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Chronic Lead Exposure Attenuates Ethanol-Induced Hypoalgesia

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BURKEY, R. T., J. R. NATION AND G. R. BRATTON. *Chronic lead exposure attenuates ethanol-induced hypoalgesia.* PHARMACOL BIOCHEM BEHAV 47(2) 227–231, 1994. – Adult male rats were exposed to drinking fluid containing either 500 ppm lead acetate (group lead), or an equivalent concentration of sodium acetate (group control) for 61 days prior to pain reactivity testing using a tail-flick procedure. Rats were placed in restraining tubes for a 20 min acclimation period, and then baseline tail-flick latencies in response to a radiant heat source were measured. Subsequently, half the animals from each group were serially injected IP with either 1.0, 2.0, or 3.0 g/kg body weight of a 20% v/v ethanol solution, and the other half were injected with an equivalent volume of saline. Tail-flick latencies were reassessed at 20-min intervals over the next 2 h. Results indicated dose-dependent ethanol-induced hypoalgesia at all doses, but at the two higher doses the magnitude of the hypoalgesic response was significantly greater in the group control animals than in the group lead animals across the 2-h postinjection period. Results are discussed in terms of an attenuation of the pharmacological properties of ethanol by lead.

Lead Ethanol Hypoalgesia Tail-flick

LEAD, an ubiquitous environmental contaminant, places the health of increasingly greater numbers of people at risk (18). In addition to lead-related physiological damage (e.g., renal, hepatic, and hemopoietic disturbances), it is becoming clear that even low levels of lead exposure can impact the central nervous system, resulting in cognitive deficits (2), hyperactivity (4), and altered patterns of drug response (15,19,22). With respect to the latter, lead exposure has been shown to produce an increased volitional intake of ethanol under conditions of mild (13) and moderate stress (12) in the adult rat. Initially, this phenomenon was interpreted as an increase in the hedonic valence of the drug among lead-treated animals. However, a subsequent study (14) revealed that when lead-treated rats were required to lever press for access to a 6% v/v ethanol solution, these animals pressed at lower rates relative to controls. Moreover, as the ethanol concentration was systematically increased to 32%, lead-treated animals displayed a shift to the right in the dose-response function relative to controls.

The most recent data on lead/ethanol interactions suggest that lead exposure may produce a pharmacological attenuation of the drug. Within this interpretive framework, the increased intake in a free access situation may be viewed as compensatory. That is, lead-treated animals may require more ethanol to achieve the same pharmacological effect as the control animals. In confirmation of the pharmacological attenuation hypothesis, rats deprived of water were injected with ethanol prior to permitting the animals access to tap water via a drinking tube (15). Lick responses were punished with electric shocks to the tongue every 20th lick. Typically, ethanol administration produces an antipunishment effect, whereby ethanol-treated animals will lick at higher rates than their control counterparts that did not receive ethanol prior to testing. The results indicated that lead-treated animals were less responsive to the ethanol treatment (the antipunishment effects of ethanol were diminished) compared to control animals. Additionally, it has been demonstrated that chronic lead exposure antagonizes the rate-depressant effects of ethanol in an operant preparation (10). While these studies provide evidence supporting a lead-induced pharmacological attenuation rationale, the possibility that lead alters the motivational or learning aspects of these appetitive tasks cannot be dismissed. In this regard, one effect of ethanol, hypoalgesia, can be used to measure the intoxicating effects of ethanol, yet avoid potential confounds associated with motivational and learning phenomena. It is within this context that the current study was designed. In this experiment, lead-treated and control animals were tested for differential reactivity to ethanol in a hypoalgesia preparation that employed tail-flick latency as a dependent measure.

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MATERIALS AND METHODS

Animals

Thirty-two male Sprague-Dawley rats (Holtzman Company, Madison, WI), approximately 50 days old and weighing between 180 and 200 g were used in this study. Sixteen animals were administered distilled water containing 500 ppm lead acetate, while the remaining 16 animals received water containing 210 ppm sodium acetate to equalize acetate exposure for the two groups. One animal from group lead-ethanol (see below) died on the final day of testing, and data from this animal were not included for that test day.

Apparatus

Hypoalgesia was assessed with a radiant heat tail-flick device, described in detail elsewhere (3). Latency to respond was recorded to the nearest 0.01 s.

Procedure

The room lights in the animal holding area were on a 12/12 light/dark cycle. Throughout the experiment all animals were individually housed, and appropriate drinking fluid and food were available ad lib. Animal body weights as well as total fluid consumption were recorded weekly. At the beginning of the experiment, rats were stratified into four groups based on body weight: control-saline, control-ethanol, lead-saline, and lead-ethanol (N = 8/group).

On the 61st day of exposure to their respective drinking fluids, at 1700 hours, testing commenced. To measure ethanol-induced hypoalgesia in rats, a tail-flick latency procedure was employed which measures the latency of the tail-flick reflex after applying an intense radiant heat source to the tail [cf. (3)]. Since large doses of ethanol also produce hypothermia, tail temperature was recorded immediately prior to each tail-flick test. A temperature sensor (Radio Shack 277-0123) was taped to the base of each animal's tail prior to each test session, and left in place for the duration of the session.

Animals were divided into eight squads of four animals per squad, counterbalanced such that each squad was composed of one member of each condition. Further, animals within each squad were randomly assigned to one of four restraining tube positions to prevent any possible order effect within each squad. Animals were placed in the restraining tubes for a 20-min acclimation period, and then three baseline tail-flick latencies were measured at 2-min intervals. The mean of the last two tail-flick latencies was used as a baseline index of the animals' sensitivity to aversive stimuli for that day of testing.

Following baseline recordings, animals within each dietary condition were injected IP with either 1.0 g/kg (20% v/v) ethanol (group control-ethanol and group lead-ethanol) or the equivalent volume of saline (group control-saline and group lead-saline), and tail-flick latencies were assessed successively at 20-min intervals for the next 2 h. Animals were then returned to their home cages and allowed to rest for three days. This procedure, including the rest interval, was then repeated serially in an ascending order for each animal at ethanol doses of 2.0 and 3.0 g/kg body weight. Previous research indicates that the three-day rest period is sufficient to avoid acute tolerance effects with repeated ethanol administrations (8). Again, saline-treated animals received an equivalent volume of the vehicle based on dose comparisons with body weight.

Chemical Analysis

Twenty-four hours after the final day of testing, animals were rendered unconscious in a bell jar with CO_2 and then were decapitated. After each animal had been sacrificed, trunk blood was collected. The concentration of lead in blood was accomplished via dry ashing and atomic absorption spectrophotometry as described elsewhere (16).

RESULTS

Body Weight

The mean body weights for control and lead-treated animals are shown in Fig. 1. A 2 (Group: Control, Lead-Treated) × 10 Weeks (1-10) repeated-measures analysis of variance (ANOVA) test performed on this measure failed to reveal either a significant main effect of group, $F(1, 30) = 0.06$, $p > .05$, or a significant Group × Week interaction, $F(9, 270) = 0.58, p > .05$.

Fluid Intake

A 2 (Group: Control, Lead-Treated) × 10 Weeks (1-10) repeated-measures ANOVA test of mean fluid intake showed no significant main effect for group, $F(1, 30) = 1.17$, $p > .05$. Similarly, there was no significant Group × Weeks interaction, $F(9, 270) = 1.29, p > .05$.

Tail-Flick Latency Data

Ethanol-induced hypoalgesia was observed at all three doses of the drug. Evidence was obtained which indicated that recurrent exposure to lead attenuated the hypoalgesic properties of ethanol at the 2.0-g/kg and 3.0-g/kg doses.

Separate Group (Control, Lead-Treated) × Injection (Saline, Ethanol) ANOVA tests performed on mean preinjection baseline latencies for each dose (see Table 1) failed to reveal any group differences in reaction to aversive stimuli at any phase of the testing program (Fs < 1.66).

Statistical analysis of the tail temperature data indicated no systematic group differences in tail temperature (ps >

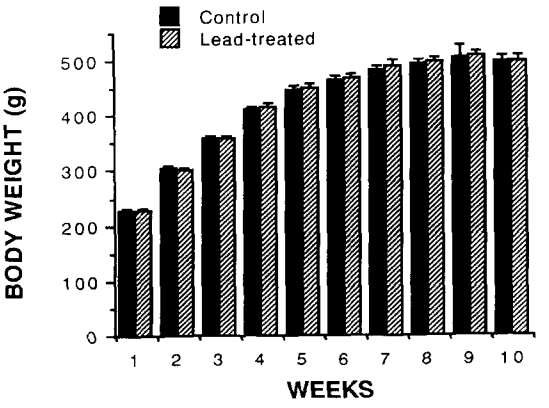

FIG. 1. Mean (+SE) body weight over the 10 weeks of dietary exposure to water containing sodium acetate (control, n = 16) or water that contained 500 ppm lead acetate (lead-treated, n = 16).

TABLE 1
MEAN BASELINE TAIL-FLICK LATENCIES AT EACH DOSE LEVEL ACROSS GROUPS

Condition	Ethanol Dose (g/kg, IP)		
	1.0	2.0	3.0
Control-saline	3.42	3.87	4.18
Control-ethanol	3.96	3.94	4.36
Lead-saline	3.78	3.86	3.99
Lead-ethanol	3.71	4.07	4.20

.05). Figure 2 shows the tail-flick latencies for each group at each dose across the 2-h postinjection test period. Separate 2 (Group: Control, Lead-Treated) × 2 (Injection: Saline, Ethanol) × Interval (1-6) repeated-measures ANOVAs performed on postinjection tail-flick latencies at each dose revealed that the 1.0-g/kg dose of ethanol produced a significant increase in tail-flick latencies, $F(1, 28) = 5.59$, $p < .05$ (Fig. 2A). However, neither the group, $F(1, 28) = 0.89$, $p > .05$, nor any interaction with group were significant.

Analysis of the 2.0-g/kg dose of ethanol revealed both a main effect for injection, $F(1, 28) = 58.91$, $p < .05$, and a significant Group × Injection interaction, $F(1, 28) = 14.98$, $p < .05$. No other interaction reached an acceptable level of significance. Pairwise analysis (Newman-Keuls) on the Group × Injection interaction means confirmed what is visually apparent in Fig. 2B—that is, latencies for control-ethanol-injected rats were longer than all other groups ($ps < .05$) and latencies of lead-ethanol-injected rats were longer than both saline-injected groups ($ps < .05$).

Similarly, a Group × Injection repeated-measures ANOVA on tail-flick latencies at the 3.0-g/kg dose yielded a main effect for injection, $F(1, 27) = 344.68$, $p < .05$, and a Group × Injection interaction, $F(1, 27) = 7.24$, $p < .05$. Again, no other interaction with group was significant. Newman-Keuls pairwise analysis on the Group × Injection interaction means revealed that control animals receiving an ethanol injection (group control-ethanol) had significantly longer latencies than the remaining three groups ($ps < .05$), and group lead-ethanol animals exhibited significantly longer latencies than the saline groups ($ps < .05$).

Lead Residues in Blood

A group t test on lead concentrations in blood revealed that group-lead animals ($\bar{X} = 0.247$ ppm) had significantly higher concentrations of lead in their blood than group-control animals ($\bar{X} = 0.017$), $t(24) = 16.62$, $p < .05$.

DISCUSSION

The findings from this experiment indicated that animals chronically exposed to 500 ppm lead acetate in their drinking water, and injected with high doses of ethanol, had shorter tail-flick latencies than animals exposed to an equivalent concentration of sodium acetate and injected with ethanol. Specifically, at ethanol doses of 2.0 g/kg and 3.0 g/kg control animals displayed a significant increase in tail-flick latency compared to all other groups, and lead-ethanol animals evinced greater tail flick latencies than the saline control groups, but this increased latency to respond reflexively was less than that observed for group control-ethanol. The data from the 1.0-g/kg dose of ethanol displayed in Fig. 2A show a similar visual trend to the higher doses, but the pattern was not statistically confirmed, possibly due to an increase in subject variability at this threshold dose of ethanol.

In hypoalgesia preparations of the type used here, it could be argued that increased tail-flick latency results from ethanol-induced motor impairment. Of course, a lead-mediated decrease in ethanol-induced motor impairment would still support the basic notion that lead attenuates the pharmacological properties of ethanol. In any case, we are inclined here to adopt the more straightforward position that chronic exposure

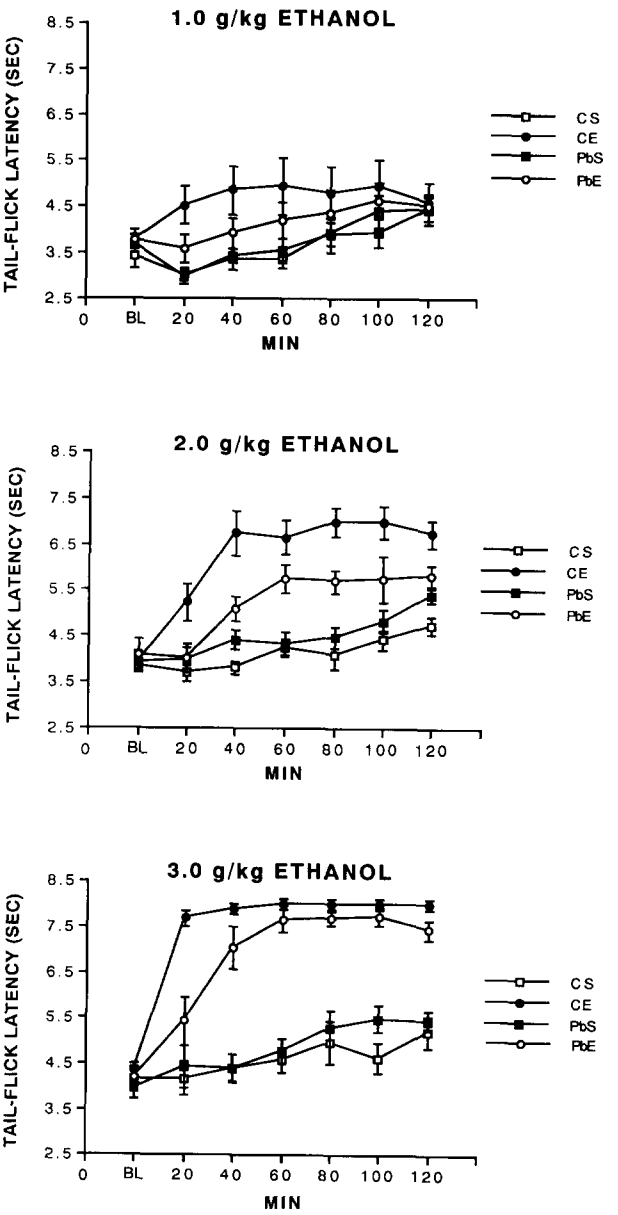

FIG. 2. Baseline (BL) tail-flick latency and latency after IP injections of either saline or ethanol. CS = control-saline, CE = control-ethanol, PbS = lead-saline, PbE = lead-ethanol.

to lead results in a displacement to the right in the dose-response function associated with the hypoalgesic properties of ethanol.

The finding that lead attenuates ethanol-induced hypoalgesia is consistent with the effects of an acute administration of lead on the hypnotic effects of ethanol (20). In this study, lead-exposed animals exhibited shorter sleep time and longer latency to lose the righting reflex than controls after an injection of 3.5 g/kg of ethanol. The present data also agree with previous research from our laboratory which shows that the toxicant decreases the antipunishment properties of the drug (15). The fact that a single dose of ethanol (1.5 g/kg) results in less punished licking in lead-treated rats, coupled with the present findings which indicate that lead masks the ethanol-induced increases in tail-flick latency, is especially instructive with respect to clarifying the nature of lead/ethanol interactions. It is increasingly apparent that lead toxicity diminishes the overall pharmacological profile of alcohol.

To the extent that lead retards the psychoactive effects of ethanol, it would be reasonable to expect changes in preference and self-administration patterns. That is, a compensatory increase in the consumption of ethanol in a free access situation among lead-exposed animals would be expected, inasmuch as more drug would be needed for the animal to achieve the same psychoactive effect as a control animal (12,13). Conversely, diminished lever responding for ethanol in a self-administration context may derive from the decreased reinforcement effectiveness of the drug (14).

While the assertion that lead toxicity decreases the overall pharmacological potency of ethanol seems reasonable, characterizing the precise mechanism(s) responsible for the attenuation is necessarily more speculative. In the mammalian system, lead has been shown to act as an L-type calcium channel antagonist (1). These findings are especially relevant to the present investigation, since calcium ostensibly plays a central role in many of the effects of ethanol. For example, elevated calcium levels have been shown to increase ethanol-induced sleep time in mice (7). Moreover, calcium antagonists such as verapamil have been found to reduce the impact of a set dose of centrally administered ethanol as defined by changes in locomotor activity (6). Furthermore, calcium channel blockage has been shown to decrease the stimulated release of dopamine (17), which is an important substrate in ethanol intoxication (21). Congruent with this overall rationale, decreases in dopamine bioavailability have been reported in lead-treated animals (11). It follows that insofar as lead restricts calcium activity and related changes in cell function and transmitter availability such as dopamine, the observed attenuation of the pharmacological impact of ethanol would be expected, at least insofar as ethanol operates on similar neural substrates.

While the mechanism or mechanisms for the reduced effects of ethanol remain to be fully elucidated, it appears that this exogenous environmental contaminant plays a significant role in the behavioral effects of acute ethanol exposure. Although lead has been shown to increase volitional ethanol intake and reduce behavioral markers of intoxication, there is evidence to show that ethanol-induced histopathology and other health risks associated with alcohol consumption are enhanced by the contaminant (5,9). Considering the possibility that lead may mask the overt behavioral signs of ethanol intoxication and yet exacerbate the internal toxic effects of the drug, greater efforts should be made to investigate the pathologic consequences of lead and ethanol cotreatment.

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REFERENCES

1. Audesirk, G.; Audesirk, T. Effects of inorganic lead on voltage-sensitive calcium channels in N1E-115 neuroblastoma cells. Neurotoxicology 12:519–528; 1991.
2. Bellinger, D.; Leviton, A.; Needleman, H. L.; Waternaux, C.; Rabinowitz, M. Low-level lead exposure and infant development in the first year. Neurobehav. Toxicol. Teratol. 8:151–161; 1986.
3. Burkey, R. T.; Nation, J. R.; Grover, C. A.; Bratton, G. R. Chronic cadmium exposure attenuates ethanol-induced hypoalgesia in the adult rat. Alcohol. Clin. Exp. Res. 17:423–427; 1993.
4. Chester, A. E.; Meyers, F. H. Central sympathoplegic and norepinephrine-depleting effects of antioxidants. Proc. Soc. Exp. Biol. Med. 187:62–68; 1988.
5. Dhawan, M.; Flora, S. J. S.; Tandon, S. K. Biochemical changes and essential metals concentration in lead-intoxicated rats preexposed to ethanol. Alcohol 9:241–245; 1992.
6. Engel, J. A.; Fahlke, C.; Hulthe, P.; Hard, E.; Johannessen, K.; Snape, B.; Svensson, L. Biochemical and behavioral evidence for an interaction between ethanol and calcium channel antagonists. J. Neural Transm. 74:181–193; 1988.
7. Erickson, C. K.; Tyler, T. D.; Harris, R. A. Ethanol: Modifications of acute intoxication by divalent cations. Science 199:1219–1221; 1978.
8. George, F. R.; Ritz, M. C.; Meisch, R. A. Rate-depressant effects of ethanol on fixed-ratio responding in AIKO AA and ANA rats. Adv. Alcohol Subst. Abuse 9:31–42; 1990.
9. Grandjean, P.; Hollnagel, L.; Hedegaard, L.; Christensen, J. M.; Larsen, S. Blood lead-blood pressure relations: Alcohol intake and hemoglobin as confounders. Am. J. Epidemiol. 129: 732–739; 1989.
10. Grover, C. A.; Nation, J. R.; Burkey, R. T.; McClure, M. C.; Bratton, G. R. Lead/ethanol interactions I: Rate depressant effects. Alcohol 10:355–361; 1993.
11. Missale, C.; Battaini, F.; Govoni, S.; Castelletti, L.; Spano, P. F.; Trabucchi, M. Chronic lead exposure differentially affects dopamine transport in rat striatum and nucleus accumbens. Toxicology 33:81–90; 1984.
12. Nation, J. R.; Baker, D. M.; Fantasia, M. A.; Ruscher, A. E.; Clark, D. E. Ethanol consumption and free operant avoidance performance following exposure to dietary lead. Neurotoxicology 8:561–568; 1987.
13. Nation, J. R.; Baker, D. M.; Taylor, B.; Clark, D. E. Dietary lead increases ethanol consumption in the rat. Behav. Neurosci. 100:525–530; 1986.
14. Nation, J. R.; Dugger, L. M.; Dwyer, K. K.; Bratton, G. R.; Grover, C. A. The effects of dietary lead on ethanol-reinforced responding. Alcohol Alcohol. 26:473–480; 1991.
15. Nation, J. R.; Grover, C. A.; Bratton, G. R. Lead attenuates the antipunishment effects of ethanol. Alcohol 8:1–5; 1991.
16. Nation, J. R.; Grover, C. A.; Bratton, G. R.; Salinas, J. A. Behavioral antagonism between lead and cadmium. Neurotoxicol. Teratol. 12:99–104; 1990.
17. Pani, L.; Kuzman, A.; Diana, M.; De Montis, G.; Gessa, G. L.; Rossetti, Z. L. Calcium receptor antagonists modify cocaine effects in the central nervous system differently. Eur. J. Pharmacol. 190:217–221; 1990.
18. Perrotta, D. M. Surveillance of children's blood lead levels—United States, 1991. JAMA 268:1654; 1992.

19. Rosen, J. B.; Young, A. M.; Beuthin, F. C.; Louis-Ferdinand, R. T. Discriminative stimulus properties of amphetamine and other stimulants in lead-exposed rats. Pharmacol. Biochem. Behav. 24: 211–215; 1986.
20. Swartzwelder, H. S. Altered responsiveness to alcohol after exposure to organic lead. Alcohol 1:181–183; 1984.
21. Yoshimoto, K.; McBride, W. J.; Lumeng, L.; Li, T.-K. Ethanol enhances the release of dopamine and serotonin in the nucleus accumbens of HAD and LAD lines of rats. Alcohol. Clin. Exp. Res. 16:781–785; 1993.
22. Zenick, H.; Goldsmith, M. Drug discrimination learning in lead-exposed rats. Science 212:569–571; 1981.