190 THE EFFECT OF LEAD ON NEURITOGENESIS AND CHOLINERGIC ENZYME ACTIVITY.

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Sponsor: M Kolta.

The effects of lead on neurite outgrowth and on the cholinergic enzyme activities of acetylcholinesterase (AChE) and choline acetyltransferase (ChAT) were studied in monolayer cultures of mouse neuroblastoma cells (NB41A3) obtained from ATCC. In the course of NGF-induced differentiation of the PC-12 cell line, some of the late events that are frequently transcriptionally dependent and occur within 24–48 hours are the induction of AChE and the outgrowth of neurites. NB41A3 cells do not need NGF to induce neurite outgrowth and this property presents an interesting model for looking at lead toxicity on NEURITOGENESIS and cholinergic enzyme activity when treated with or without NGF. Cells were grown, subcultured, seeded in 6-well plates for neurite studies and 75 cm^3 for enzyme studies and exposed to various concentrations of lead. NGF was used at a concentration of 50ng/ml to observe its effect, if any, on neuritic extensions and enzyme activity. A 24 hour study using 0–50μg/dl lead levels showed no significant changes in neurite outgrowth, elongation, nor enzymatic activity of AChE and ChAT. The effect of high lead levels on AChE, ChAT, and neurite outgrowth are presently under study at 24 and 48 hour exposure. Higher concentrations of lead will include 50μM and 100μM.

191 EFFECTS OF LEAD ON PROTEIN TYROSINE KINASE PHOSPHORYLATION IN VIVO.

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Lead (Pb) is a well known neurotoxic metal which has been shown to cause mental retardation in young children and behavioral abnormalities in rodents. Our earlier *in vitro* studies have shown that Pb inhibits insulin-like growth factor - I (IGF-I) mediated autophosphorylation of IGF-I receptors. Pb also competitively inhibits phosphorylation of the artificial substrate Poly (Glu:-Tyr) 4:1. IGF's have been shown to stimulate nervous system development and differentiation of the neurons. Therefore, the present study was initiated to determine the *in vivo* effects of Pb on protein tyrosine kinase phosphorylation. Adult male Sprague-Dawley rats were treated with 500 ppm of lead acetate in drinking water for 15 days. The control group received only distilled water. The animals were sacrificed and brains were removed and processed for the determination of protein tyrosine kinase (PTK) activity. PTK activity was determined as a measure of phosphorylation of poly(Glu: Tyr) 4:1. There was no significant change observed in brain PTK activity in Pb treated animals as compared with the control group. These data suggest that young animals may be more susceptible to Pb toxicity than adults and/or longer duration of exposure to Pb is warranted to test our hypothesis.

192 ROLE OF PKC IN MEDIATING EFFECTS OF LEAD ON VITAMIN D-DEPENDENT PRODUCTION OF OSTEOCALCIN.

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In lead poisoned children the circulating levels of osteocalcin are decreased. Lead decreases both basal and vitamin D- dependent production of osteocalcin in osteoblast like cells, ROS 17/2.8. Several studies have suggested a negative controlling effect of PKC in the actions of vitamin D. The purpose of our study is to characterize the signal transduction processes mediating the effects of lead. Our hypothesis is that lead activates PKC-β thereby exerting a negative feed back control on the actions of vitamin D, including production of osteocalcin via phosphorylation of the vitamin D receptor. To characterize the effects of lead on osteocalcin production ROS cells were treated with PKC inhibitors or activators in the presence of various concentrations of lead (≤20μM). Osteocalcin was measured by RIA 24 hours after the addition of vitamin D (100 pM). Activity of PKC was determined by ELISA. Time- and dose- response experiments with several PKC inhibitors did not change basal or vitaminD- induced osteocalcin production. In contrast, activation of PKC by 4hr TPA treatment significantly reduced basal and vitamin D-induced osteocalcin production. Down regulation of PKC by 24 hr TPA treatment increased osteocalcin synthesis. Treatment with both lead and TPA had additive effects in reducing osteocalcin levels. Our data suggest that lead acts like a PKC activator in ROS cells. The exact mechanism of action is not known, but phosphorylation of the vitamin D receptor, as a substrate for activated PKC, and consequently reduction in binding of the vitamin D receptor to the vitamin D responsive element in the osteocalcin gene is one possible mechanism under exploration. Supported by NIH ES04040.

193 INHIBITION OF TYROSINE HYDROXYLASE ACTIVITY BY LEAD.

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Previous studies from our laboratory reported reduction in dopamine (DA) contents in various regions of rat brain (Neurotox. 16(2) : 297–308, 1995) and reduced K^+-induced release of DA in nucleus accumbens of rats exposed to 50 ppm lead acetate for 90 days (J. Neurochem. 65 : 1631–1635, 1995). Although similar reductions in dopaminergic activity have been reported by other investigators, the mechanisms involved in these reductions remain unclear. Autoreceptor agonism and inhibition of tyrosine hydroxylase (TH), a key regulatory enzyme in the biosynthesis of DA, by Pb have been implicated in reductions in central DA activity in Pb-exposed animals. This study was designed to assess the ability of Pb to affect TH in vitro as well as in vivo. 21.3, 22.8 and 56% inhibition of TH activity in vitro was observed in presence of 5, 50, and 500 ppm Pb respectively. Similarly, the TH activity was found to be 43% lower in brain homogenates obtained from rats exposed to 50 ppm lead for 30 days as compared to the controls. The alterations in TH activity were further confirmed by Western blot analysis. These results are consistent with our previous studies and indicate that Pb-induced inhibition of TH activity in rat brain may contribute to the reductions in dopaminergic activity observed in Pb-exposed animals. Supported by ATSDR/MHPF grant No. 398948–05.

194 RABBIT RENAL ORNITHINE DECARBOXYLASE (ODC) ACTIVITY IS INHIBITED IN A DOSE-DEPENDENT MANNER AS BLOOD LEVELS OF LEAD INCREASE.

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As part of a multidisciplinary research project to evaluate the relevance of the rabbit as a male reproductive toxicology model, the effect of lead exposure on renal ODC activity was determined. At sacrifice, kidneys were removed from rabbits that had been treated with lead acetate to produce experimentally established blood lead levels of 0, 20, 40 and 80 ug Pb/Dl. Cytosolic ornithine decarboxylase (ODC) activity was measured in kidneys from each treatment group by determining ^{14}C liberated as CO_2 from radiolabled ornithine. Kidney ODC activity was decreased in a dose-dependent manner: Control = 2137 ± 268 dpm; 20ug/Dl = 1821 ± 275 dpm; 40ug/Dl = 1332 ± 131 dpm and 80ug/Dl = 1262 ± 66.2 dpm. It was recently reported that blood levels of Pb, even within a range considered low, impaired kidney function in adult men. To our knowledge, the need for and function of ODC in rabbit kidney has not been established; however, the dose-dependent inhibition may be related to tissue toxicity. Studies are currently underway to determine if specific forms of ODC are selectively inhibited.

195 PYRROLOQUINOLINE QUINONE (PQQ) IS AN HIGHLY EFFECTIVE CHELATOR OF LEAD (Pb) IN OSTEOBLASTIC BONE CELLS (ROS 17/2.8).

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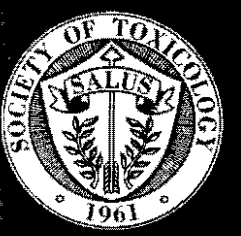
PQQ is an essential nutrient (vitamin) in mammalian organisms, present in plants, milk and beer, a redox cofactor, and a selective target for mitochondrial complex I inhibitors, such as Pb. In hepatocytes, PQQ selectively reverses the inhibitary effects of Pb on complex I, thereby normalizing mitochondrial energy metabolism. In view of these observations, PQQ's chelating properties were assessed in ROS 17/2.8: 1) By ^{19}F NMR, affinities of PQQ and DMSA for Pb, expressed as dissociation constants, were similar: 7 x 10^{-8}M and 1 x 10^{-9}M, respectively; affinities of PQQ and DMSA for Ca and trace metals were similarly low; 2) At 4,6 ad 24 hours of PQQ treadment (.083–.33 μM) with ^{210}Pb, cellular and medium ^{210}Pb (cpm/mg cell or medium protein, expressed as a ratio of treated divided by control values) were similar in control and PQQ-treated ROS 17/2.8 ($p > .05$); 3) After 24 hours of loading with ^{210}Pb, PQQ treatment (.083–.33 μM) for 24 hours produced a maximal decrease in cellular ^{210}Pb of 238 ± 12% ($p < .001$); 4) When ROS 17/2.8 were pre-labeled with ^{210}Pb for 24 hours and then treated with PQQ or DMSA



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Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the symposium, platform, poster / discussion, workshops, roundtables, and poster sessions of the 36th Annual Meeting of the Society of Toxicology, held at the Cincinnati Convention Center, Cincinnati, Ohio, March 9-13, 1997.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 371.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 395.

The abstracts are reproduced as accepted by the Program Committee of the Society of Toxicology and appear in numerical sequence.

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