Abstract

Theme: Neurobehavioural Toxicity

- Fitsanakis VA., Piccola G., Aschner JL., Aschner M (Department of Pediatrics, B-3307 Medical Center North, Vanderbilt University School of Medicine, Nashville, TN 37232-2495, USA): Characteristics of manganese (Mn) transport in rat brain endothelial (RBE4) cells, an in vitro model of the blood-brain barrier. Neurotoxicology, 27(1), 2006, 60-70. [85 Ref]

Manganese (Mn), an essential elemental nutrient, is known to be neurotoxic at high occupational levels. We examined the transport of Mn across a monolayer of rat brain endothelial cell (RBE4) to evaluate whether an electromotive permeability mechanism is responsible for Mn transport across the blood-brain barrier (BBB). The 54 Mn2+ apparent permeability and flux showed significant temperature, energy- and pH dependence, as well as partial sodium-dependence. Additionally, iron (Fe)-rich and Fe-deficient media significantly increased the apparent permeability of 54Mn2+. Finally, Mn flux and permeability decreased when RBE4 cell were grown in astrocyte-conditioned media (ACM), compared to standard alpha-media. These data reinforce observations that transport of Mn across the BBB occurs in part through active transport process.

- Heidmets LT., Zharkovsky T., Jurgenson M., Movits KJ., Zharkovsky A (University of Tartu, Department of Pharmacology, 19 Ravila Street, 51014, Tartu, Estonia): Early post-natal low-level lead exposure increases the number of PSA-NCAM expressing cells in the dentate gyrus of adult rat hippocampus. Neurotoxicology, 27(1), 2006, 39-43. [32 Ref]

Although lead is widely known as a potent neurotoxin, the effect of lead exposure on the expression of the polysialic acid linked neural cell adhesion molecule (PSA-NCM) remains unclear. We exposed Wistar rat pups to 0.2 percent lead acetate from postnatal day (PND) 1 to PND 30. This exposure protocol resulted in pup blood lead levels, which increased to 29.3+-5.0 mg/dl on PND 15, and subsequently rose to 34.2+-5.8 mg/dl at weaning. Corresponding brain tissue lead levels were 456+-23 ng/g on PND 15 and 781 +-87 ng/g on PND 30. Animals were sacrificed on PND 80, when the blood and brain lead concentrations did not differ from those of the control group. Lead exposure induced a significant increase in the total number of PSA-NCAM expressing cells, compared to the control group (P<0.01), and did not change the proportion of cells co-expressing PSA-NCAM with glial or neuronal markers (calbindin, TuJI, GFAP). These results suggest that early post-natal lead exposure induces persistent changes in the number of PSA-NCAM expressing cells, which could be, at least, partly the basis of impairments in the learning and memory formation, which follows low-level lead exposure.


Exposure to non-nutritional food additives during the critical development window has been implicated in the induction and severity of behavioral disorders such as attention deficit hyperactivity disorder (ADHD). Although the use of single food additives at their regulated concentrations is believed to be relatively safe in terms of neuronal development, their combined effects remain unclear. We therefore examined the neurotoxic effects of four common food additives in combinations of two (Brilliant Blue and L-glutamic acid Quinoline Yellow and aspartame) to assess potential interactions Mouse NB2a neuroblastoma cells were induced to differentiate and grow neurites in the presence of additives. After 24 h, cells were fixed and stained and neurite length measured by light microscopy with computerized image analysis. Neurotoxicity was measured as an inhibition of neurite outgrowth. Two independent models were used to analyze combination effects: effect additivity and dose additivity. Significant synergy was observed between combinations of Brilliant Blue with L-glutamic acid, and Quinoline Yellow with aspartame, in both models. Involvement of N-methyl-D-aspartate (NMDA) receptors in food additive-induced neurite inhibition was assessed with a NMDA antagonist, CNS-1102. L-glutamic acid- and aspartame induced neurotoxicity was reduced in the presence of CNS-1102; however, the antagonist did not prevent food color-
induced neurotoxicity. Theoretical exposure to additives was calculated based on analysis of content in foodstuff, and estimated percentage absorption from the gut. Inhibition of neurite outgrowth was found at concentrations of additives theoretically achievable in plasma by ingestion of a typical snack and drink. In addition, Trypan Blue dye exclusion was used to evaluate the cellular toxicity of food additives on cell viability of NB2a cells; both combinations had a straightforward additive effect on cytotoxicity. These data have implications for the cellular effects of common chemical entities ingested individually and in combination.

- **Lurie DI., Brooks DM., Gray LC** (Department of Biomedical and Pharmaceutical Sciences, School of Pharmacy and Allied Health Sciences, The University of Montana, Skaggs Building Room 304, 32 Campus Drive, Missoula, MT 59812-1552, USA): *The effect of lead on the avian auditory brainstem*. Neurotoxicology, 27(1), 2006, 108-117. [39 Ref]

  Lead (Pb) continues to be a significant environmental toxin and remains an integral part of many industrial processes, hobbies, and tobacco smoke. Pb has been shown to be a potent toxin to the CNS and low levels of Pb (below the CDC established toxic blood level of 10 ug/dl) have been correlated with decreases in the IQ of children. The current study was undertaken to identify the cellular changes induced by Pb exposure in the auditory brainstem of chickens that are likely anatomical correlates of the observed deficits in backward masking. We found Pb exposure had no effect on neuron number or glial cells within the auditory brainstem. However, Pb exposure does result in significant decreases in the amount of the medium weight neurofilament protein (NFM) as well as decreased NFM phosphorylation within the axons connecting auditory nuclei in the avian brainstem. Because the amount of neurofilament can affect the conduction velocities of axons, these results may provide an anatomical link between Pb exposure, auditory temporal processing deficits, and dyslexia.

- **Mundy WR., Freudenrich TM** (Neurotoxicology Division, National Health and Environmental Effects Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, B105-06, Research Triangle Park, NC 27711, USA): *Apoptosis of cerebellar granule cells induced by organotin compounds found in drinking water: involvement of MAP kinases*. Neurotoxicology, 27(1), 2006, 71-81. [50 Ref]

  Mono- and dialkyl organotin compounds are used primarily as heat stabilizers in polyvinyl chloride (PVC) plastics. Recently, monomethyltin (MMT), dimethyltin (DMT), monobutyltin (MBT), and dibutyltin (DBT) have been detected in water from homes and business served by PVC pipes. While trialkyl organotins such as trimethyltin (TMT) and triethyltin (TET) are well known neurotoxicants, the toxicity of the mono-and dialkyl organotins is not well described. The present study compared the cytotoxicity of organotins found in drinking water with the known neurotoxicant TMT in primary cultures of cerebellar granule cells, and examined the role of MAP kinase signaling in organotin-induced cell death. Twenty-four hour exposure to TMT resulted in a concentration-dependent decrease in cell viability with an EC50 of 3 uM. Exposure to MMT, DMT, and MBT at concentrations up to 10 uM had no effect. DBT, however, was very potent, and decreased cell viability with an EC50 of 0.3 uM. Staining of organotin-treated cerebellar granule cells with the nuclear dye Syto-13 revealed that TMT and DBT, but no MMT, DMT, or MBT, produced condensation and fragmentation of chromatin characteristic of apoptosis. TMT and DBT-induced apoptosis was confirmed using TUNEL staining and measurement of PARP cleavage. Activation of MAP kinase pathways was examined after 6 h of exposure to the organotins which induced apoptosis. Both TMT and DBT activated ERK/2, but only TMT activated the JNK/c-Jun and p38 pathways. Pharmacologic blockade of JNK/c-Jun and p38 activation significantly decreased apoptosis produced by TMT, but not by DBT. These results show that DBT is a potent neurotoxicant in vitro, but unlike TMT, does not induce cell death via activation of MAP kinase signaling.

- **Virgolini MB., Bauter MR., Weston DD., Cory-Slechta DA** (Environmental and Occupational Health Sciences Institute, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, 170 Frelinghuysen Road, Piscataway, NJ 08854, USA): *Permanent alterations in stress responsivity in female offspring subjected*
to combined maternal lead exposure and/or stress. Neurotoxicology, 27(1), 2006, 11-21. [68 Ref]

levated lead (Pb) exposures preferentially impact low socioeconomic status (SES) populations, the same groups thought to sustain the highest levels of environmental stress. As co-occurring risk factors, therefore, Pb and stress could interact, a possibility further supported by the fact that both act on mesocorticolimbic dopamine systems of the brain. We recently demonstrated in rats that maternal Pb exposure could permanently increase basal corticosterone levels of offspring consistent with altered hypothalamic pituitary adrenal (HPA) axis function. The current study was thus designed to test the hypothesis that stress responsivity of offspring should likewise be altered, with the outcome differing in response to Pb, stress or Pb+stress. The impact of intermittent variable stress challenges (restraint, novelty, cold) on behaviour sensitive to Pb exposure (fixed interval (FI) schedule-controlled responding) and on stress-induced corticosterone changes were evaluated in adult female offspring of dams that had been exposed to Pb (150 ppm) in drinking water from 2 months prior to breeding through lactation with or without maternal restraint stress on days 16 and 17 of gestation. This design yielded four treatment groups: (NS/o, no maternal Pb, no maternal stress; S/O, no maternal Pb, maternal stress; NS/150, maternal Pb, no maternal stress; and S/150, maternal Pb exposure and maternal stress. While maternal Pb alone and stress alone each altered components of stress responsivity, the greatest number of effects was seen in response to Pb+stress. This included alteration in FI performance following both restraint and cold stress and in the corticosterone response to cold stress. Collectively, these studies reveal that maternal Pb exposure alone can permanently alter stress responsivity and that the profile of effects produced by maternal Pb differ from those produced by maternal Pb in conjunction with stress, findings which have both mechanistic and risk assessment significance.


Chronic exposure to n-hexane may result in peripheral neurophathy. 2,5-Hexanedion (2,5-HD) has been identified as toxic metabolite of n-hexane. The CYP2E1, CYP1A1 and GST genes are involved in the formation of 2,5-hexanedione from n-hexane as well as the elimination of 2,5-HD-formed electrophile, and these genes are highly polymorphic in the general population. A nested case-control study in an industrial cohort was conducted to evaluate the associations between polymorphisms in these metabolic genes and n-hexane-induced peripheral nerve damage. The study subjects included 22 cases, who worked in a printing factory with symptoms of peripheral nerve damage, and 163 controls, who came from the same factory of cases. DNA was extracted from blood samples and genotyping was conducted for CYP2E1 Pst, CYP2E1 Dra, CYP2E1 Ins96, CYP1A1 Msp, GSTT1 null, GSTM1 null and GSTP1 105V. Unconditional logistic regression was applied to estimate the odds ratio and 95 percent confidence intervals. There were no significant differences between the two groups regarding age, sex, smoking and alcohol status. A significant association between Dra polymorphism and peripheral nerve damage was found. The frequency of CYP2E1 Dra homozygous mutation in the case group (18.2 percent) was higher than that in the control group (3.7 percent, p=0.015). Individuals with homozygote genotype (CC) of CyP2E1 Dra had a significantly higher risk of peripheral nerve damage compared with those with DD genotype (adjusted OR=5.58, 95 percent CI = 1.32-23.65)after n-hexane exposure duration, sex, age, smoking and alcohol status were adjusted. No significant association was found that CYP2E1 Pst, CYP2E1 Ins96, CYP1A1 Msp, GSTT1, GSTM1, GSTP gene polymorphisms associated with the susceptibility of peripheral nerve damage. These findings suggested that CYP2E1 gene might increase the susceptibility to n-hexane-induced peripheral damage.