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The effects of developmental cadmium exposure on morphine sensitization and challenge with selective D_1 and D_2 antagonists

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Abstract

The purpose of this investigation was to determine the effects of developmental (perinatal) cadmium exposure on the development and expression of behavioral sensitization to morphine. Adult female rats were maintained ad libitum on diets containing 0, 25, or 50 ppm added cadmium (administered as cadmium chloride) for 30 days prior to breeding with nonexposed males. This exposure regimen continued throughout the gestational period and for 15 days postnatally during lactation, at which time regular rat chow was provided. On postnatal day (PND) 21, male pups from the respective litters were weaned and placed on an unadulterated food supply (no added cadmium) and tap water for the remainder of the study. Beginning on PND 70, animals from each exposure condition (0, 25, 50 ppm exposure conditions) received, for 21 consecutive days, either vehicle (distilled water) or 10 mg/kg morphine sulfate injections (ip) prior to being monitored for locomotor activity during 80-min test sessions. Following this 21-day period of morphine sensitization training, dose–effect profiles were determined for each exposure condition with successive daily challenges of 0, 10, and 20 mg/kg morphine. Subsequently, different doses of the D_1 antagonist SCH 23390 (0.01, 0.056, and 0.10 mg/kg) and the D_2 antagonist eticlopride (0.01 and 0.056 mg/kg) were presented prior to administration of the training dose of morphine (10 mg/kg). The results of the investigation revealed that developmental cadmium exposure attenuated the development/expression of morphine sensitization. Furthermore, it was found that the suppressive effects of the D_2 antagonist eticlopride were decreased by early cadmium exposure.

Keywords: Activity; Cadmium; Developmental; Morphine; Sensitization

1. Introduction

Cadmium is a metal toxicant that accumulates in the leaves of tobacco plants (Benedetti et al., 1999; Ellingen et al., 1997; Maranelli et al., 1990). Because cadmium is known to be a placental toxicant in mammals, including humans (Shiverick and Salafia, 1999), and because cadmium is readily transferred to offspring during lactation (Andersson et al., 1997), there has been increasing interest in the scientific community over the long-term consequences of smoking during pregnancy or breastfeeding and the attendant developmental cadmium exposure. Given that cadmium is estimated to have a biological half-life of approximately 30 years (Klaassen, 1990), relatively permanent anatomical and neurochemical disturbances could be observed long after developmental exposure to cadmium is discontinued.

A possible consequence of these anatomical and neurochemical disturbances resulting from early cadmium exposure is an alteration in the impact of a set delivery of psychoactive drugs. Cadmium alters μ-opioid receptor function (Tejwani and Hanissian, 1990), glutamate activity (Legendre and Westbrook, 1990), and dopamine availability (Olivier et al., 1999), all of which are linked to the development and expression of drug effects. Yet, only a nominal literature on cadmium/drug interactions exists. With respect to the few relevant reports that are available, studies investigating the effects of adult cadmium exposure on drugs such as morphine and cocaine show a decreased sensitivity to the drugs. It has been reported that adult cadmium exposure results in a decrease in behavioral sensitization to morphine (Nation et al., 1997a,b) and cocaine (Nation et al., 1995). Behavioral sensitization is

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defined as an increased sensitivity to a drug with chronic drug administration, and it has been proposed as an animal model for drug-seeking and -taking behaviors (Kalivas and Alesdatter, 1993; Kuribura, 1995). Other investigations of cadmium exposure in the adult organism have shown that the metal reduces the conditioned reinforcement properties of morphine (Miller and Nation, 1997), and the discriminative stimulus properties of morphine (Nation et al., 2000) and cocaine (Nation and Miller, 1999).

Although the aforementioned investigations provide insight into the effects of adult cadmium exposure on drug responsiveness, research to date on the effects of developmental cadmium exposure on drug sensitivity has been limited. Even though evidence does exist that prenatal/postnatal cadmium exposure alters neurobehavioral and neurophysiologic functions that are known to modulate drug effects (Ali et al., 1986; Desi et al., 1998), few investigations of developmental metal/drug interactions have been published.

In this study, we examined alterations in behavioral sensitization to morphine after perinatal exposure to cadmium by measuring changes in locomotor activity associated with repeated morphine administration. Additionally, because dopamine has been shown to be involved in the expression of morphine sensitization (Jeziorski and White, 1995), the role of dopamine D_1 and dopamine D_2 receptor subtypes in the potential alteration of drug responsiveness by developmental cadmium exposure was examined. This was accomplished by presenting the D_1 antagonist, SCH 23390, or the D_2 antagonist, eticlopride, against a morphine challenge, after the establishment of behavioral sensitization to morphine.

2. Method

2.1. Animals and exposure regimen

All aspects of the research reported here were approved by the University Laboratory Animal Care Committee. Adult female Sprague–Dawley female rats (Charles River, Boston, MA) were matched on initial body weight across groups. Each female rat was exposed to cadmium via an adulterated food supply containing added cadmium (presented as cadmium chloride), or to a control diet with no added cadmium. Dyets (Bethlehem, PA) specially formulated the diets. Following 30 days of exposure ad libitum to their respective cadmium doses, females were bred to nonexposed males. Males were removed from the home cage once females tested positive for copulatory plugs. Females continued to receive their respective diets throughout the gestational period and for the first 15 days of lactation (see below). Tap water was available ad libitum in the home cage.

No significant differences were observed among exposure groups in the presence of copulatory plugs, the number of dams that delivered pups, number of pups per litter, pup weights on postnatal day (PND) 1, or pup mortality, and none of the available animals were discarded nor were their data excluded from analysis. Litters were culled to 10 pups 7 days after parturition rather than earlier to permit a more reliable sex determination of pups via visual inspection of genital spacing. All male pups remained in the litter, and enough females remained to maintain consistent litter size across all dams. Male littermates from each cadmium exposure group were sacrificed at PND 1 for blood-cadmium analysis.

Sixteen male pups from 10 dams exposed to a food supply containing 0 ppm cadmium, 13 male pups from 6 dams exposed to a food supply containing 25 ppm cadmium, and 15 male pups from 4 dams exposed to a food supply containing 50 ppm cadmium, were included in the investigation. The remaining pups were used in other research projects. During the first 15 days of lactation, dams continued to be exposed to their respective diets. Dams were placed on standard rat chow at PND 15 to ensure that pups did not begin feeding on cadmium-adulterated rat chow. The resulting procedure permitted perinatal cadmium exposure within an experimental framework wherein pups were unable to gain access to cadmium postnatally via routes other than the maternal milk supply.

On PND 21, pups were weaned and for the remainder of the study placed on ad libitum standard rat chow diets, and they had continuous access to a tap water supply that contained no added cadmium. All animals were individually housed from PND 21 until the study was completed. After weaning, food intake and weight measures were recorded daily for the remainder of the study for each subject.

2.2. Apparatus

The test apparatus involved an automated Digiscan-16 system (Omnitech Electronics, Columbus, OH). Activity monitors and cages were located in a soundproof room with a 40-dB [SPL] white noise generator operating continuously. A multiplexor-analyzer in an adjacent room monitored beam breaks from the optical beam activity monitors and tracked the simultaneous interruption of beams. The multiplexor-analyzer updated the animal's position in the acrylic cage ($40 \times 40 \times 30.5$ cm) every 10 ms using a 100% real-time conversion system. Computerized integration of the data obtained from the monitor afforded the recording of general activity using total distance (cm) as the dependent measure.

2.3. Procedure

The selection of cadmium doses was made on the basis of pilot investigations that yielded blood-cadmium levels in dams falling within a clinically relevant range (~ 15 μg/dl or less). Male offspring from each of the three exposure conditions (0, 25, and 50 ppm cadmium) were stratified

according to body weight. Within given weight ranges, offspring were randomly assigned to receive either vehicle or morphine injections.

To control for possible litter effects (cf. Holson and Pearce, 1992), efforts were made to place male offspring from the same litter in different test conditions (no more than two offspring from the same litter were assigned to a given condition). Animals began activity testing on PND 70 and were randomly assigned to one of six test groups created by interacting cadmium-exposure condition (0, 25, 50 ppm) and type of injection (vehicle, morphine). Thus, the six groups used in this study were 0-vehicle ($n=8$), 0-morphine ($n=8$), 25-vehicle ($n=6$), 25-morphine ($n=7$), 50-vehicle ($n=7$), and 50-morphine ($n=8$). Modeling an earlier procedure (Jeziorski and White, 1995), animals receiving morphine were administered daily intraperitoneal injections of 10 mg/kg morphine sulfate expressed as the salt, while vehicle controls received distilled water (1.0 ml/kg volume). In this initial phase of the project, animals were tested during 80-min sessions each day for 21 successive days, in groups of four, counterbalancing by exposure condition. Each animal was placed in the chamber immediately following the injection, at which time the room lights were turned off. This procedure was employed in order to increase the discriminatory properties of the injections. Previous investigations (e.g., Post et al., 1981) have shown that contextual cues contribute to augmented responding associated with repeated drug administration. Insofar as administering the injections, placement in the test chambers, turning off the test room lights, and other preinjection correlates serve in a feed-forward capacity (as CSs), it is reasonable to assume that reinstatement of such events could play an additive role in behavioral sensitization (Weiss et al., 1984). We tested such a possibility by administering a vehicle-only (0 mg/kg morphine) injection following initial sensitization testing (see procedures for Day 22 of testing). In all tests conducted in this study, total distance traveled (cm) was recorded post-injection across successive 5-min intervals for 80 min.

On Days 22–24 of testing, all animals within each of the three morphine-treated groups (0-morphine, 25-morphine, 50-morphine) received successive daily injections of 0, 10, and 20 mg/kg morphine. Groups given vehicle injections (0-vehicle, 25-vehicle, and 50-vehicle) continued to receive vehicle injections during this period.

Following 1 day of vehicle or 10 mg/kg morphine exposure (Day 25 of testing), antagonist doses were administered in random order beginning on Day 26 of testing. All animals in all six groups received an intraperitoneal injection of the antagonist (SCH 23390 or eticlopride; 1.0 ml/kg volume) 30 min prior to administration of 10 mg/kg morphine or vehicle. Following their respective morphine or vehicle injections, animals were placed immediately in the activity chamber. Animals were injected with the antagonists, and returned to the home cage prior to receiving morphine or vehicle injections. SCH 23390 doses of 0.010, 0.056, and 0.100 mg/kg were presented randomly against a 10-mg/kg morphine challenge on Days 26, 28, and 30 of behavioral testing. Eticlopride was administered on Days 33 and 35 of behavioral testing. The doses of eticlopride administered randomly were 0.010 and 0.056 mg/kg. On the day following each dose of the antagonist, animals received a vehicle preinjection followed by 10 mg/kg morphine or vehicle in order to reestablish baseline responding. The vehicle (distilled water) preinjection was administered in an effort to control for the possible development of cueing properties associated with the injection of the antagonist.

2.4. Drugs

Morphine sulfate was provided gratis by the Research Technology Branch of NIDA. Both the SCH 23390 and the eticlopride were purchased from Research Biochemicals, RBI (Natick, MA). For morphine and antagonists tests, drug vehicle, route of administration, injection volume, and time of injection prior to placement in the activity chamber are presented in Table 1. Drug doses and the times between antagonist administration and morphine administration were selected on the basis of earlier morphine sensitization studies (Jeziorski and White, 1995).

2.5. Blood-cadmium determinations

Cadmium residues in blood were measured via atomic absorption spectrophotometry. To assure accuracy in measurements of blood levels, spiked samples were spaced intermittently (1 per 20 experimental samples) to ensure adequate recovery. If, at any time, values from the standards were more than 5% above or below the certified mean or outside the 99% confidence interval, analysis of samples was stopped and recommenced following diagnosis of any problem with the cadmium analysis procedures (details of the analytical procedure can be found elsewhere; Nation et al., 1997a,b).

2.6. Data analysis

Analysis of variance (ANOVA) tests were performed on the behavioral data. In all cases throughout this report, Newman–Keuls procedure for examining significant mean differences was employed as the post hoc test.

Table 1
Experimental parameters for morphine and antagonists tests

Drug	Vehicle	Route	Volume (ml/kg)	Time
Morphine sulfate	Distilled water	ip	1	Immediately
SCH 23390	Distilled water	ip	1	30 min[a]
Eticlopride	Distilled water	ip	1	30 min[a]

[a] For SCH 23390 and eticlopride, time refers to period between antagonist injection and morphine injection. All animals were placed in the activity test chambers immediately after receiving their respective morphine injections.

3. Results

3.1. Food intake and body weight

Collapsed over the 6-week period prior to commencing morphine sensitization training, analysis of mean weekly food intake showed significant group differences in food consumption ($P < .01$); group means were 193.6, 177.8, and 150.7 g for animals born to dams maintained on diets containing 0, 25, and 50 ppm added cadmium, respectively. However, during the final 3 weeks prior to testing, reliable group differences were not observed. That is, consistent separation was not evident for any of the comparisons of exposure condition across the 3 weeks immediately preceding the onset of behavioral testing ($P > .05$).

A somewhat more consistent pattern was evident for the measure of body weights. Collapsed across 6 weeks, body weights were lower in animals exposed to cadmium during perinatal development ($P < .05$); means were 264.2, 210.2, and 193.4 g for animals born to dams maintained on diets containing 0, 25, and 50 ppm added cadmium, respectively. Unlike the case for food intake, these rank-order differences tended to persist throughout the period prior to behavioral testing ($P < .05$). The exception was on the week prior to the beginning of training when animals born to 0 (control) and 25 ppm dams did not differ, but weighed more than animals born to 50 ppm dams.

3.2. Morphine sensitization

Fig. 1 presents the mean total distance traveled for animals administered either repeated vehicle injections (left panel) or repeated 10 mg/kg morphine injections (right panel). Statistical confirmation of group differences was provided by a 3 [Exposure Condition (0, 25, 50 ppm cadmium)] × 2 [Type of Injection (vehicle, morphine)] × 4 [Day (1, 7, 14, 21)] repeated-measures ANOVA performed on the data, with Day serving as the within factor. The selection of representative data points (days) avoids capitalizing on chance, which becomes an issue when variable levels greatly exceed the number of subjects per group (Stevens, 1996). With respect to main effects, both the main effect for Exposure Condition [$F(2,38) = 3.64$, $P < .05$] and Day [$F(3,114) = 7.32$, $P < .01$] were found to be significant. Post hoc analyses of means revealed that 50 ppm cadmium animals exhibited lower total distance traveled scores than either of the other two exposure conditions ($P < .05$). The Day main effect was a result of increasing overall activity over the course of testing ($P < .05$).

Of the various tests for interaction effects, the most instructive was the finding of a significant Exposure Condition × Type of Injection × Day interaction [$F(3,114) = 2.46$, $P < .05$]. Post hoc analyses of group means of animals administered morphine injections revealed that each of the three exposure conditions significantly increased total dis-

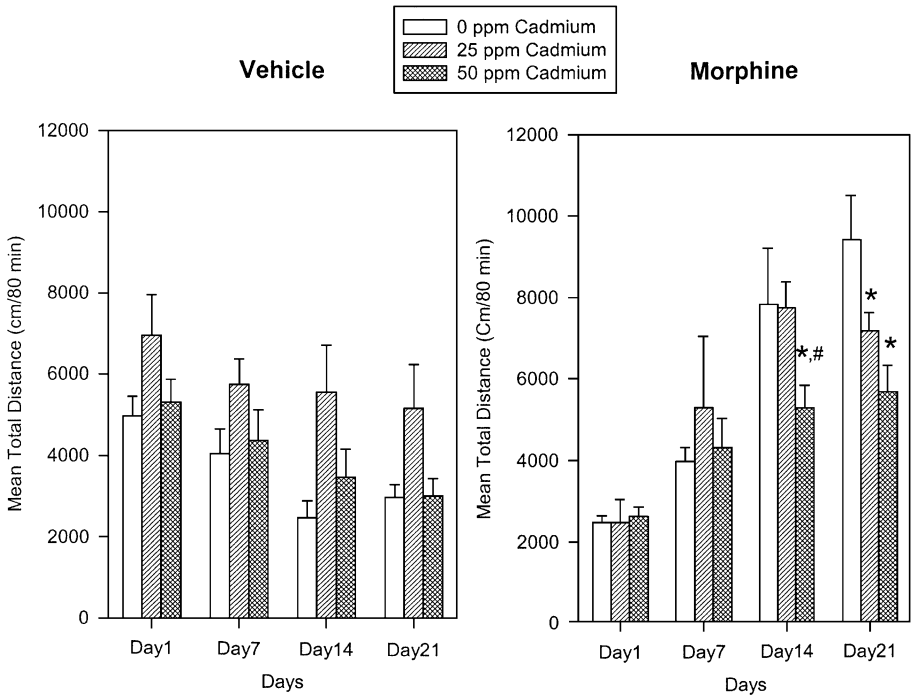

Fig. 1. The mean total distance traveled (cm) and standard error across 80-min test sessions at Days 1, 7, 14, and 21 of morphine sensitization training. Animals perinatally exposed to dams maintained on diets containing 0, 25, or 50 ppm added cadmium were administered repeated daily injections (ip) of vehicle (left panel) or 10 mg/kg morphine (right panel). The symbol * denotes significant difference from controls (0 ppm group) on the day of morphine sensitization training ($P < .05$). The symbol # denotes that Group 50-morphine was significantly different from Group 25-morphine ($P < .05$).

tance traveled from Day 1 to Day 21 ($P<.05$), i.e., behavioral sensitization to repeated morphine injections was evident in Groups 0-morphine, 25-morphine, and 50-morphine. Additional comparisons showed that Group 50-morphine animals exhibited lower levels of locomotor responding than either of the other two exposure conditions on Day 14, and on Day 21, activity levels were lower for both Group 25-morphine and Group 50-morphine relative to Group 0-morphine ($P<.05$).

Because early cadmium exposure may have compromised motor ability per se, it is useful to examine group performances on Day 21 in greater detail. Day 21 is of special interest because it was on this day that the greatest group differences were observed. Fig. 2 profiles individual group performances across successive 5-min intervals for the entire 80-min test session. Close inspection of the initial 5-min bins reveals that activity levels were high for all groups administered morphine. Separation among groups seemingly occurred because of the more enduring effects of the drug in Group 0-morphine, a pattern suggestive of pharmacokinetic differences or reduced potency in cadmium-exposed animals. Thus, the differences observed here would seem to derive from disturbances in responsiveness to the opiate rather than cadmium-induced locomotor toxicity.

3.3. Dose–effect testing

The mean total distance traveled for each exposure condition at morphine doses of 0, 10, and 20 mg/kg is presented in the right panel of Fig. 3. The left panel of Fig. 3 reflects the mean locomotor activity for groups continuing to receive vehicle-only injections. A 3 [Exposure Condition (0, 25, 50 ppm cadmium)] × 2 [Type of Injection (vehicle, morphine)] × 3 [Day (22, 23, 24)] repeated-measures ANOVA was performed on these data. The results of this analysis revealed that the Type of Injection × Day interaction reached an acceptable level for statistical significance [$F(2,76)=7.74$, $P<.01$]. Individual comparisons of means showed that while the pattern of responding did not change across days for animals receiving vehicle-only injections ($P>.05$), for animals receiving increasing doses of morphine, activity increased from Day 22 to Day 23 (from 0 mg/kg morphine to 10 mg/kg morphine; $P<.05$) and declined from Day 23 to Day 24 (from 10 mg/kg morphine to 20 mg/kg morphine; $P<.05$). However, of the various tests involving exposure condition, only the Exposure Condition × Type of Injection interaction was statistically significant [$F(2,38)=4.71$, $P<.05$]. With respect to animals administered vehicle-only injections, post hoc examination of mean differences showed that both metal-exposed conditions (25 ppm cadmium, 50 ppm cadmium) exhibited greater locomotor activity than controls (0 ppm cadmium), collapsed across all days of dose–effect testing ($P<.05$). By way of contrast, for animals given increasing doses of morphine, 25 and 50 ppm cadmium animals exhibited lower total distance traveled scores than control animals, collapsed across all days of dose–effect testing ($P<.05$).

3.4. SCH 23390 testing

Fig. 4 depicts the suppressive effects of increasing doses of the D_1 antagonist SCH 23390. The results of a 3 [Exposure Condition (0, 25, 50 ppm cadmium)] × 2 [Type of Injection (vehicle, morphine)] × 3 [Dose (0.010, 0.056,

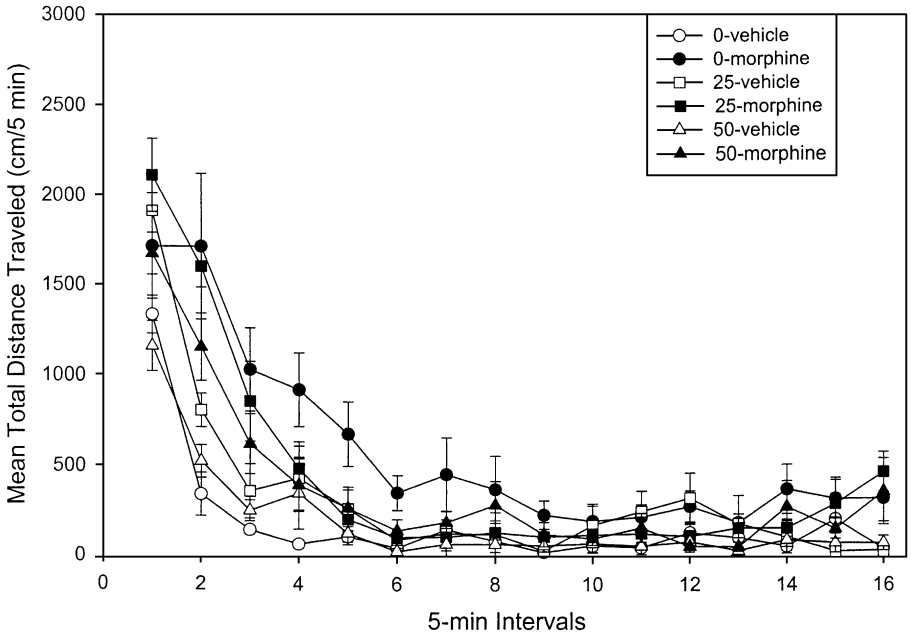

Fig. 2. The mean total distance traveled (cm) and standard error for each group across successive 5-min intervals on Day 21 of morphine sensitization training (see text).

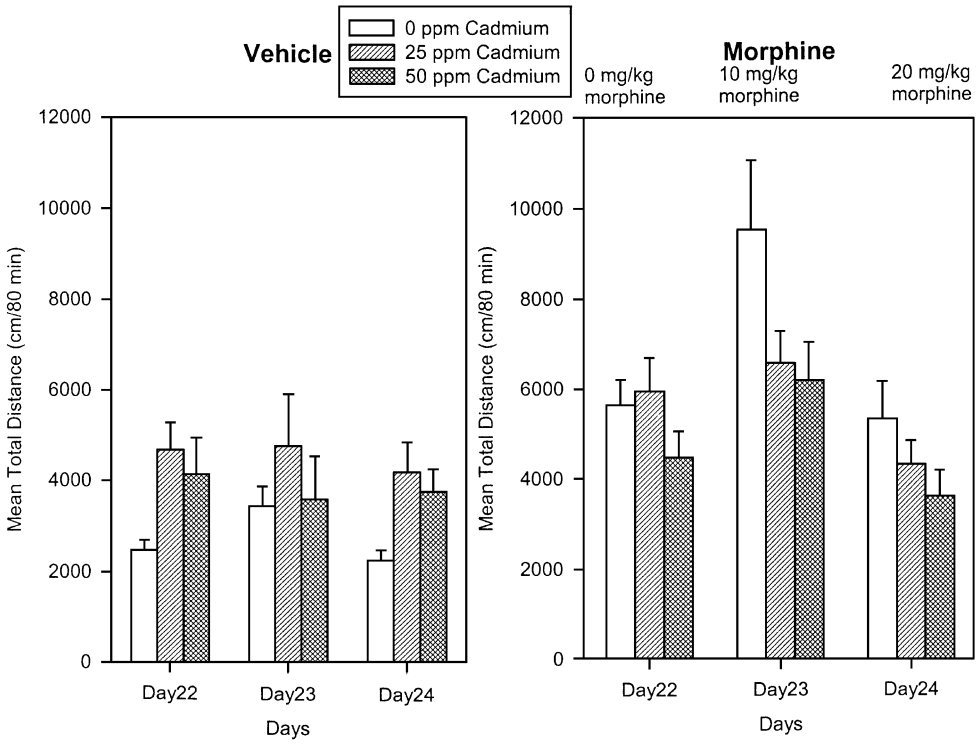

Fig. 3. The mean total distance traveled (cm) and standard error across 80-min test sessions at morphine doses of 0, 10, and 20 mg/kg. Following the initial period of morphine sensitization training, animals perinatally exposed to dams maintained on diets containing 0, 25, or 50 ppm added cadmium were administered successive daily injections (ip) of vehicle (left panel) or the indicated morphine dose (right panel).

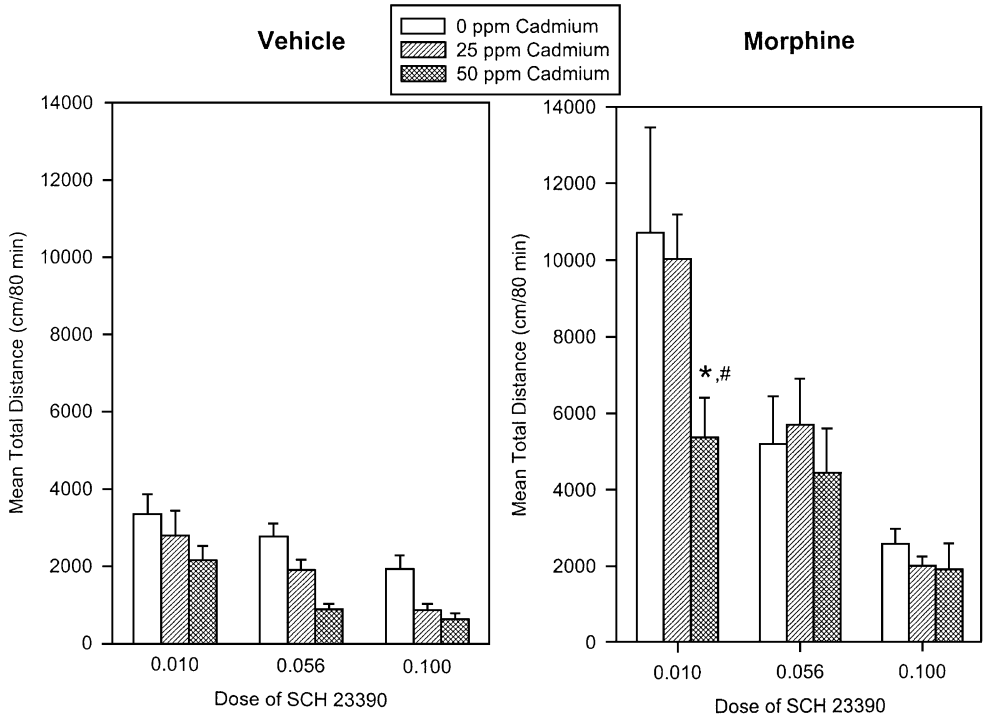

Fig. 4. For animals perinatally exposed to dams maintained on diets containing 0, 25, or 50 ppm added cadmium, effects of increasing doses of the D_1 antagonist SCH 23390 on mean total distance traveled (cm) and standard error across 80-min test sessions where the antagonist was presented against intraperitoneal vehicle injections (left panel) or 10 mg/kg morphine injections (right panel). These tests were conducted following morphine sensitization training. The symbol * denotes significant difference from controls (0 ppm group) on the day of morphine sensitization training ($P < .05$). The symbol # denotes that Group 50-morphine was significantly different from Group 25-morphine ($P < .05$).

0.100 mg/kg)] repeated-measures ANOVA performed on the SCH 23390 data showed significant main effects for Exposure Condition [$F(2,38) = 4.79$, $P < .05$], Type of Injection [$F(1,38) = 37.02$, $P < .01$], and Dose [$F(2,76) = 47.17$, $P < .01$]. Post hoc analyses revealed that activity levels were lower ($P < .05$) in animals exposed to dams receiving 50 ppm cadmium daily relative to the other two exposure conditions which did not differ significantly ($P > .05$). The Type of Injection main effect resulted from greater total distance traveled among animals administered SCH 23390 and morphine relative to animals receiving vehicle-only injections ($P < .01$). The Dose main effect was due to significant reductions in locomotor responding in a dose-dependent manner ($P < .01$).

The Exposure Condition × Type of Injection × Dose interaction also was found to be significant [$F(4,76) = 2.84$, $P < .05$]. Subsequent comparisons of means indicated that at the lowest dose of SCH 23390 (0.010 mg/kg), responding in sensitized animals was less for Group 50-morphine than it was for Group 0-morphine or Group 25-morphine which were not different. None of the other comparisons involving exposure condition were significant ($P > .05$).

3.5. Eticlopride

The data associated with presenting the D_2 antagonist eticlopride against 10 mg/kg morphine are shown in Fig. 5. The three-way analysis of the eticlopride test data showed significant main effects for Type of Injection [$F(1,38) = 46.78$, $P < .01$], and Dose [$F(1,38) = 21.47$, $P < .01$]. Individual comparisons of group means indicated that animals receiving combined eticlopride and morphine injections were overall more active than their vehicle-only counterparts ($P < .01$). In addition, locomotor responding was lower at the higher dose of eticlopride than the lower dose ($P < .01$).

As was the case with the SCH 23390 analysis, a significant Exposure Condition × Type of Injection × Dose interaction also was found with the eticlopride test data [$F(2,38) = 6.74$, $P < .01$]. Post hoc comparisons revealed that at the 0.010-mg/kg dose of eticlopride, locomotor activity was greater for Groups 0-morphine and 25-morphine compared to Group 50-morphine ($P < .01$). Groups were not different when administered vehicle-only injections ($P > .05$). At the eticlopride dose of 0.056, the suppressive effects of this dopamine antagonist were profound. However, the 0.056-mg/kg dose of eticlopride had less of an effect in cadmium-exposed animals. That is, both Groups 25-morphine and 50-morphine, although not different from one another, exhibited greater locomotor responding to the 10-mg/kg morphine challenge than the control condition (Group 0-morphine; $P < .01$).

3.6. Blood-cadmium levels

The atomic absorption assays performed on the dams at weaning showed that blood-cadmium levels were ordered by exposure regimen (< 1, 7.50 ± 1.50, 12.33 ± 0.33 μg/dl) for dams exposed to diets containing 0, 25, and 50 ppm cad-

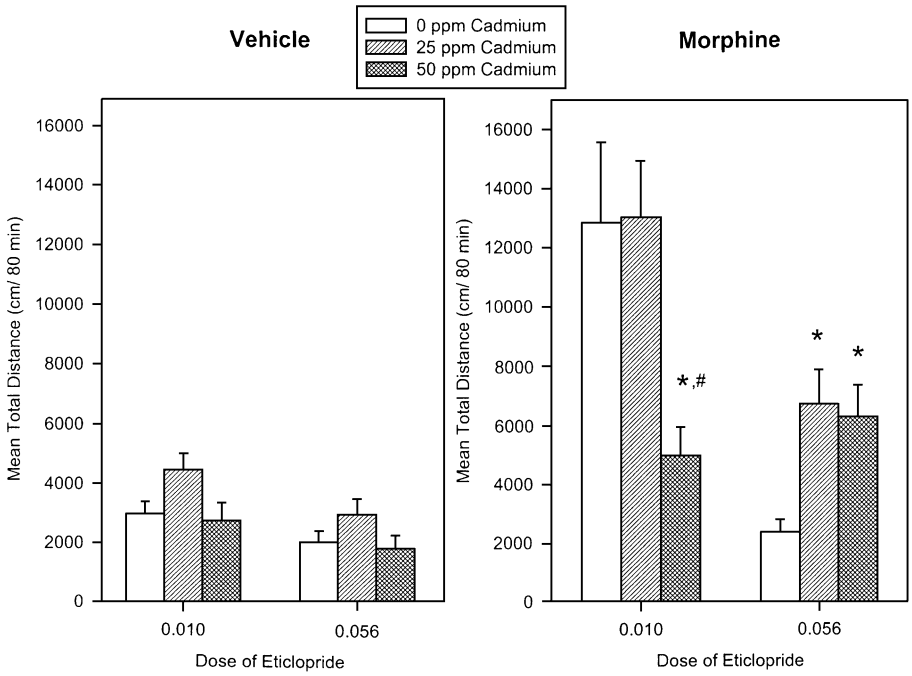

Fig. 5. For animals perinatally exposed to dams maintained on diets containing 0, 25, or 50 ppm added cadmium, effects of increasing doses of the D_2 antagonist eticlopride on mean total distance traveled (cm) and standard error across 80-min test sessions where the antagonist was presented against intraperitoneal vehicle injections (left panel) or 10 mg/kg morphine injections (right panel). These tests were conducted following morphine sensitization training. The symbol * denotes significant difference from controls (0 ppm group; $P < .05$). The symbol # denotes that Group 50-morphine was significantly different from Group 25-morphine ($P < .05$).

mium daily ($P < .05$). For littermates sacrificed at weaning, mean blood-cadmium values were < 1, 6.33 ± 0.33, and 11.34 ± 3.56 for the 0-, 25-, and 50-ppm exposure conditions, respectively ($P < .05$). For those animals tested in this investigation, in all cases (0, 25, 50 ppm exposure), blood-cadmium levels measured in trunk blood taken 24 h after the completion of testing were below the limits of detection (< 1 μg/dl). It would be of interest to know more precisely what the blood-cadmium levels were at specific points along the time course for sensitization testing, but it was not possible to collect these data in this investigation because littermates were used in other experiments.

4. Discussion

The results of this investigation showed that animals born to dams chronically exposed to a diet containing 25 ppm cadmium, or 50 ppm cadmium, exhibited an attenuated response to repeated morphine administration. Specifically, when tested as adults, a 10-mg/kg morphine challenge at 21 days of repeated morphine administration occasioned augmented locomotor responding in male controls (Group 0-morphine) and male metal-exposed animals (Groups 25-morphine and 50-morphine), relative to the initial day of exposure to this same dose. This behavioral sensitization effect was reduced among animals that were born to dams exposed to either regimen that presented dams with diets containing added cadmium. Finally, the suppressive effects of the D_2 antagonist eticlopride, but not the D_1 antagonist SCH 23390, were significantly diminished by developmental cadmium exposure, i.e., when sensitized animals were presented with a dopamine antagonist coincident with a 10-mg/kg morphine challenge, Groups 25-morphine and 50-morphine exhibited greater activity than controls (Group 0-morphine) at the highest dose of eticlopride.

4.1. Morphine sensitization

For the adult literature on metal/drug interactions, on the whole lead exposure and cadmium exposure produce parallel effects. With respect to opiates for instance, both lead and cadmium attenuate morphine sensitization when the toxicants are exposed during the adult cycle (Miller et al., 2000; Nation et al., 1997a,b). However, the situation seems to be different when lead or cadmium are exposed developmentally. That is, in a previous investigation where animals were perinatally exposed to lead, repeated experience with morphine was associated with increased sensitivity to the locomotor stimulating properties of the drug (Miller et al., 2000). This finding with developmental lead contrasts markedly with the results from the present study where it was found that developmental cadmium exposure attenuated morphine sensitization. Because information on mechanisms underlying metal/drug interactions is lacking, it is not possible to speculate about why these directional differences would occur following developmental lead or cadmium exposure.

It would be worthwhile to suggest some possible neural substrates for the effects observed here. One model of morphine sensitization suggests dopaminergic as well as nondopaminergic modulation of the phenomenon (cf. Jeziorski and White, 1995). Speculation has it that nondopamine inhibitory (perhaps GABAergic) neurons originating in the region of the ventral tegmental area (VTA) regulate *N*-methyl-D-aspartate (NMDA) activation of dopamine neurons projecting to the nucleus accumbens. It is known that these nondopaminergic VTA inhibitory interneurons express μ-opioid receptors, and when opiates bind to these receptors, these same inhibitory neurons are rendered less excitable. The resulting disinhibition promotes NMDA-mediated activation of dopamine projection neurons that is ultimately necessary for the expression of morphine sensitization. It is further established that neurochemical disruption at any point along this cascade results in diminished morphine sensitization. For example, opioid antagonists (Kalivas and Duffy, 1987), the noncompetitive NMDA antagonist MK-801 (Jeziorski et al., 1994), and dopamine D_1 and D_2 antagonists (Jeziorski and White, 1995; Kuribura, 1995), all prevent or retard behavioral sensitization to morphine. It follows that the application of other external events that accomplish the same sort of disturbance(s) would attenuate behavioral sensitization as well.

It has long been known that cadmium, along with numerous other divalent cations, selectively inhibits the binding of μ-opioid ligands to their receptors (Tejwani and Hanissian, 1990). In addition, single-channel NMDA activation is inhibited by cadmium as well (Legendre and Westbrook, 1990). Regarding the final dopamine pathway integral to the expression of morphine sensitization, both prepubertal and adult cadmium exposure result in decreased dopamine synthesis and content (e.g., Lafuente et al., 2000; Olivier et al., 1999). Thus, given that one or all of these mechanisms may have been affected by perinatal exposure to cadmium in the present study, it is not really surprising that animals born to dams maintained on a diet containing 25 or 50 ppm added cadmium exhibited a reduced response to repeated morphine administration (Fig. 1).

It is noteworthy that there was no evidence on Day 22, when a vehicle-only injection was administered to all animals, that animals repeatedly exposed to morphine were different than animals that previously had received vehicle-only injections throughout training. This finding of no context-induced enhancement of locomotor activity among controls or animals developmentally exposed to cadmium agrees with previous morphine investigations (Jeziorski and White, 1995; Nation et al., 1997a,b), and in this regard distinguishes morphine from other drugs such as cocaine where conditioning features have been shown to play a prominent role in the development and expression of sensitization (Post et al., 1981). Also, it is noted that the vehicle-only injections on Day 22 failed to alter cadmium-based

disturbances in morphine sensitivity, i.e., cadmium-exposed groups continued to be less responsive to morphine challenges during dose–effect testing than controls (Fig. 3).

4.2. Dopamine antagonists

As mentioned, both D_1 and D_2 receptor antagonists reduce the ambulation increases associated with repeated morphine administration, and this effect is produced in a dose-dependent fashion (Jeziorski and White, 1995; Kuribura, 1995). Because it further has been shown that the attenuation of morphine sensitization by dopamine antagonists derives from blockade of the expression of morphine sensitization rather than the development of the phenomenon (Jeziorski and White, 1995), it must be considered that opiate sensitization may still develop when exogenous factors differentially affect dopaminergic function. This is an important issue, but not one that can be addressed in this study inasmuch as it is not possible separate the selective impact of cadmium on development/expression. That is, because the consequences of cadmium exposure cannot be systematically manipulated, it necessarily remains uncertain whether the antagonism of morphine sensitization by developmental exposure to the metal results from toxicant-induced compromise of one or both aspects of sensitization training.

Still, the present results regarding D_2 antagonist challenges to morphine are of interest. Recall that with the D_1 antagonist, SCH 23390, group differences were apparent at the lowest dose (0.010 mg/kg) of the antagonist (Fig. 4). However, given that the overall profile at this low dose essentially replicates Day 21 of sensitization training (Fig. 1) for control animals and animals born to dams exposed to diets containing 50 ppm added cadmium, the most straightforward explanation would be that at this lowest dose of the antagonist no effects of any kind were produced. The remaining comparisons with SCH 233390 document the suppressive impact of the D_1 antagonist on the stimulatory properties of morphine, but the drug was mutually potent across exposure groups, therein questioning the significance of this receptor subtype in the determination of the effects observed here.

The situation with the D_2 antagonist eticlopride is perhaps more compelling. It is apparent from Fig. 5 that the high dose of eticlopride (0.056 mg/kg) had more of a suppressive effect on control than cadmium-exposed animals when presented against the training dose of morphine (10 mg/kg). Indeed, locomotor activity levels in Group 50-morphine at this highest dose were not significantly different from Day 21 levels or from the low eticlopride dose (0.010 mg/kg) that apparently had no effect in the other groups. The diminished impact of eticlopride on morphine sensitization among cadmium-exposed animals implicates the D_2 receptor subtype as potential site for cadmium-based alterations in morphine sensitivity. Insofar as conformational changes in receptor characteristics, receptor numbers, binding affinity, or other determinants of D_2 receptor function are altered by developmental cadmium exposure, it is reasonable to expect that the potency of agonists and antagonists that act at this site would be reduced in a similar manner. This is not to say that other receptor operations known to be affected by cadmium presence (μ-opioid receptors, NMDA glutamate-receptor subtypes) are uninvolved in developmental cadmium/morphine interactions. Rather, given the essential role of the D_2 receptor in the expression of morphine sensitization (Jeziorski and White, 1995), it is arguable that disruption of this system by cadmium, as evidenced by the pattern of results reported here, is at least partly responsible for cadmium-related decreases in morphine sensitivity.

4.3. Implications

The behavioral data reported here indicate that developmental cadmium exposure results in altered drug responsiveness later in life. Also, the possible long-lasting consequences of early cadmium exposure with respect to changes in patterns of opiate self-administration should not be overlooked. Examination of the interface between toxicology and pharmacology mandates careful inspection of the unique molecular linkages among xenobiotic contaminants and drugs possessing substantial abuse liability. Only with a more complete understanding of underlying mechanisms can we fully appreciate the extent to which our external chemical environment delimits/augments drug action, and therein contributes to a complex matrix that determines drug use/abuse.

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