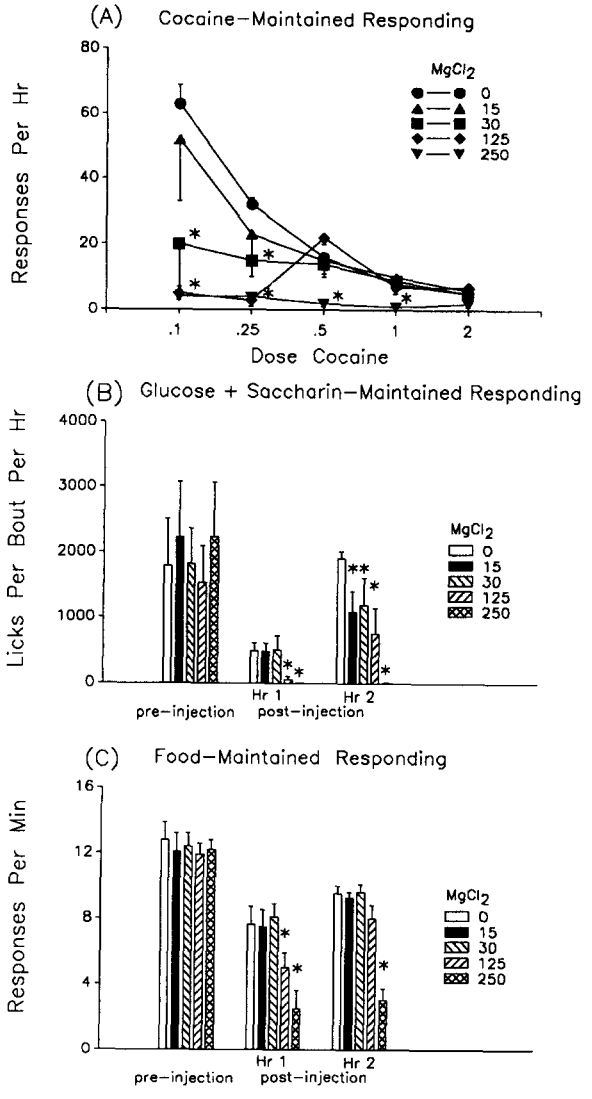

FIG. 1. Mean ± S.E.M. responses maintained by 0.1–2.0 mg/kg/infusion cocaine (A); 3% glucose + 0.125% saccharin (B); and 45 mg food pellets (C) during the first and second hours postinjection of acutely administered $MgCl_2$ in a dose range of 0–250 mg/kg. *$p<0.05$ when compared to the 0 mg/kg dose of $MgCl_2$.

with the dose of cocaine changed daily in a descending order to determine the dose-effect curve. During the first cocaine dose-response determination, the daily dose of cocaine continued to be available for the two hours postinjection of $MgCl_2$. During the second cocaine dose-response determination, the daily dose of cocaine was discontinued and 0.9% NaCl was available for the two hours postinjection of $MgCl_2$. The same measures as above were recorded.

Experiment 3. Acute and multiple injection effects of $MgCl_2$ on glucose + saccharin-maintained responding. Using a FR 1 schedule of tongue contacts for access to a 3% glucose + 0.125% saccharin solution (G+S), rats (n=4) that were maintained on ad lib water and 16 g per day food, were first exposed to the G+S solution for 5 days in daily 3-h baseline sessions. In order to independently assess the acute effects of $MgCl_2$ from potential multiple injection effects of $MgCl_2$ on G+S-maintained responding, in one experiment injections of $MgCl_2$ (0 to 250 mg/kg) were given no more than twice weekly (Tuesdays and Fridays) and were made after 1-h access to G+S. In order to assess the multiple injection effects of $MgCl_2$ on G+S-maintained responding, in a second experiment 4 daily injections of 30 mg/kg $MgCl_2$ (n=4) were administered immediately after a 3-h G+S session and data were recorded the next day before the $MgCl_2$ injection. In both experiments, the number of licks per h, bouts of licking per h and licks per bout per h were measured. A bout of licking was defined as a group of licks separated by 10 or more min to the next group of licks.

Experiment 4. Acute and multiple injection effects of $MgCl_2$ on food-maintained responding. Following 5 daily 30-min baseline sessions of FR 1 food-maintained responding, rats (n=4) were injected with different doses of $MgCl_2$ (0 to 250 mg/kg), no more than twice weekly (Tuesdays and Fridays) to assess the acute effects. In order to determine the rates of food-maintained responding prior to injections and during the first and second h following $MgCl_2$ injections without having food available for a continuous 2–3-h session, injections were made on 2 separate occasions either 1) at the start of a 2-min time out after the initial 10 min of the session, and responding was recorded during the next 20 min, or 2) at the start of a 60-min time out after the initial 10 min of the session, and responding was recorded over the next 20 min. A shorter session was used for food to avoid a satiation effect. In order to independently assess the multiple injection effects of $MgCl_2$ on food-maintained responding, 4 daily injections of various doses of $MgCl_2$ (0–250 mg/kg) were administered immediately after a 10-min access to food pellets in 5 groups of rats (n=4) and data were recorded the next day before the $MgCl_2$ injection. Data are expressed as responses or pellets per min.

Experiment 5. Effects of a Mg^{2+}-deficient diet on cocaine-maintained responding. The effects of a 15% required Mg^{2+}-deficient diet (n=5) and a 100% required control diet (n=5) were examined. Initially, after 5 days of baseline access to cocaine and before diets were initiated, descending cocaine dose-response functions (2, 1, 0.5, 0.25 and 0.1 mg/kg/infusion) were determined in both groups of rats. The diets were then fed for three weeks (14) and descending dose-response functions were redetermined while rats continued on their respective diets. The number of responses per h were calculated and individual event records were generated. Cocaine-maintained responding was compared between groups during acquisition of cocaine responding (baseline), during the first prediet dose-response determination (prediet period), and during the second postdiet dose-response determination (postdiet period).

All data were analyzed by the appropriate analysis of variance and post hoc Duncan Multiple Range Tests were performed to make specific comparisons between means.

RESULTS

Experiment 1. Acute and Multiple Injection Effects of $MgCl_2$ on Cocaine-Maintained Responding

Acute effects. All $MgCl_2$ treatment groups had similar rates of responding during the baseline period. There were no significant differences between groups during baseline training; responding was maintained at 10–20 responses per h. $MgCl_2$ had a significant interaction, $F(20,95)=7.53$, $p<0.001$, with responding maintained by cocaine during the postinjection period. The administration of $MgCl_2$ caused a dose-dependent reduction in cocaine-maintained responding (Fig. 1A). The dose-dependent reductions in responding were observed at the 0.1 and 0.25 mg/kg/infusion doses of cocaine. The differences compared to the 0

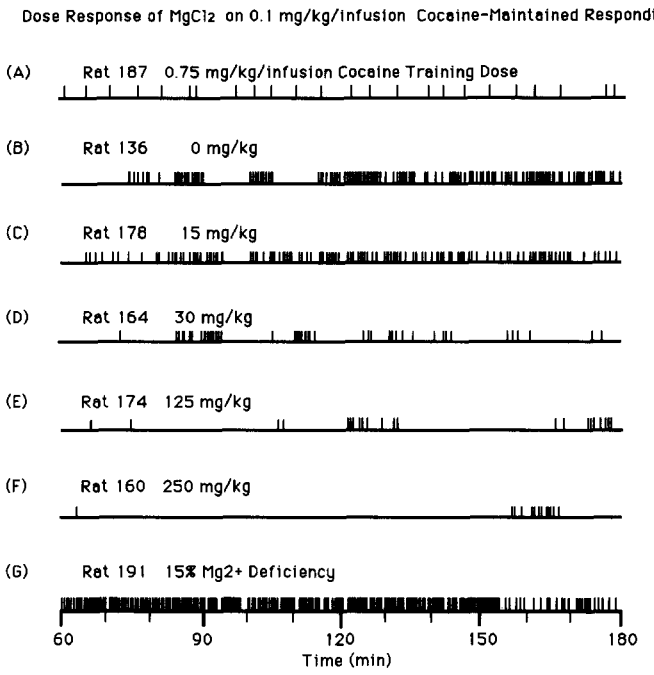

FIG. 2. Representative event records from each of 6 groups of rats undergoing cocaine dose-response determination and given different Mg^{2+} treatments of 0, 15, 30, 125 and 250 mg/kg $MgCl_2$ (B–F) or a 15% required Mg^{2+}-deficient diet (G). Data were taken from the 0.1 mg/kg/infusion dose of cocaine and are presented for the last two hours (Postinjection Period or Diet Period) of the session. For comparison purposes, an event record of cocaine-maintained responding during baseline acquisition of the 0.75 mg/kg/infusion training dose is included (A).

mg/kg control dose were significant for 30, 125 and 250 mg/kg $MgCl_2$, $p<0.05$ for both cocaine doses. A dose of 15 mg/kg $MgCl_2$ produced reductions in 0.1 and 0.25 mg/kg/infusion cocaine-maintained responding which were different neither from the 0 mg/kg control dose nor from 30 mg/kg $MgCl_2$. At higher doses of cocaine, 0.5, 1 and 2 mg/kg/infusion, there were no differences in cocaine-maintained responding between the different $MgCl_2$ doses and the 0 mg/kg $MgCl_2$ control, except for the 250 mg/kg dose which produced significant reductions in responding maintained by 0.5 and 1 mg/kg/infusion cocaine, $p<0.01$ compared to the 0 mg/kg control dose. Concomitant with these decreases in cocaine-maintained responding was a change in the pattern of responding with the different $MgCl_2$ doses (Fig. 2A–F). These data are shown for representative animals for baseline responding maintained by the 0.75 mg/kg/infusion training dose of cocaine (Fig. 2A) and for the 0.1 mg/kg/infusion dose of cocaine (Fig. 2B–F) because this dose was the most sensitive to $MgCl_2$ manipulations and showed the greatest dose-dependent effects. Representative event records were chosen for animals whose individual performance most closely resembled the mean of the group performance. On the event record for rat No. 187, regularly spaced responding maintained by the 0.75 mg/kg/infusion cocaine training dose was defined as responding which occurred throughout the test session, without the occurrence of frequent response bursts (>3 consecutive responses with relatively short interinjection intervals of <30 s) and without the occurrence of relatively long interinjection intervals (>15 min). These representative event records demonstrate that the response pattern for the 0.75 mg/kg/infusion cocaine training dose was regularly spaced and stable (Fig. 2A); that the interresponse interval decreased as dose of cocaine decreased to 0.1 mg/kg/infusion (Fig. 2B); and that the interresponse intervals increased for the 0.1 mg/kg/infusion dose of cocaine as the dose of $MgCl_2$ increased (Fig. 2C–2E). Responding was suppressed by 250 mg/kg $MgCl_2$ (Fig. 2F). At this dose, responding was inhibited for 90 min after the injection and then resumed at a normal rate

TABLE 1

MULTIPLE INJECTION EFFECTS OF $MgCl_2$ ON RESPONDING MAINTAINED BY COCAINE, GLUCOSE + SACCHARIN AND FOOD

Dose	Baseline	Days of $MgCl_2$ Injections 1	2	3	4
		(A) FR 1 Cocaine-Maintained Responding (mg intake/h)			
0	2.4 ± 1.2	2.5 ± 0.3	2.6 ± 0.2	2.1 ± 0.5	2.0 ± 0.3
15	3.1 ± 0.4	3.1 ± 0.4	3.2 ± 0.7	2.4 ± 0.7	1.9 ± 0.6*
30	2.9 ± 0.7	2.8 ± 0.8	3.9 ± 0.4	2.8 ± 0.3	1.1 ± 0.5*
125	3.7 ± 0.5	5.6 ± 0.6	3.8 ± 1.1	0.5 ± 0.1*	0.3 ± 0.1*
250	2.5 ± 0.3	2.8 ± 1.3	1.2 ± 0.4*	1.2 ± 0.6*	0.2 ± 0.1*
		(B) FR 1 Glucose + Saccharin-Maintained Responding (licks/bout/h)			
30	1393 ± 465	1232 ± 487	1383 ± 474	1449 ± 239	1603 ± 176
		(C) FR 1 Food-Maintained Responding (pellets/min)			
0	6.6 ± 1.0	8.4 ± 0.6	8.5 ± 0.7	7.3 ± 1.0	7.5 ± 1.3
15	6.3 ± 0.2	7.4 ± 0.9	5.2 ± 1.1	5.7 ± 1.4	5.2 ± 1.7
30	7.7 ± 0.6	7.2 ± 1.5	8.7 ± 0.3	8.5 ± 1.1	8.9 ± 2.0
125	8.3 ± 0.9	8.8 ± 0.6	8.0 ± 0.6	6.7 ± 1.1	7.4 ± 0.8
250	6.7 ± 0.3	7.6 ± 1.2	1.8 ± 1.2*	1.8 ± 1.3*	1.9 ± 1.1*

*$p<0.05$ compared to baseline. Baseline was the average mg intake of cocaine/h during the 5-day cocaine acquisition period prior to any experimental manipulations.

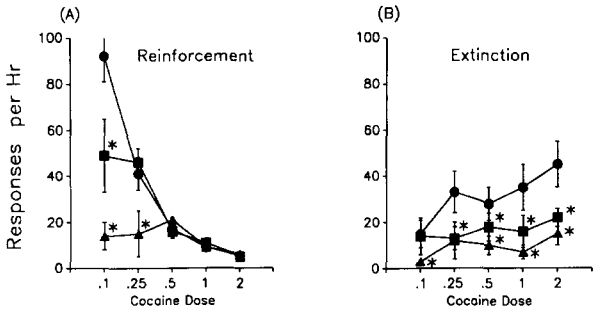

FIG. 3. Mean ± S.E.M. responses per h maintained by cocaine reinforcement (A) and during extinction from cocaine (B) during the first and second hours postinjection of acutely administered $MgCl_2$ in a doses of 0 (circles), 30 (squares) or 125 (triangles) mg/kg. *$p<0.05$ when compared to the 0 mg/kg dose of $MgCl_2$.

for the remaining 30 min of the session.

Multiple injection effects. Systematic changes in cocaine intake were present before the daily injection of $MgCl_2$ subsequent to the first injection (Table 1A). It was evident from examining the mg intake of cocaine per h during the first h preceding $MgCl_2$ injections that there was an interaction effect of multiple injections of $MgCl_2$ on cocaine-maintained responding, $F(20,95) = 1.77$, $p<0.03$. Normally, the mg intake of cocaine per h remained constant over doses of cocaine and over the 5 days of the cocaine access (data from 0 mg/kg $MgCl_2$). Compared to baseline cocaine intakes, there were decreases in cocaine intake after 4 days of injecting 15 and 30 mg/kg $MgCl_2$, $p<0.05$, after 3 days of injecting 125 mg/kg $MgCl_2$, $p<0.05$, and after 2 days of injecting 250 mg/kg $MgCl_2$, $p<0.05$. Although it is evident that the multiple injection effects of $MgCl_2$ contributed to the differences measured following the acute administration of $MgCl_2$ described above, added effects were produced by acute injections of $MgCl_2$ as evidenced by intakes which were 25–60% lower immediately following the administration of $MgCl_2$ than 24 h following the administration of $MgCl_2$.

Experiment 2. Effects of $MgCl_2$ Injections on Cocaine-Maintained Responding and Responding During Extinction from Cocaine

Acute and multiple injection effects under reinforcement conditions. During the postinjection period, $MgCl_2$ dose dependently reduced responding maintained by cocaine overall, $F(2,20) = 6.31$, $p<0.0075$, and significantly interacted with cocaine, $F(8,80) = 6.32$, $p<0.0001$. There were decreases in responding maintained by 0.1 mg/kg/infusion cocaine following 30 and 125 mg/kg $MgCl_2$, $p<0.01$ compared to the 0 mg/kg control, and decreases in responding maintained by 0.25 mg/kg/infusion cocaine following 125 mg/kg $MgCl_2$, $p<0.01$ compared to the 0 mg/kg control (Fig. 3A). During the preinjection period, $MgCl_2$ significantly interacted with cocaine, $F(8,80) = 5.21$, $p<0.0001$. The mg intake of cocaine per h remained constant over doses of cocaine and over the 5 days of cocaine access in the 0 mg/kg control group. Compared to baseline cocaine intakes, there were decreases in cocaine intake after 4 days of injecting 30 mg/kg $MgCl_2$, and after 3 days of injecting 125 mg/kg $MgCl_2$, $p<0.05$. Intakes were 25–40% lower immediately following the administration of $MgCl_2$ than 24 h following the administration of $MgCl_2$. These data on the acute and multiple injection effects of $MgCl_2$ replicate the results obtained in Experiment 1.

Acute effects under extinction conditions. In the same group of rats in which $MgCl_2$ reduced cocaine-maintained responding, $MgCl_2$ dose dependently reduced responding during extinction from cocaine, $F(2,20) = 3.92$, $p<0.04$ (Fig. 3B). This influence of $MgCl_2$ occurred at all doses of cocaine, and thus, there was no significant interaction effect. Further analyses of the 0 mg/kg control data revealed significant differences between the cocaine dose-effect curves under reinforcement and extinction conditions, $F(4,72) = 32.3$, $p<0.0001$. NaCl-injected rats had increased rates of responding during extinction from 0.5, 1.0 and 2.0 mg/kg/infusion doses of cocaine compared to reinforcement conditions, and decreased rates of responding during extinction from the 0.1 mg/kg/infusion dose of cocaine compared to reinforcement conditions, $p<0.05$. Furthermore, as preextinction dose of cocaine increased, the rate of responding under extinction conditions increased, $F(4,80) = 2.51$, $p<0.05$. Following doses of cocaine for which extinction increased rates of responding, 30 and 125 mg/kg $MgCl_2$ attenuated this accelerated extinction responding, $p<0.05$. Following doses of cocaine for which extinction decreased rates of responding, 30 and 125 mg/kg $MgCl_2$ reduced the rate of responding to an even greater extent, $p<0.05$.

Experiment 3. Acute and Multiple Injection Effects of $MgCl_2$ on Glucose + Saccharin-Maintained Responding

Acute effects. Following injections of the various doses of $MgCl_2$ there were reductions in G+S-maintained responding during the 2-h postinjection period in the number of licks per bout, whereas there were no differences in this measure during the first h preinjection, $F(2,30) = 17.8$, $p<0.0001$. During first postinjection h, the number of licks per bout (Fig. 1B) were decreased by 125 and 250 mg/kg $MgCl_2$, and during second h postinjection, the number of licks per bout (Fig. 1B) were dose-dependently decreased by 15, 30, 125 and 250 mg/kg $MgCl_2$ compared to the 0 mg/kg saline control condition, $p<0.05$. The number of licks was influenced in the same manner as licks per bout. Bouts of licking, $F(2,30) = 23.9$, $p<0.0001$, were not different during the first h preinjection or first h postinjection, but were decreased by 30, 125 and 250 mg/kg $MgCl_2$ during the second h postinjection, $p<0.05$. Upon defining a bout as a group of licks separated by 2 or more s, the number of which would reflect motor aspects of licking (7), bouts of licking were not different except during the first and second h postinjection of 250 mg/kg $MgCl_2$, $F(2,30) = 11.6$, $p<0.0003$, when large reductions occurred, $p<0.05$. These data suggest that only a dose of 250 mg/kg $MgCl_2$ was nonspecifically suppressing behavior, possibly by interfering with the motor ability to lick. The dose-effect profile of $MgCl_2$ on G+S-maintained responding was very similar to that observed on cocaine-maintained responding.

Multiple injection effects. A one-way analysis of variance was conducted to determine if there were any multiple injection effects of 30 mg/kg $MgCl_2$ on G+S-maintained responding. The results demonstrate that there were no multiple injection effects of 30 mg/kg on the number of licks, bout of licking or licks per bout per h (Table 1B) over 4 days of multiple injections.

Experiment 4. Acute and Multiple Injection Effects of $MgCl_2$ on Food-Maintained Responding

Acute effects. Following injections of the various doses of $MgCl_2$ there were interaction effects on food-maintained re-

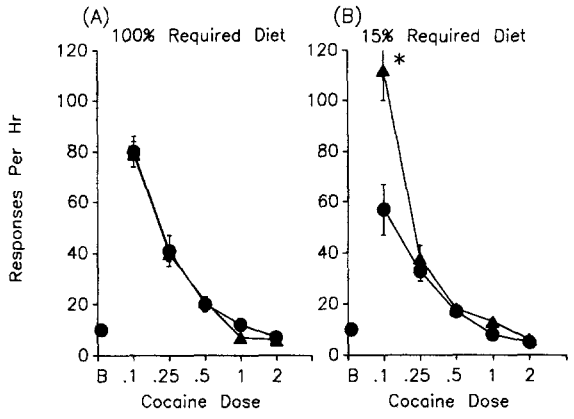

FIG. 4. Mean ± S.E.M. responses per h maintained by cocaine during baseline (B) acquisition of the 0.75 mg/kg/infusion training dose and different infusion doses of cocaine (0.1, 0.25, 0.5, 1 and 2 mg/kg/infusion) during the Prediet Period (circles) and Postdiet Period (triangles) in 2 groups of rats fed either the control diet (A) or the 15% required Mg^{2+}-deficient diet (B) for 3 weeks between the Prediet and Postdiet dose-response determinations. *$p<0.05$ when compared to the control diet or Prediet Period.

sponding during the 2-h postinjection period, $F(8,30)=6.72$, $p<0.0001$. The results demonstrate that food-maintained responding was markedly suppressed only by the highest dose of 250 mg/kg $MgCl_2$ which suppressed responding during both the first and second h postinjection, $p<0.05$ (Fig. 1C). In addition, there was a small transient decrease in food-maintained responding during the first h after the injection of 125 mg/kg $MgCl_2$, $p<0.05$.

Multiple injection effects. Following multiple injections of $MgCl_2$, a significant interaction effect was found on food-maintained responding, $F(16,60)=4.21$, $p<0.0001$. Further analysis revealed that the only effect of multiple injections of $MgCl_2$ on food-maintained responding was by 250 mg/kg which decreased the number of responses or pellets consumed per min, $p<0.05$ (Table 1C).

Experiment 5. Effects of a Mg^{2+}-Deficient Diet on Cocaine-Maintained Responding

Dietary Mg^{2+} had a significant interaction with varying doses of cocaine during the prediet and postdiet periods, $F(4,32)=12.86$, $p<0.01$. Both groups had similar rates of responding maintained by cocaine during the 0.75 mg/kg/infusion cocaine acquisition period (baseline), and during the prediet cocaine (Prediet Period) dose-response determination (Fig. 4A and B). During the postdiet period, a 15% required Mg^{2+}-deficient diet (Fig. 4B) caused an increase in the rate of responding maintained by 0.1 mg/kg/infusion of cocaine, $p<0.05$ compared to the control diet (Fig. 4A) and the group's own prediet period responding maintained by the 0.1 cocaine dose. The representative event record shows an altered pattern of responding that is characterized by frequent response bursts throughout the test session (Fig. 2G).

DISCUSSION

The results of Experiment 1 demonstrated that acute and multiple injections of $MgCl_2$ decreased the rate of cocaine self-administration during cocaine dose-response determinations. The decreases in cocaine-maintained responding and intake of cocaine were measured during both the preinjection period and the postinjection period, with bigger decreases during the postinjection period (acute injections) than during the preinjection period (multiple injections). However, because reductions in cocaine-maintained responding were observed during the preinjection period in Experiment 1, the changes observed during the postinjection period should be more appropriately regarded as a combined acute and multiple injection effect. Although there is a lack of independence of the acute effects of $MgCl_2$ from the multiple injection effects of $MgCl_2$ on responding maintained by cocaine in this study, the data are systematic and are interpretable in terms of specificity and selectivity relative to G+S- and food-maintained responding.

The dose-response functions obtained during the $MgCl_2$ postinjection periods were shifted downward from the 0 mg/kg control dose-response function. These effects of $MgCl_2$ dose manipulations were not observed on all cocaine doses, but primarily on doses below the 0.75 mg/kg/infusion training dose of cocaine. It is not entirely clear why there was no effect of the various doses of $MgCl_2$ on responding maintained by doses of cocaine greater than the training dose, but there was an effect on lower doses of cocaine. A similar influence was observed on the dose-effect curves of $MgCl_2$ on the dose-response to apomorphine-induced stereotypy (17), where an enhancement of stereotypy by $MgCl_2$ was only observed with the lower doses of apomorphine. Others (5) have demonstrated that the 5-HT reuptake inhibitor fluoxetine reduced responding maintained by lower doses of cocaine (0.1 and 0.2 mg/kg/infusion) but not a higher dose (0.4 mg/kg/infusion). A lack of an effect of fluoxetine on responding maintained by a high training dose of 1 mg/kg/infusion cocaine has been reported as well (29). It may be that the responding maintained by cocaine can not be easily decreased when the dose of cocaine that is self-administered by the animal is more potent than the treatment drug that is injected by the experimenter, and hence self-administration responding is maintained at a rate which is normal for that particular dose of cocaine. Such an influence of cocaine potency has been previously shown where the availability of a higher self-administered dose of cocaine shifted the chlordiazepoxide dose-effect curve on cocaine-maintained responding to the right (10). In that experiment, chlordiazepoxide (10 mg/kg) decreased responding maintained by a low dose of cocaine (0.5 mg/kg/infusion), but not by a higher dose of cocaine (1.0 mg/kg/infusion).

During the preinjection period without any injections of $MgCl_2$, there was no multiple injection effect of repeated cocaine administration on daily intake. In a previous study on mouse aggression, no multiple injection effects with these same doses of $MgCl_2$ were detected over a 2-week period when mice were tested prior to the daily injection (13). Thus the behavioral effects of multiple injections observed in the present study were not likely a result of injecting $MgCl_2$ alone or injecting cocaine alone, but more likely they were a result of the two drugs having been administered together.

The acute and multiple injection effects of $MgCl_2$ in reducing responding did not appear to be nonspecific. In high doses (500–1000 mg/kg), $MgCl_2$ is known to have sedative and paralytic properties (27). Consequently, the dose-dependent reductions in cocaine-maintained responding might be related to nonspecific behavior suppression or to a motor disturbance. This interpretation might be true of the effects observed with 250 mg/kg $MgCl_2$ because the large reductions in cocaine-maintained responding occurred over the entire cocaine dose range, were not dependent on dose of cocaine, and responding was inhibited for most of the test session. This dose of $MgCl_2$ completely inhibited aggression in mice and caused them to be sedated (13). There is also evidence from Experiment 3 that 250 mg/kg $MgCl_2$

interferes with the motor ability to lick, whereas other doses of $MgCl_2$ do not.

Acute effects of $MgCl_2$ were evident on G+S-maintained responding in Experiment 3. The $MgCl_2$ dose-effect functions were similar for cocaine and G+S. Thus it is likely that if injections of $MgCl_2$ were made biweekly in Experiment 1, rather than daily, similar acute decreases in cocaine-maintained responding would have been obtained which would indicate a distinct acute effect. Since food-maintained responding was minimally influenced by acute $MgCl_2$ injections in Experiment 4, these data indicate that the changes in responding were specific, but not selective for cocaine-maintained responding. In Experiment 2, which replicated the acute and multiple injection effects of $MgCl_2$ on cocaine-maintained responding obtained in Experiment 1, $MgCl_2$ decreased responding during extinction from higher doses of cocaine. These data also suggest that the rate decreasing effects of acute $MgCl_2$ were not selective for cocaine-maintained responding. Using a DRL 20-s schedule of food reinforcement which produced the same overall rate of responding maintained by food as an FR 5 schedule produced for responding maintained by cocaine, injections of bromocriptine in rats decreased the rate of responding maintained by cocaine, but not food (12). Similarly, injections of bromocriptine in rhesus monkeys reduced cocaine-maintained responding and had minimal effects on food-maintained responding (21). These data have been interpreted to indicate that bromocriptine can selectively reduce responding maintained by cocaine. Clearly, the use of an alternative reinforcer such as G+S is beneficial for more precisely determining the selectivity of a treatment drug on cocaine-maintained responding. It has been demonstrated that the 5-HT reuptake blocker fluoxetine, which reduced cocaine-maintained responding, also reduced G+S-maintained responding without influencing food consumption (5). A treatment effect on G+S-maintained responding is not always found. Tryptophan, which reduced cocaine-maintained responding, had no effect on G+S-maintained responding (6). These data also suggest that treatments which reduce responding maintained by both cocaine and G+S might be acting indirectly with cocaine to alter the rate of responding, thus resulting in nonselective effects on highly rewarding substances. The multiple injection effects of $MgCl_2$ do appear to be more selective for cocaine because neither G+S- nor food-maintained responding was affected by the repeated administration of $MgCl_2$. Thus, following repeated exposure to $MgCl_2$, the changes in behavior might be related to a more direct interaction between $MgCl_2$ and cocaine.

The decreases in cocaine self-administration with injections of $MgCl_2$ are similar to what is observed with psychomotor stimulants (12, 21, 36, 39). Such changes are generally interpreted to indicate that the injected drug is increasing or substituting for the reinforcing properties of the drug being self-administered [see (38) for review]. If a substance increases the reinforcing action of a self-administered drug, then the intake of that drug should diminish, as was observed. This decrease in cocaine-maintained responding occurred without any instances of accelerated responding which can be indicative of a reduction in or blockade of reinforcement. The results from Experiment 2 suggest that $MgCl_2$ is not blocking the effects of cocaine because accelerated extinction responding was not potentiated by $MgCl_2$. The increase in cocaine self-administration with the Mg^{2+}-deficient diet is comparable to changes in cocaine-maintained responding following administration of pimozide [a D_2 antagonist, (8)], SCH 23390 [a D_1 antagonist, (22)], 6-OHDA [a catecholamine neurotoxin, (30)], haloperidol [a D_2 antagonist, (31)], and other selective dopamine antagonists (3). An increase in cocaine-maintained responding following dopamine blockade is thought to be indicative of a blockade of the reinforcing effects of cocaine. Since responding was maintained at a high level throughout the 3-h session, these effects of a Mg^{2+}-deficient diet are more similar to low doses of DA antagonists because with higher doses, accelerated responding is followed by an extinguished response. Although there was a significant difference between the control-diet and deficient-diet groups, this significance was primarily due to differences at a single cocaine dose of 0.1 mg/kg/infusion. In an earlier study of mouse aggression (14), exposure to Mg^{2+}-deficient diets for 7 weeks produced greater decrements in aggression than exposure for 3 weeks. These decrements in aggression were coupled with normal levels of locomotion, and thus were not associated with nonspecific hyperactivity. It is unlikely that the increase in responding maintained by 0.1 mg/kg/infusion cocaine following the Mg^{2+}-deficient diet which was fed for 3 weeks was a result of a nonspecific hyperactivity produced by the diet. Perhaps if a longer diet exposure was used in the present study, a more extreme deficiency of Mg^{2+} might have been more capable of increasing cocaine-maintained responding.

Although the above interpretation that $MgCl_2$ increases the effectiveness of reinforcing substances other than food is consistent with the evidence showing that self-administered $MgCl_2$ appears to substitute for cocaine (19) and that $MgCl_2$ can induce a conditioned place preference (24) and can potentiate cocaine-induced conditioned place preference (23), other viable interpretations exist. Another possible explanation for these data is that $MgCl_2$ is suppressing responding by serving as an aversive, negative or punishing stimulus. It has been shown that as the intensity of a behavioral suppressing stimulus such as shock increases, cocaine-maintained responding decreases (2,11). Consistent with the interpretation that $MgCl_2$ has possible suppressing effects on responding are the data from Experiment 2 which show that accelerated responding under cocaine extinction conditions is also attenuated by $MgCl_2$, and the data showing that in cocaine-naive rats, $MgCl_2$ does not maintain responding beyond day 1 of access (18). It may just be that in the $MgCl_2$ substitution experiments in cocaine-trained rats (19), cocaine experience blocks the suppressing effects of $MgCl_2$ and responding is maintained by $MgCl_2$ because of its stimulant properties. It has been shown, however, that cocaine and d-amphetamine do not attenuate the punishing effects of shock or pressurized air on responding as do pentobarbital and chlordiazepoxide (34). Even though $MgCl_2$ most likely maintains responding in cocaine-trained rats in a manner unrelated to a blockade of punishing effects of $MgCl_2$ by cocaine experience, others have shown that a drug can simultaneously have positive and negative behavioral directing consequences. Stimulants such as cocaine (33) and d-amphetamine or apomorphine (37) can maintain responding that leads to their injection and can either suppress behavior leading to reinforcement (37) or cause an animal to engage in behavior that postpones reinforcement (33). Clearly, more work is need to determine if $MgCl_2$ can have aversive, negative or punishing effects on responding, and if so, to determine under what experimental conditions $MgCl_2$ negatively controls behavior and under what experimental conditions $MgCl_2$ positively controls behavior.

These data have implications for the clinical management of cocaine-addicted humans. The present data show that injections of $MgCl_2$ can decrease cocaine-maintained responding in rats, while under other conditions it is self-administered and can maintain responding after cocaine is no longer available (19). Studies in mice (15) and squirrel monkeys (16) demonstrate that $MgCl_2$ attenuates the effects of behavior-increasing doses of cocaine, and it can increase behavior associated with ineffective doses of cocaine. Given the similarities across different species to the dose range of $MgCl_2$ that produces these effects (15–125

mg/kg), it is likely that this dose range would also be efficacious in altering the effects of cocaine in humans. One concern, however, is whether or not toxicity could develop from the repeated administration of magnesium in this dose range in humans. Although Mg^{2+} is a mineral nutrient, it is widely abundant in the body and brain (1). A dose of 280 mg (approximately 4 mg/kg) per day is needed to maintain a positive Mg^{2+} balance (28). This average intake of Mg^{2+} maintains serum Mg^{2+} in its normal range of 1.5–2.5 mEq/liter. Signs of Mg^{2+} toxicity are manifested when serum levels rise over 5 mEq/liter (28). At serum concentrations of 5–9 mEq/liter, hypotension, nausea and vomiting are commonly observed, while at serum concentration of 10–15 mEq/liter, ECG changes, respiratory depression, coma and cardiac arrest occur. Renal effects of excess Mg^{2+} are inconsistently observed. Intramuscular injections of 60 mg/kg/day (4 g) $MgSO_4$ have been shown to maintain serum concentrations of Mg^{2+} at 3 mEq/liter during repeated treatment (9). Since it appears that toxic reactions to excess magnesium would not be associated with doses within this range, then magnesium might safely and effectively treat cocaine addiction [see (20) for review].

ACKNOWLEDGEMENTS

This work was supported by NIDA grant RO1-04325 to K.M.K. The cocaine hydrochloride was also generously supplied by NIDA. The authors wish to thank Dr. James E. Smith for extending his time and his laboratory to teach us the methods to enable us to conduct these experiments. We are also grateful to Dr. Henry Marcucella for his extensive preview and comments on this manuscript.

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