49 LEAD MAY AFFECT HORMONAL INDUCTION OF TYROSINE AMINOTRANSFERASE IN CULTURED HEPATOMA CELLS THROUGH INHIBITION OF PROTEIN KINASE C

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Previous results have shown that exposure (24-48 hr) of hepatoma cells (H4-II-E-C3) to 1–10 μM lead (Pb) acetate inhibited dexamethasone induced tyrosine aminotransferase (TAT) specific activity by 52-61%. A possible mechanism of the Pb-induced inhibition may be perturbation of calcium/phospholipid dependent protein kinase (PKC), a family of isozymes which mediate signal transduction responses to a variety of growth factors, neurotransmitters and hormones. To determine whether PKC plays a role in glucocorticoid-induced TAT levels, hepatoma cells were incubated with dexamethasone (10 nM) in the absence or presence of the PKC inhibitor H-7 (10-300 nM). TAT specific activity was dose-dependently inhibited from 17.3 to 81.4%. Treatments with the PKC-activating agent phorbol myristate acetate (PMA) 30 nM–1 μM, alone did not induce TAT. Extended treatment with PMA alone or with dexamathasone decreased basal and induced TAT specific activities, respectively. When cells were incubated with dexamethasone for 18 hr, then 2 hr with 30, 100 or 300nM PMA, TAT specific activity was potentiated by 19.6, 37.1 and 46.5%, respectively. In 48 hr Pb-exposed cells, potentiation by those same PMA treatments was reduced to 0.6, 21.7 and 23.4%, respectively. Pb exposure for 24 hr likewise resulted in a significant loss of PMA-induced potentiation of enzyme activity. These results suggest that PKC is involved in the hormonal signal transduction events leading to TAT induction, and exposure of cells to the environmental pollutant Pb may inhibit PKC activity. One or more PKC isozymes may be molecular targets of Pb. (Supported by ATSDR and NIH 03320).

50 DIFFERENTIAL MODULATORY EFFECTS OF HEAVY-METAL TOXICANTS ON CONSTITUTIVE NITRIC OXIDE SYNTHASE ACTIVITY

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We have recently demonstrated that constitutive nitric oxide synthase (cNOS) requires superoxide anion for conversion of L-arginine to nitric oxide (NO) and is inhibited by pretreatment with calcium ion [BBRC. 193, 126–132 (1993) and BBRC. 203, 8–15 (1994)]. Since cNOS is a Heme(Fe^{2+}) containing flavoprotein which also exhibits electron transport properties, the present studies were designed to evaluate the in vitro effects of transition heavy-metals on brain cNOS activity. NOS activity was determined in cytosolic fractions of rat cerebral hemispheres by conversion of ^{3}H-L-arginine to ^{3}H-L-citrulline. Different concentrations of mercury (Hg^{2+}), manganese (Mn^{2+}), nickel (Ni^{2+}), zinc (Zn^{2+}) and cadmiun (Cd^{2+}) were tested on NOS activity. All the metals caused significant inhibition of cNOS activity. However, significant differences were observed in the apparent Ki values for various ions [Hg^{2+} = 23 μM; Mn^{2+} = 3.5 mM; Ni^{2+} = 0.36 mM; Zn^{2+} = 0.25 mM; Cd^{2+} = 0.22 mM]. In contrast to calcium ion-mediated inhibition which requires preincubation in the absence of sustrates, the transition metals caused cNOS inhibition without preincubation treatment. Also, the preincubation of cytosolic preparations with calcium ion in combination with heavy-metals led to additive inhibitory response. These data indicate that while calcium ion modulate cNOS activity at regulatory sites, inhibitory influence of heavy-metals is exerted directly on the catalytic site(s). [Supported by RCMI 2G12RR03045-09 and MBRS 2S06 GM08061 from NIH].

51 EFFECTS OF LEAD ON DOPAMINE β HYDROXYLASE ACTIVITY IN THE PC12 CELL MODEL

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Lead poisoning following chronic exposure is injurious to the learning abilities of exposed children. The mechanisms surrounding this toxic effect are still unclear. We hypothesize that lead influences the optimal functioning of the enzyme system involved in neurotransmitter synthesis and thereby, alters the levels and or activities of both dopamine and norepinephrine. We report studies on the effects of lead on commercial grade and PC12 cells derived dopamine β hydroxylase, the enzyme involved in the formation of norepiniphrine from dopamine. Lead was shown to have marginal effects on cell viability at lead ions concentrations relevant to those found in chronically exposed children. There was no significant effect on dopamine β hydroxylase enzyme activity at 0.1–1000 μg/dL lead, but a 20-40% reduction in Km in the presence of lead. Lead's neurotoxic effects, therefore, may not be solely dependent on its effects on dopamine β hydroxylase per se. We are currently investigating its influence on the rate limiting enzyme tyrosine hydroxylase. This work was supported by an ATSDR Grant No. U50/ATU39894802.

52 THE EFFECT OF LEAD (PB) ON INTERLEUKIN-6 (IL-6) PRESENCE IN MOUSE SPLEEN AND BRAIN

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IL-6 is a cytokine that has diverse biological activities including induction of acute phase proteins, stimulation of T cells, differentiation of B cells to plasma cells, and activation of the hypothalamic-pituitary-adrenal (HPA) axis. The heavy metal Pb has immunomodulatory effects, and Pb-induced suppression of host defense mechanisms at low doses in laboratory animals is well documented. However, the effect of lead on the production of IL-6 has not been characterized. The *in vivo* effects of Pb on the production of IL-6 in the saline-perfused spleen and brain upon LPS challenge were assessed. B6C3F1 female mice (5 mon) that were exposed to 2 mM Pb in their drinking water for 3 mon were used for the experiments. ELISA quantification of IL-6 demonstrated that both Pb-exposed and age and sex matched control mice exhibited the same kinetic patterns upon i.v. challenge with 25 μg LPS. IL-6 peaked 2 hr and 3 hr after LPS challenge in spleen and brain, respectively. The highest amounts of IL-6 found were approximately 480 pg and 240 pg/mg protein in spleen and brain, respectively. When the amounts of IL-6 detected were compared at 2 and 6 hr post-LPS, Pb-exposed and non-exposed spleen samples did not show any difference. However, substantially higher amounts of IL-6 were detected at both time points in the brains of mice exposed to Pb. Since IL-6 activates the HPA axis and this activation results in the induction of glucocorticoids that have a profound suppressive effect on the immune system, the enhanced IL-6 level observed in the brain of Pb-exposed mice could be, in part, the mechanism responsible for Pb-induced immunosuppression upon infection. (Supported by NIH grant ES03179)

53 HEAVY METAL LEAD AFFECTS THE EXPRESSION OF CYTOKINES IN HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMC)

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Lead has been implicated as a causative agent for several immune function abnormalities. In order to find some direct evidence for lead poisoning effects on the human immune system, PBMC isolated by Ficoll-hypaque from healthy donors were cultured with medium, 50 μM lead chloride (Pb), 1% (vol/vol) phytohemagglutinin (PHA), PHA + Pb, 1 ng/ml lipopolysaccharide (LPS), and LPS + Pb. After 24h, the supernatants were assayed for cytokines using enzyme linked immunosorbent assays (ELISAs), and the cells were used for mRNA isolation. RNA-polymerase chain reaction (PCR) was done to detect the mRNA levels of different cytokines. The secretion of interferon γ (IFN-γ), interleukin 2 (IL-2), and interleukin 4 (IL-4) were significantly increased by Pb in the PHA-activated PBMC. The secretion of tumor necrosis factor α (TNF-α) was significantly increased by Pb in the LPS-activated PBMC. There was no detectable IL-2, IL-4, or TNF-α in the supernatants of non-treated and Pb-treated PBMC. Without PHA or LPS treatment, the mRNA levels of IL-1β and IL-2 were upregulated by Pb. We did not see any change at the mRNA levels of IL-4, IL-10, IFN-γ, and TNF-α. In the PHA or LPS activated PBMC, Pb did not substantially alter the mRNA levels of these cytokines except IFN-γ which was downregulated by Pb. These results suggest that Pb can modulate cytokine secretion at both transcriptional and post-transcriptional levels. Supported by ES03179.

54 NERVOUS SYSTEM DAMAGE PRECEDES IMMUNE SYSTEM INVOLVEMENT IN MALE MICE EXPOSED TO PB

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Previously we presented evidence that Pb may provoke an immune response against nervous system (NS) structures. However, it is not known whether Pb-induced neural damage must precede the immune response. To resolve this question, male and female CBA/J mice were exposed to 820 ppm Pb in the drinking water. Endpoints included: serum autoantibody (AuAb) titers to nervous system proteins (NSP): neurofilaments (NF), glial fibrillary acidic protein (GFAP) and myelin basic protein as well as interleukin 2 and 6 (IL-2,IL-6) levels from supernatants of lectin-stimulated splenocytes. GFAP was used as a

marker of neurotoxicity. GFAP levels were elevated in the hippocampus and cerebellum of male mice at 5d but recovered to baseline afterward. In males, serum AuAbs to NSP were mostly directed against the NFs and peaked predominately at 10d. These early AuAb changes accompanied IL-6 elevations (10d), followed by IL-2 elevations (28d). Analogous effects were not seen in female mice. These results suggest that NS damage, as indicated by elevated GFAP levels, precedes AuAb production in male mice suggesting that Pb-induced neural damage precedes the immune response. This study also confirms that males are more sensitive to Pb neurotoxicity. However, these differences cannot be accounted for on the basis of Pb exposure since weight gain and water consumption did not differ between the genders. (Sponsored by ES-04895).

55 MORPHOMETRIC ANALYSIS OF CORTICAL ASTROCYTES OF JUVENILE RATS FROM LEAD TREATED DAMS

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The objective of this study was to determine the maternal effect of very low doses of lead on the area of astrocyte of juvenile rats as determined by morphometeric analysis. Female Sprague-Dawley rats were treated orally with 25 ppm and 50 ppm lead acetate (PbAc) in drinking water for 5 weeks. Treatment was completed prior to mating with untreated males. Litters were culled and 21 day pup brains were sectioned and Golgi-stained. The sections were then analyzed using the BioQuant Advanced Image Analysis System. Parameters measured were the area of the astrocyte and its perimeter. Preliminary data collected and analyzed showed a clear and significant difference in the statistical population distribution of the astrocyte area with a shift from larger to smaller areas with treatment. Detailed analysis using more sections showed a significant trend.

56 COMPARISON OF LEAD UPTAKE IN MATURE AND IMMATURE ASTROGLIA

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There is a growing concern about the effects of low level lead (Pb) exposure on the developing central nervous system in children. Cognitive, attentional and behavioral deficits have been reported, and a no-effect level for Pb has not been determined. As a result, current research is focused on identifying cellular markers of toxicity. Because astroglia have been shown to accumulate Pb and are necessary for maintaining the brain microenvironment, our studies have focused on these cells. The present study reveals a differential uptake of Pb by astroglia that is dependent upon age in culture at which Pb exposure is initiated. Primary rat astroglia were exposed to 1 μM Pb beginning at 7 days (D7), 14 days (D14) and 21 days (D21) in culture. By seven days of exposure the D7 and D14 cells showed a significantly greater accumulation of Pb when compared to the D21 cells. Also, an additional 10 μM glutathione in the medium had no effect on Pb accumulation except in the D14 astroglia which showed a significant reduction in Pb content. In association with this, only the D14 calls showed an elevated Pb to calcium ratio when compared to both D7 and D21 astroglia. Future studies will be directed at exploring these age-dependent differences in astroglia in order to better understand the toxicological implications of Pb exposure.

57 VISUALIZATION OF LEAD UPTAKE IN BRAIN CAPILLARY ENDOTHELIAL CELLS USING A FLUORESCENT DYE

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The mechanism by which lead crosses the blood-brain barrier is not known. The blood-brain barrier is comprised of brain capillary endothelial cells which form tight junctions with each other; lead must traverse these cells to reach the brain. We have developed a new method to monitor lead transport into cultured bovine brain capillary endothelial cells using Indo-1, a fluorescent dye commonly used as a Ca^{2+} indicator which is quenched upon binding to lead. Uptake was monitored using spectrofluorimetric and digital fluorescence imaging techniques. For spectrofluorometric measurements, Indo-1-loaded cells were monitored at ex 336 and em 450 nm. Addition of 1–10 μM lead caused quenching which was time- and concentration-dependent. Fluorescence was only partially recovered by the addition of the cell-impermeable heavy metal chelator diethylenetriaminepentaacetic anhydride, and was fully recovered upon the addition of the permeable chelator tetrakis(2-pyridylmethyl)ethylenediamine, indicating that quench was due to intracellular lead. Single-cell imaging confirmed this observation. Dye-loaded cells grown on glass coverslips were placed in a chamber on a microscope, and images were recorded before and after the addition of lead. Lead caused quench of fluorescence first in the nucleus, then in cytoplasm of endothelial cells. Fluorescence was recovered by the addition of heavy metal chelators. This novel approach to studying lead uptake may facilitate investigations of the transport mechanism in brain capillary cells, and may be extended to other cell types as well. Supported by ES05855, ES07026 and ES06484.

58 LEAD TOXICITY TARGETS TO PYRROLOQUINOLINE QUINONE (PQQ) IN MITOCHONDRIAL COMPLEX I AND IN RED CELLS

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We have demonstrated that lead toxicity involves inhibition of mitochondrial complex I; higher levels of lead causes hemolysis of red cells. Our focus is on the prevention of both of these effects by the addition of the natural product PQQ. PQQ is a redox cofactor originally discovered in methylotrophic bacteria and more recently shown by our laboratory to be present in mammalian systems in protein-free dialysates of biological fluids, tissue homogenates, red and white cells and mitochondrial sonicates. In mitochondria, PQQ is the metabolic target for a variety of complex I inhibitors. In a diaphorase assay, electron transport inhibited at complex I by rotenone, iodonium compounds or *N*-methylphenylpyridinium (MPP+) is restored with PQQ. Lead inhibits oxygen uptake in intact mitochondria in the presence of complex I NADH-generating substrates, but not with the complex II FADH-generating substrate. We demonstrated the reversal of rotenone inhibition by addition of a lipoidal agonist of PQQ. This approach is currently being applied to mitochondria inhibited with lead. We have also shown that high levels of lead cause hemolysis of red cells by sequestering endogenous PQQ. This action is prevented by adding exogenous PQQ. These findings may lead to novel approaches for treatment of lead toxicity using PQQ and PQQ agonists. Research Support: National Dairy Promotion & Research Board Grant, MRC and AHFMR (JM).

59 EFFECT OF LEAD ON INSULIN BINDING AND RESPONSIVENESS

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The effect of lead exposure on the binding and cellular responsiveness of the hormone insulin to its cell surface receptor has been investigated using 3T3-C2 mouse fibroblasts, an insulin-responsive cell line. Specific binding of insulin to cell surface receptors increased with 48 h treatment of 10^{-4} M lead acetate. Data from Scatchard analysis of equilibrium binding isotherms indicate that the increased binding is not due to a change in affinity, but to a change in the number of receptors. To assess changes in insulin sensitivity of lead treated cells, glucose uptake and the ability of treated cells to downregulate their insulin receptor was investigated. No significant change in glucose uptake was observed. However, data from downregulation studies indicate that treated cells decrease receptor number to the same steady state level but the time required to attain the steady state level is shorter in lead treated cells. Results from these studies will increase the understanding of the cellular effects of toxic metals and aid in delineating whether the effects of lead on insulin receptor binding and metabolic processing impacts the health status of an exposed population.

60 CHARACTERIZATION STUDIES OF HUMAN BRAIN LEAD-BINDING PROTEINS (PBBPS)

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The recognition that metal-binding proteins may exert homeostatic control over the intracellular bioavailability of metal ions is of central importance for both essential and toxic elements. Our group has previously reported the existence of PbBPs in several species and in various tissues. In this study we present results of Pb-binding studies which confirm that the PbBPs isolated from human brain cytosol (Quintanilla-Vega et al., 1994, The Toxicol. 14, 255) bind Pb with a K_d of $\cong 10^{-9}$ M. Similar chemical, chromatographic and Pb-binding

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