5th International Conference on Phospholipase A_2-Mediated Signaling in Translational Medicine

20-21 May 2013

Conference Venue:

Neuroscience Center of Excellence,

School of Medicine

Louisiana State University Health Sciences Center

New Orleans, LA, USA

From La Pavillon Hotel to The Little Gem Saloon and LSU Neuroscience Center of Excellence

International Conference on Phospholipase A_2 (PLA_2) and Lipid Mediators

1st PLA2: May 26-29, 1999 (Berlin, Germany);
S Nigam and E Dennis

2nd PLA2: October 6-9, 2004 (Berlin, Germany);
S Nigam and E Dennis

3rd PLA2: May 9-12, 2007 (Sorrento (Naples) Italy);
G Goracci, V Di Marzo, ML Balestrieri and M Triggiani

4th PLA2: May 25-28, 2009 (Chiyoda-ku, Tokyo, Japan);
H Arai

5th PLA2: May 20-21, 2013 (New Orleans, LA, USA);
NG Bazan

Awardees

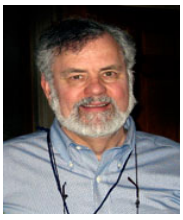
LSUHSC Chancellor Award Lecture in Neuroscience and Medicine
Edward Dennis
Evolution of Phospholipase A_2s in Catalysis and Cellular Function and Membranes
Departments of Chemistry/Biochemistry and Pharmacology
School of Medicine, University of California at San Diego
La Jolla, California, 92093-0601 USA

Journal of Lipid Research Lectureship Award
Charles N. Serhan
Pro-Resolving Mediators and Their Role in the Resolution of Infection
Center for Experimental Therapeutics and Reperfusion Injury
Department of Anesthesia, Perioperative and Pain Medicine
Harvard Institutes of Medicine, BWH and Harvard Medical School

Lifetime Achievement Award Lecture
Takao Shimizu
Cytosolic Phospholipase A_2 in Health and Disease
Department of Biochemistry and Molecular Biology, Faculty of Medicine,
The University of Tokyo, Bunkyo, Tokyo,
Group on Lipid Signaling, National Center for Global Health and Medicine,
Shinjuku, Tokyo

Neuroscience Center of Excellence Award Lecture
Gérard Lambeau
From Toxic to Therapeutic $sPLA_{2s}$
Institute of Molecular and Cellular Pharmacology, CNRS and
University of Nice Sophia Antipolis, Valbonne, France

LSUHSC Dean's Award Lecture
Jesper Z. Haeggström
The Leukotriene Cascade, Recent Insights to Signaling and Regulation
CERIC, Center of Excellence for Research on Inflammation and Cardiovascular Disease
Department of Medical Biochemistry and Biophysics Division of Physiological Chemistry 2,
Karolinksa Institutet, S-171 77 Stockholm, Sweden



Innovator Award Lecture
Robert Murphy
Lipid Imaging and Lipid Identification in Tissues by Mass Spectrometry
University Distinguished Professor, Department of Pharmacology
University of Colorado Denver, Aurora, CO 80045

Neuroscience Frontier Award Lecture
Wei-Yi Ong, Singapore
Distribution and Behavioral Effects of Brain $iPLA_2$ and $sPLA_2$
Department of Anatomy, and Neurobiology and Ageing Research Programme,
National University of Singapore, Singapore

Program

Sunday, May 19, 2013

6:00-8:00 PM *WELCOME RECEPTION*

Monday, May 20, 2013

9:00-9:40 AM **Welcome by Nicolas G. Bazan**
Edward Dennis, San Diego, CA, USA
LSUHSC Chancellor Award Lecture in Neuroscience and Medicine
Evolution of Phospholipase A_2s in Catalysis and Cellular Function and Membranes

9:40-10:10 AM **Grace Sun**, Columbia MO, USA
Regulation of Cytosolic PLA_2 by Oxidative and Inflammatory Signaling Pathways: Implication to Alzheimer's Disease

10:10-11:05 AM *COFFEE BREAK AND POSTERS*

11:05-11:35 AM **Paul Kotzbauer**, St. Louis, MO, USA
Disease Mechanisms and Therapeutic Approaches in Neurodegenerative Disorders Caused by PLA_2G6 Mutations

11:35 AM-12:05 PM **Nicolas G. Bazan**, New Orleans, LA, USA
PLA_2 and Lipid-Derived Mediators in Stroke, Alzheimer's Disease, Parkinson's Disease and Age-Related Macular Degeneration

12:05-13:00 PM *LUNCH*

13:00-13:40 PM **Introduction of Charles N. Serhan by Edward Dennis**
Charles N. Serhan, Boston, MA, USA
Journal of Lipid Research Lectureship Award
Pro-Resolving Mediators and Their Role in the Resolution of Infection

13:40-14:10 PM **Sasanka Ramanadham,** Birmingham, AL, USA
$iPLA_2\beta$ and Diabetes

14:10-15:10 PM *COFFEE BREAK AND POSTERS*

15:10-15:40 PM **Suzanne E. Barbour**, Richmond, VA, USA
Regulation of Hepatic Lipid Metabolism by $iPLA_2\beta$

15:40-16:20 PM **Wei-Yi Ong,** Singapore
Neuroscience Frontier Award Lecture
Distribution and Behavioral Effects of Brain $iPLA_2$ and $sPLA_2$

16:20-16:50 PM **James AK Shayman,** Ann Arbor, MI, USA
Group XV (Lysosomal) Phospholipase A_2: Identifying a Role in Adaptive Immunity, Autoimmunity, and Drug-induced Phospholipidosis

ADJOURN

18:00 PM *AWARDS DINNER at RIO MAR*

Tuesday, May 21, 2013

9:00-9:40 AM	**Takao Shimizu**, Tokyo, Japan ***Lifetime Achievement Award Lecture*** *Cytosolic Phospholipase A_2 in Health and Disease*
9:40-10:10 PM	**George Kokotos**, Athens, Greece *Synthetic Selective Inhibitors of Phospholipases A_2 as tools and New Medicinal Agents*
10:10-10:50 AM	**Gérard Lambeau**, Nice-Sophia Antipolis, France ***Neuroscience Center of Excellence Award Lecture*** *From Toxic to Therapeutic $sPLA_2$s*
10:50-11:20 AM	*COFFEE BREAK AND POSTERS*
11:20-11:50 AM	**Michael H. Gelb**, Seattle, WA, USA *Role of Phospholipase A_2 in Asthma and Arthritis*
11:50 AM-12:20 PM	**Makoto Murakami**, Tokyo, Japan *Deciphering the Physiological Functions of $sPLA_2$s*
12:20-13:00 PM	**Jesper Z. Haeggström**, Stockholm, Sweden ***LSUHSC Dean's Award Lecture*** *The Leukotriene Cascade, Recent Insights to Signaling and Regulation*
13:-00-13:55 PM	*LUNCH*
13:55-14:35 AM	**Robert Murphy**, Denver, CO, USA ***Innovator Award Lecture*** *Lipid Imaging and Lipid Identification in Tissues by Mass Spectrometry*
14:35-15:05 PM	**Christina Leslie**, Denver, CO, USA *$cPLA_2$, Eicosanoids and Macrophages: Regulators of Inflammation and Immune Responses*
15:05-15:35 PM	*COFFEE BREAK AND POSTERS*
15:35-16:05 PM	**Jesús Balsinde**, Valladolid, Spain *Phospholipase A_2-regulated Lipid Droplet Formation of Leukocytes*
16:05- 16:35 PM	**Arambakkam Janardhanam Vanisree**, Tamil Nadu, India *Naringenin Challenges PI3K Mediated Cyclooxygenase Activation and Key Metabolites Implicated in Aggressiveness of Glioma.*
16:35-17:05 PM	**Walter J. Lukiw**, New Orleans, LA, USA *Herpes Simplex Virus Type-1 (HSV-1) Infection of Human Primary Brain Cells Induces miRNA-146a, $cPLA_2$ and Alzheimer's Disease-type Pro-inflammatory Signaling*
	ADJOURN
18:00 PM	*DINNER and MUSIC EVENT at the LITTLE GEM SALOON (TO WELCOME PARTICIPANTS FROM OUT-OF-TOWN)*

Abstracts

PHOSPHOLIPASE A_2-REGULATED LIPID DROPLET FORMATION IN LEUKOCYTES

Jesús Balsinde

Institute of Molecular Biology and Genetics (IBGM-CSIC),
University of Valladolid School of Medicine,Valladolid, Spain.

Background – Lipid droplets (LD) are cytosolic inclusions present in most eukaryotic cells that contain a core rich in neutral lipids such as triacylglycerol (TAG) and cholesteryl esters (CE) and are surrounded by a phospholipid monolayer decorated with a variety of proteins.

Objective – We have examined the pathways for LD biosynthesis in human monocytes exposed to free arachidonic acid (AA), and studied the signaling cascade and intracellular events leading to LD formation in human monocytes.

Methods – Mass spectrometry analyses of neutral lipids were conducted to delineate the composition of LD in monocytes exposed to AA.

Results – Exposure of human peripheral blood monocytes to AA results in the rapid induction of LD formation by these cells. This effect appears specific for AA in that it is not mimicked by other fatty acids, whether saturated or unsaturated. LD are formed by two different routes, namely (i) the direct entry of AA into triacylglycerol and (ii) activation of intracellular signaling leading to increased triacylglycerol and cholesteryl ester formation utilizing fatty acids coming from the de novo biosynthetic route. LD formation can be completely inhibited by selective inhibition of the group IVA cytosolic phospholipase $A_{2\alpha}$ ($cPLA_{2\alpha}$), pointing out this enzyme as a key regulator of AA-induced signaling. LD formation in AA-treated monocytes can also be blocked by the combined inhibition of the mitogen-activated protein kinase family members p38 and JNK, which correlates with inhibition of $cPLA_{2\alpha}$ activation by phosphorylation.

Conclusions – These results suggest that concomitant activation of both p38 and JNK by AA cooperate to activate $cPLA_{2\alpha}$, which is in turn required for LD formation possibly by facilitating biogenesis of this organelle, not by regulating neutral lipid synthesis.

This work was supported by the Spanish Ministry of Science and Education and the Spanish National Network on Diabetes and Associated Metabolic Disorders (CIBERDEM).

Key words: Phospholipase A_2; Lipid Droplet; Arachidonic Acid

Regulation of Hepatic Lipid Metabolism by iPLA$_2$β

Suzanne E. Barbour*, W. Palmer Wilkins III*, Gregorio Gil*, Mamatha Kambalapalli*, Xiaoyong Lei@, Charles E. Chalfant*, and Sasanka Ramanadham@

*Department of Biochemistry & Molecular Biology, Virginia Commonwealth University
@Department of Cell, Developmental, and Integrative Biology and Comprehensive Diabetes Research Center, University of Alabama at Birmingham

The Group VIA phospholipase A$_2$ (iPLA$_2$β) is highly expressed in metabolically active tissues and recent studies have connected the enzyme to a variety of metabolic diseases. Our studies are focused on the role of the enzyme in diabetes mellitus and fatty liver disease. iPLA$_2$β is highly expressed in β-cells in the pancreas and we have demonstrated that this enzyme pool is involved in ER stress-induced apoptosis of β-cells, which contributes to pathogenesis of both Type 1 and Type 2 diabetes mellitus. We will present evidence that the underlying molecular mechanism of this apoptotic response is related to iPLA$_2$β-regulated alternative splicing of the pre-mRNAs encoding Bcl-x, caspase 9, and the receptor for advanced glycation endproducts (RAGE). In each splicing event, iPLA$_2$β biases splice site selection to favor events that promote β-cell apoptosis (increased caspase 9a, decreased Bcl-xL, and increased expression of full length RAGE on the cell surface). Exogenous unsaturated fatty acids (UFA) suppress expression and processing of sterol regulatory element binding protein-1 (SREBP-1), a transcription factor that regulates lipogenic gene expression in the liver. We compared hepatic lipid metabolism in iPLA$_2$β$^{-/-}$ and wild type mice, to test the hypothesis that the iPLA$_2$β might be a source of endogenous UFA that regulate SREBP-1 and thereby modulate fatty liver. As expected, iPLA$_2$β$^{-/-}$ livers contained more SREBP-1c and exhibited increased processing of this protein, compared to wild type livers. The changes in SREBP-1 expression/processing correlated with increased lipogenic gene expression, synthesis of fatty acids and triacylglycerols (TAG), and TAG mass in iPLA$_2$β$^{-/-}$ livers. We also observed evidence of reduced secretion of TAG, cholesterol, and cholesterol ester in iPLA$_2$β$^{-/-}$ hepatocytes, suggesting that TAG accumulation in iPLA$_2$β$^{-/-}$ livers is the result of both increased synthesis and reduced secretion. Our studies indicate that iPLA$_2$β-derived lipids contribute to pathogenesis of at least two metabolic diseases. Identification of the bioactive lipids and their mechanisms of action may uncover novel ways to treat diabetes mellitus and fatty liver disease.

PLA_2 AND LIPID-DERIVED MEDIATORS IN STROKE, ALZHEIMER'S DISEASE, PARKINSON'S DISEASE AND AGE-RELATED MACULAR DEGENERATION

Nicolas G. Bazan

Boyd Professor, Ernest C. and Yvette C. Villere Endowed Chair of Retinal Degenerations,
Professor of Ophthalmology, Biochemistry and Molecular Biology, and Neurology
Director, Neuroscience Center of Excellence
Chair, Executive Research Council, Translational Research Initiative

The significance of the selective enrichment in omega-3 essential fatty acids (docosahexaenoyl–DHA-chains of membrane phospholipids, 22C and 6 double bonds) in the nervous system (eg. synaptic membranes and dendrites) has remained, until recently, incompletely understood. While studying mechanisms of cell survival in neurodegenerations, we found PLA2 activating followed by DHA release. In turn docosanoid is synthesized from by 15-lipoxygenase-1, which we dubbed neuroprotectin D1 (NPD1,10R,17S-dihydroxy-docosa-4Z,7Z,11E,13E,15E,19Z hexaenoic acid). This mediator is a docosanoid because it is derived from a 22C precursor (DHA), unlike eicosanoids, which are derived from the 20 C arachidonic acid family of essential fatty acids not enriched in the nervous system. We found that NPD1 is promptly made in response to oxidative stress, seizures and brain ischemia-reperfusion, and in the presence of neurotrophins. NPD1 is neuroprotective in experimental brain damage, oxidative-stressed retinal pigment epithelial (RPE) cells, and in human brain cells exposed to amyloid-β peptide. Thus we envision NPD1 as a protective sentinel, one of the very first defenses activated when cell homeostasis is threatened by neurodegenerations. We provide here recent experimental examples that highlight the specificity and potency of NPD1, spanning beneficial bioactivity during events critical during the initiation and early progression of neurodegenerations:

1) We used epileptogenesis as a model to explore key mechanisms that sustain neuronal network integrity under adverse conditions. Using LC-MS/MS-based mediator lipidomic analysis we found that NPD1 increases during seizures in the hippocampus, and when we administered this docosanoid during pharmacologically–induced epileptogenesis it elicited a remarkable attenuation of pathological brain oscillations. This effect reflects attenuation of aberrant neuronal network activities that lead to spontaneous recurrent seizure. We used multi-microelectrode arrays in freely moving mice. Thus, docosanoid-mediated signaling rescues neuronal network disruptions.

2) Since protein misfolding and proteotoxic stress is involved from early stages of neurodegenerative disease we have explored these events as a possible NPD1 target in cell culture models (human RPE cells and primary neuronal mix cultures). We have studied Ataxin-1 PolyQ and at to some extend huntingtin 72 Q. We found that NPD1 decreased phospho-Ser -776 in Ataxin-1. We speculate that in agreement with our previous findings that NPD1 may work by increasing PP2A activity. Thus the lipid mediator may counteract PP2A inhibition, allowing the 82Q form to be de-phosphorylated and cleared or relocated into the spliceosome. The fact that Anp32 was proposed to have a stronger interaction with the expanded form rather than with the wild type Ataxin-1 makes this protein an excellent target candidate for NPD1 signaling. Impairments to neuronal circuitry likely also involves overexpression of the normal part of the misfolded protein. Thus in addition to the expansions in the poly-glutamine tract, AXH has an important role in the functionality of Ataxin-1. AXH, a self-folding domain present in Ataxin-1, is responsible for the

protein-protein interactions between Ataxin-1 and other transcription factors, such as the capicua homolog CIC protein. The sequestration of the complex partners formed by Ataxin-1 by its inactive counterpart may be involved in the loss of function observed in neurodegenrations. Brother of Ataxin-1 (Boat), another member of the AXH domain-containing protein family, is an example of the proposed loss of function. Boat is an *in vivo* binding partner of Ataxin-1 that is also affected by the malfunction of Ataxin-1 82Q. Thus the expression of AXH alone in our cells resulted in increased apoptosis. Furthermore, it aggravated the cytotoxicity induced by Ataxin-1 82Q. Unlike the sequestration scenario, in which the complexes are formed but are inactive, AXH induces toxicity in this case by increasing disassembly of the complex, thus promoting inactivation of its partners. NPD1 signaling promotes survival by modulating a set of genes that homeostatically control cell fate. NPD1 reversed the toxicity of both AXH and Ataxin-1 82Q in our cells (J. Calandria *et al.*, *J Biol Chem.* 2012)

3) We found that NPD1 is drastically reduced in CA1 areas from Alzheimer's patients (Lukiw *et al.*, *J.Clin Inv.* 2005). Therefore we have explored the significance of NPD1 in cellular models that recapitulate part of the Alzheimer's pathology. Human neurons and astrocytes challenged by amyloid-β or by overexpressing APPsw (double Swedish mutation that causes familial forms of the disease) show that NPD1 downregulates amyloidogenic processing of amyloid-β precursor protein, switches off pro-inflammatory gene expression (TNF-α, COX-2 and B-94-TNF-α inducible pro-inflammatory element), and promotes neural cell survival. Moreover, anti-amyloidogenic processing by NPD1 targets α- and β-secretases and PPARγ receptor activation (Y. Zhao *et al.*, *PLOsOne,* 2011). Currently we are also using imaging MALDI/TOF-MS to further unravel the lipidome in specific brain regions. The availability of anti-apoptotic BCL-2 proteins is positively modulated by NPD1, whereas pro-apoptotic BCL-2 proteins are negatively regulated, as is microglial activation. The cell survival cascade and the events that sustain neuronal network homeostatic integrity involves multiple checkpoints and signaling networks. NPD1 regulation targets upstream events of cell survival as well as neuroinflammatory signaling, in turn promoting homeostatic regulation of synaptic and neural circuitry integrity.

(Supported by NIH: NINDS R01 NS046741, NEI R01 EY005121)

Evolution of Phospholipase A_2s in Catalysis and Cellular Function on Membranes

Edward Dennis

LSUHSC Chancellor Award Lecture in Neuroscience and Medicine

Departments of Chemistry/Biochemistry and Pharmacology
School of Medicine, University of California at San Diego
La Jolla, California, USA

The phospholipase A_2 (PLA_2) superfamily consists of sixteen groups and many subgroups, and constitutes a diverse set of enzymes that have a common catalytic activity due to convergent evolution. These enzymes exhibit a large array of functions, but of special interest is the inflammatory cascade which is initiated by the release of free arachidonic acid by some types of phospholipase A_2, all of which interact with membrane phospholipids [Review: 1]. However, different PLA_2 types have unique three-dimensional structures and catalytic residues as well as specific tissue localization, distinct biological functions, and unique allosteric interactions with membranes. Understanding how the different PLA_2s associate with phospholipid membranes, specific phospholipid substrate molecules, and inhibitors on a molecular basis has advanced in recent years due to the introduction of hydrogen/deuterium exchange mass spectrometry approaches [Review: 2]. We will review the current state of knowledge about PLA_2 enzymes with an emphasis on very recent results utilyzing hydrogen/deuterium exchange approaches and molecular dynamics on the major types of PLA_2, especially on the interaction of lipoprotein-associated PLA_2 with lipoproteins [3] and $iPLA_2$ with inhibitors [4] and the nature of the interaction of these enzymes with specific substrate phospholipids in membranes.

1. Dennis EA, Cao J, Hsu YH, Magrioti V, Kokotos G (2011) Phospholipase A_2 enzymes: physical structure, biological function, disease implication, chemical inhibition, and therapeutic intervention. *Chem Rev*, 111:6130-85. [PMCID3196595]

2. Cao J, Burke JE, Dennis EA (2013) Using Hydrogen-Deuterium Exchange Mass Spectrometry to Define the Specific Interactions of the Phospholipase A_2 Superfamily with Lipid Substrates, Inhibitors and Membranes. *J Biol Chem*, 288:1806-13. [PMCID354890]

3. Cao J, Hsu YH, Li S, Woods VL, Jr., Dennis EA (2013) Structural Basis of Specific Interactions of Lipoprotein-Associated Phospholipase A_2 with HDL Revealed by Hydrogen Deuterium Exchange Mass Spectrometry. *J Lipid Res*, 54:127-33. [PMCID3520519]

4. Hsu YH, Bucher D, Cao J, Yang SW, Kokotos G, Woods VL, Jr., McCammon JA, Dennis EA (2013) Fluoroketone inhibition of Ca^{2+}-independent phospholipase A_2 through binding pocket association observed by hydrogen/deuterium exchange and molecular dynamics. *J Am Chem Soc*, 135:1330-37. [PMCID3561773]

THE LEUKOTRIENE CASCADE, RECENT INSIGHTS TO SIGNALING AND REGULATION

Rinaldo-Matthis, A., Stsiapanava, A., Niegowski, D., Salvado, M.D., Wan, M., Agerberth, B., Sun, J., Di Gennaro, A., and **Haeggström, J.Z.**

LSUHSC Dean's Award Lecture

Division of Chemistry 2, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, S-171 77 Stockholm, Sweden.

Downstream of $cPLA_2$, leukotrienes (LT) can formed along the 5-lipoxygenase pathway of arachidonic acid metabolism. This cascade involves the highly unstable intermediate LTA_4, which may either be enzymatically hydrolyzed by the bifunctional LTA_4 hydrolase/aminopeptidase (LTA4H), into the chemotaxin LTB_4 or conjugated with glutathione by a specific synthase (LTC4S) to produce the spasmogenic agent LTC_4. Recent work has demonstrated that LTA4H plays a role during the resolution of inflammation by removal of the endogenous aminopeptidase substrate, the chemotactic tripeptide Pro-Gly-Pro. The results of efforts aimed at generating an "aminopeptidase-sparing" inhibitor of LTA4H will be presented. We have shown that leukotrienes may act in synergy with certain anti-inflammatory peptides, in particular LL-37. This peptide binds to the ALX receptor, activates $cPLA_2$ and causes assembly of the leukotriene biosynthetic complex at the nuclear membrane of neutrophils and lipid bodies within eosinophils, to produce LTB_4 and LTC4, respectively. We have also found that LL-37 exerts a direct proangiogenic effect on endothelial, through activation of $cPLA_2$ via unorthodox signaling pathways, followed by PGE_2synthesis. Apparently, $cPLA_2$ is a key signaling hub in the cross-talk between LL-37 and the eicosanoid cascades.

Synthetic Selective Inhibitors of Phospholipases A_2 as Tools and New Medicinal Agents

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Background – Among the various groups of phospholipases A_2 (PLA_2), cytosolic GIVA PLA_2, secreted PLA_2, calcium-independent GVIA PLA_2 and lipoprotein-associated PLA_2 have attracted the medicinal interest. Each PLA_2 type seems to play distinct roles and thus, there is a great interest in developing potent and selective PLA_2 inhibitors as tools to understand the biological role of each particular PLA_2 and as novel agents to treat inflammatory diseases.

Objective – Our objective is to design, synthesize and study new PLA_2 inhibitors.

Methods – New synthetic methods have been developed for the synthesis of the various inhibitors. Computer-assisted drug design methods have been employed for the design of new inhibitors and understanding the binding mode of the inhibitors to enzymes.

Results – Lipophilic 2-oxoamides based on (S)-γ- or δ-amino acids were found to be selective GIVA $cPLA_2$ inhibitors presenting interesting *in vivo* anti-inflammatory activity. The location of the 2-oxoamide inhibitor AX007 in the active site of GIVA $cPLA_2$ was studied by a combination of molecular dynamics and deuterium exchange mass spectrometry. A model which may be a useful tool for the design of new inhibitors with improved inhibitory properties toward GIVA $cPLA_2$ has been developed. Pentafluoroethyl and trifluoromethyl ketones, for example FKGK11 and FKGK18, were found to be potent and selective inhibitors of GVIA $iPLA_2$. FKGK11 inhibitor showed strong reduction in the clinical severity and progression of the experimental autoimmune encephalomyelitis (EAE), the widely used animal model for multiple sclerosis. Further optimization studies led to the identification of GK187 which is more potent and selective in comparison to the previously reported fluoroketones. Lipophilic 2-oxoamides based on (S)-α-amino acids are potent and selective inhibitors of GIIA and GV $sPLA_2$. The binding mode of such 2-oxoamides with GIIA $sPLA_2$ has been studied by molecular docking calculations.

Conclusions – We have developed various classes of new synthetic inhibitors which selectively inhibit cytosolic GIVA PLA_2, secreted PLA_2 and calcium-independent GVIA PLA_2.

Key words: fluoroketones, inhibitors, oxoamides, synthesis

Disease Mechanisms and Therapeutic Approaches in Neurodegenerative Disorders Caused by *PLA_2G6* Mutations

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Mutations in the *PLA_2G6* gene cause infantile neuroaxonal dystrophy (INAD), a genetic subtype of neurodegeneration with brain iron accumulation (NBIA). This progressive neurodegenerative disorder affects the central and peripheral nervous system and is defined pathologically by the presence of spheroids containing accumulated membranes in distal axons. The *PLA_2G6* gene encodes the enzyme known as group VIA calcium-independent phospholipase A_2 (Pla_2g6). Our studies demonstrate that human Pla_2g6 hydrolyzes both phospholipids and lysophospholipids to produce free fatty acids, and that disease-associated mutations dramatically impair the catalytic function of the protein. Disease-associated mutations also interfere with the normal localization of Pla_2g6 protein to neuronal axons and dendrites. These results indicate that Pla_2g6 plays an important role in the production of fatty acids from phospholipids in neuronal processes, and predict two pathological pathways in INAD/NBIA: accumulation of Pla_2g6 substrates and deficiency of products. Accumulation of Pla_2g6 substrates explains membrane accumulation within neuroaxonal spheroids in INAD. The corresponding defect in free fatty acid production caused by loss of Pla_2g6 function in INAD likely restricts rates of new lipid synthesis and remodeling, but may also restrict the availability of fatty acids for other processes such as beta-oxidation. Neuroaxonal spheroids are recapitulated in our Pla_2g6-KO mouse model and accompany the progressive neurological impairment observed in these mice. Further studies in Pla_2g6-KO mice are examining the hypothesis that the defect in free fatty acid and subsequent acyl CoA production caused by *PLA_2G6* mutations can be addressed by approaches that increase acyl CoA production through other pathways.

FROM TOXIC TO THERAPEUTIC $sPLA_2$s

Gérard Lambeau

Neuroscience Center of Excellence Award Lecture

Institute of Molecular and Cellular Pharmacology, CNRS and
University of Nice Sophia Antipolis, Valbonne, France

Secreted phospholipases A_2 ($sPLA_2$s) are low molecular mass (14-19 kDa), Ca^{2+}-dependent enzymes with a His-Asp catalytic dyad. These enzymes were first discovered in snake venoms where they usually exert digestive and toxic functions towards preys. More than two decades ago, we started to work on toxic venom $sPLA_2$s and discovered specific $sPLA_2$ protein receptors (M and N) including the so-called M-type receptor or PLA_2R1, a 180 kDa C-type lectin membrane receptor.

Based on the large structural diversity of venom $sPLA_2$s, we next hypothesized that there might be a similar diversity of $sPLA_2$s in mammals, which would act as endogenous ligands of the above receptors identified with venom $sPLA_2$s and would also exert enzymatic roles, bringing the concept of $sPLA_2$s acting as both ligands and enzymes. This led us to clone a number of novel mammalian $sPLA_2$s, bringing the total number of human and mouse $sPLA_2$s to 11 and 12 members, respectively.

Since a decade or so, the major and still current challenge is to identify the respective biological roles of the different $sPLA_2$s and their receptors in different tissues and settings. It is now known that the individual mammalian $sPLA_2$ enzymes exhibit unique tissue and cellular localizations and enzymatic properties, suggesting distinct biological roles. Several of them also bind to specific proteins including PLA_2R1, which may serve to inhibit or promote $sPLA_2$ action in some specific tissues. It is now also clear that individual $sPLA_2$s are involved in diverse biological events through enzymatic-dependent and -independent processes, act redundantly or non-redundantly in the context of physiopathology, and may represent potential drug targets or bioactive drugs in certain situations. Moreover, PLA_2R1 may be a polymodal receptor with multiple ligands and functions, beyond its interaction with $sPLA_2$s. In this talk, I will present novel biological roles of some $sPLA_2$s and PLA_2R1 in host defense, atherosclerosis, fertility, cancer and membranous nephropathy, a human auto-immune kidney disease.

Keywords: phospholipase A_2, C-type lectin receptor, human diseases.

Sources of funding: CNRS, ANR and ARC among others.

cPLA$_2$, Eicosanoids and Macrophages: Regulators of Inflammation and Immune Responses

Saritha Suram and **Christina C. Leslie**

Department of Pediatrics, National Jewish Health, Denver, CO

Resident tissue macrophages are sentinel cells that are important in first sensing and responding to microbial invasion. An early response of resident peritoneal macrophages to the opportunistic pathogen *Candida albicans* is the activation of Group IVA cytosolic phospholipase A_2 (cPLA$_2\alpha$) that releases arachidonic acid for the production of eicosanoids. Cell wall polysaccharides of *C. albicans* engage multiple receptors that differentially regulate cPLA$_2\alpha$ activation. β-glucans and mannans engage dectin-1 and dectin-2, respectively, that together with MyD88, trigger activation of mitogen-activated protein kinases and calcium mobilization that promote cPLA$_2\alpha$ activation. Arachidonic acid released by cPLA$_2\alpha$ couples to cyclooxygenase-1 for rapid production of prostaglandins. A comparison of *C. albicans*-infected cPLA$_2\alpha^{+/+}$ and cPLA$_2\alpha^{-/-}$ macrophages revealed that the early production of prostaglandins promotes an autocrine loop resulting in increases in cAMP production that globally effects expression of genes involved in host defense and inflammation. The results suggest that cPLA$_2\alpha$-mediated prostaglandin production represents a negative feedback loop that acts to balance the pro-inflammatory host defense responses to dampen inflammation.

HERPES SIMPLEX VIRUS TYPE-1 (HSV-1) INFECTION OF HUMAN PRIMARY BRAIN CELLS INDUCES miRNA-146A, cPLA$_2$ AND ALZHEIMER'S DISEASE-TYPE PRO-INFLAMMATORY SIGNALING

Lukiw WJ[1], Jones BM[1], Cui JG[1], Li YY[1], Bhattacharjee PS[2], Bhattacharjee S[1], Corkern M[1], Clement C[1], Kammerman EM[1], Ball MJ[3], Zhao Y[4], Sullivan PM[5], Hill JM[1]

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[3]Department of Pathology, Oregon Health & Science University, Portland, OR,
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Background and Objective - Herpes simplex virus type-1 (HSV-1) infection of primary human brain cells induces changes in gene expression favorable to the propagation of the infecting agent and detrimental to the vitality of the host cells. We report that infection of a human primary neural brain cell line with a high phenotypic reactivator strain of HSV-1 (17syn+) induces significant up-regulation of the brain-enriched micro-RNA 146a (miRNA-146a). This small, 22 nucleotide miRNA is associated with altered inflammatory- and immune-signaling in stressed human brain cells, and is also up-regulated in brain tissues obtained from Alzheimer's disease (AD) patients.

Methods - bioinformatics, DNA arrays; ELISA, LED-Northern assay; miRNA arrays; primary co-culture of human neurons and glia; RT-PCR; Western analysis.

Results - The expression of cytoplasmic phospholipase A_2 (cPLA$_2$) and the inducible pro-inflammatory prostaglandin synthase cyclooxygenase-2 (COX-2) were each up-regulated. A known miRNA-146a target in the brain, complement factor H (CFH), was significantly down-regulated. These data suggest a role for HSV-1-induced miRNA-146a in the evasion of HSV-1 from the complement system, a major first-line host defense mechanism.

Conclusions - These results further support the hypothesis that brain cells infected with HSV-1 (17syn+) activates key elements of the arachidonic acid cascade and increases the expression of pathogenic markers known to contribute to AD-type change.

Acknowledgements - Thanks are extended to Drs. C Eicken, P Dua and C Hebel for miRNA array work and initial data interpretation, and to D Guillot and AI Pogue for expert technical assistance. Research on miRNA in the Lukiw laboratory involving neurotrophic viruses, the innate-immune response in AD, amyloidogenesis and neuroinflammation was supported through an Alzheimer Association Investigator-Initiated Research Grant IIRG-09-131729 and NIH NIA Grants AG18031 and AG038834.

Keywords: Alzheimer's disease (AD), bioinformatics, cytoplasmic phospholipase A_2, (cPLA$_2$); HSV-1, micro RNA (miRNA), senescence

DECIPHERING THE PHYSIOLOGICAL FUNCTIONS OF sPLA$_2$S

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About one third of the phospholipase A_2 (PLA_2) enzymes belong to the secreted PLA_2 ($sPLA_2$) family, which consists of low-molecular-weight, Ca^{2+}-requiring enzymes with a His-Asp catalytic dyad and contains 10 catalytically active isoforms. Individual $sPLA_2$s exhibit unique tissue and cellular distributions and enzymatic properties, suggesting their distinct biological roles. Although $sPLA_2$s have been implicated in inflammation, atherosclerosis and host defense, precise roles of individual $sPLA_2$ subtypes still remain largely unknown. Our recent studies using transgenic and knockout mice for nearly a full set of $sPLA_2$ subtypes, in combination with lipidomics approaches, have revealed the distinct contributions of individual $sPLA_2$s to various pathophysiological events by producing pro- and anti-inflammatory lipid mediators, by promoting membrane remodeling, by modifying extracellular non-cellular lipid components such as lipoproteins and surfactant, or by degrading foreign phospholipids such as microbes and dietary lipids. Emerging roles of $sPLA_2$s, including reproductive $sPLA_2$s that regulate sperm maturation and function, metabolic $sPLA_2$s that control local and systemic nutrition states and thereby affect metabolic disorders, anaphylactic $sPLA_2$ that facilitates mast cell maturation and anaphylaxis, resolving $sPLA_2$ that sequesters inflammation, or epidermal $sPLA_2$ that regulates epidermal barrier function, will be discussed.
(Supported by Grants-in Aid for Scientific Research from the Ministry of Education, Science, Culture, Sports and Technology of Japan)
Key words: $sPLA_2$, knockout mouse, lipidomics

Lipid Imaging and Lipid Identification in Tissues by Mass Spectrometry

Robert C. Murphy, Ph.D.

Innovator Award Lecture

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Advances in mass spectrometry, specifically matrix assisted laser desorption ionization (MALDI), have enabled imaging of biomolecules present in tissues by rastering the laser beam across the tissue, which has been covered with an organic matrix compound that absorbs the wavelength of the MALDI laser photon and the generation of secondary ions in each x,y coordinates where the laser is fired. The collection of all ion data (m/z and intensity) at each point can be considered as a pixel of mass spectral data information. The majority of ion current generated by MALDI imaging mass spectrometry (MALDI IMS) of animal tissues is related to desorption of lipids, specifically phospholipids from tissue sites. This is not surprising given the abundance of these hydrophobic molecules that are present membranes of cellular structures and their relatively low molecular weight (400-1500 Daltons) which is well matched to the currently available mass spectrometric devices for IMS. These images are molecular species specific and the polarity of the ions collected (positive or negative ions) determines to large extent the class of phospholipid (PC, SM-positive ions; PE,PS,PA,PI, PG and CL-negative ions). This information provides insight into the regional location of specific molecular species of phospholipids and the underlying biochemical events engaged in synthesis and metabolism of specific lipids. Recent experiments have used this technique to discover alterations to the normally present lipids in the tissues such as the retina, lung and brain that might reflect processes of injury or pathology occurring at local tissue sites.

DISTRIBUTION AND BEHAVIORAL EFFECTS OF BRAIN IPLA$_2$ AND SPLA$_2$

Wei-Yi Ong

Neuroscience Frontier Award Lecture

Department of Anatomy, and Neurobiology and Ageing Research Programme,
National University of Singapore, Singapore

Background – The phospholipase A$_2$ superfamily includes cytosolic PLA$_2$ (cPLA$_2$), calcium-independent PLA$_2$ (iPLA$_2$) and secretory PLA$_2$ (sPLA$_2$) enzymes. Brain PLA$_2$s do not function interchangeably but act on different phospholipids to generate lipid mediators; hence tight regulation of different isoforms is essential. Upregulation of cPLA$_2$ is involved in generation of arachidonic acid-derived lipid metabolites that are involved in neuroinflammation and neurodegeneration. On the other hand, gene polymorphisms or decrease in iPLA$_2$ expression are linked to brain iron accumulation, Parkinson's disease and Alzheimer's disease.

Objective – To determine the distribution and behavioural effects of iPLA$_2$ and sPLA$_2$ in the brain.

Methods – RT-PCR, immunohistochemistry, electron microscopy, intracerebral antisense oligonucleotide injection, lipidomic analyses, and behavioural analyses are used to study the distribution and behavioural effects of brain iPLA$_2$ and sPLA$_2$.

Results – Quantitative RT-PCR shows significantly higher mRNA expression of iPLA$_2$ than cPLA$_2$ in all regions of the rat brain. iPLA$_2$ activity is essential for the prevention of vacuous chewing movements; and induction of prefrontal cortical iPLA$_2$ is important in antidepressant- and antinociceptive effects of the selective noradrenaline reuptake inhibitor (NRI) antidepressant, maprotiline. Lipidomic analyses shows decreases in phosphatidylcholine species containing long-chain PUFAs and increases in lysophosphatidylcholine after maprotiline treatment, suggesting *endogenous release* of docosahexaenoic acid (DHA) after maprotiline treatment. The alpha 1 adrenergic receptor plays a role in maprotiline induced iPLA$_2$ expression. iPLA$_2$ inhibition negatively modulates long-term potentiation in the hippocampal-prefrontal cortex pathway, and induces deficits in the attention set-shifting test. Certain sPLA$_2$ isoforms are also expressed in the CNS, and could have a physiological role. sPLA$_2$-IIA is present in a postsynaptic location in the spinal cord. The enzyme is packaged in fusion-competent vesicles and released upon stimulation by AMPA and kainate receptors, suggesting regulated secretion. The signal peptide of sPLA$_2$-IIA is required for its vesicular localization and exocytosis. sPLA$_2$-IIA itself induces exocytosis in PC12 cells and neurons. Another isoform, sPLA$_2$-XIIA, is localized to axon pre-terminals in the cerebral cortex, and inhibition produces deficits in attention.

Conclusions – The above findings support the view that PLA$_2$ activity may not only be important during neurodegeneration, but depending on the isoform, could also be essential in prevention of neurological disorders, and for the therapeutic effects of certain antidepressants.

Acknowledgements – Supported by the National Medical Research Council of Singapore.

Key words: iPLA$_2$, lipidomics, prefrontal cortex

iPLA$_2$β AND DIABETES

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Diabetes mellitus arises from beta-cell dysfunction resulting from loss of beta-cells due to apoptotic cell death. If the evolution of diabetes is to be delayed or prevented, mechanisms that contribute to beta-cell death need to be better understood. Our laboratory has been studying the role of lipid signals in causing beta-cell death and our work has led to the identification of the Group VIA Ca^{2+}-independent phospholipase A_2 (iPLA$_2$β) as a participant in this process. Our collection of observations reveal that various diabetogenic stresses promote expression and activation of iPLA$_2$β, that in turn induces neutral sphingomyelinase leading to hydrolysis of sphingomyelins to generate ceramides, which activate the intrinsic apoptotic pathway. Decreases in iPLA$_2$β activity (chemically, siRNA, or knockout) significantly blunt stress-induced beta-cell apoptosis, raising the possibility that inhibition of iPLA$_2$β could ameliorate the development of diabetes. Here, we will describe our most recent findings arising from studies that examined this possibility. Our work was supported by grants from The NIH (DK69455) and American Diabetes Association.

Pro-Resolving Mediators & Their Roles in Resolving Inflammation & Infection

Charles N Serhan

Journal of Lipid Research Lectureship Award

Center for Experimental Therapeutics and Reperfusion Injury
Department of Anesthesia, Perioperative and Pain Medicine
Harvard Institutes of Medicine, BWH and Harvard Medical School, Boston MA, USA

This presentation updates our recent advances on the novel genus of n-3 specialized pro-resolving mediators (SPM) that include the resolvins, protectins and maresins. These novel mediators possess potent anti-inflammatory, pro-resolving actions and enhance microbial clearance in animal models. SPM demonstrate stereoselective biosynthesis as well as actions that involve specific pro-resolving G-protein-coupled receptors. Temporal metabolipidomics with self-limited resolving inflammatory exudates revealed that Resolvin D3 (RvD3) displayed a distinct time frame from other mediators with RvD3 accumulating in the late resolution phase. We established the complete stereochemistry of this third member of the D series and that of its aspirin-triggered 17R-epimer (AT-RvD3) with materials prepared by total organic synthesis (Dalli *et. al., Cell Chemistry & Biology* 2013). Both RvD3 and AT-RvD3 display potent actions in mouse inflammation and with human leukocytes. Together these findings open the potential for resolution-based pharmacology and the control of local inflammatory responses.

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Group XV (Lysosomal) Phospholipase A_2: Identifying a Role in Adaptive Immunity, Autoimmunity, and Drug-Induced Phospholipidosis

James Shayman, M.D.

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Group XV phospholipase A_2 (GXVPLA$_2$) was discovered and characterized as an acidic transacylase that catalyzes the formation of 1-*O*-acylceramide. This broad specificity phospholipase is a ubiquitous, mannose rich enzyme with an acidic pH optimum. GXVPLA$_2$ is localized to the lysosome and secreted. GXVPLA$_2$ is 49 percent identical to LCAT, another phospholipase A_2 with transacylase activity. Our recent work has focused on studying the role of this lysosomal phospholipase A_2 in health and disease. Amiodarone is a potent inhibitor of GXVPLA$_2$ activity, but not through direct binding to the enzyme. GXVPLA$_2$ catalysis requires an electrostatic charge interaction with anionic phospholipids in cell membranes. In addition to amiodarone, this interaction is inhibited by approximately half of the FDA approved cationic amphiphilic drugs known to cause phospholipidosis. GXVPLA$_2$ knockout mice develop an early lung phenotype with alveolar macrophage foam cell formation and surfactant accumulation, consistent with a role for the enzyme in surfactant catabolism. By one year of age the knockout mice develop an autoimmune phenotype with features of systemic lupus erythematosus. This phenotype includes nephritis, splenomegaly, lymphadenopathy, and anti-nuclear antibodies. Immuno-phenotyping of these mice has failed to reveal a primary B or T cell defect. However, macrophages and dendritic cells exhibit a generalized inability to degrade apoptotic bodies. GXVPLA$_2$ null mice have an impaired adaptive immune response to tuberculosis, an infectious disease in which the processing of apoptotic bodies by macrophages and dendritic cells is critical. Thus either acquired or inherited loss of GXVPLA$_2$ activity may potentially be basis for a significant spectrum of disease. Current efforts are being directed to find the clinical phenotypes associated with human deficiencies in GXVPLA$_2$.

CYTOSOLIC PHOSPHOSLIPASE A_2S IN HEALTH AND DISEASES

Takao Shimizu

Lifetime Achievement Award Lecture

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Cytosolic phospholipase A_2 ($cPLA_2$, also termed Group IV PLA_2) is an enzyme family consisting of 6 different molecular species. Among them, $cPLA_2\alpha$ (Group IVA) is well known and a number of literatures indicate its importance in both physiology (synaptic plasticity, reproduction) and pathologies. Either genetic ablation or specific inhibitors ameliorates various inflammatory and immune disorders (Ref. 1). $cPLA_2\alpha$ or its downstream metabolites are involved in bronchial asthma, bleomycin-induced fibrosis, ARDS, ischemia-reperfusion injury and thrombosis. To investigate the downstream lipid mediators in disease models and patients, we established lipidomics techniques to measure more than 100 lipid mediators in a single run, and profile the phospholipid composition. By combination of lipidomics data together with other omics studies, for example, we proposed that PGE_2 in dendritic cells play a pivotal role in initiation and progression of autoimmune encephalomyelitis. This concept was confirmed by knockout mice of PGE_2-related enzymes and receptors. Similarly, we found that PAF and $PGF_2\alpha$ are important in various disease models and also in patients (2). Summarized was herein the importance of $cPLA_2$ studies (enzyme character, knockout mice, and inhibitors), including those of other family members (b, d, e).

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Key words: $cPLA_2$, lipidomics, phospholipids

Regulation of Cytosolic PLA_2 by Oxidative and Inflammatory Pathways: Implication to Alzheimer's Disease

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Following the novel discovery by Dr. Bazan showing the release of arachidonic acid (AA) from brain membrane phospholipids in response to neural excitatory events including epilepsy and cerebral ischemia, we and others have embarked on the quest to search for the source and mechanism for AA release, as well as the physiological consequences of this intriguing phenomenon. It is now understood that many types of PLA_2s can release fatty acids from membrane phospholipids under different physiological and pathological conditions. However, much attention has been placed on the role of cytosolic PLA2 (cPLA2), an enzyme activated by intracellular Ca^{2+}, and receptor signaling pathways associated with activation of protein kinases (including PKC, ERK, CAMK II and MINK). In addition, there is evidence that $cPLA_2$ can interact with nitric oxide causing cysteine nitrosylation. In the central nervous system, $cPLA_2$ are ubiquitously present in neurons and glial cells, although regulation of its activity may vary depending on the receptor agonists and signaling pathways that govern intracellular Ca^{2+} homeostasis and protein kinase activity. Studies with neurons demonstrated that neuronal excitation through activation of the ionotropic NMDA receptor led to increased production of reactive oxygen species (ROS) through activation of NADPH oxidase, which in turn, activate PKC and MAPK pathway and phosphorylation of $cPLA_2$. Although not completely understood, a number of studies have implicated the involvement of $cPLA_2$ in the pathophysiology of Alzheimer's disease (AD). In our studies, oligomeric amyloid beta (Abeta) was shown to stimulate ROS production in neurons and activate ERK1/2, which subsequently led to phosphorylation of $cPLA_2$ and AA release. In addition, NMDA-induced ROS production was inhibited by inhibitors for $cPLA_2$ (and possibly $iPLA_2$), suggesting a feedback loop for regulation of ROS by PLA_2. Since many phenolic compounds from botanical source exhibit anti-oxidative and anti-inflammatory properties, our studies have been extended to further test effects of polyphenols, such as epigallocatechin-gallate (EGCG) from green tea and honokiol from the Magnolia bark, on ameliorating excitotoxicity in neurons. These studies place the role of $cPLA_2$ among oxidative and inflammatory pathways in brain cells and provide new insights for therapeutic potential for these botanicals to prevent and mitigate the progress of many neurodegenerative diseases including AD.

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Naringenin Challenges PI3K Mediated Cyclooxygenase Activation and Key Metabolites Implicated in Aggressiveness of Glioma

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Background - Glioma remains a challenge for oncological researchers and clinicians owing to its high invasiveness and chemoresistance. In India, a recent epidemiological study had revealed an alarming increase in the incidence of this tumor of glial cells. PI3k/Akt and IGF-1 receptor signaling are said to play significant roles in glioma; cyclooxygnase-2 (COX-2) and phosphoinositides (IP3,IP2) are also thought to contribute to the aggressiveness of glioma.

Objective - The aim of study was to analyse the purported sinister pathway in C6 induced glial tumor in rats and to evaluate potential of a natural intervention, Naringenin, the flavanone, against cerebrally implanted glioma.

Methods - The study utilized C6 model of glioma in rats; animals with and without naringenin treatment (50mg/kg bw for 21 days) and control rats were maintained. Brain tissues were collected and the factors influencing the expression of cyclooxygenase-2 in malignant condition were analysed. The tissues were examined for the expressions of mRNA and proteins viz PI3K, Akt, PTEN,COX-2,IGF-1R,Erk,PKC. The levels of Prostaglandin E_2 and phosphoinositides (IP3) were also estimated using HPLC analysis and commercial kit respectively.

Results - The expressions of the protoncogene, kinases and oxygenase which were up-regulated in C6 group were found to be significantly ($p<0.05$) reduced in naringenin treated group. A reverse scenario was observed in the expression of tumor suppressor gene. The drug, however, did not cause any significant change in the expressions in brain of normal ones. The levels of small molecules (PGE_2 & IP3) were also altered significantly among the groups ($p<0.05$).

Conclusion - Together, the study support that the natural intervention that act as inhibitors of COX-2 and dysregulated PI3K/PTEN/Akt would be beneficial for future clinical development of candidate drugs against this deadly tumor.

Key Words: Glioma, Cyclooxygenase-2, phosphoinositides.

Poster Presentations

LIPIDOMICS PROFILING UNVEILS THE MOLECULAR PHENOTYPE OF "OMEGA-3" TRANSGENIC MICE

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Essential fats, such as omega-3 and omega-6 fatty acids, must be obtained through the diet and cannot be synthesized *de novo* in mammals. In 2004, the fat-1 transgenic mouse model was developed, enabling the mouse to endogenously convert omega-6 to omega-3 fatty acids. Research has demonstrated that the fat-1 mouse is protected against a wide variety of diseases and conditions related to inflammation including colitis, pancreatitis, asthma, hepatitis, liver disease, atherosclerosis, insulin resistance, and several types of cancer (breast, colon, pancreatic, liver).

Although a large number of studies have demonstrated reduced disease risk and health benefits in fat-1 mice, a comprehensive comparison of lipids profiles in fat-1 and wild-type mice has not been previously feasible due to lack of a sensitive and comprehensive analytical technique capable of simultaneously quantifying high-abundance (e.g., phospholipids) and low abundance lipids (e.g., oxylipins).

In this study, we used a state-of-the-art, high-throughput assays for the analysis of bioactive lipid species in plasma and liver samples from fat-1 and wild-type mice, providing new clues to the pathways and mechanisms that may be involved in the health benefits associated with alterations of the omega-6/omega-3 fatty acids ratio.

Lipin-1 Controls Phospholipase A_2 Activation in Human Macrophages

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Introduction - The lipin family of proteins plays multiple roles in cells by regulating lipid biosynthesis and cellular signaling. Lipins possess phosphatidic acid (PA) phosphatase activity that hydrolyzes PA to yield diacylglycerol (DAG). Both PA and DAG are important signaling lipids, having the capacity to modulate key signaling events. Most cellular studies on lipin have been conducted in metabolically active tissues such as adipose tissue. As a matter of fact, Lpin-1 is known as an obesity-related gene because its absence promotes the lack of adipose tissue and its overexpression promotes obesity in mice. However, little is known on the possible roles of lipin in inflammation. Before the nucleotide sequence of the PAP1 enzymes was revealed, studies with pharmacological inhibitors showed that PAP1 activity is involved in the regulation of the mobilization of free arachidonic acid (AA), the precursor of the inflammatory mediators known as the eicosanoids. However, identification of the actual PAP1/lipin form involved in AA mobilization has not been elucidated.

Objectives - The aim of the present study was evaluate the role of lipin-1 in Phospholipase A_2 (PLA_2) activation.

Methods - Human macrophages derived from blood monocytes, siRNA technology, mass-spectrometry and confocal microscopy was used.

Results - Human macrophages made deficient in lipin-1 by siRNA mobilized significantly less AA than did control cells upon treatment with innate immunity and metabolic stimulus. AA mobilization under these conditions was abolished by treating the cells with the selective inhibitor for Group IVA PLA_2, pyrrophenone (1 mM). Furthermore, Group IVA PLA_2 phosphorylation was found to be significantly reduced ($p<0.05$) in homogenates from cells deficient in lipin-1, as measured by immunoblot using an Ab specific for the phosphorylated protein. In keeping with these observations, measurements of PGE_2 formation demonstrated that cells deficient in lipin-1 manifest a marked defect in eicosanoid generation. The distribution of AA among glycerophospholipids in untreated versus lipin-1–deficient cells was studied by mass spectrometry. The results demonstrated that lipin-1 deficiency did not significantly alter the distribution of AA among phospholipids in resting cells, thus suggesting that diminished Group IVA PLA_2 activation in the lipin-1–deficient cells is unlikely due to altered availability of substrate.

Conclusions - Collectively, these data suggest that lipin-1 regulates the activation of cytosolic group IVA phospholipase A_2 in human monocyte-derived macrophages.

Key Words: Phospholipase A_2; Lipin; Human macrophage

Infection of Human Primary Brain Cells with Herpes Simplex Virus Type-1 (HSV-1) Up-Regulates miRNA-146a, Cytoplasmic Phospholipase A_2 ($cPLA_2$) and Pro-Inflammatory Signaling Patterns Characteristic of Alzheimer's Disease (AD)

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Background and Objective - The cytosolic, calcium-dependent group IVA cytoplasmic phospholipase ($cPLA_2$) hydrolyzes arachidonyl phospholipids at the sn-2 position releasing arachidonic acid, and as such is a major initiator of the arachidonic acid cascade. Primary human neuronal-glial (HNG) cells infected with herpes simplex virus type-1 (HSV-1) induces $cPLA_2$ expression and the stimulation of $cPLA_2$-mediated inflammatory signaling, including a downstream up-regulation of cyclooxygenase-2 (COX-2). Here we report that infection of a human primary neural brain cell line with a high phenotypic reactivator strain of HSV-1 (17syn+) further induces significant up-regulation of the brain-enriched micro-RNA-146a (miRNA-146a) associated with altered inflammatory- and immune-signaling in stressed human brain cells. Interestingly $cPLA_2$, COX-2 and miRNA-146a are also found to be up-regulated in Alzheimer's disease (AD) affected brain.

Methods - bioinformatics, DNA arrays; ELISA, LED-Northern assay; miRNA arrays; post-mortem analysis; primary co-culture of human neurons and glia; RT-PCR; Western analysis.

Results - Up-regulation in the expression of $cPLA_2$, increases in the inducible pro-inflammatory prostaglandin synthase COX-2 levels and up-regulation of the pro-inflammatory miRNA-146a were observed in HSV-1-stressed HNG cells. A known miRNA-146a target in the brain, complement factor H (CFH), was significantly down-regulated. The data indicate a role for HSV-1-induced miRNA-146a in the evasion of HSV-1 from the complement system, a major first-line host defense mechanism, and an inter-related up-regulation in pro-inflammatory signaling.

Conclusions - These results further support the hypothesis that brain cells infected with HSV-1 activates key elements of the arachidonic acid cascade and increases the expression of pathogenic markers such as COX-2, $cPLA_2$ and miRNA-146a, factors that are known to contribute to AD-type change.

Acknowledgements - Thanks are extended to Drs. P Dua, C Hebel and C Eicken, for miRNA array work and initial data interpretation, and to D Guillot and AI Pogue for expert technical assistance. Research on miRNA in the Lukiw laboratory involving neurotrophic viruses, the innate-immune response in AD, amyloidogenesis and neuroinflammation was supported through an Alzheimer Association Investigator-Initiated Research Grant IIRG-09-131729 and NIH NIA Grants AG18031 and AG038834.

Keywords: Alzheimer's disease (AD), bioinformatics, cytoplasmic phospholipase A_2, ($cPLA_2$); HSV-1, micro RNA (miRNA), senescence

The PEDF Neuroprotective Domain Plus DHA Selectively Induces Corneal Nerve Regeneration After Experimental Surgery

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Purpose – Treatment with pigment epithelial-derived factor (PEDF) in association with docosahexaenoic acid (DHA) after lamellar keratectomy increases the regeneration of corneal nerves. We have also shown that corneal sensation returns to normal levels in treated animals at 8 weeks after surgery (Cortina *et al*, *IOVS*, 2010). Some therapeutic advantages of using smaller peptides include better tissue penetration, a narrower spectrum of action with reduced side effects, and ease of synthesis in reproducibly large-scale quantities. The purpose of this study was to compare the effect of two PEDF derivatives: a 44 mer-PEDF that has neurotrophic activity and a 34 mer-PEDF with antiangiogenic properties in association with DHA in corneal nerve regeneration after experimental surgery.

Methods – An 8 mm corneal stromal dissection was performed in the left eyes of adult New Zealand rabbits. Treatment groups received topical PEDF+DHA; 34 mer-PEDF+DHA or 44 mer-PEDF+DHA by means of a 72 h collagen shield for 6 weeks. The control group received vehicle. Corneal sensitivity was assessed weekly with a Cochet-Bonnet aesthesiometer. Rabbits were sacrificed at 8 weeksand corneas were processed for immunohistochemistry. Corneal nerves were stained with βIII tubulin. The βIII tubulin-positive area at the subepithelial nerve plexus was calculated and compared to the total area using an image analysis program.

Results – Six weeks after surgery there was a 73% recovery of corneal sensitivity in the 44 mer-PEDF+DHA-treated animals, while in the 34 mer-PEDF+DHA and vehicle-treated groups only 54 and 43% of corneal sensitivity was recovered, respectively. Subepithelial corneal nerve area in the 44 mer-PEDF+DHA-treated group was increased over two-fold compared to the 34 mer-PEDF+DHA- and vehicle-treated groups (8.65 +/- 0.6; 4.19 +/- 0.7; 4.45+/- 0.9). This difference was statistically significant with a p value of 0.003.

Conclusions – The 44 mer-PEDF, with neurotrophic activity in combination with DHA, promotes functional regeneration of damaged corneal nerves after experimental surgery while 34 mer-PEDF does not have significant activity in corneal nerve regeneration. 44 mer-PEDF could be a novel therapeutic agent of easier synthesis and better bioavailability than the complete PEDF molecule for the treatment of neurotrophic keratitis and dry eye that develops as a result of corneal nerve damage.

Support – NH Grant R01 EY 019465

Changes in Corneal Innervations After Herpes Simplex Virus Type 1 (HSV-1) Latency Established with Different Reactivation Phenotypes

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Purpose – HSV-1 is a neurotrophic virus that establishes latency in sensory ganglia. Recurrent herpetic stromal keratitis (HSK) is the most common cause of infectious blindness in developed countries. Clinically, patients with HSK often have decreased corneal sensitivity, which is strongly correlated with decreased corneal innervation after HSV infection. However, the exact pathogenesis remains unclear. Here we used a rabbit model infected with high phenotypic reactivators as well as recombinant HSV-1 with deletions to study their effect on corneal innervation after latency was established.

Methods – Adult NEZ rabbits were inoculated with 50 µl (200,000-300,000 PFUs) of HSV-1 McKrae, 17 Syn+ or recombinant mutants with gK deletion or an immediately early protein 0 (ICP0) deletion. The rabbits were sacrificed and the corneas removed between 124 to 125 days post-infection. After fixation, the corneas were stained with anti-βIII tublin antibody. Images were acquired with a fluorescence microscope in time-lapse mode and merged together to build a whole view of the corneal nerve architecture. Corneal subbasal nerve density was calculated on the basis of the whole mount view of the central area. Differences between the HSV infected eyes, as compared with normal control, were analyzed by ANOVA.

Results – In comparison with the normal corneas, all the HSV-infected corneas showed significant decrease in corneal nerve density. The corneas infected with ICP0 and gK deletion showed mild to moderate nerve damage while those infected with 17Syn+ and McKrae were seriously damaged. In the ICP0 deletion eyes, there were reduced subbasal nerve bundles, but most of the corneas kept a normal stromal network; in gK deletion eyes, both epithelial and stromal nerves were involved. Corneas infected with 17Syn+ and McKrae displayed destroyed nerve structures and formation of a scar tissue in the central cornea, in which only a few nerve fibers could be detected. Around the scar tissue, there were some newly-regenerated nerves. In addition, a dense infiltration of inflammatory cells was detected.

Conclusions – The results suggest that HSV infection seriously damages corneal innervations and persist after four months of infection. Reduction of axonal transport (by gk deletion) or virus replication (by ICP0 deletion) significantly attenuates the nerve damage induced by HSV-1.

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Angiogenesis of Choroid-Retinal Endothelial Cells Modulated by Lipid Mediators, Retinal Pigment Epithelial Cells, and Leukocytes Under Inflammatory Activation

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Background - Choroidal neovascularization is a process that both angiogenesis and inflammation are involved. Inflammation is a self-defensive response of the body against pathogens and injuries. The inflammation process in the posterior of the eye is orchestrated by endothelial cells, leukocytes, retinal pigment epithelium, retina neurons, glial cells, and other types of cells present. These cells interact with each other and communicate through signaling molecules, such as lipid mediators and cytokines.

Objective - To determine lipidomes in the promotion of angiogenesis of choroid-retinal endothelial cells (CRECs) by retinal pigment epithelial (RPE) cells and monocytes on CREC angiogenesis including migration and vasculature formation.

Methods - CREC migration was carried out in 24-transwell plate with RPE cells and/or monocytes in low chambers stimulated with IL-1β and TNF-α. Tube formation was performed on Matrigel with media conditioned with RPE cells and CRECs. Lipidomes of CREC, RPE and monocytes were analyzed by LC-UV-MS/MS.

Results and Conclusions - We found that inflammatory stimulation promoted RPE cells, CRECs, and monocytes to produced PGE_2, 12HETE, and many other lipid mediators. CRECs and leukocytes produced much more lipid mediators than RPE. COX-2 inhibitor NS398 and 12-lipoxygenase inhibitor Baicalein suppressed production of PGE_2 and 12-HETE respectively. After activation by inflammatory stimulation, RPE cells, CRECs, and monocytes promote CREC angiogenesis. More experimental replications are needed to define the roles of these lipid mediators in CREC angiogenesis.

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Neuroprotectin D1 Stimulates the Expression and Secression of Nerve Growth Factor in Corneal Epithelial Cells

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Purpose – Nerve growth factor (NGF) is a neurotrophic factor expressed in the corneal epithelium that promotes cell proliferation and wound healing. It is responsible for the axonal growth and survival of sensory neurons. Neuroprotectin D1 (NPD1) is a lipid mediator derived from docosahexaenoic acid (DHA) with anti-inflammatory and neuroprotective actions. Synthesis of NPD1 is stimulated in corneal epithelial cells treated with pigment epithelial derived factor (PEDF) in conjunction with DHA. Recent studies in our laboratory showed that topical treatment of NPD1, applied to rabbit corneas after experimental surgery, increase corneal nerve regeneration and enhanced neurite outgrowth of trigeminal ganglion neurons in culture (ARVO 2012). The exact mechanism by which NPD1promotes nerve regeneration is not understood. The purpose of this study was to investigate if NPD1 and/or its precursors PEDF plus DHA stimulate NGF synthesis.

Methods – First passage rabbit corneal epithelial cells (RCEC) were used. The cells were grown in serum-free medium (CnT-20). Once they reached 70-80 % confluence, the cells were starved overnight and then stimulated with 50nM NPD1 or with the combination of 50ng/ml PEDF and 50nM DHA for different times. Changes in NGF mRNA expression were assayed by PCR performed using Taq PCR Master Mix Kit (Qiagen) with specific primers for rabbit. Secretion of NGF peptide was measured in the tissue culture supernatant by ELISA.

Results – NGF gene expression increased significantly after 3h and 6h of NPD1 stimulation; at 16h gene expression started to decrease. There was an increase of NGF secreted into the medium from the cells in the presence of NPD1 or PEDF+DHA. After 48h, NGF stimulation increased between 40 and 50% when NPD1 was added to the medium; in presence of PEDF plus DHA, the NGF increment was around 30%.

Conclusions – The results suggest that NPD1 and its precursors PEDF plus DHA promote regenerative corneal innervation by modulating NGF gene expression, followed by its synthesis and secretion.

Support – NH Grant EY019465

Manipulation of Long-Chain ACYL-COA Synthetase Activity Affects Arachidonic Acid Metabolism in Rat Fibroblasts.

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Long-chain acyl coenzyme A synthetases (Acsl) are family of enzymes that convert free long-chain fatty acids into their acyl-CoAs, and play an important role in fatty acid metabolisms. In human and rodents, five Acsl isozymes have been identified until now: Acsl1, Acsl3, Acsl4, Acsl5, and Acsl6. The aims of this study are to assess the role of Acsl in the regulation of arachidonic acid (AA) metabolism. Treatment of 3Y1 cells with triacsin C, an inhibitor of Acsl, markedly enhanced IL-1β-induced prostaglandin (PG) biosynthesis. Small interfering RNA-mediated knockdown of endogenous Acsl4 expression increased significantly the release of AA metabolites, including PGE_2, PGD_2, and $PGF_{2\alpha}$, compared with replicated control cells, whereas knockdown of Acsl1 expression reduced the IL-1β-induced release of AA metabolites. Experiments with double knockdown of Acsl4 and intracellular phospholipase A_2 (PLA_2) isozymes revealed that group IVA PLA_2, but not group VI PLA_2s, is involved in the Acsl4 knockdown-enhanced PG biosynthesis. Electrospray ionization mass spectrometry of cellular phospholipids bearing AA revealed that the levels of some, if not all, phosphatidylcholine (PC) and phosphatidylinositol species in Acsl4 knockdown cells were decreased after IL-1β stimulation, while those in control cells were not so obviously decreased. In Acsl1 knockdown cells, the levels of some AA-bearing PC species were reduced even in the unstimulated condition. Collectively, these results suggest that Acsl isozymes play distinct roles in the control of AA remodeling in rat fibroblasts: Acsl4 acts as the first step of enzyme for AA remodeling following IL-1β stimulation, and Acsl1 is involved in the maintenance of some AA-containing PC species.

INHIBITORS OF SECRETED PHOSPHOLIPASE A_2 INHIBIT THE RELEASE OF PGE_2 IN RAT MESANGLIAL CELLS

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Background – The phospholipase A_2 (PLA_2) superfamily consists of various groups and subgroups. Of these, cytosolic GIVA PLA_2 and secreted PLA_2 have been reported to play a major role in the production of arachidonic acid and various eicosanoids, for example PGE_2, in cells.

Objective – Our objective is to study the role of each of cytosolic GIVA PLA_2, secreted PLA_2 and calcium-independent PLA_2 in the release of PGE_2 in rat mesanglial cells.

Methods – A variety of synthetic inhibitors, mainly developed in our labs, which are known to selectively inhibit each of GIVA $cPLA_2$, GVIA $iPLA_2$, and GII and GV $sPLA_2$ were used as tools in this study. Cultures of rat renal mesangial cells were stimulated for 24 h with interleukin 1 plus forskolin to trigger a huge increase of PGE_2 synthesis. Cells were treated in the absence or presence of increasing concentrations of the various inhibitors. Supernatants were collected and taken for a PGE_2-ELISA to quantify PGE_2 released from the cells.

Results – GIVA $cPLA_2$ oxoamide inhibitor AX109 and GVIA $iPLA_2$ fluoroketone inhibitor FKGK11, as well as oxoamide AX048 which inhibit both GIVA $cPLA_2$ and GVIA $iPLA_2$ were not found to inhibit PGE_2 release even at 10 μM. On the contrary, inhibitors of GIIA and GV $sPLA_2$, oxoamide GK126 and amide GK115, presented interesting inhibition of the release of PGE_2. Based on these observations, analogs of GK115 were synthesized and the evaluation of their effect is in progress.

Conclusions – Using a variety of synthetic PLA_2 inhibitors, we have demonstrated that secreted PLA_2 plays a predominant role in the production of PGE_2 in rat mesanglial cells. Synthetic inhibitors of secreted PLA_2 present interesting inhibition of PGE_2 release.

Acknowledgements – This research has been co-financed by the European Union (European Social Fund – ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) - Research Funding Program: Heracleitus II. Investing in knowledge society through the European Social Fund.

Key words: inhibitors, PGE_2, rat mesanglial cells

POTENT AND SELECTIVE POLYFLUOROKETONE iPLA$_2$ INHIBITORS

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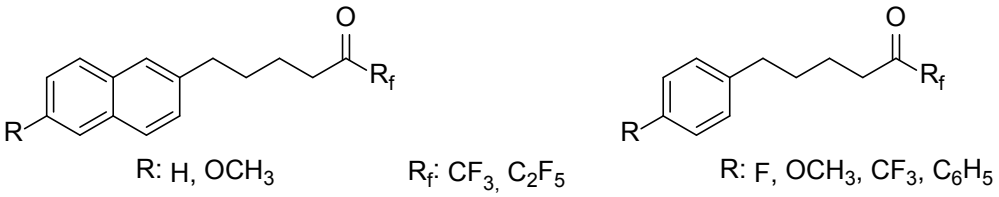
Background – The phospholipase A_2 superfamily currently consists of 16 different groups and various subgroups. Three of the most important types of PLA$_2$s that can be found in human tissues are the secreted (such as GIIA and GV sPLA$_2$), the cytosolic GIVA cPLA$_2$ and the calcium-independent GVIA iPLA$_2$. Group VIA calcium-independent phospholipase A_2 (GVIA iPLA$_2$) has recently emerged as an important pharmaceutical target as it has been found to be involved in severe conditions such as multiple sclerosis, diabetes and ovarian cancer.

Objective – Selective and potent GVIA iPLA$_2$ inhibitors can be used to study its role in various neurological disorders. In the current work, we explore the significance of the introduction of a substituent in previously reported potent GVIA iPLA$_2$ inhibitors.



Methods – For the synthesis of trifluoromethyl and pentafluoroethyl ketones, a Wadsworth-Horner-Emmons olefination reaction of the corresponding commercially available substituted aromatic aldehydes with triethyl phosphonocrotonate yielded the unsaturated esters. Hydrogenation in the presence of 10% Pd/C, followed by saponification afforded the corresponding acids, that were treated with oxalyl chloride, followed by trifluoroacetic or pentafluoropropionic anhydride and pyridine to yield trifluoromethyl and pentafluoropropyl ketones. Furthermore, Grignard reagents were prepared from corresponding bromides and then were coupled with ethyl difluoroacetate to afford difluoromethyl ketones.

Results – 1,1,1,2,2-Pentafluoro-7-(4-methoxyphenyl)heptan-3-one (GK187) is the most potent and selective GVIA iPLA$_2$ inhibitor ever reported with a XI(50) value of 0.0001, and with no significant inhibition against GIVA cPLA$_2$ or GV sPLA$_2$.

Conclusions – New potent and selective polyfluoroalkyl ketones are presented for the inhibition of GVIA iPLA$_2$.

Acknowledgements – This research was partially funded by the University of Athens Special Account of Research Grants no 10812.

Keywords: GVIA iPLA$_2$, inhibitor, polyfluoroalkyl ketone

PHOSPHOLIPASE A_2 SUPERFAMILY OF INTERFACIAL ENZYMES: INSIGHT INTO SUBSTRATE AND INHIBITOR BINDING

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Phospholipase A_2 (PLA_2) constitutes a superfamily of enzymes which are characterized by their ability to catalyze the hydrolysis of the ester bond at the sn-2 position of phospholipid molecules (*Chem. Rev.* 2011, 111:6130-6185). The products of PLA_2 activity include free fatty acids, predominantly arachidonic acid (AA), and lysophospholipids. The AA is further metabolized by downstream enzymes (COX-1, COX-2 and 5-LO) to form a variety of pro-inflammatory lipid mediators including prostaglandins, leukotrienes and thromboxanes. Among the members of the PLA_2 superfamily, the calcium-independent group VIA-2 (GVIA-2 $iPLA_2$) and the cytosolic group IVA (GIVA $cPLA_2$) have similar sizes (~ 85 kDa) and they function through a Ser/Asp catalytic dyad located in a patatin-like *α/β*-hydrolase catalytic domain. GIVA $cPLA_2$ contains a C2 domain where the intracellular calcium binds resulting in the activation and localization of the enzyme on the membrane. GVI-2 $iPLA_2$ contains 7 ankyrin repeats (ANK) and a linker region that connects the ANK and catalytic domain. GIVA $cPLA_2$ shows high specificity for phospholipids that contain arachidonic acid at the *sn*-2 position, while the GVIA $iPLA_2$ shows low specificity. The crystal structure of GIVA $cPLA_2$ (PDB ID: 1CJY) is available but there is none available for GVIA-2 $iPLA_2$, so a homology model was created since the enzyme shows 40 % homology with patatin (PDB ID: 1OXW) and we modeled inhibitor binding (*J. Am. Chem. Soc.* **2009**, 131:8083-8091 and *J. Am. Chem. Soc.* **2013**, 135:1330-1337). These two enzymes have been associated with inflammatory diseases including diabetes, Barth syndrome and cancer, and thus they are attractive targets for inhibitor development. Covalent and Induced Fit docking methods (*J. Med. Chem.* **2006**, 49:534-53) have now been employed to predict the binding mode of the substrate and inhibitors of the enzymes. The substrate-enzyme and the ligand-enzyme complexes revealed key residues that play important roles in binding. Further studies of the resulting complexes with molecular dynamics simulations using NAMD have been employed to interpret experimental results from Deuterium/Hydrogen Exchange Mass Spectrometry (DXMS) (*J. Biol. Chem.* **2013**, 288:1806-1813). This information will be useful in developing new inhibitors with improved properties (NIH grant GM 20,501).

Key words: Phospholipase A_2, DXMS, Molecular dynamics.

Neuroprotectin D1 Attenuates Aberrant Neuronal Networks in Epileptogenesis

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Acquired epileptogenesis triggers molecular and cellular mechanisms after a brain injury that leads to spontaneous recurrent seizures, the hallmark of epilepsy. We determined the role of neuroprotectin D1 (NPD1), a derivative of the omega-3 fatty acid docosahexaenoic acid (DHA), in the development of seizures in a post status epilepticus model of epileptogenesis. Multi-microelectrodes were implanted in the dorsal hippocampus in adult male mice after status epilepticus (SE) induced by pilocarpine. NPD1 or vehicle was administered systemically during the 5 days after SE. Local field potentials from hippocampal regions were recorded using data acquisition systems at different time points. Time-dependent changes of the oscillatory activity of hippocampal networks were analyzed, including interictal spikes (IS), microseizures, high frequency oscillations, and spike unit activity. Recurrent seizure activity was video-recorded during the three weeks after SE. Brains were dissected and dendrites of principal cells from hippocampal regions were analyzed using Golgi staining. NPD1 reduced spontaneous microseizures, bursts of high frequency oscillations and dendritic spine loss in different hippocampal layers during epileptogenesis. As a result, NPD1 limited the appearance of spontaneous recurrent seizures. Also, NPD1 attenuated evoked hyper-excitability in the dentate gyrus, limiting interneuronal cell loss and microgliosis. Our results indicate neuroprotective bioactivity by NPD1 on hippocampal circuits during epileptogenesis. Understanding the mechanisms of NPD1 on neuronal network activities following injury-induced epilepsy may contribute to development of new anti-epileptogenic therapeutic strategies. Supported by: NIH P20RR016816.

Platelet Activating Factor Antagonism Attenuates Epileptogenesis

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Limbic epilepsy is associated with previous history of brain injury, such as status epilepticus (SE). Epileptogenesis that takes place within the period between the precipitant injury and the first occurrence of spontaneous seizures involves several articulated cellular and molecular inflammatory processes whereby the neuronal network develops spontaneous recurrent seizures. Platelet activating factor (PAF), a potent phospholipid messenger, increases after seizures, activates hippocampal excitatory synapses and becomes a pro-inflammatory and neurotoxic mediator. Therefore the PAF receptor is an excellent target for regulation of neuroinflammation-mediated excitability in epileptogenesis. We tested the bioactivity of the new platelet activating factor (PAF) antagonist series against acute seizures using the pentylenetetrazol (PTZ) model and after SE induced by pilocarpine in freely moving C57BL/6 adult mice. Seizures were evaluated using a multi-channel synchronization approach for continuous recordings of local field potential in hippocampal layers. Neuronal hyper-excitability was tested following administration at different time points of SE following PTZ administration. Also, neuroinflammation and neuronal architecture were evaluated using combined Golgi, immunohistology staining and biochemical techniques, including mass spectroscopy of hippocampal tissue from treated mice. We observed that new PAF antagonists limited epileptogenesis, reducing recurrent spontaneous seizures and aberrant dendritic spines in the hippocampus. These changes were correlated with decrease of concentration of neuroinflammatory markers following SE. In addition, PAF antagonism reduced epileptic hippocampal seizure susceptibility. Our results show that PAF receptor over activation contributes to hippocampal hyper-excitability and seizure susceptibility in the early stage of epileptogenesis. Therapeutic strategies focusing on new PAF antagonists on inflammation could attenuate certain initial harmful inflammatory signaling pathways to prevent seizures. Supported by: NIH P20RR016816.

SELECTIVE INHIBITION OF HUMAN GROUP IIA PLA$_2$ SIGNALLING

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Background – Human group IIA secreted phospholipase A_2 (hGIIA) promotes tumor growth and inflammation, and can act independently of its well-described catalytic lipase activity *via* an alternative poorly understood signaling pathway.

Objective – We aimed to use a combination of pharmacological, structural and cell biological approaches to shed light on the enzyme activity-independent function of hGIIA in cells human cells relevant to inflammation.

Methods – With six chemically diverse inhibitors we show that it is possible to selectively inhibit hGIIA signaling over catalysis and X-ray crystal structures illustrate that signaling involves a pharmacologically distinct surface to the catalytic site.

Results – We demonstrate in rheumatoid fibroblast-like synoviocytes that non-catalytic signaling is associated with rapid internalization of the enzyme and colocalization with vimentin. Trafficking of exogenous hGIIA was monitored with immunofluorescence studies, which revealed that vimentin localization is disrupted by inhibitors of signaling that belong to a rare class of small molecule inhibitors that modulate protein-protein interactions.

Conclusions – This study provides structural and pharmacological evidence for an association between vimentin, hGIIA and arachidonic acid metabolism in synovial inflammation, avenues for selective interrogation of hGIIA signaling and new strategies for therapeutic hGIIA inhibitor design.

TOWARDS THE MODULATION OF PHOSPHOLIPASE A_2 BY PARASITE INFECTION

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Trypanosomatid transmission by blood-sucking arthropods still causes a huge damage on human populations worldwide. Salivary anti-hemostatic factors are important to keep blood fluidity during feeding of hematophagous arthropods. In *Rhodnius prolixus*, vector of the agent of Chagas Disease *Trypanosoma cruzi*, we demonstrated that the major lipids present in the saliva were phosphatidylcholine and lysophosphatidylcholine (LPC). LPC found in *R. prolixus* saliva was able to inhibit platelet aggregation, and increases the production of nitric oxide, an important vasodilator. The mechanism of generation of LPC in saliva is still unknown. Phospholipase A_2 (PLA_2) hydrolyzes phospholipids yielding lysophospholipids and fatty acids. In insects, phospholipases are related to poison, digestion, immunity and reproduction. Our hypothesis is that PLA_2 is present in salivary glands and its action generates bioactive lipids, as LPC, that contributes to the repertoire of anti-hemostatic molecules present in saliva. We found seven different PLA_2s in *R. prolixus* genome (group VIA, VIB, XIIA, III and three unclassified). In salivary glands, gene expression for PLA_2 VIA and XIIA were detected by reverse transcriptase-PCR (RT-PCR). PLA_2 activity was also measured in both isolated saliva and salivary gland epithelium using the fluorogenic substrate 12-NBD-PC. In *Glossina morsitans*, a vector of African trypanosomiasis, PLA_2 XIIA gene family was previously identified. In the present study we have determined a phospholipase activity in saliva whose activity is inhibited upon the infection with *Trypanosoma brucei* or *Trypanosoma congolense*. Western blotting and quantitative PCR analysis showed that the PLA_2 expression level is downregulated in infected flies. These results support the data that *Trypanosoma* infection modifies salivary protein composition, including the modulation of PLA_2. This finding may indicate that LPC levels can be reduced which implies on a decrease of anti-haemostatic potential and culminate in hampered feeding performance, which may enhance parasites transmission.

Supported by: CNPq, CAPES, FAPERJ and INCT-EM.

Keywords: Saliva, Trypanosomatids, Bioactive lipids, LPC.

cPLA$_2$α Regulates TNF-Induced Effectors of Synovitis and Joint Destruction in Fibroblast-Like Synoviocytes

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*This work was supported by Central Norway Regional Health Authority Grants and The Research Council of Norway (NFR)

Background – In rheumatoid arthritis (RA), chronic synovitis causes pain, swelling and loss of joint function by cartilage and bone destruction, largely due to excessive production of pro-inflammatory cytokines such as TNF. NSAIDS represent an effective treatment for RA, thus demonstrating the central role of prostaglandins in RA pathology.

Objectives – The aim of this study was to elucidate the role of cPLA$_2$α in regulating TNF-induced expression of selected effectors of synovitis and destruction of cartilage and bone in RA.

Methods – In SW982 fibroblast-like synoviocytes stimulated with TNF, involvement of cPLA$_2$α was analyzed by performing arachidonic acid (AA) release assay, gene expression analysis by real-time PCR and ELISA method for the detection of PGE$_2$ and protein production.

Results – Inhibition of cPLA$_2$α by specific inhibitors (AVX002, ATK) significantly reduced cellular release of AA, PGE$_2$, IL-8 and MMP-3. This reduction was evident both at transcriptional and metabolite or protein levels. Interestingly, cPLA$_2$α inhibition affects several key points of the arachidonyl cascade; AA-release, COX-2 expression and PGE$_2$ production. Furthermore, the current study led to the novel finding that cPLA$_2$α is subject to transcriptional auto-regulation as inhibition of cPLA$_2$α resulted in reduced PLA$_2$G4A expression in TNF-stimulated synoviocytes.

Conclusions – cPLA$_2$α functions downstream TNF in regulating central effectors of inflammation and joint destruction, namely PGE$_2$, IL-8 and MMP-3. Decreased transcription of the PLA$_2$G4A and COX-2 genes in response to cPLA$_2$α inhibitors further suggest a self-reinforcing effect of cPLA$_2$α inhibition in response to TNF. Collectively, these results support the comprehension that cPLA$_2$α is a contributor to synovitis, and its inhibition reduces multiple pro-inflammatory factors representing important features in RA pathogenesis.

Key words: cPLA$_2$α, synoviocyte, TNF

GROUP III PHOSPHOLIPASE A_2 REGULATES MAST CELL MATURATION AND ANAPHYLAXIS THROUGH A NOVEL PARACRINE PGD_2 LOOP

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Background – Microenvironment-based alterations in mast cell phenotypes influence the susceptibility to anaphylaxis, yet the mechanisms underlying proper maturation of mast cells toward an anaphylaxis-sensitive phenotype are incompletely understood. As Group III $sPLA_2$ ($sPLA_2$-III) is the sole mammalian homolog of bee venom PLA_2, a potent inducer of anaphylaxis, we were interested in the role of $sPLA_2$-III in allergy.

Objective – We examined the roles of $sPLA_2$-III in mast cells and anaphylaxis using $sPLA_2$-III-deficient (*Pla$_2$g3$^{-/-}$*) and –transgenic (*Pla$_2$g3-Tg*)-mice. We also analyzed 23 knockout mouse lines for other PLA_2s, eicosanoid-biosynthetic enzymes or eicosanoid receptors to identify a $sPLA_2$-III-specific lipid pathway that dictates the mast cell fate.

Results – Mast cell-associated passive and active anaphylactic responses were markedly attenuated in *Pla$_2$g3$^{-/-}$* mice and augmented in *Pla$_2$g3*-Tg mice. *Pla$_2$g3$^{-/-}$* bone marrow-derived mast cells (BMMCs) failed to reconstitute the anaphylactic response after transfer into mast cell-deficient *Kit$^{W\text{-}sh/W\text{-}sh}$* mice, indicating that the defects caused by $sPLA_2$-III deficiency is mast cell autonomous. Dermal or skin mast cells in *Pla$_2$g3$^{-/-}$* mice were numerically normal but morphologically and functionally immature, showing impaired degranulation. *Pla$_2$g3$^{-/-}$* BMMCs exhibited defective fibroblast-directed maturation *in vitro*. Strikingly, similar mast cell abnormalities were also evident in mice lacking lipocalin-type PGD_2 synthase (L-PGDS) or those lacking the PGD receptor DP1, suggesting their functional linkage. Indeed, ablation of DP1 in mast cells or L-PGDS in fibroblasts impairs mast cell maturation and anaphylaxis.

Conclusion – $sPLA_2$-III secreted from immature mast cells is coupled with fibroblastic L-PGDS to provide microenvironmental PGD_2, which in turn facilitates mast cell maturation via DP1 induced on mast cells. Thus, a novel lipid mediator-driven $sPLA_2$-III/L-PGDS/DP1 paracrine loop that drives mast cell maturation may be a novel drug target for mast cell-associated diseases.

(Supported by JSPS KAKENHI and MEXT KAKENHI)

Key words: $sPLA_2$, PGD_2, Mast cells

GROUP IIF PHOSPHOLIPASE A_2 MAINTAINS SKIN HOMEOSTASIS

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Background – Secretory phospholipase A_2 ($sPLA_2$) enzymes exhibit unique tissue and cellular localizations and enzymatic properties, suggesting their distinct physiological roles. Although perturbed lipid metabolism can often lead to skin abnormality, the role of PLA_2 in skin homeostasis is poorly understood. Recently, we have reported that transgenic (Tg) overexpression of $sPLA_2$-X in mice resulted in skin abnormalities characterized by alopecia, and epidermal and sebaceous gland hyperplasia (*J. Biol. Chem.* 286:11616; 2011).

However, endogenous $sPLA_2$-X is expressed in mouse skin only minimally, raising the possibility that there would be the other skin-intrinsic $sPLA_2$ that participates in skin homeostasis. We found that the expression of $sPLA_2$-IIF, an isoform whose *in vivo* functions have currently been unknown, was uniquely upregulated in the hyperplastic epidermis of $sPLA_2$-X Tg mice as well as in that of humans with skin pathology.

Objective – To elucidate potential *in vivo* functions of $sPLA_2$-IIF in skin, we analyzed Tg and knockout (KO) mice for $sPLA_2$-IIF in combination with lipidomics approaches.

Results – $sPLA_2$-IIF was dominantly expressed in the suprabasal layers of mouse epidermis. Tg overexpression of $sPLA_2$-IIF, where its expression reached a level relevant to that seen in skin pathology, led to marked epidermal hyperplasia with hair loss, accompanied by increased expression of genes related to terminal epidermal differentiation. On the other hand, $sPLA_2$-IIF KO mice exhibited age-associated epidermal abnormalities manifested by defective epidermal barrier, impaired acidification, and fragile cornified envelope with decreased mechanical integrity. *Ex vivo* culture of primary keratinocytes revealed that $sPLA_2$-IIF was secreted from differentiating keratinocytes, that it hydrolyzed phospholipids secreted from keratinocytes to provide polyunsaturated fatty acid-oxygenated metabolites that could affect keratinocyte differentiation, and that its deficiency caused a keratinocyte differentiation defect which was restored by supplementation with recombinant $sPLA_2$-IIF protein. Moreover, skin inflammation and cancer were ameliorated in $sPLA_2$-IIF KO mice.

Conclusions – Our results highlight the unexplored role of $sPLA_2$-IIF as an "epidermal $sPLA_2$" in skin biology and provide a rationale for treating patients with epidermal disorders with an agent targeting this enzyme.

(Supported by JSPS KAKENHI and MEXT KAKENHI)

Key words: $sPLA_2$, lipidomics, skin.

DEVELOPMENTAL DEFECTS CAUSED BY OVEREXPRESSION OF MOUSE GROUP VIB Ca^{2+}-INDEPENDENT PHOSPHOLIPASE A_2

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Group VIB Ca^{2+}-independent phospholipase A_2 ($iPLA_2\gamma$) is a membrane-bound $iPLA_2$ enzyme with four potential translation initiation sites, which produce distinct sizes (88-, 77-, 74- and 63-kDa) of its protein. $iPLA_2\gamma$ has mitochondrial and peroxisomal localization signals and preferentially distributed in the mitochondria and peroxisomes. In an effort to elucidate the functions of membrane-associated calcium-independent PLA_2 ($iPLA_2\gamma$) enzymes *in vivo*, we generated transgenic (Tg) mice overexpressing $iPLA_2\gamma$, which act potently on cardiolipin and plasmalogen phospholipids. The cDNA for mouse $iPLA_2\gamma$ was inserted into the the pCALNL5 vector which contains the expression-switching reporter consisting of a cytomegalovirus immediate early enhancer-chicken β-actin hybrid (CAG) promoter and a neomysin resistance gene cassette with Cre/loxP sites at both ends (LNL cassette). This construct was microinjected into 0.5-day fertilized eggs of C57BL/6 females and then the eggs were transferred into oviducts of ICR pseudopregnant females. The offspring were examined for expression of the transgene by PCR genotyping. The male founders were mated with female C57BL/6 mice to confirm germ line transmission by PCR genotyping, and those with successful germ line transmission were then crossed with female CAG-Cre Tg mice, which carry the Cre recombinase transgene under the CAG promoter. This step resulted in removal of the LNL cassette from the transgene, thereby allowing activation of $iPLA_2\gamma$ transgene in the whole body of offspring. As the results, we found that $iPLA_2\gamma$ Tg mice showed developmental defect by E11.5 and embryonic lethality from E12.5. These results suggest that $iPLA_2\gamma$ may have important physiological role during embryonic development.

Key words: $iPLA_2\gamma$, transgenic, embryonic development

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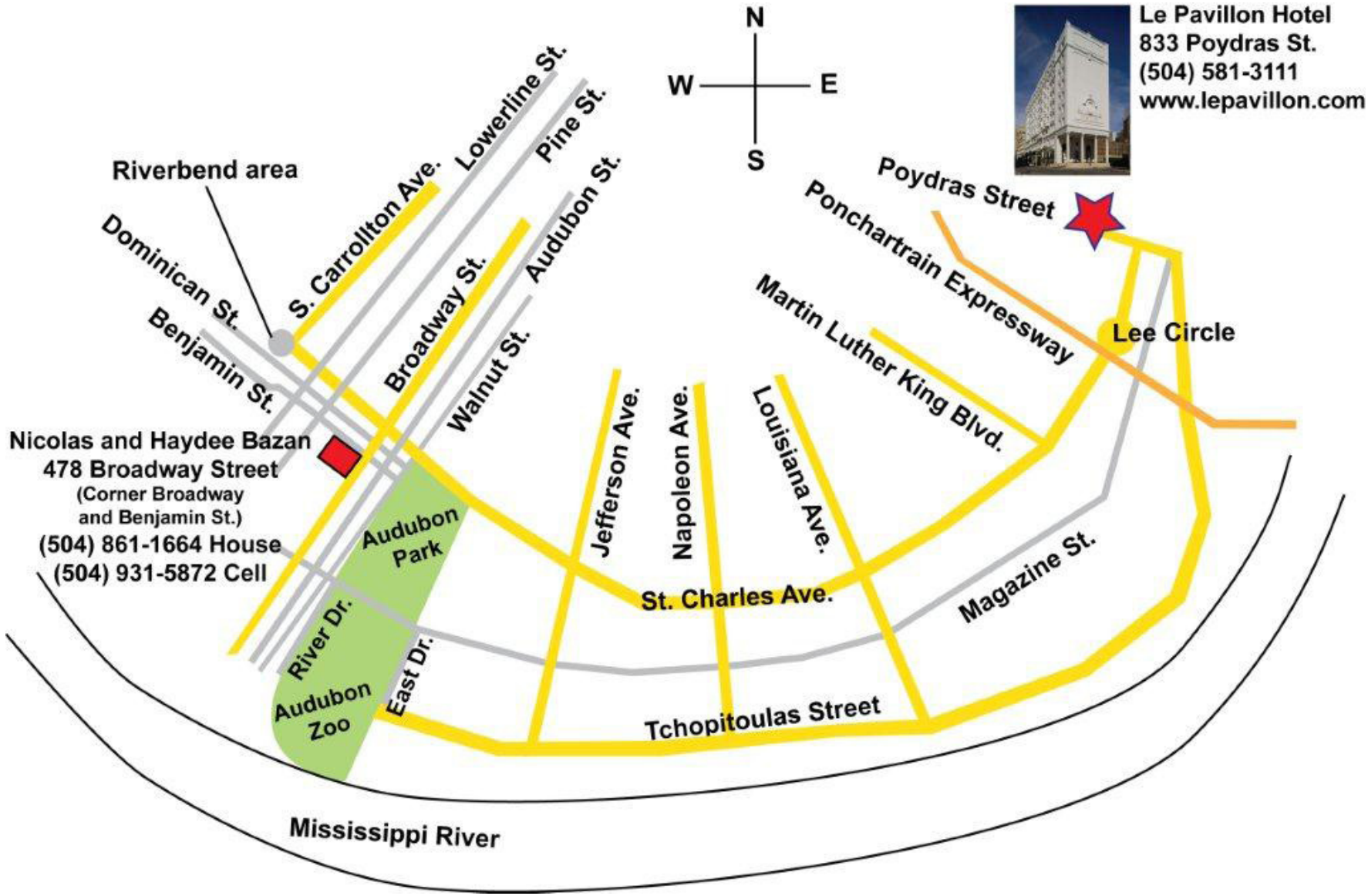

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