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Dietary cadmium exposure attenuates D-amphetamine-evoked [^{3}H]dopamine release from striatal slices and methamphetamine-induced hyperactivity

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Abstract

Prolonged exposure to environmentally relevant amounts of $CdCl_2$ results in cadmium accumulation in dopamine-rich brain regions, such as striatum. Exposure to these low levels of cadmium also diminishes cocaine-induced hyperactivity and conditioned reinforcement. The goal of the present study was to assess the effect of cadmium on amphetamine pharmacology. Direct application of cadmium (0.1–100 μM), within the concentrations reported in brain after chronic exposure, to preloaded rat striatal slices did not alter D-amphetamine-evoked [^{3}H]dopamine release. To determine the effect of dietary cadmium exposure on amphetamines, rats received ad libitum access to diet containing $CdCl_2$ (10 or 100 ppm) or to control diet for 30 days and then D-amphetamine-evoked [^{3}H]dopamine release and methamphetamine-induced hyperactivity were measured. Dietary $CdCl_2$ exposure produced a marked increase in cadmium blood and brain levels, approximate to environmental metal exposure. Dietary cadmium exposure was associated with decreased potency of D-amphetamine to evoke [^{3}H]dopamine release. Cadmium-exposed rats were also less sensitive to the locomotor-activating effect of acute methamphetamine (0.3 or 1.0 mg/kg) injection. The present findings demonstrate that the presence of cadmium in brain is not sufficient for the inhibition of D-amphetamine-evoked dopamine release. This suggests that cadmium does not directly interfere with the mechanism of action for amphetamine pharmacology; rather, it suggests that long-term cadmium exposure induces a change in the number and/or function of striatal neurons.

Keywords: Amphetamine; Cadmium; Dopamine; Methamphetamine; Striatum

1. Introduction

The industrial uses of cadmium have increased distribution of this "heavy" metal into the environment. People are exposed to cadmium through air, water, and food from cadmium-contaminated crops (Gutenmann et al., 1982; Bache et al., 1987). *Nicotiana tabacum* is also prone to cadmium accumulation (Scherer and Barkemeyer, 1983; Yue, 1992), such that tobacco smokers may have significantly elevated levels of the metal compared to non-smokers. Cadmium accumulates in neural tissue in the central and peripheral nervous systems (Arvidson, 1986). For example, Clark et al. (1985) treated rats with an environmentally relevant concentration (0, 20 or 100 ppm) of $CdCl_2$ via diet for 30 days. Metal-exposed rats had greater (~5 μM) cadmium residues than control rats (<0.01 μM) in multiple brain regions, including the striatum. Therefore, prolonged exposure to relatively low amounts of $CdCl_2$ results in cadmium accumulation in a dopamine-rich brain region.

Prolonged cadmium exposure has been shown to attenuate the behavioral activation produced by acute

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injection of cocaine to rats (Nation et al., 1991, 1995). For 60 days rats received water containing $CdCl_2$ (100 ppm) or water without added $CdCl_2$. Subsequently, cadmium-exposed and control rats were injected with cocaine (10 mg/kg) or saline and locomotor activity was measured. Cocaine injection produced hyperactivity in both control and cadmium-exposed rats, but the magnitude of the behavior was decreased in the latter group. Cadmium-exposed rats that were administered saline did not display hyper- or hypoactivity. Thus, dietary cadmium exposure did not alter basal locomotor activity, but decreased the efficacy of cocaine to induce hyperactivity.

A possible mechanism of action for cadmium to inhibit the behavioral effects of cocaine is diminishment of the cocaine-induced increase in extracellular dopamine that is observed after injection (Rothman, 1990; Uhl et al., 2002). Cocaine does not directly release presynaptic dopamine, but rather prevents dopamine uptake by inhibiting the dopamine transporter (Ritz et al., 1987; Carroll et al., 2002). Cadmium has been demonstrated to both increase and decrease the basal concentration of dopamine in brain (Hrdina et al., 1976; Olivier et al., 1995; Lafuente et al., 2000, 2003), actions that are likely mediated through a calcium-mediated process. Cadmium (>100 μM) is an efficacious, but non-selective, inhibiter of calcium channels (Hinkle et al., 1987). Furthermore, higher concentrations (≥250 μM) of cadmium have been demonstrated to inhibit [^{3}H]dopamine uptake in rat striatal synaptosomes, suggesting that cadmium intrinsically inhibits the function of the dopamine transporter (Lai et al., 1981). The effect of dietary cadmium exposure on the function of the dopamine transporter has not been determined.

Similar to cocaine, administration of amphetamines results in an increase in extracellular dopamine levels and hyperactivity. However, amphetamines are a substrate for the dopamine transporter and cause dopamine to be released from presynaptic stores (Sulzer et al., 1995; Rothman et al., 2001). Thus, amphetamine and cocaine have similar behavioral outcomes, but different mechanisms of action. Based on the previous research on cadmium and cocaine, the present hypothesis is that dietary cadmium exposure will attenuate the neurochemical and behavioral effects of amphetamines. In the first experiment, an environmentally relevant range (0.1–100 μM) of cadmium concentrations were directly applied to striatal slices and D-amphetamine-evoked [^{3}H]dopamine release was measured. In the second experiment, rats received dietary exposure to $CdCl_2$ (10 or 100 ppm) or to a control diet, and subsequently the concentration–response for D-amphetamine-evoked [^{3}H]dopamine release was determined. Cadmium-exposed and control rats were injected with methamphetamine and locomotor activity was measured in the third experiment to determine if dietary cadmium exposure alters the behavioral effects of amphetamines.

2. Materials and methods

2.1. Subjects

All animal procedures were approved by the Institutional Animal Care and Use Committee of the University of Missouri. The subjects were male Sprague Dawley rats (Harlan, Indianapolis IN; 175–200 g upon arrival to the laboratory) that were double-housed with ad libitum access to tap water under a 12/12-h light/dark cycle. Rats used in Experiment 1 received standard rodent chow (#5008; LabDiet, Richmond IN). Rats in Experiments 2 and 3 received a specially prepared purified diet containing $CdCl_2$ (10 or 100 ppm) or a control diet that did not contain added $CdCl_2$ (Dyets, Bethlehem PA). Analysis (vide infra) of samples of the diet revealed elevated levels of cadmium (mean 100 ppm $CdCl_2$ diet=54.6 ppm Cd, mean 10 ppm $CdCl_2$ diet=4.9 ppm Cd) in the diets with added $CdCl_2$, and levels below the detectable limit (0.5 ppm Cd) in the control diet. Levels of cadmium in tap water also were below the detectable limit. Rats in Experiments 2 and 3 received their respective diet for at least 30 days before determination of evoked [^{3}H]overflow or behavioral testing. The concentrations of $CdCl_2$ in the diet and the duration of the metal exposure regimen has been demonstrated to significantly increase blood levels of cadmium to levels that approximate pack-a-day tobacco smokers (Nation et al., 1997b; Yue, 1992).

2.2. Drugs and chemicals

D-Amphetamine sulfate and methamphetamine HCl were purchased from Sigma Chemical Company (St. Louis MO). Pargyline and $CdCl_2$ were purchased from Acros Organics (Fairlawn NJ). Radiolabeled dopamine (dihydroxyphenylethylamine 3,4-[ring-2,5,6-^{3}H], specific activity=54–59 Ci/mmol) was purchased from PerkinElmer Life Sciences (Boston MA). TS-2 tissue solubilizer was purchased from Research Products International (Mt. Prospect IL). All other chemicals were purchased from Fisher Scientific (Fairlawn NJ).

2.3. Experiment 1: effect of cadmium on amphetamine-evoked [^{3}H]dopamine release

The rats used in this experiment were not exposed to cadmium via diet. Rats were euthanized via rapid decapitation and striata were dissected and sliced (750 μm thick slices) using a Stoelting (Wood Dale IL) tissue chopper. Slices were incubated in oxygenated buffer (in mM, 108 NaCl, 25 $NaHCO_3$, 11.1 glucose, 4.7 KCl, 1.3 $CaCl_2$, 1.2 $MgCl_2$, 1.0 NaH_2PO_4, 0.11 ascorbic acid, 0.004 EDTA) in a metabolic shaker at 37 °C for 30 min. Slices were transferred to fresh buffer, [^{3}H]dopamine (0.1 μM) was added, and slices were incubated for an additional 30 min. Each slice was transferred to 1 of 12 reaction chambers (0.2 ml) bounded by glass microfiber filters (GF/B, Whatman, Madistone England) in an automated superfusion system (Suprafusion 2500, Brandel, Gaithersburg MD) (Delle Donne and Sonsalla, 1996). Pargyline (10 μM) was added to buffer to inhibit monoamine oxidase (Zumstein et al., 1981) and slices were superfused with buffer at a rate of 0.75 ml/min. After 60 min, sample collection commenced at a rate of 1 sample/5 min. After the collection of 3 baseline samples, the intrinsic activity of 0.1, 3, 10 or 100 μM cadmium on [^{3}H]overflow was

determined for 30 min. In each rat, one concentration of cadmium was added to buffer for 6 of the 12 slices and the remaining 6 slices were superfused with buffer that did not contain cadmium. These cadmium concentrations were selected based on the reported level (~5 μM) of cadmium in striatum after prolonged exposure to water containing $CdCl_2$ (100 ppm) (Clark et al., 1985). To determine the concentration–response for D-amphetamine in the presence and absence of cadmium, each slice was then superfused with 1 of 5 concentrations of D-amphetamine (0.1–30 μM) or with buffer for 30 min. At the completion of the experiment, slices and filters were removed from the reaction chamber and incubated with tissue solubilizer (0.25 ml/sample). Radioactivity in superfusate samples and slices/filters was measured by liquid scintillation spectroscopy (LS 6500 Scintillation Counter, Beckman-Coulter, Fullerton CA; counting efficiency ≈ 45–55%).

2.4. Experiment 2: D-amphetamine-evoked [^{3}H]dopamine release in slices from rats exposed to cadmium via diet

Rats received diet containing $CdCl_2$ (10 or 100 ppm) or control diet for at least 30 days. Rats were euthanized via rapid decapitation and trunk blood (~3 ml) was collected immediately. Striatal slices and buffer were prepared as described for Experiment 1, and the remaining brain tissue was collected. Brain and blood samples were frozen for subsequent analysis (vide infra). After superfusion for 60 min and the collection of 3 baseline samples, one slice per rat was superfused with 1 of 9 concentrations of D-amphetamine (0.01–30 μM) or 1 of 2 concentrations of KCl (10–30 mM). One slice was superfused in the absence of D-amphetamine and KCl. Thus, the concentration–response for D-amphetamine and KCl was determined in slices from each rat. After addition of D-amphetamine or KCl, samples were collected for 60 min. At the completion of the experiment, slices and filters were prepared and radioactivity was measured.

2.5. Behavioral apparatus

Locomotor activity was monitored automatically using Med Associates' (St. Albans VT) Open Field Test Environments (ENV-515), comprised of a 16×16 horizontal grid of infrared sensors and a bank of 16 vertical sensors. Each monitor surrounded an acrylic cage (43.2×43.2×30.5 cm), and each monitor and cage was housed in a large sound-resistant cubicle (ENV-017M). Data were collected in 5 min intervals using Med Associates' Open Field Activity Software (SOF-811) that records the number of sensor breaks and computes these data to measures of distance traveled (cm).

2.6. Experiment 3: effect of dietary cadmium exposure on methamphetamine-induced hyperactivity

The rats used in this experiment were exposed to diet containing $CdCl_2$ (10 or 100 ppm) or to control diet for at least 30 days before the start of behavioral testing. Testing was conducted during the light phase of the light/dark cycle. On Acclimation Days 1 and 2, rats were transported from the colony room to the laboratory, weighed, injected (sc, injection volume=1 ml solution/kg body weight) with saline (0.9% w/v) and placed in the apparatus for 60 min. At the completion of each session the apparatus was cleaned with a mild soap solution. On the following day (Test Day) rats were injected (sc) with methamphetamine (0.3 or 1.0 mg drug/kg body weight, free base weight) or vehicle (saline) and placed in the apparatus for 60 min. These doses of methamphetamine have been demonstrated to produce hyperactivity after acute injection to rats (Itoh et al., 1984; Szumlinski et al., 2000).

2.7. Determination of cadmium in blood and brain

One milliliter of blood was added to 3 ml of matrix modifier (0.2% nitric acid, 0.5% Triton X-100 and 0.2% ammonium phosphate in distilled water) (Miller et al., 1987) then frozen at 4 °C until analysis for cadmium. Brain samples were stored at minus 80 °C until subsamples were processed for cadmium analysis. One gram of tissue was placed in a 5 ml Teflon container (Cole-Parmer, Vernon Hills, IL) to which 2 ml of trace metal concentrated nitric acid (Fischer Scientific, Pittsburgh, PA) was added. The mixture was incubated at 90 °C overnight, cooled and diluted to a final volume of 10 ml with deionized distilled water.

Blood and brain samples were analyzed by graphite furnace atomic absorption spectroscopy (GFAAS) with Zeeman background correction using a THGA tube. Standard conditions from the Perkin-Elmer Analyst 800 (PerkinElmer Corp, Norwalk, CT) manual were used as a starting point; however, these instrument parameters were optimized for this project. The analytical sequence followed the standard U.S. EPA contract laboratory (CLP) procedures, with internal and continuing calibration verification analyses every 10 samples, spikes every 15 and recalibration every 15 samples. All tissue samples were analyzed in duplicate, and a preparation duplicate analysis was performed every 15 samples. Because of limited blood volume, these samples were analyzed once and a duplicate performed every 20 samples.

2.8. Data analysis

Fractional release for each superfusate sample was calculated by dividing the [^{3}H] collected in each 5-min sample by the total [^{3}H] present in the tissue at the time of sample collection. Basal [^{3}H]outflow was calculated from the average of fractional release in the three samples just before addition cadmium and/or D-amphetamine to the superfusion buffer. The fractional samples greater than baseline were summed and basal outflow was subtracted to determine total [^{3}H]overflow.

In Experiment 1, total [^{3}H]overflow data from the 30 min period of superfusion with D-amphetamine were analyzed via 2-way repeated measures analysis of variance (ANOVA) in which amphetamine concentration was a within-subject factor and cadmium concentration was a between-groups factor (SPSS for Windows version 11.0, SPSS, Chicago IL). For all analyses significance was established as $P<0.05$ and simple main effect analyses and Tukey post hoc tests were performed when appropriate. EC_{50} and EC_{100} values for each rat were determined by nonlinear regression fit of the total [^{3}H]overflow data to a sigmoidal dose response equation (Prism version 3.03, Graph Pad, San Diego CA). The log EC_{50} and log EC_{100} values were analyzed via 1-way ANOVA with cadmium concentration as a between-groups factor.

In Experiment 2, total [^{3}H]overflow data from the 60 min period after D-amphetamine was added to buffer were analyzed via

2-way repeated measures ANOVA with amphetamine concentration as a within-subject factor and cadmium diet as a between-groups factor. The EC_{50} and EC_{100} values were calculated for each rat. For four rats, D-amphetamine did not produce a concentration-dependent increase in [^{3}H]dopamine release and EC_{50} and EC_{100} values of 30 μM were assigned for these rats. The value of 30 μM was selected because it was the highest D-amphetamine concentration tested. Kruskal–Wallis tests were performed on the EC_{50} and EC_{100} values to determine group differences. Spearman's Rho analyses were performed between the EC_{50} value and the respective concentration of cadmium in blood and in brain. For 7 of the 10 rats in the control condition the concentration of cadmium in blood was below detectable limits (0.02 micro-g/l) and the value of the lower detection limit was assigned for these rats. Similarly, for 4 of the 10 rats in the control condition the concentration of cadmium in brain was below the detectable limit (0.02 ng/g) and the value of the lower detection limit was assigned for these rats. Total [^{3}H]overflow data from the 60 min period after KCl was added to buffer were analyzed via 2-way repeated measures ANOVA with KCl concentration as a within-subject factor and cadmium diet as a between-groups factor. Neither EC_{50} nor EC_{100} values were determined for KCl because only 2 concentrations of KCl were tested.

For Experiment 3, distance traveled (cm) was the dependent measure, consistent with previous studies of cadmium inhibition of cocaine-induced hyperactivity (Nation et al., 1991, 1995). The measure of total distance traveled was calculated by summing the distance from the twelve 5-min intervals on each day. Activity on Acclimation Days 1 and 2 was analyzed via 2-way repeated measures ANOVA with cadmium diet as a between-groups factor and time (twelve 5-min intervals) as a within-subject factor. Activity on the Test Day was analyzed via 3-way repeated measures analysis of covariance with cadmium diet and methamphetamine dose as between-groups factors, Time as a within-subject factor, and total distance traveled on Acclimation Day 2 as a covariate. Spearman's Rho analyses were performed for each methamphetamine dose between the total distance traveled and the respective concentration of cadmium in blood and in brain.

For Experiments 2 and 3, the concentrations of cadmium in blood and in brain were analyzed via Kruskal–Wallis tests. When the concentration of cadmium in blood or brain for a rat was below detectable limits (0.02 micro-g/l or 0.02 ng/g, respectively) the lower detection limit was assigned as the value for that rat. For Experiment 3, body weights also were analyzed via 1-way ANOVA with cadmium diet as a between-groups factor.

3. Results

3.1. Experiment 1: cadmium does not alter D-amphetamine-evoked [^{3}H]dopamine release

In Experiment 1, the concentration–response for D-amphetamine (0.1–30 μM) to evoke [^{3}H]dopamine release was determined in the presence and absence of cadmium (0.1, 3, 10 or 100 μM) added to the superfusion buffer. Total amphetamine-evoked [^{3}H]dopamine overflow in the absence and presence of cadmium is presented in Table 1. A significant main effect of amphetamine concentration was found ($F(5,70)=91.84$, $P<0.01$). Neither the main effect of cadmium concentration ($P=0.69$) nor the amphetamine concentration × cadmium concentration interaction ($P=0.52$) were significant. In the absence of cadmium, D-amphetamine produced a concentration-dependent increase in total [^{3}H]dopamine release (control EC_{50} value=5.5 μM, S.E.M.=1.6 μM; control EC_{100} value=26.7 μM, S.E.M.=2.5 μM). Addition of cadmium did not significantly change the EC_{50} or EC_{100} values, relative to the control condition ($P>0.05$). Thus, addition of a range of environmentally relevant concentrations of cadmium to slices did not alter D-amphetamine-evoked [^{3}H]dopamine release.

3.2. Experiment 2: dietary cadmium exposure attenuates D-amphetamine-evoked [^{3}H]dopamine release

Rats received diet containing $CdCl_2$ (10 or 100 ppm) or control diet for at least 30 days before the determination of the concentration–response for D-amphetamine and KCl to evoke [^{3}H]dopamine release. This cadmium exposure regimen produced a significant increase in the concentration of cadmium in blood and brain ($T(2)=127.2$, $P<0.05$ and $T(2)=118.3$, $P<0.05$, respectively; Table 2). Rats that received the diet containing 100 ppm $CdCl_2$ had cadmium

Table 1
Cadmium does not inhibit D-amphetamine-evoked [^{3}H]overflow from rat striatal slices preloaded with [^{3}H]dopamine

Cadmium concentration (μM)	D-Amphetamine concentration (μM)					
	0	0.1	1	3	10	30
Control	0.10±0.06	0.42±0.19	6.24±0.60	11.1±1.05	19.3±2.21	26.7±2.54
0.1	0.11±0.09	0.28±0.28	9.52±3.01	13.1±1.60	16.3±3.66	27.8±3.24
3	0.16±0.10	0.68±0.52	5.89±1.98	6.80±1.32	8.83±2.07	19.5±4.33
10	0.03±0.03	0.61±0.28	7.66±1.30	10.8±2.68	18.6±2.23	34.8±4.18
100	0.30±0.17	0.18±0.11	5.50±1.05	15.1±0.78	22.1±4.32	24.4±5.86

The concentration–response for D-amphetamine (0.1–30 μM)-evoked [^{3}H]dopamine release was determined in the presence and absence of one concentration cadmium (0.1, 3, 10 or 100 μM) in slices from each rat (Experiment 1).
Data represent mean (±S.E.M.) total [^{3}H]overflow from the 30 min period of superfusion with D-amphetamine. Control represents superfusion in the absence of cadmium for each rat, (n=4 rats/cadmium concentration).

Table 2
Dietary cadmium exposure produces a concentration-dependent increase in levels of cadmium in blood and brain

Dietary group	Experiment 2 ([^{3}H]dopamine release)		Experiment 3 (behavior)	
	Blood	Brain	Blood	Brain
Control	0.4±0.2	0.5±0.1	0.3±0.1	0.4±0.1
10 ppm $CdCl_2$	13.0±2.5*	10.0±1.6*	12.8±2.0*	10.2±1.3*
100 ppm $CdCl_2$	64.8±3.8*	151.4±13.2*	70.7±2.6*	147.3±8.2*

Rats received diet containing $CdCl_2$ (10 or 100 ppm) or control diet for at least 30 days before the [^{3}H]dopamine release (Experiment 2) or behavior experiments (Experiment 3) were conducted.
Data represent mean (±S.E.M.) concentration of cadmium in blood (μg/l) or brain (ng/g). Asterisks designate significant (P<0.05) difference from the control group, (n=8–10 rats/dietary group).

levels significantly greater than rats that received diet containing 10 ppm $CdCl_2$ or the control diet. Rats that received diet containing the lower concentration of $CdCl_2$ had cadmium levels in blood and brain that were significantly greater than the control group. A significant, positive relationship was found between the concentration of cadmium in blood and in the concentration of cadmium in brain (ρ=0.99; T(30)=90, P<0.025).

For the total [^{3}H]overflow measure, a significant main effect of amphetamine concentration was found (F(9,243)= 31.66, P<0.01; Table 3) and post hoc tests determined that the effect of D-amphetamine in this assay was concentration-dependent. However, neither the main effect of cadmium diet (P=0.55) nor the cadmium diet × amphetamine concentration interaction (P=0.99) were significant.

Table 3
Dietary cadmium exposure does not alter D-amphetamine- or KCl-evoked total [^{3}H]overflow from rat striatal slices preloaded with [^{3}H]dopamine

		Dietary group		
		Control	10 ppm $CdCl_2$	100 ppm $CdCl_2$
D-amphetamine concentration (μM)	0	0.00±0.00	0.13±0.09	0.55±0.31
	0.01	0.00±0.00	0.37±0.24	1.15±1.08
	0.1	0.59±0.36	3.19±2.19	1.48±1.33
	0.3	7.97±4.20	4.74±2.33	2.47±0.84
	0.6	4.34±1.25	9.94±4.01	8.52±1.62
	1	5.16±1.29	13.3±4.55	11.9±4.58
	3	14.6±2.95	16.3±3.87	14.3±2.88
	6	21.3±5.92	24.4±4.21	14.2±3.24
	10	26.3±2.82	31.2±6.30	23.1±3.88
	30	46.7±12.7	48.2±13.0	40.2±9.41
KCl concentration (mM)	10	0.59±0.16	0.86±0.41	1.54±1.34
	30	35.1±6.14	38.3±9.34	47.0±15.8

The concentration–response for D-amphetamine (0.01–30 μM) and KCl (10–30 mM) was determined in rats that had received diet containing $CdCl_2$ (10 or 100 ppm) or control diet for at least 30 days (Experiment 2).
Data represent mean (±S.E.M.) total [^{3}H]overflow from the 60 min period of superfusion with D-amphetamine or KCl, (n=10 rats/group).

EC_{50} and EC_{100} values from the D-amphetamine concentration–response curves were determined for each of the rats in this experiment. Analysis of the EC_{100} values did not reveal significant differences among the groups (P>0.05). However, analysis of the EC_{50} values revealed significant differences among the groups (T(2)=18.6, P<0.05). Subsequent multiple comparisons revealed that the EC_{50} values for rats that received diet containing 100 ppm $CdCl_2$ (mean EC_{50} value=12.6 μM, S.E.M.=±1.5 μM) were greater than those for rats that received diet containing 10 ppm $CdCl_2$ (mean EC_{50} value=8.5 μM, S.E.M.=±1.5 μM) or for rats that received the control diet (mean EC_{50} value=3.5 μM, S.E.M.=±1.3 μM). The mean EC_{50} value for the group of rats that received 10 ppm $CdCl_2$ was significantly less than that for rats that received 100 ppm $CdCl_2$. Thus, dietary cadmium exposure produced a decrease in the potency of D-amphetamine to evoke [^{3}H]dopamine release.

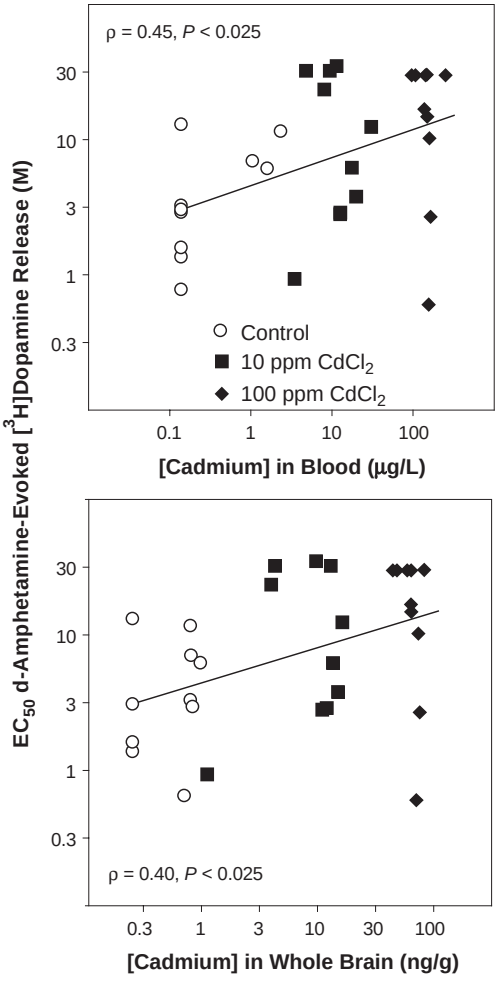

Fig. 1. Dietary cadmium exposure attenuates D-amphetamine-evoked [^{3}H]dopamine release from preloaded rat striatal slices. The concentration–response for D-amphetamine (0.01–30 μM) was determined in rats that had received diet containing $CdCl_2$ (10 or 100 ppm) or control diet. Data represent the EC_{50} value for D-amphetamine against the concentration of cadmium in blood (top panel) or whole brain minus striatum (bottom panel), (n=10 rats/group).

The EC_{50} values for D-amphetamine-evoked release for each rat were compared to their respective concentration of cadmium in blood and in brain (Fig. 1). A significant, positive relationship was found between the EC_{50} values and concentrations of cadmium in blood (ρ=0.45; T(30)=2479, P<0.025; Fig. 1, top panel). A significant, positive relationship also was found between the EC_{50} values and concentrations of cadmium in brain (ρ=0.40; T(30)=2698, P<0.025; Fig. 1, bottom panel). Thus, these results indicate an inverse relationship between the concentration of cadmium in blood and brain and the potency of D-amphetamine to evoke [^{3}H]dopamine release.

Regarding KCl-evoked [^{3}H]dopamine release, a significant main effect of KCl concentration (F(2,54)=41.29, P<0.01) was found for the total [^{3}H]overflow measure (Table 3). KCl produced a concentration-dependent increase in total [^{3}H]dopamine overflow. However, neither the main effect of cadmium diet (P=0.49) nor the cadmium diet × KCl concentration interaction (P=0.62) was significant.

3.3. Experiment 3: dietary cadmium exposure attenuates methamphetamine-induced hyperactivity

At the start of behavioral testing, rats that received diet containing 100 ppm $CdCl_2$ (mean=358 g, S.E.M.=±6 g) weighed significantly less than rats that received diet containing 10 ppm $CdCl_2$ (mean=384 g, S.E.M.=±8 g) or control diet (mean=382 g, S.E.M.=±6 g; F(2,71)=13.31, P<0.01). There was no significant difference between rats that received diet containing 10 ppm $CdCl_2$ and rats that received the control diet.

On Acclimation Days 1 and 2 all rats were injected with saline and placed in the activity monitor for 60 min. Analysis of distance traveled data from these days (Table 4) revealed significant main effects of cadmium diet (F(2,69)=3.55, P<0.05 and F(2,69)=3.35, P<0.05, respectively). On each day rats that received diet containing 100 ppm $CdCl_2$ traveled less than rats that received diet containing 10 ppm $CdCl_2$ or control diet. There was no significant difference between the control group and the group that received 10 ppm $CdCl_2$. Thus, the high concentration of $CdCl_2$ produced hypoactivity.

Table 4
Dietary cadmium exposure attenuates basal locomotor activity

Dietary group	Acclimation day	
	1	2
Control	9728±400	8640±430
10 ppm $CdCl_2$	9840±370	8770±450
100 ppm $CdCl_2$	8680±530*	7420±390*

Rats received diet containing $CdCl_2$ (10 or 100 ppm) or control diet for 30 days before locomotor activity was measured on Acclimation Days 1 and 2. Data represent mean (±S.E.M.) total distanced traveled (cm) during a 60 min period after saline injection (sc) and placement in the activity monitor. Control condition represents rats that received diet without added cadmium. Asterisks designate significant (P<0.05) difference from the control and 10 ppm $CdCl_2$ groups, (n=24 rats/dietary group).

After the completion of the acclimation days, rats were injected with methamphetamine (0.3 or 1.0 mg/kg) or saline and placed in the activity monitor for 60 min (Test Day, Fig. 2). A significant main effect of methamphetamine dose (F(2,63)=74.87, P<0.01) was found and post hoc tests determined that methamphetamine produced dose-dependent hyperactivity. Although the main effect of cadmium diet was not significant (P=0.11), a significant cadmium diet × methamphetamine dose interaction (F(4,63)=2.47, P<0.05) was found. The covariate of total distance traveled on Acclimation Day 2 did not significantly influence activity following methamphetamine injection on the Test Day.

For rats administered saline (Fig. 2, left panel), a significant main effect of cadmium diet was found (F(2,21)=4.39, P<0.05), although the cadmium diet × time interaction was not significant (P=0.44). Consistent with the results observed on Acclimation Days 1 and 2, rats that received diet containing 100 ppm $CdCl_2$ were less active than rats that received diet containing 10 ppm $CdCl_2$ or the control diet. For the low dose (0.3 mg/kg) of methamphetamine (Fig. 2, middle panel), neither the main effect of cadmium diet (P=0.17) nor the cadmium diet × time interaction (P=0.44) was significant. For the high dose (1.0 mg/kg) of methamphetamine (Fig. 2, right panel), both the main effect of cadmium diet (F(2,21)=2.75, P<0.05) and the cadmium diet × time interaction (F(22,231)=9.15, P<0.01) were significant. Rats that received diet containing 10 ppm $CdCl_2$ were less active than rats in the control group at the 25–35 min time points. Rats that received diet containing 100 ppm $CdCl_2$ were less active than rats in the control group at the 20–60 min time points. Thus, dietary cadmium exposure attenuated the locomotor-activating effect of the high dose (1.0 mg/kg) of methamphatmine.

For control rats, a significant main effect of methamphetamine dose (F(2,21)=33.25, P<0.01) was found and post hoc tests determined that control rats administered 0.3 or 1.0 mg/kg methamphetamine were more active than control rats injected with saline (compare across panels in Fig. 2). Control rats injected with 1.0 mg/kg methamphetamine were more active than those injected with the lower (0.3 mg/kg) methamphetamine dose. For rats that received diet containing 10 or 100 ppm $CdCl_2$, significant main effects of methamphetamine dose also were found (F(2,21)=21.90, P<0.01 and F(2,21)=24.58, P<0.01, respectively; compare across panels in Fig. 2). For both cadmium exposure groups, rats administered 0.3 mg/kg or 1.0 mg/kg methamphetamine were more active than rats in their respective cadmium exposure group that were injected with saline. However, for both cadmium exposure groups there were no differences in activity between rats administered 0.3 mg/kg methamphetamine and rats administered 1.0 mg/kg methamphetamine.

Analysis of blood and brain samples from Experiment 3 revealed results similar to those for Experiment 2 (Table 2). Dietary $CdCl_2$ exposure produced a significant concentration-dependent increase in levels of cadmium in blood and

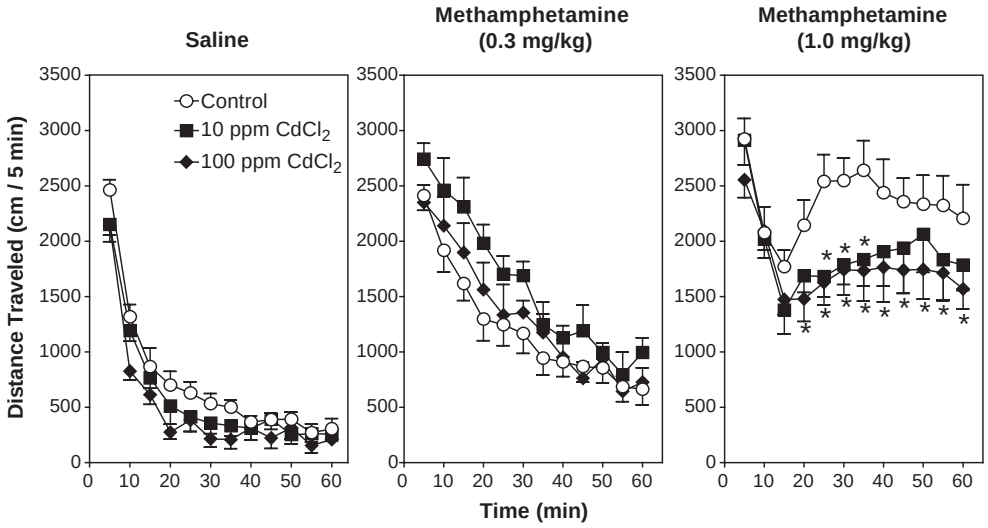

Fig. 2. Dietary cadmium exposure attenuates methamphetamine-induced hyperactivity. Rats that received diet containing $CdCl_2$ (10 or 100 ppm) or control diet were injected (sc) with methamphetamine (0.3 or 1.0 mg/kg) or saline (vehicle) and placed in an automated activity monitor for 60 min. Data represent distance traveled (cm) in 5-min intervals after injection. Asterisks designate significant ($P<0.05$) difference from the control group ($n=8$ rats/group).

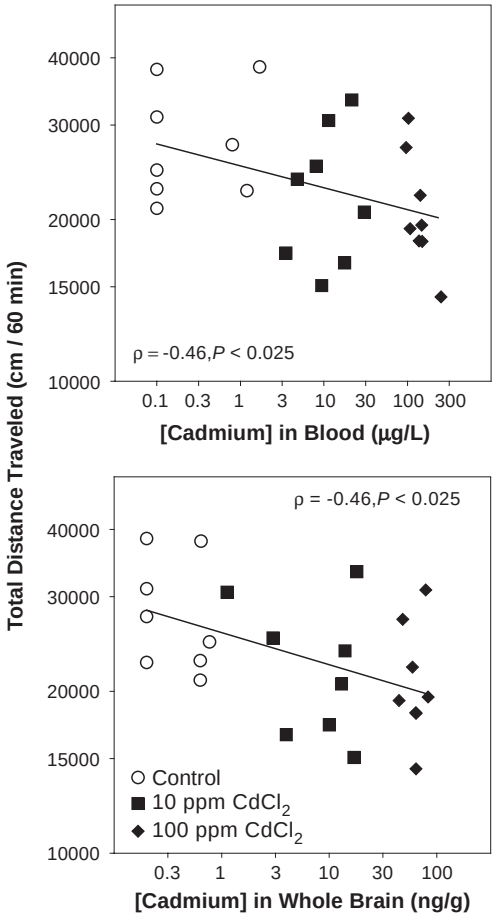

Fig. 3. Dietary cadmium exposure attenuates methamphetamine (1.0 mg/kg)-induced hyperactivity. Rats that received diet containing $CdCl_2$ (10 or 100 ppm) or control diet were injected (sc) with methamphetamine (1.0 mg/kg) or saline (vehicle) and placed in an automated activity monitor for 60 min. Data represent the total distance traveled (cm) after methamphetamine injection against the concentration of cadmium in blood (top panel) or whole brain minus striatum (bottom panel), ($n=8$ rats/group).

brain ($T(2)=97.2$, $P<0.01$ and $T(2)=86.0$, $P<0.01$, respectively). A significant, positive relationship between the levels of cadmium in blood and brain also was found ($\rho=0.96$; $T(56)=1048$, $P<0.025$).

Analyses also were performed to compare the total distance traveled values for methamphetamine for each rat to its respective concentration of cadmium in blood and in brain. For saline and the low dose (0.3 mg/kg) of methamphetamine, significant relationships between the total distance traveled and concentration of cadmium in blood or the concentration of cadmium in brain were not found ($P>0.05$, data not shown). However, for the high dose (1.0 mg/kg) of methamphetamine a significant, negative relationship was found between the total distance traveled and concentration of cadmium in blood ($\rho=-0.46$; $T(24)=3364$, $P<0.025$; Fig. 3, top panel). A significant linear relationship also was found between the total distance traveled and concentration of cadmium in brain ($\rho=-0.46$; $T(24)=3352$, $P<0.025$; Fig. 3, bottom panel). Thus, elevated levels of cadmium in blood or brain were negatively associated with methamphetamine (1.0 mg/kg)-induced hyperactivity.

4. Discussion

Consistent with previous research (Clark et al., 1985; Nation et al., 1997b), exposure to $CdCl_2$ via diet produced a marked increase in cadmium blood and brain levels approximate to environmental metal exposure (Scherer and Barkemeyer, 1983; Yue, 1992; Shaham et al., 1996). The results of the present study demonstrate that this cadmium exposure regimen produces a concentration dependent decrease in the potency of D-amphetamine to evoke [^{3}H]dopamine release from striatum. Dietary $CdCl_2$

exposure also attenuated the potency of acute methamphetamine injection to increase locomotor activity. Interestingly, direct application of cadmium to striatum did not alter the potency of D-amphetamine to evoke [^{3}H]dopamine release, suggesting that long-term $CdCl_2$ exposure induces neuroadaptations of striatal neurons.

Previous studies, in which rats received chronic access to $CdCl_2$, determined that cadmium accumulates in brain to concentrations (~5 μM) (Clark et al., 1985) that are lower than the concentrations (≥100 μM) typically applied to slices or cells to inhibit calcium channels (Hinkle et al., 1987). Thus, the dietary exposure regimen used in the present study results in levels of cadmium in brain that are below those which inhibit calcium channels. Dietary $CdCl_2$ exposure did not inhibit KCl-evoked [^{3}H]dopamine release (Experiment 2), a calcium-mediated process. Moreover, the application of cadmium (0.1–100 μM) to striatal slices (Experiment 1) did not alter D-amphetamine-evoked [^{3}H]dopamine release, suggesting that an environmentally relevant range of cadmium concentrations does not affect the mechanism of action for amphetamine in striatal neurons.

In contrast to the results from Experiment 1, decreased sensitivity to D-amphetamine was observed when rats were exposed to cadmium via diet (Experiment 2). Concentrations of cadmium in brain were negatively correlated with the potency of D-amphetamine to evoke [^{3}H]dopamine release. This indicates that dietary cadmium exposure, but not direct application of cadmium to striatal slices, alters the neuropharmacology of D-amphetamine. Thus, the presence of cadmium in brain is not sufficient for the inhibition of D-amphetamine-evoked dopamine release. This suggests that cadmium does not directly interfere with the mechanism of action for D-amphetamine to evoke dopamine release. Rather, it suggests that long-term cadmium exposure induces a change in striatal neurons. Previous experiments have reported that long-term cadmium exposure results in an alteration of extracellular dopamine levels in brain (Hrdina et al., 1976; Olivier et al., 1995; Lafuente et al., 2000, 2003) and alters the discriminative stimulus properties of ligands selective for dopamine receptors (Nation and Miller, 1999). It is also possible that chronic cadmium exposure results in the loss of striatal neurons and/or a change in the number or function of vesicular monoamine and dopamine transporters, the primary pharmacological targets for amphetamines.

Cadmium produced a concentration-dependent decrease in the potency of D-amphetamine to evoke dopamine release (Experiment 2); however, the magnitude of this effect was relatively small. The present results suggest that dietary $CdCl_2$ exposure produces a subtle neurotoxic effect on striatal neurons over a prolonged period of time, which may be more profound with prolonged metal exposure. The current $CdCl_2$ exposure regimen (~30 days via diet) was based on previous studies which demonstrated sub sensitivity to morphine and cocaine with month-long metal exposure (Nation et al., 1991, 1995, 1997a). Future experiments should investigate the time-dependent effects of cadmium on the number and/or function of striatal neurons.

Dietary $CdCl_2$ exposure produced attenuation, but not a complete inhibition, of methamphetamine-induced hyperactivity (Experiment 3), a pattern of results consistent with previous findings in which cadmium exposure (100 ppm) attenuated the behavioral activation produced by acute cocaine injection to rats (Nation et al., 1991, 1995). In the present study, acute methamphetamine (0.3 and 1.0 mg/kg) injection produced an increase in locomotor activity in all rats. However, control rats were more active than cadmium-exposed rats after administration of 1.0 mg/kg methamphetamine. Furthermore, methamphetamine produced a dose-dependent increase in locomotor activity in control rats. In contrast, in rats exposed to 10 and 100 ppm $CdCl_2$, there was no difference in the magnitude of locomotor activity induced by 0.3 and 1.0 mg/kg methamphetamine, indicating that cadmium prevented the dose-dependency of methamphetamine to induce this behavioral change.

The inhibitory effect of cadmium on methamphetamine-induced behavior was dependent on the concentration of $CdCl_2$ in diet. The attenuation of methamphetamine (1.0 mg/kg)-induced hyperactivity by 10 ppm $CdCl_2$ was shorter lived than the attenuation produced by 100 ppm $CdCl_2$. Perhaps the accumulation of methamphetamine in brain ~30 min after injection was able to surmount the inhibitory effect associated with 10 ppm $CdCl_2$. Similar to the results observed with D-amphetamine-evoked [^{3}H]dopamine release, statistical analyses revealed significant negative correlations between the concentration of cadmium in blood and brain and distance traveled following methamphetamine (1.0 mg/kg) injection. This indicates that the inhibitory effect of cadmium on drug-induced behavior is related to both the concentration level of $CdCl_2$ received and the accumulation of cadmium in each rat.

Rats that received the high concentration (100 ppm) of $CdCl_2$ via diet had lower body weights and were less active than rats that received the control diet or the diet containing 10 ppm $CdCl_2$. Previous studies have shown that rats which received diet containing 100 ppm $CdCl_2$ consume less than rats that received diet without added $CdCl_2$ (Nation et al., 1997a). Thus, the inhibitory effect of 100 ppm $CdCl_2$ on D-amphetamine-evoked [^{3}H]dopamine release and methamphetamine-induced hyperactivity could be the result of decreased vitamin and caloric intake and utilization, such that a nutritional deficiency contributes to the altered neuronal and behavioral functioning. The effect of dietary $CdCl_2$ exposure on nutrition could also be cadmium concentration-dependent because the lower concentration (10 ppm) of $CdCl_2$ did not significantly decrease body weights or produce an intrinsic change in locomotor activity.

The significant statistical correlations between the concentration of cadmium in the body and neuropharmacology indicate the importance of looking at differences among groups of rats that receive diet containing the same

concentration of $CdCl_2$. A relatively large degree of variability in the EC_{50} values for D-amphetamine-evoked [^{3}H]dopamine release was observed for rats that received 100 ppm $CdCl_2$, as in some cases the EC_{50} values were comparable to or less than those for control rats. The negative correlations were found for cadmium in both blood and brain. However, in these experiments the brain was not perfused before tissue collection or sample freezing, such that cadmium in brain could reflect residual cadmium in blood. A significant positive correlation was found between the concentration of cadmium in blood and in brain.

In the present study, dietary cadmium exposure was associated with decreased D-amphetamine-evoked dopamine release and methamphetamine-induced hyperactivity. Thus, these findings suggest that dietary cadmium exposure could diminish the reinforcing properties of amphetamines. Previous research has shown that chronic cadmium exposure attenuates the development of conditioned place preference to cocaine and opiates, such that greater drug doses were necessary to produce reinforcement (Miller and Nation, 1997; Miller et al., 1999). Collectively, these preclinical findings suggest that a clinical population of concern is cigarette smokers who also use amphetamines. Tobacco smoking produces a long-lasting increase in the body burden of cadmium (Scherer and Barkemeyer, 1983; Yue, 1992), and therefore this population might administer higher doses of amphetamine than non-smokers to achieve comparable reinforcing effects. Self-administration of higher amphetamine doses would increase the risk of drug toxicity or mortality.

A second implication for the present research is the possible contributory role of cadmium toxicity in the development of neurological or psychiatric disorders associated with dysfunction of central dopaminergic pathways, such as schizophrenia or Parkinson's disease (Evans and Lees, 2004; Remy and Samson, 2003). The present findings suggest that an environmentally relevant body burden of cadmium is associated with alteration in the number and/or function of striatal dopamine neurons. As such, those exposed to cadmium via tobacco smoke (Scherer and Barkemeyer, 1983; Yue, 1992) or industrial pollution (Thun et al., 1985) may be at greater risk for the development of dopamine-related disorders or may display a decreased sensitivity to antipsychotic medications or drugs used in Parkinsonism.

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