Down-regulation of Inducible Nitric Oxide Synthase Gene Expression by 4-Nonylphenol in Macrophages

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4-Nonylphenol (NP) is a degradation product of a widely used non-ionic surfactant group, alkylphenol polyethoxylates that are mainly found as an intermediate in the chemical manufacturing industry. In this study, we investigated the effect of NP on the regulation of inducible nitric oxide synthase (iNOS) in murine macrophages. NP alone did not affect the expression of iNOS, in contrast, suppressed the LPS-induced gene expression of iNOS, in a dose-dependent manner as determined by RT-PCR analysis. NO production was assessed by measurement of nitrites in the medium. The level of NO was found to correlate well with a decrease in transcripts of iNOS. Since the promoter in iNOS gene contains binding motifs for NF-kB, the effect of NP on the inactivation of this transcripts factor was determined by transient transfection assay. Employing a transfection and reporter gene expression system with p(NF-kB)3-Luciferase, the treatment of NP produced a dose-dependent inhibition of luciferase activity in RAW 264.7 murine macrophages cell line. These results suggest that suppression of iNOS gene expression by NP might be mediated by the inhibition of NF-kB activation.

[PA4-18] [10/18/2001 (Thr) 14:00 - 17:00 / Hall D]

Differential Effects of Glutamine Synthase in Cell-free Brain Homogenate, Cultured Mixed Gilal Cells and Brain Regions of Rats Exposed to Methylmercury

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Glutamine synthase (GS), known as a glial-specific enzyme catalyzes the synthesis of glutamine from glutamate and ammonia and has been reported to be associated with ischemic injury and several neurological diseases. Central nervous system is a known target for methylmercury (MeHg). In this study, we investigated whether MeHg exposure in the cell-free brain homogenate, cultured mixed glial cells and rat models has adverse effects on GS. Cell-free brain homogenates were prepared from dissected brain regions of untreated rats. Primary cultures of mixed glial cells were obtained from postnatal day (PND) 1 cerebral cortex of rats, and MeHg (0-10 μM) was exposed to subcultured glial cells for 6 days from 5 days in vitro. To Sprague-Dawley rats (PND 36), multiple dosage of MeHg (0, 1, 4, and 10 mg/kg) was intraperitoneally administered for 3 days. Body weight was measured for administration period. In each experimental model, GS activity was measured spectrophotometrically based on the γ-glutamyl transfer reaction. MeHg exposure (0.1 to 100 μM) to cell-free brain homogenate produced dose-dependent decreases of GS activity in cerebellum, hippocampus and frontal cortex. In cultured mixed glial cells, MeHg exposure (0-10 μM, for 6 days) resulted in dose-dependent increases of GS activity. Cell viability, total cell number, and protein content were significantly decreased in primary culture of mixed glial cells. Western blot with GS antibody showed a qualitative increase of GS protein. In the glial cells exposed to 5 μM MeHg for 6 days, GS activity was significantly increased (2-fold), but MeHg exposure from 6 to 48 hr was not affected on GS activity. GS activity was significantly increased in frontal cortex and caudate nucleus of 4 or 10 mg/kg MeHg-treated rats, but not the entorhinal cortex, hippocampus and cerebellum. GS activity, however, was significantly decreased in liver tissue at 4 and 10 mg/kg MeHg doses. These results showed that MeHg exhibited differential effects on GS at the relatively low concentrations of MeHg, indicating that MeHg may potentiate GS activity in living organisms against MeHg-induced stresses.

[PA4-19] [10/18/2001 (Thr) 14:00 - 17:00 / Hall D]

In utero sumithrin exposure affects postnatal reproductive development in rat offspring

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Sumithrin is one of the synthetic pyrethroid insecticides, developed classes of insecticides, due to its high activity against insects, and relatively low mammalian toxicity compared to other insecticide classes. Sumithrin is commonly used insecticide for in-door pest control, providing for human exposure. Our uterotrophic assay using immature SD female rats demonstrated that sumithrin acts like an estrogen agonist. Estrogen or antiestrogen clearly influence reproductive development. Therefore, We determined the effects of in utero exposure to sumithrin on postnatal body weight, reproductive development (anogenital distance(AGD), vaginal opening, organ weight) in rat offspring. Pregnant SD rats were intraperitoneally injected with sumithrin (300 ㎎/㎏/day) from gestation day(GD) 6 to 18. Male and female offsprings were examined at postnatal days(PND) 3, 15 and 1, 22, respectively. Rat exposed to sumithrin had a statistically significant increase in body weight on PND21(male) and 22 (female) and brought significant decreas in male AGD on PND 15 and 21. Also, vaginal opening was accelerated significantly ($P < 0.05$). These results indicate that persistent exposure to this compound may contribute to reproductive developmental dysfunction.

[PA4-20] [10/18/2001 (Thr) 14:00 - 17:00 / Hall D]

Identification and Cloning of Multiple Forms of Neutral Sphingomyelinase in Bovine Brain

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Neutral form of sphingomyelinase (N-SMase) is a family of enzymes which hydrolyze sphingomyelin to produce a lipid-derived tumor suppressive second messenger ceramide. N-SMase exists as multiple forms in brain and seems to transmit different signals and to give rise to different pools of ceramide, eliciting cellular responses ranging from apoptosis and cell cycle arrest to cell survival and cell proliferation. In previous study, we have identified at least seven forms of N-SMase activities termed N-SMase α, β, ɣ, δ, ε, ζ, λ in bovine brain based on extraction patterns, column profiles and biochemical properties (*J. Neurochem. 75, 1004-1014, 2000, Jung et al.*). Here we first report the purification of 68 kDa N-SMase λ, a cytosolic form of Mg^{2+}-independent N-SMase. Second, we report cDNA cloning of the 30 kDa forms of SMase α, β, ɣ, δ using a specific antibody against the 30 kDa protein from rat brain λZAP II cDNA library expressing proteins. The resulting three positive clones were identified as an identical gene encoding one of isoforms of a signaling protein playing a crucial role in cell survival and death. Third, we also identified the 60 kDa N-SMase ε as a known stress protein whose role has not been fully defined by MALDI-TOF analysis. We are underway to confirm these proteins as the respective N-SMase enzymes through overexpression of these proteins in eukaryotic cells and immunoprecipitation experiments.

[PA4-21] [10/18/2001 (Thr) 14:00 - 17:00 / Hall D]

Role of Ceramide in Hypoxia-induced Neuronal Cell Death

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Ceramide is an important lipid messenger involved in mediating a variety of cell functions including proliferation, differentiation, growth arrest and apoptosis. This study was undertaken to determine