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MeHg effects on caspase-3 levels: MeHg increased activated caspase-3 in hippocampus at 8 hours, suggesting the toxicant induced programmed cell death. These data suggest that organomercury compounds rapidly and directly alter brain development by modulating regional neurogenesis. (NIH ES11256, ES05022, EPA R82939101).

1784 A ROLE FOR P53 IN MOUSE MIDBRAIN NEURAL PRECURSOR CELL (NPC) CELL CYCLE ARREST AND PREMATURE NEURONAL DIFFERENTIATION FOLLOWING METHYLMERCURY EXPOSURE

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The cyclin kinase protein inhibitors p19, p21, and p27 and the tumor suppressor protein p53 have been demonstrated to play important roles in facilitating cell cycle arrest prior to differentiation in several neuronal cell types including oligodendrocytes and cortical precursor cells. These same proteins are often upregulated in the stress response, although the distinction or overlap of these two uses of the same class of proteins during brain development remains poorly explored. We have previously characterized a mouse midbrain neural precursor cell (NPC) culture where cells proliferate and differentiate into GABAergic, cholinergic, and dopaminergic neurons over 9 days. In these cells, increases in p27 and p53 proteins were temporally associated with cell cycle exit and differentiation. To explore the role of p53 in normal and toxicant perturbed neuronal proliferation and differentiation, we treated cycling midbrain NPCs from p53+/+ and p53-/- ED11 mouse embryos for 24h with sub-cytotoxic concentrations of methylmercury (0.1 µM, 0.5 µM) that caused significant cell cycle arrest but not significant decreases in cell viability. We hypothesized that MeHg induced cell cycle inhibition would lead to premature neuronal differentiation in a p53 dependent manner. We first demonstrated significant upregulation of nuclear p53 protein in MeHg treated p53+/+ cells at 20 minutes through 24h. Following 24h treatment with 0.5 µM MeHg, a statistically significant increase in [3H]GABA uptake (2.2-fold) and acetylcholinesterase activity (2-fold) in p53+/+ but not p53-/- NPCs was observed. Our results demonstrate p53 may play a dual role in normal midbrain development and toxicant defense, and support the hypothesis that toxicant-induced cell cycle inhibition can lead to premature neuronal differentiation. Supported by R01ES10613, P01ES09601, R826886010, P30ES07033, P30HD02274.

1785 THE EFFECT OF ETHYL MERCURY ON WILDTYPE AND METALLOTHIONEIN-2 KNOCKOUT *Caenorhabditis elegans*

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Ethyl mercury (EtHg) in the form of thimerosal is used in some vaccines as a preservative. Since prior research has indicated that EtHg is a neurotoxicant, its presence in vaccines has led to suspicions that it may be involved in the etiology of autism. In these studies, the nematode *Caenorhabditis elegans* (*C. elegans*) was used to determine EtHg's effect on the nervous system in the absence of metallothionein-2 (MT2), a protein involved in the sequestration of metals. The worms were treated either acutely (30 min.) or chronically (24 hrs.) with concentrations of EtHg ranging from 0 to 5 mM. Mercury (Hg) content and the lethality associated with the various concentrations were determined using inductively coupled plasma-mass spectrometry (ICP-MS) and live vs. dead counting techniques. Data obtained from analyses of Hg content indicate that at the end of chronic treatment, MT2-knockout worms have similar levels of Hg as compared to wildtype (WT) worms. This suggests that the lack of MT2 does not affect the accumulation of Hg in the MT2-knockout as compared to WT *C. elegans*. Acute treatment with EtHg demonstrated a protective effect of the MT2-knockout compared to WT. These studies suggest the expression of MT2 enhances the sensitivity of *C. elegans* to EtHg. Future experimentation will include analysis of the Hg content of *C. elegans* following an acute treatment course as well as the effect of over-expression of MT-2 on EtHg content and *C. elegans* viability.
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1786 NEUROTOXICITY STUDIES OF DEPLETED URANIUM

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DU is an extremely dense metal with high chemical and low radiological toxicity that has become prevalent in armor-piercing munitions, aviation counter-balances, and radiation shielding. Questions regarding potential toxic health effects have arisen due to this expanded use of DU. However, there is limited data on the specific effects of DU in the central nervous system (CNS). We have exposed CNS cell cultures and various strains of the nematode *C. elegans* to DU to evaluate its toxicity, and are utilizing a number of functional genomic and proteomic analysis tools to test the hypothesis that DU acts as a cytotoxic agent that induces the formation of ROS and induction of apoptosis. In primary mixed and neuronal cultures treated with DU concentrations ranging from 0 to 100 µM for up to 48 hours, DU results in a concentration- and time-dependent cytotoxic response, as measured by the lactate dehydrogenase cytotoxicity assay. Microscopic inspection and cell staining with Hoechst 33342 and propidium iodide confirmed this observed cytotoxicity. Mixed cultures demonstrated a more pronounced response compared to neuronal cultures. In *C. elegans*, the LC50 for the N2 strain (wild-type) was 104.1 µM, while a notable leftward shift of the concentration-response curve was seen to 40.6 µM in the metallothionein-2 knockout strain VC128, compared to wild-type worms. Together, our data indicates that CNS cells are sensitive to DU and that metallothionein-2 may be involved in the cellular response to DU exposure. Further understanding the biological effects of DU in the CNS will aid in the development of methodologies and pharmacotherapies to treat neurological conditions resulting from DU exposure.

1787 NEUROTOXIC EFFECTS OF LEAD ON HUMAN NEUROBLASTOMA CELLS IN CULTURE

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Lead (Pb) is a toxic heavy metal that causes adverse health effects in humans and animals. The developing central nervous system is especially more sensitive and vulnerable to Pb-toxicity. In the present study, the effects of low-levels of Pb-exposure on human SH-SY5Y neuroblastoma cell cultures were assessed. The cells were exposed to Pb (0.01 µM - 10 µM) for 48 hrs and cell proliferation was determined. Pb significantly inhibited the proliferation of neuroblastoma cells in a concentration-dependent manner. A 50% inhibition (IC_{50}) in cellular proliferation was observed with 5 µM Pb. A significant decrease in glutathione (GSH) levels, a critical intracellular antioxidant, was observed when the cells were exposed to different Pb concentrations (0.01 µM -10 µM) for 48 hrs. These two observations also coincided with a multifold increase in caspase-3 activity, a key executioner of apoptosis and prostaglandin E_2 (PGE_2) levels. Our results suggest that the neurotoxic effects of Pb may be mediated by apoptosis and PGE_2 release that could be potentially detrimental to neuronal survival (This research work supported by NIGMS/NIH grant # GM 60853).

1788 MODULATION OF CA2+-INDEPENDENT, PB2+-INDUCED EXOCYTOSIS FROM RAT PC12 CELLS BY CAM KINASE II

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Exocytosis is essential for intercellular communication and consists of several steps, including vesicle filling, priming, docking, fusion and the subsequent release of vesicle contents. Many environmental toxicants, with Pb2+ being one of the most hazardous pollutants, are known to disturb exocytosis. In this study vesicular catecholamine release from intact and ionomycin-permeabilized dexamethasone-differentiated PC12 cells has been measured at the single cell level using amperometry to reveal whether Pb2+ is able to trigger exocytosis, and which signaling pathways are involved. Additional confocal laser scanning microscopy (CLSM) was used to determine the intracellular [Pb2+] required for exocytosis.
The results demonstrate that superfusion with Pb2+-containing saline evokes exocytosis after a concentration-dependent delay. Thresholds to induce exocytosis are between 1 and 10 µM Pb2+ in intact cells and between 10 and 30 nM Pb2+ in permeabilized cells. Using membrane-impermeable and -permeable chelators and CLSM it is demonstrated that 1) intracellular Ca2+ is not required for Pb2+-induced exocytosis and 2) cytoplasmic components buffer Pb2+ with high affinity. Pharmacological manipulation of intracellular signaling pathways in permeabilized cells revealed that Ca2+-evoked exocytosis is modulated mainly by PKC and calcineurin. Pb2+-evoked exocytosis is strongly reduced by inhibition of CaM kinase II, but is insensitive to modulation of PKC and calcineurin activity. Inhibition of calmodulin only affects the delay to onset of Pb2+-evoked exocytosis. None of the treatments has significant effects on vesicle contents. It is concluded that 1) Pb2+ acts as a high-affinity substitute for Ca2+ to trigger essential steps leading to exocytosis, 2) CaM kinase II provides a novel and plausible target for the direct intracellular action of Pb2+ leading to exocytosis, 3) Pb2+-evoked exocytosis occurs when only ~20% of the intracellular high-affinity binding capacity (~2 attomol/cell) is saturated with Pb2+.