Journal of Neurochemistry, **94** (Suppl. 1), 1–51

Plenary Talk P01: Experience-dependent modification of neural circuits – cellular and molecular mechanisms

Mu Ming Poo

Division of Neurobiology, Department of Molecular & Cell Biology, Berkeley, CA, USA

Correlated spiking of pre- and postsynaptic neurons can result in strengthening or weakening of glutamatergic and GABAergic synapses, depending on the order of and/or the time interval between the pre- and postsynaptic spiking. The functional significance of this synaptic modification is illustrated by the experience-dependent development of Xenopus retinotectal system. Activity-induced modification of retinotectal synapses underlies the refinement of the receptive field of tectal neurons, a process involving a progressive matching of excitatory (retinotectal) and inhibitory (intratectal) inputs to the tectal neuron. Furthermore, the receptive field properties can also be rapidly 'trained' by conditioning visual stimuli through a selective and persistent potentiation and depression of specific retinotectal and intratectal synapses. Interestingly, activity-induced synaptic modifications *in vivo* are quickly reversed by either subsequent spontaneous activity in the tectum or exposure to random visual inputs. Such reversal depends on the activation of the NMDA subtype of glutamate receptors and postsynaptic protein phosphotases. However, stabilization of synaptic modifications can be achieved by an appropriately spaced pattern of visual stimuli. Taken together, these findings underscore the importance of spike timing-dependent synaptic plasticity and suggest a temporal constraint on the pattern of sensory inputs for effective modification of developing neural circuits.

Symposium S01: Molecules and mechanisms in Schwann cell development

Chair: K.R. Jessen & R. Mirsky

S01-01

The function of Notch and stress kinase signals in early Schwann cell development

KR JESSEN, R MIRSKY, A WOODHOO, D PARKINSON and A BHASKARAN

Department of Anatomy and Developmental Biology, University College London, London, UK

Embryonic Schwann cell development involves two main transitions: (1) the emergence of Schwann cell precursors (SCPs) from neural crest cells, (2) the generation of immature Schwann cells from SCPs. The third major transition in the lineage is the largely postnatal diversification of immature Schwann cells to form myelinating and non-myelinating Schwann cells. This talk will focus on the function of Notch signalling in controlling the SCP/Schwann cell transition, proliferation of immature Schwann cells and the onset of myelination. The function of Notch and stresskinase singalling,in particular the JNK/c-Jun pathway, as negative regulators of myelination will be discussed, as well as the interactions between these pathways and the pro-myelin transcription factor Krox-20.

S01-02

Krox-20/Egr2 collaborators and regulation of gene expression during myelination of the peripheral nervous system

J SVAREN*, SE LEBLANC*, R SRINIVASAN*, C FERRI†, GM MAGER*, AL GILLIAN-DANIEL* and L WRABETZ†

**University of Wisconsin, Madison, WI, USA, †San Raffaele Scientific Institute, DIBIT, Milan, Italy*

Myelination of peripheral nerves by Schwann cells is coordinated by transcriptional regulatory proteins that trigger various genetic events in the synthesis and compaction of myelin. One central aspect of myelin formation is the requirement for a large amount of lipid and cholesterol biosynthesis, and recent experiments have suggested that Egr2/Krox-20 may trigger induction of cholesterol and lipid biosynthetic enzymes at the transcriptional level. To elucidate the control of cholesterol and lipid biosynthetic genes during peripheral nerve myelination, we investigated how expression of the Sterol Regulatory Element Binding Protein (SREBP) pathway components is affected by the absence of Egr2. During myelination of sciatic nerve, there is a very significant induction of SREBP1 and SREBP2, as well as their target genes, suggesting that the SREBP transactivators are important regulators in the myelination process. In addition, the expression levels of lipid/cholesterol biosynthetic genes are dramatically reduced in sciatic nerves from mice with a targeted disruption of Egr2, and we found that Egr2 and SREBP2 can coordinately activate expression of several SREBP target genes, including HMG CoA reductase. In summary, our data indicate that Egr2/Krox-20 interacts with SREBP activators to directly regulate cholesterol/lipid biosynthetic genes as part of the myelination program.

S01-03

Purinergic signaling molecules regulating schwann cell development in response to impulse activity in axons

RD FIELDS

National Institutes of Health, NICHD, Bethesda, MD, USA

Development of myelinating glia is regulated by a complex variety of interactive and dynamically regulated signals from developing axons, including neural impulses. Impulse activity is detected by premyelinating glia in part by the activity-dependent release of ATP from axons, which is hydrolyzed by ectonucleotidases to ADP, AMP, and adenosine. These extracellular signaling molecules activate different purinergic receptors on Schwann cells regulating various second messengers and protein kinases, including cAMP and Ca^{2+}, erk 1/2 and p38 MAPK. In comparison with growth factors, the developmental effects of purinergic signaling on Schwann cell development have received much less attention. Our laboratory is exploring how these activity-dependent purinergic signaling molecules function in concert with other axonal signals to regulate proliferation and development of Schwann cells. The results indicate that the effects of impulse activity, growth factors, and purinergic receptor activation are context-dependent. Opposite effects of adenosine on SC proliferation and erk/MAPK activation are seen in the presence and absence of growth factors, for example. This suggests that the biological effects of impulse activity and growth factors are dependent upon the complement of different signals impinging upon the cell at any given time. These considerations may help explain how axonal signals can have different biological effects at different stages of development.

S01-04

Tyrosine kinase signaling in peripheral nerve tumorigenesis

N RATNER, KR MONK, J WILLIMAS, B LING, S MILLER and J WU

Dept. Pediatrics, Cincinnati Children's Hospital, University of Cincinnati, Cincinnati, OH, USA

Benign peripheral nerve tumors called neurofibromas are a major source of morbidity for patients with the inherited disease neurofibromatosis type 1 (NF1). We developed a tissue culture model of peripheral nerve tumorigenesis in NF1. NF1–/–TXF Schwann cells have loss of function mutations at NF1, and, like Schwann cells in neurofibromas, lose contact with axons and aberrantly express the epidermal growth factor receptor (EGFR). When the human EGFR is expressed in Schwann cells (CNPase-hEGFR mice), a neurofibroma-like phenotype develops (Ling *et al*. *Cancer Cell* 2005; 7: 65–75). CNPase-hEGFR mice also show hypoalgesia, consistent with disruption of neuron-glial interaction we described in CNPase-hEGFR mouse nerves. We used the CNPase-hEGFR mouse model to test response to the EGFR antagonist Cetuximab (ImClone Systems). We administered Cetuximab at various times beginning at birth. Nerve hypertrophy, mast cell accumulation, collagen deposition and disrupted axon-glial interactions characteristic of this model were all normal at three months of age only when mice were treated early in life. The results suggest that application of anti-EGFR therapeutics in young animals blocks tumorigenesis by halting a crucial step (perhaps Schwann cell proliferation) in a cascade of events that leads to complex changes characteristic of neurofibroma formation.

Acknowledgement: Supported by NIH-NS28840 and DAMD-17-02-1-0679.

Symposium S02: Progress on the pathogenesis of hereditary neurodegenerative disorders

Chair: L. Notterpek

S02-01

Presenilins in the pathogenesis of Alzheimer's disease

G THINAKARAN

The University of Chicago, Chicago, IL, USA

Sequential processing of amyloid precursor protein (APP) by β- and γ-secretases releases the 39–42 amino acid long beta-amyloid (Aβ) peptides, which accumulate in the brains of aged individuals and patients with Alzheimer's disease (AD). The major β-secretase is an aspartyl protease termed BACE-1, which cleaves APP within the ectodomain. Aβ is then released by intramembraneous cleavage by γ-secretase, a multiprotein complex made of four essential components, presenilin (PS) 1 (or PS2), nicastrin, PEN-2 and APH-1. Mutations in PSEN1 and PSEN2, which PS1 and PS2 respectively, cosegregate with the majority of cases of autosomal dominant familial early-onset AD (FAD). FAD-linked PS1 and PS2 variants elevate the production of highly fibrillogenic Aβ42 peptides. Exactly how multiple FAD-linked single amino acid substitutions within PS1 or PS2 alter APP processing in a manner that selectively elevates the levels of Aβ42 peptides is still not clear. Genetic, biochemical and pharmacological evidence suggests that γ-secretase is an aspartyl protease with PS1 constituting the active site. In addition to APP, a select number of type I membrane proteins including Notch, ErbB4, CD44, LRP, DCC etc., are also cleaved by γ-secretase. Emerging evidence implicates γ-secretase processing of diverse substrates at spatially distinct sites within intracellular membranes. In the adult nervous system BACE-1, γ-secretase, and ectodomain cleaved APP localize to cholesterol-and sphingolipid-rich membrane microdomains, consistent with a role for lipid rafts in amyloidogenic processing of APP. Such spatial segregation of secretases and substrates might be exploited for the development of therapeutic strategies aimed at reducing Aβ burden.

S02-02

Formation and clearance of amyloid pathology in mouse models of Alzheimer's disease

DR BORCHELT*‡, JL JANKOWSKY†, A SAVONENKO‡, T MELINKOVA‡, HH SLUNT‡, LH YOUNKIN§, NG COPELAND¶, NA JENKINS¶ and SG YOUNKIN§

**Dept Neuroscience, University of Florida, Gainesville, FL, †Div. of Biology, Calif. Inst Tech., Pasadena, CA, ‡Dept Pathology and Neuroscience, Johns Hopkins Univ Sch of Med, Baltimore, MD, §Mayo Clinic, Dept. Neuroscience, Jacksonville, FL, ¶National Cancer Inst, Mouse Genetics, Frederick, MD, USA*

Studies of mice that express amyloid precursor protein (APP) and presenilin have demonstrated that the rate at which amyloid pathology develops is regulated by the amount of A-beta 42 that is generated from proteolytic processing of APP. Mutations in APP and presenilin, a component of one processing protease, directly influence this process by enhancing cleavage at sites that generate A-beta 42. Analyses of mice harboring mutant APP and presenilin genes demonstrate that deposition of amyloid is sufficient to induce cognitive dysfunction. Recently, we have generated a transgenic mouse model that over-express mutant APP (swe/ind) from a vector that can be regulated by doxycycline. Under induced conditions, high level expression of APPswe/ind quickly leads to fulminant amyloid pathology. We show that administration of doxycycline inhibits transgenic APP expression by 95% and abruptly halts the progression of amyloid pathology. However, existing amyloid plaques require a far longer interval to disperse than to assemble and associated pathologies likewise persist. Our findings suggest that arresting A-beta production in AD patients should halt the progression of pathology, but that early treatment may be imperative as repair of amyloid induced damage appears to be a very slow process.

S02-03

Protein quality control of P0 glycoprotein in hereditary neuropathies

L WRABETZ

San Raffaele Scientific Institute, DIBIT, Milan, Italy

P0 is the most abundant glycoprotein of peripheral myelin. In human, diverse MPZ mutations result in various neuropathies, ranging from mild Charcot-Marie-Tooth (CMT) disease type 1B to the more severe forms Dejerine-Sottas syndrome (DSS) and Congenital Hypomyelination (CH). The dominant inheritance of most MPZ mutations suggests gain of function mechanisms. Deletion (S63del) or conversion of serine 63 to cysteine (S63C) in the extracellular domain results in CMT1B or DSS, respectively. Premature truncation in the cytoplasmic domain (Q215X) produces CH. Utilizing transgenic mouse models that express these mutant P0s, we have shown that each mutant protein is synthesized and produces a phenotype similar to its human counterpart through a gain of abnormal function. However, intracellular trafficking differs among mutants. P0S63C is mostly trafficked to myelin, whereas P0S63del is retained intracellularly in the endoplasmic reticulum (ER), and P0Q215X is partially retained intracellularly after the ER, but before arrival to the myelin sheath. Protein quality control ensures that only correctly synthesized proteins are delivered to their final destination. For example, accumulation of P0S63del in the ER triggers an unfolded protein response (UPR) in a dose-dependent fashion, indicating a toxic gain of function, and genetic ablation of a UPR mediator restores neuromuscular defects in S63del mice, suggesting that the UPR is pathogenetic in CMT1B due to P0S63del. Thus, the diversity of MPZ-related neuropathies results from various toxic gain of function mechanisms deriving from various intracellular locations.

S02-04

Protein aggregation and aggregate clearance in PMP22-associated neuropathies

L NOTTERPEK, J FORTUN and WA DUNN

McKnight Brain Institute of the University of Florida, Gainesville, FL, USA

Peripheral myelin protein 22 (PMP22) is a hydrophobic membrane glycoprotein whose abnormal expression is linked to hereditary demyelinating peripheral neuropathies. In normal cells, the majority of the newly-synthesized PMP22 has a short-half life and is rapidly degraded by the ubiquitin-proteasome pathway. Only a small fraction of the protein is transported to the Schwann cell membrane, where it is incorporated into myelin. When the proteasome is inhibited, or PMP22 is mutated or overexpressed, it accumulates in perinuclear, cytosolic aggresomes. The presence of PMP22 aggregates is associated with an impairment of proteasome activity and the accumulation of ubiquitinated substrates and chaperones, in and around the aggregates. Since in cell culture models, and in nerves of neuropathy mice, PMP22 aggregates are surrounded by lysosomes, we hypothesized that their formation represents a pathway for the removal of misfolded PMP22. Indeed, under permissive conditions, Schwann cells have the ability to clear preexisting cytosolic PMP22 aggregates by a mechanism that is assisted by autophagy and chaperones. Using electron microscopy, we found autophagosomes engulfing protein aggregates of Trembler J neuropathy nerves. Furthermore, by stimulating the autophagic and chaperone responses of Schwann cells, the formation of PMP22 aggregates can be hindered. Our studies demonstrate that the intracellular fate of PMP22 can be altered in Schwann cells by modulating autophagy and the cellular levels of chaperones. Furthermore, these studies provide a novel approach for the treatment of PMP22-linked neuropathies.

Acknowledgements: Supported by the Muscular Dystrophy Association and the NIH-NINDS.

Colloquium C01: Mechanisms of HIV-induced neuropathology: a critical role for astroglia, microglia, and HIV–opiate interactions

Chair: P. Knapp

C01-01

Mechanisms of neuropathology with HIV infection: a clinical overview

A NATH and J MCARTHUR
Johns Hopkins University, Baltimore, MD, USA

HIV infection can involve the entire neuro-axis, causing a dementia (HIVD), myelopathy or neuropathy. In spite of antiretroviral therapy, the prevalence and incidence of neurological complications is rising. While peripheral neuropathy is the most common neurological complication, neurocognitive impairment is the most fearsome and most well studied. In the pre-HAART era, it was described as a subacute, subcortical dementia. In the HAART era, new subtypes have been characterized that include reversible, chronic inactive and chronic active dementia. Although pathological correlates of these subtypes are not yet available, HIVD is often accompanied by an encephalitis characterized by multinucleated giant cells and microglial nodules. HIV causes a productive infection in brain macrophages/microglia and a restricted/latent infection in astrocytes. Infection of other cell types in brain remains uncertain. Loss of neurites and neuronal cell loss is also present which is indirectly mediated by viral and host products released by HIV infected or activated glial cells. There is also a breakdown in the blood brain barrier. All pathological findings are more prominent in drug abusing populations. Antiretroviral drugs used for treating the neurological complications are not ideal. Drawbacks include poor penetration into the CNS, lack of effect on the production of some neurotoxic viral proteins once the virus is integrated and mitochondrial toxicity caused by the drugs. Hence significant effort has been devoted to developing neuroprotective strategies. Although none is currently in clinical use for HIVD, approaches include blocking excitotoxicity, antioxidants, blocking platelet activating factor, immunophilin ligands, growth factors, chemokine receptor antagonists and modulation of matrix metaloproteinases.

C01-02

HIV alters astrocyte gene expression and disrupts glutamate homeostasis: parallels with HIV dementia and HIV brain disease in a mouse model

DJ VOLSKY
Columbia University, New York, USA

The potential role of astrocytes in HIV neuropathogenesis is not well understood. We have developed an integrated program involving molecular, cell biology, animal model, and patient studies to elucidate glial functions following HIV-1 infection. Exposure of fetal astrocytes to HIV-1 *in vitro* was found to alter cellular gene expression and impair glutamate transport. The EAAT2 glutamate transporter was down-modulated in brain tissues from patients with HAD, indicating that HIV-1-infected astrocytes can contribute to excitotoxicity. To better understand the overall impact of HIV-1 on human astrocyte biology, we determined genome wide transcript expression profiles of HIV-1-infected human fetal astrocytes using the Affymetrix HG-U133A/B high-density oligonucleotide array set. Probe level data analysis revealed over 1000 transcripts that were significantly altered in a temporal manner in infected compared to uninfected cells ($P \leq 0.01$). Upregulated transcripts included chemokines, IFN-induced genes, cytokines, and complement C3. Elevated expression of these genes was confirmed by QPCR and protein assays *in vitro*, and some of the altered gene products localized specifically to astrocytes in brain tissues from patients with HAD by IHC. Selected genes upregulated by HIV in human astrocytes were also elevated in parallel to HIV neuroinvasion in brain tissues of HIV infected mice. Thus interaction of HIV-1 with astrocytes leads to a global and extensive reprogramming of cellular gene expression and cell functions that may contribute to neuropathogenesis in multiple ways, including aberrant innate immune responses and disruption of glial neuroprotective functions.

C01-03

HIV-1 brain infection, blood-brain barrier (BBB) and co-morbidity factors in HIV-1 associated neurodegeneration

Y PERSIDSKY

Depts Pathol Microbiol & Pharmacol, University of Nebraska Med. Center, Omaha, NE, USA

Oxidative and inflammatory brain injury plays an important role in a number of neurodegenerative diseases (including HIV-1 encephalitis, HIVE), and alcohol abuse could be a co-factor in their progression. Given multiple confounding factors in human host, such processes can be investigated only in relevant *in vitro* and *in vivo* models. Using *in vitro* BBB model, we demonstrated that ethanol (or its metabolite, acetaldehyde) decreased BBB tightness (measured by transendothelial resistance) that was reversed by ethanol or acetaldehyde withdrawal. Ethanol activated myosin light chain (MLC) kinase (MLCK) leading to phosphorylation of MLC and tight junction (TJ) proteins. Cytoskeletal and TJ alterations resulted in enhanced monocyte migration across the BBB. Inhibition of MLCK, anti-oxidant treatment or suppression of ethanol metabolism in brain endothelium prevented these changes. Thus, ethanol may impair TJ assembly promoting leukocyte infiltration into the brain. Role of alcohol abuse in HIV-1 brain infection was next studied in small animal model for HIVE that reproduces neuroinflammatory and acquired anti-viral immune responses (Poluektova *et al*. *J Immunol* 2002). HIVE mice, chronically exposed to ethanol, demonstrated increased levels of viremia (HIV-1 p24), ineffective elimination of HIV-1 infected macrophages by cytotoxic lymphocytes in the brain, enhanced microglial reaction and prominent BBB damage. Our data acquired in diverse experimental systems proved that alcohol could be an exacerbating factor in HIV-1 CNS infection. Understanding of mechanisms underlying combined toxic effects of HIV-1 brain infection and alcohol abuse will help to design neuro-protective strategies.

Acknowledgements: AA13846, MH65151.

C01-04

A central role of astroglia in opioid-mediated neuroplasticity and in the pathology of drug-HIV interactions

KF HAUSER, N EL-HAGE, S BUCH, AJ BRUCE-KELLER and PE KNAPP

Department of Anatomy & Neurobiology, University of Kentucky, Lexington, KY, USA

Astroglia can express mu-opioid receptors (MOR) and are strategically positioned to integrate opioid neurochemical signals with other physiologic/pathophysiologic events during maturation, trauma, or disease. This is revealed by opiate abuse, which causes aberrant changes in astroglial maturation and in their response to disease. In combination with HIV Tat, MOR activation causes synergistic increases in glial precursor death. In mature astrocytes, opiates and HIV are only lethal to a small percentage of cells; however, astroglial function is synergistically disrupted, which includes a loss of Ca^{2+} homeostasis, increased chemokine (MCP-1, RANTES) and cytokine (IL-6) production. The above effects were prevented by MOR antagonists. Chemotaxis assays showed that N9 microglial cell motility to Tat exposed astrocytes *in vitro*, or macrophage numbers near sites of Tat injection *in vivo*, were significantly greater in the presence of morphine compared to either substance alone. Chronic disruptions in astroglial function combined with losses in glial precursors may contribute to neurocognitive defects seen with opiate abuse and to the exaggerated CNS pathology seen in HIV-infected individuals who abuse opiates.

Acknowledgements: NIH DA13559 and DA13728.

Colloquium C02: Mechanisms and regulation of metal transport into the CNS

Chair: J. Connor

C02-01

Mechanism and regulation of brain iron transport

JR CONNOR

Penn State University, Hershey, PA, USA

The concentration of iron varies greatly among different brain regions but there is no understanding of how this regional heterogeneity is initiated or maintained. Studies on the mechanism of delivery of iron to the brain are conflicting and almost no data exist on regulation of the mechanism. Endothelial cells forming the blood-brain-barrier must integrate signals regarding plasma iron status, brain iron status and their own iron requirements. We examined the profile of iron management proteins in microvasculature isolated from different brain regions and found that regional brain iron heterogeneity is not reflected in microvascular iron management protein levels. The levels of ferritin observed in the microvasculature indicate that the endothelial cells have significant iron storage capacity. To determine if brain iron or systemic iron status was responsible for the iron protein profile in the microvasculature rats with normal or low brain iron status were made iron deficient. In both groups the profile changed but the changes differed based on brain iron status suggesting that brain iron status dictates the profile in the microvasculature and not systemic iron status. To begin to understand the mechanism of iron transport into the brain, we investigated transport of fluorescein-transferrin-59Fe in a bovine retinal endothelial cell culture system. Our results indicate that there is both transferrin mediated and non-transferrin mediated transcytosis of iron and that the process is influenced by the iron status of the cells. We also obtained evidence that ferritin is transported into the brain. The long-term goal of this line of research is to understand the dynamics of brain iron acquisition and apply this knowledge to the maladaptions that may contribute to neurological disease.

C02-02

Manganese transport, toxicity and speciation in the CNS

M ASCHNER

Department of Pediatrics, Vanderbilt University Medical Center, Nashville, TN, USA

Manganese (Mn) is an essential mineral that is found at low levels in food, water, and the air. Under certain high-dose exposure conditions elevations in tissue Mn levels can occur. Excessive Mn accumulation results in adverse neurological, reproductive, and respiratory effects both in laboratory animals and humans. In humans, Mn-induced neurotoxicity (manganism) is the overriding concern since affected individuals develop a motor dysfunction syndrome that is recognized as a form of Parkinsonism. This lecture will focus on the essentiality and toxicity of Mn and consider contemporary studies evaluating Mn transport across the blood-brain barrier, and specifically the relationship between brain Mn levels and iron (Fe) deficiency. Specific mechanisms for the transport of Mn across the blood-brain barrier via the divalent metal transporter-1 (DMT-1) and the transferrin (Tf) receptor will be discussed. Since Mn3+ can be produced by oxidation of Mn2+ by superoxide radical and a Mn3+ complex has been shown capable of oxidizing dopamine, attention will also be directed at recent studies with X-ray Absorption Near Edge Structure (XANES) spectroscopy investigating the hypothesis that cell damage in Mn toxicity is caused by oxidation of important cell components by Mn3+.

C02-03

The role of multi-copper oxidases in the central nervous system

ZL HARRIS, X XU and S PIN

Johns Hopkins University and School of Medicine, Baltimore, MD, USA

To further delineate and characterize the roles of the multicopper oxidases in iron metabolism, a mouse lacking ceruloplasmin and hephaestin was generated. Total tissue iron, hematologic profiles, serum iron measurements, northern blot analysis, western blot analysis, in-situ hybridization, histologic staining, electron microscopy, neurobehavioral testing and rodent magnetic resonance (MR) imaging were performed. Multicopper oxidase (MCO) deficient mice manifest a neurodegenerative phenotype at approximately 6 months of age (5–9 months). Presenting symptoms included gait abnormalities, weakness and poor grooming. Tissue iron content studies (mcg Fe/dry weight tissue) reveal a significant contribution by hephaestin in regulating iron homeostasis in all tissues except the liver. The neurodegenerative phenotype coupled with the increased whole brain tissue iron content suggested an essential role for multicopper oxidases in central nervous system iron homeostasis. Histologic analysis of brain tissue by Perls and modified DAB-Perls staining demonstrate enhanced iron accumulation within the specific brain regions. MR imaging on a 9.4T scanner non-invasively confirm pathologic iron deposition. Neurobehavior testing identifies neurologic deficits consistent with the regional iron deposition. Electron microscopy is suggestive of a mitochondrial defect associated with the increased iron deposition. The multicopper oxidases appear to regulate the redox state of the central nervous system and protect tissues from iron mediated oxidative damage.

C02-04

Regulation of metal transporters in brain

G GEORGIEFF

Graduate Program in Neuroscience, Minneapolis, MN, USA

This presentation will compare and contrast the expression, localization and potential mechanisms of action/regulation of metal transporters in three systems of vectoral metal transport; the intestine, the placenta and the brain. Using iron transport as a model, the presentation will address how studies in intestinal and placental iron transport provide clues into brain iron transport mechanisms. In particular, work in human placental tissue and its relationship to brain will be presented because of the ability to study human tissue directly. Data will be presented on the localization of metal importers on the apical (serum facing) surfaces and exporters on the basal surfaces intestine, placenta and brain.

Colloquium C03: Modeling brain metabolism: challenges and controversies

Chair: S. Hutson

C03-01

Lactate muscles its way into consciousness: Influence of brain activation on CMRO2/CMR carbohydrate metabolic ratio

GA DIENEL

Univ. Arkansas Med. Sci, Little Rock, AR, USA

The possibility that lactate might be an important fuel for activated neurons has reached a high level of awareness during the past decade but remains unproven *in vivo*. Oxidative metabolism of glucose provides most of the energy for working brain, but the pathways of glucose utilization and energy metabolism appear to shift during activation. For example, under some, but not all, stimulation paradigms in different laboratories, the CMRO2/CMRglc ratio falls below the resting value of 5.5–6 in different brain structures and retina; the global metabolic ratio also falls in humans during strenuous exercise. Thus, non-oxidative metabolism of glucose appears to increase, but lactate accumulation in brain and efflux to blood does not fully account for the 'excess' utilization of glucose, indicating involvement of other processes. Increased lactate production tightly coupled to local lactate oxidation requires stoichiometric increases in oxygen and glucose utilization, which is not observed. In activated retina, most of the glucose taken up is released to blood as lactate. Vigorous muscular exercise raises the blood lactate level and increases lactate uptake into brain; this lactate does not accumulate in brain and is presumably oxidized to support brain functional activity. Inclusion of blood lactate in the total carbohydrate consumed by brain further reduces the oxygen/carbohydrate ratio to as low as 3.5, suggesting that more of the blood-borne glucose is processed by non-oxidative pathways in brain. The contributions of endogenously- and exogenously-generated lactate to brain energy production are not known and are very difficult to quantify *in vivo* due, in part, to technical difficulties of quantitatively measuring complete metabolic balances.

C03-02

Validation of *in vivo* measurements of neuronal/astroglial glutamate trafficking

DL ROTHMAN

Yale University, New Haven, CT, USA

Over the last decade a variety of novel isotopic methods and metabolic modelling strategies have been introduced to measure the rate of neuronal/glial amino acid neurotransmitter trafficking. However these studies have not been without controversy due to the question of how well can neuronal and glial metabolism be distinguished by isotopic labeling methods. To address this question several strategies have been used to validate the measurement of glutamate neurotransmitter trafficking. These strategies may be broadly broken down into three classes: (1) measurement of glutamate trafficking as a function of neuronal activity, (2) comparison of measurements of glutamate trafficking from isotopically labeled compounds (15NH4, 14CO2, [1–13C] glucose, [2–13C] glucose, [1–13C] acetate, [2–13C] acetate, [2–13C] beta hydroxy butyrate) that introduce label at different points in the neuronal and glial TCA and glutamine/glutamate synthesis pathways, and (3) comparison with measurements of glutamate labeling in the extracellular fluid and isolated nerve terminals. The consistency of the findings of these studies strongly support the importance of neuronal/glial glutamate trafficking in maintaining normal glutamatergic function and as a major metabolic pathway. However important questions remain including the role of glutamate oxidation and pyruvate recycling in glutamate neurotransmitter repletion, the effects of pathology on trafficking pathways, and the development of optimal isotopic strategies for quantitating these pathways.

C03-03

The energetics of glutamate/glutamine and GABA/glutamine cycling *in vivo*

KL BEHAR

Department of Psychiatry and Magnetic Resonance Research Center, New Haven, CT, USA

Glutamatergic and GABAergic neurons represent the majority of neocortical neurons. The contribution these neurons make to overall glucose oxidative metabolism in the cerebral cortex and how their neurotransmitter fluxes relate to changes in activity is unknown. Studies of rat cortex *in vivo* using NMR spectroscopy in conjunction with infusions of [1-^{13}C] glucose have revealed that the cycling flux of glutamate (Glu) and glutamine (Gln) between neurons and astroglia is substantial, more than 60% of neuronal glucose oxidation. Measurements in the anesthetized rat at different levels of neuronal activity induced pharmacologically from isoelectricity to bicuculline-seizures, showed that Glu/Gln cycling varied linearly with neuronal glucose oxidation and in a proportional manner above isoelectricity. Studies combining [1,6-$^{13}C_2$] glucose with the glial substrate, [2-^{13}C] acetate, has permitted separate estimates of the glutamatergic and GABAergic neurotransmitter cycling fluxes. We found in anesthetized rats (1% halothane/70% nitrous oxide), that GABAergic neurons comprised ~20% of total (GABA plus glutamate) cycling and glucose oxidation of cortical neurons and that both fluxes had increased above isoelectricity. Because GABA metabolism in the glia directly involves the glial TCA cycle, activity dependence of GABA/Gln cycling indicates that glial oxidation is likely to play a central role in this process.

C03-04

Relationship between malate/aspartate shuttle and glucose consumption in the mammalian brain

KF LANOUE*, DA BERKICH*, Y XU* and SM HUTSON†

**Penn State College of Medicine, Hershey, Pa, †Wake Forest University School of Medicine, Winston-Salem, NC, USA*

Evaluation of brain energy consumption *in vivo* has been a matter of controversy for decades. The problem is a lack of a 'gold standard', such as an accurate measure of brain O2 consumption. Some commonly used methods employ 13C-NMR and trace carbon 13 as it passes from glucose to glutamate where its appearance can be monitored by NMR. Some metabolic models assume rapid equilibrium between glutamate and intramitochondrial α-ketoglutarate in order to justify equating glutamate turnover with citric acid cycle flux. Since equilibration requires extraordinarily high rates of transport across the mitochondrial membrane, this assumption has been challenged. We measured these transport rates using isolated brain mitochondria and found they are of the same order of magnitude as the citric acid cycle flux. Using isolated retinas we also measured 14CO2 production from [U-14C] glucose and initial rates of carbon 14 incorporation into retinal glutamate. The rates are surprisingly similar. Glucose oxidation requires transport of cytosolic reducing equivalents from NADH into mitochondria. This reducing equivalent transport is supplied by the malate/aspartate (M/A) shuttle. However our studies and those of others show there is no M/A shuttle in brain astrocytes. Because there is a 1 : 1 correlation between glucose oxidation and the rate of the M/A shuttle, we conclude that glutamate turnover as measured by 13C NMR closely monitors oxidation of glucose but not citric acid cycle flux especially not citric acid cycle flux in astrocytes.

Acknowledgements: This work was supported by grant NIH R01NS38641.

C03-05

Clairvoyance and confusion: outcome and limits of quantitative TCA cycle flux measurements

R GRUETTER*†, PG HENRY†, G OZ† and K UGURBIL†

**Ecole Polytechniqe Federale de Lausanne, Lausanne, Switzerland, †University of Minnesota, Minneapolis, MN, USA*

The predominantly glial localization of glutamine synthetase, pyruvate carboxylase and glutamine on one hand, and the predominantly neuronal localization of brain glutamate on the other imply that ^{13}C label incorporation into glutamate and then into glutamine by the glutamate-glutamine cycle reflects neurotransmission. However, this is not the only pathway by which label can be incorporated into glutamine, as oxidative metabolism in glia can be substantial as judged from the fact that the labeling of glutamate relative to glutamine is not constant for the C2, C3 and C4 carbons. In addition, the accurate measurement of TCA cycle rates, V_{tca}, needs to consider the fact that isotopic exchange (V_x) across the inner mitochondrial membrane can profoundly influence the labeling of Glu C4. Unfortunately, the magnitude of this exchange V_x is poorly represented in data derived from label incorporation into a single molecule position. The rate of label turnover into glutamate C4 from acetyl-CoA depends on V_x and V_{tca}, whereas the turnover of Asp C3 is almost independent on V_x. Recent advances in sensitivity have shown that V_x can be on the order of V_{tca}, especially in metabolically depressed states, which implies a major regulatory role for e.g. the malate-aspartate shuttle in the *in vivo* brain. In conclusion, an accurate measurement of neuroglial energy metabolism is possible in the *in vivo* brain, provided that the methods address glial oxidative energy metabolism, pyruvate carboxylase and energy-dependent subcellular substrate transport.

Workshop W01: *In vivo* MR tracking of stem cell transplants in the CNS

Chair: J. Bulte

W01-01

Principles and methods for the preparation of magnetically labeled cells

JW BULTE

Johns Hopkins University School of Medicine, Baltimore, MD, USA

As stem cell tracking using MR imaging is now widely pursued, an overview of the currently available methods for intracellular labeling of cells with superparamagnetic iron oxide (SPIO) nanoparticles will be presented. Basic assays for evaluating the effectiveness of SPIO-labeling procedures will also be discussed. Ideally, SPIO-labeling meets the following six criteria: (1) Reliable and fast procedure that can be easily performed by someone not skilled in the art; (2) Internalization of label; (3)Starting iron content >5 pg iron per cell; (4) Unaltered viability and proliferation; (5) Unaltered stem cell differentiation; and (6) Method suitable for clinical SPIO formulations. Initially, the labeling challenge had been to induce sufficient SPIO uptake in non-phagocytic cells. Internalizing antibodies and peptides can be used to meet this challenge but have drawbacks including bioconjugation and specificity. The use of transfection agents (TAs) allows a universal, non-specific SPIO labeling of cells and is currently the preferred technique. The latest development is the use of magnetoelectroporation (MEP); this technique appears most promising as cells can be labeled within seconds without the need for initiating cell cultures. Several methods for determining the cellular iron concentration will be compared, as well as (immuno) histochemical techniques for visualization of SPIO particles. Recent stem cell differentiation studies prove the need of careful cellular functional studies that must be tailored to the specific needs when conducting stem cell therapy. In conclusion, SPIO-labeling of cells is easy to perform and non-toxic when carefully pursued, paving the way for MRI cell tracking studies including its use in clinical cell therapy trials that have recently started in Europe.

W01-02

MR imaging of transplanted cell migration in EAE

T BEN-HUR* and J BULTE†

**Hadassah Hebrew University Medical Center, Jerusalem, Israel,*
†Johns Hopkins University School of Medicine, Baltimore, MD, USA

Central to the future success of stem cell transplantation in demyelinating diseases is the ability of transplanted cells to migrate from the site of transplantation to relevant disease foci. Magnetic resonance (MR) tracking of superparamagnetic iron oxide labeled cells enables to non-invasively determine the biodistribution, speed of migration and survival of transplanted cells. We transplanted magnetically labeled neural stem cells into the ventricles of rodents with experimental allergic encephalomyelitis (EAE), the animal model of multiple sclerosis (MS). High-resolution (microscopic) *ex vivo* MR images showed the migration patterns of newborn rat neural precursor cells into white matter structures of rats, with a good correlation of the corresponding histopathology. Serial *in vivo* MR imaging was then applied to assess the biodynamics of migration of mouse neural stem cells and human embryonic stem cell (hESC)-derived neural precursors in a chronic mouse EAE model. Magnetic labeling did not affect the differentiating potential of the cells *in vitro* and *in vivo*. Transplanted cell migration occurred mainly during the early acute phase of disease, with asymmetric dissemination along white matter tracts. The extent of cell migration correlated well with disease severity, indicating that inflammatory signals modulate transplanted cell migration in a positive manner. In correlation, an increased number of microglia was found in white matter tracts that contained transplanted cells. hESC-derived neural precursors responded to tissue signals of EAE similar to rodent cells. The findings should aid in designing and optimizing cell therapies for (MS) and to obtain better understanding of *in vivo* cell dynamics and cell–tissue interactions.

W01-03

Stem cells for stroke regeneration: an *in vivo* MRI study

M HOEHN, R WEBER, S WEGENER, U HIMMELREICH and P RAMOS-CABRER

Max-Planck-Institute for Neurological Research, Cologne, Germany

Conventional therapeutical strategies for stroke have so far only demonstrated limited success. With the rapid recent advances in stem cell biology, the potential of these cells for tissue replacement and regeneration of cerebral lesions is explored. For such chronic therapeutical approaches, longitudinal studies using noninvasive imaging modalities are attractive in order to assess the effect of the therapeutical intervention. Here, murine embryonic stem (ES) cells were labeled with iron oxide nanoparticles, an MRI contrast agent, using a lipofection procedure. Following implantation of these labeled ES cells into the rat brain two weeks after stroke, their migrational dynamics towards the ischemic lesion was monitored over weeks, leading to massive accumulation of ES cells in the target zone. This process was resolved with high resolution MRI at spatially isotropic 78 μm resolution at scan times of 70 min. At the target zone, the ES cells differentiate into neurons and glia, as demonstrated by immunohistochemical studies. During the spontaneous evolution of the lesioned tissue, delayed degradation of cerebral vessels is observed in the ischemic territory several weeks after stroke, leading to leakage of erythrocytes and resulting in a pronounced hypointensity in T2*-weighted MR images. To discriminate T2*-weighted MR contrast induced by localized vascular origin and stem cell migration, a modulation of the inhalation gas mixture was developed which permits contrast separation of vessels (BOLD effect) and labeled stem cells. This experimental protocol, incombination with fMRI and behavioral tests, will now allow to investigate the therapeutical potential of cell replacement therapy and assess improvement of outcome following this approach.

W01-04

Cell tracking in spinal cord grafts

C SPENGER

No abstract available

W01-05

The use of bimodal contrast agents in the tracking of transplanted stem cells

M MODO

Institute of Psychiatry, King's College London, London, UK

The use of stem cells to replace lost cells and recover impaired functions in neurodegenerative disease is an attractive future therapeutic approach. Nevertheless, to date little progress has been achieved on the vivo monitoring of these transplants. Especially, the non-invasiveness and deep tissue-penetration of magnetic resonance imaging (MRI) is apposite to probe the dynamic nature of stem cell therapy repeatedly *in vivo*. In order to visualize the presence of transplanted stem cells, it is, however, necessary to pre-label stem cells *in vitro* with contrast agents before transplantation, ideally with a bimodal contrast agents that has the added advantage of also being visualized by fluorescence histology. This contrast agent-enhanced approach allowed us to detect the transhemispheric migration of neural stem cells from the contralateral hemisphere into peri-infarct lesion, but also provided evidence of differential graft survival of fetal transplants in immunocompetent and immunsuppressed hosts. The fluorescent properties of bimodal agents further allowed us to corroborate these *in vivo* findings by an independent imaging modality and afford further characterization of grafted cells revealing their phenotypic differentiation. These studies highlight how MRI can be used to monitor stem cell therapy and provide novel insights into the mechanisms responsible for transplant-mediated recovery.

Workshop W02: Oligodendrocyte cell culture models

Chair: A.I. Boullerne & D. Osterhout

W02-01

How related are oligodendrocyte cell lines to primary oligodendrocyte cultures?

J DE VELLIS

No Abstract available

W02-02

Glutamate receptors in oligodendroglia and their progenitors – good, bad or indifferent?

JA BENJAMINS, L NEDELKOSKA, M JOHNSTON and DM STUDZINSKI

Wayne State University School of Medicine, Detroit, MI, USA

Ionotropic and metabotropic glutamate receptors mediate excitotoxicity in cells of the oligodendroglial lineage. Differences in receptor expression *in vivo* versus *in vitro*, and between one culture method versus another, indicate that these receptors are sensitive to regulation by a number of extrinsic signals. We find that mature MBP+ oligodendroglia (OLs) from both mouse and rat exhibit excitotoxicity via low affinity but not high affinity kainate receptors. The mature OLs abundantly express the Group I metabotropic glutamate receptors mGluR1 and mGluR5, which mediate protection from kainate in OLs. Our results agree with several studies *in vitro* and *in vivo*, but differ from other studies that find no evidence of excitotoxicity via either high or low affinity kainate receptors in mature OLs in culture, and very low levels of Group I mGluRs. In our studies, OL progenitors, maintained in a proliferating state by FGF and PDGF, are sensitive to high concentrations of kainate, but not concentrations below 500 *u*M kainate, again indicating the presence of low affinity kainate receptors only. However, other studies report kainate excitotoxicity in OL progenitors via high affinity kainate receptors. We conclude that expression and function of both ionotropic and metabotropic glutamate receptors in OLs can be regulated independently of expression of myelin basic protein and other markers of OL differentiation.

Acknowledgement: Supported by NIH grant NS13143 (JAB).

W02-03

Molecular, functional and developmental properties of NG2-expressing progenitors *in situ* and in culture

V GALLO, R CHITTAJALLU and A AGUIRRE

Ctr. for Neuroscience Res., Children's Res. Institute, Children's Natl. Med. Ctr., Washington, DC, USA

NG2-expressing cells are the largest proliferative progenitor population in the postnatal CNS. Recent studies have raised questions as to the lineage identity of subpopulations of these cells. It remains unclear whether this heterogeneity is paralleled by differing functional properties. Using a transgenic mouse in which EGFP was expressed in NG2+ cells under the control of the CNP gene promoter, we performed patch-clamp analysis of NG2+ cells of the subcortical white (wm) and cortical gray matter (c) regions in P4–P7 brain slices. In the wm, 95% of EGFP+ cells with a 10–30 pF capacitance expressed NG2. Only 10% and 0% of these cells were O4+ or CNP+ , respectively. In contrast, approximately 92% of cells with a 60–90 pF capacitance were O4+, with only 0% and 9% expressing NG2 or CNP, respectively. At this age all cEGFP+ cells were NG2+ (20–70 pF). wm and cNG2+ cells display distinct physiological properties. A subpopulation of cNG2+ cells displayed properties of immature neurons, i.e. expression of b-tubulin III and TTX-sensitive spikes upon depolarization. This latter phenomenon was associated with a significantly higher density of voltage-activated Na+-channels and a lower density of the delayed outward rectifying K+-channels, as compared to non-spiking cNG2+ and wmNG2+ cells. FACS-purified cNG2+ cells generated a significant percentage of TUJ+ and NeuN+ neurons after 4 days in culture, thus indicating the potential to give rise to a neuronal population. Our data demonstrate that cNG2+ cells display properties that are distinct form those expected in wmNG2+ cells and therefore raise questions as to the role and identity of a subpopulation of gray matter cells expressing NG2.

W02-04

Molecular differences between neonatal and adult oligodendrocytes

DJ OSTERHOUT

Department of Neurosurgery, SUNY Upstate Medical University, Syracuse, NY, USA

Oligodendrocyte progenitor cells exist in the adult brain, and can migrate, differentiate and form myelin similar to neonatal progenitors. Adult progenitor cells can also be isolated and maintained in culture; however, they exhibit different growth properties, and do not differentiate as easily as their neonatal counterparts. This difference is also observed *in vivo*, where studies have demonstrated the presence of adult progenitor cells that do not readily form myelin near demyelinating lesions. The reluctance of older progenitor cells to myelinate may be attributed to age-related molecular changes that hinder their differentiation. We have examined the molecular differences between neonatal and adult oligodendrocytes. Primary cultures of oligodendrocyte precursor cells were established from rat brain at several ages. These were further expanded *in vitro* in serum-free defined media, in the presence and absence of growth factors. The purity of the cultures was assessed using immunocytochemical analysis of surface markers prior to biochemical studies. In our work, we have observed changes in the expression and activation of Src tyrosine kinases as a function of cellular age. There are differences in the expression of growth factor receptors, including the platelet derived growth factor receptor (PDGFR) and fibroblast growth factor receptors (FGFR). Moreover, long-term culture in growth factors alters the expression of certain molecules in adult cells, but not the neonatal cells. These findings suggest that cellular signaling can change with age, which may impact the biology and behavior of adult oligodendrocyte progenitor cells.

W02-05

Biology of adult human oligodendrocytes

AI BOULLERNE*, DM FRIM† and BG ARNASON*

**Department of Neurology, University of Chicago, †Section of Neurosurgery, University of Chicago, Chicago, IL, USA*

Purified adult human oligodendrocyte progenitor cells (OPC) are able to regenerate myelin when grafted into mouse brain and to proliferate when exposed to growth factors *in vitro*. However, OPC from adults also express markers found on mature oligodendrocytes (OLG) such as galactocerebroside (GalC) and myelin oligodendrocyte glycoprotein (MOG) that are not expressed by newborn rodent or fetal human OPC. We set out to characterize the cycling and maturation properties of cultured OPC isolated from drug-intractable epileptic patients. 15% of isolated cells expressed the OPC marker chondroitin sulfate proteoglycan NG2 as revealed by immunostaining, and the other 85% the maturing OLG marker GalC. Further maturation of OLG proceeded at a slow pace and a fully mature phenotype with flat membrane extensions and positivity for GalC, myelin basic protein (MBP), myelin associated glycoprotein (MAG) and MOG was not reached before 2 weeks in culture. Most cycling OPC, defined by BrdU incorporation, expressed NG2 and Notch-1, but only rarely GalC or MBP as determined by immunodetection. Surprisingly, unlike fetal human OPC, they never expressed platelet-derived growth factor receptor (PDGFα-R). Rather, PDGFα-R was found at early stages of maturation in bi-to multipolar cells, and was lost on more mature OLG that expressed O4 sulfatide. This finding points to a substantial difference between fetal OPC and OPC from mature human brain. After a month in culture we consistently found two third mature OLG and one third small round cells that expressed NG2, GalC, MBP or PDGFα-R. These cells appear to comprise a pool of slowly proliferating NG2+ OPC that have not yet entered the maturation path.

W02-06

Inter-regional differences of neonatal and adult oligodendrocytes

RH MILLER

Department of Neurosciences, Case Western Reserve University School of Medicine, Cleveland, OH, USA

The localized origin of oligodendrocyte precursors is a characteristic of all regions of the CNS so far assayed. In caudal regions such as spinal cord local signaling mechanisms have been extensively studied. Here Sonic hedgehog (Shh) derived initially from the notochord induces ventral cell fate in the floorplate and motor neurons. Continued expression of Shh facilitates the subsequent appearance of oligodendrocyte precursors. Recent studies demonstrate a second source of spinal cord oligodendrocytes that develop from dorsally derived cells. These cells have a different developmental profile and response to growth factors. Whether they are targeted to distinct functions has yet to be determined. In more rostral CNS regions a similar phenomena of multiple restricted locations of oligodendrocyte precursors is evident. Whether similar signaling mechanisms mediate their appearance is unknown. One attractive hypothesis is that local cell–cell interactions mediate the induction of oligodendrocytes. For example, the precursors for a subset of optic nerve oligodendrocytes develop from a group of cells in the floor of the third ventricle directly dorsal to the optic chiasm. A variety of studies indicate that the initial appearance of these cells is dependent on cues from retinal ganglion cell axons that include Shh and neuregulin. It seems likely the generation of adult oliogodendrocyte precursors will be mediated by multiple mechanisms and the development of appropriate therapeutic interventions will require a detailed understanding of both intrinsic oligodendrocyte precursor biology and the local environmental cues.

Special Session SS01: Cutting edge discoveries and scientific advances

Chair: R. Miskimins

SS01-01

Expression Arrest™ short hairpin RNA libraries: solutions for transient, stable and *in vivo* RNA interference

G FEWELL

Open Biosystems Inc., Huntsville, AL, USA

Expression Arrest™ whole genome human and mouse short hairpin RNA (shRNA) libraries from Open Biosystems are already cloned into retroviral vectors and are ready-to-use for gene silencing studies. shRNA constructs are expressed as microRNA-30 precursors which has been shown to greatly increase knockdown efficiency. This vector-based system is amenable to *in vitro* and *in vivo* applications such as the creation of stable knockdowns. Molecular barcodes are included in each shRNA vector enabling RNAi screens with pools of shRNA. Possible approaches for screening such whole genome RNAi collections will also be discussed. These include but are not limited to (1) screening individual shRNAs in multiwell format for activation or repression of a reporter or activity in a cell based or biochemical assay, (2) infecting cells with pools of shRNA followed by positive selection screens, (3) monitoring in mass transduced pools of cells, via molecular barcodes contained within the shRNAs, by microarrays containing the complement of barcode sequences to detect relative changes in shRNA representation following application of a selective stimulus.

SS01-02

The HaloTag™: a novel technology for cellular analysis

GV LOS*, C ZIMPRICH*, MG MCDOUGALL†, N KARASSINA*, R LEARISH*, DH KLAUBERT†, A DARZINS*, RF BULLEIT* and K WOOD*

**Promega Corporation, Madison, WI, †Promega Biosciences, Inc., San Luis Obispo, CA, USA*

The HaloTag™ Interchangeable Labeling Technology is a novel tool for (1) imaging live or fixed mammalian cells that express the HaloTag™ Protein or protein fusions, (2) analyzing posttranslational modification of labeled proteins, (3) capturing and immobilizing protein fusions and protein complexes generated in living cells or *in vitro*. In live cell imaging the HaloTag™ Technology works in a manner similar to GFP except that the fluorophore can be interchanged among a variety of standard dyes. This allows imaging of live cells at different wavelengths without requiring changes to the underlying genetic constructs. The color can be rapidly switched to allow temporal and spatial analysis of protein fate. The dyes can also be exchanged with other functional groups, such as biotin to serve as an affinity handle for capturing and/or immobilization of proteins. Because all functional groups are covalently attached to the HaloTag™ Protein, labeled protein can be analyzed under denaturing conditions. The interchangeability allows visualization of proteins in living cells before alternative groups are attached to the HaloTag™. The HaloTag™ Technology complements existing methods and provides new options for cellular analysis.

Oral Session O01: Neurotoxicology

Chair: R. Wiggins and N. Banik

O01-01

Estrogen treatment and immunoselection of basal forebrain cholinergic neurons

K BENNETT, C HOELTING and J STOLL

Texas Tech School of Pharmacy, Amarillo, TX, USA

Women have a 2–3 fold greater risk of developing Alzheimer's disease (AD) than men, an effect that may be related to low post-menopausal estrogen levels. This project investigated the effect of estrogen on the function of basal forebrain cholinergic neurons (BFCNs), which degenerate in AD. We hypothesized that estrogen stimulates high-affinity choline transport (HACT). HACT is considered to be the rate-limiting step in acetylcholine synthesis. Primary neuronal cultures were prepared from the basal forebrain of embryonic rats, and the BFCNs were identified by the presence of cells immunostained for the vesicular acetylcholine transporter (VACht). These cultures were further characterized by flow cytometry. HACT levels were determined by the measurement of hemicholinum-3-sensitive choline uptake. Cells were either treated with a series of estrogen concentrations, or estrogen treatment was administered for varying time periods. Estrogen treatment increased HACT and/or cholinergic cell number (30–100%) in some but not all experiments. The variable presence of non-cholinergic cells between preparations could modulate BFCN response to estrogen, which may explain the negative results in some experiments. Thus in some instances estrogen treatment can stimulate HACT and cell survival, but the conditions that determine this variable effect have yet to be elucidated. Immunoselection experiments were also conducted in order to generate enriched cultures of cholinergic neurons. Magnetic separation techniques were performed using an antibody for the low-affinity nerve growth factor receptor, p75. Experiments showed up to a 5.5-fold increase in cholinergic neurons. After optimization, the immunoselection of BFCNs promises to be a valuable technique in the study of these neurons.

O01-02

Cytokines, lipid metabolism, and CDP-choline in stroke

RM ADIBHATLA, J HATCHER, K TUREYEN, F TSAO and R DEMPSEY

Univ. of Wisconsin, Madison, WI, USA

TNF-α and IL-1α/ß are rapidly up-regulated in the brain following stroke, and contribute to ischemic injury. Phosphatidylcholine (PC), a major membrane phospholipid, is hydrolyzed by phospholipase A_2 (PLA_2) and PC-phospholipase C (PC-PLC). PC loss is sufficient in itself to induce cell death. TNF-α and IL-1α/ß activate PLA_2 and PC-PLC to hydrolyze PC. PC hydrolysis by PLA_2 releases free fatty acids including arachidonic acid, and lyso-PC, an inhibitor of CTP-phosphocholine cytidylyltransferase (CCT), the rate-limiting enzyme in PC synthesis. Arachidonic acid oxidative metabolism is a significant source of ROS. Brain PC levels are regulated by a balance between synthesis and hydrolysis. TNF-α/IL-1ß may disrupt PC homeostasis by increasing its hydrolysis (PLA_2, PC-PLC) and inhibiting its synthesis (CCT). The beneficial effects of CDP-choline in CNS injury may be elicited by attenuating TNF-α/IL-1 mediated events, integrating cytokine biology-lipid metabolism. We have shown that CDP-choline treatment attenuated TNF-α and IL-1ß levels, PLA_2 activity, loss of CCT activity, hydroxyl radical generation, lipid peroxidation, and phospholipid hydrolysis. A tentative mechanism has been proposed. CDP-choline stroke clinical trials in Europe and Japan showed significant improvement, while the USA trials gave ambiguous results. Liposome encapsulation of CDP-choline greatly increased brain uptake over i.v. and oral routes in rats. Our data show that liposome encapsulated CDP-choline significantly reduced infarct after transient middle cerebral artery occlusion in rat. CDP-choline is non-xenobiotic, safe, well tolerated, and its use in stroke treatment may still be achievable by more efficient delivery methods.

Acknowledgements: Supported by NIH & VA.

O01-03

Manganese-induced astrocyte swelling: role for the low grade brain edema in chronic hepatic encephalopathy

KV RAMA RAO*, AS HAZELL† and MD NORENBERG*

**Dept. Pathology, Univ. Miami School of Medicine, Miami, USA,*
†Dept. Medicine, Univ. Montreal, Montreal, Canada

Manganese in excess is neurotoxic. While the mechanisms of manganese neurotoxicity are incompletely understood, oxidative as well as nitrosative stress and mitochondrial dysfunction have been shown to play major roles. One neurological disorder associated with increased manganese deposition is chronic hepatic encephalopathy (CHE). Low-grade brain edema has been reported in CHE, that is believed to be due to astrocyte swelling. However, the mechanisms of brain edema in CHE are not well understood. We have reported that in cultured astrocytes manganese dose-and time-dependently induced the mitochondrial permeability transition (MPT), which was blocked by cyclosporin A (CsA). We hypothesized that oxidative stress and/or the MPT may lead to astrocyte swelling. Accordingly, we examined the effect of manganese on cell volume in cultured astrocytes. Cells were exposed to manganese (Mn^{3+}; 25 μM) for 2 days and the cell volume was determined by the [^{3}H]-3-O-methylglucose method. Manganese caused a significant (42%, $P < 0.05$) increase in cell volume in cultured astrocytes. Pretreatment with antioxidants (vitamin E, desferroximine, PBN) as well as the nitric oxide synthase inhibitor L-NAME, completely blocked manganese-induced astrocyte swelling, suggesting the involvement of oxidative and nitrosative stress in this process. Likewise, CsA completely inhibited the astrocyte swelling by manganese suggesting a role for the MPT as well. These data suggest that oxidative stress and the MPT contribute to the brain edema of conditions associated with increased brain manganese levels, such as chronic hepatic encephalopathy.

Acknowledgements: VA Merit Review & NIH (DK063311).

O01-04

Hydrogen sulfide as a scavenger of HOCl

TM JEITNER*, MA HORSWILL* and G MORAN†

**Medical College of Wisconsin,†University of Wisconsin at Milwaukee, Milwaukee,WI, USA*

Hypochlorous acid (HOCl) is increasingly being recognized as an important mediator of cell death in the brain. This powerful oxidant is formed by myeloperoxidase, as the enzyme reduces hydrogen peroxide to water. Myeloperoxidase is found in both neurons and glial cells. Moreover, the amount of myeloperoxidase and its reaction products are increased in Alzheimer Disease brains. Glutathione (GSH) provides most cells with sufficient protection against HOCl-mediated damage. This protection is based on the rate at which the thiol of GSH reacts with HOCl (>10e7 M/s) and the rapid turnover of GSH. The brain has a very slow turnover of GSH (60–80 h) and consequently is at risk of damage from HOCl. The brain also produces 50–160 mM concentrations of hydrogen sulfide. Given that the scavenging of HOCl by GSH is due to the thiol group, we hypothesized that hydrogen sulfide also scavenges HOCl. The aim of our study was to test this hypothesis. Physiological concentrations of hydrogen sulfide protected retinoic acid-differentiated SH SY5Y cells from the toxicity of HOCl. Moreover, hydrogen sulfide prevented the inactivation of glyceraldehyde 3-phosphate dehydrogenase and the alpha-ketoglutarate dehydrogenase complex, as well as, the oxidation of GSH by HOCl. The addition of hydrogen sulfide to inactivated glyceraldehyde 3-phosphate dehydrogenase also restored a significant proportion of enzyme activity. Stopflow studies indicated that hydrogen sulfide and HOCl react at a rate comparable to that of HOCl and GSH. Thus, hydrogen sulfide may act to scavenge and thereby, protect the brain from the toxicity of HOCl.

O01-05

Neuroprotection by noradrenaline: effects on neuronal IkB-alpha and PPAR receptors

JM MADRIGAL, C DELLO RUSSO, V GAVRILYUK and DL FEINSTEIN

Department of Anesthesiology, University of Illinois at Chicago, Chicago, IL, USA

Previous studies have shown that neuronal cultures are damaged if incubated with media from activated microglial cells. In the current study, we have investigated the factors that mediate this type of damage, and tested if the neurotransmitter noradrenaline (NA) could reduce neuronal damage. Primary neurons were prepared from embryonic day 16 cerebral cortices, and cultured in neurobasal medium to reduce glial contamination. Primary microglial cells were prepared from post natal day 1 rats, and conditioned media (CM) obtained from cells activated for 24 h with 1 μg/ml bacterial endotoxin lipopolysaccharide (LPS). Incubation of neurons with microglial CM results in significant damage assessed by LDH release. However, neuronal damage was significantly less if we used CM activated with LPS plus NA (NA-CM), or if NA was directly added to the neurons. The factors present in CM that induce neuronal damage are not yet known, but initial data using IL1R antagonist and TNFα blocking antibodies indicates a role for these two cytokines, both known to be potent activators of the transcription factor NFkB. Analysis using QPCR and western blots showed that NA increased neuronal mRNA and protein levels of the inhibitory IkBα subunit of NFkB, suggesting that one possible mechanism of action of NA could be inactivation of NFkB, and therefore reduced NOS2 expression. Neuronal damage was also induced if cells were treated with aggregated oligomeric Amyloid-β (Aβ)1-42, or with glutamate 10 μM, and in both cases damage was reduced by NA, or by incubation with the anti-inflammatory drug pioglitazone which binds to the PPARγ receptor. We therefore tested if the protective effects of NA could be due in part to changes in neuronal PPARγ expression, observing increased levels of PPARγ and δ mRNA in neurons treated with NA. Our results suggest that neuroprotective effects of NA may be due to multiple effects, including changes in IkBa and activation of neuronal PPARs.

O01-06

Comparison of pharmacological profiles of volume-regulated Cl-currents and excitatory amino acid release in cultured astrocytes

AA MONGIN*, IF ABDULLAEV*†, A RUDKOUSKAYA* and HK KIMELBERG*

**Albany Medical College, †Ordway Research Institute, Albany, NY, USA*

Ubiquitously expressed volume-regulated anion channels (VRACs) are permeable to a variety of inorganic and small organic anions. VRACs are critical for cell volume regulation and participate in many other cell functions. In the brain, VRACs are thought to mediate a substantial portion of the pathological release of excitatory amino acids (EAAs) from swollen astrocytes in ischemia and other neurological disorders. In the present study we used a pharmacological approach to screen for anion channels and transporters contributing to EAA release from cultured rat astrocytes, and compared the pharmacological profiles of swelling activated EAA release and VRAC currents measured by a patch-clamp technique. The plasmalemmal VDAC inhibitor Gd^{3+} (30 μM), the ClC-2 blocker Cd^{2+} (300 μM), an antagonist of calcium-sensitive chloride channels (CLCA) niflumic acid (100 μM), and an inhibitor of MDR-1 pump verapamil (100 μM) had little or no effect on swelling-activated EAA release and VRAC currents. In contrast, both EAA release and VRAC currents were potently suppressed by the broad spectrum anion channel blockers NPPB (100 μM) and tamoxifen (10 μM). 100 μM phloretin, which at this concentration inhibits VRAC but not CFTR, strongly inhibited both EAA release and VRAC currents. These data imply that VDAC, ClC-2, CLCA, MDR-1 and CFTR do not contribute to either astrocytic volume-regulated EAA release or VRAC currents. The similar pharmacological profiles support the view that EAA release and VRAC currents are mediated by the same membrane permeability pathway.

Acknowledgements: Supported by NINDS grant NS35205.

O01-07

Presence of MPP^+ and motoneuron apoptosis with calpain activation in spinal cord of mice with MPTP-induced parkinsonism

S SAMANTARAY, SK RAY and NL BANIK

Department of Neurology, Medical University of South Carolina, Charleston, SC, USA

Parkinsonism is a major movement disorder characterized by progressive degeneration of the dopaminergic neurons in the substantia nigra (SN). Existing therapies are symptomatic and unable to prevent progression of the disease. An understanding of the scope and mechanism of this disease may lead to development of new therapeutic strategies. So, we explored a possibility of involvement of spinal cord (SC) in the pathophysiology of MPTP-induced parkinsonism in C57BL/6N mice. Injection of 25 mg/kg of MPTP (ip twice, 6 h apart) could render moderate toxicity with apoptosis in SN at 24 h. Then, we investigated generation of the active toxin MPP^+ from MPTP in SC. Using sensitive HPLC-photodiode array, we measured levels of MPP^+ in whole SC homogenate as well as in striata after administration of single dose of MPTP (25 mg/kg). We detected generation of MPP^+ in the SC, starting 1 h and getting cleared 4 h after the injection of MPTP. MPP^+ levels in SC ranged from one-fifth to one-eighth of that found in the striata. Detection of MPP^+ in SC indicated a possibility of neurodegeneration in SC. TUNEL assay identified more apoptosis in ventral than dorsal horns of lumbar SC. Western blottings showed increased expression and activation of calpain in SC in mice with MPTP-induced parkinsonism by day 1. Further, TUNEL assay and double immunofluorescent labeling specifically detected apoptosis of ChAT-positive motoneurons in SC by the 7^{th} day of MPTP neurotoxicity. Taken together, results showed generation of MPP^+ and degeneration of motoneurons with activation of calpain in SC in mice with experimental parkinsonism.

Acknowledgements: Supported in part by the grants from the NIH and the state of South Carolina.

Public Forum PF01: Focus on autism

Chair: M. Carson

PF01-01

Is there brain inflammation in autism?

CA PARDO

No abstract available.

PF01-02

Immune proteins in normal brain development and plasticity: possible implications for autism

L BOULANGER

No abstract available.

Poster Session PSM01: Neurogenesis and regeneration

PSM01-01

Critical involvement of CD4+ T-lymphocytes in axotomy-induced PACAP gene expression in mouse facial motor neurons

JA WASCHEK, BD ARMSTRONG, C ABAD, S CHHITH, WI RODRIGUEZ, G CHEUNG-LAU and D NGO

University of California at Los Angeles, Los Angeles, CA, USA

The neuropeptide pituitary adenylyl cyclase activating peptide (PACAP) is strongly induced in neurons following several types of injury, and exhibits neuroprotective and regenerative actions *in vitro* and *in vivo*. It is thought that changes in expression of neuropeptides and other molecules in injured neurons are mediated by new factors produced in Schwann and immune cells at the injury site and/or alternatively, loss of target-derived factors. To begin to determine the role of the inflammatory mediators, we investigated axotomy-induced changes in PACAP gene expression in the facial motor nucleus in severe combined immunodeficient (SCID) mice, and in mice replaced with lymphocytes and lymphocyte subset populations. In normal mice, PACAP mRNA was strongly induced in facial motor neurons 4 days after axotomy. The increase in PACAP mRNA was blocked in SCID mice, but fully reversed by an infusion of normal splenocytes, suggesting that the induction of PACAP mRNA requires inflammatory mediators. Because CD4+ lymphocytes appear to be neuroprotective in facial nerve and other injury models, we also studied PACAP gene expression in SCID mice preinfused with CD4+ enriched splenocytes. Infusion of CD4+ enriched splenocytes restored the number to a value not significantly different than controls. The CD4+ cell-dependent induction of PACAP in motor neurons may thus be a factor in the cascade of events triggered by immune cells that ultimately leads to nerve regeneration.

PSM01-02

Neurogenesis in the lesions of multiple sclerosis

MC SMITH, A CHANG, SM STAUGAITIS and BDTRAPP

Department of Neurosciences, The Lerner Research Institute, The Cleveland Clinic Foundation, Cleveland, OH, USA

Subcortical white matter (WM) in the human CNS contains a population of resident neurons, which are believed to serve several functions, including regulation of blood flow. By MAP2 immunocytochemistry, WM neurons have pyramidal or fusiform soma with one to three major dendrites oriented parallel to the myelinated fibers. Synaptophysin positive dots closely oppose neural perikarya and dendrites, suggesting that WM neurons receive synaptic input. Similar to cortical interneurons, subsets of WM neurons are positive for nNOS, somatostatin, and calbindin. The density of WM neurons is highest close to the cortex and gradually decreases the further one goes into the deep WM. To determine whether inflammatory demyelination affects these neurons, we investigated their density in subcortical WM from control and multiple sclerosis (MS) brains. In acute MS lesions, WM neurons were absent or dystrophic, with short, fragmented dendrites, indicating that these neurons were destroyed during the demyelinating process. While most chronic lesions also lacked neurons, a subset of chronic lesions contained dense clusters of MAP2-positive neurons with extensive dendritic projections that were opposed by substantially increased punctate synaptophysin immunoreactivity as compared with control WM neurons. In this subset of lesions there was also a 57.1% increase in NeuN-positive/MAP2-negative cells, suggesting an increase in cells committed to the neuronal lineage that have not yet differentiated into mature neurons. These data support the hypothesis that neurogenesis occurs in a subset of MS lesions.

PSM01-03

A recombinant human IgM promotes remyelination at doses analogous to a growth factor

AE WARRINGTON*, AJ BIEBER*, V VAN KEULEN†, B CIRIC†, LR PEASE† and M RODRIGUEZ*

**Dept of Neurology, †Dept of Immunology, Mayo Clinic, Rochester, MN, USA*

The promotion of remyelination is an important therapeutic goal in the treatment of demyelinating diseases. rHIgM22 is a recombinant human IgM that localizes *in vivo* to CNS lesions and promotes the synthesis of new myelin in models of demyelination. The minimum dose of remyelination-promoting mAbs required for a biologic effect has a direct bearing on the proposed mechanism of action. Prior studies administered 25 mg/kg of rHIgM22 intraperitoneally. A dose-ranging study using rHIgM22 was performed in mice with chronic virus-induced (TMEV) demyelination, a model of multiple sclerosis. Five weeks following a single mAb dose spinal cords were assessed histologically. Doses of rHIgM22 as low as 250 μg/kg resulted in significantly more remyelination ($P = 0.006$). A time course study performed in chronically demyelinated mice revealed that remyelination is significantly increased by rHIgM22 three weeks following mAb administration and plateaus by five weeks. An additional dose of rHIgM22 did not increase the degree of remyelination over a single dose. This study demonstrates that rHIgM22 is efficacious at *in vivo* concentrations typically observed with growth factors. The data suggests that the mechanism of action of rHIgM22 is direct, for example binding to cells rather than indirect, such as blocking pathogenic factors or opsinizing debris. The action of rHIgM22 is likely amplified by CNS immune or glial cells. Because a single low dose is required for activity, human remyelination-promoting mAbs are potentially practical and safe therapeutic agents to increase endogenous tissue repair.

PSM01-04

Neural stem cell transplantation reduces brain GM2 and GA2 content in a mouse model of Sandhoff disease

RC BAEK*, JP LEE†‡, TN SEYFRIED* and EY SNYDER‡

**Department of Biology, Boston College, Chestnut Hill, MA, †Department of Pediatrics, UCSD School of Medicine, ‡The Burnham Institute, La Jolla, CA, USA*

Sandhoff disease is a glycosphingolipid (GSL) lysosomal storage disease that arises from an autosomal recessive mutation in the gene for the β-subunit of β-Hexosaminidase A (*Hexb* gene), the enzyme that helps catabolyze GM2 within lysosomes. Accumulation of GM2 and asialo-GM2 (GA2) occurs primarily in the CNS, leading to progressive neurodegeneration and brain dysfunction. Neural stem cell (NSC) transplantation aims to cross-correct the deficient lysosomal enzyme thereby reducing ganglioside storage. *Hexb* –/– (129/SV) mice were injected intracerebroventricularly with NSCs from clone C17.2 at postnatal day 1 (p-1), and the mice were analyzed at p-30. Hexosaminidase A activity in the *Hexb* –/–mice was about 2-fold higher in the NSC transplanted mice than in the non-transplanted mice. The concentrations (μg/100 mg dry wt) of brain GM2 and GA2 in the non-transplanted *Hexb* –/–mice were 309 ± 26 μg and 975 ± 37 μg, respectively, but were undetectable in the *Hexb* +/–mice. Brain GM2 and GA2 content in the NSC transplanted *Hexb* –/–mice was 197 ± 22 μg and 709 ± 86 μg, corresponding to a 36% and a 27% reduction compared to the untreated mice, respectively. These results suggest that NSC may be an effective cross-correctional therapy for the management of GSL storage diseases.

Acknowledgements: Supported by NIH grant (HD39722), NICHD funds, and NTSAD.

PSM01-05

LPA, endocannabinoids, and their receptors in the development of neural progenitors

SI SVETLOV*, VG KUKEKOV†, KK WANG* and RL HAYES*

**University of Florida, Neuroscience, Gainesville, FL, †University of Tennessee, Memphis, TN, USA*

Identification of molecular mediators for directed manipulation of stem/progenitor cells is essential for cell therapy and stimulation of endogenous neurogenesis. We have shown that pro-survival lipid lysophosphatidic acid (LPA) instigated clonal generation of mouse neurospheres via LPA receptor-dependent proliferation of AC133 positive neural progenitors. Given a structural and receptor homology between LPA and endocannabinoids anandamide(AEA) and 2-arachidonoyl glycerol (2-AG), we suggested that the endocannabinoid system may play a role in development of neural progenitors. A strong expression of cannabinoid receptors CB1 was found in mouse clonal neurospheres generated by EGF/FGF2 or LPA. CB1 receptor was expressed in the core of neurosphere and partially co-localized with LPA receptor and AC133. In neurospheres differentiated on an adherent matrix with EGF/FGF2, CB1 expression was restricted to cells of neuronal lineage. In contrast, neurospheres developed in the presence of LPA accumulated CB1 receptor in clusters of AC133 positive primitive progenitors. Interestingly, there was a partial co-localization of CB1 and GFAP in the core of the LPA neurospheres, suggesting a possible role of CB1 in astrocyte precursors. Finally, synthetic endocannabinoids sustained growth of neurospheres from dissociated mouse and rat postnatal forebrain, while AM251, a selective CB1 receptor antagonist, abolished the generation of clonal neurospheres. Because AEA, 2-AG, LPA, and their receptors are up-regulated upon traumatic brain injury, and appear to be pro-survival and mitogenic for neural progenitors, the endocannabinoid/LPA system may play a role linking neuroprotection and neurogenesis during brain injury.

PSM01-06

Controlling transgene expression by lentiviral infection of human neural progenitor cells

CR SEEHUS, BL SCHNEIDER, EE CAPOWSKI and CN SVENDSEN

University of Wisconsin, Waisman Ctr, Madison, WI, USA

Human neural progenitors (hNPCs) derived from the fetal cortex are a source of primary, neurally committed tissue capable of proliferation *in vitro*, as well as differentiation into neurons and astrocytes. They represent both a useful model for studying neurological diseases and a potential therapeutic tool. We are interested in establishing transgenic lines over-expressing disease genes or growth factors. As a first step, we present a molecular analysis of lentiviral delivery in hNPCs, using the green fluorescent protein (GFP) transgene driven by the constitutive mouse phosphoglycerate kinase promoter. We present a method to infect ≥80% of hNPCs using a lentiviral vector, and expand them as neurospheres. Lentivirus was used in concentrations ranging from 11 to 300 ng of p24 per million hNPCs. The number of inserted transgene copies and their relative transcription levels were determined by quantitative PCR. Following infection, cultures were expanded for up to 75 days, and transgene expression monitored by flow cytometry. Transgene stably integrated and GFP expression directly correlated with the dose of virus administered to the hNPCs. Data collected 40 and 75 days post infection showed remarkable stability of GFP expression in the transgenic hNPCs, which retained their ability to grow and differentiate. GFP expression was maintained in neurons and astrocytes differentiated for three weeks from long-term expanded hNPCs. Given the lentiviral infection efficiency and stability of transgene expression shown in NPCs, there is great promise for this system in neurobiological applications. Such applications include therapeutic gene delivery and disease modeling, in which genetic analysis in hNPCs could provide greater understanding of neuropathogenesis.

PSM01-07

Perinatal hypoxia/ischemia enhances EGF responsiveness of SVZ neural stem/progenitors

D ALAGAPPAN*, RJ FELLING* and SW LEVISON*†

**Departments of Neural and Behavioral Sciences, Penn State College of Medicine, Hershey, PA, †Neurology and Neurosciences, UMDNJ-NJMS, Newark, NJ, USA*

Perinatal hypoxia/ischemia (H/I) is the leading cause of neurologic injury during birth complications resulting in sequelae such as cerebral palsy, epilepsy, and cognitive deficits; thus, we investigated the effects of this injury on the subventricular zone (SVZ). The neural stem cells in SVZ form colonies called neurospheres *in vitro*. Using the Vannucci 6-day-old rat pup H/I paradigm, 3 days recovery from perinatal H/I shows twice as many neurospheres generated from the injured SVZ, and the neurospheres are noticeably larger after an identical time *in vitro* compared to uninjured control cells. The larger spheres are observed in the presence of EGF and the combination of EGF and FGF-2, but not in the presence of FGF-2 alone. A cumulative thymidine incorporation assay reveals that the SVZ cells from the damaged brain have a shorter cell cycle time and greater proportion of proliferation vs. controls. EGF receptor expression within damaged SVZ is increased twofold at the mRNA and protein level. By contrast, the levels of transforming growth factor alpha mRNA are reduced by 50% and levels of EGF and heparin-binding EGF mRNAs remain unchanged. These data demonstrate that H/I injury enhances the sensitivity of SVZ neural stem/progenitor cells to EGF ligands to stimulate their proliferation and could account for mobilizing the neural stem cells following perinatal H/I. Understanding the molecular mechanisms underlying this expansion may allow therapeutic interventions to restore normal pattern of development following perinatal H/I.

Acknowledgements: Supported by MH 59950 (SWL) and NS0469903-01 (Felling).

PSM01-08

FGF-2 is sufficient but not completely necessary for neurogenesis from hNPCs

AD NELSON*† and CN SVENDSEN*†‡

**Neuroscience Training Program, †Waisman Center, ‡Department of Anatomy, University of Wisconsin-Madison, Madison, WI, USA*

Human neural progenitor cells (hNPCs) derived from 8–15 week old cortices require cell division to produce neurons. Multiple exogenous factors have been suggested to promote the final round of cell division necessary for neurogenesis to occur. However, the availability of these factors being endogenously produced remains unknown. We examined the expression of FGF-2 during periods of neurogenesis from hNPCs because FGF-2 is produced by the hNPCs. We found that FGF-2 levels remain low during the peak of neurogenesis but rise following the peak of neurogenesis. hNPCs responded to low level FGF-2 stimulation by phosphorylating CREB. Additionally, hNPCs plated at low densities responded to low concentrations of FGF-2 by increasing neuronal output. This effect was only partially blocked by the addition of FGF-2 neutralizing antibodies. Therefore, we conclude that low concentrations of FGF-2 are sufficient for promoting neurogenesis yet are not completely necessary for neuronal production from hNPCs. These results implicate a role for FGF-2 in neurogenesis but suggests that other factors are playing a role in the birth of new neurons from hNPCs.

Acknowledgements: Supported by the UW Foundation and Children's Neurological Solutions.

PSM01-09

Spatiotemporal expression of GM1 in murine medial pallial neural progenitor cells

RK YU, MB DINKINS, CY SU and SS LIOUR

IMMAG, Medical College of Georgia, Augusta, GA, USA

The expression of gangliosides is developmentally regulated in the central nervous system. The expression of GM1 in the neural progenitor cells of telencephalonic ventricular zone (VZ) has been reported in several studies. However, information of the spatial and temporal regulation of GM1 expression in VZ is still lacking. In this study, we characterized the expression of GM1 in the developing mouse telencephalon. At E13, in addition to the expression of GM1 in neuronal cells, the expression of GM1 in the VZ is also detected. The initial expression of GM1 in the VZ is restricted to regions close to the medial pallium. Fluorescent activated cell sorting (FACS) analysis and characterization of E14 GM1 positive cells showed that they contain progenitor cells that proliferate in response to EGF and/or bFGF stimulation. The results obtained from quantitative gene expression analysis using real time PCR suggest that FACS of GM1-expressing cells in the fetal forebrain enriches the medial pallial neural progenitor cells.

PSM01-10

EAAT2 expression by neural progenitor cell derived astrocytes

SM KLEIN and CN SVENDSEN

University of Wisconsin-Madison, Madison, WI, USA

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by progressive loss of spinal cord, motor cortex, and brainstem motor neurons. The mechanism of selective motor neuron degeneration is unknown but recent insight has shown motor neuron dependence on mediators of the direct extracellular environment. Astrocytes may be a critical link in the support of motor neuron health. Dysfunction or loss of the glial glutamate transporter EAAT2 in ALS may be involved in promoting motor neuron degeneration. Therefore, preservation of motor neurons may rely on the addition of glutamate transporters to the ALS spinal cord. Neural progenitor cells (NPC) are a renewable source of astrocytes. They can be expanded in culture and transplanted into the spinal cord of ALS animal models. The derivation of mature, functional astrocytes from NPC is not well characterized. We will describe the creation of appropriate NPC derived astrocytes for future therapeutic transplantation in ALS. Astrocytes derived from human, wild-type rat, and ALS mutant (superoxide dismutase 1, $SOD1^{G93A}$) rat NPC will be compared in their expression of EAAT2. New evidence shows defects in embryonic motor neuron function in ALS, but it is not know whether there are any changes in NPC or astrocytes this early in the disease. Therefore, adult spinal cord astrocytes will be compared to the NPC derived astrocytes in the $SOD1^{G93A}$ rat. Loss of EAAT2 in ALS is involved in the progressive degeneration but addition of non-ALS astrocytes to the spinal cord may support endogenous motor neurons. We describe the production of fresh astrocytes derived from NPC and their ability to express EAAT2. Furthermore, comparison of ALS with wild type NPC and astrocytes will give insight to the early stages of the disease process.

Poster Session PSM02: Alzheimer's and aging

PSM02-01

Amyloid-β enhances purinotoxicity by Caspase3 mediated cleavage of the P2X4 C-terminal

YF CHAI and NJ HAUGHEY

Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD, USA

Objective. To determine the contribution of P2X4 receptor overactivation in amyloid-b1-42 (Aβ)-induced neuronal cell dysfunction and death. Methods. GT1-7 neuronal cells and rat hippocampal neurons were transfected with full length P2X4, P2X4D280Q (where the aspartic acid in position 280 was mutated to glutamine; a putative caspase cleavage site in the C-terminal), SiRNA directed to knock down P2X4 or GFP. Cells were exposed to lethal amounts of the neurotoxins glutamate, starurosporine, potassium cyanide, iron, or pre-aggregated Aβ. P2X4 expression was determined by Western blot and immunochemistry, cell survival by nuclear morphology and P2X4 function by calcium imaging. Results. The neurotoxins tested increased P2X4 receptor expression with the following potency: staurosporine > Aβ > glutamate > potassium cyanide > iron. P2X4 expression increased prior to nuclear fragmentation. P2X4 was cleaved by caspase 3 but not by caspase 4 at low pH. Consistent with C-terminal cleavage, pretreatment of neurons with staurosporine decreased ATP-induced internalization of P2X4 receptors. The neuronal death induced by staurosporine, Aβ, glutamate and potassium cyanide decreased after the neurons were treated with SiRNA. Conclusions. The purinergic receptor P2X4 is involved in staurosporine, glutamate, potassium cyanide and Aβ-induced neuronal cell death. Activation of caspases by neurotoxins may result in the cleavage the P2X4 C-terminal, preventing agonist-induced internalization and enhancing calcium flux. Modulators of the P2X4 receptor may be neuroprotective in Alzheimer's and other neurodegenerative disorders.

PSM02-02

Effect of aged garlic extract on Morris water maze performance in Tg2576

NB CHAUHAN and JC SANDOVAL

UIC, JBVAMC, Chicago, IL, USA

Aged garlic extract is know to exert beneficial effects to human health. It is prepared by soaking crushed garlic in 20% aqueous ethanol for %7E 20 months. The 'aging' enriches the extract with many sulfur-containing compounds that exert antioxidant, anti-inflammatory and anti-tumor effects. The neuroprotective effects of aged garlic extract have begun to be recognized. Aged garlic extract is shown to protect hippocampal neurons from Abeta-induced toxicity, and prevent physiological aging in senescence-accelerated mice. We have demonstrated that feeding of Swedish mutant Alzheimer's transgenic mice with aged garlic extract reduced cerebral amyloid, and reduced Abeta-induced tau phosphorylation. Further, we wished to investigate whether or not chronic treatment with dietary aged garlic extract improves hippocampal-based learning and memory task in Tg2576. 2-month-old Tg2576 animals (Tgs) were fed with a diet containing 2% aged garlic extract for 5 months. Untreated littermates (Lts) before and after the treatment served as controls. Animals were subjected to Morris water maze testing before and after the treatment. Results show that water maze performance was deteriorated in age-matched untreated Tgs compared respective controls. Feeding of aged garlic extract restored these changes toward normal after 5 months of treatment. These results indicate that even at very young age, in absence of Abeta-plaques, Tg2576 animals showed behavioral deficits, most likely due to subtly increased cerebral Abeta levels. Restoration of these deficits by aged garlic extract indicate that aged garlic extract may have an ability to regulate amyloidogenic processing of APP, and to regulate signaling mechanisms underlying hippocampal-based learning and memory task.

Acknowledgements: Supported by NIH [AT001812 & NS044538 (N.B.C.)].

PSM02-03

In vivo beta-amyloid plaques visualized through MRI in mouse model of Alzheimer's disease

M MARTIN* and C READHEAD†

**Dept of Physics, University of Winnipeg, Winnipeg, Canada,*
†Biological Imaging Center, Caltech, Pasadena, CA, USA

Beta-amyloid plaques in the brain characterize Alzheimer's disease, which affects 4.5 million people worldwide. Until recently, these plaques could not be detected in patients, and confirmation of the disease could only be made by post-mortem histology. This has made it difficult to conduct longitudinal studies and clinical trials or to make prognoses. Contrast agents, chemicals that cross the blood-brain-barrier (BBB) and adhere to the plaques causing contrast in images, have been shown to make beta-amyloid plaques visible *in vivo* using positron emission tomography (PET), and magnetic resonance imaging (MRI). Weakening of the BBB to allow the passage of contrast agents might be hazardous for patients and make them vulnerable to opportunistic infections. Thus it is advantageous to image beta-amyloid plaques without the use of contrast agents, as has been done in fixed brains using MRI and recently in live mice. Here we show images of beta-amyloid plaques in mice without the use of BBB weakening contrast agents, using T2*-weighted MRI. Here we show three-dimensional MRIs of transgenic mice with beta-amyloid plaques. The plaques cause a loss of signal in a T2*-weighted image in the form of a black spot. The locations of the plaques were confirmed using histology. This method has opened the way for detailed longitudinal studies that will provide a better understanding of the disease. It may also lead to early pre-symptomatic diagnosis that may allow for preventative therapies and is a valuable step toward making an important tool for clinical trials.

Acknowledgement: The authors would like to acknowledge funding from NSERC.

PSM02-04

Gene expression profiles in aging rhesus monkey brain

C CHEN*, JA DUCE*, W HOLLANDER*, D KIPLING‡, DL ROSENE† and CR ABRAHAM*

**Department of Biochemistry, †Department of Anatomy and Neurobiology, Boston University School of Medicine, Boston, MA, USA, ‡Department of Pathology, University of Wales College of Medicine, Cardiff, UK*

We have shown that age-related degenerative changes in the white matter of the rhesus monkey are associated with cognitive dysfunction. Biochemical studies have shown that the levels of certain proteins in myelin and oligodendrocytes (CNPase, MOSP and MAG) are altered with age. We hypothesize that these changes are associated with proteolytic and inflammatory mediators such as calpain and complement. To confirm these findings and further understand the mechanisms of age-related changes in brain, DNA microarray analysis was used to study gene expression profiles of white and gray matter obtained from young and old, behaviorally tested, rhesus monkeys. Results from the white matter microarray data analysis support the biochemical and immunohistochemical findings of inflammation and myelin dystrophy but also suggest the participation of additional inflammation-related genes as well as other proteins. Specifically, there was a decrease in the expression of genes involved in mitochondrial respiration, signal transduction, cell replication and neurotransmission. Genes controlling transcription and translation as well as several senescence and cognitive related genes previously implicated in aging were also altered in aged animals. In conclusion, the genomic findings support an important role for the complement system, ROS and other inflammatory mediators in initiating and enhancing age-related changes in the brain. Our microarray results open new avenues for the study of normal aging pathways and dysmyelination.

PSM02-05

Activation of the antioxidant response element in the prefrontal cortex of Tg2576 mice expressing mutant amyloid precursor protein

DW SIRKIS*, AD KRAFT*, TD STEIN‡, DA JOHNSON* and JA JOHNSON*†

**School of Pharmacy, †Molecular and Environmental Toxicology, ‡Neuroscience Training Program, UW-Madsion, Madison, WI, USA*

Alzheimer's disease (AD) is pathologically characterized by extracellular deposition of beta amyloid (Aβ), intracellular elevation of phosphorylated tau, and neuronal cell death. Increasing evidence implicates oxidative stress in this pathogenesis. Tg2576 mice overexpress a familial Alzheimer's disease gene mutation in the amyloid precursor protein (APPsw), resulting in heightened Aβ levels in the hippocampus and cerebral cortex. These mice display increased oxidative stress and Aβ plaque deposition, but little neuronal loss. The lack of neuronal cell death in these mice alludes to the possible activation of cellular defense mechanisms. One plausible explanation for this observation is activation of a cis-acting regulatory element known as the antioxidant response element (ARE) in these animals. To correlate the oxidative stress observed in the brain of APPsw mice with activation of the antioxidant response element, APPsw mice were crossed with transgenic reporter mice expressing the core ARE coupled to a human placental alkaline phosphatase (hPAP) reporter gene. At twelve months of age, APPsw positive mice had significantly enhanced expression of the ARE-hPAP reporter construct as well as ARE-driven NADPH : quinone oxidoreductase (NQO1) in the prefrontal cortex. These data implicate the ARE system in the AD-like progression of Tg2576 mice.

PSM02-06

Visualization of homodimeric and heterodimeric interactions between APP and Notch2 proteins in living cells using BiFC analysis

C CHEN, S OHAND and CR ABRAHAM

Department of Biochemistry, Boston University School of Medicine, Boston, MA, USA

We reported previously that the amyloid precursor protein (APP) interacts with Notch receptor proteins. To investigate potential homodimeric and heterodimeric interactions between APP and Notch2 proteins that may result in signaling by these two proteins, we have visualized the subcellular localization of the complexes formed by these two proteins in living cells using bimolecular fluorescence complementation (BiFC) analysis. We fused the N-terminal and C-terminal of YFP fragments to APP, Notch2, and a C-terminal truncated form of Notch2, respectively. When expressed in COS cells, these proteins alone did not produce a fluorescent signal. The APP homodimer produced a weak fluorescent signal. Expression of either Notch2 full-length or its truncated form did not produce a visible fluorescent signal, suggesting Notch2 receptor may not form homodimers. The strongest flurescent signal was obtained with co-expression of the C-terminus of YFP fragment fused to APP and the N-terminus of YFP fragment fused to the truncated form of Notch2. The heterodimer formed by APP and truncated form of Notch2 localized to plasma membrane, intracellular vesicles and subcellular compartments. These results confirmed our previous proteomic and biochemical findings of APP and Notch2 interactions. This method of specifically visualizing APP/Notch interaction will have diverse application in understanding the roles of APP and Notch signaling.

PSM02-07

Roles of the different beta-amyloid molecular species in cellular binding and pathology in Alzheimer's disease

DC FERRARI, KZ BOURNE and JR PEREZ-POLO

University of Texas Medical Branch, Gavleston, TX, USA

β-Amyloid peptide (Aβ) is the primary constituent of the extracellular amyloid deposits in the brain, one of the main neuropathological characteristics of Alzheimer's disease. The activity of the β-site amyloid precursor protein cleaving enzyme type 1 (BACE1) is required for the formation of the Aβ peptide. Our goal is to characterize the effect of extracellular or intracellular Aβ molecular species in the regulation of the transcription of BACE1. We used the rat pheochromocytoma PC12 neuronal cell line to incubate with a final concentration of 10 μM $A\beta_{1-42}$ for 24 and 96 hours. Using denaturing SDS-PAGE we show that the Aβ molecular species incubated correspond to the monomer and soluble oligomers (trimer and hexamer). Western blot analysis showed that $A\beta_{1-42}$ significantly decreases endogenous BACE1 protein levels after 96 hours of incubation, compared to cells not exposed to the peptide. BACE1 protein levels are not affected by $A\beta_{1-42}$ after 24 hours. PC12 cells incubated with 10 μM $A\beta_{1-42}$ show a significant decrease of the peptide found in the media after 72 hours. This decrease could be due to $A\beta_{1-42}$ incorporation or degradation. Western blot studies here showed $A\beta_{1-42}$ incorporation into the cell, specifically to the endoplasmic reticulum and nuclear fractions. These results would suggest a role for intracellular Aβ in the regulation of transcription of BACE1.

Acknowledgement: This work has been supported in part by grants from the Sealy Center for Aging.

PSM02-08

Beta-amyloid dependent expression of inducible nitric oxide synthase in neurons: prevention by α2-adrenergic receptor antagonist

PE POLAK*, S KALININ*, JM MADRIGAL*, V GAVRILYUK*, M MARIEN† and DL FEINSTEIN*

**University of Illinois at Chicago, Chicago, IL, USA, †Pierre Farbres, Castres, France*

The neurotransmitter noradrenaline (NA) exerts important anti-inflammatory effects on glial cells including suppression of the inducible form of nitric oxide synthase (NOS2). We examined the consequences of manipulating NA *in vivo* by treating adult rats with the neurotoxin DSP4 which selectively lesions noradrenergic neurons of the locus ceruleus (LC), and reduces cortical NA levels. Rats received two i.p. injections (one week apart) of either DSP4 (5 mg/kg) or PBS. Four weeks later, Aβ1–42 oligomers (2 uL at 0.5 μg/μL) or saline was injected into each cortical hemisphere. On the same day, animals received three i.p. injections (–0.5, +4, and +8 h relative to Aβ injection) of the α2-AR antagonist F14413 (0.16 mg/kg) or saline. Following LC lesion, injection of Aβ1–42 caused appearance of NOS2 within neurons and increased neuronal damage assessed by staining for non-phosphorylated neurofilament proteins with antibody SMI-32. Co-treatment with F14413 reduced neuronal NOS2 staining as well as SMI-32 staining. Neuronal damage was dependent upon NOS2 expression since injection of Aβ1–42 into DSP4-treated NOS2 deficient mice did not result in neuronal damage. These results demonstrate that perturbation of NA levels *in vivo* exacerbates inflammatory responses and neuronal damage due to inflammatory stimuli such as Aβ. Current studies are underway to examine NA influence on other inflammatory markers. These findings suggest that peripherally administered α2-AR antagonists could provide benefit in neurological diseases such as AD or PD where LC loss occurs.

PSM02-09

Select oxidants produce changes in endoplasmic reticulum Ca^{2+} stores reminiscent of those in patients with Alzheimer

GE GIBSON, HM HUANG and HL CHEN

Burke Med. Res. Inst. Weill Med. Coll. Cornell Univ., White Plains, New York, USA

Abnormalities in calcium homeostasis and oxidative processes occur in fibroblasts from Alzheimer's disease (AD) patients and in fibroblasts and neurons of transgenic mice bearing a presenilin-1 mutation. Bombesin-releasable calcium stores (BRCS) from the endoplasmic reticulum (ER) are exaggerated in fibroblasts from patients with AD compared to controls. We hypothesize that alterations in specific reactive oxygen species (ROS) underlie the exaggerated BRCS in AD, and that appropriate antioxidants may treat this abnormality. Different oxidants selectively induced various ROS in different patterns and uniquely modified basal Ca^{2+} and/or ER Ca^{2+}. Among the oxidants tested, tert-butyl-hydroxyperoxide (*t*-BHP) and H_2O_2 were the most specific for exaggerating BRCS without affecting basal calcium in fibroblasts from young controls. The ability of fibroblasts from AD patients to handle *t*-BHP was tested. *t*-BHP-induced ROS production was greater in fibroblasts from AD patients than in those from aged controls. *t*-BHP further exaggerated BRCS in AD fibroblasts. Several antioxidants were tested to determine whether they could ameliorate the deficit in AD. Among the antioxidants, α-keto-β-methyl-n-valeric acid (KMV) was most selective for: (1) diminishing the ROS that modified the BRCS, (2) reducing BRCS either with or without exogenous oxidants and (3) diminishing the AD-related exaggeration in the BRCS. ROS production that was not diminished by KMV did not affect BRCS. Other antioxidants that diminished *t*-BHP-induced ROS did not alter BRCS. The results suggest the possibility of choosing appropriate antioxidants that may alter specific oxidant-induced changes in a cell system.

PSM02-10

Possible mechanisms of noradrenergic action in TgAPP mice

SA KALININ*, N CHAUHAN†, VG GAVRILYUK*, E GALEA* and DL FEINSTEIN*

**University of Illinois at Chicago, †VA Health Care System, Chicago, USA*

Noradrenaline (NA) is an endogenous regulator of brain inflammatory responses. In Alzheimer's disease (AD), loss of noradrenergic locus ceruleus (LC) neurons occurs which could be an important etiological factor. Previously we showed that chemical lesion of the LC in transgenic TgAPP mice over-expressing mutated human APP (Indiana mutation V717F), using the selective neurotoxin DSP4 caused an increase in plaque number. This was accompanied by a significant increase in microglial activation (determined by Mac-1 staining), and neuronal damage (determined by staining for non phosphorylated neurofilament proteins with antibody SMI32). Here we test two possible mechanisms to explain increased plaque formation: increased APP processing and reduced amyloid removal. Western blot analysis using antibody G369 against APP(645–694) showed higher levels of the APP C99-B stub peptide in brain homogenates from DSP4 treated vs control mice. However, DSP4 did not modify BACE1 protein levels or β-or γ-secretase mRNAs. To test effects of NA on microglial phagocytosis, we fluorescently labeled Ab1-42 with CY3 (Ab42-Cy3). NA increased phagocytosis of Ab42-Cy3 in both primary microglial cells and the BV2 cell line. Furthermore, mRNA levels of neprilysin which can degrade Ab were decreased by DSP4 treatment. These data show that loss of LC neurons can increase or accelerate plaque deposition and exacerbate neuronal damage. The effect on plaques is most likely through decreased Ab removal rather than increasing amyloidogenic processing. The use of DSP4 in current animal models of AD could provide additional information to AD pathogenesis.

Acknowledgements: This work supported in part by grant from the NIH and the VA.

PSM02-11

Peroxisomal proliferation prevents β-amyloid-neurotoxicity in rat hippocampal neurons

RA QUINTANILLA, JA GODOY, A TORO, MJ SANTOS and NC INESTROSA

Centro de Regulación Celular y Patología 'Joaquin V. Luco' (CRCP), MIFAB, Unidad de Bioquímica Celular y Genética, PUC, Santiago, Chile

Alzheimer's disease (AD) is a degenerative process that leads to severe cognitive impairment as a consequence of selective death of neuronal populations. The molecular pathogenesis of AD, involved the participation of the β-amyloid (Aβ), and oxidative stress. We report here, that peroxisomal proliferation induced by Peroxisomal Proliferator Activated Receptor-α (PPARα) agonist Wy-14.463 (Wy) and 4-phenyl butyric (4-PB), attenuated Aβ-dependent toxicity in rat hippocampal neurons. Pre-treatment with Wy and 4-PB prevent the neuronal cell death and the neurite network loss induced by Aβ peptide. Moreover, the hippocampal neurons treated with these compounds, showed an increase in the number of peroxisomes, with a concomitant increases in catalase activity. Additionally, we evaluate the Wy protective effect in the β-catenin levels, production of intracellular reactive oxygen species (ROS), and cytoplasmic calcium changes in hippocampal neurons exposed to H_2O_2. Results show that the PPARα agonist, protects from H_2O_2 cytotoxicity, prevented β-catenin degradation, ROS production, and cytoplasmic calcium increase. In conclusion, we demonstrate that the activation of PPARα prevents β-amyloid induced neuronal cell death and morphological changes. Our result shows a novel role of the PPARα in the prevention of the neuronal damage mediated by the oxidative stress.

PSM02-12

The role of the alternative NF-kB signaling pathway in the induction of AD pathogenesis

G BRITTAIN and JR BETHEA

University of Miami, Miller School of Medicine, The Miami Project to Cure Paralysis, Miami, FL, USA

CD40-ligand (CD40L) receptor signal transduction has been previously shown to induce amyloidogenic processing of the amyloidal precursor protein (APP) whereas TNF-alpha and CD95 signal transduction did not. The primary characteristic of CD40L receptor signal transduction that may account for such a difference in function is that CD40L activates both the classical and alternative NF-kB signaling pathways, while TNF-alpha and CD95 only activate the classical pathway. For this reason, we are investigating activation of the classical and alternative pathway of NF-kB signaling is responsible for the induction of amyloidogenic processing of APP. Interestingly, we found that the alternative pathway (p52) is constitutively active in both primary cortical neurons and neuronal cell lines. For this reason, it is our present belief that constitutive activity of the alternative pathway and NIK have important roles in regulating normal neuronal physiology and that aberrations in such activity may result in the induction of neuropathogenic responses, such as the pathogenesis of Alzheimer's disease (AD). Therefore, current research in our laboratory is focused on characterizing the role of the alternative NF-kB pathway and NIK in the regulation of amyloidogenic APP processing, as well as identifying potential regulatory proteins and signaling components that may be involved in amyloidogenic processing of APP.

PSM02-13

Effects of minocycline on Alzheimer's disease pathogenesis

TJ SEABROOK, L JIANG, M MAIER and CA LEMERE

Center for Neurologic Diseases, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

Alzheimer's disease (AD) is characterized by beta amyloid (Aß) plaques, surrounded by activated microglia which produce nitric oxide (NO), Tumour Necrosis Factor (TNF) or Interleukin-6 (IL-6). Minocycline has been demonstrated to decrease microglial activation therefore it may be a potential therapeutic in AD. In the present studies, we examine minocycline's effect on microglia in response to Aß 42. BV-2 cells, a mouse microglial cell line, did not produce N0, IL-6 or TNF when stimulated with Ab42. However, when combined with lipopolysaccharide, there was increased production of NO and TNF; minocycline reduced this by one-half ($P < 0.05$). Cultures of primary mouse microglia stimulated with 0.1 or 1.0 mM Aß produced IL-6, the levels of which were reduced by 10 mM minocycline, 50 and 57% respectively according to Aß dose. However, in a mixed culture of astrocytes and microglia, this reduction was not observed. There was no decrease in the production of Aß when HEK cells, that were transfected to overproduce Aß were incubated in the presence of minocycline (0.1–2 mM). We conclude: 1) BV-2 cells respond differently to Aß compared to primary microglia; 2) minocycline can decrease IL-6 production in primary microglia but not in mixed glial culture; and 3) minocycline does not affect the production of Aß directly. Currently, we are treating J20 APP tg mice (an AD model) with minocycline. Data on cerebral Aß levels, behavior and pathology will be presented. The signaling mechanisms induced by Aß in microglia and astrocytes and the impact of minocycline will be discussed.

PSM02-14

Ectopic localization of active Smad2 in the vulnerable neurons in Alzheimer disease

HG LEE, M UEDA, X ZHU, G PERRY and MA SMITH

Department of Pathology, Case Western Reserve University, Cleveland, OH, USA

Transforming growth factor-β (TGF-β), a multifunctional cytokine, has been widely suggested to play a role in the pathogenesis of Alzheimer disease (AD). Supporting this, levels of TGF-β are elevated in the cerebrospinal fluid and sera, and TGF-β is also detected in the senile plaques of patients with AD. Since TGF-β is neuroprotective, whereas AD is typified by neurodegeneration, we speculated that there could be defects in TGF-β signaling that abrogate its neuroprotective properties. To test this, as well as to further define the role of TGF-β in AD, we investigated downstream TGF-β signaling events by examining the expression of the phosphorylated active form of Smad2. Consistent with an increase in TGF-β in AD, we found significant increases in phospho-Smad2 in the hippocampal neurons of AD compared to age-matched control. However, in contrast to an expected nuclear localization, phosphorylated Smad2 in AD was predominantly and ectopically found in the cytoplasm, specifically co-localized with neurofibrillary tangles and granular vascuolar degeneration. This ectopic localization of phosphorylated Smad2 in AD suggests a defect in the Smad-mediated signaling pathway of TGF-β. Therefore, although TGF-β is increased in AD, it is likely that the TGF-β-mediated neuroprotection pathways become uncoupled and may lead to, or contribute to, the pathogenesis of AD.

Acknowledgements: Supported by NIH (NS38648) and the Alzheimer Association (IIRG-03-6263).

PSM02-15

Beta-amyloid stimulated microglia induce neuron death via synergistic stimulation of tumor necrosis factor and NMDA receptors

CK COMBS and AM FLODEN

Department of Pharmacology, Physiology and Therapeutics, University of North Dakota, Grand Forks, ND, USA

A variety of studies have documented the ability of Abeta fibrils to directly stimulate microglia *in vitro* to assume a neurotoxic phenotype characterized by secretion of a plethora of proinflammatory molecules. Collectively, these data suggest that activated microglia play a direct role in neuron death in Alzheimer's disease (AD) rather than simply a role in clearance following plaque deposition. Although it is known that Abeta-stimulated microglia acutely secrete toxic oxidizing species, the identity of longer-lived neurotoxic agents remains less defined. We have utilized Abeta-stimulated conditioned media from primary mouse microglia to identify more stable neurotoxic secretions. The N-methyl-D-aspartate (NMDA) receptor antagonists, memantine and 2-amino-5-phosphopetanoic acid, as well as soluble tumor necrosis factor alpha (TNFalpha) receptor protect neurons from microglial conditioned media-dependent death implicating the excitatory neurotransmitter, glutamate, and the pro-inflammatory cytokine, TNFalpha, as effectors of microglial-stimulated death. Neuron death occurs in an oxidative damage-dependent fashion requiring activity of inducible nitric oxide synthase. Toxicity results from coincident stimulation of the TNFalpha and NMDA receptors since stimulations of either alone, are insufficient to initiate cell death. These findings suggest the hypothesis that AD brains provide the appropriate microglial-mediated inflammatory environment for TNFalpha and glutamate to synergistically stimulate toxic activation of their respective signaling pathways in neurons as a contributing mechanism of cell death.

PSM02-16

Pharmacokinetic analysis of BBB transport of 125I-AB40 in WT/AD transgenic mice and its implication for amyloid plaque formation

KK KANDIMALLA, GL CURRAN, SS HOLASEK, EJ GILLES, TM WENGENACK and JF PODUSLO

Mayo Clinic College of Medicine, Depts. Neurology, Neuroscience, & Biochem./Molec. Biol., Molecular Neurobiology Lab, Rochester, MN, USA

Amyloid plaques are formed in the extracellular space of Alzheimer's disease (AD) brain due to the accumulation of amyloid β proteins (Aβ) such as Aβ40. The relationship between Aβ40 pharmacokinetics and its accumulation within and clearance from the brain in both wild type (WT) and AD transgenic mice (APP, PS1) was studied to understand the mechanism of amyloid plaque formation and the potential use of Aβ40 as a probe to target and detect amyloid plaques . In both WT and APP, PS1 mice, the ^{125}I-Aβ40 tracer exhibited bi-exponential disposition in plasma with very short first and second phase half-lives. The ^{125}I-Aβ40 was significantly metabolized in the liver >>> kidney > spleen. Co-administration of exogenous Aβ40 inhibited the plasma clearance and the uptake of ^{125}I-Aβ40 at the BBB in WT animals but did not affect its elimination from the brain. The ^{125}I-Aβ40 was shown to be metabolized within and effluxed from the brain parenchyma. The rate of efflux from APP, PS1 brain slices was substantially lower compared to WT brain slices. Since the Aβ40 receptor at the BBB can be easily saturated, the blood-to-brain transport of Aβ40 is less likely to be a primary contributor to the amyloid plaque formation in APP, PS1 mice. The decreased elimination of Aβ40 from the brain is most likely responsible for the amyloid plaque formation in the brain of APP, PS1 mice. Furthermore, inadequate targeting of Aβ40 to amyloid plaques despite its high BBB permeability is due to the saturability of Aβ40 transporter at the BBB and its metabolism and efflux from the brain.

PSM02-17

Development of novel amyloid imaging agents based upon thioflavins

CY WU*, JJ WEI*, PE POLAK†, AR SHARP†, JL MAO*, DL FEINSTEIN†, RM GOULD†, L NICHOLS‡, K PARK‡, L CAI‡, RB INNIS‡, VW PIKE‡ and YM WANG*

**Department of Medicinal Chemistry and Pharmacognosy, †Department of Neuroanesthesiology College of Medicine, Cell Biology and Anatomy, University of Illinois at Chicago, Chicago, IL, ‡Molecular Imaging Branch, National Institute of Mental Health, National Institutes of Health, Bethesda, MD, USA*

Objective: To develop thioflavin based molecular probes for imaging *in vivo* amyloid deposits and neurofibrilary tangles.
Methods: Analogs of thioflavins were synthesized and radiolabeled with positron or single photon emitting radionuclides. The binding affinity for Aβ was evaluated using isolated amyloid fibrils from human brain tissue. Binding specificity was assessed using fluorescent tissue staining. *In vivo* brain uptake was evaluated in mice.
Results: Neutral, lipophilic analogs of thioflavins capable of radiolabeling with C-11 or I-125 were synthesized. These compounds bound to human Aβ with affinities in the nanomolar range. Tissue staining showed selective binding to Aβ deposits *in vitro*. Biodistribution of selected compounds displayed high brain permeability at early time points. At later points, the compounds were cleared from the normal brain, indicating low non-specific binding *in vivo*.
Conclusion: Small molecular probes have been developed that readily entered the brain and selectively bound to Aβ deposits and neurofibrilary tangles. Potential applications of these amyloid binding agents include facilitating drug screening in animal models and use as *in vivo* markers of early and definitive diagnosis of AD.

PSM02-18

Development of Aβ40 derivatives that do not form fibrils as MRI contrast agents for detecting alzheimer's disease amyloid plaques

KE GILES, KK KANDIMALLA, M RAMIREZ-ALVARADO and JF PODUSLO

Depts. Neurology, Neuroscience, & Biochemistry/Molecular Biology, Mayo Clinic College of Medicine , Rochester, MN, USA

Formation of plaques by amyloid (Aβ) proteins, including Aβ40, in the extracellular space of the brain parenchyma is considered the primary pathology of Alzheimer's disease (AD). Currently, no method exists for pre-mortem diagnosis of AD. Our lab is developing molecular probes that are derived from Aβ40 to image amyloid plaques by MRI. The probes are chemically modified to increase their permeability at the blood brain barrier (BBB) and enhance plaque binding. This is accomplished by either modifying Aβ40 with putrescine (PUT-Aβ40) or truncating the neurotoxic domain to the first 30 amino acid residues and synthesizing the protein with 5 diamine inserts ([N-4ab/Q-4ab] Aβ30). Moreover, a DTPA arm that can chelate gadolinium (Gd) is attached to the N-terminal position with a 6-aminohexanoic acid (HEX) linker to provide contrast for MRI imaging. These modifications are expected to minimize the ability of the probes to form fibrils. We studied the fibril formation of various Aβ40 derivatives by thioflavin-T (THT) assay. Our results show that only Aβ40 and PUT-Aβ40 form fibrils after 6 h of agitation in TRIS-EDTA buffer. However, PUT-Aβ40+HEX, PUT-Aβ40+HEX+DTPA, Aβ30, [N-4ab/Q-4ab] Aβ30, [N-4ab/Q-4ab] Aβ30+HEX, [N-4ab/Q-4ab] Aβ30+HEX+DTPA, and [N-4ab/Q-4ab] Aβ30+HEX+Gd[DTPA] do not form fibrils even after 120 h of agitation. Additionally, [N-4ab/Q-4ab] Aβ30+HEX+Gd[DTPA] does not enhance fibril formation of Aβ40 when agitated without seeding. These results suggest that [N-4ab/Q-4ab] Aβ30+HEX+Gd[DTPA] does not contribute to fibril formation and can be used as a contrast agent for imaging amyloid plaques in AD transgenic animals and eventually patients.

Poster Session PSM03: Death and survival

PSM03-01

Dexamethasone decreases while acetazolomide increases temozolomide induced apoptosis in human glioblastoma U87MG cells

A DAS, S KARMAKAR, A SAHA, NL BANIK and SK RAY

Department of Neurology, Medical University of South Carolina, Charleston, SC, USA

Use of dexamethasone (DXM) is a long-standing practice for controlling vasogenic edema and pain associated with glioblastoma, which is the deadliest brain tumor of astrocytic origin. Acetazolomide (ACZ), an inhibitor of carbonic anhydrase, alleviates edema in pseudotumor cerebri and intracranial pressure in brain tumor patients. Temozolomide (TMZ), a DNA akylating agent, is now a promising chemotherapy for glioblastoma. We compared the modulatory effects of DXM and ACZ in TMZ induced apoptosis in human glioblastoma U87MG cells. Cells were treated with DXM, ACZ, or TMZ for 6 h followed by incubation in a drug-free medium for 48 h. Wright staining and ApopTag assay showed no apoptosis in cells exposed to 40 μM DXM or 100 μM ACZ whereas significant number of apoptosis in cells treated with 100 μM TMZ. Apoptosis occurred with increased intracellular free [Ca^{2+}], as determined by fura-2 assay. Western blot analyses showed changes in Bax and Bcl-2 levels resulting in an increased Bax: Bcl-2 ratio and also detected over activation of calpain and caspase-3, which cleaved 270 kD α-spectrin at specific sites to generate 145 and 120 kD spectrin break down products (SBDPs), respectively. Pretreatment of cells with 40 μM DXM for 1 h decreased TMZ induced apoptosis, decreasing Ca^{2+} influx, Bax: Bcl-2 ratio, and SBDPs. Conversely, pretreatment of cells with 100 μM ACZ caused an increase in TMZ induced apoptosis with increasing Ca^{2+} influx, Bax: Bcl-2 ratio, and SBDPs. So, treatment of glioblastoma patients with ACZ prior to chemotherapy with TMZ might control edema and enhance apoptosis.

Acknowledgements: This study was supported in part by the grants from the NCI, NINDS, and the State of SC.

PSM03-02

Curcumin activated mutiple molecular mechamisms for apoptosis in human glioblastoma T98G cells

S KARMAKAR, A SAHA, A DAS, NL BANIK and SK RAY

Department of Neurology, Medical University of South Carolina, Charleston, SC, USA

Glioblastoma is the deadliest brain tumor of astrocytic origin. Current therapies are mostly ineffective. So, there is an urgent need for developing new strategies for the management of glioblastoma. The therapeutic effect of curcumin (CCM), a polyphenolic compound from the rhizome of *Curcuma longa,* has not yet been explored in glioblastoma. So, we used human glioblastoma T98G cells to explore the efficacy of CCM for inducing apoptosis and identifying mechanisms involved. Trypan blue dye exclusion test showed decrease in cell viability with increasing dose of CCM. Wright staining and ApopTag assay showed, respectively, morphological and biochemical features of apoptosis in T98G cells exposed to 25 μM and 50 μM of CCM for 24 h. Treatment with CCM activated extrinsic pathway of apoptosis as Western blot analyses showed activation of caspase-8, cleavage of Bid to tBid, and down regulation of the nuclear factor kappa B (NFκB). Also, CCM activated intrinsic pathway of apoptosis increasing Bax: Bcl-2 ratio, caspase-9 activity, and calpain and caspase-3 activities that cleaved 270 kD α-spectrin at specific sites to generate 145 and 120 kD spectrin break down products (SBDPs), respectively. Also, inhibitor of caspase-activated DNase (ICAD) was cleaved by caspase-3 activity, which was further confirmed by a colorimetric assay. Besides, CCM caused over-expression of apoptosis-inducing factor (AIF) that could contribute to caspase-independent apoptosis. These results strongly suggest the involvement of multiple molecular mechanisms in CCM induced apoptosis in T98G cells. Thus, CCM can be an effective therapeutic agent against deadly glioblastoma.

Acknowledgements: This research was supported in part by the grants from the NCI, NINDS, and the State of SC.

PSM03-03

Phosphodiesterase inhibitors protect against apoptotic cell death induced by nitric oxide or rotenone in NG108-15 cells

RR FISCUS, J TSIM, CY WONG and LH LEUNG

Chinese University of Hong Kong, Dept. Physiol. (Fac. Medicine) & Mol. Cell. Gerontol. (Ctr. Gerontol. & Geriatrics), Shatin, Hong Kong

Previously, our lab showed that elevation of cGMP and activation of protein kinase G (PKG) by atrial and brain natriuretic peptides (ANP & BNP) or cGMP analogs can protect against apoptosis in neural cells. Like cGMP, cAMP also has neural anti-apoptotic effects. Phosphodiesterases (PDEs) metabolize cAMP/cGMP, thus regulating cAMP/cGMP levels. The present study determined if inhibition of cAMP-PDE (PDE4) with Ro 20-1724 or cGMP-PDEs (PDE5, PDE9, PDE10 & PDE11) with dipyridamole or zaprinast can protect NG108-15 cells against pro-apoptotic effects of nitric oxide (NO) or rotenone. Apoptosis (24 h, determined by Cell Death ELISA measuring cytosolic histone-associated DNA fragments) was induced by NO donor S-nitroso-N-acetylpenicillamine (SNAP, 0.5 mM) or rotenone (10 μM). PDE inhibitors were added 2 h before SNAP or rotenone. SNAP-induced apoptosis was inhibited by 48% with dipyridamole (1 μM), 44% with Ro 20-1724 (1 μM) and 40, 48 & 70% with zaprinast (1, 10 & 100 μM, respectively). Rotenone-induced apoptosis was inhibited by 42% with dipyridamole (1 μM), 26 & 52% with Ro 20-1724 (1 & 10 μM, respectively) and 25% with zaprinast (1 μM). Results indicate that PDE4 and PDE5/9/10/11 inhibitors prevent apoptosis induced by neurotoxic concentrations of NO or rotenone. Thus, PDE inhibitors may be useful therapeutic agents for preventing neural apoptosis of neurodegenerative diseases like Alzheimer's disease and Parkinson's diseases.

Acknowledgements: RGC Competitive Earmarked Grant (CUHK4169/02M) and Direct Grant to RRF.

PSM03-04

Estrogen attenuates glutamate-induced death in a spinal motoneuron cell line

NL BANIK, EA SRIBNICK and SK RAY
Med Univ of S Carolina, Charleston, SC, USA

To examine a possible mechanism of action for 17β-estradiol(EST)-mediated cytoprotection, VSC4.1 cells were divided into 4 groups: control, 100 nM EST for 30 h, 1 mM L-glutamic acid (LGA) for 24 h, and EST (6 h pretreatment) + 24 h LGA cotreatment. Cell death was examined by Wright staining, TUNEL assay, and MTT assay. Treatment with 17α-estradiol was performed to examine if cytoprotection was EST receptor (ER)-dependent, and conjugated-EST was used to examine the role of putative membrane ER. Calpain and caspase-3 activities were measured by Western blotting of protease-specific spectrin breakdown products. Whole cell recording was performed to measure membrane potential and capacitance, and intracellular free Ca^{2+} was measured using Fura-2. Potential sites of Ca^{2+} entry were blocked with or without EST. LGA-induced cell death was significantly attenuated with EST but not with either 17α-estradiol or conjugated-EST, indicating that protection is ER-dependent but not through membrane ER. EST attenuated both calpain and caspase-3 activities. Both membrane potential and capacitance were altered in LGA-treated cells but were restored with EST treatment. EST significantly attenuated Ca^{2+} levels, and the L-type Ca^{2+} channel blocker nifedipine showed comparable results. To further characterize EST-mediated cytoprotection, cells were treated with a voltage gated Ca^{2+} channel (VGCC) agonist FPL-64176, which, at 100 μM, caused comparable cell death, and EST pretreatment caused comparable cytoprotection. These results suggest that EST-mediated cytoprotection in VSC4.1 cells is ER-dependent and may be related to VGCC, preventing pathologic Ca^{2+} influx and downstream protease activation.

Acknowledgement: Supported by NIH grants.

PSM03-05

Bax and reactive oxygen in cytochrome C-depleted neurons

JL FRANKLIN and RA KIRKL
University of Georgia, Athens, GA, USA

Sympathetic neurons undergo apoptotic death when deprived of nerve growth factor (NGF). This death is caused by caspases activated by Bax-induced release of cytochrome c from mitochondria. A Bax-dependent increase in mitochondrial-derived reactive oxygen species (ROS) occurs prior to detectable release of cytochrome c in these cells. Both depleting the electron transport chain of cytochrome c and caspase activity can increase ROS production by mitochondria. However, the timing of the ROS burst in relation to that of cytochrome c release in these neurons suggests that these mechanisms cannot be the sole cause for the increased ROS after NGF withdrawal. Cytochrome c degrades rapidly once it is released into the cytoplasm of sympathetic neurons. To further investigate the effects of Bax on ROS in these cells, we used this degradation to deplete bax+/+ and bax+/–neurons of cytochrome c. Apoptosis was blocked in cultures deprived of NGF with the broad-spectrum caspase inhibitor, BAF. By 48 h after NGF withdrawal, immunocytochemistry and immunoblotting revealed that both bax+/+ and bax+/–neurons had lost about 80% of their cytochrome c stores. ROS levels in these neurons were determined with the redox-sensitive dye, CM-H2DCFDA. Changes in CM-H2DCFDA fluorescence were monitored by confocal microscopy. CM-H2DCFDA fluorescence was significantly lower (36%; $P < 0.001$) in the bax+/–neurons than in the bax+/+ cells at 48 h after NGF deprivation. Therefore, ROS levels were higher in cells containing more Bax but having the same concentration of cytochrome c. These data indicate that Bax can affect ROS production independently of effects on cytochrome c release.

Acknowledgement: NIH NS37110.

PSM03-06

Combination of TUNEL and double immunofluorescent labeling demonstrated calpain in neuronal apoptosis in rat spinal cord injury

SK RAY, EA SRIBNICK, DD MATZELLE and NL BANIK
Department of Neurology, Medical University of South Carolina, Charleston, SC, USA

An abrupt Ca^{2+} influx in neurons after spinal cord injury (SCI) may upregulate Ca^{2+}-dependent proteases including calpain leading to neuronal death. A direct evidence for calpain involvement in neuronal apoptosis in SCI is needed. We examined (1) internucleosomal DNA fragmentation as evidence of apoptosis, (2) level of calpain expression specifically in neurons, and (3) apoptosis of the neurons after induction of SCI (40 g.cm force) at T12 in rats and therapy immediately (within 15 min) with either vehicle or calpain inhibitor E-64-d (1 mg/kg). Sham animals underwent laminectomy only. After 24 h treatment, animals were sacrificed to collect the 1-cm long rostral, lesion, and caudal segments of spinal cord. Agarose gel electrophoresis of genomic DNA from segments indicated apoptosis with internucleosomal DNA fragmentation that was prominent in lesion, moderate in caudal, and slight in rostral segments from vehicle-treated SCI rats, compared to sham animals. Treatment with E-64-d reduced apoptosis in all segments of SCI rats, compared to vehicle-treated SCI rats. The lesion and caudal segments were further investigated. *In situ* double immunofluorescent labeling detected overexpression of calpain in neurons in SCI rats treated with only vehicle and reduced expression of calpain in SCI rats treated with E-64-d. Combination of *in situ* TUNEL and double immunofluorescent labeling specifically detected neuronal death in SCI rats and neuroprotection in SCI rats treated with E-64-d. Results directly showed calpain involvement in neuronal apoptosis and efficacy of calpain inhibitor for neuroprotection in SCI.

Acknowledgements: This study was supported in part by the grants from the NIH and the State of SC.

PSM03-07

Thiazolidinediones induce glioma toxicity: involvement of mitochondria and ROS

A SPAGNOLO*, T LICHTOR†, R GLICK†, C DELLO RUSSO*, P MURPHY*, H LIN†, V GAVRILYUK* and DL FEINSTEIN*
**Dept. of Anesthesiology, University of Illinois at Chicago, †Dept. of Neurosurgery, Rush University, Chicago, IL, USA*

Thiazolidinediones (TZDs) are synthetic agonists of the peroxisome proliferator activated receptor (PPAR) gamma. TZDs exert anti-proliferative effects in several transformed cell lines making them potential candidates for tumor treatment. Our previous findings showed that two TZDs (troglitazone and pioglitazone) selectively induced cell death in mouse GL261 glioma cells, while causing little damage to primary cultures of mouse astrocytes. Examination of metabolic parameters and mitochondrial function showed that TZDs induced more profound changes in GL261 cells than in astrocytes, which might contribute to differential cell susceptibility. TZDs increased superoxide production to a greater extent in glioma cells than astrocytes, which may be due to more efficient removal of ROS in astrocyte since we found that SOD levels are higher in astrocytes than glioma cells. Interestingly, we also found that TZDs increase expression of the tumor suppressor p53 gene and the cyclin dependent kinase inhibitor p21. To determine if TZDs could be effective *in vivo*, we tested the effects of pioglitazone on animal survival following intracerebral implantation of GL261 cells. We found that oral treatment with pioglitazone (100 ppm provided ad libitum in chow) tended to increase survival; while a combination of oral together with intraventricular injection of pioglitazone significantly increased mean survival time. Although troglitazone was withdrawn due to hepatotoxic effects, we are now testing this TZD for ability to reduce tumor size and increase animal survival.

PSM03-08

Cisplatin-induced delayed cell death in drg neurons is associated with altered mitochondrial function

JL PODRATZ, AM KNIGHT and AJ WINDEBANK
Mayo Clinic School of Medicine, Rochester, MN, USA

Cisplatin (Pt) is a chemotheraputic drug that binds to DNA and induces apoptosis in Dorsal Root Ganglion neurons (DRG). In DRG, Pt induces upregulation of p53, bax translocation, cytochrome-c release and caspase activation leading to apoptosis. In Bax deficient DRG, cell death is significantly delayed but not prevented. The present experiments were designed to characterize the secondary delayed cell death. Dissociated Bax deficient mouse DRG were cultured in medium containing 8–10 ng/ml NGF +/–2 μg/ml Pt or 76 μM FMK-ZVAD. Cell survival was determined by imaging the same area of the dish over time and comparing the number of live cells to the number of cells at time zero Using laser confocal microscopy, mitochondrial membrane potential was observed using 10 nM tetramethylrhodamine, methyl ester, perchlorate (TMRM) and mt-DNA replication was observed using 10 μM 5-bromo-2′-deoxy-uridine (BrdU). Cell survival at 72 h in Bax+/+ DRG was 53% when treated with Pt and was increased to 82% with the addition of FMK-ZVAD. Survival of Pt treated Bax–/–DRG at 72 h was 90%. Delayed death was seen in the Bax–/–with 54% cell survival at 168 h that was increased by addition of FMK-ZVAD to 95%. Increased fluorescence in Pt treated DRG was observed using TMRM at 24 h in Bax+/+ DRG that was delayed to 72 h in Bax–/–DRG. Labeling of mt-DNA with BrdU suggested changes in mt-DNA synthesis. Cisplatin induces two different pathways that lead DRG neurons to apoptotic cell death. The first pathway involves DNA damage, p53 and Bax. The second may involve damage to mt-DNA and caspase dependent apoptosis.

PSM03-09

Slips versus rafts: A novel mechanism regulating cell fate decisions by sphingolipid-induced remodeling of cell signaling platform

E BIEBERICH
Medical College of Georgia, Augusta, GA, USA

The metabolism of sphingolipids is strictly regulated during the mitotic cell cycle. Before the G1-to-S transition, the ceramide and glucosylceramide concentration is elevated. Ceramide induces apoptosis synergistically with pro-apoptotic proteins that may be asymmetrically inherited during cell division. Only one daughter cell dies shortly after mitosis, a mechanism we suggested to regulate the number of neural stem cells during embryonic development. The progeny cells, however, may protect themselves by converting ceramide to glycosphingolipids or sphingomyelin. The cell cycle may thus be a turning point for sphingolipid metabolism and explain rapid changes of the sphingolipid composition in cells that undergo mitotic cell-fate decisions. In the proposed model termed 'Shiva cycle', progression through the cell cycle, differentiation, or apoptosis may rely on a delicate balance of sphingolipid second messengers that modulate the G1-to-S transition or G1-to-apoptosis program. Ceramide-induced cell cycle delay at G0/G1 is either followed by ceramide-induced apoptosis or by conversion of ceramide to glucosylceramide or sphingomyelin, a proposed key regulatory rheostat that rescues cells from re-entry into a life/death decision at G1-to-S. We show experimental evidence suggesting a novel mechanistic model for sphingolipid-induced protein scaffolds ('slips') that regulate cell-fate decisions and will discuss the biological consequences and pharmacological potential of manipulating the sphingolipid-dependent cell fate program in cancer and stem cells. In particular, we will discuss the significance of sphingolipids for the emergence and characterization of tumor stem cells.

Acknowledgement: This work was supported by NIH grant R01NS046835.

PSM03-10

Ceramide induces apoptosis selectively in pluripotent stem cells and promotes neuronal differentiation of neuroprogenitors

G WANG*, J SILVA*, K KRISHNAMURTHY*, BG CONDIE† and E BIEBERICH*
**Medical College of Georgia, Augusta, †University of Georgia, Athens, GA, USA*

The formation of stem cell-derived tumors (teratomas) is observed when engrafting undifferentiated embryonic stem (ES) cells, embryoid body-derived cells (EBCs) or mammalian embryos, and is a significant obstacle to stem cell therapy. We show that in tumors formed after engraftment of EBCs into mouse brain, expression of the pluripotency marker Oct-4 co-localized with that of PAR-4, a protein mediating ceramide-induced apoptosis during neural differentiation of ES cells. We tested the ability of the novel ceramide analog N-oleoyl serinol (S18) to eliminate mouse and human Oct-4(+)/PAR-4(+) cells and to increase the proportion of nestin(+) neuroprogenitors in EBC-derived cell cultures and grafts. S18-treated EBCs persisted in the hippocampal area and showed neuronal lineage differentiation as indicated by the expression of β-tubulin III. Untreated cells, however, formed numerous teratomas that contained derivatives of endoderm, mesoderm and ectoderm. Our results show for the first time that ceramide-induced apoptosis eliminates residual, pluripotent EBCs, prevents teratoma formation and enriches the EBCs for cells that undergo neural differentiation after transplantation.

Acknowledgements: Supported by NIH grants R01NS046835 (to E.B.) and R01MH064794 (to B.G.C.).

PSM03-11

Modulation of NMDA-induced calcium transients by NO applications simultaneous or prior to the stimulus: cell death or survival

G WOLF, A SCHROETER, S and RABI and T HORN
University of Magdeburg, Institute of Medical Neurobiology, Magdeburg, Germany

Traditionally, mitochondria have been viewed as the 'powerhouse' of the cell, i.e., the site of the oxidative phosphorylation machinery involved in ATP production. But in recent years a large body of knowledge have come to recognize that mitochondria also participate in other important processes such as the intracellular calcium levels, the initiation and performance of cell death programs and aging. Moreover, mitochondria constitute a primary locus for the intracellular formation and conversion of reactive oxygen and nitrogen species (ROS, RNS). These processes are pivotal for the modulation of critical cellular functions, where nitric oxide (NO) forms the crossroads for oxydative and nitrosative pathways and for damaging and protective actions as well. The NO-related formation and opening behavior of the mitochondrial permeabilty transition pore (mtPTP) is suggested to play a particular role in this context. As to brain tissue, preconditioning induced by NO pre-adaption can increase the tolerance to nitrosative/oxidative stress, although the underlying neuroprotective mechanisms are not fully understood. Accordingly we show that not only the concentration but also the time point of the NO exposure determines the outcome of NO actions as demonstrated by the intracellular calcium dynamics and mtPTP opening in response to the activation of glutamate receptors (NMDA-subtype).

PSM03-12

Focussed microarray analysis reveals induction of TRAIL in cerebral cortex following fluid-percussion injury in the rat

R HERRERO, JH YI and AS HAZELL

Neuroscience Research Unit, Department of Medicine, University of Montreal, Montreal, Canada

The role of neuronal apoptosis in the pathophysiology of traumatic brain injury (TBI) is being increasingly recognized. Its relevance and the pathways by which it occurs in TBI, however, are not well defined. The aim of our study was therefore to identify genes that may contribute to the development of this type of cell death following TBI. Male Sprague-Dawley rats (300–350 g) underwent moderate lateral fluid-percussion injury and were sacrificed 6 h and 24 h post-injury. Focussed microarray analysis of the injured cortex involving 96 apoptosis-related genes (Oligo GEArray for Rat Apoptosis, SuperArray, Frederick, MD, USA) identified altered expression of diverse pro-apoptotic (Bid, Tnfrsf1b, TRAIL) and anti-apoptotic (Bcl2, Bcl-w, Bcl2a1) genes in this brain region. In particular, expression of TRAIL (tumor necrosis factor-related apoptosis-inducing ligand) was dramatically increased by 5.2-fold and 10-fold compared to shams at 6 h and 24 h following injury, respectively. Immunoblot analysis of TRAIL confirmed these changes at the protein level at 6 h and 24 h post-TBI. Immunohistochemical studies revealed this protein to be predominantly localized in neurons of the ipsilateral cortex. Since TRAIL is a potent inducer of apoptosis, our findings suggest an important role for it in the development of neuronal cell death following TBI.

Acknowledgements: Supported by CIHR and the Marie Robert Head Trauma Foundation (Quebec).

PSM03-13

GD3 induces apoptosis in U-1242 MG glioma cells through a caspase-8 dependent pathway

HE SAQR, OM OMRAN, JL OBLINGER and AJ YATES

The Ohio State University, Columbus, OH, USA

An increasing amount of evidence indicates that the disialoganglioside GD3 is involved in apoptosis in many cell lines. Our previous studies demonstrated that endogenous GD3 expression induced apoptosis in U-1242 mg glioma cells transfected with the GD3 synthase gene (U1242MG-GD3+ cells). We present evidence that GD3-induced apoptosis occurs through a caspase-8 dependent pathway since the membrane-permeable caspase-8 inhibitor zIETD-fmk was very effective at reducing apoptosis in GD3-expressing U1242MG-GD3+ cells compared to GD3-induced cells with functional caspase-8. We found that endogenously synthesized GD3 localizes to the caveolae of U1242MG-GD3+ and caspase-8 was also translocated to the caveolar fraction and cleaved; the cleavage products of caspase-8 then re-located into the dense fractions. GD3 also induced the aggregation of caspase-8 into small patches on the plasmalemma, where they later developed into apoptotic blebs. In addition, GD3 caused the translocation of several death receptors (including DR5, Fas, and TNF-R1) to the caveolae, where they also aggregated into the apoptotic blebs. In conclusion, we found that GD3 induces apoptosis through a caspase-8 mediated pathway which is initiated by the aggregation of death receptors within the caveolae.

Acknowledgement: This work was supported by a grant from the NCI CA85799.

PSM03-14

Inhibitors of Bcl-2/x(L) function elicit glutathione-sensitive neuronal death and oxidation of the mitochondrial ANT

AK ZIMMERMANN*, FA LOUCKS†, RJ BOUCHARD*, KA HEIDENREICH*† and DA LINSEMAN*†

**University of Colorado Health Sciences Center, †Veterans Affairs Medical Center, Denver, CO, USA*

Apoptosis plays a significant role in neurodegeneration and often occurs via an intrinsic death pathway. Bcl-2 proteins are key regulators of this mitochondrial apoptotic cascade. Pro-survival family members, like Bcl-2 and Bcl-x(L), protect neurons from diverse apoptotic insults. Herein, we investigated the pro-survival function of Bcl-2/x(L) in cerebellar granule neurons by defining the mechanism of death elicited by inhibition of these proteins. Inhibition of Bcl-2/x(L) with either of two uniquely structured Bcl-2-homology-3 (BH3) domain mimetics, HA14-1 or Compound 6, induced CGN apoptosis that was prevented by the antioxidant, glutathione (GSH). Similarly, antisense-mediated depletion of Bcl-2 triggered GSH-sensitive CGN apoptosis. These data indicate that suppression of oxidative stress is a key pro-survival function of Bcl-2/x(L) in CGNs. Moreover, blockade of this pro-survival activity is sufficient to induce oxidative stress-dependent neuronal apoptosis. Utilizing a dinitrophenylhydrazine (DNPH) assay to detect free carbonyls on oxidized proteins, we identified the mitochondrial adenine nucleotide translocase (ANT) as a major target of oxidation in Bcl-2/x(L)-suppressed CGNs. We are currently investigating if the oxidation of ANT disrupts its ATP/ADP translocase function and contributes to opening of the permeability transition pore complex and subsequent mitochondrial depolarization. These findings suggest that protecting ANT from oxidative inactivation is an essential neuroprotective function of Bcl-2/x(L).

PSM03-15

SEQUENTIAL activation of guanylate cyclase, PKG and cGMP-degrading phosphodiesterase in LTP. alterations in hyperammonemia

P MONFORT and V FELIPO

Fundación Valenciana de Investigaciones Biomédicas, Valencia, Spain

Long-term potentiation (LTP) in hippocampus is a long-lasting enhancement of synaptic transmision efficacy and is considered the basis for some forms of learning and memory. Hyperammonemia is a main responsible for the neurological alterations in and hepatic encephalopathy, including decreased intellectual function. LTP is impaired in hyperammonemia. We showed that sequential activation of soluble guanylate cyclase (sGC), cGMP-dependent protein kinase (PKG) and cGMP-degrading phosphodiesterase (PDE) is necessary for proper LTP induction in hippocampus. Application of the tetanus induces a rapid rise in cGMP, reaching a maximum at 10 seconds. sGC activity increased at 10 seconds after tetanus remaining high at 5 min and returning to basal levels at 60 min. This leads to an initial transient increase in cGMP. A subsequent decrease in cGMP was due to sustained (at least 60 min.) tetanus-induced increase in PDE activity which is mediated by activation of PKG. This indicates that the initial increase in cGMP activates PKG that phosphorylates (and activates) PDE, which, in turn, degrades cGMP. Proper induction of LTP requires activation of this pathway. We found that hyperammonemia impairs LTP in hippocampus by altering the modulation of this sGC-PKG-PDE pathway. Exposure of hippocampal slices to 1 mM ammonia completely prevents tetanus-induced decrease of cGMP by imparing PKG-mediated activation of PDE. LTP is also impaired in hippocampal slices from hyperammonemic rats without liver failure and more severely in rats with portacaval anastomosis, a model of hepatic failure. These alterations in LTP may be involved in the cognitive impairment in patients with hepatic encephalopathy.

PSM03-16

Neurotrophic growth factors up-regulate neuroprotectin D1 (NPD1) synthesis and anti-apoptotic signaling in human retinal pigment epithelial (RPE) cells

NG BAZAN*, VL MARCHESELLI*, PK MUKHERJEE, J HU†, D BOK† and M HARDY*

**Neuroscience Center and Department of Ophthalmology, LSU Health Sciences Center School of Medicine, New Orleans, LA,*
†Jules Stein Eye Institute, UCLA, Los Angeles, CA

Neuroprotectin D1 (NPD1) is synthesized from docosahexaenoic acid (DHA) by enzyme-mediated oxygenation in brain and in RPE cells, and displays potent cytoprotective bioactivity against oxidative stress, ischemia-reperfusion, and neurodegeneration (PNAS 101:8491, 2004; JBC 278:43807, 2003; Brain Pathol 15:159, 2005). Here we have explored growth factors as potential modulators of NPD1 synthesis in RPE cells and aimed to define the mechanisms involved. Human RPE cells seeded onto Millicell-HA culture plates displayed transepithelial resistance of at least 500 $\Omega \times cm^{-2}$. After adding neurotrophic growth factors (GDNF, rCNTF, rhNGF, hFGF, rhBDNF, persepin, cardiotrophin, LiF, NT3, or PEDF), apical and basal media were separately collected and measured by LC-PDA-ESI-MS-MS-based lipidomic analysis. All neurotrophic factors, except cardiotrophin, activated NPD1 synthesis and release through the apical surface of the cell. PEDF was by far the most potent when added to the apical medium, and not to the basal medium. Dose-dependent NPD1 synthesis occurred when cells were treated on the apical side with DHA, but when co-cultured with PEDF, a 4-fold increase in NPD1 level was found. We also used ARPE-19 cells confronted with oxidative stress. We found that DHA synergized with PEDF to down-regulate cytokine-mediated pro-apoptotic Bcl-2, Bax, Bid, and Bad proteins and to up-regulate anti-apoptotic Bcl-2 and Bfl-1A expression. At the same time, caspase-3 was down-regulated, NPD1 was synthesized, and oxidative stress-mediated apoptosis was down-regulated. The concentration-dependency of this effect (50–200 pM of the growth factor) and the specificity of the apical cell surface suggest that growth factor-mediated regulation of NPD1 synthesis may be either autocrine or paracrine, and that the NPD1 release to the interphotoreceptor matrix may exert survival actions. Similar mechanisms may operate in brain cells when confronted with oxidative stress or neurodegeneration. Supported by NIH EY05121.

Poster Session PSM04: Gene expression and signaling I

PSM04-01

Regulation of cholesterol/lipid biosynthetic genes by Egr2/Krox-20 during peripheral nerve myelination

SE LEBLANC*, R SRINIVASAN*, C FERRI†, GM MAGER*, AL GILLIAN-DANIEL*, L WRABETZ† and J SVAREN*

Affiliations: *University of Wisconsin-Madison, Madison, WI, USA, †San Raffaele Scientific Institute, Milan, Italy*

Myelination of peripheral nerves by Schwann cells requires a large amount of lipid and cholesterol biosynthesis. To elucidate the transcriptional coordination of the myelination process, we have investigated the developmental relationship between Egr2/Krox-20, a pivotal regulator of peripheral nerve myelination, and the Sterol Regulatory Element Binding Protein (SREBP) pathway, which controls expression of cholesterol/lipid biosynthetic genes. During myelination of sciatic nerve, there is a very significant induction of SREBP1 and SREBP2, as well as their target genes, suggesting that the SREBP transactivators are important regulators in the myelination process. Egr2/Krox-20 does not appear to directly regulate the levels of SREBP pathway components, but rather, we found that Egr2/Krox-20 and SREBP transactivators can synergistically activate promoters of several SREBP target genes, indicating that direct induction of cholesterol/lipid biosynthetic genes by Egr2/Krox-20 is a part of the myelination program regulated by this transactivator.

PSM04-02

Nab2 represses transcription by recruiting the nucleosome remodeling and histone deacetylase complex

GM MAGER, R SRINIVASAN, RM WARD, J MAYER and JP SVAREN

Affiliations: *University of Wisconsin-Madison, Madison, WI, USA*

The EGR2/Krox-20 transcriptional activator plays a critical role in vertebrate hindbrain development and in peripheral nerve myelination. Our previous studies revealed that EGR2 expression in Schwann cells regulates genes for a number of myelin proteins, as well as genes involved in the lipid/cholesterol synthesis required for myelin formation. Recently, several independent mutations have been associated with human peripheral neuropathies. One such mutation highlights the importance of regulation of EGR2 activity by the NAB1 and NAB2 (NGFI-A/EGR1-binding) corepressors, which are recruited to EGR2 target promoters by direct interaction with EGR2. Using protein interaction assays, we have found that NAB2 interacts with the CHD4 (Chromodomain Helicase DNA-binding protein 4) subunit of the NuRD (Nucleosome Remodeling and Deacetylase) chromatin remodeling complex, and dominant negative mutants of CHD4 have revealed that NAB2 represses via interaction with the NuRD complex. In addition, we have also characterized the domains required for interaction between NAB2 and CHD4. This unexpectedly revealed that EGR2 activity is modulated by at least two repression domains within NAB2, one of which uniquely requires interaction with CHD4 in order to repress transcription. Finally, the interaction with CHD4 is regulated by alternative splicing of the NAB2 mRNA. Overall, our studies provide the first evidence implicating a chromatin remodeling complex in the transcriptional regulation of the myelination program in Schwann cells.

PSM04-03

6-Hydroxydopamine activates the antioxidant response element through oxidative, excitotoxic, and structural factors

RJ JAKEL*†, JT KERN†, DA JOHNSON† and JA JOHNSON*†

Affiliations: *Neuroscience, †Pharmacy, UW-Madison, Madison, MI, USA*

Parkinson's disease (PD), a progressive neurodegenerative disorder, is characterized by loss of midbrain dopaminergic neurons. The etiology of sporadic PD is unknown; however, oxidative stress is thought to play a role in disease pathogenesis. The antioxidant response element (ARE) is a cis-acting enhancer upstream of many phase II detoxification and antioxidant genes. Transgenic reporter mice engineered to express ARE-driven human placental alkaline phosphatase (hPAP) have been shown to be sensors of oxidative stress. 6-hydroxydopamine (6OHDA), a mitochondrial complex I inhibitor and oxidative stressor, is a toxin used to model sporadic PD. 6OHDA-induced toxicity can be attenuated by exogenous antioxidants. Additionally, 6OHDA shares some structural similarities with HQ and tBHQ, two potent activators of the ARE. We hypothesize that 6OHDA activates the ARE due to generation of ROS as well via structural activation. Primary cortical cultures prepared from ARE-hPAP mice exposed to 6OHDA demonstrate ARE activation in a dose-dependent fashion. Pretreatment with high-dose antioxidants reduced, but did not abolish ARE activation, indicating a component of ARE activation that is oxidative stress independent. Pretreatment with MK-801 also reduced ARE-activation at low concentrations of 6OHDA suggesting that the oxidative stress caused by 6OHDA is caused, in part via an excitotoxic mechanism. When administered stereotactically to ARE-hPAP mice, 6OHDA induces ARE activity *in vivo*. The data suggest that ARE-mediated gene activity may be a mechanism to cope with oxidative stress due to 6OHDA.

PSM04-04

Gene expression in a retinal model of age-related susceptibility

JE ROYL and AM GELLER
USEPA, Neurotox. Div., RTP, USA

Our study of susceptibility in the aged population, examines changes in constitutive gene expression in the retinas of young (4 mos), middle-aged (11 mos) and aged (23 mos) male Long Evans rats. The retina displays age-related anatomical changes and a lifetime accumulation of non-degradable metabolic by-products. Age is also a co-factor in retinal degeneration caused by fungicides and organophosphate insecticides. Gene expression in retinas from 7–8 rats/age was assessed using the Affymetrix rat 230A gene chip. A number of genes were found to change with aging. Expression of members of the crystallin family decreased significantly ≥ 2 fold). Crystallin alpha A gene expression decreased 9 fold from 4 to 11 mos and a further 29 fold from 11 to 23 mos. Crystallin alpha A belongs to the small heat shock protein family of molecular chaperones. It binds to denatured proteins, preventing their aggregation, and may play a role in cell proliferation and genomic stability. It can prevent apoptosis by inhibiting caspases and possesses autokinase activity. This suggests that it plays a protective role in the retina and its loss could contribute to age-related susceptibility. In contrast, genes associated with the immune response (mucins, protein kinase C gamma, and keratin) were up-regulated in the aged retina. Gene expression of glial fibrillary acidic protein (GFAP), a known marker for neuronal degeneration, increased 2.3 fold from 4 to 11 mos and 2.6 fold from 11 to 23 mos. These data present a picture of the aged retina with enhanced susceptibility to environmental injury via loss of protective mechanisms and functional cells. This abstract does not necessarily reflect US EPA policy.

PSM04-05

Gene expression profiles in the developing rat cerebellum and hippocampus

PR KODAVANTI and JE ROYL
Neurotoxicology Division, NHEERL/ORD, USEPA, Research Triangle Park, NC, USA

Development of the nervous system is a complex program. In rodents, rapid brain growth occurs during early postnatal development. At this time, dendritic and axonal outgrowth and the establishment of neural connections occur. Simultaneously, animals acquire many new motor and sensory abilities. In this study, we compared the steady state genomic expression profiles in the hippocampus (Hip) and cerebellum (Cb) during the rapid brain growth period. RNA was extracted from male Long-Evans rat brains on postnatal days (PNDs) 7 and 14. Gene expression was determined on Affymetrix rat 230A chips containing 15 923 genes. Preliminary analysis shows that ≈ 3500 and 4000 genes are ≥1.5 fold differentially expressed between the Hip and Cb at PND7 and 14, respectively, with ≈ 2300 genes in common to both ages. Expression levels of ≈ 2100 genes in the cerebellum and ≈ 1600 genes in the hippocampus are changed by >1.5 fold from PND7 to 14. Of those genes, 680 were common to both brain regions. Interestingly 9 of the 10 top genes upregulated from PND7 to 14 in both the Cb and Hip were related to myelination. This correlates with the rapid increase in myelin known to occur during the brain growth spurt. Genes down regulated dealt with neuronal migration and axon outgrowth (e.g. cerebroglycan, retinoic acid binding protein), suggesting these processes were largely complete by PND14. Data indicate that (1) differences in gene profiles are greater due to brain area than developmental age based on number of affected genes, (2) gene profiles reflect changes during brain development and (3) careful baseline studies are necessary for accurate interpretation of genomic data. (The abstract does not necessarily reflect USEPA policy).

PSM04-06

Transcription of brain creatine kinase in U87-MG glioblastoma is modulated by factor AP2

GR MOLLOY, D WILLIS and Y ZHANG
University of Delaware, Newark, DE, USA

In astrocytes, brain creatine kinase (CKB) is essential in regenerating the ATP needed to drive the morphological change from a stellate to a flat morphology, an event mediated by cyclic AMP. CKB may function to maintain the rapid cytoskeletal rearrangements that mediate changes from stellate to flat morphology which are known to be essential to astrocyte functioning for both the uptake of glutamate from the synaptic cleft and in supporting the formation and maintenance of neuronal synapses. Despite the absence of a cAMP response element (CRE) in the CKB proximal promoter, we showed previously that elevated cAMP increased transcription of CKB. Here we show transcription of CKB in U87 cells is induced by transcription factor AP2 alpha, which is known to be activated by cAMP. Systematic mutagenesis of the four potential AP2 elements within the CKB proximal promoter showed induction of CKB by AP2 was mediated principally through the AP2 element located at −50 bp in the promoter. Electromobility shift assays revealed a protein in U87 nuclear extracts that bound to a consensus AP2 alpha element as well as to the (−50) AP2 element in CKB. Interestingly, the CKB (−50) AP2 element contains GCCAATGGG which also bound NF-Y, the CCAAT-binding protein, suggesting that interplay between AP2 and NF-Y may modulate CKB transcription. This is the first report of a role for AP2 in the regulation of CKB transcription and of an AP2 element within which an NF-Y site is also located.

PSM04-07

Regulation of myelin proteolipid protein gene expression: molecular dissection of the antisilencer/enhancer region in intron 1

BT GREUEL*, B PEREIRA*, M SAMPLE* and PA WIGHT†
**John Brown Univ., Siloam Springs, †Univ. of Arkansas for Medical Sciences, Little Rock, AR, USA*

Expression of the myelin proteolipid protein (*Plp*) gene is intimately tied to oligodendrocyte differentiation and peaks during the active myelination period of CNS development. Several regulatory elements/regions have been implicated in the spatiotemporal regulation of *Plp* gene expression. One region, designated antisilencer/enhancer (ASE), is present within mouse intron 1 DNA positions 1093–1177 and appears to mediate the dramatic upsurge in *Plp* gene activity during the active myelination period. To characterize the functional domains within the ASE, eleven linker scanning (LS) mutants containing 8 bp substitutions were generated, which span the entire ASE, and inserted into a *Plp-lacZ* fusion gene lacking the ASE. The constructs were transiently transfected into N20.1 (oligodendroglial) cells and analyzed for β-gal activity. Five of the mutants with linkers inserted near the center of the ASE demonstrated reduced activity (10–50% of wild-type levels). The LS mutant with the largest negative effect overlaps putative binding sites for AP-1, Oct-1, NRF2, p53, MEIS1, BACH1, HOXA9, MIBP1/RFX1, and TCF11/MafG suggesting that these sites may be important for *Plp* gene regulation. Surprisingly, mutants with linkers inserted on either side of the central region increased β-gal activity by 2–3-fold, relative to wild-type levels, even though our previous results suggest that the entire sequence is necessary for the enhancer to function in an orientation-independent manner. These results demonstrate the critical importance of sequences within the central domain of the ASE in the up-regulation of *Plp* gene expression.

PSM04-08

Homology modelling and site directed mutagenesis of pyroglutamyl peptidase II. Omega-versus amino-peptidase specificy

L CHÁVEZ-GUTIÉRREZ*, E MATTA*, J OSUNA*, P JOSEPH-BRAVO*, B MAIGRET† and JL CHARLI*

**IBT, UNAM, Cuernavaca, México, †Université Henri-Poincaré, Nancy, France*

Pyroglutamyl peptidase II (PPII) is an omegapeptidase that removes N-terminal pyroglutamate from thyrotropin releasing hormone. PPII is a member of the M1 family of metallopeptidases, which includes aminopeptidase N (APN) or leukotriene A4 aminopeptidase (LTA4H). These homologous exopeptidases hydrolyse peptides with different structures. Recognition of the NH2-group of substrates by M1 aminopeptidases is by hydrogen-bonding of two well conserved residues with the amine moiety. For PPII (no free amino terminus), the recognition mechanism has not yet been clarified. While one of the NH2 recognizing residues is conserved (Glu408, exopeptidase motif), the other one is replaced by a serine (Ser269). Given that the structural determinants ensuring the specificity of M1 peptidases are not established, we built a 3D-model for the catalytic domains of PPII and APN, using the crystallographic structure of LTA4H as template. Unlike for APN, we found a salt-bridge in PPII active site (E408-K463). Multiple alignments in the family showed that the S269 and K463 are specific for PPII. We predicted that S269Q-K463N PPII double mutation should transform PPII from an omega to a true aminopeptidase. This hypothesis was supported by site-directed mutagenesis, showing that E408-K463 salt bridge is crucial for PPII activity while the S269Q/K463N PPII mutant displayed amino peptidase activity. Therefore, interactions of the Glu within the exopeptidase motif with various alternative residues play a key role in determining specificity in the M1 family.

Acknowledgement: Supported in part by CONACYT 39931.

PSM04-09

Characterization of the enhanceosome formed on the myelin proteolipid gene by DNA affinity chromatography and mass spectrometry

A DOBRETSOVA*, CF LICHTI† and PA WIGHT*

**Department of Physiology and Biophysics, †Department of Biochemistry and Molecular Biology, University of Arkansas for Medical Sciences, Little Rock, AR, USA*

The myelin proteolipid protein (Plp) gene encodes the principal protein present in CNS myelin, accounting for nearly 50% of the total protein. In oligodendrocytes, expression of the gene is regulated in a temporal fashion, peaking during the active myelination period of CNS development. Data from our laboratory suggest that the dramatic upswing in Plp gene activity during this period is mediated by a potent transcription regulatory element located within intron 1 DNA. This element, termed ASE for antisilencer/enhancer, has been delimited to an 85-bp sequence located approximately 1 kb downstream of exon 1 sequences. Previous results from our laboratory suggest that assembly of a higher-order, multi-protein complex (enhanceosome) must form on the ASE, for it to function. In order to identify the factors that constitute the complex, nuclear extracts prepared from mouse brains were subjected to several chromatographic separation steps, including DNA-affinity purification, followed by mass spectrometry analysis. A number of proteins involved in transcriptional regulation including PARP-1, USF-2, TAR DNA binding protein, NF-45 and JunD demonstrated selective binding to the ASE sequence suggesting that these factors may be important in up regulating Plp gene expression in oligodendrocytes.

Acknowledgement: Supported by NIH grant NS037821.

PSM04-10

Thrombin enhances osmosensitive release of taurine from human 1321N1 astrocytomas: role of volume sensitive organic anion channel

TA CHEEMA, C WARD and SK FISHER

Department of Pharmacology, University of Michigan, Ann Arbor, MI, USA

The homeostatic mechanism for regulation of cell volume is crucial in the central nervous system due to restrictions imposed by the skull. A mechanism known as regulatory volume decrease helps to normalize cell volume after hypo-osmotic swelling by the release of organic osmolytes, such as taurine via a volume-sensitive organic osmolyte anion channel (VSOAC). In this study, the ability of thrombin to regulate the osmosensitive taurine efflux from human 1321N1 astroyctoma cells has been examined. Addition of thrombin resulted in an enhancement (3–4 fold) of taurine efflux in hypoosmolar buffers increasing the threshold of release from 270 to 340 mOsm. The effect of thrombin(EC_{50} = 60 pM) was primarily mediated via the PAR-1 receptor and could be blocked by anion channel inhibitors such as 1,9-dideoxyforskolin, DIDS, NPPB and SITS indicating the involvement of a VSOAC. Thrombin receptors can couple to G_q, $G_{i/o}$ or $G_{12/13}$ signaling pathways. However, pretreatment of cells with pertussis toxin did not significantly inhibit stimulated taurine efflux. Addition of thrombin to 1321N1 cells elicited both a rise in $[Ca^{2+}]_i$ and an increase in phosphoinositide turnover. Elevation of $[Ca^{2+}]_i$ with ionomycin partially mimicked the ability of thrombin to stimulate taurine efflux. Although removal of extracellular Ca^{2+} had no effect on thrombin stimulated taurine efflux, depletion of intracellular Ca^{2+} with BAPTA-AM resulted in a 60% inhibition. The results suggest that low neuroprotective concentrations of thrombin enhance the volume-dependent efflux of osmolytes from 1321N1 astrocytoma cells via a VSOAC.

Acknowledgement: Supported by NIH NS 23831 to SKF.

Poster Session PSM05: Glia and inflammation I

PSM05-01

GFAP mutations account for all forms of Alexander disease

M BRENNER*, R LI*, M SU*, AB JOHNSON†, MS VAN DER KNAAP‡, GS SALOMONS‡, J GOLDMAN§, R QUINLAN¶ and A MESSING**

**Dept Neurobiol, UAB, Birmingham, AL, †Dept Pathol, Albert Einstein College of Medicine, NY, USA, ‡Dept Child Neurol and Clinic Chem, Vrije Univ Medical Center, Amsterdam, the Netherlands, §Dept Pathol, Columbia Univ, NY, ¶Univ Durham, Sch Biol & Biom Sci, Durham, NC, **Univ Wisconsin, Waisman CTR, Madison, WI, USA*

Alexander disease is a fatal leukodystrophy that primarily affects infants, but also occurs in juveniles and adults. Although the clinical signs differ markedly for the different age groups, they are united by the abundant presence of astrocytic protein aggregates that contain GFAP. We recently discovered heterozygous missense mutations in GFAP for 12 of 13 infantile cases of Alexander disease tested. We have now expanded our analyses to another 41 cases, including 10 patients with the juvenile form and 3 with the adult form. Heterozygous missense mutations in GFAP have been found in all forms, demonstrating that later onset Alexander disease is also due to GFAP alterations. Nearly all the mutations arise de novo on the paternal chromosome, indicating that they occur during gametogenesis. We have investigated the functional consequences of several of the GFAP mutations by transfecting expression vectors into SW13vim-cells. The mutant protein formed aggregates, whereas the wild type protein yielded normal appearing filaments. Preliminary results with a monoclonal antibody specific for one of the mutations indicates that the mutant protein is present at significantly lower levels than the wild type in Alexander disease brain.

Acknowledgements: Supported by NINDS grant P01NS42803, MRRC grant P30HD38985, and the Lei Foundation.

PSM05-02

Regulated glutathionylation in astrocytes: a new aspect of neuroinflammation

K HENSLEY, M MHATRE, S MOU, N PYE and L SZWEDA

Oklahoma Medical Research Foundation, Oklahoma City, OK, USA

Glial inflammation (neuroinflammation) has gained credence in recent years as a genuine phenomenon that may contribute to the pathogenesis of diverse neurolgical diseases. Neuroinflammation is manifest by a plethora of glial alterations occurring both in astrocytes and microglia. Operationally glial inflammation comprises cytokine hyperexpression, increased production of reactive oxygen and nitrogen species, and alterations in signal transduction including hyperactivity of multiple protein kinase cascades. These aspects are inter-related in a complex and yet poorly understood fashion. Recently our laboratory has begun investigating glutathionylation as a redox-sensitive signal transduction modality that is altered in the G93A-SOD1 mouse model of amyotrophic lateral sclerosis (ALS), a motor neuron disease possessing a strong component of glial inflammation. Using monoclonal antibodies and biotinylated glutathione probes we identify a major 45 kDa protein that is glutathionylated in astrocytes, in response to tumor necrosis factor alpha, and which appears to be hyperglutathionylated in spinal cords from the G93A-SOD1 mouse. Data suggests this protein to be actin. The relationship of glutathionylated proteins especially glial cell actin is the subject of ongoing investigations within our labs.

Acknowledgements: Supported in part by the Oklahoma Center for Advancement of Science and Technology, the ALS Association and NIH NS0144154.

PSM05-03

Generation and characterization of mice carrying Alexander disease-associated mutations in GFAP

A MESSING, JX CONNOR and TL HAGEMANN

Waisman Center, University of Wisconsin, Madison, WI, USA

Alexander disease is a rare leukodystrophy caused by mutations in the gene for the astrocyte specific intermediate filament glial fibrillary acidic protein (GFAP). To develop an animal model for this disorder, we have generated knock-in mice with missense mutations homologous to those found in humans. Mice with a GFAP-R236H mutation corresponding to R239H in humans, develop Rosenthal fibers, the hallmark protein aggregates observed in astrocytes in Alexander disease, in the corpus callosum, periventricular and subpial regions, hippocampus and olfactory bulbs. Histochemical staining for myelin, and western analysis of MBP show no apparent differences between mutant and wildtype mice. However, when crossed with an anti-oxidant response element reporter line, the mutant mice show a distinct pattern of reporter gene induction that is especially prominent in the corpus callosum. Histochemical staining also reveals an accumulation of iron in the corpus callosum. The GFAP-R236H mice are smaller than wildtype littermates, but have a normal lifespan and show no overt behavioral defects. Although the mice do not display the full spectrum of pathology observed in Alexander disease of humans, these studies provide formal proof linking GFAP mutations with Rosenthal fibers and oxidative stress.

PSM05-04

Transcription factor activity in astrocytes: interactions between HIV-1 tat and opiates

SP ZOU, MH ADAMS, TY ZHAO, N EL-HAGE, KF HAUSER, AJ BRUCE-KELLER and PE KNAPP

Dept. Anat. & Neurobiol., Univ. KY, Lexington, KY, USA

Opiates can act synergistically with the HIV protein Tat to destabilize astrocyte (AS) function. Our previous work has shown that Tat alone drives changes in $[Ca^{2+}]i$, ROS production, and secretion of inflammatory chemo/cytokines. Combined exposure to Tat+morphine can enhance many of these responses. We have hypothesized that opiate-induced dysregulation of HIV-exposed ASs may drive aspects of the neuropathology seen in some HIV infected individuals, and may accelerate HIV neuropathogenesis in individuals who abuse opiates. TransSignal' Protein/DNA Arrays (Panomics) were used to identify signaling pathways and transcriptional events that may be involved in AS responses. Murine ASs were exposed for 4 or 24 h to Tat, morphine, or Tat+morphine. Nuclear extracts were probed with biotinylated DNA-binding oligonucleotides. Protein-DNA complexes were hybridized to arrays with consensus-binding sequences for over 150 transcription factors and detected with chemiluminescence. Most factors were unchanged or depressed by morphine alone at 4 and 24 h. Tat alone, or Tat+morphine upregulated proteins in the AP-1/NFAT, forkhead (FKHR, FOXO4, Freac), GATA, ATF/CREB, and Rel/NFkB families in temporally specific patterns. Many of these modulate pathways involved in inflammation or cell survival, with downstream effects through PI3-kinase/Akt or MAPK. Results suggest that HIV activates multiple transcriptional events in ASs and that the disruptive effects in this cell type are exacerbated by concurrent opiate abuse.

Acknowledgements: Support by DA15097 (PEK) & DA13728 (KFH).

PSM05-05

Caveolin isoform expression during differentiation of C6 glioma cells

WI SILVA*, G VELÁZQUEZ*, M RUBIO-DÁVILA*, JD MIRANDA*, HM MALDONADO†, J JARDÓN*, E AQUINO*, N MAYOL† and A CRUZ*

**Physiology and Microbiology Departments, UPR Medical School, San Juan, †Pharmacology Department, UCC Medical School, Bayamon, PR, USA*

Caveolae and their constituent caveolins play a role in potocytosis, endocytosis, transcytosis and signal transduction. This study expands the analysis of the expression of cav1, 2 and 3 in C6 glioma cells, and evaluates their expression during db-cAMP-induced differentiation. Three distinct approaches unveiled the expression of cav3 in C6 cells, in addition to cav1 and cav2. In C6, cav3 was associated with light-density, detergent-insoluble membrane fractions. In addition, during differentiation real-time RT-PCR revealed a parallel up-regulation at T48 of cav1 134% ($P < 0.01$) and cav2 117% ($P < 0.05$). In contrast, cav3 mRNA levels gradually decrease, with a maximum decline of 85% ($P < 0.001$) at T48. Immunoblots revealed a similar pattern, with up-regulation at T48 of cav1 30% and cav2 (82%) ($P < 0.05$), and a decrease in cav3 levels at T24 ($P < 0.05$) and T48 ($P < 0.01$) (maximum decline of 37%). Indirect immunofluorescence analysis via laser scanning confocal microscopy reveals that cav1 and cav2, display similar subcellular distribution patterns and co-localization in undifferentiated and differentiated C6 cells. A similar profile was revealed when cav1 and cav3 distribution and co-localization were analyzed. Therefore, the C6 astroglial cells model system permits the assessment of the role of caveolae and caveolins in glial cell maturation and differentiation, and the signal transduction processes that these may mediate.

Acknowledgements: Supported in part by NIH grants GM08224 (WIS), GM50695 (HMM), and GM61838 (RISE students GV and JJ).

PSM05-06

Intracellular signaling pathways in ammonia-induced astrocyte dysfunction

AR JAYAKUMAR, KS PANICKAR and MD NORENBERG

Dept of Pathology, VA Medical Center & University of Miami Miller School of Medicine, Miami, FL, USA

Hepatic encephalopathy (HE) is a major complication in patients with severe liver failure. Ammonia is a major pathogenetic factor in HE and astrocytes appear to be the primary target of ammonia toxicity. Astrocyte swelling and neurotransmitter dysfunction are major findings in HE, but the mechanisms of ammonia-induced astrocyte dysfunction are unclear. We investigated the role of various intracellular signaling pathways potentially involved in cell swelling and glutamate uptake in rat primary cultured astrocytes. Cultures were exposed to 5 mM ammonium chloride and phosphorylation (activation) of mitogen-activated protein kinases (MAPKs) was examined by western blots. Erk-1/2 phosphorylation was increased by 50% and 200% at 1 and 24, hr respectively ($P < 0.05$ vs. cont) after ammonia treatment. Ammonia also increased the phosphorylation of p38 and SAPK/JNK by 110% at 3 and 6 h ($P < 0.05$). Effect of inhibitors of MAPKs on ammonia-induced cell swelling was assessed by 3-OMG method. Cultures were treated with ammonia for 48 h with or without MAPK inhibitors (UO126, SB239063 and SP600125 for Erk1/2, p38 and JNK, respectively). UO126 and SB239063 (but not SP600125) prevented ammonia-induced cell swelling by 90% and 70% respectively ($P < 0.05$). Ammonia-induced glutamate uptake inhibition was reversed by 91% and 79% by inhibitors of p38 and JNK respectively ($P < 0.05$), but inhibition of Erk had no effect. Further, cultures treated with the above kinase inhibitors significantly protected ammonia-induced cytopathological changes. These studies indicate that ammonia-induced activation of MAPKs contributes to astrocyte dysfunction. These findings may assist in identifying novel molecular targets for the treatment of HE.

PSM05-07

Activation of ERK1/2 stimulates NHE1 activity in astrocytes in response to *in vitro* ischemia

DB KINTNER*, A LOOK*, GE SHULL† and DD SUN*‡

**Dept. Neurosurg., Univ. Wis., Madison, Madison, WI, †Dept. Mol. Gen., Biochem. and Microbio., Univ. Cin., Cincinnati, OH, ‡Dept. Physiol., Univ. Wis. Madison, Madison, WI, USA*

Na/H exchanger (NHE1) in astrocytes is stimulated and leads to intracellular Na^+ loading following oxygen and glucose deprivation(OGD) (Kintner *et al.*, 2004). However, the mechanisms for stimulation of NHE1 activity are unknown. Here, we investigated a role for the ERK1/2 pathway in NHE1 activation. NHE1 activity was elevated by ≈75% in $NHE1^{+/+}$ astrocytes following 2 h OGD and 1 h reoxygenation (O/R). The O/R-mediated stimulation of NHE1 was blocked by 30 μM PD98059. Increased expression of phosphorylated ERK 1/2 was detected in $NHE1^{+/+}$ astrocytes following O/R. Moreover, stimulation of NHE1 activity not only disrupted Na^+ but also Ca^{2+} homeostasis via reverse-mode operation of Na^+/Ca^{2+} exchange (NCX). O/R led to 103% increase in $[Ca^{2+}]_i$ in $NHE1^{+/+}$ astrocytes in the presence of thaspsigargin. Inhibition of NHE1 activity with HOE 642 decreased O/R-induced elevation of $[Ca^{2+}]_i$ by 73%. To further investigate changes of Ca^{2+} signaling, bradykinin (BK)-mediated Ca^{2+} release was evaluated. BK-mediated $Ca^{2+}{}_i$ transient in $NHE1^{+/+}$ astrocytes was increased by ≈ 84% following O/R. However, in $NHE1^{-/-}$ astrocytes or $NHE^{+/+}$ astrocytes treated with HOE 642, the BK-induced Ca^{2+} release was only increased by ≈34%. Inhibition of the reverse mode of NCX abolished O/R-mediated Ca^{2+} rise. Taken together, our data suggest that ERK1/2 is involved in activation of NHE1 in astrocytes following *in vitro* ischemia. NHE1-mediated Na^+ accumulation subsequently alters Ca^{2+} homeostasis via NCX.

Acknowledgements: NIH & AHA.

PSM05-08

Role of aquaporin-4 in the mechanism of ammonia-induced astrocyte swelling

KV RAMA RAO and MD NORENBERG

Department of Pathology, University of Miami School of Medicine, Miami, FL, USA

Brain edema is a lethal complication of hepatic encephalopathy (HE) in the setting of fulminant hepatic failure (FHF) and ammonia is a neurotoxin strongly implicated in HE. The edema in FHF is believed to be largely cytotoxic due to swelling of astrocytes. It was shown earlier that cultured astrocytes exposed to pathological concentrations of ammonia undergo swelling. However, the mechanisms of ammonia-induced astrocyte swelling are not completely understood. Aquaporins (AQP) are integral membrane proteins present in various cells, including astrocytes, that facilitate transmembrane water movement. Since altered aquaporin expression has been implicated in brain edema in various neurological conditions, we examined the role of aquaporin-4 (AQP-4), the principal AQP in astrocytes, on astrocyte swelling after exposure to NH_4Cl (5 mM). Cell volume was determined by the [^{3}H]-O-methylglucose method, and AQP-4 protein by immunoblot analysis. Astrocytes exposed to ammonia displayed an increase in AQP-4 protein as early as 9 h, progressively increased up to 24 h and persisted until 48 h. The increased AQP-4 expression preceded astrocyte swelling by ammonia. The degree of increase in AQP-4 expression correlated strongly with the severity of astrocyte swelling, suggesting a causal relationship between AQP-4 upregulation and astrocyte swelling by ammonia. Further, treatment of astrocytes with cyclosporin A, an inhibitor of the mitochondrial permeability transition (MPT) which was shown to block ammonia-induced astrocyte swelling, completely normalized the AQP-4 expression, suggesting the involvement of the MPT in the upregulation of AQP-4. These data suggest that increased AQP-4 expression may contribute to the development of brain edema in fulminant hepatic failure.

PSM05-09

Role of aggrecan in astrocyte differentiation

MS DOMOWICZ, MM MUELLER, JG HENRY and NB SCHWARTZ

Department of Pediatrics, University of Chicago, Chicago, IL, USA

Aggrecan expression is developmentally regulated in chick brain at both the protein and mRNA levels. Whole mount *in situ* hybridization detects aggrecan mRNA by E6 in the ventricular zone (VZ) of the ventral midbrain; by E8 expression progresses rostral to caudal through the VZ of the optic tectum and is observed in the VZ of the telencephalon and hindbrain in a characteristic pattern. When E11 optic tectum VZ was cultured in EGF/bFGF-containing media, cells expressing predominantly aggrecan, tenascin C, SOX9 and transitin, but not markers of differentiated neurons, astrocytes or oligodendrocytes, were established. These cells differentiate into astrocytes and oligodendrocytes when cultured in glia differentiation medium, suggesting that the cells which express aggrecan represent a sub-set of glia precursor that may give rise to astrocytes and/or oligodendrocytes *in vivo*. The nanomelic (nm) chick, which bears a mutation in the aggrecan gene that introduces a stop codon in the translated sequence, leading to synthesis of an unglycosylated truncated core protein, was used to analyze the consequences of a lack of aggrecan during brain development. Expression of several glial cell markers in the nm brain was monitored by *in situ* hybridization and Northern analysis: up-regulation and mislocalization of the astrocytic markers glutamine synthase, GFAP and GLAST at E20 in mesencephalon and cerebellum was observed, while markers of oligodendrocyte precursors and neurons remained unchanged. When cultures established from nm VZ were allowed to differentiate, levels of the astrocytic markers GFAP and GLAST were also increased compared to wt cultures, consistent with our observations *in vivo*. These results suggest a function for aggrecan in regulation of astrocyte differentiation.

PSM05-10

Neuronal and astroglial response to a long abstinence period after a low, chronic ethanol exposure in the adolescent rat

S MIROCHNIC, SG EVRARD, M DUHALDE VEGA, P TAGLIAFERRO, L CALTANA and A BRUSCO

Instituto de Biología Celular y Neurociencia, Fac. de Medicina, Univ. de Buenos Aires, Buenos Aires, Argentina

Chronic ethanol (EtOH) exposure (CEE) alters neurons and glia function. Long after the cessation of a low CEE we studied some morphological parameters of neurons and astrocytes. Adolescent male Wistar rats were exposed to EtOH 6.6% v/v in drinking water for 6 weeks and studied after ending exposure or after a 10-week recovery period drinking water. Brain sections were immunostained using antibodies to gliofibrillary acidic protein (GFAP, the main cytoskeletal astrocytic protein); S-100b (a cytosolic astocytic protein secreted under 5-HT_{1A} receptor stimulation); microtubule associated protein-2 (MAP-2, expressed mainly in dendrites); 200 kDa neurofilaments (Nf-200); neuronal nitric oxide synthase (nNOS) and serotonin (5-HT). We studied by image analysis three cognitive-related prosencephalic areas (CA1 hippocampal area, striatum and frontal cortex) and the mesencephalic dorsal and median raphe nucleus (DRN; MRN). In all the prosencephalic areas astrocytic cell area ($GFAP^+$-cells) was increased after exposure and tended to return to normality after abstinence; the immunoreactivity (-ir) of S100b protein, the relative area of $MAP\text{-}2^+$- and $Nf\text{-}200^+$-fibers were decreased, and later partially recovered. In the striatum and frontal cortex nNOS-ir was decreased after abstinence. 5-HT-ir was decreased in the DRN and recovered after abstinence and was not changed in the MRN. In conclusion: to stop drinking can partially ameliorate EtOH-induced morphological changes in neurons and astroglia but cannot fully return them to the basal state. In the recovery, astrocytes may play a role and S-100b may be involved.

Acknowledgements: Grant UBACYT M-072.

PSM05-11

Microglial iron status influences survival of oligodendrocytes

JR CONNOR and X ZHANG

Penn State University, Hershey, PA, USA

During normal development in white matter, iron accumulates first in microglia followed by oligodendrocytes and then myelination ensues. Following injury, microglia remain enriched for iron longer but eventually iron is detected in oligodendrocytes and myelination proceeds. Iron positive microglia and iron positive oligodendrocytes do not overlap. The *in vivo* observations prompted us to develop a cell culture model to test the relationship between iron status of microglia and survival of oligodendrocytes. Herein we report the effect of conditioned medium from microglia that were either iron loaded or not iron loaded on oligodendrocytic survival. Conditioned media from iron loaded microglia increases the survival of mature oligodendrocytes. Because of the involvement of iron and microglia in inflammatory reactions, a second experiment was performed in which iron loaded and non-iron loaded microglia were exposed to cytokines or lipopolysaccharide (LPS). Cytokines are lethal to oligodendrocytes but conditioned media from iron loaded microglial cells protected the oligodendrocytes from cytokine exposure. Finally, direct exposure to LPS has no effect on oligodendrocytes but conditioned medium from LPS activated microglial cells is lethal to oligodendrocytes. This lethal conditioned medium effect is inhibited if the microglial cells are treated with an iron chelator before activation. Iron loading the oligodendrocytes increases the lethal effect of the conditioned medium from LPS activated microglial cells. These results indicate that microglia play an important role in iron homoeostasis and mediating growth and survival of oligodendrocytes. These results also indicate that inflammation can alter the iron mediated trophic influence of microglia.

PSM05-12

NAAG, glutamate and NO-induced block of action potential generation and propagation in the crayfish nerve fiber

RA KHAIROVA and EM LIEBERMAN

School of Medicine, East Carolina University, Greenville, NC, USA

Previous investigations suggest that electrical stimulation of medial giant nerve fibers (MGNF) at high frequency causes the release of N-acetylaspartylglutamate (NAAG) into the perineural space and activation of the NAAG peptidase to yield glutamate and N-acetylaspartate. We have shown that the stimulation-induced hydrolysis of excess exogenous and endogenous NAAG with the release of glutamate activates glial NMDA receptors (NMDAR) and NO synthase (NOS) to generate a toxic quantity of NO that contributes to reduced axon excitability. Exogenous glutamate, similarly to NAAG and NMDA, decreased axon excitability in a dose-dependant manner. L-cysteic acid (10^{-3} M), a glutamate uptake inhibitor, potentiated the effect of glutamate on the block of action potential generation. The effect of NAAG, NMDA and glutamate was prevented by 10^{-5} M MK-801, an antagonist of NMDAR, while the effects of NAAG were also abolished by 10^{-7} M 2-phosphonomethyl-pentanedioic acid, NAAG peptidase inhibitor. Inhibition of guanylyl cyclase by 10^{-5} M ODQ or adenylyl cyclase by 10^{-5}M MDL-12,330 completely prevented the effect of 10^{-7} M NAAG on axon excitability. Superfusion of MGNF with cell-impermeable cAMP (10^{-6} M) or cGMP (10^{-6} M) had no effect on axon excitability. The results of these experiments suggest that activation of glial NMDAR by exogenous glutamate or glutamate released from NAAG hydrolysis triggers stimulation of NOS and production of NO. NO-induced activation of guanylyl and adenylyl cyclases is an essential step in the mechanisms of glutamate and NO neurotoxicity in MGNF of crayfish, as indicated by the block of action potential generation in the giant axon.

Poster Session PSM06: Metabolism

PSM06-01

Lactate originating from glycogen is compartmentalized from glucose derived lactate in cultured astrocytes

HM SICKMANN*, A SCHOUSBOE*, K FOSGERAU† and HS WAAGEPETERSEN*

**Department of Pharmacology, Danish University of Pharmaceutical Sciences, Copenhagen, †Novo Nordisk A/S, Pharmacology Research 1, Maaloev, Denmark*

Brain glycogen located in astrocytes has been shown to be degraded during high neuronal activity, indicating a functional role. The objective of this study was to examine glycogen metabolism in cultured mouse neocortical and cerebellar astrocytes, respectively. Isofagomine, a inhibitor of glycogen phosphorylase, was used as a pharmacological tool to investigate the association between glycogen metabolism and other metabolic parameters. The cultures were incubated for 4 hours in media containing [U-13C] glucose (6 mM) in the absence or presence of isofagomine and subsequently cell extracts and incubation media were analyzed with regard to 13C labeling using mass spectrometry. The amount and labeling of glycogen was determined, as well as the effect of inhibiting glycogen breakdown on the metabolic levels of lactate and citrate as indicators of glycolytic and TCA cycle activity, respectively. The percentage of glycogen labeling in cerebellar and neocortical astrocytes was 48% and 31%, respectively, and in the presence of isofagomine it was decreased to 20% and 8%, respectively. The total percentage of lactate labeling was much higher extracellularly (92%) than intracellularly (24%) in both cell types. Surprisingly, the percentage of lactate labeling intracellularly was also decreased in the presence of isofagomine. The results demonstrate that glycogen in cultured astrocytes is continuously synthesized and degraded. Moreover, in astrocytes lactate originating from glycogen is compartmentalized from that derived from glucose, indicating a functional compartmentation of glycolytic and glycogenolytic enzymes.

PSM06-02

Inhibition of glutamine hydrolysis in the interstitial fluid of the rat brain by a glutaminase inhibitor, GPI-20767

HR ZIELKE*, CL ZIELKE*, PJ BAAB*, T TSUKAMOTO†, D FERRARIS†, C ROJAS†, K WOZNIAK† and B SLUSHER†

**University of Maryland Sch. of Med., †Guilford Pharmaceuticals, Baltimore, MD, USA*

When glutamine (GLN) was infused into the interstitial fluid of the rat brain by reverse microdialysis, glutamate (GLU) increased in the recovered dialysate. If 14C-GLN was infused or injected at the site of the microdialysis, 14C-GLU was recovered with a specific activity that was not statistically different from 14C-GLN. We hypothesized that GLN hydrolysis was catalyzed by phosphate activated glutaminase (PAG) and/or by the ectoenzyme maleate activated glutaminase (*J Neurosc Res* 2000; 60: 632). In these studies we tested if addition of a specific PAG inhibitor (GPI-20767, IC50 = 1 uM; Guilford Pharmaceuticals) affected the formation of GLU. Reverse microdialysis with 5 mM GLN in the absence of GPI-20767 increased GLU in the microdialysate from 0.31 ± 0.03 mM to 0.90 ± 0.05 mM (n = 3). Reverse microdialysis with 80 uM GPI-20767 by itself had no significant effect on the level of 19 amino acids in the dialysate. In a paradigm where 5 mM GLN + 80 uM GPI-20767 was infused subsequent to aCSF + 5 mM GLN, the 3-fold increase in baseline GLU levels due to GLN infusion was reduced by 47% following inclusion of GPI-20767. Pretreatment with GPI-20767 in the perfusate, followed by 5 mM GLN plus 80uM GPI-20767, prevented the GLU increase (0.20 ± 0.03 and 0.23 ± 0.07 uM, respectively, n = 4). These data support a major role of PAG in the hydrolysis of GLN to GLU in the interstitial space and suggest that PAG may be released following trauma and that inhibition of PAG may decrease potentially toxic extracellular GLU levels. (HD16596, Guilford Pharmaceuticals.)

PSM06-03

Branched-chain α keto-acid dehydrogenase is a neuronal enzyme in brain

JT COLE*, AJ SWEATT*, R WALLIN*, KF LANOUE†, CJ LYNCH† and SM HUTSON*

**Wake Forest University School of Medicine, Winston Salem, NC, †Penn State College of Medicine, Hershey, PA, USA*

The brain expresses cytosolic and mitochondrial branched-chain aminotransferases (BCATc and BCATm) which use α-ketoglutarate as substrate and produce glutamate and branched chain α-keto acids (BCKAs). Transamination is made irreversible by oxidation of the BCKAs by the dehydrogenase (BCKD) enzyme complex. Defects in BCKD lead to accumulation of BCKAs, resulting in Maple Syrup Urine Disease (MSUD). BCATs are thought to participate in a keto-acid nitrogen shuttle that provides nitrogen for synthesis of glutamate from α-ketoglutarate. BCKD activity would regulate the supply of BCKAs for such a shuttle. Immunolocalization of the BCATs and BCKD in rats revealed first that BCATm is present in astrocytes in white matter and in neuropil, while both BCKD and BCATc are found only in neurons. Second there is a relative segregation of BCATc to the mossy fiber region of the hippocampus while BCATm and BCKD appear in neuronal cell bodies in the granule cell layer of the dentate gyrus. Additionally, in the cerebellum, all three enzymes appear in the cell bodies of Purkinje cells, with BCATc also residing in the dendritic tree. BCATm also localizes to astrocytes in the white matter of the cerebellum and spinal cord. The segregation of BCATm to astrocytes and BCKD to neurons provides further support for the existence of a BCAA-dependent glial-neuronal nitrogen shuttle since the data show that BCKAs produced by glial BCATm must be exported to neurons. Additionally, the neuronal localization of BCKD suggests that MSUD is a neuronal defect involving insufficient oxidation of BCKAs, with secondary effects extending beyond the neuron.

Acknowledgement: NIH NS38641.

PSM06-04

Expression of monocarboxylic acid transporter mRNA in the developing rat brain cortex

ML SCHROEDER*, M SNEVE† and LR DREWES†

**Department of Chemistry, †Department of Biochemistry and Molecular Biology, University of Minnesota Duluth, Duluth, MN, USA*

Monocarboxylic Acid Transporters (MCT) are members of the solute carrier 16 (*Slc16*) family of transporters. MCT1-4 are generally characterized by their role in lactate, pyruvate, and ketone body influx and efflux across the cell membrane. Specific organs, such as the brain, can use these metabolites as a major fuel source in addition to glucose. This is particularly evident in newborn and suckling infants that exhibit increased levels of blood lactate and ketone bodies. Therefore, MCT1-4 expression may play an important role during brain development and metabolism. Other *Slc16* family members have unknown substrates or function in transporting amino acids or thyroid hormones. This study examined the transcript levels of various members of the *Slc16* family at different times during development of the rat cortex. Cortex samples ($n = 5$) were taken at 8 developmental time points (E18, P1, P5, P10, P18, P27, P35, and P90). Transcripts were analyzed with Real-Time PCR using gene specific primers. Results showed a sharp decrease in transcript levels of MCT1 between P10 and P18. MCT2 transcript levels increased between P10 and P18, while MCT5 levels increased between P18 and P27. Other MCTs that were investigated that had no statistical change during development were MCT3, MCT4, and MCT7. However, MCT4 was robustly expressed throughout all the time points. These results demonstrate that transcription of various MCT family members are developmentally regulated. The changes in MCT transcripts correlate with changes in diet during development and the maturation of neurons and astrocytes.

Acknowledgement: Supported by NIH grant NS37764.

PSM06-05

Diurnal regulation of brain fatty acid binding protein (fabp7) mRNA and poly(A) tail length in the rodent brain

JR GERSTNER, WM VANDER HEYDEN and CF LANDRY

Department of Psychiatry, University of Wisconsin, Madison, WI, USA

Fabp7 is the brain-specific member of a family of small, fatty-acid binding proteins that transport fatty acids and retinoids. Fabp7 is important in radial glial cell development, is expressed in astrocytes in the adult brain and binds with high affinity to fatty acids like docosahexaenoic acid that are major components of membrane phospholipids in the brain. We identified fabp7 in a screen for diurnally regulated genes in the mouse hypothalamus. Northern blotting revealed a diurnal regulation of fabp7 mRNA in adult mouse brain that was evident in the cortex, hypothalamus, and brain stem. Fabp7 mRNA levels were highest at ZT4 (4 h after lights on) and lowest at ZT16. A 30 minute light-pulse delivered 4 h after lights off (ZT16) did not affect fabp7 expression. *In situ* hybridization histochemistry further indicated that the diurnal regulation of fabp7 mRNA occurred throughout the brain. In addition to changes in mRNA abundance, diurnal variations in mRNA size were also observed. These size differences were found to result from diurnal changes in poly(A) tail length that were also evident throughout the brain. The presence of cytoplasmic polyadenylation binding elements within the fabp7 mRNA and the identification of cytoplasmic polyadenylation element-binding (CPEB) proteins 1 and 4 in astrocytes suggest that changes in fabp7 poly(A) tail length may result from cytoplasmic polyadenylation in a manner similar to that observed in neurons. The diurnal oscillation of fabp7 in brain may be related to the turnover of phospholipids in glial or neuronal membranes. Further, these results provide evidence that cytoplasmic polyadenylation occurs in glial cells.

PSM06-06

Alpha-synuclein gene-ablation decreases brain palmitate uptake and alters palmitate metabolism in brain phospholipids

MY GOLOVKO*, NJ FAERGEMAN†, NB COLE‡, PI CASTAGNET*, RL NUSSBAUM‡ and EJ MURPHY*

**Department of Pharmacology, University of North Dakota, Grand Forks, ND, USA, †Department of Biochemistry, University of Southern Denmark, Odense, Denmark, ‡NIH, Genetic Diseases Research Branch, Bethesda, MD, USA*

α-Synuclein is an abundant protein in the central nervous system that is associated with a number of neurodegenerative disorders. Its physiological function is poorly understood, although recently it was proposed to function as a fatty acid binding protein. To better define a role for α-synuclein in brain fatty acid uptake and metabolism, we infused awake, wild-type or α-synuclein gene-ablated mice with [1-^{14}C]palmitic acid (16 : 0) and assessed fatty acid uptake and turnover kinetics in brain phospholipids. α-Synuclein deficiency decreased brain 16 : 0 uptake 35% and decreased its targeting to the organic fraction. The incorporation coefficient for 16 : 0 entering the brain acyl-CoA pool was significantly decreased 36% in a-synuclein gene-ablated mice. Because incorporation coefficients alone are not predictive of fatty acid turnover in individual phospholipid classes, we calculated kinetic values for 16 : 0 entering brain phospholipid pools. α-Synuclein deficiency decreased the incorporation rate and fractional turnover of 16 : 0 in a number of phospholipid classes, but also increased the incorporation rate and fractional turnover of 16 : 0 in the choline glycerophospholipids. No differences in incorporation rate or turnover were observed in liver phospholipids, confirming that these changes in lipid metabolism were brain specific. Using titration microcalorimetry, we observed no binding of 16 : 0 or oleic acid to α-synuclein *in vitro*. Thus, these data demonstrate that ablation of α-synuclein expression *in vivo* has an impact on brain fatty acid uptake and metabolism due to changes in fatty acid metabolism because α-synuclein does not bind palmitate.

PSM06-07

Receptor independent effects of thiazolidinediones in astrocytes

CA AKAR*, J COLCA†, C DELLO RUSSO*, A SPAGNOLO*, V GAVRILYUK* and DL FEINSTEIN*

**University of Illinois at Chicago, Chicago, IL, †Pfizer Inc., New York, NY, USA*

Agonists of peroxisome proliferator-activated receptor gamma (PPARg), thiazolidinediones (TZDs), exert anti-inflammatory, neuroprotective effects in animal models of neurological disease including AD and MS. TZDs also have PPARg independent effects, including an increase in anaerobic metabolism in astrocytes. To characterize receptor independent metabolic actions of TZDs, we investigated their interaction with recently discovered mitochondrial protein, mitoNEET. Primary mouse and rat astrocytes were infected with adenovirus containing siRNA against mitoNEET mRNA. After 72 h, astrocytes were treated with TZDs and effect on glucose and lactate metabolism determined at various times. Q-PCR analysis of total RNA from infected vs control samples showed efficiency of mitoNEET knock down was %BB 50%. Glucose consumption remained approximately the same in mitoNEET knocked down cells compared to controls whereas lactate production increased by 62% after 6 h. Pioglitazone (Pio) treatment increased lactate production %BB 42% in controls, and over 100% in the mitoNEET knocked down cells. These results show, decreasing mitoNEET levels caused a significant switch towards anaerobic metabolism in normal astrocytes, suggesting, mitoNEET normally regulates oxidative phosporylation.

Pio treatment of astrocytes with reduced mitoNEET levels caused a greater shift toward anaerobic metabolism, suggesting a common target site of action for Pio and mitoNEET. Further studies to determine if mitoNEET is a direct target of Pio are in progress, including characterization of PPARg agonist effect on astrocytes from PPARg conditional null mice.

PSM06-08

Transglutaminase activity in non-synaptosomal mouse brain and liver mitochondria

BF KRASNIKOV*, RR RATAN*, GE GIBSON*, S IISMAA†, AJ COOPER*

**Weill Med. Coll. Cornell U., Burke Med. Res. Inst., White Plains, NY, USA, †Victor Chang Cardiol. Res. Inst., NSW, Australia*

Mitochondrial dysfunction and increased transglutaminase (TGase) activity occur in neurodegeneration. TGases catalyze the Ca^{2+}-dependent linking of a K residue to a Q residue in protein/peptide substrates with the formation of an N^{ε}-(γ-L-glutamyl)-lysine (GGEL) isopeptide bridge. Excessive cross linking may lead to neural dysfunction. At least three TGase isoforms are present in brain, namely TGase 1, TGase 2 (tissue TGase) and TGase 3. Free GGEL is elevated 3-fold and 8–10-fold in the CSF and autopsied brain of Huntington disease patients, respectively. GGEL linkages are also increased in Alzheimer disease brain and CSF. Increased TGase activity may contribute to the downward spiraling in neurodegenerative diseases. To investigate a possible link between mitochondrial dysfunction and increased TGase activity in neurodegenerative diseases, we tested whether TGase activity occurs in mitochondria. TGase activity was previously known to be present in the cytosolic, nuclear and extracellular compartments of brain. We have now shown that highly purified mouse brain non-synaptosomal mitochondria, mouse liver mitochondria and mitoplast fractions derived from these preparations possess TGase activity. Western blotting and experiments with TGase 2-knock out mice ruled out the possibility that most of the mitochondrial/mitoplast TGase activity is due to TGase 2, a major TGase isoform in brain. The possibility that the mitochondrial TGase activity is due to an isoform other than 1–3 is under investigation. The mitochondrial TGase may play a role in regulation of mitochondrial function both in normal physiology and in disease, and perhaps may be a target for drug intervention.

PSM06-09

Different enantioselectivity of 4-hydroxy-trans-2-nonenal oxidation in rat brain and liver mitochondria

J BRICHAC, A HONZATKO and MJ PICKLO

Department of Pharmacology, Physiology, and Therapeutics, Department of Chemistry, University of North Dakota, Grand Forks, ND, USA

4-hydroxy-trans-2-nonenal (HNE) is a product of lipid peroxidation that is implicated in the pathogenesis of numerous neurodegenerative disorders. HNE possesses a chiral center at the C4 carbon. The oxidation of HNE to 4-hydroxy-trans-2-nonenoic acid (HNEA) is a major route of mitochondrial HNE detoxification. Mitochondrial aldehyde dehydrogenases (ALDHs) play an important role in HNE detoxification. HNE is oxidized in brain mitochondria predominantly by ALDH5A, while ALDH2 plays a greater role in liver mitochondria oxidation. We tested the hypothesis that brain and liver mitochondria oxidize HNE in an enantioselective manner. We used mitochondrial lysate and pure HNE enantiomers. In brain mitochondria R-HNE and S-HNE were oxidized with Km 31.0 ± 3.0 and 58.1 ± 4.2 μM, respectively and with Vmax 9.4 ± 0.3 and 5.5 ± 0.2 nmol/min/mg, respectively. In liver mitochondria, for R-HNE and S-HNE oxidation Km was 66.2 ± 8.8 and 22.5 ± 2.0 μM, respectively and Vmax was 52.9 ± 3.1 and 43.5 ± 1.2 nmol/min/mg, respectively. These results demonstrate that R-HNE is selectively oxidized by brain mitochondria and S-HNE is selectively oxidized by liver mitochondria. To test, if the absolute configuration of the HNE C4 carbon remains the same during oxidation, we developed HPLC method for determination of HNEA enantiomers using chiral stationary phase Chiralcel OB. Prior to HPLC analysis two solid phase extractions (SPE) were used to remove HNE and to transfer HNEA into mobile phase hexane-2-propanol (97 : 3, v/v). However no racemization was observed during oxidation of HNE enantiomers with liver mitochondria lysate.

PSM06-10

Stereoselective detoxification of trans-4-hydroxy-2-nonenal by rat brain mitochondria

A HONZATKO, J BRICHAC, TC MURPHY, DM MOSLEY and MJ PICKLO

Dept. of Pharmacology, Physiology, and Therapeutics,UND School of Medicine, Grand Forks, ND,USA

Trans-4-hydroxy-2-nonenal (HNE) is a neurotoxic product of lipid peroxidation with many cellular effects. HNE possesses a chiral center at the C4 carbon that affects its metabolism. In this study we tested the hypothesis that freshly isolated rat brain mitochondria metabolize HNE in an enantioselective manner. We used a novel method for indirect chiral separation of HNE enantiomers by reverse phase chromatography and LC-MS-MS method for determination of the major anticipated product of the HNE metabolism, 4-hydroxy-2-nonenoate (HNEAcid) and glutathione-HNE conjugates (GSHNE). When racemic HNE was used, (R)-HNE was consumed with a VMAX of 11 ± 1.4 nmol/min/mg and a KM of 78 ± 16 mM HNE and (S)-HNE with a VMAX of 4.2 ± 0.40 nmol/min/mg and a KM of 25 ± 6.0 mM HNE. When pure enantiomers were applied, (R)-HNE was consumed with a VMAX of 36 ± 5.6 nmol/min/mg and a KM of 72 ± 19 mM HNE and (S)-HNE with a VMAX of 14 ± 2.4 nmol/min/mg and a KM of 55 ± 18 mM HNE. (R)-HNE was a preferred substrate for rat brain mitochondrial aldehyde dehydrogenases over (S)-HNE to form HNEAcid. HNEAcid was formed from (R)-HNE with a VMAX of 14 ± 1.7 nmol/min/mg and a KM of 24 ± 7 mM HNEAcid and from (S)-HNE with a VMAX of 3 ± 0.4 nmol/min/mg and a KM of 7 ± 4.5 mM HNEAcid. The concentration of GSHNE formed was fifty times lower than concentration of HNEAcid formed, and we did not observe enantioselective GSHNE formation. These studies demonstrate that the chirality of HNE affects its metabolism in rat brain mitochondria and that aldehyde dehydrogenase activity is a major determinant of mitochondrial HNE detoxification.

PSM06-11

Protein–protein interaction technologies for identifying mct1 regulatory proteins

JA SPANIER and LR DREWES

University of Minnesota-Duluth, Duluth, MN, USA

Bidirectional transport of monocarboxylates such as lactate, pyruvate, and ketone bodies across cellular membranes is facilitated by a family of monocarboxylate transporters (MCTs). In the adult rat brain MCT1 is primarily expressed in endothelial cells and to a lesser extent in astrocytes. MCT1 is known to interact with CD147, an ancillary protein which is necessary for MCT1 translocation to the plasma membrane. MCT1 function is tightly regulated at both the RNA and protein levels. It is hypothesized that MCT1 interacts with proteins, in addition to CD147, to regulate its function. Recently, there have been major advances in the development of technologies used to identify and characterize protein-protein interactions. A more classical approach to identify protein-protein interactions is the use of the yeast-two-hybrid. This approach has proved to be extremely valuable, but it poses many limitations. To overcome these limitations we are employing the recently developed technique of tandem affinity purification (TAP) to identify proteins that interact with MCT1 within rat brain endothelial cells. Currently, we have amplified the entire coding region of MCT1 by PCR and cloned it into the N-TAP vector. To demonstrate the feasibility of expression of the fusion protein construct, we have cloned green fluorescent protein into the N-TAP vector and demonstrated its expression in TR-BBB cells. These results suggest that the TAP technology may be very useful for identifying novel protein interactions with membrane transport proteins expressed by brain endothelial cells.

Acknowledgement: Supported by NIH grant NS37764.

PSM06-12

Withdrawn.

PSM06-13

Reduced excitotoxic neuronal degeneration in mice deficient in cytosolic phospholipase A2

SJ HEWETT*, JS SILAKOVA*, JV BONVENTRE† and JA HEWETT*

**University of Connecticut School of Medicine, Farmington,CT,*

†Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

Phospholipase A_2 (PLA_2) comprises a superfamily of extracellular and intracellular esterases that catalyze the hydrolysis of sn-2 fatty acyl bonds of phospholipids. Recent work by Bonventre and colleagues demonstrated that animals with a targeted deletion of cytosolic phospholipase $A_2\alpha$ (PLA2g4a) have a 34% reduction in brain infarct volume and suffer fewer functional neurological deficits than wild type control animals following transient middle cerebral artery occlusion (Bonventre *et al. Nature* 1997; 390: 622). Whether this specific reduction in post-ischemic brain injury could be due to decreased susceptibility to glutamate mediated damage was assessed herein. Chloral hydrate anesthetized BALB/c male mice (24–30 g) with or without disruption of the gene encoding for $cPLA_2\alpha$ were injected with the glutamate agonist NMDA (350 nl) into the CA1 region of the left hippocampus. Three days later, brains were removed and 30 µm coronal sections, taken at 150 µm intervals, were processed and imaged for assessment of cell loss. In wild-type mice, hippocampal microinjection of NMDA (12 nmol) resulted in marked loss of primary neurons in all layers of the hippocampus ipsilateral to the injection. Neuronal loss in the CA1, CA3 and dentate gyrus layers was also detected in contralateral hippocampi of wild-type mice. By comparison, there was less damage to the ipsilateral hippocampi of $cPLA_2\alpha$ null mice with little or no contralateral involvement. Quantitative assessment of the hippocampal damage demonstrated that $cPLA_2$ null mutant animals had ≈35% less neuronal injury than wild-type littermate controls. These results suggest that the relative resistance of $CPLA_2\alpha$ null mutant mice to cerebral ischemic damage could be attributed to the direct attenuation of glutamate neurotoxicity.

Acknowledgement: Supported by NS36812-07.

PSM06-14

Retinal glycosphingolipid abnormalities in Sandhoff and GM1 gangliosidosis mice

CA DENNY, JR CHALIFOUX, YP KIM and TN SEYFRIED

Department of Biology, Boston College, Chestnut Hill, MA, USA

Retinal abnormalities have been well-documented in patients with ganglioside storage diseases. However, no previous studies have analyzed retinal gangliosides in normal mice or in mouse models of gangliosidoses. The content and distribution of retinal glycosphingolipids (GSL) was studied in normal mice and in mouse models of Sandhoff disease (SD) and GM1 gangliosidosis. Similar to previous findings in rat retina, GD3 was the major ganglioside (49%) in normal adult mouse retina, while GM2 and GM1 were minor species. Total retinal ganglioside content (μg sialic acid/100 mg dry wt) was significantly greater in the SD mice (208 μg, $n = 2$ independent pooled retina samples) and in the GM1 mice (201 ± 9 μg, $n = 4$) than in the normal mice (144 ± 21 μg, $n = 3$). Retinal GM2 content was 12-fold greater in the SD mice than in the normal mice, whereas retinal GM1 content was 53-fold greater in the GM1 mice than in the normal mice. Although asialo-GM2 (GA2) and asialo-GM1 (GA1) were undetectable in normal mouse retina, high concentrations (μg/100 mg dry wt) of GA2 (566 μg) and GA1 (587 ± 19 μg) were found in the SD mouse retina and in the GM1 mouse retina, respectively. The retinal GSL abnormalities in the SD and GM1 mice were associated with significant reductions in retinal β-hexosaminidase A and β-galactosidase enzyme activities, respectively. Our findings suggest that the mouse retina may serve as a new model system for assessing retinal pathobiology and combinatorial therapies for the gangliosidosis.

Acknowledgements: Supported by NIH grant (HD39722) and NTSAD.

PSM06-15

Caloric restriction extends longevity without altering brain lipid composition or cytoplasmic neuronal vacuoles in Sandhoff mice

CA DENNY*, JL KASPERZYK*, KN GORHAM*, RT BRONSON† and TN SEYFRIED*

**Department of Biology, Boston College, Chestnut Hill, †The Dana-Farber Cancer Institute/Harvard Medical School, Boston, MA, USA*

Caloric restriction (CR) improves health and increases longevity without causing nutritional deficiencies. CR was studied as a therapy in Sandhoff disease (SD) mice, which accumulate brain ganglioside GM2 and asialo-GM2 (GA2). Adult mice were fed a rodent chow diet either *ad libitum* (AL) or restricted to reduce body weight by 15–18%. Under normal feeding conditions, brain GM2 and GA2 content was significantly higher in the *Hexb* knockout (–/–) mice than in the *Hexb* heterozygous (+/–) mice. Cytoplasmic neuronal vacuoles were abundant in the *Hexb* –/– mice, but were undetectable in the *Hexb* +/– mice. No significant differences were seen between the *Hexb* –/– and the *Hexb* +/– mice for phospholipids and cholesterol, but cerebrosides and sulfatides were reduced in the *Hexb* –/– mice. In addition, rotarod performance was significantly worse in the *Hexb* –/– mice than in the *Hexb* +/– mice. CR, which decreased circulating glucose and elevated ketone bodies, had no significant effect on brain lipid composition or on cytoplasmic neuronal vacuoles, but significantly improved rotarod performance and extended longevity in the *Hexb* –/– mice. Our results show that CR is effective in improving health and motor performance in SD mice without altering brain lipids or cytoplasmic neuronal vacuoles. We suggest that the CR delays disease progression in Sandhoff disease and possibly other ganglioside storage diseases through anti-inflammatory mechanisms.

Acknowledgement: Supported by NIH grant (HD39722) and NTSAD.

PSM06-16

Suppression of NMDA receptor function in hibernating Arctic ground squirrels

HW ZHAO*, AP ROSS*, SL CHRISTIAN*, JN BUCHHOLZ† and KL DREW*

**University of Alaska Fairbanks, Fairbanks, †Depts. of Physiol. and Pharmacol. School of Med. Loma Linda Univ. Loma Linda, USA*

Arctic ground squirrels (AGS) hibernate to survive food shortages and cold temperatures during winters. Hibernation torpors are periodically interrupted by arousal bouts to state of euthermy throughout hibernation season. During torpor, AGS show suppressed metabolism, upon arousal, AGS return to normal level without any adverse effects. The fluctuations of ATP and blood flow during arousal from torpor have some similarities to ischemia and reperfusion in human stroke but there is no observed neurological damage in AGS. NMDA receptors (NMDAR) are highly permeable to Ca^{2+} and play a key role in neuronal survival and neuroexcitotoxicity. To address the function of NMDAR, we used Ca^{2+} imaging to determine effects of glutamate (Glu) and AP5 on changes of intracellular Ca^{2+} ($[Ca^{2+}]i$) of AGS and rat hippocampal slices. Our results show that Glu induces a smaller $[Ca^{2+}]i$ increase in AGS compared with rats ($P < 0.05$). Interestingly, AP5 can block Glu-induced $[Ca^{2+}]i$ increase in rats and euthermic AGS (ibeAGS) ($P < 0.05$) but not in hibernating AGS (hAGS).This suggests that NMDAR function is suppressed in hAGS. We used western blot to examine possible mechanisms responsible for the reduced NMDAR function in hAGS. The ratio of phospho-NMDAR1 (Ser897, pNR1) over NMDAR1 (NR1) subunit of NMDAR was compared in membrane fractions of AGS and rats hippocampus. pNR1/NR1 was significantly lower in hAGS compared with ibeAGS and rats ($P < 0.05$). There was no NR1 detected in cytosolic fractions of AGS or rats. We conclude that NMDAR are suppressed in hAGS compared to ibeAGS and rats due to decreased phosphorylation of NMDAR in the membrane. NMDA channel specific suppression may be one of neuroprotective mechanisms in hAGS.

Poster Session PSM07: Spinal cord

PSM07-01

Differential expression of nociceptive genes influence pain behavior following spinal cord injury in adult rats

GS MIRANPURI*, R VEMUGANTI*, MG DOMBOURIAN*, NA TURNER*, TA GEROVAC*, K TUREYEN*, JW ISAACSON*, V MILETIC* and DK RESNICK*

**Neurological Surgery, UW Medical School, †Comparative Biosciences, UW School of Veterinary Medicine, Madison, WI, USA*

In humans, spinal cord injury (SCI) is known to cause chronic neuropathic pain (NP). To identify if the pain behavior has a molecular basis, we analyzed mRNA expression of pain-related genes in adult Sprague-Dawley rats subjected to moderate SCI induced by a MASCIS impactor. 18 rats were tested pre-injury and post-injury at regular intervals for the presence of thermal allodynia using a hind foot withdrawal latency test. Based on pain latency, the rats were divided into 2 groups (8 with higher, 10 with lower allodynic responses between days 28 to 42 after SCI). A decrease of >3 sec latency time from baseline was considered allodynia. At days 28 to 42 post-injury, spinal cords were harvested from all rats and mRNA was isolated from the injury epicenter and rostral segments. The expressions of bradykinin receptor B1 and vanilloid receptor TRPV1 were estimated using real-time PCR analysis. Compared to sham ($n = 5$), B1 expression increased 3-fold in the SCI rats showing greater allodynia ($P < 0.05$). Rats exhibiting less allodynia after SCI did not show any change in B1 expression. TRPV1 was down-regulated after SCI in both the pain and no pain groups. However, the no pain group showed a 7-fold decrease of TRPV1 which was significantly different from the 3-fold decrease observed in the pain group. This study indicates that chronic NP following SCI is associated with molecular changes. Identifying target genes that prevent pain perception in the subset of animals that showed less pain after SCI might help in developing novel therapies to control NP.

PSM07-02

Regulation of innate immunity after spinal cord injury in mice: involvement of toll-like (TLR) receptors

KA KIGERL*, S RIVEST† and PG POPOVICH*

**Ohio State University College of Medicine and Public Health, Columbus, OH, USA, †Laval University, Quebec, Canada*

Spinal cord injury (SCI) triggers an inflammatory response with divergent effects on the nervous system. Specifically, cells of the innate immune system (e.g., macrophages) release inflammatory cytokines and proteases that can exacerbate tissue loss at the injury site. However, some of these mediators may also promote neuroprotection and axon regeneration. Unraveling the mechanisms that are responsible for this functional heterogeneity could reveal novel strategies for limiting post-traumatic neurodegeneration or promoting tissue repair. Toll-like receptors (TLRs) are part of an evolutionarily-conserved signaling pathway involved in triggering NF-κB transcription and cytokine production in innate immune cells (e.g., macrophages) in response to pathogens. Here we show regulation of TLRs in a sterile and controlled model of SCI. Using *in situ* hybridization we find that CD14 mRNA expression (surrogate for TLR4) is increased at 6 hours and 3 dpi, while TLR2 mRNA peaks 3–14 dpi. These times correspond with the onset and peak of CNS macrophage activation. CD14 and TLR2 mRNA co-localize with enhanced CD11b staining, suggesting selective expression of TLRs on CNS macrophages. Furthermore, mRNA for TNF-α and IκB-α, downstream products of NF-κB activation, are up-regulated at the injury site. Preliminary data indicate that macrophage activation via TLR4 may impart neuroprotective effects after SCI. Indeed, locomotor recovery is impaired and demyelination is enhanced after SCI in TLR4 mutant mice (C3H/HeJ) compared to wild-type control mice. Together, these data indicate modulation of post-traumatic injury/repair by TLR-mediated activation of CNS macrophage function.

Acknowledgement: Supported by NINDS NS037846.

PSM07-03

Alterations in sympathetic nervous system and hypothalamic-pituitary-adrenal axis function after experimental spinal cord injury

KM LUCIN, VM SANDERS, TB JONES, WB MALARKEY and PG POPOVICH

The Ohio State University, College of Medicine and Public Health, Columbus, OH, USA

Maintenance of normal immune function (e.g., cytokine release, antibody synthesis) may be impaired after spinal cord injury (SCI) due to sympathetic nervous system (SNS) and hypothalamic-pituitary adrenal (HPA) axis dysfunction. Indeed, systemic norepinephrine (NE) and cortisol can be elevated chronically after SCI leaving patients susceptible to infection. To model these changes in rodents, we have used both mid-and high thoracic spinal contusion and transection injuries in rats and mice. Following spinal contusion (T8/9) in rats, serum corticosterone (CORT) is elevated up to 28 days post-injury (dpi). In SCI mice, an identical lesion produces similar acute elevations of CORT (1–3 dpi) but with a return toward baseline by 7 dpi. However, circadian cycling of CORT release remains disrupted up to 28 dpi. Similarly, because the SNS innervates the spleen and because SCI damages SNS preganglionic neurons at the level of injury and in nearby spinal segments, we predicted SCI would induce an abrupt spike in splenic NE. However, after T9 spinal contusion or transection injury in mice, splenic NE levels were unchanged. Conversely, when supraspinal input was removed at higher spinal levels (e.g. T3 transection), splenic NE was markedly increased. Associated with this NE spike was an upregulation of splenocyte β_2-adrenergic receptor mRNA expression. Together, these data indicate that the SNS and HPA axis are disrupted after SCI, with the dysregulation being more prominent after higher level injuries. Additional studies are needed to determine how these changes influence the initiation, progression and resolution of immunological function.

PSM07-04

Spinal synaptic plasticity following intermittent hypoxia improves respiratory function after chronic cervical spinal cord injury

FJ GOLDER and GS MITCHELL

Department of Comparative Biosciences, University of Wisconsin, Madison, WI, USA

Respiratory failure is the leading cause of death following high cervical spinal cord injuries (SCI). Although respiratory motor recovery can occur after SCI, the magnitude of spontaneous recovery is limited. We hypothesized that weak respiratory motor recovery after cervical SCI could be strengthened using a known stimulus for spinal synaptic plasticity, intermittent hypoxia. Phrenic motor output was recorded before and after intermittent hypoxia from anesthetized, vagotomized and pump ventilated control and C2 spinally hemisected rats at 2, 4, and 8 weeks post-SCI. Weak phrenic motor recovery was present in all C2-injured rats via crossed spinal synaptic pathways. Intermittent hypoxia caused augmentation of crossed synaptic pathways (phrenic long-term facilitation; pLTF) for up to 60-min post-hypoxia at 8 weeks (change in phrenic burst amplitude from baseline: 24 ± 5%, of hypercapnic maximal amplitude), but not 2 weeks (–0.6 ± 4%), post-SCI ($P < 0.05$). Ketanserin, a 5-HT2A receptor antagonist, administered prior to intermittent hypoxia prevented pLTF at 8 weeks post-SCI. Serotonergic innervation near phrenic motoneurons was assessed at similar time intervals post-injury. The limited magnitude of pLTF at 2 weeks was associated with an injury-induced reduction in serotonin-containing nerve terminals in the vicinity of phrenic motoneurons ipsilateral to C2 injury. Thereafter, pLTF magnitude correlated with the recovery of serotonergic innervation in the phrenic motor nucleus ($r^2 = 0.35$; $P < 0.01$). Intermittent hypoxia (or pLTF) has intriguing possibilities as a therapeutic tool since its greatest efficacy would be in patients with chronic SCI at a time when most patients have already achieved maximal spontaneous functional recovery.

PSM07-05

Peptide attachment to biodegradable polymers for axonal guidance in spinal cord injury

AM KNIGHT*, S GEORGI*, A ISSA*, M DE RUITER*, MJ YASZEMSKI† and AJ WINDEBANK*

**Mayo Clinic Rochester. Neurology, †Othopedic Research, Rochester, MN, USA*

We are currently developing multi-channel, biodegradable, poly (lactic-co-glycolic acid) (PLGA) and poly (caprolactone fumarate) (PCLF) scaffolds to provide guidance for axon regeneration in transected spinal cord. To improve the nerve regeneration microenvironment, we incorporated Schwann cells or Neuronal stem cells into the guidance channels. Using this approach axonal growth was achieved over a 3 mm gap. Within these scaffold tubes a thick fibrous ring surrounded the central core of axons. When the diameter of the guidance channels was increased, so to was the thickness of this fibrous layer, which also often separated from the channel wall. Similar observations were seen in peripheral nerve in the absence of preloaded cells. To improve interaction between polymer and tissue we attached peptides derived from N-CAM and laminin to the polymer surface via a streptavidin/biotin link using carbodiimide chemistry and measured attachment and neurite growth with PC12 cells and Dorsal Root Ganglion (DRG) neurons. Neither PC12 cells nor primary neurons attached directly to the polymer surfaces. Biotinalyted N-CAM peptides and Laminin derived peptides promoted attachment and neurite formation in both in PC12 and DRG cells. We conclude that the surface of biodegradable guidance tubes can be manipulated to promote neuronal attachment and neurite growth. The biotin/streptavidin system provides a versatile link for a variety of polypeptides. Single polypeptides or combinations can be tested *in vitro* and in *in vivo* in peripheral nerve, prior to the spinal cord injury model.

PSM07-06

Estrogen attenuates neurodegeneration and improves motor function in the chronic model of spinal cord injury

NL BANIK, EA SRIBNICK, DD MATZELLE, GG WILFORD and SK RAY

Medical University of South Carolina, Charleston, SC, USA

Although, spinal cord injury (SCI) often occurs in young adults and can cause permanent damage, drug therapy is limited to methylprednisolone, which has limited efficacy. Estrogen-mediated neuroprotection has been demonstrated in several disease models, so we used estrogen as a possible therapy for SCI in rats. Adult, male Sprague-Dawley rats were divided into three groups: sham, vehicle, and estrogen. Sham rats received a laminectomy, while vehicle and estrogen-treated animals received laminectomy and a 40 gcf SCI. Estrogen-treated animals were given 4 mg/kg intravenous estrogen at 15 min and 24 h post-injury and five additional intraperitoneal doses. Vehicle-treated rats received equal volumes of dimethylsulfoxide, and all rats were sacrificed at 42 days post-SCI. Motor function was assessed by Basso, Beattie, Breshnehan (BBB) scoring, and tissue histology was assessed by immunohistolabelling of 68 kD neurofilament protein (NFP), dephosphorylated NFP (dephosNFP), and NeuN for neurons. Tissue damage was also assessed by Fluoro-Jade staining, transmission electron microscopy, and luxol fast blue staining (LFB). Survival rates for vehicle-treated animals were significantly lower than for estrogen-treated animals, and estrogen-treated animals showed significantly better BBB scores. We are in the process of evaluating data gathered from NFP, dephosNFP, NeuN, and Fluro-Jade. However, serial sections stained with LFB demonstrated a significantly decreased lesion volume in estrogen-treated animals. These findings indicated that estrogen treatment could improve histology, survival, and motor function in the chronic SCI model.

Acknowledgement: Funding provided by NIH grants.

PSM07-07

Clinical anatomical studies on the spinal cord of the Egyptian goat

AC KARKORA

Anatomy Department, Alexandria University, Alexandria, Egypt

The present work was carried out on twenty specimens of adult healthy goats of both sexes fixed by formalin 10% then the spinal cord was exposed by laminectomy to study the morphology of the cord and its meninges. The spinal cord measurements were studied on both dorsal and ventral root attachment, dorsal and ventral interroot lengths, segment length, as well as transverse and dorsoventral diameters of each spinal segment.

This work also studied the demonstration of the topographic anatomy and relationships between the spinal cord segments and the vertebrae transverse and spinous processes studies the cross sections of spinal cord segments to demonstrate the amount of both gray and white matter and total cross sectional area of each spinal segment and the petrcentage between them. The site of epidural anaesthesia were studied, the ideal site can easily carried out at, sacrococcygeal space, lumbosacral and first interlumbar space.

PSM07-08

Acute injury is required for Neurotrophin-3 induced axonal plasticity in the spinal cord

Q CHEN, L ZHOU and HD SHINE

Cell & Gene Therapy, Neurosurgery, Neuroscience, Mol. & Cell. Biology, Baylor College of Medicine, Houston, TX, USA

Adenoviral vector-mediated over-expression of Neurotrophin-3 (NT-3) promoted axonal sprouting after acute spinal cord injury but not in the unlesioned spinal cord. This suggests that neuroplasticity is dependent upon processes associated with injury such as Wallerian degeneration (WD) in addition to NT-3 over-expression. WD is a complex cascade of events caused by axotomy that include macrophage and microglia recruitment, oligodendrocyte death, and myelin clearance. If WD is involved, then delaying NT-3 over-expression until the processes of WD have resolved would result in failure of axonal sprouting. We lesioned the corticospinal tract (CST) unilaterally at the level of the medulla and over-expressed NT-3 by delivering an adenoviral vector carrying the NT-3 gene to the spinal motoneurons by retrograde transport through the sciatic nerve in rats. To uncouple NT-3 over-expression from WD we delayed vector delivery 4 months after CST lesion. We measured the number of CST axons that arose from the intact CST, traversed the midline, and grew into the lesioned side of the spinal cord where the NT-3 was over-expressed. No spouting was measured when NT-3 over-expression was delayed 4 months. Since the processes of WD would have resolved within 4 months after injury, these data demonstrate that products of WD are a likely source of the co-inducing signals that support neuroplasticity. In contrast, sprouting was measured if NT-3 was delivered 2 weeks after CST lesion and persisted for up to 6 months, suggesting that functional connections had been established with target neurons.

Acknowledgements: Supported by the NIH, Christopher Reeve Paralysis Foundation, and Mission Connect of TIRR.

PSM07-09

Inflammatory cytokines regulate nociceptive peptides through nerve growth factor

AM SKOFF and JE ADLER

Wayne State University, Detroit, MI, USA

Nerve injury frequently results in development of chronic, dysesthetic pain and allodynia. We have previously demonstrated that partial transection of sciatic nerve induces a shift of nociceptive peptides within the spinal cord. There is a reduction of peptide content in superficial laminae of the dorsal spinal cord, where substance P is normally found, with a corresponding increase in deeper laminae, where it is not. However, following nerve injury, spinal cord glia become activated and secrete inflammatory cytokines including interleukin-1 (IL-1) and tumor necrosis factor-a (TNF-α). To investigate whether neuropeptide distribution could be altered by exposure to these cytokines, spinal sensory neurons from mature rats were grown in culture and substance P expression was analyzed. Both TNF-α and IL-1 increased neuronal content of substance P. Moreover, with IL-1 exposure, neurons secreted substantially more substance P after stimulation than did control cultures, supporting a physiologic role for this inflammatory cytokine after nerve injury. The effect of IL-1 on substance P expression was highly dependent of dose. There was minimal stimulation at a concentration of 10 pg/ml. Concentrations of 0.1–5 ng/ml elicited a maximal response while stimulation began to decline at 10 ng/ml. To determine if inflammatory cytokines were acting directly on sensory neurons or whether effect was secondary, TNF-α and IL-1 stimulated neurons were grown with an antibody to nerve growth factor (NGF). Anti-NGF eliminated effects of both TNF-α and IL-1 but had no effect on neuronal survival or viability. Accordingly, it appears that inflammatory cytokines can influence expression of nociceptive peptides but that their effect is mediated by NGF.

Poster Session PSM08: Oligodendroglial biology

PSM08-01

Specific deficiency of kappa-opioid receptors in oligodendrocytes in the CNS of jimpy mice

PE KNAPP, SP ZOU, YV ALIMOVA, KF HAUSER and VV ADJAN
Dept. Anat & Neurobiol, Univ. KY College of Med., Lexington, KY, USA

Oligodendrocytes (OLs) in the CNS of jimpy (jp) mice die prematurely starting shortly after birth. The loss of most OLs leads to severe hypomyelination and death by 25 days. Our previous work showed that cultured jp OLs have defects in opioid receptor expression. They fail to express kappa opioid receptors (KORs) at any stage of development, although mu opioid receptors (MORs) appear normal. Brain sections from normal and jimpy mice at 5–8 and 15–18 days were immunostained for KORs, MORs and either OL or neuron markers to determine if KOR deficiency occurs *in situ*. We found a specific decrease in KOR expression in jp OLs. At 5–8 days, 67% of normal vs 9% of jp OLs expressed detectable KORs. At 15–18 days, 85% of normal vs 10% of jp OLs expressed KORs. MOR expression was equivalent in normal and jp brains at both ages. The KOR deficiency was OL specific since levels of KORs were similar in jp and normal neurons. The jp mutation is an amino acid substitution in the proteolipid protein/DM20 gene that results in excision of an exon, a frameshift, and truncation of predicted proteins. It is uncertain how this defect in a myelin protein gene reduces KORs in jp OLs. However, it is probably not due to general downregulation of protein synthesis in dying OLs since (1) the defect is seen in jp corpus callosum at a time when most OLs are phenotypically normal; and(2) the defect is specific to KORs, not affecting MOR expression. We previously showed that KOR signaling protects OLs against glutamate toxicity, so the loss of KORs may adversely affect jp OL survival.

Acknowledgement: Support by DA15097.

PSM08-02

Phosphodiesterase-Ialpha/autotaxin's matricellular properties facilitate process formation in oligodendroglial cells

J DENNIS, MA WHITE, MA FOX, FS AFSHARI and B FUSS
Virginia Commonwealth University, Richmond, VA, USA

During development oligodendrocytes undergo substantial morphological changes. This morphological remodeling is thought to require oligodendroglial cells to remain in an intermediate state of adhesion. In the central nervous system (CNS), phosphodiesterase-Ia/autotaxin [PD-Ia/ATX (NPP-2)] is released by oligodendrocytes at the initial stages of myelination. Our previous data demonstrated that PD-Ia/ATX is present at these initial stages of myelination as an extracellular component in the CNS and antagonizes adhesion of post-migratory oligodendrocytes to otherwise adhesive ECM molecules. An approximately 40 kD C-terminal fragment of the full length (approximately 130 kD) PD-IaATX protein mediates adhesion-antagonism, and reorganized assembly of cytoskeletal elements, in particular vinculin and paxilin. This novel functional PD-Ia/ATX domain appears to confer matricellular protein-like activity that promotes intermediate adhesive states in oligodendroglial cells. Here we demonstrate that the C-terminal fragment of PD-Ia/ATX facilitates process outgrowth in oligodendroglial cells in addition to antagonizing adhesion and reorganizing focal adhesions. Furthermore, these effects of PD-Ia/ATX on oligodendroglial cells are eliminated upon removal of the EF-hand-like motif present in PD-Ia/ATX. These data strongly suggest a pivotal role for the EF-hand-like motif of PD-Ia/ATX in regulating oligodendroglial cell-ECM interactions.

Acknowledgements: Supported by grants from the NIH, the National Multiple Sclerosis Society and the Wadsworth Foundation.

PSM08-03

Myelin/oligodendrocyte glycoprotein: receptor endocytosis, membrane targeting, and an intracellular partner

MV GARDINIER, C ALLAMARGOT, MS KOCH, Y LEE and K MENON
Department of Pharmacology, University of Iowa, Iowa City, IA, USA

Myelin/oligodendrocyte glycoprotein (MOG) is an Ig-like membrane protein targeted to the surface of oligodendrocytes (OLs). It is localized on the cell body, its processes, and the outermost surface of myelin. Due to this accessibility on OLs, it is a major antigen attacked during immune-mediated demyelination (e.g., multiple sclerosis). MOG's cytoplasmic domain has dileucine (L_{195}II) and tyrosine (Y_{199}NWL) motifs that are implicated in basolateral targeting and endocytosis. Site-directed mutagenesis of these motifs disrupts basolateral targeting when MOG mutants are expressed in polarized MDCK cells. In N20 OLs, these MOG mutations also affect their ability to endocytose. Alternative splicing of *human* MOG mRNAs results in translation of MOG isoforms with variable cytoplasmic domains. MOG25.1 and MOG25.6 retain basolateral and endocytosis motifs; however, other isoforms lacking these sequence elements show altered cellular distribution and fail to endocytose. Lastly, MOG's cytoplasmic domain (residues 198–218) was used to screen a yeast two hybrid brain cDNA library for a cytosolic interacting partner. Stathmin, a phosphoregulatory protein involved in microtubule dynamics, was found. It colocalizes with MOG at the cell surface, and the two proteins can be reciprocally co-immunoprecipitated from OLs. Thus we propose that MOG acts as a receptor with restricted membrane localization on OLs, undergoes endocytosis that may be up-regulated, and interacts with stathmin just beneath the plasma membrane. A MOG:stathmin interaction at the periphery of OLs may modulate the availability of free tubulin heterodimers, which may then impact microtubule dynamics (growth vs. catastrophe).

Acknowledgements: Research supported by NINDS and NMSS.

PSM08-04

Developmental partitioning of MBP into myelin microdomains

L DEBRUIN, J HAINES, A LAFOREST and G HARAUZ

Department of Molecular and Cellular Biology, University of Guelph, Guelph, Canada

Myelin is a complex membrane comprising various microdomains including lipid rafts, which are involved in signalling, pathogen entry, and membrane trafficking in other cell types. Coalesced lipid rafts can be isolated as detergent-resistant membranes (DRMs) that float to a low buoyant density on a sucrose gradient. We have studied the partitioning of myelin basic protein (MBP), and its post-translationally modified forms, into lipid rafts in developing and mature bovine myelin, in order to further our understanding of this protein's function. DRMs from early myelin are enriched in Golli-MBP, CNP, and several Src tyrosine kinases. As myelin further develops, MAG, MOG, PLP, and MBP become localized in myelin rafts, whereas Src tyrosine kinases and Golli-MBP are no longer detectable in them. Furthermore, phosphorylated, citrullinated, and methylated MBP are found in 'non-raft' microdomains at this stage. In mature myelin, phosphorylated MBP is now partitioned into DRMs. This developmental localization of specific modified forms of MBP into different microdomains supports the thesis that MBP is multifunctional and may participate in signalling events, potentially in myelin maintenance and turnover. We are continuing these studies using a transgenic mouse line that overexpresses the proteolipid variant DM20, and which exhibits spontaneous demyelination after 3 mo of age. Our preliminary data indicate differences in the temporal distribution of MBP and its modified forms into microdomains in the demyelinating phenotype compared to normal controls. Such aberrant partitioning of MBP, and disruption of its structural and potentially signalling roles, may contribute to demyelination and failure to remyelinate in multiple sclerosis.

PSM08-05

Two types of low density detergent-insoluble membrane domains from myelin

JM BOGGS, DN ARVANITIS, W MIN and Y GONG

Research Institute, Hospital for Sick Children and University of Toronto, Toronto, Canada

Two different types of low density detergent-insoluble glycosphingolipid-enriched fractions (DIGs) were isolated from myelin by extraction with TX-100 in 50 mM Na phosphate at room temperature (procedure 1), in contrast to a single low density fraction obtained by extraction with TX-100 in Tris buffer with 150 mM NaCl, 5 mM EDTA at 4°C (procedure 2). The two DIG fractions obtained by procedure 1 gave two opaque bands, B1 and B2, at somewhat lower and higher sucrose density, respectively, than myelin itself. The single DIG fraction obtained by procedure 2 gave a single opaque band at similar sucrose density as B1. Both B1 and B2 had characteristics of lipid rafts, i.e. high galactosylceramide and cholesterol content and enrichment in GPI-linked NCAM120 and flotillin. However, they differed significantly in their protein composition. B2 contained 41% of the actin, 100% of the tubulin, and most of the caveolin in myelin while B1 contained more CNP, MBP, MAG and NCAM120 than B2. The single low density DIG fraction obtained by procedure 2 contained only low amounts of actin and tubulin. B1 and B2 also had size-isoform selectivity for PLP, MAG, and MAPK suggesting specific interactions and different functions of the two membrane domains. We propose that B1 may come from non-caveolar raft domains while B2 may come from caveolin-containing raft domains associated with cytoskeletal proteins. Some kinases present were active on myelin basic protein suggesting that the DIGs may come from signaling domains.

Acknowledgements: Supported by the Canadian Institutes for Health Research and Multiple Sclerosis Society of Canada.

PSM08-06

Alpha6beta4 integrin confers stability to peripheral myelin

ML FELTRI*, SC PREVITALI*, D ZAMBRONI*, G DATI*, S OCCHI*, G DINA*, U DEL CARRO*, K CAMPBELL†, F SAITO‡, A QUATTRINI*, L WRABETZ* and A NODARI*

**San Raffaele, Milan, Italy, †Univ. of Iowa, HHMI, Iowa City, IA, USA, ‡Teyko Univ., Tokyo, Japan*

Signals from laminins are required for Schwann cell-axon interaction and myelination. To investigate the role of laminin receptors α6β1 and α6β4 integrin, dystroglycan, DG, we have ablated them in Schwann cells using the Cre/LoxP system in mice. β4 integrin protein is synthesized at birth and polarized to the outer surface of Schwann cells after myelination. Additionaly, β4 integrin mRNA expression increases in the adult, suggesting a role in myelin maintenance. β4 integrin conditional null mice develop normally, and show normal motor performance, conduction velocity, myelinogenesis and regeneration. However, at 12 months of age β4 integrin null nerves show an increase in the number of fibers containing myelin infolding, suggesting myelin instability. Infoldings appear to originate near paranodes, and to form by invagination of abnormally folded myelin sheaths. DG inactivation in Schwann cells causes abnormally folded myelin sheaths in adult, indicating also a defect in myelin maintenance. To ask if the role of β4 integrin and DG are redundant, we generated β4 integrin/DG double null mice. In double mutants the number of abnormally folded sheaths is reduced, while the number of in and outfoldings is increased. This suggests that in the absence of α6β4 integrin, abnormally folded myelin sheaths evolve into myelin infolding. Thus, α6β4 integrin promotes myelin stability, and this function is partially redundant with that of DG.

PSM08-07

Trafficking of wild type and cytoplasmic domain-mutated myelin protein zero-GFP in living Schwann cells

J EICHBERG, VB KONDE, V GARGA and MA REA

University of Houston, Houston, TX, USA

A contribution of the myelin protein zero (MPZ) cytoplasmic domain to its homophilic adhesive interactions in myelin is suggested by domain truncation or certain point mutations, which impair or abolish MPZ adhesive properties. Previously, we reported that wild type (WT) MPZ-green fluorescent protein (GFP) transfected into immortalized Schwann cells (RSC96) accumulates at the plasma membrane, whereas MPZ-GFP mutated at a protein kinase C phosphorylation site (S204A), or at a nearby PKC consensus motif, remains partly in the cytoplasm and co-localizes with markers for endoplasmic reticulum and the Golgi. Using confocal microscopy, we compared the time course of appearance and distribution of newly synthesized WT and S204A MPZ-GFP. Four hours after WT transfection began, fluorescence is barely visible, but protein, visualized using an anti-GFP antibody, is present in the cytoplasm and at the plasma membrane. With time, the amount of membrane-associated MPZ-GFP increases, while protein in the cytoplasm disappears. In contrast, S204A MPZ-GFP trafficking to the plasma membrane is delayed. The mutated fluorescent protein at the cell surface displays a diffuse and irregular pattern, as compared to the sharp, smooth line formed by WT MPZ-GFP. Whereas fluorescence recovery after photobleaching (FRAP) indicates MPZ-GFP diffuses laterally within the plasma membrane, repeated photobleaching (FLIP) suggests interconnectivity exists between the plasma membrane and intracellular pools of the protein. These results further support a role for S204 phosphorylation in normal trafficking of MPZ-GFP and suggest that mutated protein reaching the plasma membrane may be incorrectly inserted.

Acknowledgement: Research supported by NIMH 62490.

PSM08-08

The myelin proteome II: updated functional proteomic mapping of the myelin membrane

CM TAYLOR*†, M KARIM†, CB MARTA†, D HAN†, MN RASBAND† and SE PFEIFFER†

**Dana-Farber Cancer Institute, Boston, MA, †University of Connecticut Health Center, Farmington, CT, USA*

Myelin is a remarkably active membrane involved in intense, bi-directional, interactive signaling with axons. Disruption of myelin results in many debilitating neurological diseases, including Multiple Sclerosis. To elucidate biological processes of myelin biogenesis, maintenance and functional interactions with neurons, we have undertaken the identification and characterization of the complex community of proteins in mouse myelin. Using enhanced methods for solubilizing transmembrane proteins for 2D gel electrophoresis, coupled with mass spectrometry and immunoblotting, we previously developed a high-resolution 2D proteomic map of proteins present in myelin, identifying 98 proteins corresponding to 130 of the approx. 200 spots. In addition to known myelin proteins, many classes of metabolically active proteins are represented in the map. These include proteins related to the cytoskeleton and scaffolding (e.g., tubulin, actin, ERM proteins), signaling (e.g., heterotrimeric G-proteins, Rho), mitochondria and energy metabolism (e.g., cytochrome C oxidase, ATP synthase), and vesicle transport, docking and fusion (e.g., NSF, Munc-18, VAT-1). More recently, we have used 1D gel electrophoresis and mass spectrometry to identify 278 proteins, 80 of which were not identified using 2D gel electrophoresis. We are currently applying this proteomic map to analyses of the localization and function of selected proteins, finding that it provides a powerful tool for the investigation of the diverse functions of myelin.

PSM08-09

Development of molecular probes for *in vivo* studies of myelin

Y WANG*, RM GOULD‡, B STANKOFF§, C LUBETZKI§, C WU*, PE POLAK†, J WEI*, J MAO*, DC LANKIN*, DL FEINSTEIN† and B ZALC§

**Departmet of Medicinal Chemistry and Pharmacognosy, †Department of Anesthesiology, ‡Department of Cell Biology and Anatomy, University of Illinois at Chicago, Chicago, IL, USA, §Biologie des Interactions Neurones/Glie, UMR-711 INSERM/UPMC, Paris, France*

Objectives: The goal of this study is to develop small-molecule probes that can be used to follow myelin changes *in vivo* in patients with neurodegenerative diseases.

Methods: (1) spectrophotometric binding assays with subcellular fractions of myelin to identify binding component(s) associated with myelin sheaths; (2) fluorescent staining of brain tissue sections to evaluate specificity of binding to normal and remyelinated sheaths; and (3) *ex vivo* fluorescent microscopy in normal and EAE mouse models to determine the brain permeability and characterize the binding specificity *in vivo*.

Results: To date, we have identified a lead compound, termed BMB, as a molecular probe with specificity towards myelin. The *in vitro* and *in vivo* binding properties of BMB have been demonstrated in the following ways: (1) BMB selectively binds to a myelin subcellular fractionation; (2) BMB selectively stains intact myelinated regions in normal control mouse brain and detects demyelinated lesions in EAE mouse models and postmortem tissue sections of MS brain; and (3) BMB readily penetrates the blood-brain barrier, selectively stains intact myelin sheaths in the brain, and detects demyelinated lesions in living EAE mice.

Conclusions: A myelin-specific probe has been developed that could be used to monitor myelin changes *in vivo*.

PSM08-10

Evolution of myelin proteins: homologs identified in the ascidian (*Ciona intestinalis*) genome

RM GOULD*†, HG MORRISON†, E GILLAND† and RK CAMPBELL‡†

**University of Illinois at Chicago, Chicago, IL, †Marine Biological Laboratory, Woods Hole, ‡Serono Reproductive Biology Institute, Rockland, MA, USA*

Many common proteins are used to form and maintain central (CNS) and peripheral nervous system (PNS) myelin sheaths in different vertebrate species. One to several different alternatively-spliced myelin basic protein (MBP) isoforms are present in all sheaths, proteolipid protein (PLP) and DM20 (except in amphibians) are major proteins in tetrapod CNS myelin sheaths and one or two protein zero (P0) isoforms are dominant in fish CNS and all vertebrate PNS myelin sheaths. In addition, several other proteins including 2′,3′-cyclic nucleotide 3′-phosphodiesterase (CNP), myelin and lymphocyte protein (MAL), plasmolipin, and PMP22 (prominent in PNS myelin) are localized to myelin and myelin-associated membranes and important for myelin sheath formation/stability. Databases with MBP, P0, PLP/DM20, CNP, MAL, plasmolipin and PMP22 sequences from teleost and cartilaginous fishes, amphibians, reptiles, birds and mammals were made and used as base to search the Ciona intestinalis genome for potential myelin protein homologs. Protein relatives of DM20/M6, MAL/plasmolipin and PMP22 proteins were identified in the Ciona genome, whereas homologs of MBP, P0 or CNP were not. All of the proteins present in the Ciona genome are tetraspan proteins indicating the evolutionary importance of these proteins. These studies provide a framework for understanding how proteins that participate in myelination were recruited and structural adaptations that took place to enable them to function in myelination.

Acknowledgement: Supported by NSF grant #ISB 0402188 (RMG).

PSM08-11

cDNA array in two oligodendroglial cell lines overexpressing transferrin show enhanced neurosteroids and mitochondrial activity

CI GARCIA, PM PAEZ, EF SOTO and JM PASQUINI

Department of Biological Chemistry, School of Pharmacy and Biochemistry, IQUIFIB-CONICET, University of Buenos Aires, Buenos Aires, Argentina

In the CNS transferrin (Tf) is produced by oligodendrocytes (OLG) and is essential for their survival. We have recently shown that in two OLG lines, N19 and N20.1, representing different OLG differentiation stages (Verity *et al.*, 1993), the addition of apoTf promotes their differentiation (Paez *et al.*, 2004). Clones that overexpress human Tf in both cell lines were used to study the genes involved in the maturational effect of Tf by cDNA array. In the less mature cells (N19) overexpressing Tf there was a significant increase in key enzymes of neurosteroid metabolism: 3β-HSD that mediates progesterone synthesis from pregnenolone and 5α-reductase type 1, that converts progesterone into 5α-dihydroprogesterone. We also analyzed in this cell line the activity of 3β-HSD and neurosteroids production in isolated mitochondria. Recent reports have described an important role for progesterone in OLG maturation (Gago *et al.*, 2001). In addition, in Tf-overexpressing cells of both lines there was a rise in the expression of several genes related to mitochondrial function and to complex lipid metabolism. It is known that these are crucial steps for myelin synthesis. Some of these results were confirmed by RT-PCR or by Western blot. Summarizing, aTf induced differentiation in both cell lines modulating different genes according to the maturational stage of the cells.

PSM08-12

A decrease in proteasome activity induces an activation of the myelin basic protein promoter

CA CALATAYUD, CI GARCÍA, PM PAEZ, EF SOTO, JM PASQUINI and LA PASQUINI

Dept. of Biological Chemistry-IQUIFIB, School of Pharmacy and Biochemistry-UBA-Conicet, Buenos Aires, Argentina

The Ub-proteasome system (UbPS) participates in the oligodendroglial cell (OLGc) differentiation. The addition of a proteasome inhibitor, lactacystin (Lc), to a primary OLGc culture, produces arrest of the cell cycle and cell differentiation. The regulation of the mbp gene takes place particularly at the beginning of transcription. The mbp promoter contains numerous binding sites to different transcription factors, among them Sp1. The level of expression of some of these factors is regulated through the UbPS. To elucidate the mechanism and the signal transduction pathways participating in the accelerated maturation of OLGcs induced by a decrease in proteasome activity, we used an OLGc line (N20.1), transiently transfected with the mbp promoter bound to a reporter gene (β-galactosidase) and different inhibitors of signal transduction pathways. Proteasome inhibitors (Lc and MG132) were able to significantly increase the expression of the mbp promoter. Regarding the signaling pathways participation, our results seem to indicate that tyrosine kinase and PI3K-Akt participate positively in the effect of Lc and MG132. PKA, Ras-Ras-MAPK-MEK/ERK1 and Ca-CaM seem to contribute negatively to such effect. When a PKC inhibitor and an Sp1-DNA binding inhibitor were tested, the expression of the mbp promoter was markedly decreased and it was reverted by Lc or MG132. The decrease in proteasome activity also produced a significant increase in p27 and Sp1 levels and an increased binding of Sp1 to DNA. These results indicate that the increased activation of the mbp promoter by partial inhibition of the proteasome could be due in part to the stabilization of p27 and Sp1.

PSM08-13

Angiogenic expression profile of neurofibromin-deficient Schwann cells and regulation by neurofibromin GAP related domain

GH DE VRIES*† and S THOMAS†‡

**University of Illinois Chicago, Chicago, †Hines VA Hospital, Hines, ‡Loyola University Chicago, Maywood, Il, USA*

Neurofibromatosis Type 1 (NF1) tumors are highly vascularized and contain Schwann cells (SC) that lack neurofibromin (Nf) which contains a GAP related domain (GRD). Nf-deficiency results in increased Ras activation. We have found that conditioned medium from Nf-deficient SC is a potent stimulator of endothelial cell proliferation and migration. Using an angiogenesis gene array followed by ELISA analysis for genes that are found to have significant expression changes in Nf-deficient SC compared to normal human SC, we found increased gene expression of several pro-angiogenic factors in Nf-deficient Schwann cells: angiopoietin-1 (ANGPT1), basic fibroblast growth factor (bFGF), hepatocyte growth factor (HGF), vascular endothelial growth factor C (VEGFC), and urokinase plasminogen activator (uPA). The anti-angiogenic factor secreted protein acidic and rich in cysteine (SPARC) was found to have decreased gene expression in Nf-deficient SC compared to normal human SC. Stable transduction of the GRD using a retroviral vector, resulted in the decreased proliferation rate of Nf-deficient SC. These studies provide evidence for pro-angiogenic changes in the angiogenic expression profile of Nf-deficient SCs. In addition, restoration of the Nf GRD in Nf-deficient SCs decreases cell growth rate and may modulate the angiogenic expression profile.

Acknowledgement: Supported by Minn NF Inc.

PSM08-14

Peripheral myelin protein 22 forms a complex with beta4 integrin in the Schwann cell membrane

SA AMICI and L NOTTERPEK

Department of Neuroscience, College of Medicine, McKnight Brain Institute of the University of Florida, Gainesville, FL, USA

Myelination in the PNS is dependent on precise contact between Schwann cells and the extracellular environment. We recently found that mice deficient in peripheral myelin protein 22 (PMP22), a Schwann cell glycoprotein, show morphological and biochemical changes in the sciatic nerve basal lamina. In an epithelial model, overexpression of PMP22 slows the migration of cells and alters the distribution of actin (Roux *et al.*, 2005). Based on these findings we hypothesized that PMP22 may interact with the integrins, molecules known to link the basal lamina and the cytoskeleton. In nerve lysates of PMP22 knockout mice, the levels of beta4 integrin are drastically reduced, while beta1 integrin levels are unaltered. Furthermore, we immunoprecipitated PMP22 from nerve lysates of wild type mice and found that PMP22 and beta4 integrin interact and are in a complex with alpha6 integrin and laminin. Reciprocal immunoprecipitation and Western blotting experiments confirmed the presence of PMP22 in the laminin-integrin protein complex. In clone A cells, which express high levels of beta4 integrin, overexpressed myc-tagged PMP22 is targeted to the beta4 integrin-positive cell surface. In agreement with the biochemical studies, in dorsal root ganglion neuron and Schwann cell explant cultures from wild type embryos we found the partial colocalization of PMP22 with beta4 integrin during the early stages of myelination. These results identify a novel role for PMP22 in the plasma membrane and could explain in part the delayed myelination observed in PMP22 deficient mouse nerves.

Acknowledgements: This work was supported by the State of Florida Brain and Spinal Cord Injury Rehabilitation Trust Fund.

PSM08-15

A glycosynapse in myelin?

JM BOGGS, W GAO, M WANG, Y HIRAHARA, Y GONG, DN ARVANITIS and W MIN

Research Inst., Hospital for Sick Children, and University of Toronto, Toronto, Canada

In myelin, contact between extracellular surfaces of membrane from the same cell occurs. The two major glycosphingolipids (GSLs) of myelin, GalC and sulfatide, can interact with each other by trans carbohydrate-carbohydrate interactions across apposed membranes. They may be located in membrane signaling domains which may transiently contact each other across apposed extracellular membranes of myelin, thus forming 'glycosynapses'. Multivalent forms of these carbohydrates, GalC/Sulfatide-containing liposomes, or galactose conjugated to albumin, have been added to cultured oligodendrocytes (OLs) to mimic interactions which might occur between these signaling domains when the extracellular surfaces of myelin come into contact. They cause co-clustering or redistribution of myelin GSLs, GPI-linked proteins, several transmembrane proteins, and signaling proteins to the same membrane domains. They also cause depolymerization of the cytoskeleton, indicating that they cause transmission of a signal across the membrane. Their effects are similar to those of anti-GSL antibodies on OLs, shown by Dyer and Benjamins, suggesting that the multivalent carbohydrate interacts with GalC/sulfatide in the OL membrane. Its effects on OLs were prevented by several kinase inhibitors suggesting which signal transduction pathways are involved. Participation of transient GalC/sulfatide interactions in glycosynapses between the apposed extracellular surfaces of mature myelin might allow transmission of signals throughout the myelin sheath and thus facilitate myelin-axonal communication.

Acknowledgement: Supported by the Multiple Sclerosis Society of Canada.

PSM08-16

The exocyst regulates myelin formation and maintenance

M ANITEI*, MF IFRIM†, MA EWART‡, R BANSAL†, JH CARSON*† and SE PFEIFFER*†

**Department of Molecular Biology and Biochemistry, University of Connecticut, †Department of Neuroscience, University of Connecticut Health Center, Farmington, CT, USA, ‡University of Glasgow, Division of Molecular Biology and Biochemistry, Glasgow, Scotland, UK*

Membrane biogenesis requires a complex apparatus that includes scaffolding and tethering proteins for the transport and assembly of specific proteins and lipids to distinct membrane sub-domains. We show that oligodendrocytes (OLs), cells in the central nervous system that produce myelin membrane, express components of the mammalian exocyst (Sec8, 6) and multiple domain scaffolding proteins (CASK, Mint1). Sec8 and CASK are co-localized, co-immunoprecipitate and co-fractionate with OSP/Claudin11 in OLs, suggesting that they may be involved in its trafficking to/retention at the myelin membrane. Functional studies strongly suggest that Sec8 is essential for the formation and maintenance of the myelin-like membranes. Sec8 overexpression promotes the formation of large myelin-like membranes in OLs in enriched cultures; intracellular antibody-mediated perturbation of Sec8 function leads to retraction of OL processes and myelin-like membranes. We propose that in OLs, the exocyst complex is involved in the capture and anchorage of vesicles transporting myelin proteins (e.g., OSP/Claudin11) to sites of membrane growth and is involved in local cytoskeletal rearrangements.

PSM08-17

Regulation of oligodendrocyte process formation via PD-Ialpha/ATX and LPA

L NOGAROLI*‡, SG PAYNE†, S SPIEGEL† and B FUSS*

**Dept. Anatomy and Neurobiology, †Dept. Biochemistry, Virginia Commonwealth University, Richmond, VA, USA, ‡Dept. Anatomy, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil*

During development of the central nervous system, oligodendrocytes (OLGs) send out processes that direct migration at progenitor (OPC) stages and that establish neuronal contact at differentiating stages. This process formation is regulated by extracellular cues, one of which appears to be phosphodiesterase Iα/autotaxin (PD-Iα/ATX). PD-Iα/ATX, via its adhesion-antagonizing domain, facilitates process formation in differentiating OLGs. PD-Iα/ATX also possesses lysophospholipase D activity potentially generating the lipid signaling molecule lyso-phosphatidic acid (LPA). In OLGs, LPA induces process retraction at the OPC but not differentiated stages, despite the expression of LPA1, one of the cell surface receptors mediating cellular responses to LPA. Using the OLG cell lines CIMO and N19, we provide here further insight into the potential dual function of PD-Iα/ATX. In CIMO cells, similar to OPCs, LPA treatment induces process retraction. However, in N19 cells, similar to differentiating OLGs, LPA has no cytoskeletal effect. Both cell lines express LPA receptors and are responsive to PD-Iα/ATX-mediated adhesion-antagonism, but only N19 cells express PD-Iα/ATX endogenously. Most importantly, in CIMO cells the LPA-induced effect is inhibited in the presence of the adhesion-antagonizing domain of PD-Iα/ATX. Thus, our data suggest that (1) signaling induced by the adhesion-antagonizing domain of PD-Iα/ATX interferes with LPA-induced process retraction and (2) PD-Iα/ATX may regulate process formation at different stages of the oligodendrocyte lineage via different functional domains.

Acknowledgements: Supported by grants from the NIH, the National Multiple Sclerosis Society and the Wadsworth Foundation.

PSM08-18

A solid state NMR study of MBP interaction with lipid bilayers

R ZAND*‡, CD POINTER-KEENAN†, DK LEE*†, K HALLOK†, A TAN*† and A RAMAMOORTHY*†

**Biophysics Research Division, †Chemistry Department, ‡Biological Chemistry Department, University of Michigan, Ann Arbor, MI, USA*

A study of the interaction of bovine MBP charge isomers (C1–C3) with phospholipid bilayers was carried out using solid state NMR experiments on model membrane systems. P-31 NMR experiments on multilamellar vesicles and aligned bilayers were used to ascertain the degree of lipid headgroup disorder in the headgroup region induced by MBP incorporation. H-2 NMR data provide the information on the disorder induced by the protein in the hydrophobic core of the bilayers. We have interpreted the data from these studies as indicating that the MBP charge isomers do not fragment or disrupt bilayers formed from DMPC, DOPC, POPC, POPC : POPG and POPE. The data are viewed as demonstrating that MBP induced fragmentation of POPC bilayers is a consequence of the freeze thaw cycles used in the preparation of multilamellar vesicles and is not the result of intrinsic protein lipid interactions.

Acknowledgements: This research was supported in part by NIH grant (AI054515) and NSF grant career development awards to A.R. and a National Multiple Sclerosis grant RG 3167 to R.Z.

PSM08-19

Myelin lipid deficiency in aspartoacylase-null (tremor) rats, model for Canavan disease: specific deficit of cerebrosides

J WANG*, G WU*, Z LU*, P LEONE† and RW LEDEEN*

**New Jersey Medical School-UMDNJ, Newark, †Robert Wood Johnson Medical School-UMDNJ, Camden, NJ, USA*

N-acetylaspartate (NAA) is localized in neurons and has become widely used as a non-invasive *in vivo* indicator of neuronal viability. However, its biological function remains obscure, the enigma being compounded by the fact that aspartoacylase (ASPA), the enzyme that releases acetyl from NAA, occurs in myelin. This correlates with the fact that labeled acetyl groups from NAA within axons of rat optic nerve/tract are incorporated into myelin lipids of the optic system (*J Neurochem* 2001; 78: 736–745). The tremor rat (*J Neurochem* 2000; 74: 2512–2519) is an ASPA-null mutant that is used as a model of Canavan disease (CD). Examination of the myelin lipids from such rats has revealed an abnormal TLC pattern with marked reduction of cerebroside. The greatest deficit occurred with cerebrosides containing unsubstituted fatty acids (in contrast to those with 2-hydroxyfatty acids). There was also evidence of reduced level of one subgroup of ethanolamine phosphoglycerides. These results resemble our lipid results with ASPA-null mice (Ledeen *et al*., in press), another CD model (Matalon *et al*. *J Gene Med* 2000; 2: 165–175).

Acknowledgement: Supported by National Multiple Sclerosis Society, USA.