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In the brain, glial cells serve in the role to sequester metal from the neural microenvironment and therefore play an important role as a cellular deposition site. The central nervous system is highly vulnerable to oxidative stress, and free iron can stimulate oxidative stress by the Fenton reaction. Aluminum may upregulates the transferrin-independent iron uptake system and stimulate oxidative stress. Nramp2, also known as DMT1, is a 12-transmembrane(TM) domain protein responsible for dietary iron uptake as well as metal ions such as iron, lead, manganese, zinc, copper, and cobalt. In regulation of metals levels in the brain, the interaction between metals is as yet unknown. The molecular mechanism of upreglation of iron uptake by aluminum is also unknown. We investigated whether aluminum influence on uptake of lead and iron into astrocytes(SV-FHA cells) and V373 cells. We did also whether DMT1 was influenced by aluminum. SV-FHA cells were cultured in high-glucose DMEM and V373 cells cultured low-glucose DMEM. Cells were treated with Aluminium chloride. Lead uptake were done in incubation condition of pH 5.5 and 7.4, as the previous used method. Iron uptake was done in 20mM HEPES buffer containing serum-free, 6μM NTA, 2mM CaCl2, 2mM MgCl2, 15μM Ascorbic acid, and 1.5μM FeCl3. Lead uptake into astrocytes increased concentration-dependently by aluminum treatment, but uptake into V373 cells decreased. Iron uptake increased time- and concentration-dependently by aluminum treatment, and the effects of aluminum were blocked by citrate. The effects of aluminium on lead and iron uptake were not related with DMT1.

[PA4-24] [10/18/2002 (Fri) 09:30 - 12:30 / Hall C]

The effects of estradiol and its metabolites on the regulation of CYP1A1 expression.

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The effects of estradiol and its metabolites on the regulation of CYP1A1 expression.
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2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is the most potent halogenated aromatic hydrocarbon congener that induces expression of several genes including CYP1A1. Exposure to TCDD results in many toxic actions such as carcinogenesis, hepatotoxicity, immune suppression, and reproductive and developmental toxicity. Dramatic differences in dioxin toxicity have been observed between the sexes of some animal species, suggesting hormonal modulation of dioxin action. Many studies have been reported and propose several mechanisms of anti-estrogenic effects of TCDD. In contrast, the effect of estrogen on the regulation of CYP1A1 are not clear at present. There are several reports showing conflicting results. It seems that induction/inhibition of CYP1A1 may be dependent on cell-type and concentration.
The purpose of this study was to investigate the regulation of TCDD-induced CYP1A1 gene expression by estradiol and its metabolites. We examined whether estradiol and its metabolites altered TCDD-mediated induction of CYP1A1 enzyme activity. 17β estradiol and 16α estriol at non cytotoxic concentrations caused a significant concentration dependent decline of TCDD-induced EROD activity. To determine whether reduced EROD activity reflected altered CYP1A1 mRNA expression, we measured CYP1A1 mRNA level by RT-PCR. And to examine whether estradiol and its metabolites have effects on TCDD-induced CYP1A1 gene expression at the transcription level, we also performed transient transfection with an AhR responsive reporter plasmid containing the 5' flanking region of the human CYP1A1 gene to examine whether estradiol and its metabolites have effects on TCDD-induced CYP1A1 gene expression at the transcription level.

[PA4-25] [10/18/2002 (Fri) 09:30 - 12:30 / Hall C]

Role of phospholipid metabolism in Methylmercury-induced Cytotoxicity

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Methylmercury (MeHg; CH3HgCl) is a ubiquitous environmental toxicant that readily bioaccumulates in aquatic foodchains. This toxicant is most highly exposed to humans through the ingestion of contaminated food, and

thus is an ongoing health concern. Thus far, MeHg has been suggested to exert its toxicity through its high reactivity to thiols of bioactive proteins, elevation in intracellular Ca2+ concentration, and generation of reactive oxygen species, but its mechanism remains poorly understood. The present study was designed to investigate a relationship between various cytotoxic mediators such as PLA2, PC-PLC, SMase and Ca2+ and the cytotoxicity of MeHg in MDCK cells.
Here we show that MeHg induced AA release by activating cPLA2 through multiple mechanisms including calcium, phosphorylation and oxidative stress. AACOCF3, a specific inhibitor of cPLA2 , blocked MeHg-induced AA release and intracellular ROS generation, but not LDH release. N-acetyl cysteine, an antioxidant, could not protect against the cytotoxicity of MeHg despite a significant inhibition of the AA release.
MeHg induced a slight increase in DAG production, and ceramide generation with concomitant hydrolysis of SM. The activity of A-SMase, not N-SMase is markedly activated by MeHg. Monensin and NH4Cl, indirect inhibitors of A-SMase inhibited ceramide generation but not LDH release. Inhibition of PC-PLC, a well-known upstream activatior of A-SMase, inhibited the MeHg-induced DAG generation, A-SMase activation, ceramide generation, and LDH release.
MeHg increased intracellular calcium in a bimodal pattern with a sharp peak at 1 min and sustained increase up to 10 min. Chelation of extracellular calcium partially attenuated a short-term cytotoxicity of MeHg with the abolishment of the sharp peak at 1 min and significant reduction in the sustained Ca2+ increase. Interestingly, D609, PC-PLC inhibitor, completely decrease not only MeHg-induced calcium increase but also LDH release. This suggests that MeHg-induced response is composed of PC-PLC mediated Ca2+ mobilization component and a Ca2+ influx component, with the influx component being dependent on mobilization component and therefore relating with cell death.
Taken together, the present study indicates that MeHg activates cPLA2 through Ca2+-dependent and oxidative pathways. However, the resulting AA and ROS may not be implicated in its cytotoxicity, rather PC-PLC pathway is likely to play an important role in the cytotoxicity by MeHg through [Ca2+]i increase by the Ca2+ mobilization and influx.

[PA4-26] [10/18/2002 (Fri) 09:30 - 12:30 / Hall C]

Metallothionein gene expression in different tissues of Crucian carp (Carassius auratus) exposed to cadmium chloride

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Metallothioneins (MTs) are a goup of heavy metal-binding proteins characterized by cystein-rich low molecular weight(6000 - 10,000 Da). They plays a major role in the detoxification of heavy metals and also in scavenging of superoxide radicals. They are known to be induced by heavy metals in various organs of different species and represent a potential biomarker of aquatic heavy metal contamination. In this study, effect of cadmium accumulation on the metallothionein gene expression in the different tissues of crucian carp was investigated using reverse transcription (RT)-PCR method. Crucian carp were exposed to cadmium chloride with the concentrations of 0.01, 0.1, 0.5㎎/L, respectively. Gills, livers, and kidnies were quickly removed for RNA extraction and PCR was done using primers based on the known gold fish cDNA sequence. As results, mRNAs of MT were induced in all the tested organs with dose-dependant manner and gills were the most sensitive organ of fish, Carassius auratus, in the metallothionein induction by cadmium exposure.

[PA4-27] [10/18/2002 (Fri) 09:30 - 12:30 / Hall C]

Sexual Dimorphic Effects of Terbufos on Acetylcholinesterase and Lethality

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An organophosphate pesticide terbufos (S-t-butylthiomethyl-O,O-diethyl phosphorodithioate; TBF) has been extensively used as an insecticide. A sexual dimorphism in TBF toxicity was not reported and remains unclear. Objective of the work is to investigate the influence of TBF on sexual dimorphism in rats by using acetylcholinesterase (AchE). *Method*: TBF treatments were conducted as followings: In *experiment I*, 72-days-old