demonstrated by knockdown of beclin1 by siRNA or pretreatment with wortmannin, a phosphatidylinositol 3-kinase inhibitor that inhibits autophagy. Inhibition of autophagy reduced cyanide toxicity in the tet-on UCP-2 cells. In contrast, stimulation of autophagy by a mTOR inhibitor (rapamycin) enhanced the sensitivity to cyanide. The involvement of BNIP3, a BH3-only Bcl-2 protein, in the UCP-2 enhancement of cyanide toxicity was then determined. Increased UCP-2 expression in tet-on cells upregulated BNIP3 expression and stimulated BNIP3 translocation to mitochondria. Knockdown of BNIP3 by siRNA blocked UCP-2-mediated autophagy and enhancement of cyanide toxicity. On the other hand, increased BNIP3 expression in tet-on BNIP3 cells induced autophagy and sensitized the cells to cyanide. It is concluded that UCP-2 enhancement of cyanide neurotoxicity involves the autophagic mode of cell death and BNIP3 is a mitochondrial mediator of the neurotoxicity (Supported by NIH grant ES04140).

## PS 1270 THE HUMAN DOPAMINERGIC NEURONAL CELL LINE LUHMES AS *IN VITRO* MODEL FOR PARKINSONS DISEASE.

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Parkinsons disease is characterized by a gradual degeneration of dopaminergic neurons in the substantia nigra. Dopaminergic neurons are continuously exposed to elevated oxidative stress conditions due to the unstable neurotransmitter dopamine that can easily undergo oxidation to form superoxide and a quinine-form capable to react with cysteine residues in proteins or with glutathione to form dopamine-conjugates. For investigations on the molecular events occurring under these conditions, as well as for the validation of potential pharmacological interventions, an experimental human in vitro model that closely resembles the characteristics of dopaminergic neurons in vivo was established.
LUHMES cells are conditionally immortalized human fetal mesencephalic cells that acquire a dopaminergic phenotype following differentiation for 6 days.
LUHMES were characterized with respect to their response toward the parkinsonian toxin MPP+ that not only inhibits mitochondrial complex I but can also trigger the release of vesicular dopamine. In LUHMES, MPP+ caused a time-dependent degradation of neurites, accompanied by a loss of cellular ATP and GSH, and increased formation of radical species. The neurodegenerative effects observed were partially prevented by co-incubation with the mixed lineage kinase inhibitor CEP1347, by inhibition of poly-ADP-ribose polymerase (PARP), or by desferoxamine and ascorbic acid. Inhibition of the proteasomal system or the siRNA-mediated knockdown of alpha-synuclein also demonstrated protective effects, suggesting that the LUHMES/ MPP+ model reflects the major in vivo features of parkinsonian brains such as energy impairment, oxidative stress, and proteolytic stress.

## PS 1271 PROTEIN MODIFICATION AND ADVERSE FUNCTIONAL CONSEQUENCES MEDIATED BY 3, 4-DIHYDROXYPHENYLACETALDEHYDE.

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Parkinson's Disease (PD) is a progressive neurodegenerative disorder, characterized by the loss of dopaminergic neurons. Dopamine (DA), an important neurotransmitter, undergoes catabolism to form 3,4-dihydroxyphenylacetaldehyde (DOPAL). DOPAL is structurally analogous to DA, but is a reactive intermediate; therefore, it has the potential to interact with proteins containing DA-binding sites. Recent studies have shown that DOPAL, at pathological levels, modifies proteins in dopaminergic cells. Currently, the identity of these target proteins and the effect on function are unknown. Therefore, it is hypothesized that DOPAL modifies and inhibits enzymes that are important to dopamine biosynthesis and trafficking. Tyrosine hydroxylase (TH) catalyzes the rate-limiting step in DA synthesis, converting tyrosine to 3,4-dihydroxyphenylalanine (L-DOPA). Nerve growth factor differentiated PC6-3 cell lysate was treated with varying concentrations of DOPAL (10-100 μM) and western blot analysis for TH was performed. There was a decrease in antibody recognition and staining that was concentration-dependent. Furthermore, TH activity was studied using PC6-3 cell lysate. Lysate was treated with either tyrosine or tyrosine and DOPAL and the formation of L-DOPA was followed using HPLC over a 120 minute time course. Results showed a smaller increase in L-DOPA formation when DOPAL was present, as compared to controls. To study DOPAL metabolism and toxicity, PC6-3 cells were incubated in the presence of DOPAL (5-50 μM) for 2 hours and aliquots were obtained at 30 minute intervals. HPLC analysis indicated metabolism to both the acid 3,4-dihydroxyphenylacetic acid, (DOPAC), as well as the alcohol, 3,4-dihydroxyphenylethanol (DOPET), with DOPET found in excess. Furthermore, toxicity studies showed a decrease in cell viability with increasing concentrations of DOPAL present. Overall, these results indicate that DOPAL may adversely affect not only cell viability, but the function of important DA biosynthesis enzymes, such as tyrosine hydroxylase.

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## PS 1272 CHRONIC EXPOSURE TO CORT PRIMES THE CNS PROINFLAMMATORY RESPONSE IN MPTP AND METH MODELS OF NEUROTOXICITY.

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Upregulation of proinflammatory cytokines and chemokines in the brain ("neuroinflammation") accompanies CNS injury as well as, e.g., systemic infections, depression, pain and sleep disorders. Previously, we documented neuroinflammatory responses following nerve terminal damage due to acute exposure to the dopaminergic neurotoxicants, MPTP and METH. Elevations in these varied proinflammatory mediators may serve as modulators glial activation, i.e., cell responses associated with all types of brain injury. Activated glia may have neuroprotective roles or may exacerbate neural damage. As such, glia are potential targets for manipulation in order to effect neuroprotection. Our prior genetic (knockouts) and pharmacological (minocycline) interventions resulted in partial suppression of MPTP- and METH-induced neuroinflammatory responses without affecting neurotoxicity and gliosis. Because glucocorticoids are widely regarded as potent anti-inflammatory agents, we pretreated mice with corticosterone (CORT) prior to administration of MPTP or METH and then assessed striatal content of a variety of cytokines/chemokines by qPCR, and examined dopaminergic terminal damage and astrogliosis by tyrosine hydroxylase (TH) and GFAP immunoassay, respectively. Acute CORT pretreatment (20 mg/kg, s.c.) 30 minutes prior to MPTP or METH reduced, but did not completely suppress the expression of LIF, CCL2, or IL-1β in striatum, whereas decreases in TH and increases in GFAP remained unaffected. Chronic (1 week) CORT pretreatment then was employed to achieve a protracted level of anti-inflammatory therapy. Surprisingly, this CORT regimen appeared to prime the neuroinflammatory response to MPTP and METH, as all proinflammatory mediators showed highly exacerbated (e.g., >200-fold for LIF) responses. As with acute pretreatment with CORT, TH and GFAP remained unaffected. Because the levels of chronic CORT approached or exceeded those associated with high physiological stress levels, our data suggest that chronic CORT therapy or sustained physiological stress primes the CNS neuroinflammatory response to injury.

## PS 1273 TANESPIMYCIN PROTECTS CULTURED RAT DORSAL ROOT GANGLIA FROM BORTEZOMIB TOXICITY.

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INTRODUCTION: Tanespimycin (T), an inhibitor of Hsp90, is in phase 3 clinical trials in combination with bortezomib (B) in patients with relapsed/refractory multiple myeloma (MM). B-induced peripheral neuropathy (PN) is the key dose-limiting toxicity in patients. In a rat model T reversed B-induced PN. OBJECTIVE: To explore the mechanism of T-mediated neuroprotection in primary rat dorsal root ganglion (DRG) cells. METHODS: Differentiated DRG cultures (d17 rat fetuses) were treated for 24 hours with T (10 - 5000 nM), or B (1 - 1000 nM) alone, or in combination. ATP, caspase 3/7 induction, calpain activity, proteasomal chymotrypsin-like activity, and HSp70 induction (in-cell Western) were used as measures of cell viability, apoptosis, neuron-specific protease activity, proteasome function, and Hsp90 inhibition, respectively. Neuronal morphology was evaluated by light microscopy. RESULTS: At concentrations ≥100 nM, both B and T induced 5-fold or greater increases in caspase activity. Cell viability (ATP) was reduced to 20% control values. B (≥100 nM) reduced DRG neurite extensions. When DRG cells were co-exposed to both T (>500 nM) and B, concentration-dependent decreases in viability were abrogated and neurite extensions were preserved. T, but not B, induced both calpain and proteasome activity (5- and 3-fold, respectively, and these increases were reversed by combinations of T and B. Hsp70 induction by T (100 nM) was more than doubled in combination with B (10 nM). CONCLUSION: In primary cultures of rat DRG neurons, T ameliorated B-induced apoptosis and loss of viability, and restored neuronal morphology. These neuroprotective effects of T are consistent with the effects observed in a rat model of B-induced PN and with the lack of severe PN observed in patients in the phase 1/2 study of T combined with B in MM. Possible mechanisms for the protective effect of T on B-induced neuronal toxicity in MM include super-induction of Hsp70 and abrogation of calpain activity.