

Neurobiology of Lipids

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Society for Neuroscience Annual Meeting 2002

EDITOR'S CHOICE NEUROBIOLOGY OF LIPIDS SESSIONS AT NEUROSCIENCE 2002

Editorial Material

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This article alerts interested readers about editors' choice neurobiology of lipids sessions of the 32nd Society for Neuroscience (SfN) Annual Meeting, 2-7 November, 2002, Orlando, Fla. The article quotes original Neuroscience 2002 abstracts, provides the references for proceedings articles (published in *Neurobiology of Lipids* and elsewhere) and related bibliography. The entire collection of the neurobiology of lipids sessions at Neuroscience 2002 is also available (*Neurobiology of Lipids*, 2002, Vol. 1, 5, <http://neurobiologyoflipids.org/content/1/5>).

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EDITOR'S PLATINUM CHOICE*

Program number 420.11

ABNORMAL CHOLESTEROL PROCESSING IN ALZHEIMER'S DISEASE PATIENT'S FIBROBLASTS

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Cholesterol has recently received attention as a potentially important factor in Alzheimer's disease etiology. Caveolin, which binds cholesterol, plays a

prominent role in cellular cholesterol transport. This protein is mainly located in plasma membrane invaginations called caveolae, which help determine cholesterol-dependent clustering of proteins involved in specific signal transduction pathways. Here, we report new clues about the involvement of caveolin in Alzheimer's disease. Cell fractions were collected for Western blotting with fibroblasts isolated from Alzheimer's patients (AD) or age and sex matched controls (AC). While AC cells demonstrated higher levels of caveolin in membrane-enriched fractions compared with AD cells ($p < 0.05$; $n = 6$), more concentrated cholesterol and caveolin signals were found in the caveolae-enriched fractions from AD. Consistent immunocytochemistry results showed that caveolin was preferentially clustered on the plasma membrane in AD cells, whereas a more scattered signal was observed in AC cells. Furthermore, a cross-linking activation of the prion protein, which is known to link to signal transduction of caveolin, increased caveolin in AC membrane fractions ($p < 0.01$; $n = 6$), and induced the formation of clustered caveolin observed with immunocytochemistry. This stimulation had no effect on AD caveolin distribution. Our results suggest a dysregulation of cholesterol processing in AD fibroblasts. Preliminary results also suggest that caveolin-dependent signal transduction is also altered in AD. Similar dysregulation of cholesterol processing in the brain may contribute to the pathogenesis of AD.

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(14 March 2003) Available at: <http://neurobiologyoflipids.org/content/1/7>

EDITOR'S GOLD CHOICE

Program number 191.3

CORRELATION OF DIET-INDUCED AORTIC ATHEROSCLEROSIS AND CEREBRAL AMYLOIDOSIS IN A MOUSE MODEL OF ALZHEIMER'S

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Recent studies have linked many risk factors for cardiovascular disease with Alzheimer's disease (AD). High fat/high cholesterol diets have been shown to exacerbate β -amyloidosis in the brain of transgenic mouse models of AD. The study of the relationship between atherosclerosis and β -amyloidosis in such transgenic mice has not been possible because the transgenic mice were on atherosclerosis-resistant genetic backgrounds. In this study, Tg2576 mice on a mixed SJL/C57BL/6 background, overexpressing human β -amyloid precursor protein (APP) with the Swedish double mutation, were backcrossed to the C57BL/6 background for 10 generations. At the age of 7 to 8 months, 18 transgenic mice were divided into two groups receiving either an atherogenic diet or a normal diet. After 14 weeks of diet feeding, these mice were sacrificed for analyses of plasma cholesterol profiles, aortic atherosclerotic lesions, and cerebral β -amyloidosis. The results indicated that Tg2576 mice on C57BL/6 background developed age-dependent amyloid plaques in the brain as those on the mixed SJL/C57BL/6 background. The atherogenic diet induced hypercholesterolemia and atherosclerosis in these mice. The mice receiving the atherogenic diet had more amyloid deposition in the brain than the mice on the normal diet ($P < 0.05$). The area of aortic atherosclerotic lesions was positively correlated with the amyloid load in the brain (Spearman $r = 0.75$, $P < 0.05$). Our data show for the first time that the degree of aortic atherosclerosis correlates with that of cerebral amyloidosis in APP transgenic mice.

Supported by: Alzheimer's Association (NIRG-00-2281)

NOI REFERENCE:

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Program number 686.15

EXPRESSION AND REGULATION OF THE CHOLESTEROL EFFLUX MOLECULE, ABC-A1, IN NEURONAL CELLS

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Cholesterol efflux from cell membranes to high density lipoproteins is mediated by the ABC-A1 transporter, which is mutated in Tangier disease. The expression, function, and regulation of ABC-A1 has been extensively examined in peripheral tissues, but only poorly studied in the brain. We have found a largely neuronal expression of ABC-A1 in normal rat brain by *in situ* hybridization. ABC-A1 message was dramatically upregulated in glia in areas of damage by hippocampal AMPA lesion after 3-7 days. ABC-A1 protein was detected in primary mouse neurons and mouse Neuro2A (neuroblastoma) cells by immunoblot analysis. We transfected an ABC-A1-GFP expression construct into Neuro2A cells, and observed fluorescence in ER and Golgi compartments, and on the plasma membrane. ABC-A1 expression in peripheral tissues is induced by ligands of the nuclear hormone receptors of the RXR and LXR family. We found that ABC-A1 was similarly induced in primary neurons and Neuro2A cells by treatment with 1-10 μ M retinoic acid and 1-10 μ M 22-hydroxycholesterol. These data suggest that ABC-A1 is active in neuronal cells, and by extension, involved in cholesterol metabolism mechanisms implicated in Alzheimer's disease.

Supported by: Harvard Center for Neurodegeneration and Repair

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- Fukumoto H, Deng A, Irizarry MC, Fitzgerald ML, Rebeck GW. Induction of the cholesterol transporter ABCA1 in central nervous system cells by liver X receptor agonists increases secreted Abeta levels. *J Biol Chem.* **277**, 48508-13 (13 Dec 2002).

Program number 723.10

A ROLE FOR LIPOPROTEIN LIPASE DURING SYNAPTIC REMODELING IN THE ADULT BRAIN

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Lipoprotein lipase (LPL) is a member of a lipase family known to hydrolyze triglyceride molecules on the surface of lipoprotein particles. This particular member also has a role in the binding of the lipoprotein particles to cell surface receptors. LPL has been identified in the brain but has no specific function yet. This study aimed at elucidating the role of LPL during the brain's response to injury. Mice subjected to an entorhinal cortex lesion were assessed for hippocampal LPL mRNA and protein levels in a time-course analysis of the degeneration/reinnervation phases. LPL protein levels peaked at day post lesion (DPL) 2 during the active phase of degeneration, without significant alteration of enzymatic activity. Cell surface receptors known to bind LPL were examined in the same time course analysis and no change was observed for the apoE/apoB (LDL) receptor family nor for the 39 kD receptor-associated protein (RAP). These preliminary results suggest that LPL is involved in the recycling of cholesterol and lipids released from degenerating terminals via a pathway that is independent of the classical apoE/apoB receptors cascade regulating lipoprotein internalization.

Supported by: CIHR (JFB, JP); FRSQ-FCAR (JFB); Alzheimer's Society of Canada (JP)

EDITOR'S SILVER CHOICE

Program number 21.11

ALZHEIMER'S DISEASE AND AMYLOID BETA PROTEIN: DOGMA IS BAD FOR SCIENCE

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There are no doubts about the perceived importance of amyloid beta protein (Abeta) for the pathogenesis of Alzheimer's and other related neurodegenerative disorders. The search of PubMed for "amyloid beta and Alzheimer's disease" performed on 24 April 2002 retrieved 5835 articles. In addition to this open science measure there are 66 US patents that have words 'amyloid and beta' in their titles.¹ The dogmatic viewpoint is that Abeta is a bad neurotoxic molecule that has to be eliminated from the brain tissue.²⁻⁶ The inability of the toxic concept to provide the disease cure become apparent this year when anti-amyloid vaccination was withdrawn⁷ and actually exacerbated the condition - a likely consequence if one considers Abeta as an endogenous protector of neural function! That amyloid might represent an epiphenomena or a compensatory response, while justified scientifically, is blindly avoided.²⁻⁵ However, there is now compelling data that Abeta is NOT the mediator of disease but rather a response (an antioxidant or a sensor of membrane lipids dynamics, for example) to an underlying etiology. We believe that the unipolar discussion of Abeta as toxic has served to severely hamper the advancement of neuroscience by hiding the accumulating evidence that Abeta is a normal and functional component of brain metabolism and synaptic function.⁷

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Program number 483.16

OMEGA-3 FATTY ACID ENRICHED DIET ATTENUATES PATHOLOGY IN AN ALZHEIMER'S DISEASE (AD) MOUSE MODEL

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High cholesterol and fat are associated with increased AD risk and promote amyloid pathology in mouse models. Low dietary intake and blood levels of the omega-3 fatty acid docosahexaenoic acid (DHA) found in fish can be a risk factor for

AD or cognitive impairment, and AD brains are deficient in DHA. Therefore, we evaluated whether diets depleted or enriched in DHA can alter neuropathology in APPsw (Tg2576) transgenic mice. Seventeen-month-old animals were placed in one of three groups: standard "breeder" chow commonly found in transgenic mouse colonies (0.09% DHA), diets with no DHA, and diets enriched with 0.6% DHA. Animals were treated until 22.5 months of age and measurements were made in the cortex. ELISA showed that amyloid was significantly reduced by 77% in mice fed DHA-enriched diet compared to mice on DHA-depleted diet. Analysis of Western blots showed that presynaptic (synaptophysin) and postsynaptic markers (PSD-95 and NR2B) were significantly reduced by 30-74% in mice fed diets with no DHA. Replenishing dietary DHA caused a significant 2-fold elevation in postsynaptic markers. Oxidized protein levels were elevated in DHA-depleted diets compared to standard breeder chow, but significantly reduced by 52% in animals fed diets enriched with DHA. Together, these results may suggest that a diet containing DHA could be protective against amyloid accumulation, oxidative damage, and synaptic degeneration. Specific mechanisms for these effects will be discussed.

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Program number 722.4

SUPPRESSION OF ALZHEIMER'S DISEASE (AD) PATHOGENESIS BY DIETARY LIPIDS AND NSAIDS

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Non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen can reduce AD pathology in APP transgenic models. Similar to results with anti-A β immunization, the effects are attenuated in older mice. In contrast, even in aged animals (17 to 22 months) a diet containing 500ppm curcumin, (a natural compound with antioxidant, NSAID and hypolipidemic activity) effectively and dramatically suppressed β -amyloid accumulation and oxidative damage. Curcumin inhibits lipid peroxidation and should reduce synaptic ω 3 polyunsaturated fatty acid loss known to occur in AD brain. Low ω 6/ ω 3 fatty acid ratios (~4:1) in high fish diets are

associated with reduced AD risk and, like curcumin, have antioxidant, NSAID and hypolipidemic activities. Standard mouse diets (SMD) have similar ~4:1 ratios that may suppress AD pathogenesis. We compared 22 mo old APPsw mice on an SMD with mice switched at ~17 mos to a "bad" American diet (BAD, ~85:1 ω 6/ ω 3 ratios) or BAD supplemented with the ω -3 docosahexanoic acid (BAD+DHA, ~4:1) and aged to 22 mos. Compared to the SMD group, BAD mice had similar amyloid, but large reductions in synaptic markers. Like curcumin, DHA supplementation to BAD resulted in major reductions in amyloid, oxidative damage and restored synaptic marker. We hypothesize elevated focal A β -induced oxidative damage causes ω 3 depletion and synaptic toxicity leading to further A β in a positive feedback loop that can be blocked by curcumin and/or replenishing DHA even with late intervention. Because of relative safety and broad spectrum efficacy, both are strong candidates for clinical application.

Supported by: AG13471, NS43946, Alz. Assoc. (GMC), AG16570 (SAF, GMC)

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Program number 883.9

BRAIN REGION-DEPENDENT INCREASES IN β -AMYLOID AND APOLIPOPROTEIN E LEVELS IN HYPERCHOLESTEROLEMIC RABBITS

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Recent studies demonstrate a link between serum cholesterol levels, β -amyloid (A β) peptide concentrations, and the incidence of Alzheimer's

disease (AD). In the present report, the effects of dietary cholesterol on A β and apolipoprotein E (APOE) levels in several brain regions were examined using diet-induced hypercholesterolemic rabbits as the animal model. Increased dietary cholesterol levels increased A β concentrations in temporal cortex ($p = 0.02$). A similar trend was observed in the frontal cortex ($p = 0.06$), yet not in the cerebellum. Interestingly, the regional levels of A β in the hypercholesterolemic rabbit paralleled the amyloid pathology observed in AD brain. Elevated APOE levels were also noticed in temporal ($p < 0.01$) and frontal ($p < 0.01$) cortices, but not in cerebellum, in the rabbit fed with cholesterol-abundant diet. These results suggest that high serum cholesterol levels could be a risk factor for AD and APOE may play a role in aggravating the A β accumulation.

Supported by: National Science Council in Taiwan (NSC90-2320-B-006-072 and NSC90-2320-B006-052)

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Program number 884.1

AMYLOID BETA PROTEIN RESTORES HIPPOCAMPAL LONG TERM POTENTIATION: A CENTRAL ROLE FOR CHOLESTEROL?

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There is no understanding of the role of amyloid beta protein (Abeta) in brain function and Alzheimer's disease.¹ In the present study we attempted to dissect out the role for Abeta in the synaptic plasticity in brain slices from adult male rat hippocampus. The prolonged maintenance of slices ex vivo (20+ hrs) in our experimental set up preserved basic synaptic physiology but abrogated tetanus-induced long term potentiation (LTP). Peptide Abeta(1-40) rescued LTP whereas cholesterol synthesis inhibition abolished LTP restoration by the Abeta peptide. Our observation implies that Abeta protein is a functional player in

cholesterol neurochemical pathways and in synaptic structure-functional plasticity.² The finding also supports our proposed hypothesis that the change in Abeta biochemistry in Alzheimer's disease and related disorders is a functional (but not pathologic) phenomenon aiming to compensate impaired cholesterol dynamics and associated neurotransmission and synaptic plasticity.²⁻⁴ Such cholesterol mediated failure of synaptic function and neural degeneration (*Science*, 22 March 2002, Vol. 295, p.2213, Ref. 5) likely causes the major sporadic form of Alzheimer disease² (also see *Neurology*, 2002, Vol. 58, p. 1135; and our other SFN2002/01 posters⁶).

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Program number 884.10

CHANGES IN SPHINGOMYELIN METABOLISM AND OXIDATIVE STRESS IN AGING AND ALZHEIMER'S DISEASE RESULT IN THE ACCUMULATION OF NEUROTOXIC CERAMIDES AND CHOLESTEROL ESTERS

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Aging is the major risk factor for Alzheimer's disease (AD), and may promote the dysfunction and degeneration of neurons by increasing oxidative stress and perturbing metabolism of amyloid precursor protein (APP). We provide evidence that sphingomyelins (SMs) accumulate in the brain with age, and this correlates with increased oxidative stress as indicated by levels of lipid peroxides and 4-hydroxynonenal. Addition of sphingolipid substrates to primary hippocampal neurons enhances ceramide accumulation and vulnerability to amyloid beta-peptide (A β) toxicity. Conversely, inhibition of serine-palmitoyl-transferase reduces ceramide, cholesterol ester accumulation and protects neurons against death induced by oxidative insults and A β . We are currently investigating the possible links between sphingomyelin metabolism and A β accumulation in AD. We believe that the age-related accumulation of SMs and oxidative stress reaches a threshold that activates neutral and acid sphingomyelinases resulting in accumulation of long-chain ceramides, activation of acyl-coenzymeA:cholesterol acyltransferase activity, accumulation of cholesterol esters and alterations in lipid rafts. We propose that effective AD prevention strategies may include dietary manipulations designed to slow the age-related accumulation of sphingolipids. Our model also predicts that inhibitors of sphingomyelinases may be effective therapeutic interventions for patients already suffering from AD.

Supported by: NIA-IRP

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EDITOR'S BRONZE CHOICE*Program number 21.10***AMYLOID BETA, NEURAL LIPIDS, CHOLESTEROL AND ALZHEIMER'S DISEASE***N.V. Koudinova, A. Kontush, S. Arlt, T.T. Berezov, A.R. Koudinov*

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To date great number of articles were devoted to cholesterol (chol) but only few articles studied the role for chol in neuron function/degeneration. For decades this molecule had been known to be important for atherosclerosis and heart disease. First indication of the involvement of chol in Alzheimer disease (AD), however, come from the mid 1990s. At that time it was shown that heart disease patients develop brain deposits of amyloid beta (Ab), a major dogmatic molecule of AD; that apoE (a chol transport apolipoprotein) allele e4 is a major genetic risk factor for AD; and that lab animals fed a chol diet express brain amyloid.¹ These days it turns out that Ab, long thought to be exclusively a pathologic protein, is a normal and functional apolipoprotein constituent of high density lipoproteins in plasma and CSF. Thus, we and others showed that Ab modulates chol and phospholipid synthesis, and affects chol esterification.¹ Protection of lipoproteins and other biomolecules from oxidation may represent another important function of Ab.² We also discovered that neuronal chol homeostasis failure and the lack of chol supply to neurons by means of lipoprotein transport causes AD features, such as the failure of the neurotransmission and synaptic plasticity, degeneration of neuronal cell processes, and tau protein pathology.^{3,4}

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*Program number 122.5***ACAT REGULATES A β GENERATION: CHOLESTEROL-DEPENDENT APP CLEAVAGE AND TRANSGENIC MOUSE STUDIES***D.M. Kovacs, L.A. MacKenzie Ingano, S. Domnitz, R.E. Tanzi, M.P. Frosch, L. Puglielli*

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ACAT (acyl CoA:cholesterol acyltransferase) regulates A β generation by controlling the equilibrium between free cholesterol and cholesteryl-esters (Nat. Cell Biol. 2001; 3: 905). Here we used a genetically mutant CHO cell line deficient in ACAT activity (AC29) to study the role of ACAT in A β generation. We found that the selective disruption of ACAT activity in these cells alters proteolytic processing of APP, precluding the generation of A β . We have identified and characterized a 55-kDa APP C-terminal fragment (CTF) only present in AC29 cells, which we termed SD-APP-CTF for sterol-dependent APP-CTF. Amino-acid sequencing placed the cleavage site N-terminal of the Kunitz protease inhibitor domain. SD-APP-CTF first appears in the Golgi/endosomes. We reconstituted the sterol-dependent proteolysis of APP in an in vitro system. Remarkably, parental CHO Golgi/endosome vesicles, as well as those

isolated from the derivative AC29 cells, were able to generate the SD-APP-CTF, APP C83 and C99 in vitro. In conclusion, we report that a novel proteolytic cleavage of APP may control the generation of A β and that the access of APP to this proteolytic activity is sterol-dependent. In separate studies, we also explored the effect of ACAT inhibition on mouse brain cholesteryl-ester levels. We implanted 21-day time-release pellets of CP113,818 (Pfizer), a pharmacological ACAT inhibitor, into 3-month old mice. Five increasing concentrations of the inhibitor reduced brain cholesteryl-ester levels in a concentration-dependent manner, up to 86%. The effect of this compound on A β generation is being determined.

Supported by: AHAF and ISOA

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Program number 122.6

γ -SECRETASE INHIBITION VIA CHOLESTEROL DEPLETION INVOLVES PRESENILIN

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Cholesterol lowering reduces γ -secretase activity. Analysis of the compartments involved in cholesterol pathways revealed an important influence of subcellular cholesterol distribution. Blocking the intracellular cholesterol trafficking at the level of late endosomes resulted in parallel increase of cholesterol, PS and A β 42 in specific vesicles. The unexpected colocalization of certain ER markers indicates that block of cholesterol trafficking prevented export of APP-CTFs and PS from these vesicles. Moreover, it indicates that under these conditions γ -secretase is active in this compartment. The unexpected combination of PS with LE and ER markers indicates a complex trafficking of PS that partly overlaps with cholesterol trafficking. PS is mainly localized

distant from sites of secretase activity. Transient fusion of these vesicles may help to understand this spatial paradox. Selective influence on secretase activity is not unique to Rab7 positive compartments. Cholesterol level manipulation of late Golgi compartments specifically alters intracellular γ -secretase activity resulting in A β 40 production, believed to be mainly localized to the TGN. Our data suggest that reduced A β production may not be achieved by reducing the mean cholesterol content of neurons, but rather by alterations in cellular cholesterol distribution, uptake and storage. These results can be further extended since we found increased inhibition by combination with M1 muscarinic agonists. Taken together this will be especially challenging in vivo as our recent clinical study indicates reduced A β levels in statin treated mild AD patients.

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Program number 296.13

ASSOCIATION OF AMYLOID PRECURSOR PROTEIN WITH SPECIALIZED LIPID RAFTS IN BRAIN: POSSIBLE INVOLVEMENT IN ITS PHYSIOLOGICAL FUNCTIONS AND PROCESSING

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Lipid rafts are cholesterol-enriched membrane microdomains that act as platforms for conducting a variety of cellular functions, such as signal transduction and protein sorting, by selective concentration of discrete sets of proteins in the ordered lipid environment. Recent studies suggested that lipid rafts will be crucial sites for processing of amyloid precursor protein (APP) to beta-amyloid and also its aggregation and

degradation. We hypothesized that partitioning of APP into the lipid rafts depends on interaction between APP and a raft protein(s) in the ordered lipid environment and that it regulates amyloidogenic processing and physiological functions of APP. To search for the candidate raft proteins, we isolated lipid raft fractions from mouse brain by using a detergent method. We then performed immuno-isolation of the APP containing rafts to analyze its protein components. Immuno-isolation using anti-APP resulted in relative enrichment of a distinct set of proteins. Characterization of protein components of the immuno-isolated rafts by mass spectrometry and immunological methods suggested functional specialization of the APP containing rafts. The systematic identification of the enriched proteins in the fraction will provide new insights into the physiological functions of APP and regulation of APP processing.

Supported by: BSI, Riken

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Program number 591.8

NIEMANN-PICK C1 DEFICIENCY REDUCES THE LEVELS OF CHOLESTEROL IN DETERGENT INSOLUBLE, LOW-DENSITY MEMBRANE DOMAINS LEADING TO ACTIVATION OF MAP KINASE PATHWAY AND INCREASED PHOSPHORYLATION OF TAU

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Niemann-Pick type C (NPC) disease is characterized by an accumulation of cholesterol in most tissues and causes neurodegeneration with the formation of neurofibrillary tangles; the major component of which is the hyperphosphorylated tau. We have recently reported that hyperphosphorylated tau and enhanced MAPK activity

are found in brains of NPC model mice. Here, we examined the mechanism underlying hyperphosphorylation of tau using mutant CHO cell line (CT43) defective in NPC1. Immunoblot analysis revealed that tau was hyperphosphorylated at multiple sites in CT43 cells, but not in their parental cells (25RA) or the wild-type CHO cells. In CT43 cells, MAPK was activated, while the amount of PP2A not bound to microtubules was decreased. The specific MAPK inhibitor attenuated the phosphorylation of tau in CT43 cells. Electron microscopy and filipin staining showed elevated intracellular cholesterol accumulation in CT43 cells. However, CT43 cells but not 25RA cells were amphotericin B-resistant, indicating that cholesterol concentration in the plasma membrane of CT43 is decreased. In addition, the level of cholesterol in the detergent-insoluble, low-density membrane (LDM) fraction of CT43 cells was markedly reduced compared with the other two types of CHO cells. Since LDM domain is known to play critical role in signaling pathways, these results suggest that the reduced level of cholesterol in LDM domain due to the lack of NPC1, may result in the activation of MAPK and PP2A, which in turn promotes tau phosphorylation in NPC1-deficient cells.

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Program number 606.3

INCREASE IN CHOLESTEROL AND CHOLESTEROL OXIDATION PRODUCTS, AND ROLE OF CHOLESTEROL OXIDATION PRODUCTS IN KAINATE INDUCED NEURONAL INJURY

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Relatively little is known about changes in sterols, in particular cholesterol, and cholesterol oxidation products in oxidative injury in neural tissues. We

have therefore examined changes in sterols and sterol-oxidation products using a model of excitotoxic injury. Intraventricular injections of kainate resulted in neuronal damage reflected by a decrease in immunoreactivity to the glutamate receptor subunit GluR1, and increased cholesterol in the CA1 region of the rat hippocampus. This *in vivo* increase in cholesterol after kainate lesions was confirmed by filipin histochemical staining of the kainate lesioned hippocampus *in vivo*, and kainate treated hippocampal slice and neuronal cultures. Addition of lovastatin, an inhibitor of cholesterol synthesis abrogated the increased cholesterol observed after kainate treatment of cultured neurons, suggesting that the increase in cholesterol could be due, in part, to increased *de novo* cholesterol synthesis. Furthermore, gas chromatographic mass spectrometric (GC/MS) analysis of cholesterol and its oxidation products in kainate injected rat brain showed a marked increase in cholesterol and cholesterol oxidation products three days after kainate treatment. Cholesterol oxidation products were toxic to neuronal cultures as reflected by decreased staining of GluR1 in the CA field. The above results show that cholesterol oxidation products may play a critical role in kainate induced neuronal injury.

Supported by: The National University of Singapore

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Program number 799.24

ACTIVATION OF MICROGLIAL CELLS AND INDUCTION OF NEURONAL INJURY BY CHOLESTEROL OXIDES FOUND IN THE CEREBROSPINAL FLUID OF PATIENTS WITH MULTIPLE SCLEROSIS

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Endogenous cholesterol oxides derive from radical-mediated oxidation of cholesterol, a major component of myelin sheaths, which are severely destroyed by the attack of immune cells during multiple sclerosis (MS). Serum and cerebrospinal fluid (CSF) of patients with MS and of controls were analysed by GC/MS, where cholesterol oxides were detected in high concentrations in the CSF of MS patients. The main cholesterol oxide component identified was 5-cholesten-3 β -ol-7-one (7-ketocholesterol). 7-ketocholesterol only exhibited low, if any, toxic effects towards cultivated neurons and in the organotypic brain slice cultures, whereas after transfer of exogenous microglial cells, it contributed to a strong migration of microglial cells into the neuronal layers and to severe neuronal damage and death. 7-ketocholesterol was capable of inducing expression of the migration-regulating integrins CD11a and ICAM-1, accompanied by the nuclear translocation of NF- κ B and the activation of the poly(ADP-ribose)-polymerase-1 (PARP-1). Since 7-ketocholesterol is degraded by activated microglial cells very quickly, we assume that this activation can be metabolically terminated by itself. In this study, we describe a link between the release of cholesterol oxides in the CNS and the neuronal damage mediated by microglia activation and involving the activation of nuclear signal molecules and the expression of migration-regulating integrins.

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Program number 819.14

INVESTIGATIONS INTO THE ROLE OF LIPID RAFTS IN THE INTERACTION BETWEEN MYELIN AND NEURONS MEDIATED BY MYELIN-ASSOCIATED GLYCOPROTEIN

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Expression of myelin-associated glycoprotein (MAG) is restricted to paranodal loops and periaxonal membranes of myelin. The MAG-mediated interaction between axon and oligodendrocyte is thought to have effects on both cell types. Treatment of post-natal neurons with MAG in vitro results in inhibition of neurite outgrowth, and expression of MAG on the surface of oligodendrocytes plays a role in the maintenance of myelin integrity. We are investigating the role of cholesterol and sphingolipid-enriched microdomains ('lipid rafts') in the MAG-mediated interaction between myelin and neurons using detergent-resistant membrane preparations and immunocytochemistry. We found that MAG was not located within 'classic' lipid rafts it was soluble in Triton X100 and did not co-localise with classic raft markers caveolin and GM1. However, in whole brain, MAG was found to localize to detergent-resistant membranes prepared in the presence of Lubrol WX. Similarly, approximately 30% of the MAG in cultured oligodendrocytes and Schwann cells was found in Lubrol WX-insoluble membranes. Thus, MAG may be located in a novel type of Lubrol-insoluble lipid raft similar to that described for prominin. Furthermore, our data show that MAG binds to a component of Triton-insoluble membranes on the surface of neurons, suggesting that the interaction between myelin and neurons mediated by MAG may involve the interaction of lipid rafts on opposing cell membranes. Characterisation of the nature and components of lipid rafts containing MAG and MAG receptors may give insight into the mechanisms by which MAG mediates effects in both oligodendrocytes and neurons. Expression of myelin-associated glycoprotein (MAG) is restricted to paranodal loops and periaxonal membranes of myelin. The MAG-mediated interaction between axon and oligodendrocyte is thought to have effects on both cell types. Treatment of post-natal neurons with MAG in vitro results in inhibition of neurite outgrowth, and expression of MAG on the surface of oligodendrocytes plays a role in the maintenance of myelin integrity. We are investigating the role of cholesterol and sphingolipid-enriched microdomains ('lipid rafts') in the MAG-mediated interaction between myelin and neurons using detergent-resistant membrane preparations and immunocytochemistry. We found that MAG was not located within 'classic' lipid rafts it was soluble in Triton X100 and did not co-localise with classic raft markers caveolin and GM1. However, in whole

brain, MAG was found to localize to detergent-resistant membranes prepared in the presence of Lubrol WX. Similarly, approximately 30% of the MAG in cultured oligodendrocytes and Schwann cells was found in Lubrol WX-insoluble membranes. Thus, MAG may be located in a novel type of Lubrol-insoluble lipid raft similar to that described for prominin. Furthermore, our data show that MAG binds to a component of Triton-insoluble membranes on the surface of neurons, suggesting that the interaction between myelin and neurons mediated by MAG may involve the interaction of lipid rafts on opposing cell membranes. Characterisation of the nature and components of lipid rafts containing MAG and MAG receptors may give insight into the mechanisms by which MAG mediates effects in both oligodendrocytes and neurons.

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Neurobiology of Lipids

* Prior to the 32nd Society for Neuroscience Meeting (Orlando, Nov. 2-7, 2002) *Neurobiology of Lipids* published the editorial (2002, Vol.1, 5) that compiled the abstracts on the subject of the journal scope. *Neurobiology of Lipids* editors were invited to select noteworthy abstracts from the list of more than two hundred presentations. The short list yielded eighteen presentations, including one platinum choice abstract, and three, six, and eight gold, silver and bronze abstracts, respectively. The authors of noteworthy presentations were invited to publish in NoL proceedings articles matching the content of their Neuroscience 2002 presentations. Further details are provided in: 32nd Society for Neuroscience annual meeting neurobiology of lipids sessions. *Neurobiol. Lipids* Vol. 1, 5 (23 Sept 2002) Available at: <http://neurobiologyoflipids.org/content/1/5/>

** This footnote denotes proceedings articles for the corresponding abstracts. Please note that three proceedings articles were published in *Neurobiology of Lipids* (NoL). The references are compiled either by authors (Authors' References) or by the Journal managing editor (NoL References).