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Long-Term, Low-Dose Lead Exposure Alters the Gonadotropin-Releasing Hormone System in the Male Rat

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Lead is a male reproductive toxicant. Data suggest that rats dosed with relatively high levels of lead acetate for short periods of time induced changes in the hypothalamic gonadotropin-releasing hormone (GnRH) at the molecular level, but these changes were attenuated with increased concentrations of exposure. The current study evaluated whether exposure to low levels of lead acetate over longer periods of time would produce a similar pattern of adaptation to toxicity at the molecular and cellular levels. Adult 100-day-old Sprague-Dawley male rats were dosed with 0, 0.025, 0.05, 0.1, and 0.3% lead acetate in water. Animals were killed after 1, 4, 8, and 16 weeks of treatment. Luteinizing hormone (LH) and GnRH levels were measured in serum, and lead levels were quantified in whole blood. Hypothalamic GnRH mRNA levels were also quantified. We found no significant differences in serum LH and GnRH among the groups of animals treated within each time period. A significant dose-related increase of GnRH mRNA concentrations with lead dosing occurred in animals treated for 1 week. Animals treated for more than 1 week also exhibited a significant increase in GnRH mRNA, but with an attenuation of the increase at the higher concentrations of lead with increased duration of exposure. We conclude that the signals within and between the hypothalamus and pituitary gland appear to be disrupted by long-term, low-dose lead exposure. Key words: gonadotropin-releasing hormone, hypothalamic–pituitary axis, lead acetate.

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Materials and Methods

Adult male Sprague-Dawley rats, 92 days of age upon arrival at the laboratory, were purchased from Charles River Laboratories (Portage, MI) and housed for 8 days before being randomly distributed into study groups of 12–16 rats. The rats were housed in polycarbonate cages suspended on stainless-steel racks in a room with a 12 hr:12 hr light-dark cycle and controlled temperature. Animals were allowed free access to lead-free Purina (Ralston-Purina, St. Louis, MO) laboratory chow and were maintained in accordance with the standards set forth in the Animal Welfare Act. Acid-washed amber-glass water bottles, equipped with neoprene stoppers containing stainless dipper tubes with ball bearings, were used to dispense deionized water containing either no lead acetate (PbAc) or 0.025, 0.05, 0.1, 0.3% PbAc (Fisher Scientific, Springfield, NJ). We added...
of water consumption was measured at the beginning of study weeks 1, 4, 8, and 16. The animals were killed by decapitation after 1, 4, 8, or 16 weeks of treatment. Hypothalami were dissected and snap frozen in liquid nitrogen and stored at −70°C until processing for RNA and DNA studies. Trunk blood was collected and lead levels measured. Serum was separated and frozen for the measurement of GnRH and LH levels. Care and treatment of animals was conducted in accordance with established guidelines and approval of the University of Southern California Animal Use Committee and internal review board.

Measurement of blood lead and hormones. Lead analysis was performed by the Air and Industrial Hygiene Laboratory of the California Department of Health Services as described previously (26). We used the ultra-sensitive DELFIA rat LH assay (Wallac Oy, Turku, Finland) to measure LH in rodent serum (27). Sensitivity was 0.03 ng/mL, and the intra- and interassay coefficients of variations at 33.1 ng/mL were 3.4% and 16.0%, respectively.

We determined the GnRH concentrations in plasma by radioimmunoassay using reagents (125I-labeled GnRH and GnRH standards and antiserum) from Phoenix Pharmaceuticals, Inc. (Mountain View, CA). The kit standard was compared to a standard curve generated using GnRH peptide supplied by the National Institutes of Health, Bethesda, MD. Plasma was extracted using acetic acid and Bond Elut LRC-C18 columns (Varian, Harbor City, CA). Intra- and interassay variations were 10% and 18%, respectively. Sensitivity of the assay was 0.1 pg/mL.

Preparation of RNA and DNA. Total RNAs were extracted from frozen hypothalami (n = 3 per treatment group) by homogenization in guanidium isothiocyanate according to established protocols (28). The concentrations of purified RNA and DNA product were determined by optical density at wavelength 260 nm (Beckman DU 640, Columbia, MO).

Northern blot performance. Isolated total RNA (30 µg per lane) was electrophoresed separately using formaldehyde 1.2% agarose gels in MOPS buffer and transferred via capillary action onto Nylon membranes (MSI, Westboro, MA).

An EcoRI/Hind III fragment (0.396 kb) of the rat GnRH cDNA insert and an EcoRI/Hind III fragment (0.7 kb) of the rat β-actin cDNA insert were excised and purified through a 1% Sea Plaque low melting agarose (FMC BioProducts, Rockland, ME). Both cDNA fragments as probes were labeled with [α-32P]-dCTP (NEN Life Science Products, Inc., Boston, MA) using a random primer labeling kit (Life Technologies, Bethesda, MD). (The cDNA probe was supplied by J. L. Robertson, Mount Sinai School of Medicine, New York.)

Hybridization of blots with a 32P-GnRH probe was performed in a NaPO4 buffer with 7% SDS, 1 mM EDTA, and 1 mg/mL bovine serum albumin at 68°C for 18–20 hr. Blots were washed in 1% SDS, 50 mM NaCl, 1 mM EDTA, and exposed to X-ray film at −70°C with intensifying screen for about 4–5 days. We confirmed equivalent RNA loading by rehybridization of the blots for the β-actin probe as an internal standard.

Densitometric analysis. We analyzed autoradiograms for the relative intensity of signals by densitometry (BioRad Imaging Densitometer GS-670) using BioRad Molecular Analyst PC Version 1-1 (Life Sciences Group, Hercules, CA).

Statistical analysis. We analyzed mean data by analysis of variance and performed post-hoc comparisons with the Newman-Keuls test. Pearson’s correlation coefficient was used to evaluate the linear relationship between lead levels and the amount of GnRH transcripts. Significance level was p < 0.05. Values are means ± SEM. We analyzed data using the commercially available software package SPSS (SPSS, Inc., Chicago, IL).

Results

Body weights and water consumption. Although there was a trend toward lower body weights with higher doses of lead in the animals treated for 8 and 16 weeks, rare significant differences in body weights at the time of sacrifice between lead-treated animals and their respective control groups were observed in any experimental group (Table 1). All lead-treated animals consumed less water than did their respective controls (p < 0.001; Table 1).

Circulating lead, GnRH, and LH levels. All control animals had mean blood lead levels consistently < 3 µg/mL. All lead-treated animals had blood lead levels greater than those of controls (p < 0.001; Table 2).

No significant differences in plasma GnRH and LH were found among animals treated with increasing doses of lead acetate for any time period studied (Table 2).

Northern blot analysis. A significant, steady dose–response increase of GnRH mRNA concentrations with lead dosing was seen in animals treated for 1 week (p = 0.01; Figure 1). Animals treated for more than 1 week also exhibited a significant increase in GnRH mRNA, but with an attenuation of the increase at the higher concentrations of lead with increased duration of exposure (Figure 2). Correction of loading by use of the standard probe β-actin indicated that the concentrations of GnRH mRNA were not dependent on the amount of RNA loaded per lane (Figure 2). Correlation between lead blood levels and expression of mRNA GnRH levels for doses ranging from 0 to 0.1% was significant (r = 0.9, p < 0.05).

Discussion

The data presented here show that low doses of lead for long periods of time alter the rat hypothalamic–pituitary axis in a manner similar to that previously reported at higher doses for shorter periods of time. These

| Table 1. Water consumption and body weights in control and lead-exposed male rats (mean ± SEM; n = 10–16). |
|---|---|---|---|---|---|---|---|
| Weeks of exposure | Measurement | 0 | 0.025 | 0.05 | 0.1 | 0.3 | P-value |
| 1 | Water consumption (g/animal/day) | 41.21 ± 0.77 | 37.12 ± 0.86* | 37.80 ± 0.89* | 32.81 ± 0.63* | 27.80 ± 0.49* | 0.001 |
| | Weight (g) | 408 ± 2 | 403 ± 2 | 401 ± 2 | 401 ± 1.8* | 404 ± 1.7 | 0.109 |
| 4 | Water consumption (g/animal/day) | 43.14 ± 0.97 | 37.18 ± 1.10* | 37.38 ± 1.17* | 34.90 ± 0.84* | 28.89 ± 0.51* | 0.001 |
| | Weight (g) | 501 ± 4 | 493 ± 5 | 490 ± 3 | 489 ± 4 | 487 ± 3* | 0.015 |
| 8 | Water consumption (g/animal/day) | 40.34 ± 1.02 | 36.24 ± 1.39* | 37.65 ± 1.05* | 35.19 ± 1.04* | 30.96 ± 0.49* | 0.001 |
| | Weight (g) | 578 ± 7 | 572 ± 8 | 566 ± 6 | 564 ± 7 | 559 ± 5 | 0.052 |
| 16 | Water consumption (g/animal/day) | 37.91 ± 1.52 | 33.76 ± 1.39* | 33.48 ± 1.69* | 33.04 ± 1.10* | 27.58 ± 0.68* | 0.001 |
| | Weight (g) | 694 ± 11 | 680 ± 15 | 668 ± 11 | 672 ± 13 | 661 ± 9 | 0.083 |

*p < 0.05.
Chronically lead-exposed male monkeys revealed only subtle signs of reproductive toxicity (24).

A similar tolerance has been reported in studies evaluating the renal toxicity of lead. Hitzfeld and Taylor (33) reported the development of resistance to lead in a rat kidney cell line. Kidney cells pre-exposed to lead showed a higher rate of protein synthesis than nonadapted cells. They suggested that changes in protein synthesis after lead exposure appeared to be a potent parameter in the development of this resistance. Clinical studies also suggest an adaptation to lead’s toxic effects on the kidney (34). However, clinical studies evaluating the relationship between duration of exposure and reproductive toxicity have not been published.

Although lead exposure alters the production of GnRH in the hypothalamus at the molecular level, circulating GnRH and LH levels are not altered significantly. Previous studies evaluating higher exposure levels for shorter periods of time reported similar results (19,20,24,35). The signals within and between the hypothalamus and pituitary gland appear to be disrupted by lead exposure, but elevations of GnRH mRNA levels do not automatically translate into increased blood levels of GnRH, at least in the lead-intoxicated rat. GnRH is secreted from the hypothalamus in a pulsatile fashion and in turn stimulates LH biosynthesis and secretion (36). Our failure to document a corresponding change in serum GnRH levels may be due to the fact that we measured only one serum sample at the time of sacrifice. However, a more likely explanation is that lead interferes with the release of GnRH from the nerve terminal in the median eminence. Autoradiographic studies indicated that $^{210}$Pb accumulated in the greatest concentration in the median eminence (37). Bratton and co-workers (38) investigated the effects of lead on norepinephrine and prostaglandin E$_2$ (PGE$_2$)-induced GnRH release from the adult male rat median eminence incubated in vitro. Lead did not alter basal GnRH release, but it did block norepinephrine-induced GnRH release in a dose-related manner. Conversely, lead had no effect on the PGE$_2$-induced release of GnRH, but did block the norepinephrine-stimulated release of PGE$_2$. Therefore, lead may disrupt GnRH release at the hypothalamic level by interfering with PGE$_2$ synthesis or release. In vivo studies evaluating the effects of lead exposure on noradrenergic stimulation of GnRH release support this conclusion. Lead exposure at high doses interferes with naloxone-mediated release of LH (18) and acts synergistically to inhibit catecholamine synthesis by the tyrosine hydroxylase inhibitor $\alpha$-methyl-p-tyrosine (23). Because the hypothalamic–pituitary axis is a dynamic system and the actions of lead at these central nervous system sites are not completely suppressive (23), adequate amounts of GnRH are released to maintain LH synthesis and secretion resulting in steady-state serum levels. As exposure time increases, adaptation at the molecular level intensifies.

These findings of perturbed GnRH mRNA expression at low levels of lead exposure are of potential clinical importance. The CDC defines blood lead levels as elevated in children if they exceed 10 µg/dL (I). Cognitive results agree with previous findings that indicated a significant positive correlation between lead dose and expression of mRNA GnRH levels in the hypothalamus (17,19). Furthermore, the data support the hypothesis that lead exposure initially induces an increase in intracellular levels of GnRH mRNA in a dose-related manner, but with an attenuation in message production at higher levels of blood lead. The attenuation in GnRH mRNA levels with a greater dose of lead exposure without a significant change in the levels of plasma GnRH and LH support the conclusion that the male Sprague-Dawley rat adapts to the toxic effects of lead on the hypothalamus and that alterations in GnRH production at the molecular level do not translate to increases in circulating GnRH or LH levels.

Previous published studies in other systems support the hypothesis that adaptation to lead toxicity occurs with prolonged exposure. Increased duration of exposure to lead does not produce more profound toxic effects on circulating testosterone, sperm concentration, and sperm production rate in animals whose dosing began at the beginning of the pubertal period (22). Castrated, lead-treated male rats are able to respond to castration with an increase in LH, indicating an adaptation of the hypothalamic–pituitary axis to the toxic effects of lead (23). At lower exposure levels, a significant disruption in the reproductive axis occurs at puberty in pups exposed from gestation through the onset of puberty (9,20,29) or in adulthood after exposure during sexual differentiation (30). However, animals whose exposure continues to adulthood do not manifest as profound an alteration in the hypothalamic–pituitary–gonadal axis (20). Studies in monkeys also suggest that chronically exposed animals may develop compensatory mechanisms. In the female monkey, lead exposure during adulthood induced menstrual irregularities (31), but chronic dosing from infancy through adulthood did not alter menstrual cycles (32).
and behavioral development may be altered in children exposed to these low levels of lead (4,5,21). We report lead-induced reproductive abnormalities in male rats at lead levels as low as 10 μg/dL. A recent clinical study evaluating the impact of lead exposure on normal reproductive development suggests that boys with marginally increased blood lead levels may mature sexually at a later age and have smaller testicular volume than control subjects (39). The exact mechanism by which this occurs clinically remains to be elucidated, but our results using an animal model suggest that subtle disruptions in GnRH production and/or release may be involved.

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