

The Toxicologist

Supplement to *Toxicological Sciences*



Society of
Toxicology

46th Annual Meeting and ToxExpo™
Charlotte, North Carolina

*An Official Journal of the
Society of Toxicology*

www.toxsci.oxfordjournals.org

OXFORD
UNIVERSITY PRESS

ISSN 1096-6080

Volume 96, Number 1, March 2007

and MPT inhibitors (bongkreic acid (BA); cyclosporin A (CsA)) for 24h. Exposure to 0.25% dimethyl sulfoxide (DMSO), 10 μ M BA, and 1 μ M CsA alone showed no apparent activation of caspase 9 or 3. In contrast, cotreatment of 1,3-DNB with either MPT inhibitor reduced caspase 9 activation but significantly increased caspase 3 activation. Although caspase 9 appears to play a role in 1,3-DNB induced glial toxicity, this data suggests that it is not the primary initiating pathway. Further studies are necessary to determine if enhanced caspase 3 activation is due to effects independent of MPT inhibition such as metabolic impairment or whether induction of MPT may mediate protection in 1,3-dinitrobenzene-induced neurotoxicity. This work was supported by NIH T32 ES07062 and RO1 ES08846.

1055 ADDITIONAL INFORMATION FAVORING A NOVEL RECEPTOR ASSOCIATED WITH VOLTAGE-GATED SODIUM CHANNELS.

E. P. Gold^{1,2}, H. K. Jacocks² and D. G. Baden^{2,1}. ¹*Biology and Marine Biology, University of North Carolina Wilmington, Wilmington, NC* and ²*Center for Marine Science, UNCW, Wilmington, NC.*

Voltage-gated sodium channels (VGSC) are responsible for action potential initiation and propagation in excitable cells including neurons of the central nervous system. Typical VGSC are comprised of an α -subunit and 2 β -subunits. All known pharmacological agents that act on VGSC have receptor sites on the α -subunits. At least six distinct sites for neurotoxins and one for local anesthetics and related drugs have been identified (Cestele and Catterall 2000).

We presented preliminary results suggesting the presence of a novel receptor associated with VGSC characterized by the binding of an antitoxin, brevenal, produced by the dinoflagellate *Karenia brevis*, also known to make the site 5-specific brevetoxins (PbTx)(Gold et al., 2005). Both unlabeled brevenal and the aldehyde-reduced brevenol displaced tritiated brevenol (³H-B) in rat brain synaptosomes but PbTx does not. Here, we further examine the basic characteristics of receptor identification using tritiated receptor binding experiments to support our findings. To show saturation of specific brevenal receptors, ³H-B was bound to rat brain synaptosomes in the presence and absence of brevenal/brevenol. Saturation and distribution of receptors were studied in synaptosomal preparations made of whole brain, cerebrum and cerebellum. Results indicate binding maxima (B_{max}) or the maximum number of binding sites and equilibrium dissociation constants (K_D), the concentration at which half the receptors are occupied, for the whole brain to be lower than those for the cerebrum and cerebellum, respectively. Finally, displacement studies involving ³H-B against several competitors including tetrodotoxin (site 1 neurotoxin), veratridine (site 2 neurotoxin), *Centruroides sculpinatus* venom (site 4 neurotoxin source), aconitine (site 2) and amiloride (inhibitor of sodium transport) demonstrate no inhibition. These findings indicate that ³H-brevenol does not bind to any of the tested receptor sites on VGSC and suggest a novel uncharacterized site on VGSC.

1056 *IN VIVO* ACRYLAMIDE EXPOSURE PRODUCES CUMULATIVE ADDUCTION OF SYNAPTIC PROTEIN THIOLS.

D. S. Barber¹, R. M. LoPachin² and S. Stevens³. ¹*Center for Environmental and Human Toxicology, University of Florida, Gainesville, FL.* ²*Anesthesiology, Montefiore Medical Center, Bronx, NY* and ³*ICBR, University of Florida, Gainesville, FL.*

We have previously demonstrated that *in vivo* acrylamide (ACR) intoxication results in the formation of adducts on cysteine residues of synaptic proteins. We have also demonstrated that synaptic protein thiol adduct burden correlates with motor dysfunction and decreased neurotransmitter uptake and release. Using Isotope Coded Affinity Tag (ICAT) labeling, we examined the synaptic proteome of rats treated orally with ACR (21mg/kg/day) for 7, 14 or 21 days. During this time rats progress to slight motor impairment. After 21 days of intoxication, the abundance of most cysteine containing peptides is similar, but more than 35 proteins exhibit at least a 25% reduction in available cysteines in treated rats. Many of these proteins are critical to normal synaptic function including N-ethylmaleimide sensitive factor (NSF), synaptophysin, synaptotagmin, syntaxin-7, complexin-2 and vATPase. Because reduced ICAT incorporation can occur as a result of decreased protein abundance as well as adduct formation, we used multidimensional LC/MS/MS to demonstrate ACR adducts on affected cysteines, strongly suggesting that reduced ICAT incorporation is due to ACR adduction. Differential effects of ACR treatment were observed on individual cysteines within a protein, indicating specificity of adduct formation. Because ACR is a relatively weak electrophile, adducts form primarily on protein thiol groups with high nucleophilic reactivity due to their local environment. In glutamine synthase, Cys346 labeling is reduced by more than 55% in ACR treated animals while Cys99 is only slightly reduced. Because ACR toxicity is a cumulative process, we examined changes in ICAT incorporation over time. Of the set of proteins common to all samples, some proteins exhibited cumulative changes, including syntaxin-7, VDAC and NSF. These proteins are likely critical targets of ACR toxicity in the nerve terminal. Supported by NIEHS grant ES03830-20.

1057 LUMO ENERGIES PREDICT THE *IN VITRO* NEUROTOXIC POTENCIES OF TYPE-2 CONJUGATED ALKENES.

R. M. LoPachin¹, T. Gavin² and B. C. Geohagen¹. ¹*Anesthesiology, Albert Einstein College of Medicine, Bronx, NY* and ²*Chemistry, Iona College, New Rochelle, NY.*

Acrylamide (ACR) is a conjugated Type-2 alkene and a human neurotoxicant. Because pi electrons in a conjugated system are highly polarizable, ACR is a soft electrophile and will form adducts with soft biological nucleophiles, primarily sulfur. In a study of structure-toxicity relationships we showed that ACR and other Type-2 alkenes; e.g., acrolein (ACL), methylvinyl ketone (MVK), produced in vitro neurotoxicity (synaptosomal dysfunction) mediated by thiol adduct formation (LoPachin et al., 2006). The rank order of neurotoxic potencies (ACL>MVK>>ACR) suggested differences in softness among these conjugated carbonyl compounds. Reactions between soft electrophiles and soft nucleophiles is governed by frontier molecular orbital (FMO) interactions and thus, alkenes with low energy FMO's should more readily undergo adduct formation by accepting electrons from soft nucleophiles like thiolates. Since the relevant FMO for electrophiles is the lowest unoccupied molecular orbital (LUMO), we calculated LUMO energies of the Type-2 alkenes using the SPARTAN Molecular Modeling program (Wavefunction, Inc). Results show that for each chemical in a series of Type-2 conjugated alkenes the respective LUMO energies were directly correlated to the corresponding IC50's for inhibition of synaptosomal transmitter uptake and sulfhydryl loss. In contrast, non-conjugated alkene or aldehyde analogs had higher LUMO energies and did not affect synaptosomal function or thiol content. Thus, the relative softness of conjugated alkenes is directly related to respective *in vitro* neurotoxic potencies. This suggests that LUMO energy calculations might be used to identify Type-2 alkenes with neurotoxic potential. Such screening is important because: 1) our findings indicate that, conjugated alkenes, as a chemical class, might produce neurotoxicity via a common mechanism involving thiol adduct formation and presynaptic toxicity and, 2) the neurotoxic risks associated with human Type-2 alkene exposure have been poorly delineated. (Sponsored by NIEHS ES03830-20).

1058 CHARACTERIZATION OF THE N27-D_{2L} CELL LINE: ASSESSMENT AS A VIABLE CELL MODEL FOR INVESTIGATING DOPAMINERGIC D_{2L} RECEPTOR-COUPLED FUNCTIONS.

J. D. Urban¹, K. Thornley⁴, M. Wightman^{4,5} and R. B. Mailman^{2,1,3}. ¹*Curriculum in Toxicology, UNC, Chapel Hill, NC.* ²*Psychiatry, UNC, Chapel Hill, NC.* ³*Pharmacology, UNC, Chapel Hill, NC.* ⁴*Chemistry, UNC, Chapel Hill, NC* and ⁵*Curriculum in Neurobiology, UNC, Chapel Hill, NC.*

Currently there is no dopaminergic cell model that genuinely reflects typical dopaminergic neurons as they would be found in the CNS. This is an impediment to many lines of research, including understanding of the functional mechanisms of action of intrinsic dopamine D₂ receptors. In this study we assess the characteristics of the N27 cell line in order to determine its utility as such a model system. First, we verified previous reports that this cell line synthesized dopamine and endogenously expressed the dopamine transporter. Using radioligand-based competition isotherms it was determined that the N27 cell line does not endogenously express any type of dopamine receptor. A FLAG-tagged hD_{2L} receptor was stably transfected into the cell line, and was found to couple to adenylyl cyclase inhibition (a G $\alpha_{i/o}$ -mediated functional response). Real time PCR revealed the endogenous expression of a number of G protein isoforms as well as low levels of tyrosine hydroxylase and dopamine transporter. The cells, however, failed to show D_{2L}-mediated effects on dopamine uptake or release assessed using biochemical and electrochemical methods. In addition, agonist-induced D_{2L} receptor internalization also did not occur. The ability of this cell line to synthesize dopamine has made it a viable model for studying certain questions regarding the mechanisms that underlie the effects of neurotoxin activity, oxidative stress, ischemia and cell death in dopaminergic cells. It is our assessment, however, that this cell line is not a practical *in vitro* cell model for the study of dopaminergic function as it is regulated by D₂ receptors.

1059 LINKING REGULATORY TOXICOLOGICAL INFORMATION ON ENVIRONMENTAL CHEMICALS WITH HIGH-THROUGHPUT SCREENING (HTS) AND GENOMIC DATA.

M. T. Martin, K. A. Houck, K. McLaurin, A. M. Richard and D. J. Dix. *U.S. EPA, Research Triangle Park, NC.*

The application of high-throughput screening (HTS) and genomic technologies to environmental chemical classification and prioritization requires reference toxicological information for characterizing the associations between toxicological properties and HTS or genomic profiles. Regulatory toxicological data has been produced for thousands of environmental chemicals, and at EPA are used primarily in