2419 A THRESHOLD NEUROTOXIC EXPOSURE TO AMPHETAMINE DISRUPTS CORTICAL SYNAPTIC NEUROPLASTICITY

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A 9 day exposure to D-amphetamine (AMPH), at a "threshold" necessary to produce histological signs of neurotoxicity, was evaluated for its effects on genes that are normally upregulated by amphetamine or methamphetamine "challenges". cDNA arrays and RT-PCR were used to evaluate the changes in gene expression. Male rats were exposed for 9 days to either 3 X 1ml/kg saline, 3 X 2 mg/kg AMPH (Low Dose AMPH) or of 3 X 7.5 mg/kg AMPH (High Dose), with 6 hr between doses, per day. The environmental temperature was held at 17°C to prevent AMPH-induced hyperthermia. The groups were challenged with either saline or 7.5 mg/kg AMPH on the 10th day, and gene expression profiles at 3 hr post challenge were determined in striatum and parietal cortex. The AMPH challenge resulted in a significantly and substantially lesser increase in the expression of activity-related cytoskeletal protein (ARC) and nerve growth-factor inducible proteins (NGFI-A & B) in the parietal cortex of the High Dose AMPH group compared to the Saline or Low Dose AMPH group challenged with AMPH. In contrast, in the striatum there was either no difference or a slight increase in the upregulation of these genes after the AMPH challenge in the High Dose AMPH animals. These results show that within the parietal cortex the disruption of genes involved in the synaptic formation processes is sensitive to threshold neurotoxic AMPH exposures, and that the learning processes in this region may be compromised.

2420 ACRYLAMIDE ALTERS SELECTIVE ISOZYMES OF PROTEIN KINASE C IN NEURONAL CELLS IN CULTURE

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While polymer acrylamide is non-toxic, monomer is a well-studied neurotoxicant in animals as well as in humans. Exposure to acrylamide produces a degenerative neuropathy closely associated with motor dysfunction. However, studies on its mechanism of action remain limited. Protein kinase C (PKC) plays a pivotal role in etiology of neurological disease and the modulation of motor behavior and cognitive function is mediated via PKC signaling pathways. Since PKC isozymes are differentially distributed in the brain cells and their roles are isozyme-specific, analysis of individual isozymes is required to better understand the mechanism of neurotoxicity.Thus, we attempted to analyze acrylamide-induced alteration of PKC isozymes in the cerebellar granule cells in culture and to identify possible molecular targets sensitive to the acrylamide exposure. Cells were exposed to 50uM and 100uM acrylamide for 12hrs, respectively, and subsequently fractionated and immunoblotted against the selected PKC monoclonal antibodies (α, β, δ, ε, λ, ι). Acrylamide exposure induced translocation of PKC- ε [cytosol (% control): 95±12 at 50 μM and 76±10 at 100 μM; membrane (% control): 151±37 at 50 μM and 218±38 at 100 μM] and PKC–β [cytosol (% control): 95±12 at 50 μM and 97±15 at 100 μM; membrane (% control): 133±23 at 50 μM and 182±25 at 100 μM] from cytosol to membrane fractions in a concentration-dependent manner. PKC- α, -δ,-λ and ι were present in these cells, but were not altered by acrylamide exposure. RT-PCR analysis showed the increased levels of neurofilament-M and GAP-43 mRNA with exposure to acrylamide. GAP-43 is the neuron-specific phosphoprotein associated with axonal development and regeneration. Since the expression of GAP-43 in neuronal cells is implicated with PKC-ε, abnormal expression of GAP-43 by the altered PKC-ε may disturb structural formation of neuronal cells. These results suggest that the effects of acrylamide on PKC are isozyme subspecies-dependent and PKC-β as well as PKC-ε may be target molecules for acrylamide in neuronal cells.

2421 ACRYLAMIDE ADDUCT FORMATION IN NERVE TERMINALS

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We have proposed that acrylamide (ACR) forms adducts with functionally important cysteine residues on presynaptic proteins that regulate neurotransmitter storage and release. A recent proteomic study provided evidence that N-ethylmaleimide sensitive factor (NSF), a rate-limiting ATPase involved in presynaptic transmitter release, was a target of in vivo or in vitro ACR exposure. ACR did not appear to form adducts with Rab3A, syntaxin or synaptobrevin (Tox. Appl. Pharmacol. 201: 120-136, 2004). To confirm and extend these initial findings, we used liquid chromatography-tandem mass spectrometry (LC/MS/MS) to identify ACR protein adducts in tryptic digests of synaptosomes isolated from moderately intoxicated rats (21 mg/kg/d x 34 days). Several high abundance proteins exhibited specific cysteine adducts; e.g., Cys 254 of GAPDH, Cys65 of the voltage-dependent anion channel and Cys870 of the clathrin heavy subunit. To increase detection of adducts on low abundance proteins during in vivo intoxication, Isotope Coded Affinity Tag (ICAT) labeling was used. Mass spectrometry of labeled samples identified more than 1000 thiol-containing proteins including NSF, synaptophysin, syntaxin binding protein and v-ATPase in synaptosomes from ACR-intoxicated rats. Preliminary ICAT analyses revealed quantitative changes in the relative abundance of many synaptosomal proteins indicating the formation of ACR adducts. We are currently using ICAT and LC/MS/MS analyses to identify protein adducts at very early in vivo intoxication times (e.g., 50 mg/kg/d x 3 d, 21 mg/kg/d x 14 d) and to follow the accumulation of these adducts during continued ACR exposure. At low in vivo ACR concentrations, initial toxicologically relevant adducts should form readily on those protein thiol groups exhibiting relatively high nucleophilic reactivity. Impaired neurotransmitter release should be causally related to the presynaptic accumulation of these adducts. Supported by NIEHS grant ESO3830-19.

2422 EFFECT OF A NONSELECTIVE PROTEASOME INHIBITOR MLN273 ON DIFFERENTIATED PC12 NEURONAL CELLS

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Neuropathy is a common adverse effect of many chemotherapeutic drugs including proteasome inhibitors (PI). To better understand PI neuropathy, differentiated PC12 cells were treated with different concentrations of the reversible proteasome inhibitor MLN273 which resulted in a gradual 0 to 98% inhibition of the 20S proteasome within 24 hours. Differentiated PC12 cells were investigated for viability, neurite loss, ubiquitin positive protein aggregate formation and for global gene expression signatures by Affymetrix at 75% 20S proteasome inhibition level, respectively. Viability and neurite loss were PI concentration and time dependent. No effect was observed at exposures below IC50 for 20S proteasome activity within 24 hours. The effects on viability are associated with the number of cells that stained positive for ubiquitinated aggregates by immunohistochemistry. Protein aggregates were present predominantly in the cytoplasm seemingly centered about the centrosome but there were ubiquitinated aggregates in the neurites also. Concentration dependent altered cell morphology and neurite loss was also observed in PC12 cells. Gene expression analysis revealed the PI dependent downregulation of genes responsible for neurite outgrowth, for cell cycle regulation and for apoptosis. At the same time, upregulation of multiple stress genes was also observed. In conclusion, PI results in a complex response in PC12 cells at multiple levels. Protein aggregates apparently associated with the centrosome potentially perturb the cytoskeleton resulting in compromised axonal transport in the neurites. Gene expression signatures indicate that the integrity of the neurites is compromised not only by protein aggregates but potential loss of structural and functional proteins at toxic PI levels.

2423 PROTEASOME INHIBITON INDUCED PERIPHERAL NEUROPATHY IN BALB/C MICE

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In order to study proteasome associated peripheral neuropathy a mouse model was established to identify early markers in the development of nerve effects. The prototypical proteasome inhibitor MLN273 was administered intravenously once daily on Days 1, 4, 8 and 11 at dosages of 0.3, 0.6, 1.0, and 2.0 mg/kg (0.9, 1.8, 3 and 6 mg/m2). Mice were sacrificed on Days 2, 8 and 15 for microscopic hematoxylin and eosin stained sections. The dose of 2 mg/kg (6mg/m2) exceeded the MTD and all animals were preterminally euthanized. Abnormal clinical observations in these were nonspecific. Animals that survived to scheduled sacrifice had no abnormalities during the study. Lesions were detected in the dorsal root ganglia (DRG) including accumulation of hypereosinophilic +/- pale eosynophilic granular to globular material in neuronal cell bodies sometimes associated with nuclear margination, neuronal death and dropout and satellite cell hyperplasia. Myelin and axon degeneration and fragmentation were seen in nerve roots/spinal nerves associated with the DRG, spinal cord dorsal funiculi, and sciatic nerves. Lesions were most severe in the 2 mg/kg dose group. No injury occured at 0.3 and 0.6 mg/kg dosages indicating a sharp dose response curve. Dosage-related proteasome inhibition was observed in the blood varied with different doses from 57% at 0.3 mg/kg up to 91%

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The Toxicologist

Supplement to *Toxicological Sciences*

An Offical Journal of the Society of Toxicology

45th Annual Meeting and ToxExpo™

San Diego, California

OXFORD UNIVERSITY PRESS

ISSN 1096-6080
Volume 90, Number 1, March 2006

www.toxsci.oupjournals.org

Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the symposium, platform, poster discussion, workshop, and poster sessions of the 45th Annual Meeting of the Society of Toxicology, held at the San Diego Convention Center, San Diego, March 5–9, 2006.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 500.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 534.

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

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