2035 OXIDATIVE STRESS-INDUCED PHOSPHORYLATION OF PKD1 ACTIVATION LOOP IS REGULATED BY PROTEIN KINASE C-DELTA PROTEOLYTIC ACTIVATION IN CELL CULTURE MODELS OF DOPAMINERGIC DEGENERATION.

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Oxidative stress is a key mechanism by which neurotoxic chemicals induce neuronal damage; however, cell signaling mechanisms underlying oxidative neuronal damage are not well characterized. Protein kinase D1 (PKD1) belongs to a novel CaM kinase family and is emerging as an oxidative stress sensor in non-neuronal cells. We previously demonstrated that proteolytic activation of PKCδ mediates oxidative stress-induced apoptotic cell death in cell culture models of Parkinson's disease; further, phosphorylation of PKCδ Tyr311 by Src tyrosine kinase is a prerequisite for proteolytic cleavage by caspase-3. Herein, we examined whether PKCδ plays any role in activation of PKD during oxidative damage in mesencephalic dopaminergic neuronal cells (N27). Treatment of N27 cells with H_2O_2 (100-300μM) induced phosphorylation of PKD1 activation loop (Ser/Thr-744/748) in a dose- & time-dependent manner. Pretreatment with either the PKCδ inhibitor rottlerin (5μM) or p60src tyrosine-specific kinase inhibitor TSKI (5μM) almost completely abolished the H_2O_2- induced phosphorylation of PKD1. Additionally, suppression of PKCδ by siRNA significantly attenuated H_2O_2-induced phosphorylation of the PKD1 activation loop, suggesting that PKCδ indeed regulates PKD1 phosphorylation. To further determine whether the proteolytically activated PKCδ catalytic fragment mediates PKD1 phosphorylation, the pan-caspase inhibitor Z-VADfmk and N27 cells stably expressing caspase-3 cleavage-resistant PKCδ mutant (PKCδ-CRM) were used. H_2O_2-induced phosphorylation of PKD1 was significantly suppressed by Z-VADfmk (100μM), as well as in N27 cells stably expressing PKCδ-CRM mutant. Together, these results demonstrate for the first time that proteolytic activation of PKCδ regulates the phosphorylation of the PKD1 activation loop. This novel interaction between PKD1 and PKCδ may have important implications in environmental neurotoxic chemicals-induced oxidative damage in dopaminergic cells (support NIH grant ES10586 & NS45133)

2036 DEFINING "NEUROINFLAMMATION": LESSONS FROM MPTP- AND METHAMPHETAMINE-INDUCED NEUROTOXICITY.

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Neuroinflammation often is described as the underlying process responsible for toxicant-induced damage to the CNS. While glial activation and the attendant expression of proinflammatory mediators often are associated with CNS damage, it is not clear that a cause-and-effect relationship exists between the presence of a neuroinflammatory process and neural damage. We explored this issue with two models of dopaminergic neurotoxicity. We used single low-dose regimens of MPTP or METH to cause selective degeneration of striatal dopaminergic nerve terminals without affecting cell bodies in the nigra. Both compounds increased the expression of the microglia associated factors, F4/80, Il-1α, Il6, Ccl2 and Tnf-α and also elicited morphological evidence of microglial activation prior to induction of astrogliosis. Pharmacological antagonism of MPTP and METH neurotoxicity prevented these proinflammatory responses, findings suggestive of a link between neuroinflammation and the observed neurotoxic outcomes. Nevertheless, when minocycline was used to suppress the expression of all these mediators, with the exception of Tnf-α, we failed to see neuroprotection. Likewise, when we examined the effects of MPTP or METH in transgenic mice lacking Il6, Ccl2 or Tnfr1/2 genes, deficiency of either Il6 or Ccl2 did not alter neurotoxicity, whereas deficiency in Tnfr1/2 was neuroprotective. Although these observations pointed to a role of the proinflammatory cytokine, TNF-α, in the neurotoxic effects of MPTP and METH, other observations did not support this argument. For example, induction of iNOS or activation of NF-κB, effects linked to inflammatory responses and free radical formation, was not observed. Moreover, immunosuppressive regimens of glucocorticoids failed to suppress TNF-α, or block neurotoxicity. Taken together, our observations suggest that MPTP and METH neurotoxicity are associated with a "neuroinflammatory" response, yet this response lacks key features of inflammation and, with the exception of TNF-α, neurotoxicity appears to be the cause rather than the consequence of proinflammatory signals.

2037 PROLYL HYDROXYLASE INHIBITION PROTECTS AGAINST MPP+ TOXICITY *IN VITRO*.

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One prevalent feature of neurodegenerative disorders is the dysregulation of iron within different brain neurons. Compared to normal brains, iron levels are higher in affected brain areas in Parkinson's disease indicating a disruption in cellular iron regulation. Recent studies have shown that iron activates prolyl hydroxylase (PHD), which promotes the degradation of the HIF-1α, an important transcription factor that regulates pro-survival gene products. Various cellular responses were measured in dopaminergic N27 cells treated with MPP+. 2 different PHD inhibitors (DHB and DMOG) as well as an iron chelator (SIH) were able to elicit the translocation of HIF-1α into the nucleus and the up-regulation of the transferrin receptor (TfR). Additionally, sustained HIF-1α accumulation in the nucleus was observed in the presence of MPP+, suggesting that the HIF pathway is also activated in the presence of an oxidative neurotoxicant. With increasing evidence of the involvement of HIF-regulated genes encoding proteins involved in iron regulation, total iron levels were also determined. MPP+ elicited an increase in total intracellular iron of cells grown in 3% oxygen that was attenuated in the presence of DHB and DMOG. One of the factors that could contribute to the MPP+-mediated elevation in intracellular iron is the up-regulation of the TfR, responsible for the uptake of iron into cells. We observed that MPP+ resulted in a concentration-dependent increase in TfR protein levels that was significantly reduced in the presence of DHB, suggesting that the reduction in iron levels may be mediated by a down-regulation of TfR, either by the HIF or IRP pathways. To our knowledge, this study is the first to demonstrate the protective effects of PHD inhibition in a cell culture model of dopaminergic cell death associated with MPP+ toxicity. We have preliminary evidence, in both in vitro and in vivo systems, to show that the HIF pathway plays an important role in regulating proteins that are involved in the antioxidant response as well as in iron homeostasis.

2038 NUCLEAR FACTOR KAPPA B MEDIATES SELECTIVE INDUCTION OF NEURONAL NITRIC OXIDE SYNTHASE IN ASTROCYTES DURING MILD INFLAMMATORY STIMULATION WITH 1-METHYL-4-PHENYL-1, 2, 3, 6-TETRAHYDROPYRADINE.

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Recent advances in understanding the progression of Parkinson's disease (PD) implicate perturbations in astrocyte function and induction of constitutively expressed neuronal nitric oxide synthase (NOS1) in both human PD and the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyradine (MPTP) disease model. Transcriptional regulation of *Nos1* is complex, however, recent data suggest that nuclear factor kappaB (NF-KB) is an important transcription factor involved in inducible expression of the gene. The data presented here demonstrate that mild activation of astrocytes with low or 'sub-optimal' concentrations of MPTP (1 μM) and the inflammatory cytokines tumor necrosis factor alpha (10 pg/ml) and interferon gamma (1 ng/ml) results in selective induction of *Nos1* mRNA and protein, an ensuing increase in intracellular nitric oxide, and a significant elevation in global protein nitrosylation. This mild inflammatory stimulus also resulted in activation and recruitment of NF-KB to a potential NF-κB response element located in the Nos1 promoter region flanking the first exon. A role for NF-KB was confirmed through overexpression of a NF-KB "super repressor" which prevented significant induction of the NOS1. The data presented here thus demonstrate a role for NF-KB in selective induction of NOS1 during early inflammatory activation of astrocytes with low-dose MPTP and TNF-α/ IFN-γ.

2039 ALTERED DOPAMINE LEVELS AND NEURODEGENERATION IN THE SUBSTANTIA NIGRA IN RESPONSE TO INTRANIGRAL INJECTION OF ROTENONE IN MICE.

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Research suggests environmental toxins and genetic predisposition are factors in the development of Parkinson's disease, a neurodegenerative disease caused primarily by the loss of dopamine neurons in the substantia nigra. Given such evidence, we examined the potential for exposure to the toxicant rotenone on neurodegeneration of dopamine neurons in the substantia nigra of mice with a mutation in the Nurr1 gene, a genotype that makes dopamine neurons more susceptible to damage. In our current experiments, adult mice received local injections of rotenone directly into the substantia nigra and were sacrificed at various intervals of up to one week. Harvested brains were used to measure neurodegeneration via immunocytochemistry, gene eXpression profiling quantitative multiplex PCR (GeXP), and high-pressure liquid chromatography (HPLC) for catecholamine analysis. Proliferation of microglial cells, glial cells, neuron apoptosis were detected with immunocytochemistry. Multiplex GeXP was used to measure the presence of genetic responses to neural damage in the substantia nigra, with detectable levels of heme oxygenase 1, capase 3 and Bax, indicating oxidative stress and apoptosis in rotenone-treated mice. Preliminary analysis of HPLC data suggests a temporary increase in dopamine in response to rotenone-induced neurodegeneration. Initial results from these experiments suggest localized neural inflammation and neurodegeneration occurs from direct rotenone exposure in the substantia nigra.

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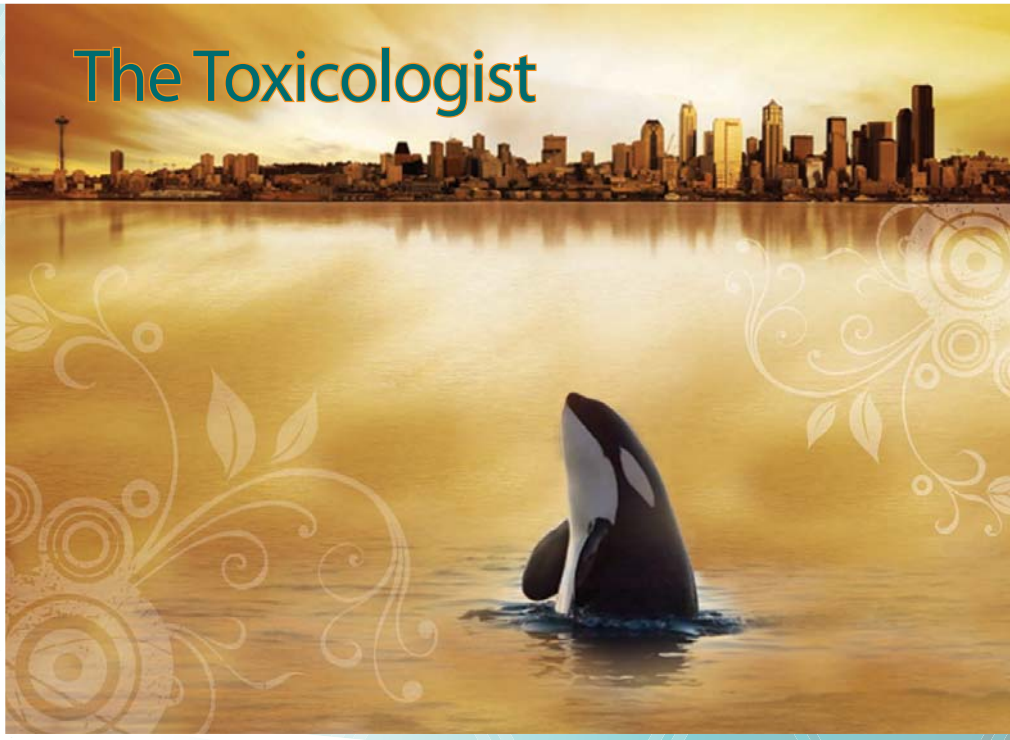
The Toxicologist

March 2008

The Toxicologist



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Supplement to Toxicological Sciences
An Official Journal of the Society of Toxicology

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OXFORD UNIVERSITY PRESS ISSN 1096-6080 Volume 102, Number 1, March 2008