and sulpiride (D2 antagonsist), melatonin can effectively prevent neuronal loss and all the pathological changes without affecting the body weight loss and hypolocomotor activity induced by rotenone. However, nomifensine, but not sulpiride, significantly reverses rotenone-induced the decrease of locomotor activity and DAT immunoreactivity in striatum. These results suggested that oxidative stress and DAT down-regulation are involved in the pathogenesis of nigrostriatal dopaminergic neurodegeneration induced by rotenone, whereas D2 up-regulation may simply represent a compensatory response.

P.227

α-SYNUCLEIN IMPACTS BRAIN PROSTAGLANDIN FORMATION

Golovko, M.Y.[1], Murphy, E.J.[1,2]
[1]*Department of Pharm., Phys., and Ther., University of North Dakota, Grand Forks, ND, USA*
[2]*Department of Chemistry, University of North Dakota, Grand Forks, ND, USA*

α-Synuclein (Snca) is an abundant protein in the CNS that is associated with a number of neurodegenerative diseases. We recently discovered that it significantly facilitates arachidonic acid (20:4n-6) turnover in brain phospholipids and modulates microglial activation, suggesting that Snca may impact prostaglandin (PG) formation. To address this question, we analyzed PG mass in *Snca*$^{+/+}$ or *Snca*$^{-/-}$ mouse brains in the presence or absence of either global ischemia or LPS treatment (i.p., 1 mg/kg). PG were extracted from brains and quantified using ion spray LC-MS/MS analysis. Baseline PG levels, which were determined after subjecting mice to head-focused microwave irradiation, were not affected by *Snca* gene-ablation. The mass of all PG analyzed in *Snca*$^{-/-}$ brains was elevated two-fold upon stimulation following either 30 s of global ischemia or LPS treatment as compared with *Snca*$^{+/+}$ brains. To determine if head-focused microwave fixation reduced PG levels, we injected mice with non-specific COX inhibitor indomethacin (Indo,10 mg/kg, i.p.) 30 min before subjecting the mice to global ischemia and then to microwave irradiation. PG levels were the same between groups subjected to head-focused microwaving, head-focused microwaving + Indo, and Indo alone, indicating that PG were not trapped or destroyed in tissue after microwaving. Collectively, our results indicate that in *Snca*$^{-/-}$ brains, LPS treatment or global ischemia results in elevated PG formation. This is consistent with our previously observed reduction in 20:4n-6 recycling through ER-localized acyl-CoA synthetase in the absence of Snca, which may result in the increased 20:4n-6 availability for PG production in the absence of Snca.

P.228

Fyn KINASE IS AN UPSTREAM REGULATOR OF PKCDELTA-MEDIATED APOPTOTIC SIGNALING IN PARKINSON'S DISEASE MODEL

Kanthasamy, A.G., Saminathan, H., Anantharam, V., Kanthasamy, A.
Department of Biomed Sci., Iowa State University, Ames, IA 50011, USA

Oxidative stress and apoptosis are two key pathophysiological mechanisms underlying dopaminergic degeneration in Parkinson's disease (PD). Recently, we identified that proteolytic activation of PKCdelta, a member of the novel PKC family, contributes to oxidative stress-induced dopaminergic degeneration in PD models and that phosphorylation of tyrosine residue 311 on PKCdelta is a key event preceding the PKCdelta proteolytic activation during oxidative damage. This study was designed to identify the tyrosine kinase involved in the proteolytic activation of PKCdelta. We found that Fyn kinase, a non-receptor tyrosine kinase, is significantly expressed in the nigral tissues and dopaminergic neuronal cells (N27 cells) and is physically associated with PKCdelta. Exposure of N27 cells to the Parkinsonian toxin dieldrin (60 μM) or the pro-oxidant H_2O_2 (100 μM) rapidly activated Fyn kinase. The Fyn activation preceded the proteolytic activation of PKCdelta and was blocked by a p60-tyrosine specific kinase inhibitor (TSKI). Additionally, TSKI effectively curtailed both dieldrin- and H_2O_2-induced PKCdelta proteolytic activation and apoptotic cell death. To further confirm Fyn's role in the proapoptotic function of PKCdelta, we adopted the RNAi approach. siRNA-mediated knockout of Fyn kinase effectively blocked dieldrin-induced PKCdelta proteolytic cleavage and activity, as well as caspase-3 activity and DNA fragmentation, suggesting that Fyn kinase regulates the proapoptotic function of PKCdelta. Collectively, these results demonstrate for the first time that Fyn kinase is a stress sensitive kinase that regulates upstream signaling of the PKCdelta-mediated apoptotic cell death pathway in dopaminergic cells.
Acknowledgments: NIH grants NS 38644 & ES10586.

P.229

TLR4 AND INFLAMMATORY RESPONSE TO NEURONAL DEGENERATION

Konat, G.W.[1], Sriram, K.[2], Kraszpulski, M.[1], O'Callaghan, J.P.[2]
[1]*Neurobiology, West Virginia University, Morgantown, USA*
[2]*CDCP, NIOSH, Morgantown, USA*

Toll-like receptor 4 (TLR4) has recently emerged as an important mediator of inflammatory responses elicited by molecules released from injured tissues. The present study was undertaken to determine the role of TLR4 in such responses following neuronal damage. Striatal dopaminergic neurodegeneration was induced in TLR4-mutant and control mice by a single subcutaneous injection of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). In both groups, the level of tyrosine hydroxylase (TH) in the striatum decreased within

72 h by ~50% as compared with untreated animals indicating extensive neuronal damage. The loss of TH was associated with glial activation as seen from the upregulation of GFAP, which was similar in both groups. The phosphorylation of STAT3, a key mediator of astrogliosis, was also increased. Moreover, the expression of glia-derived cytokines, i.e., TNFα, MCP-1 and LIF, was profoundly upregulated in both mutant and control striata. Interestingly, basal expression of MCP-1 and LIF in TLR4-mutants was approximately five fold higher than in controls. These results indicate that TLR4 does not mediate inflammatory responses associated with dopaminergic neurodegeneration. However, the receptor seems to be involved in controlling basal expression of certain inflammatory genes.

Acknowledgments: Supported by NIH grant NS051787.

Disclaimer: "The findings and conclusions in this report (abstract/presentation) have not been formally disseminated by the National Institute for Occupational Safety and Health and should not be construed to represent any agency determination or policy".

P.230

USE OF AN ORGANOTYPIC CULTURE SYSTEM TO STUDY THE ACTIONS OF NEUROGLIA IN THE PRESENCE OF NEURONS EXPRESSING MUTANT HUNTINGTIN

Kraft, A.D.[1], Turmel, G.J.[2], Lo, D.C.[2], Harry, G.J.[1]

[1]*NIEHS, RTP, USA*

[2]*Duke University, Durham, USA*

In organotypic rat brain slice cultures, neuronal expression of huntingtin (Htt) protein fragments containing a mutation linked to human Huntington's disease (HD) was achieved by transfection of a human Htt construct (N90Q73) encoding exon 1 (67 amino acids at the N-terminus) with a polyglutamine repeat of 73 glutamines (mHtt). mHtt-transfected medium spiny neurons in the striatum, visualized by expression of a co-transfected YFP visual marker, showed decreased viability over 5 days. Using this as a model of neuronal injury, we examined the response of GFAP-positive astrocytes and B4 isolectin-labeled microglia, including their contact relationship with neurons expressing mHtt, as compared with control neurons. Neighboring microglia were analyzed for deramification, "foci" clustering, process fragmentation, and spheroid formation. Astroglia associated with YFP-positive neurons were examined for hypertrophy, process length and thickness, and the formation of fibrous, "scar-like" clusters. Staining of cryosections for glia-specific markers demonstrated a differential in cells in close proximity to, or in contact with, mHtt-expressing neurons. Pharmacological manipulation of these cultures to modify glial proliferation and migration suggests a role for neuronal-glia interactions in the degeneration of mHtt-expressing cells. Manipulation of this glial activity within a system modeling the intact architecture of the brain may prove useful in developing new therapies aimed at relieving aberrant glial actions in HD.

Acknowledgments: This study was supported by NIH intramural research and the Cure Huntington's Disease Initiative.

P.231

DECREASES IN GFAP EXPRESSION BY BLUEBERRIES

Mcguire, S.O.[1], Tagge, M.J.[1], Feinstein, D.L.[2], Gavrilyuk, V.[2], Hejna M.J.[1]

[1]*Departments Pathology and CBNA, Loyola University, Chicago, USA*

[2]*Department of Anesthesiology, University of Illinois, Chicago, USA*

We have previously shown that dietary supplementation with a blueberry extract (BBE) improves efficacy of neural repair in a rodent model of Parkinson's disease, significantly increasing survival of implanted dopamine neurons as well as graft area and graft-derived innervation (McGuire *et al*., 2006). To investigate underlying mechanisms, F344 rats fed standard diets ± BBE for 6 weeks were exposed to striatal injury via insertion of a transplant needle, representing the neural repair paradigm. At 4d post-injury, rats fed BBE-supplemented diets exhibited significantly less striatal GFAP than did rats fed the control diet ($P<0.01$), revealing decreased inflammation in response to striatal injury. The effect was not accompanied by decreased immune response as the macrophage marker, CD68, as well as the expression MHCII, tended to increase ($P=0.07$) in the BBE-fed animals. Experiments in cell culture examined the direct effect of whole, freeze-dried, ground TifBlue powder (WTBBP) on GFAP expression in cultures of immortalized rat astrocytes (DITNC). Cells were exposed to increasing concentrations of WTBBP for 48 h, harvested and analyzed for GFAP by western blot. These data reveal a J-shaped curve with doses of up to 2% WTBBP (w/v) significantly decreasing GFAP expression by 35% ($P<0.02$) whereas inclusion of 4% WTBBP produced an intermediate level of GFAP. Preliminary studies in C6 glioma cells transfected with a reporter construct for PPAR response element revealed that the presence of 2% WTBBP in the media activated the PPAR RE at 15 min, implicating a possible PPAR involvement in WTBBP induced decreases in GFAP expression. We are currently investigating these and other mechanisms in primary rat astrocytes.

P.232

SKA-PD-01 SHOWS NOVEL INHIBITORY EFFECTS ON MONOAMINE OXIDASES AND NEUROPROTECTIVE EFFECTS IN A MOUSE MODEL OF PARKINSON DISEASE

Lee, J.W., Chung, J.Y., Ryu, C.H., Jee, M.K., Min, H.K., Yoon, Y.J., Park, C.H.

Drug Development Center, SK Corporation, Daejeon, South Korea

PD is one of progressive neurodegenerative diseases that need effective new therapeutic drugs. MAO is known as a potential therapeutic target for PD in early, mid and advanced stage. MAO-B inhibitors possess abilities to improve motor function and prevent neuronal loss by decreasing dopamine metabolism and oxidative stress. To find novel anti-Parkinson drugs, we synthesized new compounds and screened the inhibitory effect