Poster Session DP2: Gangliosides

DP2-01

GM1-induced activation of ERKs in the brain of young and aged rats

M. Hadjiconstantinou, L. Mo, R. Qun, N. H. Neff and A. M. Duchemin

Departments of Psychiatry & Pharmacology, The Ohio State University College of Medicine and Public Health, Columbus, OH, USA

We investigated the ability of GM1 to induce phosphorylation/activation of ERKs in the brain of young and aged rats. In brain slices *in situ* GM1 induced a rapid and transient activation of ERK1/2 in both young and aged rats, and a similar response was observed after stimulation with NGF or BDNF. The aged brain appeared to respond more robustly to neurotrophic stimulation with the pERK2 response being significantly greater in the hippocampus and frontal cortex. Acute ICV administration of GM1 resulted in short-lasting phosphorylation of ERKs in both age groups; while, chronic systemic administration of GM1induced a protracted phosphorylation of the kinases, with the pERK2 levels being significantly elevated in the aged hippocampus vs. the young. *In situ* phosphorylation of TrkA was similar in the young and aged brain, and chronic administration of GM1 increased the levels of pTrkA. *In situ* challenge with NGF considerably increased the phosphorylation of TrkA in GM1 treated young and aged animals, but had no effect on the phosphorylation of ERKs. These observations indicate that the aged brain maintains the ability to respond to neurtorophic stimuli and that GM1 utilizes the ERK cascade for some of its neurotrophic actions.

Acknowledgement: This work was supported by AG10530.

DP2-02

Exogenous GM1 ganglioside activates Src kinases in brain slices

A. M. Duchemin, N. H. Neff and M. Hadjiconstantinou

Departments of Psychiatry and Pharmacology, Ohio State University College of Medicine and Public Health, Columbus, OH, USA

GM1 is a major component of cell membrane particularly abundant in neurons. It localizes preferentially in specialized functional areas known as sphingolipid-enriched microdomains. These domains are also enriched in tyrosine kinase receptors and the membrane-anchored Src kinases. GM1 has neurotrophic and neuroprotective properties *in vivo* and *in vitro* on many neuronal systems. This activity has been attributed to a release of neurotrophins by GM1 or an interaction of the ganglioside with the neurotrophin receptors. Indeed, we have previously shown that GM1 can activate Trks, and two of their down-stream signaling molecules, Erk and PI 3-kinase in several areas of the brain. We now show that GM1 can also activate Src kinase activity in slices from rat striatum. Ten minutes after addition of 100 μM GM1, Src activity – measured by *in vitro* kinase assay – was increased by 45\%. Preincubation of the slices with the Src inhibitor PP2 completely blocked the GM1 effect. Interestingly, PP2 also blocked the increase of Erk and PI 3-kinase activity induced by GM1, suggesting that Src activation occurs upstream of these effectors. GM1 has been shown to associate rapidly with the sphingolipid-enriched microdomains after exogenous administration, and could directly interact with Src kinases, leading to activation of the signal transduction pathways mediating neuronal survival and differentiation.

Acknowledgement: This work was supported in part by NIA grant AG 10530.

DP2-03

N-butyldeoxygalactonojirimycin reduces postnatal cerebellar ganglioside content in a mouse model of GM1 gangliosidosis

J. L. Kasperzyk,* A. d'Azzo,† F. M. Platt‡ and T. N. Seyfried*

**Biology Department, Boston College, Chestnut Hill,*
†Genetics Department, St Jude Child. Res. Hosp., Memphis, USA,
‡Biochemistry Department, University of Oxford, Oxford, UK

GM1 gangliosidosis is a lysosomal storage disease caused by a deficiency in acid β-galactosidase (β-gal), resulting in accumulation of ganglioside GM1 and its asialo-form (GA1) primarily in the brain. We previously showed that substrate reduction therapy (SRT) decreases brain ganglioside and GM1 content in postnatal day 5 (p-5) β-gal knockout (–/–) mice treated with *N*-butyldeoxygalactonojirimycin (*N*B-DGJ). In this study, we examined the effect of *N*B-DGJ on postnatal cerebellar and brain gangliosides. Wildtype C57BL/6J (B6) and mutant β-gal(–/–) were injected daily from p-9 to p-15 with either vehicle or *N*B-DGJ at 600mg/kg body wt. Cerebellar and brain ganglioside content (μg sialic acid/100mg dry wt) in the p-15 untreated B6 mice was 342 ± 13 and 502 ± 4, respectively, and in the β-gal(–/–) mice was 364 ± 7 and 587 ± 17, respectively. *N*B-DGJ significantly reduced cerebellar and brain ganglioside content in the B6 mice by 22 and 16%, respectively, and in the β-gal(–/–) mice by 21 and 19%, respectively. Cerebellar and brain GM1 content was reduced in the treated B6 mice by 17 and 18%, respectively, and in the β-gal(–/–) mice by 41 and 35%, respectively, compared with untreated controls. *N*B-DGJ had no effect on brain GA1 content in the β-gal(–/–) mice. *N*B-DGJ had no effect on body wt or cerebellar/brain wt, water content, or development in either genotype. These findings suggest that SRT using *N*B-DGJ may be an effective early intervention for GM1 gangliosidosis.

Acknowledgements: This work was supported by NIH grant(HD39722) and NTSAD Assoc.

DP2-04

N-butyldeoxygalactonojirimycin reduces brain ganglioside and GM2 content in neonatal sandhoff diseased mice

R. C. Baek,* J. L. Kasperzyk,* F. M. Platt† and T. N. Seyfried*

**Biology Department, Boston College, Chestnut Hill, MA, USA,*
†Department of Biochemistry, Glycobiology Institute, University of Oxford, Oxford, UK

Sandhoff disease arises from an autosomal recessive mutation in the hexb gene and causes an accumulation of ganglioside GM2 and asialo-GM2 (GA2) primarily in the CNS. This results in defective β-hexosaminidase A that, together with the GM2 activator protein, degrades GM2 within lysosomes. Substrate reduction therapy (SRT) decreases the rate of glycosphingolipid (GSL) biosynthesis to compensate for impaired catabolism. The imino sugar, *N*-butyldeoxygalactonojirimycin (*N*B-DGJ) inhibits ceramide-specific glucosyltransferase, which catalyzes the first committed step in GSL biosynthesis. We compared the concentration and distribution of brain gangliosides between 129/SV *Hexb* +/– and *Hexb* –/– mice at postnatal day 5 (p-5). Neonatal mice were injected daily (ip) from p-2 to p-5 with either saline or *N*B-DGJ at 600 mg/kg body weight. Total brain ganglioside content (μg of sialic acid/100 mg dry weight) in saline-injected *Hexb* +/– (n = 4) and *Hexb* –/– (n = 12) mice was 397 ± 6 and 395 ± 8 μg, respectively. *N*B-DGJ significantly reduced total brain ganglioside content by about 25 and 23%, respectively. GM2 and GA2 were undetectable in the *Hexb* +/– mice. *N*B-DGJ significantly reduced GM2 content by about 36% in the *Hexb* –/– mice, but had no significant effect on GA2 content. *N*B-DGJ did not alter body weight, brain weight, or brain water content in the *Hexb* +/– and *Hexb* –/– mice. These results suggest that SRT using *N*B-DGJ may be an effective early intervention therapy for the management of GM2 ganglioside storage diseases.

Acknowledgements: This work was supported by NIH grant (HD39722) and the NTSAD Assoc.

DP2-05

Influence of imino sugars on growth and ganglioside content in an experimental mouse brain tumor

L. E. Abate, M. El-Abbadi, T. Sanderson, P. Mukherjee and T. N. Seyfried

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

Previous studies show that the imino sugar, *N*-butyldeoxynojirimycin (*N*B-DNJ), reduces growth and ganglioside content in experimental mouse brain tumors. *N*B-DNJ inhibits the ceramide-specific glucosyltransferase that catalyzes the first step in ganglioside biosynthesis. Defects in ganglioside biosynthesis may underlie the invasive and malignant properties of brain tumors. It was not clear from the initial studies with *N*B-DNJ if brain tumor growth reduction resulted specifically from ganglioside synthesis inhibition or from nonspecific effects of body weight reduction that also occurred with *N*B-DNJ treatment. In this study we evaluated the effects of the three imino sugars, *N*B-DNJ, *N*B-DGJ, and OGT2378 (2500 mg/kg/day administered in the food), together with caloric restriction (CR) as an active body weight control on growth (subcutaneous volume) and ganglioside content (μg total sialic acid/100 mg dry weight) in the CT-2A mouse astrocytoma. Tumor growth in the *N*B-DNJ-, *N*B-DGJ-, OGT2378-treated, and in CR body weight controls was 25, 98, 40, and 45% of the untreated control group, respectively. Body weight (g ± SEM) in these same groups was 20.6 ± 0.5, 25.8 ± 0.2, 22.4 ± 0.1, and 22.4 ± 0.3 g, respectively. Body weight in the untreated control group was 25.6 ± 0.6 g. Tumor ganglioside content was significantly reduced in all imino sugar-treated mouse groups, but was not reduced in the CR group. Statistical analysis showed that tumor growth was significantly correlated ($n = 17$; Pearson's $r = 0.675$, $p = 0.01$) with body weight, but not with tumor ganglioside content. These findings indicate that ganglioside synthesis inhibition is not responsible for reduced growth in this brain tumor model.

Acknowledgements: This work was supported by AICR and Oxford GlycoSciences.

DP2-06

Role of GM1 ganglioside in operation of TRPC5 calcium channels

G. Wu, Z. Lu and R. W. Ledeen

UMDNJ-New Jersey Medical School, Newark, USA

Previous studies by our group and others showed that binding of GM1 on the surface of NG108-15 and other neuronal cell lines with cholera toxin B subunit (Ctx B) induced Ca^{2+} influx together with extension of axon-like neurites. Primary neurons in culture showed the same effect. This Ca^{2+} influx was voltage-independent and insensitive to blockers of voltage-operated Ca^{2+} channels. However, it was blocked by SK this resulted in elimination of Ctx B-induced Ca^{2+} influx as well as neurite outgrowth, each nucleotide having the same effect. Our results provide evidence that Ca^{2+} influx and axonogenesis induced by Ctx B are mediated by TRPC5-containing channels, with GM1 functioning as intrinsic factor associated directly or indirectly with such channels. The natural ligand reacting with plasma membrane GM1 to trigger this process remains to be elucidated.

Acknowledgement: This work was supported by NIH grant 5RO1 NS33912-07.

DP2-07

Heightened kainate-induced seizures in ganglioside-deficient (KO) mice: function of GM1 in neuronal calcium homeostasis

R. W. Ledeen, J. Wang, Z. Lu, E. Wang, M. F. Meyenhofer and G. Wu

Department of Neuroscience, New Jersey Medical School, Newark, USA

Gangliotetraose gangliosides, including GM1 and its polysialosyl analogues, are major gangliosides of the CNS and serve a number of modulatory functions. Knockout (KO) mice lacking such gangliosides, resulting from interruption of GM2/GD2 synthase (GalNAc-T) gene, have shown several neurological disorders including spontaneous seizures and sudden death at age of 6 months. Our recent study revealed that young adult KO mice (3–4-month-old) subjected to kainic acid (KA) showed significantly higher temporal seizure activity (enhanced severity and elongated duration) and mortality than normal or heterozygous mice, in association with apoptotic deterioration of pyramidal neurons in the hippocampus. KA-induced seizures and associated neuronal degeneration in KO mice were unaffected by IP administered GM1, but were significantly reduced by LIGA20, a membrane-permeable analogue of GM1. Tracking *in vivo* distribution of [^{3}H]GM1 vs. [^{3}H]LIGA20, 4 h. after IP injection, revealed significantly more LIGA20 than GM1 in brain tissue, including isolated nuclei. Hyper-susceptibility of KO mice to KA was attributed to impaired Ca^{2+} regulation in neurons, as seen in our study of cultured cerebellar granule neurons in which elevated [Ca^{2+}]i caused apoptosis in KO- but not normal cells (*PNAS* **98**: 307–312, 2001). The latter pathogenic event was prevented to a limited extent by GM1 and more potently by LIGA20 pretreatment. Cell rescue is postulated to be due to LIGA20 potentiation of Na-Ca exchanger located in the nuclear envelope, which membrane LIGA20 is able to enter (*J. Neurochem*. **81**: 1185–1195, 2002). Administered LIGA20 may also modulate Ca^{2+}-related effects in the plasma membrane.

Acknowledgement: This work was supported by NIH grant 5RO1 NS33912-7.

DP2-08

Overexpression of gangliosides suppresses proliferation of immortalized neural progenitor cells via repression of the MAPK pathway

M. Yanagisawa, S. Liour and R. K. Yu

IMMAG, Medical College of Georgia, Augusta, USA

The central nervous system consists of neuronal and glial cells generated from common neural progenitor cells during development. Cellular events for neural progenitor cells, such as proliferation and differentiation, are regulated by multiple intrinsic and extrinsic cell signals. Although much is known on the importance of the proteineous factors in regulating the fate of neural progenitor cells, the involvement of other molecules such as glycosphingolipids (GSLs) remains to be clarified. GSLs are molecules ubiquitously expressed on the outer surface of plasma membrane of cells. The expression of GSLs, especially gangliosides, is known to change drastically during development. To elucidate the biological functions of gangliosides in neural progenitor cells, we transfected an immortalized neural progenitor cell line, C17.2, which expresses only a-series gangliosides, with a fusion protein of GD3-synthase (ST-II) and EGFP (ST-II-EGFP). Analysis of the ST-II-EGFP transfectants revealed the expression of b-series and c-series gangliosides. In the transfected cells, proliferation induced by epidermal growth factor (EGF) was severely retarded. EGF-induced proliferation of C17.2 cells was dependent on the Ras-MAPK pathway, and the EGF-induced activation of this pathway was significantly repressed in the transfected cells. Thus, ST-II overexpression retarded proliferation of C17.2 cells via repression of the Ras-MAPK pathway. The result supports the concept that GSLs play an important role in regulating the proliferation of neural progenitor cells during development.

Acknowledgements: This work was supported by USPHS NS11853 and Children's Medical Research Foundation.