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ASPET Annual Meeting at Experimental Biology 2012

San Diego, CA

The Pharmacologist

Contents

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Message from the President

Dear ASPET Members:

I can hardly believe that my year as your president is over, as I feel like I have just begun to work with several of you to develop some new initiatives. For those of you present at the business meeting in San Diego, you may recall that I spoke about plans that were developed this past October when members of Council, divisional representatives, and other invited individuals gathered at the Maritime Institute in Baltimore for a long-range planning retreat. The three action items that resulted from the retreat were to:

- enhance marketing endeavors for ASPET
- create a mentoring and professional development program for ASPET members
- partner with other societies and position ASPET at the forefront

I am so pleased that several of these initiatives have been implemented and are moving along.

As I hope you all know, Suzie Thompson was hired this past January as our Marketing Director and has hit the ground running. Suzie and Christie Carrico, our Executive Officer, have identified a firm with branding and re-imaging expertise who will be moving forward to help us ensure that anyone and everyone involved in drug development, research and translation recognizes ASPET as the 'go to' society. Related to our marketing initiative is the hiring of Gary Axelrod, our new Web and Social Media Manager. Gary began in March and will ensure that we reach all of our members, irrespective of how they receive information.

Our second goal, to create a mentoring and professional development program, kicked off at the EB meeting, and is well on its way. I'd like to thank Marcus Delatte for all of his efforts, as well as Christie Carrico and Carol Paronis for their input. Keep your eyes open for progress on this program as it continues to develop.

As I indicated at the Business Meeting in San Diego, the future of ASPET lies with our graduate students, postdoctorals and young investigators. Thus, kudos to Joanna Sandilos, who attended the mentoring session at EB and has taken the initiative to create an ASPET postdoctoral group. Thanks Joanna for stepping up to the plate!

The third retreat outcome, under the direction of Steve Lanier, Mike Jarvis, Jim Barrett, Scott Waldman, and John Lazo, is seeking how ASPET can take the lead and develop alliances with related societies. As you are hopefully aware, next year we will be hosting the British Pharmacological Society at EB in Boston. Keep your eyes open for similar joint meetings in the future.

Naturally, none of these initiatives can be realized without your input. ASPET has a tremendous professional staff that deserves our gratitude, but it is you, the members, who will move ASPET into the future.

I am pleased that we are currently financially stable, and that you have elected two solid future leaders, John Lazo for the coming year and Rick Neubig for the year following. John and Rick are highly capable individuals, but they need your help. I ask all of you to think about how you can ensure that ASPET remains a vibrant society that engages future generations.

In closing, thanks for allowing me to help move ASPET forward; it has been an honor and a privilege.

My best,

Lynn Wecker

Lynn Wecker, Ph.D.

Experimental Biology 2012 in Review

ASPET met as part of Experimental Biology 2012 from April 21 to 25 in San Diego, CA. With over 13,500 attendees, the meeting provided a great mix of important science and fun networking events.

Prior to the start of EB 2012, ASPET members took some extra time to volunteer in the San Diego area on Friday, April 20. Organized by the Behavioral Pharmacology Division of ASPET, volunteers spent the day preparing and serving breakfast and lunch and painting hallways in the residence hall of Father Joe's Villages. Started more than 60 years ago as a small chapel serving San Diego's impoverished, Father Joe's Villages is now the largest provider of homeless services in Southern California and supplies food, clothing, housing, healthcare, education, job training, and child development to more than 2,000 individuals each day.

The ASPET Business Meeting took place on Saturday, April 21. Attendees were given updates on ASPET's membership, public affairs activities, finances, publications, and other business. ASPET's awards were also presented during the meeting.

President Lynn Wecker delivers a report on ASPET's activities.

Incoming President John Lazo thanks Lynn Wecker for her services as president.

Recipients of ASPET's 2012 Awards.

Experimental Biology 2012 in Review

Following the ASPET Business Meeting, members kicked off the start of the 2012 Annual Meeting with an opening reception on the outdoor terrace of the convention center.



The WIP into Shape Networking Walk took place on Sunday, April 22. ASPET members gathered for a walk down San Diego's waterfront.

aspet On The Web

Join the conversation online! ASPET is online! Join us on Facebook, Twitter or LinkedIn for the latest in news, discussions and events. Discuss hot topics with your peers, check out pictures from ASPET events and find resources to help in finding a job or a graduate program.

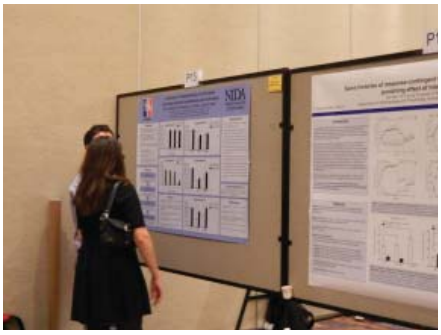
www.facebook.com/ASPETpage

www.twitter.com/ASPET

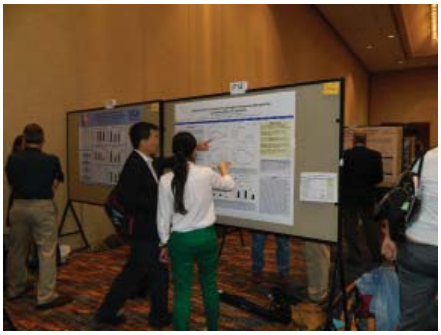
LinkedIn: Search - "ASPET"

Experimental Biology 2012 in Review

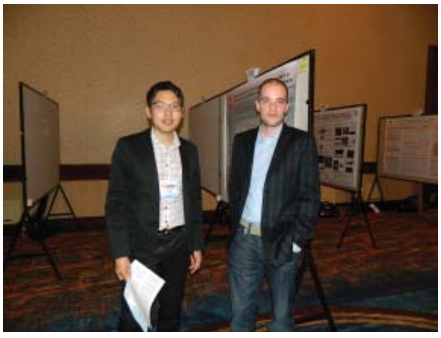
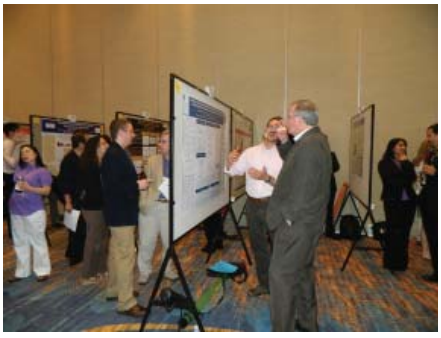
The Student/Postdoc Best Abstract Competition gave students and young scientists a chance to present their work and mingle with fellow ASPET members.










Experimental Biology 2012 in Review

ASPET students and postdocs let their hair down at the ASPET Student/Postdoc mixer. Young members enjoyed drinks, dessert, and some karaoke singing with the DJ!

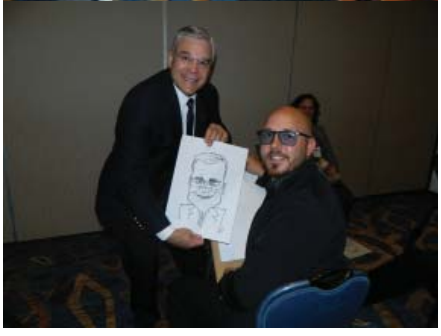


After an exciting meeting, ASPET held a closing reception on Wednesday, April 25, where members ate, drank, and had their caricatures drawn.









Experimental Biology 2012

Award Winners

ASPET Young Scientist Travel Award Winners for 2012.

ASPET Graduate Student Travel Award Winners for 2012.

Summer Undergraduate Research Fellowship Award Winners for 2012.

The PhRMA Foundation Award in Excellence in Pharmacology/ Toxicology is presented to Dr. Margarita Dubocovich.

PhRMA Foundation Postdoctoral Fellowship winner Dr. Uyen Chu.

PhRMA Foundation Predoctoral Fellowship winners John Park, Sara Nowinski, and Craig Ulrich.

PhRMA Foundation Research Starter Grant winners Drs. Seena Ajit and Travis Salisbury.

Experimental Biology 2012

Award Winners

Dolores Shockley Award

Joanne L. Peterson of the University of Oklahoma Health Sciences Center was the winner of the 2012 Dolores C. Shockley Best Abstract Award at the EB '12 meeting in San Diego, CA for her abstract entitled, *The molecular mechanism of betacellulin induced corneal epithelial wound healing*.

Dr. Shockley was the first African-American woman to earn a PhD in pharmacology and the first black woman appointed to chair a pharmacology department in the U.S. In 2009, Dr. Shockley received the Distinguished Alumni Award from her alma mater, Purdue University.

The ASPET Annual Meeting at Experimental Biology 2013
April 20 - 24, 2013
Boston, MA

Joint Annual Meeting with the British Pharmacological Society & the Canadian Society of Pharmacology & Experimental Therapeutics

http://www.aspet.org/EB2013/

- Cutting-Edge Science
- Exciting & Timely Symposia
- Oral & Poster Presentations
- Networking Opportunities
- Over 400 Exhibit Booths
- Awards Opportunities
- Volunteer Opportunties

Experimental Biology 2013®

aspet

BPS BRITISH PHARMACOLOGICAL SOCIETY
Today's science, tomorrow's medicines

CSPT SCTP

Career Center

Features

As your professional scientific society, ASPET is committed to your professional success. We see it as our job to nurture our members' professional needs. That's why we have teamed up with Boxwood Technologies and the National Healthcare Career Network to offer our members a career management solution.

When you visit our Career Center (**http://careers.aspet.org**), you have access to hundreds of jobs across the country. Because ASPET is a part of the National Healthcare Career Network, you not only have access to jobs posted on our site, but also jobs posted by over 250 other professional associations and societies (like SfN and ASN) in the healthcare industry.

But the ASPET Career Center is not just a job board—it's an online facility where you can proactively manage all aspects of your career and create a professional action plan tailored to your goals. By registering on our site, you also get access to:

FREE Career Tips – Free access to articles on job hunting, interview and negotiation techniques, networking, work-life balance, and other career related topics. Search by career stages or services you need using keywords or phrases.

Resume Critiques – Hire a professional to critique your current resume.

Resume Writing Services – Let a professional writer enhance or create a resume and cover letter that will help open the door to your next interview.

Career Coaching – Obtain individual help from an experienced and certified career coach to manage, plan, and develop career goals.

Online Profile Development – Ensure a professional presence on LinkedIn, Twitter, Visual CV, Google Profile, and Plaxo.

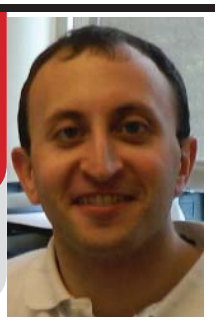
Reference Checking and Employment Verification – Identify questionable references before they speak with prospective employers.

For more information about any of these services, visit: **http://careers.aspet.org/careerdev**.

Even if you are not currently in the job market, come visit the ASPET Career Center (**http://careers.aspet.org**) today to see what we have available. Register on our site and make your curriculum vitae searchable by prospective employers, look at the jobs currently out there, and get career tips and other important career related information.

Social Media

An Introduction to Social Media



Hello everyone. My name is Gary Axelrod. I am the new Web and Social Media Manager at ASPET. We recently came up with the idea to do a monthly blog article about social media which aims to present both general ideas and some specifics as related to medical and research professionals, and of course pharmacology. To supplement the blog on our website, **http://www.aspet.org/blog/default.aspx**, most of the information from the first two articles has been reprinted here.

Social media (also known as Web 2.0) is everywhere these days. Web 2.0 expertise is a great tool for subject matter experts to have. Of the vast array of social networks, each has their own niche market, audience, and followers. ASPET is currently active on Facebook, Twitter, and LinkedIn. We aim to use Facebook and Twitter to promote ASPET events, public policy issues, and current pharmacology news. We currently use LinkedIn to connect with our members and share information on ASPET public policy updates and careers in pharmacology.

Our aim is to try to reach members who have not yet joined the fray in social media and get them involved in the conversation. To those of you who are more experienced in social media, please feel free to share ideas and help us reach the demographic of those who have not yet put forth a strong effort in social media. By connecting with and engaging ASPET, there exists the potential for mutually beneficial opportunities, as we could each post or tweet back and forth in public view. Social media outlets are great for sharing everything from quick updates to major discoveries. And of course, who doesn't appreciate those tongue-in-cheek one-liners that we all see while scrolling through updates on our computer or mobile device?

As social media is still in its first decade there are no set ways to do things. Social media promotes originality, and its main idea is to serve as a platform by which one can easily share information. Ultimately, it's up to you how helpful the information you share is. Social media is so popular that traditional media such as major magazines and TV outlets use it as a way to connect with readers and viewers and receive feedback from them. Subjectively speaking, the benefits of connecting with a broad reach of users on various social media networks are what make it all worthwhile.

Twitter Tips for 'N00bs'

We hope those of you who attended ASPET programs at EB 2012 in San Diego had a very thought-provoking meeting experience. Scicurious blogged about the ASPET Experimental Biology programming in detail (**http://blogs.scientificamerican.com/scicurious-brain/**), while we focused on live tweeting on Twitter to keep you abreast of our programs and activities throughout the day (**http://www.twitter.com/aspet**). You may have also noticed tweets about our furry friend, the ASPET Donkey, making appearances at certain ASPET events at EB.

Over the past few years, Twitter has become a go-to source to send out and receive breaking news. The often quirky snippets of information, also known as "tweets," give you details and often a link to click on within a span of 140 characters. As you are only allotted 140 characters per tweet, learning the abbreviated lingo for Twitter is a key element of getting this social network to work for you. I learned the term "n00b" (yes, those are zeros), short for neophyte, when I read some of the feedback on my initial blog post in April. I learned the term from following conversations about ASPET on Twitter, as the feedback portion of our PharmTalk blogs was not working at the time. Twitter is a useful tool to engage in dialog in that manner, and many associations use it to supplement their customer service efforts.

Social Media

Twitter Tips for 'NOObs'

A few other keys to Twitter are as follows: shortened URLs, hashtags, referencing others' Twitter handles (a handle is what a Twitter account name is called), retweets, and modified tweets. The practice of shortening URLs—a URL is another name for a Web address—allows one to write more text in a given tweet as the Web address won't take up as many spaces of your tweet after being shortened. Two of the most prominent URL shorteners are ow.ly (**http://ow.ly**) and bit.ly (**https://bitly.com**). To shorten your URL, go to one of these websites, type in the full URL, and click the button that says "shorten" or "shrink it." Then, once you have logged into your Twitter account, all you have to do is click the blue "compose new tweet" button in the upper right-hand corner of the screen and copy and paste the shortened URL into your tweet along with a message that fits within the 140 character limit.

The two most commonly used symbols on Twitter are "#" and "@." Any Twitter text that follows a "#" is referred to as a "hashtag." Following the pound sign, you must start with a letter (numbers but not symbols can follow the first letter, i.e., no apostrophes). The idea of a Twitter hashtag is to create searchable topics on Twitter. This also allows Twitter to see what particular topics are trending at any given moment. If you wanted to see recent comments about ASPET, you could type "#ASPET" into the search feature at the top of the page. You can start topics by creating your own hashtag or add to topics that already exist by using the hashtag for that topic. This spring, the #eb2012 hashtag was used in many tweets in preparation for and during Experimental Biology in San Diego in late April.

The "@" is used to reference another person's (or group's) Twitter handle when you want to refer to them in any manner, reply to, or retweet one of their messages. As such, the "@" symbol should be followed by the name of the Twitter handle you are referencing in your tweet. If you want to reply to someone's tweet, simply move your mouse so that the little hand icon on the screen is on the tweet to which you want to reply, click the link at the bottom of the tweet that says "reply" and type your message. In your reply, "@(TwitterHandleYouAreReplyingTo)" will show up and the beginning of your tweet.

When you "retweet" a message, you are sharing another's tweet with your list of followers on Twitter. ASPET shares important and interesting information generated from other groups or people in the scientific community by retweeting a handful of tweets of those whom we follow. There are three common ways to retweet a message. You can find a tweet that you would like to spread to your followers, and whether or not you are following that particular Twitter handle or they are following you, you can just hover your mouse over that particular tweet and hit the retweet button. Then, the post shows up in your tweets as a retweet with the original tweeter's Twitter handle above it. You can also retweet a message by typing "RT @(TwitterHandle)" plus the content of the message. Or if you want to send a retweet but are modifying the text of the original tweet, you should, out of common courtesy, write "MT @(TwitterHandle)" plus the content of the modified message to signal that you are posting a modification of someone else's tweet. When you type the "@" symbol plus a Twitter handle into the site's search feature, you can track recent tweets in which that particular Twitter handle is mentioned.

The above tips are in no way a comprehensive set of instructions and social mores on Twitter. This is merely an overview of the basics to help you get started. If you are still confused, we will soon be putting together a basic guide on how to use Twitter. So join us in the Twitter-verse and happy tweeting!

As mentioned above, social media works both ways. If you have suggestions for us on how we can improve our social media outreach and engagement, consider this your invitation to submit feedback to us. We will cover the "how to's" of Facebook and other social media avenues another time.

Division News

Division for Behavioral Pharmacology: Awards

At Experimental Biology 2012, the Division for Behavioral Pharmacology had 36 participants enter its Best Abstract contest. Awards were given to undergraduates for the first time this year.

Postdoctoral Fellows
First Place - Bruce Mandt, University of Colorado, Denver
Second Place - Catherine Davis, Johns Hopkins Univ School of Medicine

Graduate Students
First Place - Brandi Blaylock, Wake Forest University School of Medicine
Second Place - Yen Truong, University of Michigan

Undergraduate Students
First Place - Joel John, Temple University School of Pharmacy
Second Place - Bansi Vedia, SUNY Buffalo

Division for Cardiovascular Pharmacology: Poster/Oral Awards

Postdoctoral Fellows
First Place - Sapnil Sonkusare
Second Place - Yiang Xiang
Runner Up - Nadja Grobe

Graduate Students
First Place - John Murad
Second Place - Erin Kohler
Third Place - Jacob Myerson
Fourth Place - Alejandra Chavez
Runner Up - Tracy Thennes
Runner Up - Anna Busija
Runner Up - Shinichi Asano

Division for Drug Discovery, Drug Development and Regulatory Affairs: Awards

First Place - Adbul Khan
Second Place - Amanda Wong Hui Chyn
Third Place - E. Mitchell Seymour

Division for Molecular Pharmacology: Poster/Oral Awards

Postdoctoral Fellows
First Place - Angeline Lyon
Runner Up - Caglar Cekic
Runner Up - JimKim (Unice) Soh

Graduate Students
First Place - Yamicia Connor
First Place - Mourad Ali
Third Place - Wei Kan
Honorable Mention - Ram Naikawadi
Honorable Mention - Alex Wu

Division for Neuropharmacology: Poster/Oral Awards

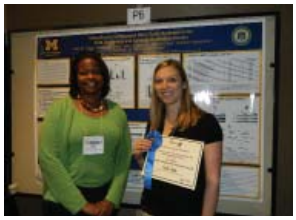
Postdoctoral Fellows
First Place - Vikas Dukhande
Second Place - Emanuela Esposito
Third Place - Anthony Hutchinson

Graduate Students
First Place - Deana Apple
Second Place - Christopher Cottingham
Third Place - Sairam Jabba

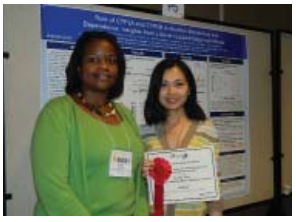
Division News

Division for Drug Metabolism

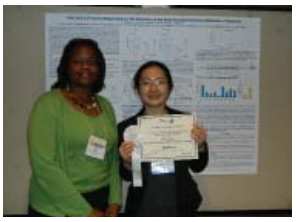
The graduate student awardees were:



First Place - Kelly Clapp, Yoichi Osawa's lab, University of Michigan; *Ubiquitination of Neuronal Nitric Oxide Synthase in the P450 Oxygenase and Calmodulin-binding Domain.*



Second Place - Lei Li, Xinxin Ding's lab, Wadsworth Center; *Characterization of a Cyp2a(4/5) bgs-null Mouse Model: Role of CYP2A and CYP2B in Nicotine Metabolism.*

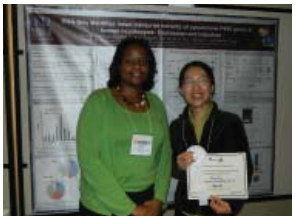


Third Place - Yanhui Lu, Namandje Bumpus' lab, Johns Hopkins University; *P450 3A5 is primarily responsible for the formation of the most abundant oxidative metabolite of maraviroc.*

The postdoc awardees were:

First Place - Manish Shah, Jim Halpert's lab, UCSD; *Dual Ligand Complexes of Human Cytochrome P450 2B6 and Rabbit Cytochrome P450 2B4 with Amlodipine Reveal Substrate Access Channels into the Active Site.*

Second Place - Aik Jiang Lau, Tom Chang's lab, University of British Columbia; *Selective Activation of Human Pregnane X Receptor, Glucocorticoid Receptor, and Constitutive Androstane Receptor by Individual Ginkgolides.*



Third Place - Dan Li, Xiaobo Zong's lab, Kansas Univ Med Center; *RNA-Seq identifies novel alternative transcripts of cytochrome P450s in human hepatocytes.*

All awardees were presented their certificates at EB 2012 by Division for Drug Metabolism Secretary-Treasurer Elect Marion B. Sewer, PhD, Associate Professor at the UC San Diego Skaggs School of Pharmacy and Pharmaceutical Sciences.

Division News

Division for Drug Metabolism

Winners of the Gillette Awards:

Drug Metabolism Category: Kyoung Noh, Seoul National University, Seoul Korea. *Farnesoid X Receptor activation by chenodeoxycholic acid induces detoxifying enzymes through AMP-activated protein kinase and extracellular signal-regulated kinase 1/2-mediated phosphorylation of CCAAT/enhancer binding protein & β.*

Pharmacokinetics Category: Xuena Lin, Metabolism and Pharmacokinetics, Novartis Institutes for Biomedical Research, Cambridge MA. *Attenuation of intestinal absorption by major efflux transporters: quantitative tools and strategies using a Caco-2 model.*

All awardees were presented their certificates at EB 2012 by Division for Drug Metabolism Chair Hollie Swanson, PhD, Professor at the University of Kentucky Department of Molecular and Biomedical Pharmacology

Division for Integrative Systems, Translational and Clinical Pharmacology

The ISTCP Division had a very successful student and postdoctoral poster competition at Experimental Biology 2012 on Sunday, April 22, in the evening. There were six student poster presentations and five postdoctoral poster presentations. The following afternoon, an oral competition for student and postdoctoral abstracts was held at the ISTCP Division Young Investigator Platform session. After the platform session, the annual ISTCP business meeting was conducted and winners were announced.

Poster Award Winners:

Student

First Place - Ocal Ozhan, UT Southwestern Medical Center
Secondnd Place - Kelly M. Thuet-Clapp, St. Louis University
Third Place - Aravind Gade, Virginia Commonwealth University

Postdoctoral

First Place - Tricia Smith, Virginia Commonwealth University
Second Place - Ross Corriden, University of Nottingham
Third Place - Randall Kimple, University of Wisconsin
Honorable mentions:
Stephanie Knebel, St. Louis University
Ling Zou, University of Kentucky
Haili Xu, University of Arizona College of Medicine
Hercules T. Maguma, Virginia Commonwealth University
Yuhua Wang, University of Louisville

Oral Award Winners:

Student

First Prize - Ocal Ozhan, UT Southwestern Med Ctr
Second Prize - Haili Xu, Univ of Arizonea Coll of Medicine
Third Prize - Ling Zou, Univ of Kentucky

Postdoctoral

First Prize - Ross Corriden, University of Nottingham
Second Prize - Yuhua Wang, University of Louisville
Third Prize - Randall Kimple, University of Wisconsin

Division News

Division for Integrative Systems, Translational and Clinical Pharmacology

First prize winners received $500. Second prize winners received $400. Third prize winners received $300 in each category and complimentary registration to EB2013 in Boston. Honorable mentions each received $100.

Following the outstanding student and postdoctoral presentations, the ISTCP business meeting was conducted by Chair **Dr. Hamid Akbarali** and Secretary/Treasurer **Dr. Jeffrey Paul**. Several issues were discussed including the changes in ASPET policy for submission of proposals and an invitation to young students/postdocs/junior faculty to get involved in Divisional activities and submission of topics for symposia at future ASPET meetings. The minutes of the meeting are available at the Division's Web page: **http://www.aspet.org/ISTCP/Home**.

The Harry Gold Travel Award in Clinical Pharmacology was presented to Dr. Zhican Wang of the University of Washington. A plaque and check for $1,000 was presented to Dr. Wang at the business meeting. The Harry Gold Travel Award is a Young Scientist Award for the best abstract in Clinical Pharmacology. Dr. Wang's study was titled *Selective induction of CYP3A4-dependent vitamin D catabolism via pregnane X receptor in human hepatocytes and healthy volunteers*.

Following the business meeting, the ISTCP Division mixer was held at the Marriott. All award winners were presented with the certificates and ribbons at the event.

Division News

Division for Toxicology

The Division of Toxicology welcomes newly elected officers **Rick Schnellmann** (Medical University of South Carolina, Chair-elect) and **Laura James** (University of Arkansas, Secretary/Treasurer-elect). We thank John Schuetz and Courtney Sulentic for their excellent leadership and service.

At EB 2012 in San Diego, the Division of Toxicology held a poster competition among graduate students and postdoctoral trainees. There were seven student entries and three postdoc entries. The first place student winner was **Jessica Morgan** (University of Tennessee). Second place went to **Stephanie Ondovcik** (University of Toronto), and **Chris Kuhlman** (University of Arizona) was the third place winner. The postdoc winner was **Smita Ghare** (University of Louisville). All winners were awarded cash prizes.

In addition, our Division symposium, *The utilization of genetically modified mice to determine mechanisms of toxicity*, chaired by **Jack Hinson**, was well attended. Several other symposia were sponsored or cosponsored by the Division. They were: *Role of pharmacogenetics in oncology*, chaired by **Penni Black** and **Hollie Swanson**; *Role of nuclear receptors in lipid dysregulation and obesity-related diseases*, chaired by **John Chiang** and Hollie Swanson; *Cognitive enhancement to improve treatment outcome and quality of life associated with neuropathologies*, chaired by **Robert Gould** and **Michael Nader**; *From structure to knockout: Common themes between CYPs and ABC transporters,* chaired by **Mary Vore**; *Protein-protein interaction (PPI) interfaces as therapeutic targets: promises and challenges*, chaired by **Haian Fu**; *NADPH-CYP450 oxidoreductase: Roles in physiology, pharmacology, and toxicology,* chaired by **Todd Porter** and **David Riddick**; and *Steroid signaling via G protein-coupled receptors*, chaired by **Eric Prossnitz**. The Division welcomes symposium proposals for future meetings. Please feel free to contact a member of the executive council for more information.

Toxicology Division Poster Award Winners

Postdoctoral Fellows
First Place - Smita Ghare

Graduate Students
First Place - Jessica Morgan
Second Place - Stephanie Ondovcik
Third Place - Christopher Kuhlman

Journals

by Rich Dodenhoff

New Editorial Board Members

Dr. Kathleen M. Hillgren in the Drug Disposition Department at Lilly Research Laboratories has joined the *Drug Metabolism and Disposition* Editorial Board. **Dr. Lee Ann MacMillan-Crow** from the Department of Pharmacology and Toxicology at the University of Arkansas is now a member of the Editorial Advisory Board for the *Journal of Pharmacology and Experimental Therapeutics*. The Board of Publications Trustees appreciates the commitment of these researchers to ASPET's journals.

Changes on Deck for the Journals

The Board of Publications Trustees met during ASPET's Annual Meeting in San Diego in April and adopted three new policies for the Society's journals.

Reporting Gender and Sex

ASPET's journals will require researchers to report the sex of experimental animals and the sex or gender of humans used in studies that are submitted for publication. This policy goes into effect for manuscripts first submitted starting July 1, 2012.

pdb Files

Journal articles that include homology models will be required to supply pdb files as data supplements. This ascii file format is a standardized convention used to stored crystallographic data describing protein structure. As **Dr. Stephen Traynelis**, Editor of *Molecular Pharmacology*, explained, "These files contain line-by-line indexing information for each atom in the crystallographic structure along with some other information. Virtually all journals require that this data be stored in one of the national databases when manuscripts are accepted for publication, and this information is immensely valuable to the scientific community. The growing number of crystallographic data sets together with ever-improving modeling software has exponentially increased the number of labs that generate homology models of their protein of interest from existing crystallographic data sets for closely related proteins. These models are used for many purposes." This policy will go into effect for new manuscripts as of July 1, 2012.

Screening Manuscripts for Plagiarism

The Board of Publications Trustees approved the adoption of CrossCheck, a plagiarism detection service from CrossRef, for ASPET's journals. CrossCheck compares manuscripts against an ever growing database of research literature from hundreds of CrossCheck member publishers. The database includes previously published content and expands daily as new articles are published and new publishers join the system.

Submitted manuscripts will be compared against all literature in the database. CrossCheck generates a report that gives the percentage of duplicated text and highlights the duplications in the manuscript with links to the suspected source. Staff and the editors will then evaluate the findings to determine if the overlapping text is harmless or constitutes a case of plagiarism.

CrossCheck will be integrated with Bench>Press, ASPET's online manuscript submission and peer review system. Announcements will be made when the system has been implemented and goes live.

Journals

by Rich Dodenhoff

Mobile Vouchers Added

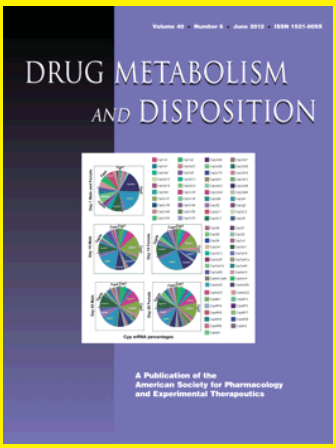
The mobile version of each ASPET journal now provides mobile vouchers. A voucher is a code that ties a mobile device to an institution's subscription and gives the user access to subscribed content while not on an institutional network. This is a useful option for those who want journal access while out of range of their institution's WiFi signal. Those with access through an institutional subscription can get a mobile voucher from any computer on the institution's network. The voucher must then be applied to a mobile device. Each device needs its own voucher.

The process is simple. While on a networked computer, a user fills out a form with three fields: first name, last name, and email address. There's also a checkbox to receive the voucher code by email. Click "submit" and the voucher code appears on-screen (and is sent by email if the box was checked). The user has 48 hours to apply the voucher, which involves entering the code in a box. That's all it takes.

Vouchers last for two months. The can be renewed for as long as the institution maintains its subscription. If a subscription expires during the two month period, access will be lost.

To request a mobile voucher, the user must first be recognized as being part of an institutional subscription. If a login page appears, the user's IP address is not being recognized via a current institutional subscription. For help connecting to your institution's WiFi access, contact your librarian or IT department. Once connected, you can get a voucher.

ASPET membership includes access to the Society's journals. A member may want to use a mobile voucher when a WiFi signal provides faster access than a smart phone's data service.



Have you activated your member subscription to ASPET's online journals?
ASPET members get online access to all ASPET journals.
Staff at info@aspet.org will be glad to help.

Integrative and Organ Systems Sciences

New Funding Opportunity

ASPET Graduate & Postdoctoral Award for Integrative Research in Pharmacology

Objective: The major goal of the ASPET Graduate & Postdoctoral Award for Integrative Research in Pharmacology (ASPET-IRP Award) is to provide support for graduate and postdoctoral trainees who are involved in active research projects that involve *in vivo* pharmacology or are focused on organ systems as an integral part of their research efforts.

Eligibility Guidelines: The ASPET-IRP Award will consider applications from research proposals submitted by graduate or postdoctoral students with demonstrated interest in *in vivo* pharmacology. Graduate and postdoctoral candidates must be members of ASPET and must conduct research at a U.S. academic institution. Postdoctoral applicants who have completed three or more years of postdoctoral training are not eligible.

Research Areas of Interest: Awards will be made to support training in *any* research area of interest. Selection of awards will be based on the depth of the *in vivo* component with clear evidence of an integrated, whole organ systems approach that also includes a pharmacological component. Applications without all of these components will not be considered.

Duration: No more than six awards will be made, each of which will be for one year duration. It is anticipated that the first awards will begin January 2013 and end December 2013.

Terms: The awards provide funding payable to the institution. The stipends may be supplemented with institutional funds or other research grants. No indirect costs are provided.

For Postdoctoral Awards:

- $30,000 stipend
- $ 2,000 supplies

For Graduate Student Awards:

- $20,000 stipend
- $ 2,000 supplies

Application Guidelines: The application deadline is October 15, 2012. Applicants will be notified by November 15, 2012 and awards will begin January 2013. Awardees will be expected to attend the Experimental Biology Meeting in the year of their award and will be honored at the ASPET Opening Awards Ceremony.

Applications should contain the following materials:

1. A letter from mentor or department chair supporting the candidate's application for the ASPET Graduate & Postdoctoral Award for Integrative Research in Pharmacology. The letter should: a) identify/acknowledge the mentor supervising the candidate's research; and b) identify existing levels of support for ongoing research.
2. If applicable, copies verifying the applicant's active (current for 2013) visa status, e.g., H1B1.
3. The applicant's curriculum vitae.
4. A research proposal no longer than 4 pages. The applicant can attach supplemental data if they wish.

Application materials should be sent (as a PDF or Word file) to **jbernstein@aspet.org**. Applications received after October 15 or incomplete applications will not be considered.

Questions? Contact **Jim Bernstein**: 301-634-7062 or **jbernstein@aspet.org**.

Public Affairs

by Jim Bernstein

How Can ASPET Members Help Sustain Funding for Biomedical Research?

ASPET's grassroots congressional education efforts are designed to help inform members to become effective public advocates for biomedical research. As a scientist, you are the most effective advocate for the biomedical science research enterprise – and the most credible too.

Toward this end, ASPET encourages its members visiting Washington, DC/Bethesda for NIH or other business to make Capitol Hill visits to your Congressional delegation. To help facilitate this, ASPET will assume the costs of an extra night hotel stay following the conclusion of your official business. So now ASPET members will be able to conclude their professional business one afternoon, stay an additional night, visit Capitol Hill offices the next day, and fly home later that afternoon or early evening. ASPET will make all Congressional meeting arrangements, provide talking points, etc., and have you well prepared for the day of advocacy.

ASPET has also created the successful *Advocacy Outreach Program*. This effort is designed to develop awareness in graduate students, postdocs, and faculty of the need for enhanced biomedical research advocacy. The discussion/presentation provides an overview of the political and economic environment impacting NIH funding and the skills needed to allow scientist-advocates to help influence the debate. ASPET has made presentations (typically one-hour duration) at the University of Louisville, UT Southwestern Medical Center, Emory University, Michigan Pharmacology Colloquium at Wayne State, Vanderbilt University Medical Center, and the Drexel College of Medicine. ASPET's Advocacy Outreach is often part of the institution's postdoctoral student association's scheduled meetings or talks. There is no financial obligation to your institution or department. ASPET assumes hotel and travel costs.

Also in development is the *ASPET Washington Fellows Program* that will allow graduate students, postdoctoral trainees and early career scientists an opportunity to become more engaged in public policy issues. Fellows will be brought to Capitol Hill to meet with their Congressional delegation, maintain contact with their local district office, write op-ed pieces in support of biomedical research, etc. In each of these activities, Fellows will be well trained by ASPET on the critical messages we need to constantly assure that NIH funding is sustained. Very shortly, ASPET members will get all the details of the Washington Fellows Program.

For information on any of the above mentioned ASPET member grassroots advocacy efforts, please contact **Jim Bernstein**, ASPET's Director of Government and Public Affairs at 301-634-7062; **jbernstein@aspet.org**. Also visit the Grassroots Advocacy Page for additional information: **http://www.aspet.org/Advocacy/Grassroots**.

Legislators need to hear from you that the investment in biomedical research should be a national priority. If you don't take the time to help, who will?

FASEB, Research!America Illustrate Sequestration's Devastating Impact to Medical Research

Passage last August of the Budget Control Act (BCA) of 2011 requires a $1.2 trillion cut from the federal budget. The BCA would require across-the-board sequestration, or cuts to all discretionary programs (and some mandatory ones too) beginning January 1, 2013.

FASEB and Research!America have published two documents that detail the potential impact that sequestration would have on biomedical research.

The FASEB analysis predicts an 11.1 percent ($2.8 billion) reduction of the NIH extramural budget. The analysis also projects extramural research funding reductions for the nation and for each state in 2013. The FASEB model analysis does not take into account inflation or the potential of shifting the burden of sequestration from defense to non-defense discretionary programs such as the NIH, potentially making for far deeper cuts: **http://www.aspet.org/PolicyUpdatesNews.aspx?id=3252**.

Public Affairs

by Jim Bernstein

Research!America's report, *Sequestration: Health Research at the Breaking Point*, looks at the damaging consequences of potential automatic spending cuts, or sequestration, to the nation's medical research enterprise and public health, and offers specific examples on how these cuts would delay scientific discoveries. The report provides an estimate of the budget cuts to the National Institutes of Health, FDA, CDC, NSF and the Centers for Disease Control and Prevention. Statements and testimonials from the leaders of these federal health agencies, as well as the patient community, academia, and industry are available at: **http://www.aspet.org/PolicyUpdatesNews.aspx?id=3379**.

House, Senate Begin Move to FY '13 Spending Decisions

House and Senate appropriators continue to move toward finishing FY 2013 spending decisions for federal agencies. Last April, the House and Senate finalized their total levels of funding available to all twelve appropriations committees. The Senate allocation of $1.047 trillion is consistent with the level enacted under the Budget Control Act last summer. However, the House Appropriations Committee approved a $1.028 trillion budget to divide among its twelve subcommittees. The House figure is $19 billion below the Senate allocation.

As a result of the lower House allocation, almost all House Subcommittees, including those funding NIH and FDA, received allocations lower than their FY 2012 level. The exceptions to this rule were the Energy & Water and Defense Appropriations Subcommittees. The higher Senate numbers allowed for some modest increases to a number of Senate Subcommittees.

The Labor, Health and Human Services, Education, and Related Agencies House Subcommittee that funds the NIH received a total allocation of $150 billion. This is a $7.1 billion reduction or 4.5% cut from the amount the Labor/HHS Subcommittee received last year. The Senate Labor/HHS Subcommittee received a total allocation of $157 billion. This is a $588 million or 0.4% increase above the amount the Subcommittee received last year.

It is expected that the Senate Labor/HHS bills will mark up the bill funding NIH sometime in mid-June. No House Labor/HHS Subcommittee markup has been scheduled and it is possible no action will be taken in that chamber. Without a final spending decision for NIH by the end of the fiscal year, September 30, Congress will once again have to enact a Continuing Resolution (CR) that funds government agencies, most likely at their current level. If a final spending decision is not made, another CR would be enacted. That will be a difficult task in a contentious election year, with highly charged political/economic decisions to be made over extending Bush-era tax cuts, raising the debt ceiling limit, and the threat of sequestration.

The House Agriculture Subcommittee that funds FDA received an allocation of $19.4 billion. This is a $375 million or 1.89% cut from the amount the Agriculture Subcommittee received last year. The Senate Agriculture Subcommittee allocation is $20.8 billion. This is a $1 billion or 5% increase over the amount the Subcommittee received last year. On May 5, the Senate Appropriations Committee passed the FY 2013 Agriculture Appropriations Bill that includes an appropriation of $2.524 billion for the FDA, an $18.7 million increase over the FY 2012 enacted level. Adding industry user fees brings the FDA to a total of $3.910 billion, a $19.8 million increase over FY 2012.

While it is not yet clear how NIH will fare, there is some hopeful news for NIH as the House and Senate approved funding increases for NSF, NASA, NOAA, and NIST.

Office of Management & Budget (OMB) Memo Directs Agencies to Cut in FY '14

OMB has advised all department and agency directors to cut FY 2014 budget requests by 5% below total FY 2013 discretionary spending levels. The memo advises agencies to control spending as dictated by the Budget Control Act of 2011 and to cut lower-priority spending to allow for continued investment in "areas critical to economic growth and job creation, including education, innovation, infrastructure, and research and development."

Public Affairs

by Jim Bernstein

Written Testimony of the American Society for Pharmacology & Experimental Therapeutics: Submitted to the House and Senate Appropriations Subcommittees for Labor, Health and Human Services, Education, and Related Agencies

Fiscal Year 2013 Appropriations for the National Institutes of Health

The American Society for Pharmacology and Experimental Therapeutics (ASPET) is pleased to submit written testimony in support of the National Institutes of Health (NIH) FY 2013 budget. ASPET is a 5,100 member scientific society whose members conduct basic, translational, and clinical pharmacological research within the academic, industrial and government sectors. Our members discover and develop new medicines and therapeutic agents that fight existing and emerging diseases, as well as increase our knowledge regarding how therapeutics affect humans.

ASPET recommends a budget of at least $32 billion for the NIH in FY 2013. Research funded by the NIH improves public health, stimulates our economy, and improves global competitiveness. Sustained growth for the NIH should be an urgent national priority. Flat funding or cuts to the NIH budget will delay advances in medical research, jeopardizing potential cures; eliminate jobs, and threaten American leadership and innovation in biomedical research.

A $32 billion budget for the NIH in FY 2013 will provide a modest 4% increase to the agency and help restore NIH to more sustainable growth. Currently, the NIH cannot begin to fund all the high quality research that needs to be accomplished. After several years of flat funding and spending cuts enacted in 2011, the NIH's funding environment has reached a critical point:

- Adjusted for inflation, the FY 2012 budget and the President's FY 2013 budget proposal are $4 billion lower than the peak year of FY 2003;
- The number of research project grants funded by NIH has declined every year since 2004, and NIH is projected to fund 3,100 fewer grants in FY 2012-2013 than in FY 2004;
- Success rates have fallen more than 14% in a decade and are projected to decline further in FY 2012 and FY 2013.

If flat funding continues or if additional cuts are mandated to the NIH budget for FY 2013 and beyond, research that improves the quality of life will be delayed or stopped, and fewer clinical trials will be conducted. International competitors will continue to gain on this highly innovative U.S. enterprise, and we will lose a generation of young scientists who see no prospects for careers in biomedical research. Flat or reduced funding for NIH will mean that the agency would have to dramatically reduce new awards, and many research projects in progress would not receive sufficient funding to complete ongoing work, thus representing a waste of valuable research resources.

An FY 2013 NIH budget of $32 billion would help to begin to restore momentum to NIH funding. A $32 billion FY 2013 NIH budget will help the agency manage its research portfolio effectively without too much disruption of existing grants to researchers throughout the country. The NIH, and the entire scientific enterprise, cannot rationally manage boom or bust funding cycles. Scientific research takes time. Only through steady, sustainable and predictable funding increases can NIH continue to fund the highest quality biomedical research to help improve the health of all Americans and continue to make significant economic impact in many communities across the country. An FY 2013 NIH budget of $32 billion will help NIH move to more fully exploit promising areas of biomedical research and translate the resulting findings into improved health care.

Public Affairs

by Jim Bernstein

Diminished Support for NIH will Negatively Impact Human Health

Diminished funding for NIH will mean a loss of scientific opportunities to discover new therapeutic targets and will create disincentives to young scientists to commit to careers in biomedical science. A difficult federal funding environment becomes more problematic as economic difficulties have led to less investment by the pharmaceutical industry and diminished venture capital needed by the biotech industry. Previous investments in NIH research have been instrumental in improving human health. However, a greater investment in research is needed to help improve the lives of many afflicted by chronic diseases:

- Parkinson's disease is estimated to afflict over one million Americans at an annual cost of $26 billion. The discovery of Levodopa was a breakthrough in treating the disease and allows patients to lead relatively normal, productive lives. It is estimated that treatments slowing the progress of disease by 10% could save the U.S. $327 million a year. Current treatments slow progression of the disease, but more research is needed to identify the causes of the disease and help to develop better therapies.
- More than 38 million Americans are blind or visually impaired, and that number will grow with an aging population. Eye disease and vision loss cost the U.S. $68 billion annually. NIH funded research has developed new treatments that delay or prevent diabetic retinopathy, saving $1.6 billion a year. Discovery of gene variations in age-related macular degeneration could result in new screening tests and preventive therapies.
- One in eight older Americans suffer from Alzheimer's disease at annual costs of more than $200 billion. It is estimated that by 2050 more than 14 million Americans will live with the disease with projected costs of $1.1 trillion (in 2012 dollars). Although there are new clinical candidates for Alzheimer's disease in development, more basic research is needed to focus on new molecular targets and potential cures for this disease. Inadequate funding will delay and prevent improved treatment of the disease.
- Heart disease and stroke are the number one and three killers of Americans, respectively. Cardiovascular disease costs the U.S. more than $350 billion annually. Death rates from cardiovascular disease have fallen by 50% since 1970. Statin drugs that reduce cholesterol help to prevent heart disease and stroke, decrease recurrence of heart attacks, and improve survival rates for heart transplant patients.
- Cancer is the second leading cause of death in the U.S. The NIH estimates that the annual cost of the disease is over $228 billion. NIH research has shown that human papillomavirus (HPV) vaccines protect against persistent infection by the two types of HPV that cause approximately 70% of cervical cancers. NIH funded researchers are using nanotechnology to develop probes that could pinpoint the location of tumors and deliver drugs directly to cancer cells. NIH funded basic research built the foundation for one of the most revolutionary FDA approved new treatments for melanoma and helped launch the era of modern personalized medicine.
- NIH-funded investigators discovered an enzyme that may act as a tumor suppressor, therapeutic target, and clinical biomarker in patients with colorectal cancer. Clinical trials are now underway to study its role as a possible novel chemoprevention approach to prevent colorectal cancer and determine the utility of the enzyme as a prognostic and predictive marker for staging patients with disease. The enzyme is also being used as a vaccine target to prevent recurrent disease. Studies are underway evaluating this enzyme's role in regulating appetite and as a possible novel therapeutic target to prevent obesity, diabetes, and metabolic syndrome.
- Finding new uses for existing drugs is difficult but could be life saving and cost effective. NIH-funded researchers using new bioinformatic approaches have discovered that a drug designed to treat heartburn also inhibited the growth of human lung tumors in laboratory mice. Without adequate support for NIH funding, this type of discovery may become impossible and potential clinical benefits will not be realized.

by Jim Bernstein

- There are almost 7,000 rare diseases, each afflicting fewer than 200,000 individuals. More than 350 drugs have been approved for rare diseases since passage of the Orphan Drug Act in 1983. The number of new drugs in development is increasing rapidly as researchers gain a better understanding of the underlying molecular and genetic causes of disease. Diminished support for NIH will prevent new and ongoing investigations into rare diseases of which the FDA estimates almost 90% are serious or life-threatening.

NIH-funded studies have also indicated that adopting intensive lifestyle changes delayed onset of type-2 diabetes by 58% and that progesterone therapy can reduce premature births by 30% in at-risk women. Historically, our past investment in basic biological research has led to many innovative medicines. The National Research Council reported that of the 21 drugs with the highest therapeutic impact, only five were developed without input from the public sector. The significant past investment in the NIH has provided major gains in our knowledge of the human genome, resulting in the promise of pharmacogenomics and a reduction in adverse drug reactions that currently represent a major worldwide health concern. Already, there are several examples where complete human genome sequence analysis has pinpointed disease-causing variants that have led to improved therapy and cures. Although the costs for such analyses have been reduced dramatically by technology improvements, widespread use of this approach will require further improvements in technology that will be delayed or obstructed by inadequate NIH funding.

Investing in NIH Helps America Compete Economically

A $32 billion budget in FY 2013 will also help the NIH train the next generation of scientists. This investment will help to create jobs and promote economic growth. Limiting or cutting the NIH budget will mean forfeiting future discoveries to other countries.

Worldwide, other nations continue to invest aggressively in science. China has grown its science portfolio with annual increases to the research and development budget averaging over 23% annually since 2000. And while Great Britain has imposed strict austerity measures to address that nation's debt problems, the British conservative party had the foresight to keep its strategic investments in science at current levels. The European Union, despite austerity measures and the severe debt problems of its member nations, has proposed to increase spending on research and innovation by 45% between 2014 and 2020.

NIH research funding catalyzes private sector growth. More than 83% of NIH funding is awarded to over 3,000 universities, medical schools, teaching hospitals, and other research institutions in every state. One national study by an economic consulting firm found that federal (and state) funded research at the nation's medical schools and hospitals supported almost 300,000 jobs and added nearly $45 billion to the U.S. economy. NIH funding also provides the most significant scientific innovations of the pharmaceutical and biotechnology industries.

Inadequate funding for NIH means more than a loss of scientific potential and discovery. As we have noted, failing to help meet the NIH's scientific potential has led to a significant reduction in research grants and the resulting phasing-out of high quality research programs and jobs lost.

Conclusion

ASPET appreciates the many competing and important spending decisions the Subcommittee must make. The nation's deficit and debt problems are numerous. However, NIH and the biomedical research enterprise face a critical moment. The agency's contribution to the nation's economic and physical well being should make it one of the nation's top priorities. With enhanced and sustained funding, NIH has the potential to address many of the more promising scientific opportunities that currently challenge medicine. A $32 billion FY 2013 NIH budget will allow the agency to begin moving forward to full program capacity; exploiting more scientific opportunities for investigation; and increasing investigator's chances of discoveries that prevent, diagnose, and treat disease. NIH should be restored to its role as a national treasure, one that attracts and retains the best and brightest to biomedical research and provides hope to millions of individuals afflicted with illness and disease.

Members in the News

Kenneth A. Jacobson, PhD

Medicinal chemist **Kenneth A. Jacobson, PhD**, Chief of the Laboratory of Bioorganic Chemistry and the Molecular Recognition Section at the National Institute of Diabetes and Digestive and Kidney Diseases, was selected for the honor of the third Philip S. Portoghese Medicinal Chemistry Lectureship. This award is funded by the ACS' *Journal of Medicinal Chemistry*. Dr. Jacobson's research interests include focusing on the structure and pharmacology of G protein-coupled receptors (GPCRs). In 2009, he received the Pharmacia-ASPET Award in Experimental Therapeutics.

Curtis D. Klaassen, PhD

Curtis D. Klaassen, PhD, University Distinguished Professor at the University of Kansas Medical Center, was presented the Merit Award by the Society of Toxicology (SOT) at their 2012 annual meeting. Since 1966, the Merit Award, SOT's greatest honor, has been presented to recognize career-long contributions to the field of toxicology in research, teaching, regulatory activities, consulting and service to SOT. Dr. Klaassen has spent the last 44 years working for the University of Kansas Medical Center.

Garrett John Gross, PhD

Garrett John Gross, PhD, Professor of Pharmacology and Toxicology at Wisconsin Medical College, received funding to study compounds that may protect the heart from tissue damage related to ischemic heart disease. The five-year, $2.2 million grant to advance the knowledge of cardiovascular disease came from the National Institutes of Health's National Heart, Lung and Blood Institute. Gross will focus his grant research on epoxyeicosatrienic acids (EETs). Previous studies on dogs, rats and mice have shown that these lipids protect animals' hearts. Dr. Gross received the Benedict R. Lucchesi Distinguished Lectureship in Cardiovascular Pharmacology in 2007.

Attention ASPET Members: What are you up to?

Share your news with fellow ASPET members!
Send news and photos to gaxelrod@aspet.org

Staff News

Gary Axelrod

Gary Axelrod joined ASPET as Web and Social Media Manager in March 2012. He is responsible for writing an autobiographical blurb for the Staff News in *The Pharmacologist* in addition to serving as the Editor of *The Pharmacologist*, maintaining the ASPET website and social media properties, and various other marketing related projects. Gary comes to ASPET after a number of years of marketing and communications experience in healthcare and the nonprofit sector, most recently with the American Kidney Fund and American Gastroenterological Association. He is a fan of Cleveland Indians baseball and great collegiate athletic rivalries, having earned his B.A. in Communication Studies from the University of Michigan after growing up in Ohio State territory. Gary is also a rabid follower of NHL hockey, having previously interned at the NHL Office in NY back in 2004, and he enjoys seeing the sights and exploring nature in and around Washington, DC with his wife Ali.

Laine Cocca

Laine Cocca, who was Director of Finance and Customer Service, left ASPET in May 2012 to pursue a career with Snyder Cohn CPAs and Business Advisors as a Manager of Outsourced Accounting at Snyder Cohn, a CPA and business advisory firm located in North Bethesda, MD. Paul Linzer has taken over her responsibilities.

Paul Linzer

Paul Linzer joined ASPET in late April 2012 as an interim replacement for Laine Cocca, Director of Finance and Customer Service. Paul, the Interim Director of Accounting, has spent much of his career in the healthcare industry, most recently with Suburban Hospital in Bethesda, MD. He and his wife Maia enjoy traveling and fawning over their grandchildren.

New ASPET Members

Regular Members

Caroline E. Bass, Univ. at Buffalo, SUNY
Bruce R. Bianchi, Abbott
Yingzi Chang, A.T. Still Univ.
Stewart D. Clark, Univ. at Buffalo, SUNY
Ryan M. Drenan, Purdue Univ.
Emine Ercikan Abali, Robert Wood Johnson Medical School-UMDNJ
Raymond Evers, Merck
Carlos A. Feleder, Albany College of Pharmacy and Health Sciences
Michael R. Garrett, Univ. of Mississippi Medical Center
Leila Gobejishvili, Univ. of Louisville
Ceren Gonen-Korkmaz, Ege Univ. Fac. Pharmacy
Samir Haj-Dahmane, Univ. at Buffalo
John Harrelson, Pacific Univ. School of Pharmacy
Yoshiyuki Horio, Sapporo Medical Univ.
Pamela J. Hornby, Janssen R&D
Paul J. Kammermeier, Univ. of Rochester Medical Center
Patricia J. Keely, Univ. of Wisconsin
Richard N. Kolesnick, Memorial Sloan Kettering Cancer Center
Hung Wen (Kevin) Lin, Univ. of Miami, Miller School of Medicine
Ioan I. Magyar, Univ. of Oradea Faculty of Medicine & Pharmacy
J. Michael M. Mathis, Louisiana State Univ. HSC
Alexander J. Mcnamara, Theravance Inc.
Patrick J. Murphy, Seattle Univ.
Raphael A. Nemenoff, Univ. of Colorado Denver
Kimberly Nixon, Univ. of Kentucky
Yan Pan, Peking Univ. Health Science Center
Dennis Pantazatos, Rhode Island Hospital Brown Medical School
Ronald T. Raines, Univ. of Wisconsin-Madison
Joseph K. Ritter, Virginia Commonwealth Univ.
Aaron M. Rowland, New Mexico State Univ.
Francesca Seta, Boston Univ.
Gautam Sethi, Yong Loo Lin School of Medicine
Misty D. Smith, Univ. of Utah
Bruno Stieger, Univ. of Zurich Hospital
Asha Suryanarayanan, Manchester College School of Pharmacy
Colin L. Willis, Univ. of New England
Jay Yang, Univ. of Wisconsin-Madison
Qingyu S. Zhou, Univ. of South Florida College of Pharmacy

Postdoctoral Members

Yubo Chai, Mayo Clinic
John W. Craft, Univ. of Houston
Khalil M. Eldeeb, Wake Forest Univ. Health Sciences
Tommaso Iannitti, Univ. of Kentucky
Xin Li, Univ. of Tennessee-HSC
Michele Paulo, Univ. of Sao Paulo
Xiulong Song, Univ. of Rhode Island
Vivek R. Yadav, Oklahoma Univ. HSC College of Pharmacy
Dmitry D. Zhdanov, Univ. of Arkansas Medical Sciences

Affiliate Members

Nakon Aroonsakool, Univ. of California-San Diego
Swati Betharia, Manchester College School of Pharmacy
Kiran K. Gogi, Vanderbilt Medical Center
Hiroyuki Kosuge, Medlet Japan KK
Soban Sadiq, Azad Jammu & Kashmir Medical Coll.
Franklin B. Shilliday, MPI Research
Larry Tam, Univ. of California-San Francisco Med. Ctr.
Jocelyn Yabut, Merck & Co.

New ASPET Members

Graduate Student Members

Tamer M. Abdelghany, The Ohio State Univ.
Risikat O. Ajibola, Portland State Univ.
Kristen A. Andersen, Univ. of Wisconsin-Madison
Elizabeth S. Barrie, The Ohio State Univ.
Amanda Binkey, Ohio Northern Univ.
Deborah L. Capes, Univ. of Wisconsin
Stephanie R. Chapelliquen, Stony Brook Univ.
Jessica L. Faulkner, Univ. of Mississippi Medical Center
Melissa Geyer, Univl of Illinois-Chicago
Girish K. Gupta, MM College of Pharmacy
Brandon G. Hillman, Creighton Univ.
Mary W. Hulin, LSU Health Sciences Center-New Orleans
Ashish K. Jena, Univ. Institute of Pharmaceutical Sciences
Mohammed Khalid, Luqman College of Pharmacy
Ja Hyun Koo, Seoul National Univ.
Vijayanarayana Kunhikatta, Manipal College of Pharmaceutical Sciences
Vivek J. Lawana
Lalit Machawal, Univ. Institute of Pharmaceutical Sciences
David A. Martin, Louisiana State Univ. HSC
Ilya Miadzvedski, The Belasian State Medical Univ.
Felix J. Nau, Louisiana State Univ. HSC
Nicole L. Nicholson, Univ. of Southern California
Ami Patel, Long Island Univ.
Jason L. Quinones, SUNY Stony Brook Univ.
Sylvia Radzikowski, Univ. of Michigan
Kellianne J. Richardson, Virginia Commonwealth Univ.
Puneet Rinwa, Univ. Institute of Pharmaceutical Sciences
Lindsey K. Roper, Univ. of Wisconsin-Madison
Andrew S. Sage, Univ. of Missouri
Roshan K. Sahu, Babasaheb Bhimrao Ambedkar Central Univ.
Nayna Sanathara, Univ. of California-Irvine
Marquitta L. Smith, Meharry Medical College
Sucharita S. Somkuwar, Univ. of Kentucky
Brittany L. Speer, Duke Univ. Medical Center
Satya Murthy Tadinada, Idaho State Univ.
Jaime E. Vantrease, Loyola Univ. of Chicago Stritch School of Medicine

Undergraduate Members

Eman Abu Alhana, Univ. of Toledo
Murad H. Al-Salamat, Jordan Univ. of Science and Technology
Lillian Assatourian, Univ. of Maryland-Baltimore County
Alexandra D. Blumer, Clemson Univ.
Kris A. Burner, Franciscan Univ. of Steubenville
Roger T. Day, Vanderbilt Univ.
Alicia A. Ericson, Robert Morris Univ.
Lindsay M. Gasper, Univ. of Toledo
Dennis Lee, New York Univ.
Caitlin L. Li, Vanderbilt Univ.
Agnieszka M. Maniak, Univ. of Illinois Urbana Champaign
Desiree Markantone, Univ. of Pittsburgh
Adam Morgenlander, Brown Univ.
Ashley C. Okonta, Univ. of Notre Dame
Rita E. Pfeiffer, Vanderbilt Univ.
Andy V. Phan, Univ. of Arizona
George Polovin, California State Univ. - Long Beach
Ari B. Romans, Univ. of Arizona
Katie A. Schappacher, Ohio Univ.
Phillip Taylor, Ohio Northern Univ.
Diana Townsend, Marine Maritime Academy
Selamawit Woldemeskel, Saint Francis College-Loretto, PA
Alison Zeccola, Univ. of Pittsburgh

In Sympathy

ASPET notes with sympathy the passing of the following Member: Amy Jo Campbell, PhD

Obituary

Amy Jo Campbell, PhD (1977-2010)

Amy passed away suddenly Friday, June 11, 2010. She was born Nov. 30, 1977. She graduated from Hatton High School, valedictorian 1996. She graduated from the University of North Alabama in May 2001, magna cum laude. Amy earned her Ph.D. at Georgia Tech in December 2005.

Her employment since graduating included Walter Reed Army Institute of Research, serving as a chemist in Silver Springs, Md., and most recently as a Battelle scientific contractor with the U.S. Army Medical Research Institute of Chemical Defense in Aberdeen, Md.

She was published six times for her discoveries in organic chemistry. She is also named on two patent submissions.

She received the Siple Award at the 26th Army Science Conference in 2008.

Survivors include her parents, Tom and Gail Campbell; two sisters, Jill White and Mary Lindley; two brothers-in-laws; four nieces and nephews; many friends and extended family.

Published in Florence Times Daily on June 17, 2010

Call for Award Nominations

John J. Abel Award

Deadline for submissions is September 15, 2012.

The John J. Abel Award in Pharmacology, named after the founder of ASPET and sponsored by Pfizer, was established to stimulate fundamental research in pharmacology and experimental therapeutics by young investigators. The annual Award consists of $5,000, a plaque, hotel, and economy airfare for the winner and spouse to the award ceremony at the annual meeting of ASPET. The winner will be invited to give a lecture at the annual meeting.

Nominees for this award shall not have passed his/her **forty-fifth birthday by September 15 (nomination deadline) of the year in which s/he is nominated**. The candidate need not be a member of the Society; however, the nomination must be made by an ASPET member. No member may nominate more than one candidate a year, and no candidate may be nominated for more than one major ASPET award in any given year.

The Award shall be made for original, outstanding research in the field of pharmacology and/or experimental therapeutics. Independence of thought, originality of approach, clarity, and excellence of data presentation are important criteria. Candidates shall not be judged in comparison with the work of more mature and experienced investigators. Quality rather than the number of contributions shall be emphasized. It shall be the responsibility of the sponsor to make clear the contribution of the candidate to any jointly authored reprints and manuscripts and the originality and independence of the candidate's research. Selection will be made by the ASPET Awards Committee, appointed by the President of ASPET.

Nominations must be submitted **electronically** to **awards@aspet.org** and shall consist of:

1. Letter of nomination with a summary that describes the importance of the candidate's work.
2. Brief biographical sketch of the candidate.
3. Candidate's curriculum vitae and bibliography.
4. Six published articles or manuscripts accepted for publication that are a representation of the candidate's work (provided as PDFs or as hyperlinks to the article). **Submit each manuscript as a separate attachment.**

Nominations for this award must be received no later than 5:00 pm (EST) on **September 15** of the year prior to the year in which it is to be awarded.

The John J. Abel Award is sponsored by Pfizer, which is pleased to support the recognition of young scientists who will provide the breakthroughs of tomorrow.

Recipients of the John J. Abel Award in Pharmacology

1947	George Sayers	1969	Ronald Kuntzman	1991	Terry D. Reisine
1948	J. Garrott Allen	1970	Solomon H. Snyder	1992	Frank J. Gonzalez
1949	Mark Nickerson	1971	Thomas R. Tephly	1993	Susan G. Amara
1950	George B. Koelle	1972	Pedro Cuatrecasas	1994	Brian Kobilka
1951	Walter F. Riker, Jr.	1973	Colin F. Chignell	1995	Thomas M. Michel
1952	David F. Marsh	1974	Philip Needleman	1996	John D. Scott
1953	Herbert L. Borison	1975	Alfred G. Gilman	1997	David J. Mangelsdorf
1954	Eva K. Killam	1976	Alan P. Poland	1998	Masashi Yanigasawa
1955	Theodore M. Brody	1977	Jerry R. Mitchell	1999	Donald P. McDonnell
1956	Fred W. Schueler	1978	Robert J. Lefkowitz	2000	William C. Sessa
1957	Dixon M. Woodbury	1979	Joseph T. Coyle	2002	Steven A. Kliewer
1958	H. George Mandel	1980	Salvatore J. Enna	2003	David S. Bredt
1959	Parkhurst A. Shore	1981	Sydney D. Nelson	2004	David P. Siderovski
1960	Jack L. Strominger	1982	Theodore A. Slotkin	2005	Randy Hall
1961	Don W. Esplin	1983	Richard J. Miller	2006	Christopher M. Counter
1962	John P. Long	1984	F. Peter Guengerich	2007	Michael D. Ehlers
1963	Steven E. Mayer	1985	P. Michael Conn	2008	Katarina Akassoglou
1964	James R. Fouts	1986	Gordon M. Ringold	2009	John J. Tesmer
1965	Eugene Braunwald	1987	Lee E. Limbird	2010	Russell DeBose-Boyd
1966	Lewis S. Schanker	1988	Robert R. Ruffolo, Jr.	2011	Laura M. Bohn
1967	Frank S. LaBella	1989	Kenneth P. Minneman	2012	Jin Zhang
1968	Richard J. Wurtman	1990	Alan R. Saltiel		

Call for Award Nominations

Julius Axelrod Award in Pharmacology

Deadline for submissions is September 15, 2012.

The Julius Axelrod Award in Pharmacology was established to honor the memory of the eminent American pharmacologist who shaped the fields of neuroscience, drug metabolism, and biochemistry and who served as a mentor for numerous eminent pharmacologists around the world. The Julius Axelrod Award is presented annually for significant contributions to understanding the biochemical mechanisms underlying the pharmacological actions of drugs and for contributions to mentoring other pharmacologists.

The award consists of an honorarium of $2,500, a medal, hotel, and economy airfare for the winner and spouse to the annual meeting. The formal presentation of this award and medal will be made at the annual meeting of ASPET. The recipient will be invited by the President of the Society to deliver the Julius Axelrod Lecture and organize the Julius Axelrod Symposium at the annual meeting a year hence. The recipient will also be invited by the Catecholamine Club to give a less formal presentation at its annual dinner meeting that year of the award.

There are no restrictions on nominees for this award. However, the nomination must be made by a member of the American Society for Pharmacology and Experimental Therapeutics (ASPET) or the Catecholamine Club. No member may nominate more than one candidate in a year, and no candidate may be nominated for more than one major ASPET award in any given year. The award shall be made on the basis of originality and uniqueness of accomplishments throughout a long career distinguished by sustained, significant contributions to research, and mentoring in pharmacology. Selection of the recipient will be made by the Axelrod Award Committee, appointed by the President of ASPET and comprised of members of ASPET and the Catecholamine Club.

Nominations shall be submitted **electronically** to **awards@aspet.org** and shall consist of:

1. Letter of nomination describing the research and mentoring contributions to pharmacology of the candidate that make him/her eligible for this Award, listing major contributions. Up to two additional letters of support would be welcome (need not be from ASPET members).
2. Brief biographical sketch of the candidate.
3. List of individuals mentored by the individual. Up to two letters from former trainees describing the quality of their training with the nominee and its impact on their careers would be welcome (need not be from ASPET members).
4. Candidate's curriculum vitae and bibliography.

Receipt date for nominations for the Julius Axelrod Award will be 5:00 pm (EST) on **September 15** of the year prior to the year in which the award is to be given.

Recipients of the Julius Axelrod Award

1991	Ullrich Trendelenberg
1992	Arvid Carlsson
1993	Norman Weiner
1994	Robert Furchgott
1995	Irvin Kopin
1998	Sidney Spector
1999	Solomon Snyder
2000	Erminio Costa
2001	Toshi Nagatsu
2002	Salomon Langer
2003	Richard Weinshilboum
2004	Richard Palmiter
2005	Marc Caron
2006	Susan Amara
2007	Tong H. Joh
2008	Randy D. Blakely
2009	Palmer W. Taylor
2010	Brian Kobilka
2011	Elaine Sanders-Bush
2012	Gavril W. Pasternak

Call for Award Nominations

Pharmacia-ASPET Award for Experimental Therapeutics

Deadline for submissions is September 15, 2012.

The Pharmacia-ASPET Award in Experimental Therapeutics is given annually to recognize and stimulate outstanding research in pharmacology and experimental therapeutics—basic laboratory or clinical research that has had, or potentially will have, a major impact on the pharmacological treatment of disease. The award is supported in perpetuity by a gift from Pharmacia (now Pfizer).

The winner will receive a $2,500 honorarium, a plaque, hotel, and economy airfare for the winner and spouse to the award ceremony at the ASPET annual meeting.

There are no restrictions on nominees for this award. The candidate need not be a member of the Society; however, the nomination must be made by an ASPET member. No member may nominate more than one candidate a year and no candidate may be nominated for more than one major ASPET award in any given year. The Award shall be made on the basis of published reprints, manuscripts ready for publication, and a two-page summary. Selection will be made by the ASPET Awards Committee, appointed by the President of ASPET.

Nominations shall be submitted **electronically** to **awards@aspet.org** and shall consist of:

1. Letter of nomination with a two-page summary that details the importance of the candidate's work.
2. Brief biographical sketch of the candidate.
3. Candidate's curriculum vitae and bibliography.
4. Six articles published or ready for publication by the candidate that have direct bearing on the Award (provided as PDFs or as hyperlinks to the article). **Submit each manuscript PDF as a separate attachment.**

Nominations for this award must be received no later than 5:00 pm (EST) on **September 15** of the year prior to the one in which the award is to be made.

Recipients of the ASPET Award for Experimental Therapeutics

1969 John A. Oates
1970 Joseph R. Bertino
1971 Elliot S. Vesell
1972 Francois M. Abboud
1973 Dean T. Mason
1974 Leon I. Goldberg
1975 Mackenzie Walser
1976 Louis Lasagna
1977 Allan H. Conney
1978 Attallah Kappas
1979 Sydney Spector
1980 Sanford M. Rosenthal
1981 David G. Shand
1982 William H. Prusoff
1983 Marcus M. Reidenberg
1984 Sir James Black
1985 Louis Lemberger
1986 Alan C. Sartorelli
1987 Albrecht Fleckenstein
1988 Jean-Francois Borel
1989 Benedict R. Lucchesi
1990 Albert Sjoerdsma
1991 Theophile Godfraind
1992 James W. Fisher
1993 V. Craig Jordan
1994 Susan Band Horwitz
1995 Henry I. Yamamura
1996 Robert F. Furchgott
1997 Michael M. Gottesman
1998 Phil Skolnick
1999 Yung-Chi Chen
2000 Salomon Z. Langer
2001 George Breese

Became Pharmacia-ASPET Award in Experimental Therapeutics

2002 Darryle D. Schoepp
2003 William C. De Groat
2004 Philip Needleman
2005 Donald P. McDonnell
2006 John C. Lee
2007 P. Jeffrey Conn
2008 Jerry J. Buccafusco
2009 Kenneth A. Jacobson
2010 Garret A. FitzGerald
2011 Jan Balzarini
2012 Angela H. Brodie

Call for Award Nominations

Robert R. Ruffolo Career Achievement Award in Pharmacology

Deadline for submissions is September 15, 2012.

The Robert R. Ruffolo Career Achievement Award in Pharmacology has been established in recognition of the contributions made to drug discovery and development by Dr. Ruffolo. The Award is presented annually to recognize the scientific achievements of scientists who are at the height of their careers (typically mid- to late-career) and who have made significant contributions to any area of pharmacology.

The award consists of a $2,500 honorarium, a commemorative medal, complimentary registration to the annual meeting, hotel, and economy airfare for the winner and his/her spouse to the award ceremony at the annual meeting.

There are no restrictions on nominees for this award. However, the nomination must be made by a member of the American Society for Pharmacology and Experimental Therapeutics (ASPET). No member may nominate more than one candidate in a year, and no candidate may be nominated for more than one major ASPET award in any given year. The award shall be made on the basis of the originality and impact of the nominee's accomplishments in pharmacology.Selection of the recipient will be made by the ASPET Awards Committee, appointed by the President of ASPET.

Nominations shall be submitted **electronically** to **awards@aspet.org** and shall consist of:

1. Letter of nomination with a summary that describes the importance of the candidate's work and his/her seminal discovery.
2. Brief biographical sketch of the candidate.
3. Candidate's curriculum vitae and bibliography.
4. Six published articles or manuscripts accepted for publication that are a representation of the candidate's work (provided as PDFs or as hyperlinks to the article), including early seminal discoveries. **Submit each manuscript PDF as a separate attachment.**

Receipt date for nominations for the Robert Ruffolo Award will be 5:00 pm (EST) on **September 15, 2012** for an award to be presented at Experimental Biology '13 in Boston, MA.

Recipient of the Robert R. Ruffolo Career Achievement Award

2012 Robert J. Lefkowitz

Recommend a Member!

Do you have a friend, colleague or student who is not yet a member of ASPET? Be sure to tell them about ASPET Membership and all the great benefits we provide including:

-Reduced registration fees for EB
-Discounted page charges to publish in our journals
-Award opportunites
-Plus much more!

Tell them to apply online at www.aspet.org

Call for Award Nominations

Division for Drug Metabolism Early Career Achievement Award

Deadline for submissions is September 15, 2012.

The ASPET Division for Drug Metabolism Early Career Achievement Award has been established to recognize excellent original research by early career investigators in the area of drug metabolism and disposition.

The award is presented biennially in odd-numbered years. The award consists of $1,000, a plaque, and complimentary registration plus travel expenses (to a maximum of $1,000) for the winner to attend the award ceremony at the annual meeting. The awardee will deliver a lecture at the annual meeting describing his/her relevant research accomplishments. The awardee will be invited to publish a review article on the subject matter of the award lecture in *Drug Metabolism and Disposition*.

Nominees for this award must have a doctoral degree (e.g., PhD, MD, PharmD, DVM) and must be within 15 years of having received their final degree, as of December 31 of the year of the award. There are no restrictions on institutional affiliation and a candidate need not be a member of ASPET. There is a requirement for two nominators, although more are acceptable. Nominators must be members of ASPET. An individual cannot nominate more than one candidate per award cycle.

Candidates who have made their research contributions in any sector (e.g., academia, industry, government) of the drug metabolism community may be nominated for the award. The primary criterion for the award is the level of excellence and originality of the research conducted by the candidate in the field of drug metabolism and disposition. Independence of thought, originality of approach, clarity of communication, and the impact of the work on the drug metabolism field are important considerations. Candidates shall not be judged in comparison with the work of more experienced investigators. Selection will be made by the Executive Committee of the Division for Drug Metabolism.

Nominations shall consist of the following components:

1. Two or more letters of nomination and support. The signed original and five copies of each letter should be submitted in hard copy format.
2. The five most significant published papers authored by the candidate. A detailed examination of these publications will form a primary basis for evaluation. Six copies of each paper should be submitted in hard-copy format.
3. A summary, limited to two pages, that describes the importance of the candidate's research contributions. This summary must include brief statements regarding the candidate's role in the five published papers and the overall significance and impact of the work. Submit as an email attachment in RTF or PDF format.
4. A brief biographical sketch of the candidate. Submit as an email attachment in RTF or PDF format.
5. The candidate's curriculum vitae and publication list. Submit as an email attachment in RTF or PDF format.

Nominations for this Award must be received no later than **September 15, 2012** by the Executive Officer, American Society for Pharmacology and Experimental Therapeutics, 9650 Rockville Pike, Bethesda, Maryland 20814-3995. Submit email attachments to **djordan@aspet.org**.

Recipients of the ASPET Division for Drug Metabolism Early Career Achievement Award

2007	Qiang Ma
2009	Wen Xie
2011	Emily E. Scott

Call for Award Nominations

Benedict R. Lucchesi Distinguished Lectureship in Cardiac Pharmacology

Division for Cardiovascular Pharmacology

Deadline for submissions is September 15, 2012.

The Benedict R. Lucchesi Award in Cardiac Pharmacology was established to honor Dr. Lucchesi's lifelong scientific contributions to our better understanding and appreciation of pharmacological treatment and prevention of cardiovascular disease and for his mentoring of countless prominent functional (in vivo) cardiovascular pharmacologists.

The Benedict R. Lucchesi Award is a biennial award, consisting an honorarium of $1,000, a custom-designed crystal bowl depicting the named Lectureship, and up to $2,000 travel expenses including registration to the annual spring ASPET meeting. A recipient will be selected and invited to deliver a state-of-the-art lecture on recent advances in the field of cardiac and electropharmacology at the spring ASPET meeting (Division's programming session). The presentation of his/her research should be of broad interest and contribute to the growth of the Cardiovascular Pharmacology Division.

There are no restrictions on institutional affiliation, nationality or age of the candidate, but the recipient must be a member of the ASPET. Nominations must be made by a member of the ASPET, and no member may nominate more than one candidate per year. Final selection of the recipient will be made by the Award Committee of the Division for Cardiovascular Pharmacology.

Nominations shall be submitted **electronically** to **awards@aspet.org** and shall consist of the following:

1. No more than five letters from nominators describing the candidate's contributions to cardiac and electropharmacology.
2. Listing of candidate's major contributions.
3. Candidate's curriculum vitae and bibliography.

Nominations for this Award must be received no later than 5:00 pm (EST) on **September 15, 2012**.

Recipients of the Benedict R. Lucchesi Award

2007	Garrett J. Gross
2009	Joan Heller Brown
2011	David J. Leffer

Keep in Touch...
Have you moved?
Changed your email address?
Changed jobs?
Keep us informed of changes to your contact information so that you don't miss out on any important ASPET news!
Email us at info@aspet.org

Call for Award Nominations

Torald Sollmann Award in Pharmacology Investigation and Education

Deadline for submissions is September 15, 2012.

The Torald Sollmann Award in Pharmacology was established to commemorate the pioneer work of Dr. Torald Sollmann in the fields of pharmacological investigation and education. The Torald Sollmann Award is presented biennially in odd years for significant contributions over many years to the advancement and extension of knowledge in the field of pharmacology.

The award consists of an honorarium of $2,500, a plaque, hotel, and economy airfare for the winner and spouse to the annual meeting. The formal presentation of this biennial award and plaque will be made at the annual meeting of ASPET.

There are no restrictions on nominees for this award; however, a nomination must be made by a member of the American Society for Pharmacology and Experimental Therapeutics (ASPET). No member may nominate more than one candidate in a year, and no candidate may be nominated for more than one major ASPET award in any given year. The award shall be made on the basis of originality and uniqueness of accomplishments throughout a long career distinguished by sustained, significant contributions to education, research, and service in pharmacology. Selection of the recipient will be made by the ASPET Awards Committee, appointed by the President.

Nominations shall be submitted **electronically** to **awards@aspet.org** and shall consist of:

1. **No more than** five letters from nominators describing the contributions to pharmacology of the candidate that make him/her eligible for this Award, listing major contributions.
2. Brief biographical sketch of the candidate.
3. Candidate's curriculum vitae and bibliography.

Nominations for this biennial Award must be received no later than 5:00 pm (EST) **September 15** of the year prior to the one in which the award is to be made.

Recipients of the Torald Sollmann Award in Pharmacology

1961	Otto Krayer
1963	Bernard B. Brodie
1966	Arnold D. Welch
1969	Earl W. Sutherland, Jr.
1973	Julius Axelrod
1975	Sidney Udenfriend
1978	Karl H. Beyer, Jr.
1981	Avram Goldstein
1984	K.K. Chen
1986	Walter F. Riker
1988	James A. Bain
1990	George B. Koelle
1992	E. Leong Way
1995	Theodore M. Brody
1997	William W. Fleming
2001	Benedict Lucchesi
2003	Palmer W. Taylor
2005	Kenneth E. Moore
2007	Sue P. Duckles
2009	S. J. Enna
2011	Marcus M. Reidenberg

Chapter News

Great Lakes Chapter of ASPET

The formation of the local Midwest affiliate of the American Society for Pharmacology and Experimental Therapeutics was spearheaded on June 10, 1987 by **Dr. William K. Riker**, Past President of ASPET, **Dr. Seymour Ehrenpreis** of the Chicago Medical School, **Dr. Toshio Narahashi** of Northwestern Medical School, and **Dr. Israel Hanin** of Loyola University Chicago. Key individuals in the Chicago area were then contacted to establish a voluntary executive working group that ultimately organized the first Scientific Day at the University of Chicago on Thursday, May 12, 1988.

Since the first Scientific Program, annual meetings have been held each spring to foster the goals of ASPET to promote scientific communication among research workers interested in pharmacology. The Chapter has consistently met the ASPET requirements of a Chapter Affiliate throughout its existence by holding at least one meeting annually and maintaining a membership roster of at least 20 ASPET members in good standing.

Program for Great Lakes Chapter 25th Annual Scientific Meeting - June 22, 2012 The Searle Conference Center; Rush University Medical Center; 1725 W. Harrison St.; Professional Building; Chicago, IL

8:30-10:30 a.m.: Registration, continental breakfast

8:30-10:30 a.m.: Poster session

8:30 a.m.-noon: Vendor Exhibit

10:45-11:45 a.m: Great Lakes Young Investigator Symposium

Speakers:

10:45-11:00 a.m.: **Jessica Loweth**, Postdoctoral Fellow, Rosalind Franklin University of Medicine and Science
Cocaine craving and AMPA receptor plasticity: Modulation by metabotropic glutamate receptors

11:05-11:20 a.m.: **Karin Ejendal**, Postdoctoral Fellow, Purdue University
The role of Gbetagamma subunits in D2 dopamine receptor-induced sensitization of Ca2+-sensitive adenylyl cyclases

11:25-11:40 a.m.: **Abdelhak Belmadani**, Research Assistant Professor, Northwestern University Feinberg School of Medicine
Uncovering new neurogenic niche in the adult mouse brain: Role for the chemokine receptor CXCR4

Noon-1 p.m.: Career Workshop (Lunch & Learn)

1-4:15 p.m.: Symposium:
Targeting GPCRs: From Traditional Pharmacology to Allosteric Modulation
Introductory remarks by GLC-ASPET President **Alejandro Mayer**

Speakers:

1:15-1:45 p.m.: **Timothy Esbenshade**, Abbott Laboratories
Drug discovery challenges of a GPCR target: Development of histamine H3 antagonists

1:55-2:25 p.m.: **Saverio Gentile**, Loyola University Medical Center
G Protein estrogen receptor (GPER) regulates hERG channel activity in ERneg breast cancer cells. Is cancer a channelopathy?

2:35-3:05 p.m.: **Annette Gilchrist**, Midwestern University
Evaluation of CCR1 antagonists for multiple myeloma

3:15-4:15 p.m.: Keynote Address, **Jeffrey Conn**, Vanderbilt University
Multiple modes of efficacy and stimulus bias of allosteric modulators of GPCRs

4:30-5 p.m.: Business meeting and poster awards

Chapter News

Mid-Atlantic Pharmacology Society

SAVE THE DATE:
Mid-Atlantic Pharmacology Society Meeting: October 25, 2012

The Mid-Atlantic Pharmacology Society is pleased to announce its annual meeting: "Epigenetic Targets and Novel Therapeutics." The 2012 meeting will be held Thursday, October 25, at the GlaxoSmithKline Upper Providence Research Campus, Collegeville, PA. GlaxoSmithKline will be our host. **C. David Allis, PhD**, Rockefeller University will be the keynote speaker. The other guest speakers are: **Victoria Richon, PhD**, Epizyme; **Peter Tummino**, GSK; and **Jonathan R. Whetstine**, PhD, Harvard Medical School/Mass. General Hospital.

The day will begin with poster presentations by undergraduate and graduate students, postdoctoral fellows, and research associates. Two trainees will be invited to give 10-minute oral presentations during the symposium. The day will end with an awards ceremony and networking reception.

Online registration and a complete schedule will be available on the MAPS/ ASPET website in early June. Please join us! For additional information, contact **Carol Beck** at **carol.beck@jefferson.edu**.

Upstate New York Pharmacology Society

UNYPS Holds Inaugural Meeting

The Upstate New York Pharmacology Society held its inaugural meeting on May 14, 2012 in the University at Buffalo Center for the Arts. UNYPS is one of five ASPET regional chapters.

UNYPS was the principal idea of **Dr. Margarita Dubocovich**, Chair of UB's Department of Pharmacology and Toxicology. Under the guidance of ASPET and the committed support from the over 100 regional members of ASPET, a successful charter for the UNYPS ASPET regional chapter was approved last year.


Dr. Margarita L. Dubocovich

Current officers are president Dubocovich, secretary-treasurer **Dr. Peter Bradford** of UB, and councilors **Dr. Aiming Yu** of UB School of Pharmacy and Pharmaceutical Sciences, **Drs. Jean Bidlack** and **Gregory Tall** of the University of Rochester, and **Dr. Carlos Feleder** of the Albany College of Pharmacy and Health Sciences.

At the inaugural UNYPS meeting, over 150 students and faculty from upstate New York and surrounding areas participated in the day-long program entitled "Signaling Pathways as Targets for Drug Discovery." Participants came from the Albany College of Pharmacy and Health Sciences, the University at Albany, Rensselaer Polytechnic Institute, Cornell University, SUNY Upstate Medical University, the University of Rochester, the University of Toronto, Roswell Park Cancer Institute, as well as the University at Buffalo.

Chapter News

Upstate New York Pharmacology Society

Dr. Michel Bouvier

In the morning session, graduate and postdoctoral students delivered oral and poster presentations. In the afternoon, the audience listened to addresses from guest scientists **Dr. Cheryl Frye** of the University at Albany, **Dr. Steven Hill** of Tulane University, **Dr. Richard Miller** of Northwestern University, and **Dr. Michel Bouvier** of the Université de Montréal. Dr. Bouvier's keynote address was entitled "Harnessing the functional selectivity of GPCRs for drug discovery: a technical challenge opening new opportunities."

A presidential symposium featuring senior graduate students selected by one of the Departments or graduate programs in the region was held in the morning. Graduate student presentations were delivered by **Michael D'Alessandro** of Albany College of Pharmacy and Health Sciences, **Wei Kan** of the University of Rochester, **Alex Morrison** of UB, **Bridget Morse** of UB, and **Laura Pitzonka** of Roswell Park Cancer Institute. All students received cash awards for excellent and well delivered presentations. Junior Scientist presentations were given by **Marc Antonyak** of Cornell University, **Yuzhuo Pan** of UB, **Melissa Perreault** of the University of Toronto, and Greg Tall of the University of Rochester.

Poster competition prizes for first and second places were awarded to **Sihem Ait-Oudhia** (UB Pharmaceutical Sciences) and **Agnieszka Lis** (UB Physiology and Biophysics) in the Junior Scientist section, to **PuiYee Chan** (University of Rochester) and **Claire Modica** (UB Pharmacology and Toxicology) in the Graduate Student section, and to **Gregory Bryman** (University of Rochester) and **Jason Ma** (UB Pharmacology and Toxicology) in the Undergraduate Student section.

Abstracts from the poster session and 12 oral presentations from the meeting are published starting on page 105 of this issue.

Election results for the chapter were announced during the business meeting: **Dr. Aiming Yu** of UB Pharmaceutical Sciences has been elected president, **Dr. Suzanne Laychock** of UB Pharmacology and Toxicology will be president-elect, Dr. Peter Bradford of UB Pharmacology and Toxicology will be secretary-treasurer, and **Drs. Kimberly Bernosky-Smith** of D'Youville College, **Paul Kammermeier** of the University of Rochester, and **Ji Li** of UB Pharmacology and Toxicology will be councilors. New officers will start their terms on July 1, 2012.

The future of the UNYPS is to continue its annual research meetings with sites rotating among academic centers in upstate New York and to expand collaborations to encompass neighboring scientific and academic centers in Pennsylvania, Ohio, and southern Ontario.

Photos from UNYPS Annual Meeting: May 14, 2012

Dr. Margarita L. Dubocovich moderates the inaugural UNYPS Annual Meeting.

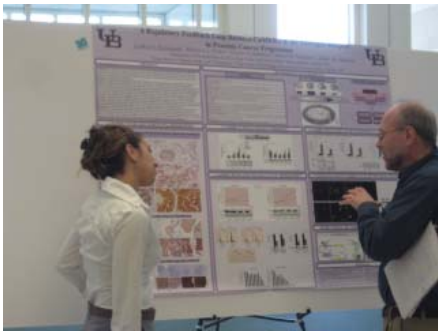
Alex Morrison presents during the Graduate Student Presidential Symposium.

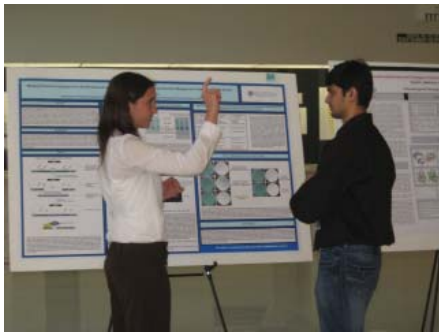
Laura Pitzonka presents at the Graduate Student Presidential Symposium.

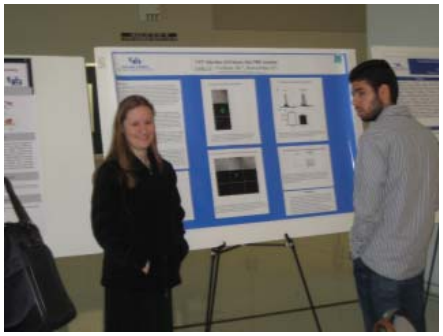
Chapter News

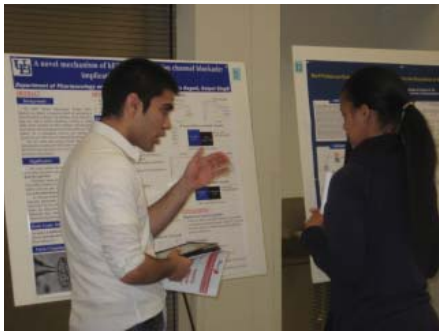
Upstate New York Pharmacology Society

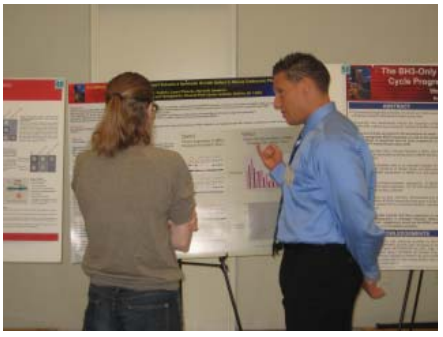
Moderators Dr. Dubocovich and Dr. Carlos Feleder with grad student presenters.

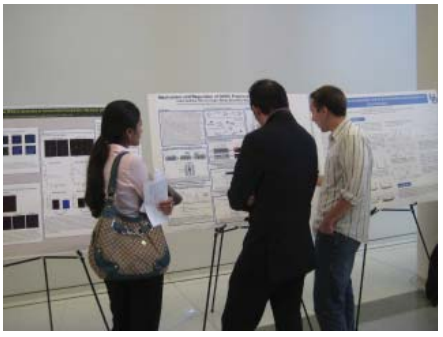






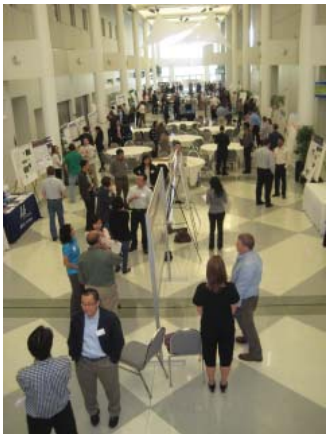


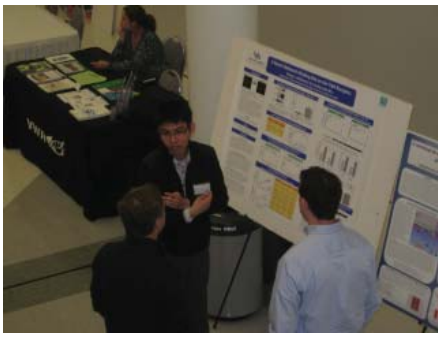




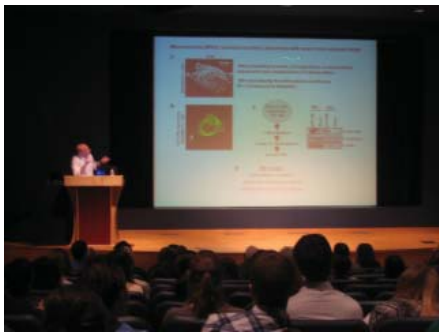
Chapter News

Upstate New York Pharmacology Society



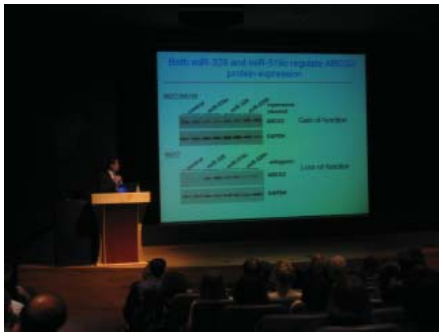


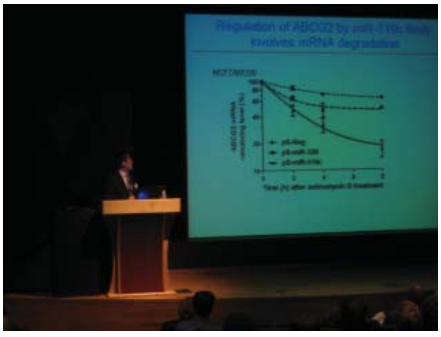
University at Buffalo student volunteers at the registration table.



Dr. Steven Hill (Tulane Univ.) speaks about melatonin and breast cancer cells.

Table with ASPET information.



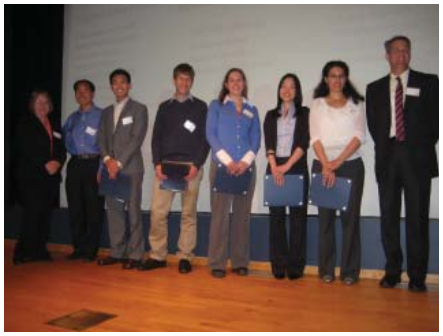


Chapter News

Upstate New York Pharmacology Society

The UNYPS meeting was held at the University at Buffalo Center for the Arts.

Dr. Michel Bouvier delivers the keynote address on GPCR functional selectivity.



Poster winners at the UNYPS Annual Meeting.

Students pose for a picture with Dr. Dubocovich.

Chapter News

Upstate New York Pharmacology Society

Abstracts Presented at the Inaugural Annual Meeting of the Upstate New York Pharmacology Society Held on May 14, 2012

Keynote Address

Abstract: Harnessing the functional selectivity of GPCR for drug discovery; A technical challenge opening new opportunities.

Michel Bouvier, PhD, FCAHS, Université de Montréal; Montreal, Quebec, Canada

In recent years, it has become clear that GPCRs are not unidimensional switches that turn 'on' or 'off' a single signalling pathway. Instead, each receptor can engage multiple signalling cascades that may or may not involve G protein activation. Individual ligands can have differential efficacies toward specific subsets of these signalling effectors. This phenomenon known as ligand-biased signalling or functional selectivity offers interesting opportunities to develop compound with increased selectivity profiles but present important challenges for the drug discovery process. Thus, methods that would allow monitoring multiples signalling pathways simultaneously would be great assets. We thus developed new assays based on luminescence and resonance energy transfer as well as label-free impedance measurements that allow monitoring multiple signalling pathways and to assess the structural determinants of ligand-biased signalling. Using these approaches, several examples of how ligand-biased signalling may be related to the physiological and therapeutic action of drugs will be discussed.

Signal Transduction Systems as Targets for Drug Discovery Symposium

Abstract: Steroids: Therapeutic challenge and promise.

Cheryl Anne Frye and Alicia Ann Walf, The University at Albany, Departments of Psychology and Biology, Centers for Life Science and Neuroscience Research

Steroids hormones have pleiotrophic effects in the brain and body, and have been considered for their potential therapeutic role. The population is aging; many factors may influence age-related phenotypes, including general health, stress effects, social supports, gender/sex related factors, and epigenetics. In rodent models, we investigated some of these effects. Isolated female rats that had been subject to chronic noise stress had higher heart rate and cholesterol levels than did their group-housed conspecifics or male rats. Estradiol and/or progesterone enhance object memory and decrease systolic and diastolic blood pressure and heart rates when administered to young intact rats, young ovariectomized rats, or mid-aged rats; albeit the effects were more modest with aging. The traditionally prescribed estrogen-mimetic therapy, conjugated equine estrogen, improved object memory, and increased heart rate and decreased blood glucose among rats that were below the median split for cognitive performance, which like ovariectomized young rats also have corticosterone levels above basal levels and have heavier adrenals. To begin to address mechanisms underlying these effects we examined expression of the traditional estrogen receptor (estrogen receptor alpha) and the more newly discovered counterpart (estrogen receptor beta). Estrogen receptor alpha is localized to hypothalamic, breast and uterine tissues, and mediates reproductive behaviors. However, estrogen receptor beta is localized to hippocampus and prefrontal cortex and mediates affective behaviors. Indeed, actions at estrogen receptor beta seem to be beneficial and not associated with pro-oncogenic effects in peripheral reproductive tissues of female and/or male rodents. How these effects relate to the challenge and promise of hormone replacement therapies will be discussed. *Supported by USAMRMC Dept. of Defense Breast Cancer Research Program (BC051001) and Karo Bio Research Grant (55850).*

Abstract: Melatonin and peripheral clocks in the regulation of breast cancer cell invasion.

Steven M. Hill, PhD, Tulane University, New Orleans, LA

Disturbed sleep-wake cycle and circadian rhythmicity is associated with cancer, but the underlying mechanisms are unknown. Employing a tissue-isolated human breast tumor xenografts nude rat model we observed that glycogen synthase kinase 3β (GSK3β), an enzyme critical in metabolism and cell proliferation/survival, exhibits a circadian rhythm of phosphorylation in human breast tumors. Exposure to light-at-night (LAN) suppresses the nocturnal pineal melatonin (MLT) synthesis, disrupting the circadian rhythm of GSK3β phosphorylation. Melatonin activates GSK3β by inhibiting Akt phosphorylation, inducing β-catenin degradation to suppress epithelial-to-mesenchymal transition (EMT), a fundamental process underlying cancer metastasis. Melatonin also induces the expression of inhibitor of DNA-binding 2 (Id2), clock controlled and dominant-negative helix-loop-helix protein that antagonizes TGF β-induced EMT by mitigating E12/E47-mediated transcriptional repression of E-cadherin. Thus, chronic circadian disruption by LAN may contribute to cancer incidence and the metastatic spread of breast cancer by inhibiting GSK3β activity and driving EMT in breast cancer patients.

Abstract: Chemokine signaling in neural development and neuropathology.

Richard Miller, PhD, Northwestern University

Chemokines are small secreted protein that signal through the activation of a family of G protein coupled receptors (GPCRs).Although originally identified due to their action as chemoattractants for immune cells, actions of chemokines have now been shown to be extremely widespread in all tissues including the nervous system. The chemokine SDF1 (CXCL12), for example, has an important role in regulating neural development through its actions on neural stem cells. Deletion of the CXCR4 receptor is associated with abnormal development of many structures in the nervous system including the dentate gyrus of the hippocampus. Stem cells that develop into the granule cells that form the dentate gyrus express CXCR4 receptors and they do not migrate appropriately in the absence of CXCR4 signaling. In the adult brain, CXCR4 is expressed in neurogenic zones such as the subventricular zone and the dentate gyrus and CXCR4 signaling helps to regulate adult neurogenesis in the normal brain and also in response to brain injury such as following stroke.

In the peripheral nervous system CXCR4 signaling is important in the development of the dorsal root ganglia (DRG) where it directs the migration of neural crest progenitors to the DRG. Chemokine signaling is also central to the function of adult DRG neurons. In particular both SDF1 and MCP1(CCL2) are important in the development and maintenance of neuropathic pain.MCP1 and its receptor CCR2 are not normally expressed by DRG neurons. However, during the development of chronic pain both MCP1 and CCR2 are expressed by DRG neurons .MCP1 can be packaged into neurotransmitter vesicles and released within the DRG where it can produce direct excitation of neighboring neurons through the activation of CCR2 receptors and transactivation of TRPV1 channels. In recent studies we have investigated the role of chemokine signaling in pain associated with osteoarthritis. Using a model of OA that reflects the progressive nature of the disease we have demonstrated that DRG neurons upregulate the expression of both MCP1 and CCR2 and that deletion of the gene for CCR2 protects mice from chronic OA pain. Our research suggests that drugs that blocking chemokine receptors may be novel therapeutic interventions in the treatment of neuropathic pain.

Graduate Student Presidential Symposium

1. The effect of deuterium labeling on the oxidative metabolism and alkylation kinetics of cyclophosphamide and ifosfamide.

D'Alessandro MA[1], Huang RS[2], Dolan ME[2], Spasojevic I[3], Ludeman SM[1]; [1]Albany College of Pharmacy and Health Sciences, Albany, NY 12208; [2]University of Chicago, Chicago, IL 60637; [3]Duke University, Durham, NC 27710

Purpose: The prodrug cyclophosphamide (CP), the most widely used anti-cancer drug, is clinically effective against more than half of all cancers. Activation occurs through a cytochrome P450-mediated oxidation at the endocyclic C-4 resulting in a 4-hydroxy metabolite and, ultimately, in the DNA-alkylating agent, phosphoramide mustard (PM). Clinically useful, structural isomer, ifosfamide (IF) has a parallel metabolism with oxidative activation at C-4 and subsequent fragmentation to cytotoxic isophosphoramide mustard (IPM). Competing with the C-4 activation pathways are enzymatic oxidations at chloroethyl side-chains which lead to neurotoxicity. The purpose of this study is to use kinetic isotope effects to minimize oxidations at the exocyclic carbons of CP and IF, thereby reducing neurotoxicity and improving therapeutic indices.

Methods: In separate experiments, CP and IF [unlabeled and labeled (deuterium at the exocyclic α and α' carbons of the chloroethyl chains)] were incubated with three, relevant, cDNA-expressed supersomes (CYP2B6, CYP3A4, CYP3A5). Metabolite concentrations produced by C-4 and side-chain oxidations were measured quantitatively using LC-MS-MS. This data was used to provide the isozyme-specific, relative ratios of C-4 versus side chain oxidations for labeled and unlabeled drugs.

Results: The partitioning of CP and IF between C-4 and side chain oxidations was isozyme-dependent among cDNA-expressed supersomes CYP2B6, CYP3A4 and CYP3A5. For CP, the prevalence of C-4 oxidation ranged from 73% (3A4) to 100% (2B6) while that for IF ranged from 36% (2B6) to 63% (3A4). Relative to unlabeled drugs, use of deuterium labeled CP and IF increased the ratio of C-4 to side-chain oxidation by 2 to 12-fold.

Conclusion: It was hypothesized that strategic labeling of CP and IF with deuterium in the chloroethyl chains would lead to kinetic isotope effects favoring oxidation at the C-4 position by disfavoring oxidations at the side chains. Initial experiments have supported this hypothesis.

2. Regulation of phospholipase Cβ3 activation via direct binding to intracellular loops of the M3 muscarinic acetylcholine receptor.

Kan W., Malik S., Burroughs M., Faibis G., and Smrcka A.; Pharmacology & Physiology, University of Rochester, NY 14642.

Background: G protein-coupled receptors (GPCRs) are seven-pass transmembrane proteins expressed at the cell surface. They are implicated in many physiological functions and constitute the largest drug target group. For example, parasympathetic responses such as airway constriction are controlled by muscarinic receptors. GPCR coupling to α and βγ G proteins, which regulate gene expression, enzyme activity, or ion channel open probability, has become a classical model for the transduction of outside signals inward to effect changes in cell physiology. There is growing recognition that GPCRs can be pre-coupled to signal transduction partners to serve a specific and efficient signaling function. In our lab, we previously found that the third intracellular loop (ICL3) of muscarinic M3 receptor (M3R), expressed as a purified protein fused with glutathione-S-transferase (GST), scaffolded purified Gαq, Gβ1γ2, and phospholipase Cβ3 (PLCβ3). GST-ICL3 was found to significantly inhibit, albeit partially, the binding of PLCb3 to full-length M3R; PLCβ3 binding surface may be comprised of multiple contacts.

Materials and Methods: The intracellular surface of M3R was broken down into GST fusion constructs to provide a high resolution mapping of PLCβ3, Gαq, and Gβ1γ2 binding sites. Furthermore, PLC activity was measured to compare samples that were immunopurified from mouse/rat lung extracts with PLCβ3, Gαq or M3R antibodies. Binding of PLCβ3 to full length M3R expressed in mammalian cell lines was compared in the presence and absence of carbachol agonist.

Results: Further definition of the PLCβ3 binding surface on M3R using purified GST fusion constructs confirmed the multiplicity of contacts with PLCβ3. Contacts close to the membrane interface were found at distinct amino and carboxyl terminal regions of ICL3, ICL2, and C terminal tail. M3R binding sites were also defined for $G\alpha_q$ and $G\beta_1\gamma_2$, which were distinct from those for PLCβ3 and from sites involved in direct coupling to the $G\alpha_q G\beta\gamma$ heterotrimer. We found that PLC could be co-purified with immunoprecipitated M3R, but not with immunoprecipitated Gαq, from native tissues. Finally, we found that there is translocation of PLCb3 to M3R upon agonist stimulation.

Discussion: Taken together, the agonist-responsive direct binding interaction between M3R and PLCβ3 represents an additional mechanism to finetune PLC signal output beyond receptor-stimulated nucleotide exchange on Gaq. (Supported in part by 10PRE3490032 [to WK] from AHA, and GM081772 and GM053536 [to AVS] from NIH)

3. Sestrin2 promotes LKB1-mediated AMPK activation in the ischemic heart.

Morrison A.[1], Tong C.[1], Lee J.H.[2], Karin M.[3], Li J.[1]; [1]Department of Pharmacology and Toxicology, School of Medicine and Biomedical Sciences, University at Buffalo (SUNY), Buffalo, NY 14214; [2]Department of Molecular and Integrative Physiology, University of Michigan Medical School, Ann Arbor, MI 48109; [3]Department of Pharmacology, School of Medicine, University of California at San Diego, La Jolla, CA 92093

Background: AMP-activated protein kinase (AMPK) has recently emerged as a pertinent stress-activated kinase shown to have substantial cardioprotective capabilities against myocardial ischemia/reperfusion (I/R) injury. However, the molecular basis behind the regulation of AMPK activity in the ischemic heart remains poorly understood. Characterizing the precise mechanism by which AMPK is activated during ischemia is fundamental to the development of new therapeutic strategies for ischemic heart disease. Recent evidence indicates that a novel stress-inducible protein Sestrin2 is involved in the activation of the AMPK signaling pathway. Therefore, we hypothesize that Sestrin2 plays an influential role during myocardial ischemia in order to promote AMPK activation and subsequent cardioprotection.

Materials and Methods: Male wild-type (WT) and Sestrin2 KO mice (all C57BL/6 background) were used to characterize the role of Sestrin2 in the heart in response to ischemic insults. In vivo regional myocardial I/R was employed by left anterior descending coronary artery (LAD) occlusion for assessing cardiac signal transduction and the extent of myocardial infarction. Ex vivo Langendorff heart preparations were used to assess differences in post-ischemic cardiac function. Cardiac hemodynamics were measured by inserting a pressure-volume loop microtransducer into the left ventricle.

Results: Immunoblots for the first time demonstrated that Sestrin2 protein levels are expressed in adult cardiomyocytes. Additionally, Sestrin2 levels were found to significantly accumulate in the heart during myocardial ischemia as compared to basal conditions ($p<0.05$). WT and Sestrin2 KO cardiac phenotype analysis indicated no significant difference in histology or left ventricular function. However, when WT and Sestrin2 KO mice were subjected to in vivo I/R, myocardial infarct size was significantly greater in Sestrin2 KO hearts ($p<0.05$ vs. WT). Furthermore, ex vivo heart perfusions indicated exacerbated post-ischemic contractile function in Sestrin2 KO hearts ($p<0.05$ vs. WT). Interestingly, AMPK activation during ischemia was significantly reduced in Sestrin2 KO hearts ($p<0.01$ vs. WT). Immunoprecipitation of Sestrin2 from WT hearts demonstrated an association between Sestrin2 and AMPK. Importantly, during ischemia, the Sestrin2 immune-complexes revealed a striking association with LKB1, the major upstream kinase of AMPK. Immunoprecipitation of LKB1 in WT hearts demonstrated AMPK association during ischemia while AMPK association in the Sestrin2 KO hearts was markedly diminished.

Discussion: The results demonstrate for the first time an important cardioprotective role of Sestrin2 against I/R injury. Furthermore, we identify a unique mechanism by which Sestrin2 is induced during ischemic stress acting as an LKB1-AMPK scaffold in order to initiate AMPK activation and elicit a cardioprotective response.

(Research Support: American Heart Association 0835169N, American Diabetes Association 1-11-BS-92, National Institutes of Health R01HL101917)

4. Combined receptor-transporter inhibition therapy for treatment of γ-hydroxybutyrate (GHB)-induced respiratory depression.

Morse B.L. and Morris M.E.; Department of Pharmaceutical Sciences, School of Pharmacy and Pharmaceutical Sciences, University at Buffalo, State University of New York, Buffalo, NY 14260

Background: Overdose of the drug of abuse γ-hydroxybutyrate (GHB) can cause respiratory depression, occasionally resulting in death; however, no pharmacologic treatment currently exists for GHB intoxication. Due to monocarboxylate transporter (MCT)-mediated renal reabsorption of GHB, increasing GHB clearance via inhibition of these transporters represents a potential therapeutic strategy in GHB overdose. The purposes of this research were to investigate the concentration-effect relationship of GHB-induced respiratory depression and assess the effect of MCT inhibition and other potential treatment strategies on this endpoint.

Materials and Methods: Respiration was measured using plethysmography in rats. Dose-dependent effects of GHB were assessed by intravenous administration of 200, 600, and 1500 mg/kg. To assess the receptors involved in GHB-induced respiratory depression, a specific GABAB receptor antagonist, SCH50911, and a specific GABAA receptor antagonist, bicuculline, were administered prior to GHB. Potential treatment strategies of receptor and MCT inhibition were assessed by inhibitor administration 5 minutes after GHB. Effects of inhibitor administration were determined using the pharmacodynamic descriptors of area below the effect curve (ABEC), maximum effect (E_{max}) and duration of respiratory depression (T_d).

Results: GHB administration resulted in a dose-dependent decrease in respiratory rate, accompanied by a compensatory increase in tidal volume. Minute volume was maintained at doses of 200 and 600 mg/kg, but was significantly decreased with 1500 mg/kg. Pretreatment with 150 mg/kg SCH50911 completely prevented the decrease in respiratory rate; 5 mg/kg bicuculline methiodide had no effect. Treatment with the MCT inhibitor L-lactate increased GHB renal and total clearances, significantly improving the ABEC and T_d for respiratory rate. Treatment with 5 mg/kg SCH50911 after GHB improved the parameters of ABEC and E_{max}, while administration of 5 mg/kg SCH50911 + L-lactate resulted in significant improvement in all pharmacodynamic descriptors.

Discussion: This is the first research to assess the effects of GHB and GHB overdose treatment strategies using direct measurement of respiratory depression, and the first to assess combined receptor-transporter inhibition using any toxicological endpoint of GHB overdose. These results indicate the primary effect of GHB on respiration to be a decrease in respiratory rate, mediated primarily by agonism at $GABA_B$ receptors. Improvement in GHB-induced respiratory depression at clinically relevant concentrations of L-lactate in this study suggests this to be a potential, practical GHB overdose treatment strategy. $GABA_B$ antagonism and combined $GABA_B$/MCT inhibition therapy represent potential therapies pending the availability of $GABA_B$ antagonists for clinical use. (Supported by NIH grant DA023223)

5. Thoc1 is required for intestinal stem/progenitor cell viability: Implications for Thoc1 as a cancer therapeutic target.

Pitzonka, L. B. and Goodrich, D.W.; Pharmacology & Therapeutics, Roswell Park Cancer Institute, Buffalo, NY 14263

Background: Thoc1 encodes an RNA binding protein (Thoc1) that interacts with the tumor suppressor Rb1 and is required for embryogenesis. Thoc1 levels are elevated, relative to normal tissue, in multiple cancers. Cancer cells, but not most normal cells, undergo apoptosis following Thoc1 deletion in vitro, suggesting Thoc1 may be a good therapeutic target. Based on these data we hypothesized cells with an extended replicative potential require Thoc1. A prediction of this hypothesis is that Thoc1 may also be required for stem cell viability. To test this, we examined the effects of Thoc1 deletion in the mouse small intestine (S.I.) in vivo. The S.I. crypts contain intestinal stem cells (ISCs) that cycle steadily to produce the rapidly proliferating transit-amplifying (TA) progenitor cells that differentiate into non-proliferative villi lineages.

Materials and Methods: Thoc1 was deleted in the adult mouse using the Tamoxifen/CreER system with Rosa26 driving CreER expression. Mice were treated with 2 mg/day Tamoxifen for 1, 2, 3, 4, 5, or 6 days. S.I. tissue was collected 24 hours after each treatment for histological, protein and mRNA analyses.

Results: Histological analysis shows Thoc1 deletion disrupts crypt architecture, induces crypt apoptosis, and decreases crypt proliferation (as detected by hematoxylin & eosin, cleaved Caspase 3 and Ki67 staining, respectively) by day 4. The villi are not directly affected by Thoc1 deletion; however, villus atrophy occurs by day 6 due to loss of crypt function. Two ISC populations in the S.I. include steady cycling, homeostatic Lgr5 positive cells and quiescent, reserve Bmi1 positive cells. Preliminary data shows Thoc1 deletion results in an initial decrease in Lgr5 and increase in Bmi1 mRNA by days 4 and 5. However, by day 6 Bmi1 mRNA also decreases. These data suggests Thoc1 deletion initially depletes Lgr5 ISCs and activates Bmi1 ISCs to expand. The now actively cycling Bmi1 ISCs may then be susceptible to Thoc1 deletion.

Discussion: This study demonstrates intestinal stem/progenitor cells with an extended replicative potential require Thoc1, whereas differentiated, non-proliferative villi cells do not. Additionally, this study suggests Thoc1 may be required for cycling ISC viability, raising the possibility that cancer stem cells may also be sensitive to Thoc1 loss. Interestingly, ISCs are thought to be the cells of origin of intestinal cancer driven by adenomatous polyposis coli (APC) mutations. Therefore, Thoc1 may be a particularly novel therapeutic target for intestinal cancers. (Work supported by NRSA T32 CA009072 and UB Mark Diamond Research Fund Grant)

Junior Scientist Symposium

1. Microvesicles: A novel form of intercellular communication with important consequences in human cancer progression.

Marc A. Antonyak and Richard A. Cerione, Department of Molecular Medicine, Cornell University

Abstract: The ability of human cancer cells to communicate with other cancer cells and with neighboring normal cell lineages that collectively make-up the tumor microenvironment is crucial for tumor expansion, as well as for promoting the invasive and metastatic activities of aggressive forms of cancer cells. One novel and unexpected form of cell-to-cell communication exhibited by cancer cells that is gaining a tremendous amount of attention involves the production of microvesicles (MVs). MVs, which are also referred to as oncosomes or shedding vesicles, are large vesicular structures (up to 3.0 μm in diameter) that are formed and shed directly from the surfaces of highly aggressive human cancer cells. They contain a variety of cargo that is not typically thought to be released from cells including cell-surface receptor tyrosine kinases, cytosolic and nuclear signaling proteins, and RNA transcripts. MVs can transfer their cargo between cancer cells, an outcome that potentiates the growth and transformed properties of these cells. Moreover, we have recently made the surprising discovery that MVs shed by the highly aggressive MDAMB231 breast cancer cells or U87 brain tumor cells, when isolated and then added to cultures of normal fibroblasts or mammary epithelial cells, confer upon the recipient cells the transformed characteristics of cancer cells. We then went on to identify two proteins in MVs, namely the protein crosslinking enzyme, tissue transglutaminase, and the extracellular matrix protein, fibronectin, that work together to elicit this cellular outcome. We have also obtained evidence that implicates members of the Rho family of small GTPases, including RhoA, Rac, and Cdc42, as playing important and distinct roles in MV formation and shedding critical for MV biogenesis. Thus, these findings underscore the important contributions that MVs have in human cancer progression, as well as begin to shed some light on the signaling mechanisms that underlie their biogenesis.

2. MicroRNA-controlled posttranscriptional regulation of breast cancer resistance protein (BCRP/ABCG2).

Yu-Zhuo Pan, Xin Li, Marilyn E. Morris, and Ai-Ming Yu, Department of Pharmaceutical Sciences, University at Buffalo, SUNY, Buffalo, NY 14260-1200

Breast cancer resistance protein (BCRP/ABCG2) is an ABC family efflux transporter that determines cellular disposition of many anticancer drugs. ABCG2 may also confer multidrug resistance of cancer cells. MicroRNAs are small noncoding RNAs that play an important role in posttranscriptional regulation of target genes, and control many cellular functions including chemosensitivity. To understand the posttranscriptional regulation of ABCG2 and the role of microRNAs (miRNAs) in control of drug disposition, we first identified that microRNA-328 (miR-328) targeted the 3'-untranslated region (3'-UTR) of ABCG2. ABCG2 protein level was down-regulated in cells when miR-328 was over-expressed, and ABCG2 protein level was up-regulated in cells when miR-328 function was inhibited. Furthermore, we compared the capacity of three miRNAs, miR-328, miR-519c and miR-520h, in modulation of ABCG2 protein expression. Our data showed that miR-519c and -328 had a more dramatic impact on ABCG2 protein expression, which involved mRNA degradation mechanism. In addition, intervention of miR-328 or -519c signaling led to a significant change in intracellular mitoxantrone accumulation, as determined by flow cytometry analyses. Together, these findings demonstrated the importance of miRNAs in modulation of cellular drug disposition through the regulation of ABC transporters. Additional studies on miRNA functions may lead to new strategies in targeting ABCG2-mediated chemoresistance and metastasis in malignancies.

Acknowledgements: This project was supported by UB Interdisciplinary Research Development Fund, and in part by the NIH award R01DA021172.

3. Dopamine D1-D2 receptor heteromer in dual phenotype GABA/glutamate-coexpressing striatal medium spiny neurons differentially regulates mesolimbic and striatonigral neurotransmission.

Perreault M.L.[1,2], Fan T.[1,2], Alijaniaram M.[1,2], O'Dowd B.F., and George S.R.[1,2,3]; [1]Centre for Addiction and Mental Health, [2]Department Pharmacology and [3]Medicine, University of Toronto, Toronto, Ontario, Canada, M5S 1A8

Background: In basal ganglia a significant subset of GABAergic medium spiny neurons (MSNs) coexpress D1 and D2 receptors (D1R and D2R). These coexpressing neurons have been shown to have a region-specific distribution throughout the mesolimbic and basal ganglia circuits, and to additionally express the dopamine D1R-D2R heteromer. The functional contribution of the D1R-D2R heteromer in regulating neurotransmission remains unknown.

Materials and Methods: Immunochemistry was performed in striatal neuronal culture and in rat nucleus accumbens (NAc) and caudate putamen (CP) to characterize the phenotype of neurons coexpressing the D1R and D2R. Levels of GABA and glutamate, and the expression of markers associated with GABA or glutamate activity, were also assessed in NAc, CP, ventral tegmental (VTA) area and substantia nigra (SN) of rats and of D1R or D5 receptor knock-out mice following systemic or intra-NAc core and shell activation of the D1R-D2R heteromer by SKF 83959 (1.5 mg/kg). Proteins examined included: BDNF, GAD67, VGLUT1, VGLUT2, pERK, pCaMKII.

Results: We identified a MSN subtype coexpressing GABA and glutamate as well as the D1R and D2R. Activation of the D1R-D2R heteromer in these neurons resulted in the simultaneous, but differential regulation of proteins involved in GABA and glutamate production or vesicular uptake in regions of the mesolimbic and striatonigral pathways. Specifically, there was increased expression of proteins involved in GABA activity in the NAc and VTA concomitant with increased expression of proteins involved in glutamate activity in CP and SN. Additionally, D1R-D2R heteromer activation in NAc shell, but not core, differentially altered protein expression in VTA and SN, regions rich in dopamine cell bodies.

Discussion: The identification of an MSN with dual inhibitory and excitatory functions provides new insights into the neuroanatomy of the basal ganglia and demonstrates a novel source of glutamate in this circuit. Furthermore, the demonstration of a dopamine receptor complex with the ability to regulate the transition between GABAergic inhibitory or glutamatergic excitatory dominance potentially will provide new insights into the regulation of dopamine neuron activity. This could have broad implications in understanding how dysregulation of neurotransmission within basal ganglia contributes to dopamine neuronal dysfunction. (Supported by the National Institute on Drug Abuse [to S.R.G. and B.F.O.] and a CIHR Postdoctoral Fellowship [to M.L.P.])

4. Ric-8 proteins are G protein α subunit GEFs in vitro and regulators of Gα biosynthesis in cells

Tall, GG. Pharmacology and Physiology, University of Rochester Medical Center at Rochester, NY 14642

Background: Ric-8 was discovered as a C. elegans mutation that was epistatic to the action of G protein α mutants in a variety of signaling pathways. The Tall laboratory first showed that mammalian Ric-8A and Ric-8B are physical binding partners of G protein α subunits. Pure Ric-8 proteins act as guanine nucleotide exchange factors (GEFs) to activate purified G protein α subunits. In cells, Ric-8A and Ric-8B are required for proper steady state expression of subsets of G proteins. Ric-8 regulates an early post-translational event during G protein biosynthesis such that nascent Gα subunits do not undergo proper initial association with an endomembrane. The current goals of our research are to understand the detailed mechanism of Ric-8 mediation of G protein biogenesis and to reconcile the in vitro GEF function of Ric-8 with this new cellular function.

Materials and Methods: Methods to prepare purified Ric-8 proteins and G proteins will be discussed. Assays to measure Ric-8 effects upon Gα subunit catalytic properties, including GTPγS binding kinetics, steady state and single turnover GTPase activities will be presented. Cell biological work will be shown including the derivation of Ric-8A$^{-/-}$ and Ric-8B$^{-/-}$ mouse embryonic stem (mES) cell lines and the use of these cells lines in metabolic pulse/chase labeling studies to measure G protein biosynthesis and fate.

Results: A new method using GST-Ric-8 as a tool to prepare unprecedented yields of all classes of purified G protein α subunits has been developed. These prepared Gα subunits were used in subsequent enzymatic studies to show that Ric-8A is a GEF for Gαi, Gα12/13, and Gαq classes and Ric-8B is a GEF for Gαs/olf class subunits. The Ric-8A and Ric-8B genes are essential for mouse embryogenesis. Ric-8A$^{-/-}$ and Ric-8B$^{-/-}$ mES cells lines were derived and used to show that Ric-8A or Ric-8B direct the biosynthesis of the same subsets of nascent Gα subunits that they are GEFs for in vitro.

Discussion: We propose that Ric-8 proteins bind newly translated Gα subunit protein chains and facilitate the final events of protein folding in concert with cellular chaperones. When the Ric-8-nascent Gα subunit complex reaches a sufficiently folded state such that a competent guanine nucleotide-binding pocket is formed, GTP binds the newly synthesized Gα subunit and dissociates it from Ric-8A. This model accounts for in vitro Ric-8 GEF activity and the cellular biosynthetic chaperoning activity of Ric-8 (Support by NIH RO1 GM088242).

Poster Abstracts (alphabetically by first author)

1. 9-cis retinoic acid induces human oligodendrocyte maturation.

Abiraman K.[1,2], O' Bara M.[2] and Sim FJ[2]. [1]Neuroscience program, University at Buffalo; [2]Department of Pharmacology and Toxicology, University at Buffalo

Oligodendrocytes, the myelin producing cells in the nervous system, are destroyed in demyelinating diseases such as multiple sclerosis. Huang and colleagues showed that transcripts encoding the retinoic acid receptor RXR gamma were differentially expressed during remyelination and that administration of the agonist 9-cis-retinoic acid (9-cisRA) to aged mice after demyelination caused an increase in remyelination (Huang et al., 2011; Nat. Neurosci 14:45-53). Human and rodent glia differ in response to environmental factors and express distinct gene expression profiles during development. As such, a conserved role of 9-cisRA in human cells cannot be assumed. Human neuronal re-aggregate cultures were established from 19-22 week

fetal brain dissociates and co-cultured with human oligodendrocyte progenitor cells (OPCs) isolated using CD140a antigen-based magnetic cell sorting. This in vitro model permits the long-term survival of OPCs and their differentiation to oligodendrocytes and astrocytes. We treated co-cultures with either 9-cisRA (50 nM) or vehicle daily for 14-21 days and assessed oligodendrocyte differentiation using immunocytochemistry for O4 antigen. The proportion of myelin sheet-forming mature oligodendrocytes (type 2) and non-sheet forming oligodendrocytes (type 0 & 1) was determined. At day 14, 9-cisRA treatment induced a significant increase in the number of type 2 O4+ cells relative to control (67 ± 17 % 9-cisRA vs. 9 ± 2 % vehicle; n=3 fetal samples, t-test $p<0.05$). Consequently, vehicle controls had a significantly higher number of type 0/1 O4+ cells (91 ± 2% vehicle vs. 33 ± 17% 9-cisRA, $p<0.05$). In preliminary studies, we characterized the total number of myelin basic protein (MBP) expressing oligodendrocytes in 9-cisRA and vehicle cultures at 21 days. We found that 9-cis RA treatment lead to a non -significant increase in the number of MBP+ oligodendrocytes (224±24 9-cis RA vs. 182±67 vehicle; n=2 fetal samples). These results suggest that 9-cisRA treatment acts similarly on human and mouse OPCs to induce oligodendrocyte differentiation. Further investigation of factors affecting oligodendrocyte differentiation using our humanized coculture model could contribute to the development of novel therapeutic strategies.

2. Effects of the melatonin receptor antagonist (MT_2)/inverse agonist (MT_1) luzindole on re-entrainment of circadian rhythms of wheel running activity and spontaneous homecage behaviors in C3H/HeN mice.

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Background: Activation of MT_1 receptors by melatonin accelerates the re-entrainment of circadian rhythms of wheel running activity after an abrupt advance of the dark cycle (Dubocovich et al., 2005). Here, we investigated the effects of luzindole (LUZ), a competitive melatonin receptor antagonist (MT2)/inverse agonist (MT1) on the re-entrainment rate of wheel running activity and spontaneous homecage behaviors after a 6hr advance of the dark onset in C3H/HeN mice.

Materials and Methods: Wheel Running activity was recorded with ClockLab (actimetrics, IL). Homecage behaviors were recorded with the HomeCageScan System (CleverSys, VA) and grouped into activity, exploratory/anxiety, ingestive and sleep behaviors.

Results: LUZ (10mg/kg, s.c.) given for three consecutive days at the new dark onset (8.4±0.4d, n=14) significantly increased the number of days (delay) necessary for re-entrainment of wheel running activity rhythms compared to vehicle (VEH) controls (6.3±0.3d, n=24; $p<0.001$). LUZ (n=8) compared to VEH (n=8) also delayed re-entrainment to dark onset of most spontaneous homecage behaviors including activity (Walk: VEH, 9.3±o.5d & LUZ, 12.3±0.7d, $p<0.005$), exploratory/anxiety (Groom: VEH, 9.1±0.6d & LUZ, 12.3±0.6d, $p<0.005$), ingestive (Eat: VEH, 9.5±0.5d & LUZ, 12.4±0.6d, $p<0.005$) and sleep (RemainLow: VEH, 9.88±0.6d & LUZ, 12.29±0.6d, $p<0.01$) behaviors.

Discussion: We concluded that LUZ, possibly acting as inverse agonist at MT_1 melatonin receptors may delay the reentrainment of wheel running and homecage behaviors. (Supported by NS 061068.)

3. Computational analysis of Paclitaxel-induced tumor priming that leads to enhanced deposition and efficacy nanoparticulate doxorubicin.

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Background: Paclitaxel (PAC) has been shown to cause tumor-priming (TP), which increases delivery of subsequently-administered drugs. PAC-mediated apoptosis results in tumor cell density reduction, interstitial space expansion, and increased vascular permeability enhancing tumor deposition of nanoparticulate formulations such as liposomal-doxorubicin (L-dox). Our aim was to develop an integrated quantitative pharmacokinetic/pharmacodynamic (PK/PD) analysis approach to optimize combination chemotherapy of PAC with L-dox.

Materials and Methods: Data were extracted from Lu et al. (J Pharmacol Exp Ther 322: 80, 2007) in which tumor xenografted mice were treated with PAC (40mg/kg), L-dox (20mg/kg), TP dosing (PAC then L-dox at 48h), or the reverse-sequence (L-dox then PAC). Data included plasma and tumor drug concentrations, PAC and L-dox induced-apoptosis kinetics, and % change in tumor-growth. Drug profiles were described with a PK model for carrier-mediated PAC, and a two-compartment model for L-dox, with first-order release of free drug from the liposome. Intratumor concentrations were described using a hybrid well-stirred model with estimated partition-coefficients (kp) for each drug. Tumor PK profiles were used to drive apoptotic responses, which were modeled using nonlinear time-dependent signal-transduction functions. The tumor-growth PD model consisted of two-transit compartments, with a first-order net-growth rate constant and the apoptotic signals from each agent driving cytotoxic effects. PAC-induced TP was modeled using a feedback loop, with the apoptosis signal of PAC enhancing the deposition of L-dox. Model fittings were performed using MATLAB. Simulations were conducted to explore priming sequences.

Results: The final model captured well the PK of PAC and L-dox in plasma and tumors, and the time course of apoptosis induction, and tumor growth for each treatment sequence. With single agent dosing, kp values for PAC and L-dox were estimated at 0.044 and 0.085. L-dox kp increased 2-fold after the TP treatment. Apoptosis signals exhibited a delayed onset that was well captured, and the intratumor concentrations producing maximum effects (Emax) and 50% Emax were 18 and 7.2µg/mL (PAC) and 17.6 and 14.3µg/mL (L-dox). The duration of drug induced-apoptosis was 27.4h for PAC and 15.8h for L-dox. Simulation with the PK/PD model suggested that earlier administration of L-dox would increase efficacy markedly.

Discussion: A model was successfully developed that captured the priming effect on PK and efficacy. Simulation suggested that administration of L-dox 24h prior to the priming peak would enhance efficacy further. This model could be adapted for evaluating other combination chemotherapies using PAC as a TP-agent. (NIH grant GM57980 and UB-Pfizer Alliance)

4. Lipoteichoic acid (LTA) induces reactive nitrogen species in lung endothelium.

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Background: Lipoteichoic acid (LTA), a cell wall ribitol polymer from Gram-positive organisms, mediates inflammation through the Toll-like receptor 2 (TLR2). TLR2 activation activates IRAK1/4; it is known that IRAK1/4activity is linked to activation of AKT. It is known that eNOS is activated by the AKT dependent phosphorylation of eNOS-ser^{1177} whereas it is inhibited by PKCδ which phosphorylates thereonine ^{495}. We propose that there is a link between IRAK1/4 activity and eNOS serine^{1177}/thereonine^{495} ratio. The effect of LTA on lung reactive nitrogen species generation is not known. We tested the hypothesis that LTA from Staphylococcus aureus induces an increase in reactive nitrogen species in pulmonary microvessel endothelial monolayers (PMEM).

Materials and Methods: PMEM were treated with LTA (10ug/ml) for 0.5 hr with and without the nitric oxide synthase (NOS) inhibitor L-NAME (100uM). The interleukin receptor-associated kinases 1 and 4 (IRAK-1 and -4) inhibitor (IRI), N-(2-Morpholinylethyl)-2-(3-nitrobenzoylamido)-benzimidazole were used to block the TLR2 signaling cascade. PMEM were pretreated for 2 hours with IRI alone (10μM) prior to co-treatment with IRI and LTA. ROS were assayed using 6-Carboxy-2', 7'-Dichlorodihydrofluorescein Diacetate, Di (Acetoxymethyl Ester) (10 μM, DCFDA). In separate studies, PMEM were assayed for the eNOS-ser^{1177}/thr ^{495} ratio which is an index of eNOS activation.

Results: LTA caused an increase in the eNOS-ser^{1177}/thr ^{495} ratio and DCFDA fluorescence which was inhibited by IRI. DCFDA fluorescence was inhibited by L-NAME.

Discussion: The data indicates that LTA causes an IRAK1/4 dependent activation of eNOS with generation of reactive nitrogen species.

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5. A novel mechanism of hERG potassium ion channel blockade: implications for drug design?

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The hERG (human Ether-à-go-go Related Gene) potassium ion channel is extremely critical for maintaining a normal heartbeat in humans. The inhibition of this channel by drugs may lead to cardiac arrhythmias resulting in sudden cardiac death. Therefore the FDA mandates a discontinuation of any drug in the development process that inhibits the hERG channel. Two key amino acid residues located intracellularly, a phenylalanine at position 656 (F656) and a tyrosine at position 652 (Y652) have been shown to be very important for binding of all drugs to the channel. This study indicates the presence of a potentially novel extracellular/alternative intracellular drug binding site, apart from Y652 and F656. Preliminary results have demonstrated that the inhibition of the hERG channel by Celecoxib, an analgesic prescribed for arthritis and acute pain, is independent of both these binding sites, a first observation of its kind. An increased understanding/identification of any novel molecular determinants of blockade of the hERG channel would allow for the refinement of the drug design process to bypass any interactions with the hERG channel. Thereby minimizing drug related cardiotoxicity (arrhythmia) and minimizing drug developmental costs.

6. Rescue of NMDA receptor hypofunction by reduction of extracellular dopamine.

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Background: Schizophrenia is a debilitating mental illness affecting 1% of the population in Canada. Despite the progress that has been made in understanding the neurobiology of schizophrenia, the aetiology of the disorder remains unclear. Current pharmacotherapy relies on antipsychotic drugs that block signaling at the post-synaptic dopamine (DA) D2 receptor. Although this approach is efficacious for some symptoms, there are significant side effects that decrease the quality of life of patients. Considering the efficacy of D2 receptor blocking, it is clear that reducing dopaminergic signaling can be of benefit in schizophrenia. However, it may be possible to dissociate benefits from side effects by finding a new way to target DA transmission. The most important factor regulating extracellular DA is the DA transporter (DAT), and increasing functional DAT would effectively result in decreasing extracellular DA. In our lab we study genetic and pharmacological NMDA receptor hypofunction mouse models that display behavioural deficits related to endophenotypes of schizophrenia, including hyperactivity and reduced social interaction. By crossing this mouse with a transgenic mouse overexpressing the dopamine transporter (DAT-tg) as well as pharmacologically manipulating extracellular dopamine and NMDA receptor function, we aim to demonstrate that increasing DAT function is beneficial in NMDA receptor hypofunction mouse models and by extension potentially in schizophrenia.

Methods: NR1-KD and wildtype mice were crossbred with DAT-TG mice and locomotor behaviour as well as social interaction behaviour was measured when the mice reached an age of three months. In separate experiments, NR1-KD mice, DAT-tg, and appropriate control mice, were treated acutely with the dopamine synthesis inhibitor alpha-methyl-para-tyrosine (AMPT) or the NMDA antagonist MK801 respectively, after which locomotor activity was measured.

Results: Preliminary data show that when crossing the NR1-KD mouse with the DAT-tg mouse, both the locomotor hyperactivity and reduced social interaction phenotypes are rescued. Furthermore, acutely reducing extracellular dopamine using AMPT partially rescues the NR1-KD locomotor phenotype. On the other hand, genetic overexpression of DAT does not seem to affect the response to acute pharmacological blockade of the NMDA receptor with MK801.

Discussion: Our results show that increasing DAT function is beneficial in the NR1-KD mouse, and that this effect is likely due to reduction of extracellular dopamine, which similarly rescues the NR1-KD phenotype. This data suggests that increasing DAT function may be a viable approach in the treatment of schizophrenia. Future experiments will further explore the relationship between extracellular dopamine and NMDA receptor function, both biochemically and behaviorally.(Supported in part by ERA to AS from Ontario Government)

Chapter News

Upstate New York Pharmacology Society

7. Up-regulation of mitochondrial proteins in mice over-expressing the dopamine transporter.

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Background: Dopamine transporter transgenic mice (DAT-Tg) are mice with an over-expression of the dopamine transporter (DAT) corresponding to a 3-fold increase in protein levels. As a result, these animals have a 40% reduction in extracellular dopamine (DA), and can be classified as a genetic model of hypodopaminergia. The aim of this study is to identify which post-synaptic proteins are altered as a result of reduced DA transmission.

Materials and Methods: The post-synaptic density (PSD) of DAT-Tg and WT animals was isolated, and a 2D-difference gel electrophoresis (2D-DIGE) was conducted to separate proteins by isoelectric focusing followed by SDS-PAGE.

Results: Three independent analytical gels were conducted and 58 protein spots were obtained ($p<0.05$, fold-change ± 1.5). All proteins obtained in the 2D-DIGE were up-regulated in DAT-Tg vs. WT. Fifty protein spots were identified by mass spectrometry and were found to be all mitochondrial related proteins. In particular, the proteins identified were from Complex I, III, and IV of the electron transport chain. Three candidate proteins identified by the mass spectrometry analyses were chosen for verification using a classical western blot approach (NADH dehydrogenase (ubiquinone) Fe-S protein 2 [NDUFS2] & NADH dehydrogenase (ubiquinone) Fe-S protein 8 [NDUFS8] (Complex I); and Ubiquinol cytochrome c reductase core protein 2 [UQCRC2] (Complex III)). Immunoblot studies verified up-regulation of all three proteins in DAT-Tg compared to WT animals. Total NDUFS2 was up-regulated by 35% ($n=6$; $p<0.0001$), NDUFS8 by 225% ($n=3$; $p<0.05$), and UQCRC2 by 152% ($n=3$; $p<0.01$). Despite the up-regulation of mitochondrial proteins, preliminary experiments indicate that mitochondrial number is not increased in DAT-Tg animals (WT= 54.6 relative units, Tg= 60.0 relative units; $n=6$).

Discussion: The magnitude of up-regulation observed through immunoblot studies is different from what was observed in the 2D-DIGE. One possible explanation for this difference may reside in post-translational modifications incurred by the tested proteins. From our initial observations, Complex I and III of the electron transport chain in DAT-Tg animals may be dysfunctional compared to WT controls.

8. Nedd42 regulation of Slick channels.

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Background: The most common cause of neuropathic pain is diabetes. Pain sensation in diabetic neuropathy is complex, consisting of negative signs such as sensory deficits, weakness and reflex changes, and positive signs such as thermal hyperalgesia and allodynia. Sensations of burning pain, spontaneous paresthesias and dysesthesia also occur. Alterations in dorsal root ganglion (DRG) excitability is known to initiate and maintain neuropathic pain, however, the principal ion conductances responsible for DRG hyperexcitability during neuropathic pain are still unresolved. Sodium-activated potassium channels (K_{Na}) are highly expressed in pain-sensing DRG neurons. Our recent findings indicate that K_{Na} channels contribute to firing accommodation in DRG neurons. There are two genes that encode K_{Na} channels namely Slack and Slick and both genes are expressed in DRG neurons. In heterologous expression systems, Slick outward currents display very fast activation kinetics. Neuronal simulations predict that homotetrameric Slick channels will prevent action potentials from forming. We performed sequence analysis of the Slick N-terminal and found a "PY" motif essential for binding by the ubiquitin-protein ligase, Nedd42. Additionally, it is known that homotetrameric Slick channels do not express well. We wanted to determine whether Nedd42 controls the expression of homotetrameric Slick channels.

Methods and Materials: Whole-cell voltage clamp electrophysiology was performed on Slick transfected CHO cells. Site-directed mutagenesis was performed to mutate the putative Nedd42 site in Slick.

Results: Nedd42 mutant Slick currents were significantly higher than wild-type Slick currents. To further confirm the regulatory role of Nedd42 on Slick channels, we recorded Slick currents incubated with or without a myristoylated Nedd42 peptide inhibitor. Endogenous Nedd42 inhibition increased Slick current expression compared to control.

Discussion: Our data indicates that Nedd42 controls Slick channel expression and this suggests that pharmacologically targeting Nedd42 could represent a novel form of analgesia. Our results may also suggest that aberrant expression of Slick channels causes the sensory deficits associated with diabetic neuropathy because ubiquitination is perturbed during diabetes.

9. Effect of GDP on potency and efficacy at the μ opioid receptor as measured by [^{35}S]GTPγS binding.

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Background: The potency and efficacy of agonists and inverse agonists at the μ opioid receptor, as well as a range of other G protein-coupled receptors, may be functionally assayed by measuring the effect of an agonist or inverse agonist on [^{35}S] GTPγS binding to cell membranes in which the receptor is expressed. The concentration of guanine 5'-diphosphate (GDP) in the assay buffer is an important determinant of ligand efficacy and potency and affects ligands to the same receptor differently. We performed a comparison of the effects of GDP on the full μ opioid receptor agonist [D–Ala^2,N-Me-Phe^4,Gly^5-ol]-enkephalin (DAMGO), the partial agonist buprenorphine, and the inverse agonist 7-benzylidenenaltrexone (BNTX).

Materials and Methods: DAMGO, buprenorphine, and BNTX were titrated against the binding of 0.08 nM [^{35}S]GTPγS to membranes harvested from Chinese Hamster Ovary (CHO) cells stably expressing the human μ opioid receptor in an assay buffer of 50 mM Tris-HCl, 3 mM $MgCl_2$, 0.2 mM EGTA, and 100 mM NaCl, pH 7.4, and varying concentrations of GDP. For inverse agonist effects, experiments were repeated with native CHO membranes to measure non-specific effects. To better characterize inverse agonist activity, the effect of substitution of K^+ for Na^+ ions in the assay buffer was studied.

Results: GDP concentration-dependently increased the stimulation of [^{35}S]GTPγS binding by 200 nM DAMGO and 100 nM buprenorphine. When DAMGO and buprenorphine were titrated at 30 μM GDP, the efficacy of DAMGO at stimulating [^{35}S]GTPγS binding increased by 70% and the potency decreased by 60% compared to titration at 3 μM GDP, while the efficacy and potency of buprenorphine were similar at both 3 μM and 30 μM GDP. Substitution of K^+ for Na^+ ions resulted in a GDP-dependent increase in basal [^{35}S]GTPγS binding compared with Na+ conditions. In K^+ conditions, GDP was required for the inhibition of [^{35}S]GTPγS binding by the inverse agonist BNTX, and, in a titration, GDP increased inhibition of 1 μM BNTX with an I_{max} value of 26% and an IC_{50} value of 4.5 μM GDP.
Discussion: Consistent with previous reports, GDP increased full agonist efficacy to a greater degree than partial agonist efficacy. However, GDP also increased the inhibitory effect of a maximally effective concentration of BNTX, suggesting that BNTX efficacy is also positively regulated by GDP. These results suggest that even for inverse agonists, efficacy in the [^{35}S]GTPγS binding assay is amplified by increasing GDP concentration.

10. Combined Rb1 and Thoc1 deletion induces a synthetic growth defect in cultured mouse embryonic fibroblasts.
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Background: Rb1 is a tumor suppressor gene frequently inactivated in cancer, whose protein product (pRb) regulates the cell division cycle and other homeostatic functions. Thoc1 encodes a protein (pThoc1) component of the THO/TREX complex mediating transcription elongation, mRNA processing and export. pThoc1 is overexpressed in breast and prostate tumors compared to normal tissue; preclinical evidence supports a particular dependency of transformed cells on Thoc1 function. Although pRb and pThoc1 physically interact, the significance of this interaction is unclear. Recent experiments in our lab have begun to investigate the genetic interaction between Rb1 and Thoc1, using Tamoxifen-induced, Cre-mediated recombination in mouse embryonic fibroblasts (MEFs).
Materials and Methods: MEFs were isolated from C57BL/6 mice containing the Rosa26Cre-ERT2 transgene and intronic LoxP sites in both alleles (F/F) of Rb1, Thoc1, or both genes. These three cell lines were spontaneously immortalized in culture, followed by incubation in the presence of 4-OH-Tamoxifen (Tam) or ethanol vehicle (Eth). Cells were harvested daily for protein and every three days for trypan blue exclusion-based viable cell counts and reseeding at 1.25 X 105 cells / 100 mm dish.
Results: Treatment of $Rb1^{F/F}$ MEFs with Tam resulted in depletion of pRb but did not affect viable cell population doubling (PD) compared to Eth treatment. $Thoc1^{F/F}$ MEFs treated with Tam were depleted of pThoc1 and showed a defect in PD compared to the Eth-treated control by day 8. $Rb1^{F/F}$:$Thoc1^{F/F}$ MEFs treated with Tam were depleted of both pRb and pThoc1, while showing a more severe PD defect than Thoc1-floxed MEFs by day 8. PD rate in Tam-treated MEFs increased to near-control level by day 11 in $Thoc1^{F/F}$ MEFs, but not in $Rb1^{F/F}$:$Thoc1^{F/F}$ MEFs.
Discussion: These results suggest that, while conditional deletion of Rb1 alone does not slow population doubling in cultured immortalized MEFs, its combination with Thoc1 deletion compounds the proliferation-retarding effects of the latter manipulation. Future work in our lab will extend these observations in primary MEFs, as well as characterize the independent and interdependent interactions of these genes with proliferation, apoptosis, and cellular stress responses. The results of this project are expected to contribute to a growing body of evidence implicating Thoc1 as a cancer-selective molecular target, by determining the effects of Rb status on cellular responses to Thoc1 depletion.

11. Ric-8 proteins regulate heterotrimeric G protein biosynthesis as a Gα subunit folding chaperone.
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Background: Proper localization and expression of heterotrimeric G proteins is crucial for cellular signal transduction. We recently demonstrated that Ric-8 guanine nucleotide exchange factors regulate an early event during heterotrimeric G protein α subunit biosynthesis. Newly made Gα subunits are defective in initial association with an endomembrane in Ric-$8A^{-/-}$ cells. To define the precise molecular events by which Ric-8 mediates Gα biosynthesis, we utilized the rabbit reticulocyte lysate (RRL) cell-free translation system to study potential Ric-8A influence of Gα subunit translation kinetics and protein folding.
Materials and Methods: The kinetics of Gα subunit translation and production of functional, folded protein from mock- and Ric-8A-(immuno) depleted RRL were compared. Gα proteins were examined using a trypsin protection assay of the activated conformation. Resolution of translated Gα proteins by gel filtration chromatography enabled evaluation of intermediate complexes of chaperones and Gα subunits during biosynthesis and folding.
Results: Endogenous Ric-8A was immunodepleted from RRL. Gα subunit translation rates and overall produced protein amounts were equivalent in the Ric-8A- and mock-depleted lysates. Resolution of translated Gα proteins by gel filtration chromatography resulted in distinct elution profiles when the Gα subunits were produced from Ric-8A- or mock-depleted RRLs. The majority of translated Gαq eluted as an apparent monomer from both lysates, while a small portion eluted with a mass ≥ 670 kDa, consistent with reports that newly translated Gα binds the ~900 kDa cytosolic chaperone complex. An intermediate Gαq peak of ~100 kDa was observed only when the mock depleted lysate was used. This species is consistent in size with the Ric-8A:Gαq heterodimer. The function of Ric-8A in Gα biosynthesis was revealed when folded Gα protein levels were quantified. Properly folded Gα subunits can adopt the active GTP-bound conformation, a form resistant to limited trypsinolysis. GDP-$AlF4^-$-bound Gα subunits produced in mock-depleted RRL had characteristic resistance to trypsinolysis. However, Gα subunits produced from Ric-8A-depleted RRL were not protected. Add back of purified Ric-8A protein to the Ric-8A-depleted RRL prior to or after Gα translation restored or enhanced protection to trypsin of GDP-$AlF4^-$ bound Gα subunits.
Discussion: This is the first report that Ric-8A serves a necessary function as a folding chaperone for Gα subunits during biosynthesis. The CCT (chaperonin containing TCP1) complex was previously shown to aid folding of G protein β and G protein αT subunits. We propose that Ric-8A works in concert with the CCT to facilitate G protein folding during biosynthesis. (Support by NIDA Grant T32 DA07232).

12. Pharmacokinetic/pharmacodynamic modeling of cocaine self-administration behavior in rats.

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To develop a predictive pharmacokinetic/pharmacodynamic (PK/PD) model to describe the concentration-effect relationship between cocaine in blood and cocaine-taking behavior in a rat model of self-administration (SA), extinction, and reinstatement.

Male Long-Evans rats were allowed to self-administer cocaine by snout-poking (0.5 mg/kg/infusion IV) for 3h session over 21 days. Each snout poke into an active (cocaine-paired) hole initiated a cocaine infusion and a cued 25-sec time-out period in which no cocaine infusions were allowed. After 3 wks, snout poking into the active hole was extinguished by 2-4 wks of exposure to the SA procedure without cocaine infusions. After extinction, the effect of a non-contingent cocaine exposure (10 mg/kg IP) on reinstatement of the active snout-poke response was evaluated. Blood cocaine profiles were simulated using a literature PK model, and number of infusions (or "sham" infusions during extinction and testing) was used as a PD biomarker. A linked PK/PD model was developed using Adapt 5 and validated with an external dataset.

The effect of cocaine on infusion (or sham) frequency was described well by a PK/PD model that used an indirect inhibitory structure incorporating a hypothetical dopamine (DA) PD compartment. The PK component was a 2-compartment model with a cocaine elimination rate constant of 331 d-1 and a volume of distribution of 1.2 L/kg. In the model, cocaine inhibits apparent DA elimination (IC_{50}=504 ng/mL) which stimulates a behavioral response rate constant (k_{in}). The model predicts that k_{in} decreases with a lag–time (26.1 d) to achieve a new response baseline (2.3 vs 11 infusions/session), which agreed well with an external dataset. A PK/PD model was developed to describe the behavioral effect of cocaine during three phases: active SA, extinction and reinstatement. The model predicts that repeated exposure to cocaine with extinction leads to a new steady-state in the behavioral response to cocaine.

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13. Effects of xenobiotics on microRNA expression in a concentration and time dependent manner in PANC-1 human pancreatic carcinoma cells.

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Abstract: MicroRNAs (miRNAs) are a new emerging class of small, noncoding RNAs in control of posttranscriptional gene regulation in humans. Aberrantly expressed miRNAs hold the promise as biomarkers for diagnosis and prognosis in clinical therapy. Pancreatic cancer is the fourth leading cause of cancer-related deaths in the US due to a late diagnosis, fast tumor progression and low response rate to chemotherapy. Our unpublished data suggest that a new miRNA miR-1291 may act as a tumor suppressor. Therefore, this study is aimed at investigating the impact of different agents (e.g., doxorubicin, bilobalide, enoxacin, ciprofloxacin, ofloxacin and retinoic acid) on miR-1291 expression in PANC-1 human pancreatic carcinoma cells. Our data revealed that miR-1291 was significantly increased by 50 μM of all tested compounds at different time points (24, 48 and 72 h). 5 μM of enoxacin and retinoic acid increased the miR-1291 expression only at 72 h, while doxorubicin, bilobalide, ciprofloxacin and ofloxacin elevated the miR-1291 expression at 24, 48 and 72 h. At a concentration of 1 μM, only doxorubicin increased the miR-1291 expression at 72 h. These results demonstrated that the tested xenobiotics including anti-cancer drugs and natural compounds altered the level of miR-1291 expression in a dose and time-dependent manner.

14. Crosstalk between NFκB and STAT3 in human multiple myeloma cells (U266).

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Purpose: To evaluate a potential interaction between NFκB and STAT3 pathways and its consequences on treatment outcome (cell survival) in a human myeloma cell line (U266).

Methods: A computational network model of signal transduction in U266 cells was used to simulate the interplay between NFκB and STAT3, suggesting that inhibition of just one of the pathways does not result in inhibition of NFκB and downstream processes, as NFκB expression is partially regulated by STAT3. To test this hypothesis, U266 cells were exposed continuously to either an IKK inhibitor (IKKi; 10 μM), a JAK inhibitor (JAKi; 10 μM), or bortezomib (Bort) (a proteasome inhibitor; 20 nM). The time-course of intracellular proteins were measured over 48 h (immunobloting), including: phospho-p65 (p-p65), p-stat3, p-IκBa, and Bcl_{XL}. Cell proliferation was measured using WST-1 reagent assay kit at 72 h. PARP cleavage was measured as an indicator of apoptosis over a 24 h period. U266 cells were also exposed transiently (1 and 2 h) to Bort treatment, and the time-course of p-p65, Bcl_{XL}, and PARP was measured. Results: Expression of p-p65 was unchanged following continuous exposure to IKKi and JAKi. However, p-p65 expression initially increased (1.7-fold) followed by a decrease below baseline following Bort exposure. Bcl_{XL} expression remained unchanged after IKKi exposure, and it decreased (80 %) either rapidly or gradually after JAKi or Bort exposure. p-stat3 expression rapidly decreased after Bort (80 %) and was transiently increased after IKKi treatment (2 fold). For short-term Bort exposure, p-p65 expression transiently increased (2.5 fold) and then returned to baseline. Bcl_{XL} expression decreased (50 %) and achieved a new steady-state baseline. Bort exposure for 2 h resulted in significant PARP cleavage (10 fold increase) in agreement with model simulations.

Conclusions: Both NFκB and STAT3 pathways need to be inhibited to fully inhibit NFκB activity. Similarly, when NFκB and STAT3 are constitutively active, both transcription factors need to be inhibited to inhibit cell proliferation. Further research is needed to better understand mechanisms of p-stat3 inhibition and p-p65 transient stimulation and long-term inhibition following Bort exposure to U266 myeloma cells.

Chapter News

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15. MT_1 or MT_2 melatonin receptor deletion abrogates methamphetamine-induced reward in C3H/HeN mice.

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Background: Methamphetamine (METH), a highly abused psychostimulant, has been extensively studied for its ability to produce and enhance reward through its action on the mesolimbic dopamine system. Deletion of both the MT_1 and MT_2 melatonin receptors blocks METH-induced locomotor sensitization in mice suggesting a role for melatonin in modulating sensitizing responses (Hutchinson et al., Program No.669.12. 2010 Neuroscience Meeting). The MT_1 and MT_2 G-coupled protein receptors (Dubocovich et al., Pharmacol Rev 2010,62:343-380) are located in several brain regions including areas of the reward pathway (Uz et al., Mol Brain Res 2005,136:45-53). The goal of this study was to determine the role of melatonin receptor activation in modulating METH-induced reward.

Materials and Methods: Reward was induced by subjecting male C3H/HeN mice to a conditioned place preference test during Zeitgeber Time [ZT] 6-8 (12h:12h light-dark cycle, ZT 0 = lights on). Chamber duration was measured during the drug-free habituation and pre-test sessions using the LocoScan System (Clever Inc, Reston, VA). Animals were conditioned for 6 days with alternating treatments of METH (1.2 mg/kg, ip) and vehicle (VEH). During conditioning METH was paired with the least-preferred compartment as determined by the duration of time spent in each compartment during the pre-test. Mice were tested for place preference 1 day after the last conditioning session. Data was analyzed using Student's t-test or Two-Way Analysis of Variance (ANOVA) with Bonferroni correction for paired comparisons.

Results: A preference score was derived by subtracting the duration spent in the VEH-paired compartment from the duration spent in the METH-paired chamber. Wild-type mice exhibited a significantly higher preference score when receiving METH (226.0+35.48 s, n=11, $p<0.001$) compared to VEH controls (-36.26+28.14 s, n=12). In contrast MT_1 knockout mice showed no significant difference in preference scores for the METH group (5.38+54.68 s, n=8) and the VEH group (7.94+105.40 s, n=6). MT_2 knockout mice displayed a similar pattern to the MT1 knockouts with no significant difference in preference scores between the METH group (15.85+47.55, n=11) and the VEH group (2.40+66.55, n=8).

Discussion: Our results suggest the rewarding properties of METH, as measured by the conditioned place preference paradigm, are dependent on either the MT_1 or MT_2 melatonin receptors. Along with reported data (Wang et al., Eur J. Neuroscience 22:2231,2005) these results suggest the presence of two alternate pathways for melatonin's effect, one linked to reward (MT_1) and the other to learning and memory (MT_2). (Supported by DA 021870 to MLD)

16. Development of in situ gelling systems for sustained drug release.

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Background: Oral administration of naltrexone is used to treat drug addiction, but its efficacy and patient compliance are compromised by the extensive drug first-pass metabolism and low bioavailability. We propose to develop bioresponsive fluid precursor formulations that can be subcutaneously injected and spontaneously form hexagonal phase gels in situ upon water absorption from the tissue for naltrexone sustained release.

Material and Methods: Monoolein, phytantriol, decylglucoside and vitamin E TPGS were investigated as structure-forming compounds to form hexagonal phase. Monoolein and phytantriol were mixed with vitamin E or oleic acid (nonpolar compounds at 10%, w/w) for hexagonal phase formation at body temperature. Propylene glycol (5-30%, w/w) was added as viscosity modifier. The influence of water uptake (10-90%, w/w) on phase behavior of systems was investigated by polarized light microscopy, and phase diagrams were plotted. The swelling kinetics of monoolein- and phytantriol-based precursor formulations containing vitamin E, oleic acid and propylene glycol (5 and 10%) were studied by weighing to determine water uptake in the precursor formulations up to 48 h.

Results: Monoolein and phytantriol formed hexagonal phases that equilibrated with excess of water once either vitamin E or oleic acid was added, and water was present at 20% or more. Propylene glycol over 20% precluded gel formation. Hexagonal phase formation was not observed in decylglucoside-based systems, whereas hexagonal phases formed by vitamin E TPGS were not stable when water content was above 60%. Water uptake by monoolein- and phytantriol- based precursor formulations reached a plateau after 24 h, and followed second-order kinetics independently on the type of non-polar compound and amount of propylene glycol.

Discussion: Monoolein and phytantriol-based precursor formulations containing oleic acid or vitamin E formed hexagonal phases over a wide range of aqueous content. The hexagonal phase was formed within 4 and 12 h for monoolein-based and phytantriol-based formulations respectively, suggesting that control over naltrexone release could be obtained faster if monoolein is used as structure-forming compound. (Study supported by PhRMA foundation and Albany College of Pharmacy and Health Sciences).

17. Chlorpyrifos bioactivation by CYP2B6 variants.

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Chlorpyrifos (CPF), a widely used organophosphorus (OP) pesticide is bioactivated to the potent cholinesterase inhibitor, chlorpyrifos-oxon (CPF-O), by cytochrome P450 enzymes (CYPs). CYP2B6 is the CYP with the lowest reported Km and highest Vmax for this reaction and is a polymorphic enzyme with variants that may impact human susceptibility to CPF. In this study, CYP2B6*1, *4, *5, *6, *7, and *18 were over-expressed in mammalian COS-1 cells to assess the impact of CYP2B6 variants on bioactivation of CPF. Cell lysates were incubated with 0-100μM CPF and the production of CPF-O was measured via HPLC analysis. CYP2B6 content was determined by western blot. CYP2B6*18 had undetectable protein

and activity. The intrinsic clearance (CLint - Vmax/Km) was not significantly different from wild-type for CYP2B6*6 but was significantly greater for CYP2B6*4,*5, and*7. Additionally, human liver microsomes (N=22) genotyped for CYP2B6*1 and CYP2B6*6 were assayed for ability to metabolize CPF at 10uM and at 0.5uM. CYP2B6*6 specimens had both reduced protein expression and CPF-O metabolite formation. Together, these data support the conclusion that variants of CYP2B6 may have altered capacity to metabolize CPF and affect individual susceptibility by altering hepatic expression of CYP2B6 protein and/or CLint for CPF-O formation. Also, the kinetic parameters generated here may be used to assess the impact of CYP2B6 genotype on current human risk assessment efforts which currently need large uncertainty factors to account for unknown interspecies and interindividual variability. (NIH R01 ES016308, EPA STAR grant R833454, and NIH F30 ES020655).

18. Profenofos metabolism and estimates of profenofos exposure in Egyptian cotton field workers.

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Profenofos is a thiophosphate organophosphorus (OP) pesticide capable of inhibiting B-esterases such as acetylcholinesterase, butyrylcholinesterase, and carboxylesterase. Detoxification of profenofos is known to be mediated by cytochrome P450s (CYPs) to form the biologically inactive metabolite 4-bromo-2-chlorophenol (BCP), which is excreted in the urine. The goal of the present study was to investigate the metabolism of profenofos by human liver microsomes and recombinant human CYPs as well as determine the concentration of BCP, a profenofos specific metabolite, in urine from Egyptian cotton field workers involved in profenofos application. Human liver microsomes and recombinant CYPs were incubated with profenofos and the rate of BCP formation was assessed. A preliminary screen with 9 CYPs found that only CYPs 3A4, 2B6, and 2C19 were able to metabolize profenofos. Subsequent kinetic analysis found that CYPs 2B6 (Km=1.02) and 2C19 (Km=0.516) have a higher affinity for profenofos metabolism than CYP 3A4 (Km=18.9), suggesting that 2B6 and 2C19 are primarily responsible for profenofos metabolism under real world exposure conditions. Daily urinary BCP concentrations were utilized as a biomarker of profenofos exposure for Egyptian cotton field workers during 8-10 consecutive days of profenofos application to cotton fields. Substantial interindividual variability was observed in urinary BCP concentrations which ranged from 0.9 - 8,053µg/g creatinine. From these BCP levels, estimates of daily absorbed profenofos were also calculated and ranged from 0.037-327µg/kg/day. This is the first report to estimate the internal dose of profenofos in humans following occupational exposure. (NIH R01 ES016308 and ES016308-02S)

19. Ion selectivity of the fusion pore.

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Background: Release of hormones and neurotransmitters occurs by exocytosis, initiated by the formation of the fusion pore. The initial fusion pore has molecular dimensions with a diameter of 1-2 nm and a lifetime on the millisecond time scale. Understanding the molecular structure of fusion is essential for a mechanistic understanding of vesicle-plasma membrane fusion and transmitter release. Bovine chromaffin cells release monovalent cationic catecholamines, norepinephrine and epinephrine. Release of cationic catecholamines through a narrow fusion pore requires compensation of the change in charge. The decrease in concentration of extracellular sodium decreases the catecholamine flux through the fusion pore as expected from electrodiffusion theory. These experiments suggest that in chromaffin cells, the release of these cationic catecholamines is accompanied by sodium influx through the early fusion pore from the extracellular side into the vesicle, and not accompanied by significant co-release of anions. However, chromaffin granules also contain anionic adenosine triphosphate (ATP). The previous evidence suggests that either the fusion pore is cation selective or the ATP is bound and can therefore not be released with the catecholamines. This study investigates the ability of extracellular anions to permeate the fusion pore and thereby accelerate catecholamine release from the vesicle through the fusion pore.
Materials and Methods: The change in catecholamine flux through the fusion pore, measured as an amperometric foot signal from primary bovine chromaffin cells with a carbon fiber electrode, is determined in the presence of extracellular solutions that vary with major anions of varying size and mobility such as chloride, HEPES, and D-glutamate.
Results: HEPES and D-glutamate have a mobility that is three times lower than chloride anions. Substitution of most of the chloride by HEPES or D-glutamate produces a significant decrease in mean foot current (p-value 0.02). There is also a change in foot duration and quantal size (p-values 0.1 & 0.07, respectively).
Discussion: A significant change in fusion pore current is affected by extracellular anionic mobility, as expected for a fusion pore permeable to anions. There is also a change in quantal size and life time of the fusion pore with anions of lower mobility but with p-values greater than 0.05. There is a possibility of extracellular monovalent anion influx accompanying the release of catecholamines through the fusion pore. Further experiments are required to determine if the anion dependence of the foot current amplitude is specifically due to anion permeability of the fusion pore. (Supported in part by NIH grants R21NS072577 and R01MH095046.)

20. Ubiquitin-dependent regulation of phospho-AKT dynamics by NEDD4-1 in IGF-1 response.

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Chapter News

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21. Resveratrol as a dual regulator of estrogen receptor alpha (ERα) and tumor suppressor p53 function in breast cancer cells.

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Background: We have reported that Estrogen Receptor α (ERα) binds to tumor suppressor p53 and represses p53's transcriptional function by recruiting corepressor complexes to p53-target gene promoters. Of significance, 17β-estradiol/estrogen promotes the formation of the p53-ERα-corepressor complex on p53-target gene promoters, while anti-estrogens prevent the recruitment of ERα and its associated corepressors. Phytoestrogens, or dietary estrogens, are naturally occurring plant compounds that share structural similarity to 17β-estradiol and modulate ERα activity. Resveratrol (RV) is a phytoestrogen found in grapes, peanuts, and red wine that has been reported to alter both ERα and p53 expression. Additionally, RV has been shown to have anti-tumorigenic properties. Based on this information, we hypothesize that a mechanism by which RV exerts its anti-tumorigenic effect is by preventing the inhibitory effects of ERα on p53.

Materials and Methods: The effect of RV on total ERα and p53 protein expression and p53 phosphorylation status was analyzed in MCF7 human breast cancer cells by western blot analysis. The effect of RV on subcellular localization of ERα and p53 was monitored by immunofluorescence. The effect of RV on cell viability was assessed using trypan blue exclusion assays. Cell cycle arrest was monitored by immunoblot analysis of CDKN1A/p21$^{CIP1/WAF1}$ levels (prototypic cell cycle arrest marker). Apoptosis was analyzed by poly-ADP ribose phosphate (PARP)-cleavage assay. Last, the effect of RV on protein-protein interactions between ERα and p53 were examined by co-immunoprecipitation assays.

Results: Dose and time-dependent experiments in MCF7 cells have shown that at 40μM, RV downregulates ERα and induces ERα nuclear accumulation, both of which are consistent with ERα activation. Interestingly, at the same dosage regimen, RV also activates p53 and causes phosphorylation of p53 at serine 15. RV halted proliferation of MCF7 cells after 24 hours by increasing the levels p21$^{WAF1/CIP1}$ without causing substantial cell death. At later time points, however, RV did promote apoptosis, as assayed by analysis of PARP cleavage.

Discussion: Previous studies on RV have focused on either its effects on ERα or p53, but this is the first study to suggest that RV modulates the activity of both ERα and p53 at the same time. Future experiments will focus on examining the effect of RV on the expression of ERα- and p53-target genes and study how RV affects the transcriptional regulation of these genes by modulating the assembly of the p53-ERα-corepressor protein complex.

Study was supported by the Hubbell Family Grant (RPCI Alliance Foundation to GMD.

22. Endoplasmic reticulum-associated erlin1/2 complex is a novel PI(3)P effector.

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Proteins containing the stomatin/prohibitin/flotillin/HflK/C (SPFH) domain are ubiquitously expressed membrane-bound proteins that play important roles in vesicle trafficking, ion channel regulation, and protein chaperoning. Typically, they share two common features: the formation of high-molecular mass ring-like complexes, and association with lipid raft-like domains, such as cholesterol-rich, detergent-resistant membranes (DRMs). Erlin1 and erlin2 are members of this family that associate into high molecular weight (~ 2MDa) complexes on the endoplasmic reticulum (ER) membrane. We have previously shown that erlin1/2 complex binds to activated inositol 1,4,5-triphosphate receptors to mediate their ubiquitination and degradation via ubiquitin-proteasome pathway (UPP). However, their distribution on the ER membrane and place in intracellular signaling pathways remain poorly characterized. Initial analysis of the lipid-binding properties of the immunopurified endogenous erlin1/2 complex in lipid-overlay assays unexpectedly demonstrated a strong interaction with phosphoinositides (PIs) phosphorylated at positions 3, 4 and 5 on inositol ring. This interaction was recapitulated with recombinant erlin2. Point mutations in its oligomerization domain did not abolish the lipid-binding ability. Titration of erlin2 with the head groups of monophosphoinositides revealed that the highest affinity was for PI(3)P, a key player in multiple intracellular signaling cascades. Interestingly, no putative lipid-binding domain was identified in erlin2 protein sequence using bioinformatic approaches. Deletion analyses of bacterially expressed erlin2 mutants revealed the SPFH domain as a region essential for interaction with PI(3)P, suggesting a potential role for erlin2 as an effector in PI(3)P-mediated signaling cascades. We are currently investigating the role of this binding in vesicle trafficking events.

23. Prostate regeneration capability of human prostate cells isolated based upon side population and ALDEFLUOR® assays.

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Background: Prostate stem cells are defined by their ability to serially regenerate prostatic epithelium. In contrast to using cell surface markers for identification of prostate stem cells, functional assays based on common stem cell phenotypes such as high ATP binding cassette (ABC) transporter mediated efflux of dye cycle violet (Side Population assay) or aldehyde dehydrogenase (ALDH)-mediated oxidation of aldehydes (ALDEFLUOR® assay) are also studied. These functional assays are based upon mechanisms that protect cells from environmental insult thus contributing to the survival of the stem cell population and thus may represent a more representative stem cell population compared to surface marker expression.

Materials and Methods: We have initially isolated and analyzed cells digested from 12 clinical prostate specimens based on side population assay and 6 clinical prostate specimens based on ALDEFLUOR® assay. Prostate stem cell properties were tested by serial recombination with rat urogenital mesenchyme.

Results: All isolated cells were capable of prostatic growth in the tissue recombination assay. The side population and ALDH^{Hi} isolated cells were enriched for cells capable of serial prostate epithelial regeneration up to third generation. Differentiation of recombinants was observed by

immunohistochemical analysis for expression of p63 in basal cells, androgen receptor and prostate specific antigen in luminal cells and chromogranin A in neuroendocrine cells.
Discussion: Our studies show that side population and ALDEFLUOR® assays could be used for enrichment of prostate stem cells from freshly digested and sorted human prostate tissues. Further studies could be aimed at using ABC transporter and ALDH activities to validate their potential as a prognostic marker to detect prostate cancer stem cells. Determining the role of ABC transporter and ALDH activity in the relapse of prostate cancer, as a consequence of cancer stem cell survival, could further be helpful in improving therapeutic outcome of patients with advanced prostate cancer. (This work was supported by NYSTEM (CO24292) and NIH (R01DK091240) grants).

24. Mechanism and regulation of DHHC proteins.
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DHHC proteins are a family of enzymes that catalyze the addition of palmitate, a 16-carbon fatty acid, to proteins via a thioester bond at cysteine residues. The enzymatic addition and removal of palmitate can dynamically regulate the properties of both soluble and integral membrane proteins. There are 23 DHHC proteins in mammalian cells (DHHC 1-24, there is no DHHC 10), all of which contain a conserved cysteine rich domain, including a characteristic Asp-His-His-Cys (DHHC) motif. The biomedical importance of DHHC proteins is highlighted by their links to several disease states, including X-linked mental retardation and Huntington's disease. DHHC proteins transfer palmitate to substrate proteins via a two-step reaction, in which the enzyme first incorporates palmitate, forming an acyl-enzyme intermediate, and then transfers it to a substrate protein. Mutation of the conserved DHHC motif to DHHS results in an enzyme that cannot incorporate palmitate or transfer it to a substrate protein in vitro. As such, the DHHC cysteine is presumed to be the palmitoylated residue in the acyl-enzyme intermediate, although this has not been directly demonstrated. Using acyl-RAC, I have discovered multiple S-linked acylation sites on DHHC3 and the catalytically inactive mutant DHHS3. My results suggest that S-acyl groups at some sites transiently modify DHHC3 as part of the catalytic mechanism, while S-acyl groups at other sites perform different functions, distinct from catalysis. Further experiments are ongoing to determine the role of S-linked acylation in the catalytic mechanism and regulation of DHHC3.

25. Understanding the role of microRNA miR-1291 in posttranscriptional gene regulation in AsPC-1 human pancreatic carcinoma cells.
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MicroRNAs (miRNAs) are small noncoding RNA molecules that play a critical role in post-transcriptional regulation of target genes via translational inhibition or mRNA degradation. There is growing evidence that some miRNAs are differentially expressed in pancreatic adenocarcinoma and normal pancreas, and several aberrantly expressed miRNAs are involved in the regulation of pancreatic cancer cell growth and tumorigenesis. Recently we have discovered a new miRNA miR-1291 that is expressed at a much lower level in human pancreatic ductal adenocarcinoma (PDAC) than that in paired adjacent normal pancreatic ductal tissue. To define the function of miR-1291 in pancreatic cancers, we established a miR-1291 stably transfected AsPC-1 human pancreatic carcinoma cell line. Stem-loop reverse transcription real time PCR analysis demonstrated an increased miR-1291 expression in miR-1291 stably transfected cells than control cells. Immunoblotting analyses showed that miR-1291 affected the protein expression of a number of genes, which are known tumor biomarkers. Characterization of miR-1291 signaling pathways in control of cancer cell proliferation, apoptosis and tumorigenesis is expected to identify new therapeutic targets or strategy for improved cancer treatment.

26. Effects of mutations at intracellular side of S6 on activation and inactivation of Kv1.4 channels.
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Background: Kv1.4 is a member of the shaker-related family of voltage-gated potassium channels encoded by gene KCNA4. This current is found in the endocardium and septum of various mammalian species, including humans and plays a very important role in cardiac repolarization. Kv1.4 is upregulated in hypertrophy and heart failure. Depolarization of Kv1.4 channels produce a rapidly activating and slowly recovering transient outward current, I_{to}. Kv1.4 is a tetramer which has four identical subunits. Each subunit has 6 transmembrane segments, with S4 being a positively charged region which senses membrane voltage. In Kv1.4 there are two distinct but interacting types of inactivation: N-type and C-type inactivation. In response to depolarizing voltage, the movement of S4 is transduced to movement of the intracellular side of S6, resulting in pore opening. Anti-arrhythmic drugs such as quinidine can bind to the intracellular side of S6 in a state-dependent manner to maintain normal heart rate. In this study, we studied the effects of mutations on intracellular side of S6 (V561) on channel activation and inactivation.
Material and Methods: Mutations were made using Agilent Quickchange site-directed mutagenesis kit. Oocytes were collected from mature female Xenopus laevis under anesthesia. Defolliculated oocytes were then injected with mRNA for a Kv1.4 clone isolated from ferret heart, fKv1.4, using Nanoject microinjection system. Oocytes were clamp with a two-microelectrode bath clamp amplifier.
Results: We mutated valine to alanine, glycine, isoleucine, leucine, aspartic acid, lysine, phenylalanine, proline, serine, methionine, cysteine, threonine at position 561. Among these mutants, glycine, leucine, aspartic acid, lysine, phenylalanine, proline and methionine mutations didn't generate any measurable currents. N-terminal-intact fKv1.4[V561A] and fKv1.4[V561T] channels inactivate at a much slower rate than wild type Kv1.4 channels. fKv1.4[V561S] and fKv1.4[V561C] have very similar phenotype as wild type Kv1.4. Both activation and inactivation rate of fKv1.4[V561I] are significantly slowed by point mutation.

Discussion: A valine to alanine mutation at intracellular mouth of the pore on S6 in human Kv1.1 channels causes episodic ataxia. In this study, we made a series of mutations at equivalent position in Kv1.4 channels. Some of these mutations significantly changed gating kinetics of this channel. Our previous data showed that quinidine can restore the stability of C-type inactivation by binding to the intracellular side of Kv1.4 channels at open state, which indicates that intracellular side of S6 may be a drug target for treatment of heart disease such as arrhythmia.

27. Dual roles of PDEF and the emergence of hormone refractory prostate cancer.

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Androgen deprivation therapy (ADT) is a mainstay of prostate cancer (CaP) management and is effective in causing widespread apoptosis in the tumor, but unfortunately it is rarely curative and often the cancer recurs and is much more aggressive. Furthermore, recent evidence it has been shown that ADT causes cells to undergo an epithelial to mesenchymal transition (EMT) and by this mechanism CaP may gain the ability to metastasize. We therefore explored the effects of ADT on prostate derived Ets transcription factor (PDEF), a protein involved in the differentiation of prostate epithelial cells. In LNCaP cells, both androgen starvation and treatment with androgen receptor (AR) inhibitor was sufficient to down-regulate PDEF. shRNA against AR recapitulated this effect and caused increased growth and migration of LNCaP cells. Ectopic expression of PDEF in cells with AR knocked down inhibited this increase in growth and migration. To further analyze the clinical significance of these observations, a tissue microarray of clinical radical prostatectomy specimens was stained for PDEF and indicated patients in the highest tertile of PDEF expression experienced significantly less metastatic disease compared to those with lower PDEF protein expression. This observation is further supported when just the patients who received ADT are analyzed, where no patients in the highest tertile developed metastatic disease compared to 33% and 40% in the lowest and mid tertiles respectively. PDEF expression may therefore predict patients who are not suitable for ADT. Furthermore, therapies to induce PDEF may present an attractive therapeutic option in combination with ADT.

28. Differential expression of peptidylarginine deiminase 2 (PAD2) during mammary tumor progression.

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Background: The peptidylarginine deiminases (PADs) are a family of calcium-dependent enzymes that post-translationally convert protein arginine residues to citrulline, thus potentially affecting the target proteins' structure and function. While PADs are expressed in a broad range of tissues, enzymatic activity (termed citrullination or deimination) is more restricted and appears to primarily be induced during the onset of inflammation-associated diseases, such as chronic obstructive pulmonary disease, rheumatoid arthritis, multiple sclerosis, and Alzheimer's disease. Inflammation has also long been recognized as a critical component of tumor progression and we have found that expression and activity of one PAD family member in particular, PAD2, is sharply elevated in several aggressive forms of breast cancer. Interestingly, my preliminary studies suggest that two isoforms of PAD2 are expressed in breast cancer cells; a ~75kDa catalytically active long form (PAD2L) and a ~50 kDa short form (PAD2S) which lacks a critical catalytic site and is therefore likely inactive. A better understanding of the mechanisms regulating the differential expression of these two isoforms in cancer cells may prove critical to our understanding of the role of PAD2 in cancer metastasis.
Materials and Methods: PCR, western blotting, immunofluorescence, immunohistochemistry, and tissue culture techniques were performed to investigate the potential mechanisms regulating PAD2 isoform expression during mammary tumor progression using the MCF10A cell line series (MCF10A [normal], MCF10AT [hyperplastic], MCF10DCIS [ductal carcinoma in situ], and invasive carcinoma/ MCF10CA1). Additional samples included, normal canine mammary tissue, BT-474 cells [ER+ and HER2+], SKBR3 cells [HER2 overexpressing], and MDA-MB231 cells [ER-, PR-, and HER2-]).
Results: Preliminary findings suggest that the PAD2L and PAD2S isoforms are differentially expressed across the breast cancer progression cell lines, with the PAD2S isoform being observed in more benign cell types and tissues while the longer isoform is expressed in several of the more malignant lines.
Discussion: This is the first report to document the presence of multiple PAD2 isoforms in breast cancer cells. Currently, I am investigating the mechanisms which regulate this change in isoform expression patterns and testing whether this switch plays a role in cancer progression.

29. Function and pharmacology of mGluR2/4 heterodimers.

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Metabotropic glutamate receptors (mGluRs) were thought until recently to function mainly as stable homodimers, but recent work suggests that heteromerization is possible. Despite the growth in available compounds targeting mGluRs, little is known about the pharmacological profile of mGluR heterodimers. Here, this question was addressed for the mGluR2/4 heterodimer, examined by coexpressing both receptors in isolated sympathetic neurons from the rat superior cervical ganglion (SCG), a native neuronal system with a null mGluR background. Under conditions that favor mGluR2/4 heterodimer formation, activation of the receptor was not evident with the mGluR2 selective agonist DCG-IV, nor with the mGluR4 selective agonist L-AP4, but full activation was apparent when both ligands were applied together, confirming that mGluR dimers require ligand binding in both subunits for full activation. Properties of allosteric modulators were also examined, including the findings that negative allosteric modulators (NAMs) have two binding sites per dimer, and that positive allosteric modulators (PAMs) have only a single site per dimer. In SCG neurons, mGluR2/4 dimers were not inhibited by the mGluR2 selective NAM Ro 64-5229, supporting the two site model. Further, application of the mGluR4 selective PAMs VU 0361737 or PHCCC and combined application of mGluR4 PAMs with the mGluR2 selective PAM BINA failed to potentiate glutamate responses through mGluR2/4, supporting the single site model and suggesting that mGluR2/4 heterodimers are not modulatable by PAMs that are currently available.

Chapter News

Upstate New York Pharmacology Society

30. A regulatory feedback loop between CaMKK2 and the androgen receptor in prostate cancer progression.

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Background: The androgen receptor (AR) plays a critical role in prostate cancer (PCa) progression, however, the molecular mechanisms by which the AR regulates cell proliferation in androgen-dependent and castrate-resistant PCa are incompletely understood. Recent studies have shown that kinases play an important role in enhancing AR transcriptional activity during PCa progression and therefore may be promising targets for PCa therapy. Involvement of Ca^{2+}/calmodulin-dependent kinase kinase 2 (CaMKK2) in PCa has been largely unappreciated. However, numerous expression profiling screens have identified CaMKK2 as a gene being overexpressed in PCa. We undertook the work reported here with three aims: to determine how the expression of CaMKK2 relates to PCa progression, to examine the regulation of CaMKK2 expression by the AR and to place this mechanism in the context of AR-dependent control of PCa cell proliferation.

Results: Immunohistochemical staining revealed that CaMKK2 increases during progression of human PCa and becomes nuclear or perinuclear in advanced disease. In a mouse model of PCa (Transgenic Adenocarcinoma of Mouse Prostate, TRAMP), CaMKK2 expression increases with progression with many cells exhibiting nuclear staining. CaMKK2 expression is higher in human castrate-resistant tumor xenografts compared with androgen–responsive xenografts and is markedly higher in the AR-expressing, tumorigenic cell line LNCaP compared to cell lines which are AR-non-expressing and/or non-tumorigenic. In LNCaP cells, dihydrotestosterone (DHT) induced CaMKK2 expression while androgen withdrawal suppressed CaMKK2 expression. Interestingly, DHT stimulation of LNCaP cells also induced nuclear translocation of CaMKK2. Knockdown of CaMKK2 expression by siRNA resulted in reduction of LNCaP cell proliferation caused by a block at the G1/S interphase. Finally, we examined whether CaMKK2 regulates AR transcriptional activity. CaMKK2 knockdown reduced 1) the expression of the AR target gene, prostate specific antigen (PSA), 2) AR transcriptional activity driven by androgen responsive elements from the prostate-specific probasin gene promoter and 3) levels of the AR-regulated cell cycle protein, cyclin D1.

Discussion: Our results suggest that in PCa progression, CaMKK2 and the AR are in a feedback loop in which CaMKK2 is induced by the AR to maintain AR activity, AR-dependent cell cycle control and continued cell proliferation. This newly described feedback loop may be one of the escape routes by which the AR maintains activity during androgen ablation therapy. Hence, a therapeutic agent targeting CaMKK2 may be effective in combination with androgen ablation to suppress a mechanism triggered in relapsed tumors by which the AR maintains control of malignant cell growth and proliferation.

31. Long-acting kappa opioid receptor antagonists failed to stimulate phosphorylation of c-Jun N-terminal kinase.

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Background: Bruchas et al. (2007), using Western blot analysis, have reported long-acting kappa opioid receptor (KOR) antagonist activation of c-Jun N-terminal kinase (JNK) in HEK293 cells transfected with rat KOR. Furthermore, they have suggested that activation of JNK is correlated with duration of action of these antagonists. In this study, we tested whether KOR agonists and long-acting antagonists increased phosphorylated JNK (pJNK) mediated by the human KOR using an ELISA.

Materials and Methods: Chinese hamster ovary (CHO) cells stably expressing the human KOR were used in Fast Activated Cell-Based ELISAs from Active Motif to measure total JNK and pJNK. Serum-starved cells were treated with varying concentrations of agonists or long-acting antagonists, and then fixed with 4% formaldehyde in PBS buffer. Fixed cells were washed, quenched, blocked, and exposed to primary and secondary antibodies according to the kit instructions. Total and pJNK levels were measured for each treatment and OD_{450} readings were corrected for cell number using crystal violet staining. Each treatment was performed in triplicate. Ratios of pJNK to total JNK for each treatment were compared to the no treatment control to determine stimulation of pJNK.

Results: Time-course results showed rapid activation of JNK (5 min after treatment) with U50,488, a KOR-selective agonist. Maximal activation was maintained for 40 min and gradually decreased to basal levels by 5 hr after treatment. Titrations with KOR-selective agonists, U50,488 and dynorphin peptides, increased pJNK levels with low nanomolar EC_{50} values. U50,488 (10 μM) did not increase pJNK in native CHO cells indicating a KOR-mediated JNK activation. The long-acting KOR antagonist, nor-BNI, completely inhibited U50,488-stimulated pJNK. We did not detect increases in pJNK levels with any long-acting KOR antagonists. Total JNK was not affected by any treatment.

Discussion: KOR agonists are potent activators of JNK, resulting in elevated pJNK levels. Compared to Western blot data from Bruchas et al. (2007), U50,488 was 2400-fold more potent in the ELISA. In contrast to Bruchas et al. (2007), we did not observe JNK activation by long-acting KOR antagonists suggesting that the long duration of action of these antagonists may not be linked to JNK activation. (Supported in part by the Paul Stark Endowed Professorship and the Margo Cleveland Fund.)

32. Dimeric two-ring scaffolds for targeting human MT_1 and MT_2 melatonin receptors.

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Background: Sleep disorders such as insomnia and circadian sleep disorders affect approximately 70 million Americans. Current treatments for sleep disorders include behavioral modifications and over the counter or prescription medication. While these medications are generally safe and effective, prominent side effects such as morning sedation, memory problems, headaches, discontinuation insomnia, and sleepwalking have been reported. Non-selective MT_1/MT_2 melatonin receptor ligands Ramelteon, Agomelatine, PD 6735 and VEC 162 have been tested in humans at doses much higher (8 to 100 mg, oral) than the doses of melatonin (0.3 to 1 mg, oral) required for the treatment of insomnia or the phase shifting

of circadian sleep onset. Such high doses of melatonin receptor agonists may potentially increase melatonin receptor desensitization and/or internalization, and hence decrease therapeutic efficacy. Discovering selective melatonin receptor ligands will significantly advance studies on the mechanism(s) of action of melatonin and the potential for identification of new drug discovery targets.
Materials and Methods: Molecular dynamics (MD) based flexible docking was employed to screen a large library of compounds. A combination of FlexX (SYBYL, Tripos), MOE (Chemical Computing Group, Inc.), INSIGHT (Accelrys), and manual structure-guided docking were employed to map receptor ligand binding surfaces. From these results, a mini library of substituted tryptophans, naphthalenics, and tetralones were synthesized. Compounds were screened in vitro by competitive 2-[^{125}I]-iodomelatonin binding to membranes of hMT_1 and hMT_2 melatonin receptors expressed in CHO cells.
Results and Discussion: Computational screening of an in-house library of 64 million compounds against pseudoreceptor-shape-based pharmacophores yielded a focused library of MT_1 or MT_2 specific compounds. From the library, 20 small molecule structures comprised of flexibly linked dimeric two- ring scaffolds were synthesized. The predicted (in silico) and observed (in vitro) MT_1/MT_2 affinity ratio had a correlation coefficient of 0.985. M20, a substituted two-ring heterocyclic compound, emerged as the top MT_2 ligand. While GTP shift assays confirmed M20 as an MT_2 agonist, removing a N-tert-butyloxy group from the indole ring of M20 results in a 80 fold reduction in its selectivity towards MT_2. In silico models in conjunction with competitive binding experiments were used to iteratively optimize M20's selectivity and affinity. Bulky alkyl groups in the spacer region connecting the two ring scaffold significantly reduced their affinity towards both MT_1 and MT_2 receptors. These studies have furthered our understanding of the structural requirements for specific binding of ligands to MT_1 and MT_2 receptors. The current compounds will serve as leads for iterative improvement of selectivity through computer aided drug discovery and chemical syntheses. Further studies will explore 2-ring scaffolds constrained by rigid linkers. Support by NS 061068.

33. TAAR1 molecular chaperone discovery with the use of a novel assay for surface expression.
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Background: Trace Amines are endogenous monoamines, structurally close to other neurotransmitters such as dopamine, serotonin, and norepinephrine. The family of G-protein Coupled Receptors (GPCR) that bind trace amines are known as the Trace Amine Associated Receptors (TAAR). TAAR1 signalling has been shown to negatively regulate dopaminergic transmission. However the study of TAAR1 has been limited due to lack of surface expression of TAAR1 in heterologous cell systems. We hypothesize that a molecular chaperone exists to help traffic TAAR1 to the plasma membrane which will allow for the screening of novel compounds for TAAR1. We have developed a novel assay for quantifying surface expression of membrane proteins by tagging the N-terminus of our receptors with the HA tagged β-lactamase enzyme. By using the cell impermeable β-lactamase substrate nitrocefin, we are able to quantify surface expression. We will compare this assay to an ELISA using β_2-Adrenergic receptor (β_2-AR) and GABAB R1(GBR1) stable cell lines.
Materials and Methods: We have generated β-lactamase fusion constructs and produced stable cell lines for the following GPCRs: β_2-AR and GBR1. The cells are seeded at a concentration of 10 000 cells per well in a 48 well plate. The cells were washed once and nitrocefin added to the wells. A mouse monoclonal anti-HA antibody was used for all ELISAs.
Results: Using this assay we were able to consistently monitor agonist induced internalization of β_2-AR in a dose and time dependent manner. An ELISA was done on the same cells and yielded poor response to agonist induced internalization. In addition pre-incubation with the antagonists (propranolol or alprenolol) was able to dose dependently block agonist mediated mediated internalization of this receptor. Lastly it has been shown in the literature that proper trafficking of GBR1 to the plasma membrane requires its dimerization with GBR2, the molecular chaperone of GBR1. In our stable cell lines for GBR1, transfection of increasing amounts of GBR2 lead to increased surface expression of GBR1 in a dose dependent manner.
Discussion: We have therefore validated a novel assay for the quantification of surface expression of membrane proteins. This assay is robust, quick, and potentially done in a high throughput manner in comparison to current cell surface expression assays. We will therefore apply this assay for the discovery of the molecular chaperone of TAAR1 using expression cloning.

34. Mapping residues important for Ric-8A interaction with G protein α subunits using a random mutagenesis yeast two hybrid screen.
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Background: Ric-8 proteins were discovered as Gα binding proteins by yeast two-hybrid (Y2H) screening. Ric-8A and Ric-8B are guanine nucleotide exchange factors (GEF) for monomeric G-protein α subunits and also serve a vital function in G protein biogenesis. Little detailed information is known about the protein-protein interaction surfaces that enable Ric-8 to facilitate Gα subunit nucleotide exchange or biogenesis, or how these two seemingly distinct activities may be related.
Materials and Methods: To identify Ric-8A amino acids involved in the Ric-8A and Gα interaction, we developed a Y2H screen to identify Ric-8A mutants that no longer interacted with Gα. A Ric-8A "gapping" Y2H prey plasmid was created. The full length Ric-8A coding sequence was then amplified by mutagenic PCR using $MnCl_2$ and suboptimal dNTP concentrations. The Ric-8A gapped prey construct and mutagenic Ric-8A PCR product were co-transformed into L40 yeast expressing various Gα baits and screened for loss of Y2H interaction. Mutant Ric-8A clones that displayed a lost Gα interaction were rescued, retested for plasmid linkage, and sequenced.
Results: The Ric-8A prey gapping construct was created by quick-change mutagenic PCR. 5′ and 3′ Xba I sites located 159 and 194 nucleotides, respectively from the 5′ Ric-8A start and 3′ Ric-8A stop codons were introduced. The 5′ mutation was silent and the 3′ mutation changed Ric-8A M467L. The construct was verified by Xba I digestion and DNA sequencing. The Ric-8A gapping prey was equally efficient at interacting with Gα

baits as the Ric-8A wild type prey. A mutagenic Ric-8A PCR library was produced using error-prone PCR. Yeast expressing Gα baits were transformed with linearized Ric-8A gapping prey and the mutagenic Ric-8A PCR library. Yeast repaired, mutant Ric-8A preys displaying attenuated interaction with Gα bait were rescued and retested. Those Ric-8A preys with attenuated interaction with Gα will be sequenced. In the case of multiple mutations, statistical comparison of multiple clones will be made to identify common mutants with attenuated interaction.
Discussion: Our Y2H screen will identify Ric-8A amino acid residues required for interaction of Ric-8A with Gα. These mutants will be useful towards understanding the mechanism of Ric-8A-mediated G-protein activation and/or folding. Many G protein GEFs have specific residues that insert into the G protein guanine nucleotide-binding pocket to disrupt contacts between the G protein and the guanine ring and/or β-phosphate group of GDP. It is anticipated that our randomized Ric-8A mutagenesis screen will elucidate these so-called Ric-8A catalytic residues.

35. VZV infection of primary rat DRG neurons.
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Background: Varicella zoster virus (VZV) is the causative agent of chicken pox (varicella) during primary infection and herpes zoster (HZ) or shingles upon reactivation from latency. During primary infection, VZV establishes latency in dorsal root ganglia (DRG) and in trigeminal ganglia. Most studies indicate that neurons are the primary if not exclusive site of latent virus. Reactivation of VZV is frequently associated with symptoms ranging from discomfort to serious, long-lasting pain known as post-herpetic neuralgia (PHN). In the United States, the number of individuals suffering from HZ is estimated to be approximately 1 million people annually. Between 25 to 50% of these affected individuals will develop PHN VZV reactivation can also result in vasculopathy, myelopathy, retinal necrosis and cerebellitis. Our goals are to establish an in vitro VZV latency model to study viral reactivation and to investigate the viral induced changes in neuronal excitability.
Methods and Materials: Cultured embryonic DRG neurons and glia were infected by a VZV recombinant virus containing green fluorescent protein (pOka VZV-GFP). Immunocytochemistry and electrophysiology analyses were conducted on infected neurons.
Results: Cultured DRG neurons were mock-infected or infected with pOka VZV-GFP for 48 hours and the expression of GFP was monitored by fluorescent microscopy. In addition to observing GFP fluorescence, we observed multinucleated neuronal syncytia. VZV is known to form syncytia in non-neuronal cells through a cell-to-cell fusion from within indicating that the virus is able to replicate within neurons also. We did not observe glial infection. We performed current-clamp electrophysiological recordings on neurons infected by pOka VZV-GFP. The rheobase, which is the minimal current amplitude required to elicit an action potential was significantly less than mock-infected neurons. Therefore action potentials are more readily fired in VZV-infected neurons. During 2.5X suprathreshold stimulus conditions over 1000 ms, we also observed no firing accommodation in VZV-infected neurons (6 of 6 neurons. All neurons fired >2 action potentials during the sustained stimulus.
Discussion: This is the first demonstration of VZV-induced neuronal syncytia. Replication in neurons is likely the manner in which HZ progresses to PHN. Because we can demonstrate replication in neurons, our in vitro model may allow us to screen novel antivirals during HZ. In our preliminary studies we provide the first electrophysiological evidence that VZV produces neuronal hyperexcitability. This could lead to the identification of putative molecular targets for analgesia in HZ-induced pain.

36. The function of Wuzhi tablet in protection of liver function may involve its action on liver-specific microRNA miR-122.
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Wuzhi (WZ) tablet is an ethanol extract from Wuweizi (Schisandra sphenanthera), a traditional Chinese medicine that has been used for thousands of years in protection of liver functions in chronic hepatitis and liver dysfunction patients. The major bioactive constituents of WZ tablet include schisandrin A, schisandrin B, schisandrin C, schisandrol A, schisandrol B, schisantherin A, schisantherin B and gomisin C, which exhibit a variety of pharmacological effects. MicroRNA (miRNA) miR-122 is a liver-specific miRNA in regulation of a number of target genes in lipid metabolism. Most interestingly, replication hepatitis C virus (HCV) is revealed to be dependent on miR-122 expression, and targeting miR-122 holds as a novel means for HCV therapy. In this study, we investigated the effects of WZ on miR-122 expression in human cell models. Our data showed that all active ingredients of WZ exhibited a time-dependent impact on miR-122 expression. Among them, schisandrin B altered miR-122 expression to the highest extent. The results may facilitate the mechanistic understanding of WZ in protection of liver functions and offer insights into the development of new therapeutic strategy for diverse liver diseases.

37. Gating differences in HERG cardiac splice variants.
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Background: HERG (human ether-a-go-go-related gene, Kv11.1) encodes a voltage gated potassium channel which plays an important role in repolarization of the cardiac action potential. Mutations in HERG give rise to the arrhythmogenic congenital long and short QT syndromes. In addition, HERG is the putative mediator of drug-induced arrhythmias for a diverse range of compounds, including many non-cardiac drugs. All new drugs seeking FDA approval require certification that they do not block HERG. Most electrophysiological research, as well as preclinical drug screening, has been performed on the HERG1a isoform. However, there are two naturally occurring cardiac splice variants, HERG1a and HERG1b. These are identical except for divergent N-termini. The HERG1a N-terminus has 396 amino acids whereas HERG1b has only 56 amino acids, of which 36 are unique to this form. HERG1b has faster activation and its deactivation kinetics is greatly accelerated. Functional HERG channels are

composed of four subunits consisting of one or more isoforms. This study design was designed to better determine the role of the N-terminus during activation and deactivation.
Materials and Methods: Defolliculated Xenopus Laevis. oocytes were digested in collagenase, and injected with up to 50 ng of cRNA. Oocytes were voltage clamped in the whole cell mode configuration using a two microelectrode oocyte clamp amplifier. Data were digitized and analyzed using pCLAMP 9.2. Further analysis was performed using Clampfit 9.2, Excel and Origin.
Results: We studied HERG1a, 1b, and channels formed from tandems with HERG1a linked to 1b. Our data show that deactivation of HERG1b is faster than HERG1a, and HERG1b steady-state activation V_{half} is shifted slightly to the less negative potentials, compared to the HERG1a. The slope factor is also modulated. We used a mathematical model of HERG to determine the transitions affected by the N-terminal. All the changes are well described by modulating the magnitude and voltage sensitivity of only the deactivation rate that communicates directly with the open state.
Discussion: Our data indicate that the N-terminal domain, which is absent in HERG1B, strongly slows the transition out of the open state of the channel which indicates channel closing. The open state is thought to be critically important as the major drug binding conformation of HERG. The N-terminus may strongly modulate the ability of the HERG channel to bind drugs. (Supported in part by NIH HL093631)

38. The combination of colistin (C) & doripenem (D) is synergistic against high inoculum pseudomonas aeruginosa in an in vitro hollow fiber infection model.
NEANG S. LY[1], GAURI G. RAO[1], PAMELA A. KELCHLIN[1], PATRICIA N. HOLDEN[1], ALAN FORREST[1], JÜRGEN B. BULITTA[1,2], PHILLIP J. BERGEN[2], ROGER L. NATION[2], JIAN LI[2], BRIAN T. TSUJI[1]; [1]Laboratory for Antimicrobial Pharmacodynamics, School of Pharmacy and Pharmaceutical Sciences, University at Buffalo, SUNY, Buffalo, NY; [2]Facility for Anti-Infective Drug Development and Innovation, Monash Institute of Pharmaceutical Sciences, Monash University, Melbourne, Australia**Background:** PA is a very problematic pathogen especially at high bacterial densities in infections such as bi-lobar pneumonia. As therapeutic options against PA are very limited, combinations of C are the last line of defense. Our aim was to evaluate combos of C & D or C & R against MDR PA.
Methods: A HFIM was used to simulate clinically relevant C, R & D regimens against three isolates of PA at an initial inoculum of 10^9 cfu/mL over 10 days. PA ATCC 27853 (C^S) (MICs to C, R & D were 1, 32, 0.25 mg/L), and two clinical isolates. C heteroresistant (C^{HR}) (MICs to C, R & D were 1, 32, 1 mg/L) and C-resistant (C^R) (MICs to C, R & D were >128, 32, 1 mg/L). C was given as 2 and 5 mg/L continuous infusion. R was dosed every 24 h (Cmax 5 mg/L, t1/2 3 h) and D every 8h (Cmax 25 mg/L, t1/2 1.5 h). Samples were collected serially over 240 h for viable counts and C population analysis profiles (PAPs) were conducted.
Results: C alone against C^S displayed initial reductions in viable counts by up to 4.2 log, followed by extensive regrowth 24h. C was inactive against the C^R & C^{HR} isolate; C monotherapy led to amplified resistance among strains. R alone was similar to growth control. D alone showed rapid initial killing up to 5.2 log followed by regrowth within 24 h. Combos of C 2 mg/L or 5mg/L with R showed initial killing up to 3.0 log within 24 h but regrowth occurred by 240 h. C 2 mg/L with D showed initial killing up to 2.5 log within 24 h, followed by regrowth in C^S & C^R isolates. C 5 mg/L with D completely eradicated (>9.1 log) C^S and C^{HR} isolates within 24 h and sustained killing to 240 h, with suppression of C-resistant subpopulations relative to baseline.
Conclusion: C & D combo demonstrated rapid synergistic killing and prevention of resistance at clinically relevant doses against PA at a high bacterial density. Further study is warranted.

39. The MT_1 melatonin receptor as a principal mediator on methamphetamine-induced sensitization in C57BL/6.
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Background: Deletion of both MT_1 and MT_2 melatonin receptors abolishes the development and expression of locomotor sensitization in melatonin-proficient C3H/HeN mice after six daily pretreatments with methamphetamine (METH; Hutchinson et al. (2010) Program 669.12. Neuroscience Meeting Planner). Locomotor sensitization has been proposed to model neuroadaptations that underlie craving, suggesting the melatonin receptors may modulate neuropharmacological processes associated with drug abuse. Sensitization was not observed in C57Bl/6 mice, which produce lower melatonin levels, after an identical regimen of six daily METH pretreatments. In contrast, one METH pretreatment (5.0 mg/kg, i.p.) induced locomotor sensitization in ddY mice (0.5 mg/kg, i.p.; Nikaido et al. Mol Pharmacol 2001;59:894-900). Thus we investigated whether a single injection of METH could induce locomotor sanitization in C57Bl/6 mice.
Materials and Methods: Male C57Bl/6 wild-type and MT_1KO mice maintained on a 12h:12h light/dark cycle were habituated to test arenas for three days. The mice were pretreated on Day 1 with an injection of saline or METH (1.2 mg/kg, i.p.). On Day 9, all mice were challenged with METH (1.2 mg/kg, i.p.). Locomotor activity was monitored for 2 hours by digital video during pretreatment and challenge trials. Trials were conducted during the light phase (Zeitgeiber time [ZT]5-7, ZT0 = lights on) and dark phase (ZT19-21).
Results: During the light phase, METH pretreatment increased locomotor activity in wild-type mice (60.6±5.5, n=12) compared to VEH (15.6±4.4, n=12, $p<0.05$) on Day 1. METH challenge on Day 9 triggered the expression of locomotor sensitization in METH-pretreated WT mice (106±5.2, n=12) but not in VEH-pretreated controls (63.2±7.5, n=12, $p<0.05$). Similar responses were observed after pretreatment and challenge in wild-type mice tested during the dark phase. In contrast, MT_1KO mice expressed an increased response to METH pretreatment (51.2±5.8, n=12) compared to VEH (11.9±1.5, n=11, $p<0.05$) only during the light phase, not during the dark, and did not express sensitization after METH challenge at either time of day.
Discussion: Our results lead to two conclusions: 1) that locomotor sensitization can be induced in C57BL/6 mice with a single METH pretreatment, and 2) that METH-induced sensitization in C57BL/6 mice requires the expression of the MT_1 receptor. Thus the MT_1 receptor is a potential target for identifying novel treatments of METH abuse based on receptor antagonism or inverse agonism. (Supported by DA021870 to MLD.)

Chapter News

Upstate New York Pharmacology Society

40. Identification of phospholipase C epsilon (PLCε) substrates in neonatal rat ventricular myocytes (NRVM).

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We have previously identified novel physiological roles for GPCR-dependent regulation of phospholipase C (PLC) activity in cardiac myocytes. Mice with knockout of a novel GPCR regulated PLC, PLCε, knockout mice show greater susceptibility to cardiac hypertrophy relative to the wild type mice, and have diminished ionotropic responses to β-adrenergic receptor stimulation (Wang et. al. 2005, Oestreich et, al., 2009). To explore a mechanistic role for PLCε in GPCR-dependent hypertrophy, we utilized an siRNA protocol in an established cellular model of cardiac hypertrophy, the neonatal rat ventricular myocyte (NRVM). Surprisingly, in this cell model, we found that PLCε appears to be required for development of cardiac hypertrophy downstream of multiple hypertrophic stimuli. To examine the subcellular localization of PLC that might lead to generation of IP3 and DAG at specific cellular sites showed that in NRVM's, PLCε is scaffolded to a member of the protein kinase A anchoring protein (AKAP). It was shown that PLCε directly binds to an isoform of the family of AKAP proteins, muscle AKAPβ (mAKAPβ), and that this scaffolding by mAKAPβ is important for the ability of PLC to regulate cardiomyocyte hypertrophy. This suggests that scaffolding of PLCε to the nuclear envelope by mAKAPβ regulates expression of hypertrophy (Zhang et. al., 2011). The substrate for most PLCs, phosphatidylinositol 4,5-bisphosphate (PIP_2), is not present at the nuclear envelope in most cells but rather localizes to the plasma membrane.

In order to identify substrates of PLCε at the nuclear envelope in NRVMs, GFP-tagged PH-domain constructs which show specificity towards PIP_2 or phosphatidylinositol 4-phosphate (PI4P) were transfected into NRVMs and visualized by confocal microscopy to determine their subcellular location. As expected, NRVMs transfected with two separate PIP_2 probes, Tubby-PH-GFP and PLCδ-PH-GFP, displayed a primarily plasma membrane distribution with no staining of the nuclear envelope. On the other hand both PH domain constructs specific for PI4P (FAPP1-PH and OSBP-PH) both had a strong and distinct peri-nuclear pattern of expression. Previously, these constructs have been shown to have localization to the Golgi but not nuclear expression in Cos cells. This is the first demonstration of a predominant location for PI4P at the nuclear envelope in any cell type. To determine if this PI4P is a substrate for PLCε peri-nuclear fluorescence of these constructs was monitored over a period of time after activating PLCε with a pharmacological activator. PI4P associated perinuclear fluorescence showed a time dependent decrease after stimulation of PLCε suggesting that PI4P is a substrate for PLCε at the nuclear envelope. Given the current data, we propose that perinuclear PI4P is the substrate of PLCε in NRVMs whose hydrolysis generates diacyglycerol locally at the nucleus to mediate activation of protein kinases involved in cardiac hypertrophy mediated through PLCε.

41. Small molecule inhibition of Mixed Lineage Kinase 3 (MLK3) attenuates cardiac fibroblast activation and pathologic cardiac remodeling.

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Background: Heart failure (HF) is a manifestation of most cardiovascular diseases whose increasing prevalence highlights the need for novel therapeutics. The extent of pathologic cardiac remodeling is correlated with clinical outcome in HF. Importantly, cardiac injury enhances cardiac fibroblast (CF) ("support cell") activation, producing myofibroblasts that release pro-fibrotic/inflammatory mediators that target cardiomyocytes (CM) ("functional cell"), CFs, and local inflammatory cells to exacerbate remodeling. Mixed Lineage Kinases (MLKs) are a family of stress-activated MAPKKKs whose functional role(s) in the heart remain largely unknown. MLK3 has been implicated in HIV-associated neurocognitive disorder (HAND), where it mediates deleterious cross-talk between microglia ("support cell") and neurons ("functional cell"), suggesting an analogous mechanism of pathologic intercellular CF-CM communication in HF. We hypothesize that MLK3 exacerbates cardiac remodeling through enhanced CF activation that contributes to pathologic cardiac intercellular communication.

Materials and Methods: We have synthesized a series of MLK3-specific small molecule inhibitors, one of which (URMC-099) was found to attenuate microglial-mediated neurotoxicity in murine models of HAND. Neonatal rat ventricular CFs were stimulated with isoproterenol (Iso), angiotensin II (AngII), TNFα, or TGFβ in the absence or presence of URMC-099. We assessed subsequent gene (RT-PCR) and protein (IF, Western blotting) expression and secretion (Luminex) of various proinflammatory cytokines (IL-1β, IL-6, MCP-1, TNFα) and of the myofibroblast marker α-smooth muscle actin (αSMA). Further, therapeutic efficacy and specificity of URMC-099 were tested in an acute pharmacologic (Iso-pump) model (7-day) and a myocardial infarction (MI) model of HF (12-week) using both wild-type (WT) and $MLK3^{-/-}$ mice.

Results: Treatment with URMC-099 was shown to attenuate TGFβ-induced JNK activation, a predominant downstream target of MLK3, in isolated fibroblasts. Concurrent treatment with URMC-099 attenuated both αSMA, indicative of myofibroblast transition, and proinflammatory cytokine production (MCP-1, IL-6) in pathologically stimulated CFs. In vivo, URMC-099 significantly attenuated cardiac hypertrophy (HW:BW) and reduced interstitial fibrosis (assessed by Masson's Trichrome staining) in an iso-pump model of HF in WT mice. Current echocardiographic data post-MI suggest cardioprotection in the MLK3-/- (shown to have no overt cardiac phenotype) and URMC-099 treated mice while highlighting the importance of appropriate timing of initiation of therapy.

Discussion: This study has elucidated a previously unappreciated role for MLK3 in cardiac pathophysiology. Our collaborative data not only suggest a role for MLK3 in cardiac remodeling through pathologic CF activation but also indicate a possibly novel paradigm of pathologic intercellular communication in multiple disease states.

Chapter News

Upstate New York Pharmacology Society

42. Neurodegeneration and Increased Sensitivity to MPTP Induced by Dopamine Transporter Over-Expression in Transgenic Mice.

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Background: While the pathological loss of nigrostriatal dopaminergic neurons in Parkinson's disease (PD) is well-established, the etiology of this neurodegeneration remains elusive in the majority of cases. Various genetic and environmental insults have been implicated in instigating selective damage to dopaminergic neurons, suggesting that these cells are inherently vulnerable. Their endogenous neurotransmitter dopamine (DA) itself can produce reactive oxygen species and quinones through metabolism, enzyme-dependent reactions and autoxidation. Thus, the presence of a constant cytosolic pool of DA could place these neurons under chronic oxidative stress. DA levels are modulated by the presynaptic dopamine transporter (DAT) which reuptakes extracellular DA. To investigate the effects of intracellular DA accumulation, we generated and characterized a novel strain of transgenic mice (DAT-tg) that over-express DAT specifically in DA neurons. Additionally, we evaluated the response of DAT-tg mice to the exogenous toxicant MPTP.

Materials and Methods: DAT-tg mice were generated by microinjection of a bacterial artificial chromosome containing the mouse DAT locus (40kb) and flanking DNA sequences (80kb). Western blots, immunohistochemistry, high-performance liquid chromatography and stereology were used to evaluate protein levels, DA tissue content and the number of DA neurons, respectively. Adult DAT-tg and wild-type (WT) mice were treated with two i.p. injections of MPTP (15 or 30mg/kg) and sacrificed after seven days. The extent of toxicity was assessed using aforementioned techniques.

Results: DAT-tg mice exhibit three times greater levels of DAT protein in comparison to WT. This over-expression is selective to DA neurons as portrayed by similar tissue localization of DAT between WT and DAT-tg mice. Furthermore, DAT-tg mice have 30-40% lower striatal DA content which is due to neurodegeneration as evidenced by a loss of 30-40% of dopaminergic neurons. DAT-tg mice are more sensitive to MPTP treatment as demonstrated by reduced tyrosine hydroxylase protein and immunoreactivity, along with lower striatal DA content versus WT treated mice.

Discussion: We report that increased DAT levels in DAT-tg mice lead to basal neurodegeneration possibly due to oxidative stress propagated by intracellular DA accumulation. This highlights the role of DAT and probably cytosolic DA as cell-specific factors that could underlie the inherent susceptibility of DA neurons to damage in PD. Moreover, the exacerbated response of DAT-tg mice to MPTP emphasizes the interplay between genetic and environmental insults. This suggests that idiopathic PD could possibly arise as a result of multiple hits on a system that is intrinsically vulnerable due to the handling of endogenous DA.

(Supported by Canadian Institutes of Health Research and Parkinson Society of Canada)

43. Sprouty1 over-expression induces oligodendrocyte differentiation from primary human oligodendrocyte progenitor cells.

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Oligodendrocytes (OLs) are glial cells in the brain which produce the myelin that insulates axons. In demyelinating diseases, such as multiple sclerosis (MS), the loss of myelin and oligodendrocytes results in chronic neurological dysfunction. Oligodendrocyte progenitor cells (OPCs) exist in the adult brain, and by studying OL development, we can formulate therapy aimed at stimulating endogenous remyelination. We have used cross-species genomic analysis of multiple data sets to identify several species-conserved pathways. Sprouty genes (SPRY) were found among those expressed in human, mouse, and rat OPCs. SPRY homologue 1 (SPRY1) is a dynamic growth factor pathway regulatory protein which has been found to interact with signaling cascades downstream of PDGF, EGF, and FGF stimulation. We hypothesized that SPRY1 might act to block MAPK signaling in OPCs and thereby induce OL differentiation. In order to test the species-conserved role of SPRY1, we developed a lentiviral construct to over-express SPRY1 in human and rat OPCs. To determine the context in which SPRY1 might regulate OL differentiation, we experimented in the presence of PDGF-AA, FGF2, or a combination of factors (10ng/ml each). In rat CG4 cells treated with PDGF-AA, we found that lentiviral SPRY1 significantly increased OL differentiation, as indicated by MBP (25±23% to 42±28%, paired t-test, $p<0.05$, $n=4$), while reducing proliferation as indicated by BrdU (34±10% to 28±8%, paired t-test, $p<0.01$). We next isolated human OPCs from fetal brain using CD140a-based magnetic cell sorting and infected cells with SPRY1 or control lentivirus (18-21 weeks, $n=4$ samples). Infected cells were cultured in the presence of PDGF-AA, EGF, FGF2, a combination of factors, and in an absence of factors (20ng/ml each). We found that lentiviral SPRY1 had a significant inductive effect on $O4^+$ OL differentiation in PDGF-AA (5±4% to 16±7%, two-way ANOVA, $p<0.01$), FGF2 (11±8% to 14±9%, paired t-test, $p<0.05$), the PDGF+FGF2 combined (7±3% to 11±3%, paired t-test, $p<0.05$), and in the absence of growth factors (8±3% to 19±10%, two-way ANOVA, $p<0.01$). Thus, SPRY1 over-expression can induce OL differentiation in both species. In future studies, we will determine whether the inductive effect of SPRY1 is mediated via a blockade of MAPK activation. As such, these results indicate that SPRY1 might be a conserved and key regulator of OL lineage fate, and serve as a novel target to therapeutically regulate OL differentiation in vivo.

44. Central cannabinoid 1 receptors control lung inflammation during endotoxic shock.

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The authors James J., Phan H. and Russo V. contributed equally to this work. Dr. A. Johnson and Dr. C. Feleder share the senior authorship.

Background: It is assumed that lipopolysaccharide (LPS) induces lung inflammation during septic shock by stimulating release of TNF-[alpha] and other mediators from macrophages and endothelial cells. Additionally, the Toll like receptor 4 (TLR-4) pathway seems to be activated and lung permeability is increased. However, recent data showed that LPS hypotension during septic shock can be prevented by inhibiting afferent impulse flow in the vagus nerve, by blocking neuronal activity in the nucleus of the solitary tract, or by blocking [alpha]-adrenergic receptors in the preoptic area/anterior hypothalamic area (POA) and brain endocannabinoid receptors CB-1. These findings suggest that the inflammatory signal is conveyed from the periphery to the brain via the vagus nerve, and that endotoxic shock is initiated through a central mechanism. In the present study, we tested a similar idea, that is, whether central cannabinoid 1 (CB-1) receptors participate in the control of pulmonary distress during endotoxemia based on preliminary evidence that lidocaine administration into the POA and NTS prevents lung inflammation. Furthermore we studied whether central CB1 receptors play a role in the activation of pulmonary TLR-4.

Materials and Methods: Male Sprague-dawley rats where injected intracerebroventricularly (ICV) with CB-1 receptor antagonist Rimonabant (250 ng), CB-1 receptor agonist WIN55 (250 ng) or the vehicle, five minutes before the administration of LPS. LPS was injected intravenous at a dosage of 1 mg/kg. After 30 minutes, animals were sacrificed and lungs were removed for perfusion studies. Pulmonary pressures and permeability data were recorded. Lung tissues were assayed using western blots for studying intracellular cascades after the activation of TLR-4.

Results: ICV administration of Rimonabant significantly inhibited the fall in pulmonary capillary pressures (Ppc's) evoked by LPS. Furthermore, Rimonabant attenuated the decrease of venous resistance and attenuates levels of interleukin-1 receptor-associated kinase 1 (IRAK-1), tank binding kinase (TBK) and phospho-IkB-α. Interestingly, while WIN55 increases the weight-dry lung ratio and permeability, no significant changes were determined in the TLR-4 intracellular pathway.

Discussion: Rimonabant prevented the associated LPS-induced activation of the TLR-4 cascade. Furthermore, Rimonabant attenuated the associated increase of lung permeability whereas WIN 55, in absence of LPS, induces lung injury. This data indicates that central CB-1 receptors may play an important role in the initiation of endotoxin induced lung inflammation. (Supported by NIH HL-059901 to Arnold Johnson and NIH grant to Carlos Feleder.)

45. Development of transcription factor-based ChIP with human primary oligodendrocyte progenitor cells.

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Background: Oligodendrocyte progenitor cells (OPCs) give rise to mature myelin-producing oligodendrocytes (OLGs) in the central nervous system (CNS). A detailed molecular understanding of OLG maturation is critical for designing therapeutic strategies to induce myelin production and repair in the CNS after demyelination by disease or injury. In the rodent, epigenetic and transcriptional regulation of OLG development plays a key role in the specification and differentiation of OLGs. Our recent genomic studies on isolated human OPCs have identified several differences in the transcriptional profiles of human and rodent cells. We hypothesized that transcription factor (TF) binding sites would also be species specific. Furthermore, as epigenetic diffe ences likely exist between primary cells and those derived from either pluripotent stem cells or cell lines, we sought to develop a technique whereby TF-based chromatin immune-precipitation sequencing (ChIP-seq) could be performed on small numbers of human primary cells.

Materials and Methods: To permit modularization to multiple TFs and to improve sensitivity, we generated a lentiviral construct to over-express each TF fused with a C-terminal HA-tag. As a proof-of-principle, we selected SOX10 as it is known to bind to highly conserved sites and is important in OLG maturation. To optimize the protocol, we first infected 1 x 10^6 rat CG4 OPC cells with lentiviral SOX10-HA virus. Expression of SOX10-HA was confirmed using both RT-PCR and western analysis. Infected and mock infected cells were fixed, sonicated, and anti-HA ChIP performed. Real-time quantitative and end-point PCR was performed on known SOX10 binding sites in the PLP1 (proteolipid protein 1) gene. We then performed the HA-based ChIP protocol using 5 x 10^5 human CD140a-sorted OPCs that were cultured for 4 days prior to infection with the SOX10-HA lenti virus.

Results: SOX10-HA infected rat pre-OLG cells were 2.2 and 8.0 fold enriched for species-conserved binding sites in the PLP1 gene (r5 and r7, respectively). Using human-specific primers, we found that both PLP1 binding sites were enriched 9.1 and 3.2 fold relative to mock infected cells. In contrast, control primers were not enriched.

Discussion: These results indicate that TF-based ChIP may be performed on limiting numbers of primary cells. In future studies, we will use high-throughput sequencing to generate genomic scale data of transcription factor binding sites in human primary OPCs.

46. Understanding the molecular mechanism of the large G-protein atlastin and its role in controlling ER morphology.

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Controlling proper membrane architecture and dynamics is an essential hallmark of eukaryotic cell biology. Therefore, it is not surprising that multiple systems exist to facilitate membrane deformation, fission, and fusion. Often, they require energy from nucleotide hydrolysis to mold the elastic lipid bilayer. My studies focus on the protein atlastin, a protein of the dynamin superfamily, which provides the mechanical forces to overcome the energetically unfavorable events during membrane fusion maintaining endoplasmic reticulum morphology. Our lab has recently determined the first structures of atlastin's soluble domains, which have provided

initial clues into atlastin's mode of action. Our preliminary crystallographic data and biophysical measurements indicate a path of conformational changes along atlastin's nucleotide hydrolysis cycle. The proposed molecular mechanism involves an initial, GTP-dependent tethering step, which is followed by a power stroke-like motion to bring two membranes into close proximity for homotypic fusion to occur. In another crystal structure, we observe lattice contacts, indicating a regulatory potential for higher-order complexes. We hypothesize that this feature may prevent unproductive fusion events and bias the system to fuse only opposing membranes. To test this model and further our understanding of the events that mediate homotypic fusion events, we study the effect of structure-guided mutations in a cell culture system. Phenotypic data will be correlated with a detailed analysis of the catalytic activity and structure of the protein in vitro. Such an integrated approach will be required to fully grasp the intricacies of membrane fusion and the underlying molecular mechanisms.

47. Identifying intervention strategies and biomarkers of Rituximab-mediated cell signaling using Boolean network modeling.
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Background: Rituximab is the first approved monoclonal antibody to be used against indolent B-cell non-Hodgkin's lymphoma (NHL) and has been shown to inhibit survival pathways (MAPK, ERK-1/2, NF-kB, and Akt signaling pathways) in B-NHL. Understanding the complexity of rituximab-mediated cell signaling and its effects are essential to identify new potential therapeutic interventions, as well as biomarkers that can help prognosis/diagnosis and improve treatment outcomes. Boolean network models represent one promising approach to achieve these goals, in which networks are qualitative representations of biological systems.
Materials and Methods: Boolean network relationships were constructed in which nodes are different system components and edges represent inter-nodal interactions and relationships. The nodes are characterized by either active (1 or ON) or inactive (0 or OFF) states, and relationships among nodes are described by either activation or inhibition. Rituximab-mediated effects on survival pathways and caspase cascades in Ramos cells were extracted from the literature. Network validation and the identification of potential intervention targets and biomarkers were guided by node steady-states. Intervention strategies and biomarkers were modeled by manipulating Boolean relationships and initial node conditions. Nodes in which inhibition, stimulation and/or removal resulted in a desired outcome were defined as intervention targets and biomarkers. In addition, a new algorithm was developed to reduce Boolean networks while preserving dynamic properties of the original network. The reduction algorithm includes a randomization process, in which the order of the node removal was selected randomly until no other node can be deleted from the network without losing the network dynamics. Odefy, a publicly available MATLAB® toolbox that transforms Boolean relations to ordinary differential equations, was used to simulate the final network.
Results: The inhibition of NF-kB-IKK-IKB protein set, YY, XIAP and the stimulation of FAS, Caspase8, Caspase3, Caspase6, and APC were identified as potential intervention targets for combination therapy. The biomarkers of rituximab-mediated cell signaling were identified as RKIP, NIK, YY and p53. Moreover, the randomized reduction algorithm was useful for determining a rank set of nodes in terms of their importance in the overall network structure.
Discussion: A Boolean network model of rituximab-mediated signaling was successfully queried with an algorithm to identify potential targets and drug activity biomarkers. A reduced network was derived that could be further used to construct enhanced pharmacodynamic models that bridge in vivo drug exposure and responses. (Supported by NIH Grant GM57980 and the UB Center for Protein Therapeutics).

48. Using a yeast two-hybrid system to measure BiP-protein interactions during oxidative stress.
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Background: The accumulation of reactive oxygen species (ROS) can lead to extensive oxidative damage to cellular macromolecules. Accumulation of cellular ROS, known as oxidative stress, has been implicated in aging, neurodegenerative diseases, diabetes, and cancer. An arguably underappreciated source of ROS is protein folding in the endoplasmic reticulum (ER), where up to one molecule of peroxide (a ROS) is generated for every disulfide bond formed. However, little is known about the redox signaling that exists to protect the cell from ROS accumulation and ROS-induced damage in the ER. Our lab has shown that a conserved cysteine residue in the molecular chaperone BiP is modified directly by peroxide during conditions of ER oxidative stress. Modification results in a decreased ATPase activity for BiP. Intriguingly, this loss-of function version of BiP serves to protect cells against oxidative stress-induced damage. In order to gain potential insight into how modification of BiP impacts BiP function, we searched for binding partners of both modified and unmodified BiP using a yeast two-hybrid system.
Materials and Methods: The sequence for human BiP (HspA5) was cloned into a vector in frame with the GAL4 DNA-Binding Domain sequence. This "bait" plasmid was transformed into the yeast strain Y2HGold and mated with yeast containing a Clontech normalized human cDNA library fused with the GAL4 DNA-Activation Domain. Library clones that interacted with BiP were isolated based on their ability to activate the GAL4 HIS3 and X-alpha-Gal reporters. We screened for interactors on media with and without hydrogen peroxide.
Results: The majority of interacting clones pulled from the screen were proteins that interacted with BiP in the absence of peroxide and lost the ability to interact with BiP in the presence of peroxide. Sequencing of the interacting clones revealed mostly short polypeptides with varying lengths (ranging from 7-64 amino acids).
Discussion: We have demonstrated that modification of the BiP cysteine can be effectively recreated in a two-hybrid screen by growth on peroxide-containing plates. The physiological significance for loss of BiP interaction with polypeptides upon peroxide treatment remains to be determined. BiP normally pulls proteins into the ER during translocation and needs to bind peptides to do so. We hypothesize that decreased binding of polypeptides by BiP upon oxidative stress may reduce the amount of proteins entering into the ER lumen. This may be beneficial for the cell as it reduces the burden of malfolded polypeptides on an already stressed ER folding environment.

Chapter News

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49. Comparative fluorescence image guided phototherapy of a near-infrared photosensitizer and its stereoisomers.

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Background: Since the Federal Drug Administrations' approval of the Photodynamic Therapy (PDT) drug Photofrin® in the 1990s for various oncologic uses, there has been a continuous interest to develop improved photosensitizers (PS) which can overcome the limitations associated with Photofrin®. The main limitations associated with Photofrin are (a) prolonged skin phototoxicity and (b) inability to treat large and deeply seated tumors. In our efforts to develop a non-toxic near infra-red (NIR) PS, we synthesized a series of stable bacteriopurpurinimide to overcome the problems associated with Photofrin®.

Materials and Methods: The bacteriopurpurinimide [3-(1-butyloxyethyl)ethyl-3-deacetyl-purpurin-18-N-butylimide methyl ester] with long wavelength absorption at λ_{max} 787 nm and the corresponding stereoisomers (R- & S-) were evaluated for both in vitro (uptake, MTT assay, intracellular localization) and in vivo (tumor uptake, biodistribution, PDT efficacy, target specificity, skin phototoxicity, fluoresce imaging) in mice bearing subcutaneous murine colon26, murine metastatic breast 4T1, or human glioblastoma U87 tumors.

Results: The bacteriopurpurinimide as an isomeric mixture or pure stereo-isomers (R- & S-) exhibit excellent tumor cure in the mouse models with 80% mice tumor-free 60 days after treatment. This was achieved with a dug dose as low as 3.55 µg/mouse, light dose of 135 J/cm^2 at 75 mW/cm^2 with no significant toxicity (including skin phototoxicity). In addition, these PS also showed great potential for fluorescence imaging ability (λ_{ex}: 787nm and λ_{em}: 805 nm). Interestingly, replacing the methyl ester functionality with a carboxylic acid group significantly reduced in vivo uptake and PDT efficacy.

Discussion: The primary focus of this study was to determine the benefits of NIR-PS over the current photosensitizer Photofrin approved by various health organizations including the US FDA. While preliminary data suggests these compounds have exceptionally high long term tumor cure rate, it was the additional discovery of the fluorescence characteristics of the PS that made it even more attractive for further investigation. The fluorescence characteristics of this PS extends its use as a single agent for image-guided therapy in which the NIR-PS can fluoresce to delineate the complete margins of tumors thereby ensuring that the full extent of the tumor is treated with no remaining cancerous cells left behind - a true "see and treat" approach. The newly developed PS provides an opportunity to modify it further by conjugating with certain target specific peptides or ligands that can act as substrates for proteins associated with cellular pathways that are deregulated in cancer thereby improving its therapeutic potential. (Work supported by NRSA T32 CA009072 and the NIH CA-127369 & PO1-CA 55791).

50. Small molecules targeting Poly Adenosine Diphosphate Ribose Polymerases (PARP) as novel anticancer agents.

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Background: Poly-Adenosine Diphosphate Ribose Polymerase (PARP) inhibitors induce DNA repair malfunction which result in cell death. PARP inhibitors have been particularly effective in the treatment of triple negative breast cancers that contain BRCA mutations. PARP has two zinc finger domains, ZN1 and ZN2, it dimerizes and binds to each end of broken DNA. This binding stabilizes the DNA strands and activates PARP. The Rossmann fold in PARP binds the cofactor NAD^+. Extensive depletion of the NAD^+ pool mediates PARP induced necrosis. PARP inhibitors target the catalytic NAD binding site. A search for a pharmacophore in our in house small molecule database resulted in several substituted indoles as top hits. Our objective is to see if these compounds will directly bind to PARP by mimicking the nicotinamide binding mode and inhibit its activity in silico and in vitro.

Materials and Methods: A combinatorial library of substituted indoles was docked against a variety of distinct PARP structures using SYBYL 8.1 and analyzed with USCF Chimera. The known PARP inhibitor 3-aminobenzamide was used as a control.

Results and Discussion: The combined approach of docking studies and QSAR analysis allowed us to gain insights into the structural basis of PARP inhibition. Docking results suggest that these indole analogues are selective for PARP-1 and PARP-2 when compared to other proteins in the PARP super family such as Tankyrase 1 and Tankyrase 2. An increase in the partition coefficient (CLOGP) and decrease in the polar surface area (PSA) of the analogues corresponds to an increase in overall binding affinity. This suggests that the PARP NAD binding site has hydrophobic residues that play a critical role in ligand binding. The top scoring ligands, L022217 (N-[[2-[2-(4-phenylbutyl)-2, 3-dihydro-1-benzofuran-4-yl] cyclopropyl]methyl]butanamide) and luzindole (Melatonin receptor antagonist) have higher binding affinity than known PARP inhibitor 3-Aminobenzamide. 3-Aminobenzamide mimics the nicotinamide site by conformational restriction and hydrogen bonding with the critical residues Gly863 and Ser 904. Luzindole and other indole analogues selectivity picked up these interactions while also making other interactions within the pocket indicatively giving higher scores. Keeping this in mind, the development of multitude of compounds using the in silico method to model binding of PARP with its inhibitors would benefit the discovery of novel hits and lead compounds selectively targeting PARP. (This research is funded by New York State, National Institute of Health (5P20MD002725), through NCMHD-RIMI Program (RVR).)

51. Genome-wide analysis of human oligodendrocyte lineage transcriptional networks reveals conserved targets for myelin repair.

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The directed induction of human oligodendrocyte fate has been impeded by a lack of understanding of the molecular similarities and differences between rodent and human. In this study, we sought to characterize the expression profiles of human oligodendrocyte progenitor cells (OPCs) during their specification and differentiation to oligodendrocytes. We first developed and characterized a novel prospective method for isolation of three stages of progenitor differentiation from fetal human forebrain using two-color FACS with PDGFαR/CD140a and O4 antigens. In vitro characterization revealed that $CD140a^+O4^+$ cells possessed an intrinsic bias to differentiate as oligodendrocytes, while $CD140a^+O4^-$ cells developed as either oligodendrocytes or astrocytes depending on their environment. We used Illumina

whole genome microarray to analyze gene expression and compared the human profiles to mouse cell isolates (Cahoy et al., J. Neurosci. 28:264-278, 2008). We identified the cholinergic receptor muscarinic type 3 (CHRM3) among the most significantly expressed receptors and found that activation of CHRM3 blocks oligodendrocyte commitment by human OPCs. In addition, we used magnetic cell sorting to isolate CD140a^{+} progenitors and analyze their gene expression profiles during oligodendrocyte commitment in vitro. To define transcriptional changes associated with oligodendrocyte differentiation, these profiles were analyzed along with matched PDGF-treated cells, a known mitogen that delays oligodendrocyte development. The human data were then compared to a similar time-course data of rat oligodendrocyte differentiation (Dugas et al., J. Neurosci. 26:10967-10983, 2006). In addition to gene-wise comparison, we used weighted gene co-expression network analysis to determine systems-level gene expression across species. These analyses revealed that, while a substantial number of transcripts were conserved between human and rodent species, there were significant differences between the two species. By identifying the specific pathways conserved between species, these data provide a valuable resource for the design of therapeutically relevant strategies for the stimulation of endogenous repair in demyelinating disease. [Supported by NYSTEM contracts #C026413 and C026428]

52. Calmodulin induced conformational changes in the estrogen receptor alpha: Implications for drug discovery.

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Background: Estrogen mediated signaling affects cell proliferation, differentiation and mammary gland function. Estrogen Receptors (hERs) are present in 70% of breast cancers. Consequently, estrogen mediated hER signaling plays a critical role in breast cancer diagnosis, prognosis, and treatment. Estrogen Receptors are ligand-triggered transcription factors. However, in the absence of cognate estrogenic ligand, hERs can be activated by a variety of other extracellular signals. It has been shown that several proteins like calmodulin (CaM) can bind to unliganded hER and induce transactivation. This is supported by the increased expression of CaM in several malignancies including breast cancer. Calmodulin antagonists have been shown to down regulate hERs and significantly inhibit hER transcriptional activity. It has been reported that melatonin, an indolic hormone produced mainly by the pineal gland, acts as a calmodulin antagonist. Melatonin induces conformational changes in the hER-CaM complex, impairs the binding of hER-CaM complex to DNA, and consequently prevents hER-dependent transcription. Moreover the mutant hER (K302G, K303G) is unable to bind CaM, and becomes insensitive to melatonin. Therefore mapping the CaM/hERα interface will provide more insight to the mechanism behind hERα unliganded activation.

Materials and Methods: Human estrogen receptor alpha (hERα) conformations were trapped and probed using chemical crosslinking in the presence and absence of Ca-CaM. Binding interfaces and folding information were collected using MALDI-FTICR Mass Spectrometry. Predictive models of the CaM/hERα interface were produced using High Ambiguity Driven biomolecular DOCKing (HADDOCK), Molecular Operating Environment (MOE; Chemical Computing Group), SYBYL (Tripos) and Chimera (UCSF).

Results/Discussion: Proteins with IQ, 1-10, 1-14 or 1-16 motifs typically bind to CaM. No known CaM interacting motifs are found in hERα. Calcium bound CaM (residues 88-91) induces a unique hERα conformation, by interacting with hERα residues 288-300. This complex was observed only in high calcium concentrations. A predictive model was generated by integrating the mass spec data with three dimensional coordinates of hER (PDB ID: 3ERD, 1A52) and CaM (1CFF, 2L1W) structures. A closer look at the binding interface reveals CaM presents a large hydrophobic pocket (FILVMLELMIAVF) and makes a critical interaction with W292 of hER, much like it does with the small molecular CaM antagonists W7 and DPD (N-(3,3,-diphenylpropyl)-N'-[1-R-(3,4-bis-butoxyphenyl)-ethyl]-propylenediamine. The model generated can be used to further drug discovery by providing a structural basis for the design and development of drugs that target CaM activated hERα conformation. (Supported by New York State, NIH (5P20MD002725) (RVR) and NSF LSAMP BD grant (BR)).

53. Biomarkers of chlorpyrifos exposure and effect in adolescent Egyptian agricultural workers: A longitudinal assessment.

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Background: Chlorpyrifos (CPF), an organophosphorus (OP) insecticide, is a public health concern due to its widespread potential for human exposure. Blood acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) levels may be used as biomarkers of effect while urinary 3,5,6-Trichloro-2-Pyridinol (TCPy) is a specific biomarker of CPF exposure. A longitudinal study was designed to assess ChE and TCPy levels in adolescent insecticide applicators in Egypt over 10 months from baseline (pre-application) to recovery (post-application). It is not known whether adolescent populations are at increased risk compared to adult workers. This longitudinal study assesses ChE and TCPy in occupationally exposed adolescent applicators and age-matched non-applicators that may have received environmental exposures.

Materials and Methods: Participants included male insecticide applicators aged 12 to 21 years (N=57). Non-applicator participants were from the same villages but did not work in the cotton fields (N=38). Preseason data served as baseline. Blood for ChE analysis was drawn five times, while urine for TCPy analysis was collected eight times for this study. Urinary TCPy was analyzed by negative ion GC-MS and normalized to urinary creatinine levels. AChE and BChE activity was measured using the EQM Test-mate kit.

Results: Pre-spray and mid-spray TCPy levels for the applicators ranged from 2.92 - 42.79 and 5.8 - 3915.16 µg/g creatinine, respectively. Non-applicator group pre-spray and mid-spray TCPy levels ranged from 2.36 – 46.17 and 9.83 – 211.94 µg/g creatinine, respectively. Both applicator and non-applicator groups showed significant inhibition of ChE during CPF application.

Discussion: The quantitative data for OP biomarkers of exposure and effect presented here are the first to be reported for adolescent agricultural workers. The biomarker data in the non-applicators mirrored that of the applicators, indicating that the adolescent non-applicators received seasonal environmental CPF exposures. This suggests that similar exposures may occur in other residents of this agricultural region during periods of agricultural pesticide application. (This research was supported by R21 ES017223 from the Fogarty Institute and the National Institute of Environmental Health Sciences.)

54. Developing new agents for tumor imaging and photodynamic therapy (PDT).

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Background: Molecular imaging can be used for early detection and localization of tumors, and "real time" monitoring of efficacy following therapeutic treatments. In recent years, Positron Emission Tomography (PET) has been included in drug development. Such studies will eventually provide the means to accomplish "personalized medicine" by monitoring individual response to drug delivery and therapy. One of the objectives of our laboratory has been to use porphyrin-based compounds for the treatment of cancer by PDT. The accumulation of certain porphyrins is higher in malignant tumors than in most normal tissues and that has provided an incentive to use these compounds. Also, the absorption wavelength of these compounds in the near infra-red region (NIR) allows for deeper penetration into tissues and provides the capability to image tumors by fluorescence before therapy. This allows for development of an effective theranostic agent allowing for real time imaging of tumor prior to therapy.

Materials and Methods: Based on a SAR study, a radioactively ^{124}I- labeled methyl 3-(m-iodobenzyloxyethyl)pyropheophorbide-a was synthesized and its capability as an imaging agent through optical imaging as well as PET was analyzed. The compound's therapeutic efficacy was analyzed in vitro in cells as well in vivo in mice with tumors. Furthermore, we utilized this newly synthesized compound to detect metastasis as well as orthotopic primary tumors.

Results: The newly synthesized compound exhibited high tumor accumulation, good PDT efficacy and was an excellent agent for tumor detection through PET as well optical imaging. The maximal uptake time for the agent was shown to be 24h and mice exhibited excellent long term cure following a single treatment. The compound was also shown to be efficient at detecting metastasis in lungs in mice with primary breast tumors as well as orthotopic tumors in brain and lungs in mice through both optical imaging and PET.

Discussion: We here report a theranostic agent which showed high in vitro/in vivo efficacy as a PDT agent. The same compound can also be used exclusively as an imaging agent through fluorescence or PET as shown by its ability to accumulate in tumors preferentially. Future direction involves addition of agents such as peptides (targeting integrin receptors) or sugars (binding to galectin receptors) or amino acids (recognizing folate receptors) to enhance its tumor specificity and allow for use of radionuclides with shorter half-lives for developing newer PET imaging agents. (The authors are thankful to NIH RO1CA127369, PO1 CA55791 (partial) and the shared resources of the Roswell Park Cancer Center support grant CA16056 for the financial assistance).

55. Small molecule G protein βγ activators.

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Background: Gβγ effectors bind to a common surface (Hotspot) on Gβγ, but each effector utilizes distinct subsets of amino acids within this surface to mediate binding. Our laboratory has previously identified a series of small molecules that bind to G protein βγ subunits and selectively block Gβγ signaling downstream of GPCRs [1]. This strategy has been shown to be beneficial in a variety of therapeutic paradigms. '12155' is one compound which we found in the screen for binding to Gβγ. Interestingly it caused released Ca^{2+}, even without activation of the receptor, by the ligand. Here we explore the mechanism for this effect.

Materials and Methods: HL60 cells were differentiated to neutrophils to express the fMLP receptor a GPCR. The cells were then treated with fura-2AM and the release of Ca^{2+} was measured by fluorimetry. ERK and AKT activation were also assayed after the neutrophils were treated directly with the compounds for 5 or 3 min respectively. Cells were harvested and blotted for either pERK or pAKT respectively, with ERK blot as the loading control. For PLC assay purified Gβγ, $mG\alpha_i$ and $PLC\beta_3$ were used and assay was done as described in [2].

Results: 12155-dependent Ca^{2+} release was blocked by a PLC inhibitor suggesting the release was dependent on PLC activation. Treatment of cells with Pertussis toxin to inhibit G protein coupling did not affect '12155'-dependent calcium release, which suggested it acts downstream of the receptor and may act on Gβγ directly. To check for a dependence on Gβγ, we used M119 a Gβγ inhibitor. M119 pre-incubation 5min prior to '12155' addition led to inhibition of its calcium release, confirming receptor-independent activation of Gβγ signaling. Using neutrophils we then tested its effect on activation other Gβγ pathways and found it selectively activates ERK, but had little or no effect on AKT activation. By an in-vitro PLC assay, using purified proteins, we found the activity of '12155' may be dependent on dissociation of Gβγ from the heterotrimer. Preliminary data also suggest '12155' may directly activate Gβγ.

Discussion: We have identified a small molecule which activates Gβγ. Since no small molecule has been developed which activates Gβγ, it will be interesting to know where it binds and how it modulates activity of Gβγ. Though it may not be used as a drug, but can be helpful in finding more pathways and functions related to Gβγ.

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56. The BH3-only protein, NOXA, is upregulated by estrogen and promotes cell cycle progression in estrogen receptor-positive breast cancer cells.

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Background: E2/17β-estradiol/estrogen stimulates cell growth and inhibits apoptosis in Estrogen Receptor (ER)-positive breast cancer cells; however, E2 also paradoxically induces the expression of NOXA/PMAIP1/APR, a BH3-only pro-apoptotic member of the Bcl-2 family. In the present study, we report on the mechanisms by which E2 upregulates NOXA expression and the associated cellular consequences in ER-positive human breast cancer cells.

Materials and Methods: MCF7 cells were treated with E2 alone, or E2 in combination with the ER-antagonists Tamoxifen and Fulvestrant, and the effects on NOXA expression were analyzed by western blot and PCR analysis. To test the involvement of ERα, p53, c-MYC, and E2F1 in the induction of NOXA expression following E2 treatment, cells were transiently transfected with specific siRNAs targeting the protein of interest. To further investigate the roles of these proteins as transcriptional regulators of NOXA expression, the recruitment of ERα, p53, c-MYC, and E2F1 to the NOXA promoter was analyzed by chromatin immunoprecipitation (ChIP) assays, following treatment with E2 and cell stress agents. The effects of E2-mediated upregulation of NOXA on cell viability, cell cycle distribution, and apoptosis were also measured using traditional assays.

Results: Treatment with ER-antagonists blocked the upregulation of NOXA by E2. In addition, siRNA-knockdown of ERα, c-MYC, and E2F1 inhibited E2-mediated induction of NOXA expression. Consistent with this result, E2 promoted the recruitment of c-MYC and ERα to the NOXA promoter in ChIP assays. However, siRNA-knockdown of p53 did not block E2-mediated induction of NOXA, and E2 treatment failed to induce the recruitment of p53 to the NOXA promoter. Interestingly, siRNA-knockdown of NOXA reduced cell viability, caused cell cycle arrest in G_0/G_1-phase, and delayed the G_1-to-S-phase transition following E2 treatment, while E2-dependent upregulation of NOXA had no effect on apoptosis.

Discussion: We demonstrate that the upregulation of NOXA by E2 is a p53-independent process that is mediated by ERα, c-MYC, and E2F1/Rb. Although p53-mediated upregulation of NOXA in response to cellular stress induces apoptosis, E2-mediated upregulation of NOXA is not associated with apoptosis under normal, unstressed cellular conditions. Rather, we demonstrate that E2-mediated upregulation of NOXA is required for cell cycle progression, suggesting a novel paradoxical role for the BH3-only protein NOXA, as a dual regulator of cell proliferation and cell death. In addition, the widely-used endocrine therapy drugs, Tamoxifen and Fulvestrant, block the upregulation of NOXA by E2 in our system, highlighting the potential clinical relevance of our findings to ER-positive breast cancer patients.

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57. Characterization of imidazoline I2 receptor agonist-induced hypothermia in rats.

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Background: Imidazoline I_2 receptors have been implicated in several central nervous system disorders. Although several I_2 receptor agonists have been described, no simple and sensitive in vivo bioassay is available for studying I_2 receptor ligands. This study examined I_2 receptor agonist-induced hypothermia as a functional in vivo assay of I_2 receptor agonism in rats.

Materials and Methods: Different groups of rats were used to examine the effects of I_2 receptor agonists on rectal temperature. The pharmacological mechanisms were investigated by combining I_2 receptor ligands and different antagonists.

Results: All the selective I_2 receptor agonists examined (2-BFI, diphenyzoline, phenyzoline, CR4056, tracizoline, BU224 and S22687, 3.2-56 mg/kg, i.p) dose-dependently produced hypothermia in rats, with varied duration of action. Pharmacological mechanism of the observed hypothermia was studied by combining the I_2 receptor agonists (2-BFI, BU224, tracizoline and diphenyzoline) with imidazoline I_2 receptor/ α_2 adrenoreceptor antagonist idazoxan or α_2 adrenoreceptor antagonist/serotonergic 5-HT_{1A} receptor agonist yohimbine. Idazoxan but not yohimbine attenuated the hypothermic effects of 2-BFI, BU224, tracizoline and diphenyzoline, supporting the I_2 receptor mechanism. In contrast, both idazoxan and yohimbine attenuated the α_2 adrenoreceptor agonist clonidine induced hypothermia.

Discussion: Together, these data suggest that imidazoline I_2 receptor agonists can produce hypothermic effects, which may be useful as a simple and sensitive in vivo assay for studying I_2 receptor ligands.

58. Regulation of prostate cancer cell migration by SRF-target genes CNN2 and SDK1.

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Background: In an effort to identify clinically relevant androgen receptor (AR) action in prostate cancer (PCa) cells, a 158 gene signature that relies on serum response factor (SRF) to achieve androgen-responsiveness was isolated. This expression profile separated benign from malignant

prostate and correlated with aggressive disease and biochemical recurrence. The manner in which the SRF-dependent gene signature conveys aggressive behavior to PCa cells is studied here by investigating two representative genes that are expressed differentially in PCa mRNA expression profiles: calponin-2 (CNN2), an actin-binding protein that is underexpressed in PCa, and Sidekick Homologue-1 (SDK1), a cell-adhesion protein, which is overexpressed.

Materials & Methods: siRNA was used to silence CNN2, SDK1, SRF or AR expression. PCa cell lines were treated with androgens (R1881, DHT) and an antiandrogen (Casodex). Protein and mRNA levels were measured by Western blot and real-time RT-PCR, respectively. A CNN2 promoter-reporter construct that harbors a SRF binding site was used in transient transfections. Rhodamine phalloidin (RP) was used to study the actin cytoskeleton. Cell migration assays were performed using culture inserts.

Results: SRF- and AR-dependency of CNN2 and SDK1 expression at the mRNA and protein level was confirmed using specific siRNAs, treatments with natural or synthetic androgens, and anti-androgens, and in time course and dose response studies. Androgen regulation of CNN2 and SDK1 was confirmed in multiple androgen-stimulated (AS) (LNCaP, VCaP) and castration-recurrent (CR) (LN-Rf, C4-2) PCa cell lines. Involvement of SRF in the observed effects was validated further in transfection experiments using a CNN2 promoter-reporter construct and in ChIP experiments. RP staining upon siRNA-mediated loss of CNN2 or SDK1 resulted in marked alterations in PCa cell morphology and cytoskeletal organization. CNN2 knockdown induced cell extensions and led to disorganization of actin filaments, which become frayed severely at the cell periphery. Conversely, SDK1 silencing induced cell rounding and stress fiber formation. As changes in cell morphology may have an impact on cell migration, wound healing assays were done. Loss of CNN2 and SDK1 resulted in promotion and inhibition of cell migration, respectively. The effects on cell migration were not due to changes in cell proliferation or apoptosis. Observations of CNN2- or SDK1-dependent effects on PCa cell actin cytoskeleton organization and cell migration were done originally in AS LNCaP cells and validated in the isogenic CR LNCaP subline C4-2.

Discussion: This work demonstrates that the SRF target genes CNN2 and SDK1 contribute to aggressive PCa behavior via effects on PCa cell migration.

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59. Isolation of CD133/CD140a-defined human neural stem and oligodendrocyte progenitors.

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Background: The specification of oligodendrocytic fate from neural stem cells (NSCs) in the human brain remains poorly understood. In order to better define the molecular mechanisms regulating oligodendrocyte development, we developed a novel immunophenotyping approach to separate human primary neural stem cells (NSCs) from committed oligodendrocyte progenitor cells (OPCs). A2B5 has been frequently used to isolate the OPCs in both rodents and human. We demonstrated here that A2B5 alone cannot give rise to OLIG2$^+$ oligodendrocyte lineage cells. As several of the markers used to isolate human NSCs are also expressed by CD140a/PDGFαR-defined OPCs (Sim et al., 2011; Nat Biotech, 29:934), we asked whether CD140a was capable of distinguishing NSCs from OPCs. As PROM1/CD133 mRNA was highly expressed by CD140a$^+$ OPCs, we hypothesized that CD133-defined human NSCs were heterogeneous.

Results: Using two-color fluorescence-activated cell sorting, we found that 24% ± 12% of CD133$^+$ cells expressed CD140a antigen in fetal forebrain dissociates (n=19). To determine the self-renewal and multilineage capacity of CD133/CD140a sorted fractions, we performed limiting dilution neurosphere assays. CD133$^+$CD140a$^-$ cells generated significantly more spheres after 3 weeks in vitro than CD133$^+$CD140a$^+$ cells (53.3 ± 5.7 and 27.6 ± 6.1 spheres per 1000 cells, respectively, n=6 fetal samples). Consistent with a NSC phenotype, the vast majority of clonogenic CD133$^+$CD140a$^-$ cells were multipotent, generating spheres containing all three neural lineages (80.7 ± 2.6% of spheres, n=6). In contrast, <21 % of CD133$^+$CD140a$^+$ cells exhibited trilineage capacity with almost one third lacking neurogenic capacity (30.2 ± 11.8%). In pro-oligodendrocyte conditions, 4 days following isolation CD133$^+$CD140a$^+$ cells rapidly differentiated into O4$^+$ immature oligodendrocytes (32 ± 4%, n=4), while <2% of CD133$^+$CD140a$^-$ cells expressed O4 antigen. Taken together, the phenotype of CD133$^+$CD140a$^+$ cells was consistent with an OPC with limited self-renewal and more restricted multilineage capacity than CD133$^+$CD140a$^-$ cells. In contrast, CD133$^+$CD140a$^-$ defined cells were multipotent neural stem cells that were not contaminated by defined oligodendroglial precursors.

Discussion: As fetal human CD133 sorted cells comprised both NSCs and OPCs, CD133 expression alone cannot be considered a specific marker of stem cell phenotype. The molecular characterization of both phenotypes will provide insight into the transcriptional regulation and signaling pathways that regulate oligodendrocyte fate from human neural stem cells.(Supported by NYSTEM contracts C026413 and C026428)

60. A novel melatonin binding site on the TrkB receptor.

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Background: Melatonin is primarily synthesized in the pineal gland and retina with high levels at night. Melatonin is involved in modulating physiological functions through activation of two G-protein coupled receptors, MT_1 and MT_2. N-acetylserotonin (NAS), the precursor of melatonin, was recently reported to trigger the TrkB receptor, a member of receptor tyrosine kinases, phosphorylation independent of Brain-Derived Neurotrophic Factor (BDNF), MT_1 and MT_2 receptors, and the MT_3 binding site.

We tested the hypothesis that melatonin and its analogues bind to the TrkB receptor. We therefore assessed and compared 2- [125I]-Iodomelatonin binding to membranes of T48-TrkB and SN56 to establish similarities and/or differences on binding capacity, affinities and kinetics with the CHO-hMT_1 and CHO-hMT_2 melatonin receptors and the MT_3 binding sites.

Materials and Methods: T48 cells stably transfected with TrkB were originally derived from SN56 cell lines which express negligible TrkB. CHO cells were stably transfected with hMT_1 or hMT_2 melatonin receptors. Radiolabeled 2- [^{125}I]-Iodomelatonin binding assays were performed to determine the pharmacological and kinetic characteristics of the receptors.

Results: Specific 2-[^{125}I]-Iodomelatonin binding to the membrane of T48 cells, but not SN56, was temperature dependent, of high affinity, saturable and low capacity (K_d =43.3 ± 2.8 pM; Bmax=7.5 ± 0.3 fmol/mg) while specific 2-[^{125}I]-Iodomelatonin bindings to CHO-hMT_1 (K_d = 48.7 ± 16.0) and CHO-hMT_2 (K_d = 108.4 ± 17.8) were of high capacity (390 ±79 fmol/mg for hMT_1, and 245 ± 56 fmol/mg for hMT_2 respectively). K_i values for melatonin analogues to the TrkB receptor and for either MT_1 or MT_2 melatonin receptors showed significant correlations (r2=0.86, p<0.05 for hMT_1, and r2=0.52, p<0.05 for hMT_2 respectively). 2-[^{125}I]-Iodomelatonin specific binding to TrkB receptor membranes was rapid and reached equilibrium at 60 min, similar to the association kinetics for MT_1 and MT_2. However, 2-[^{125}I]-Iodomelatonin specific binding to the TrkB receptor did not dissociate upon addition of 10uM melatonin or 0.1uM 2-iodomelatonin, 60 min after the radioligand.
Discussion: Neither melatonin nor 2-Iodomelatonin dissociated 2-Iodomelatonin binding to the TrkB receptor after 60 min setting up a major difference that distinguishes this receptor from the MT_1 and MT_2 melatonin receptors. Moreover, the pharmacological profile for the TrkB receptor (NAS>Mel) as well as the affinity for melatonin binding to the TrkB receptor increases with temperature (25°C>4°C) distinguishing this binding site from the MT_3 binding site. Our results suggest the presence of a novel melatonin binding site on the TrkB receptor (Supported by NS 06106801 to MLD.)

61. C-terminal di-arginine motif of Rho family GTPase Cdc42 is important for reconstitution of FcεRI-mediated Ca^{2+} mobilization in mutant RBL mast cells.
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Background: Antigen stimulation of mast cells via FcεRI, the high-affinity receptor for IgE, triggers a signaling cascade that requires Ca^{2+} mobilization for exocytosis of secretory granules during an allergic response. Mutant RBL-2H3 cells, designated B6A4C1, are defective in FcεRI-mediated Ca^{2+} mobilization and degranulation. However, our laboratory previously demonstrated that the constitutively active small GTPase, Cdc42 G12V, has the capacity to reconstitute Ca^{2+} mobilization and consequent degranulation in these cells. Recently it has been shown that a C-terminal di-arginine motif of Cdc42 is essential for the binding of Cdc42 to PIP_2-containing membranes and for 3T3 fibroblast transformation, and we hypothesized that this C-terminal di-arginine motif may also play a role in antigen-stimulated Ca^{2+} responses. Using the B6A4C1 cells as our model system, we investigated whether this C-terminal di-arginine motif plays a role in Cdc42 G12V-mediated reconstitution of Ca^{2+} mobilization in these mutant RBL mast cells.
Materials and Methods: For Ca^{2+} experiments, mutant RBL-mast cells were co-transfected with Cdc42 constructs together with the genetically encoded calcium indicator protein GCaMP3. For exocytosis experiments, cells were co-transfected with Cdc42 and a pHfluorin-tagged vesicle-associated membrane protein 8 (VAMP8-pHluorin). Both Ca^{2+} and exocytosis measurements were performed using an SLM 8000C fluorescence spectrophotometer.
Results: Our Ca^{2+} experiments confirm that Cdc42 G12V is capable of reconstituting enhanced Ca^{2+} mobilization in B6A4C1 cells. New data also shows that when the C-terminal di-arginine motif of Cdc42 G12V is mutated to di-glutamines, this construct is unable to reconstitute antigen stimulated intracellular Ca^{2+} release from stores in the mutant RBL-mast cells. Store-operated Ca^{2+} influx stimulated by antigen or thapsigargin is not inhibited by the di-glutamine mutation in these cells. Furthermore, in RBL-2H3 cells co-transfected with VAMP8-pHluorin, Cdc42 G12V showed enhanced vesicle exocytosis compared to cells transfected with the mutated C-terminal di-arginine motif.
Discussion: A charge neutralization mutation in the C-terminal di-arginine motif inhibits the capacity of Cdc42 G12V to reconstitute antigen-stimulated Ca^{2+} release from stores and consequent exocytosis in RBL mast cells. Since this di-arginine motif is important for Cdc42 binding to PIP2-containing membranes, these results improve our understanding of the relationship between Cdc42 and PIP2 in these cells. Future studies will be directed at determining the mechanism by which constitutively active Cdc42 participates in the restoration of Ca^{2+} mobilization and degranulation in B6A4C1 cells, including a possible role for Cdc42 in regulation of stimulated PIP_2 synthesis.

62. Phospholipase Cε mediates Gq-induced cardiomyocyte hypertrophy.
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Purpose: Heart disease including heart failure is a major health problem worldwide and particularly prevalent in the United States. In its initial stages, neurohumoral factors, increased during hypertension or other cardiac stress cause, hypertrophic enlargement of the heart that is maladaptive and ultimately can lead to heart failure. We have found that a novel phospholipase C isoform (PLC), PLCε contributes significantly to cardiac hypertrophy stimulated by various neurohumoral agonists through G protein coupled receptors in a neonatal rat ventricular myocyte (NRVM) cell model. Many of these receptors couple to the G protein Gq, which is a well-known hypertrophy stimulator, but they also couple to other G protein pathways. PLCε is not directly regulated by Gq, nevertheless; to determine if Gq-dependent hypertrophy is dependent on PLCε we specifically examined the role of PLCε in hypertrophy stimulated by overexpressed Gq.
Materials and Methods: Adenovirus expressing PLCε siRNA were used to knock down PLCε in NRVMs. PLCε binds the nuclear membrane anchoring protein, A-kinase anchoring protein (mAKAP), via and spectrin repeat domain. Adenoviral expression of the mAKAP-SR domain disrupts PLCε binding to mAKAP and was therefore used to examine the role of nuclear scaffolding in Gq-PLCε regulated cardiac hypertrophy. NRVMs were infected with adenoviruses expressing Gq to stimulate hypertrophy along with the constructs described above. Realtime PCR was used to measure mRNA level of ANF, a marker of cardiac hypertrophy.

Results: We found that PLCε-siRNA significantly inhibited Gq induced increased mRNA level of ANF compared to Random-siRNA (3.97 ± 0.83, PLCε siRNA v.s. 1.85 ± 0.42, ran-siRNA, P < 0.05). PLCε-siRNA also significantly inhibited Gq induced increases in myocyte area . Disruption of PLCε nuclear scaffolding with mAKAP-SR-YFP significantly inhibited Gq-induced increased mRNA level of ANF compared to YFP (3.71 ± 0.33, mAKAP-SR-YFP v.s. 1.86 ± 0.20, YFP, P < 0.05).
Conclusion: These results indicate that PLCε is a mediator of Gq-induced hypertrophy in cardiac myocytes.

63. State dependent drug binding of Kv4.2/4.3 channels.
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Background: Prolongation of cardiac repolarization is an undesired and potentially fatal side effect of many drugs, even when their intended therapeutic effect is on an organ other than the heart. It is therefore of great importance to develop new tools and methods that can identify, as early as possible, the arrhythmogenic risk of novel agents. Changes in the kinetic properties of a single ion channel caused by drugs can often be related to changes in specific rate constants in the Markov models of the channel. Kv4.2/4.3 are the major molecular bases underlying the transient outward potassium current IKtof, which plays an important role in early repolarization of the action potential (AP). A number of pharmaceutical compounds block IKtof with different mechanisms of action. It remains unexplored how drugs with similar potency, but different blocking mechanisms, differ in their ability to affect AP duration (APD) and dynamic properties such as restitution.
Materials and Methods: Starting from our previously developed endocardial and epicardial models, we changed four K+ currents from Hodgkin-Huxley type to Markov models: IKtof, IKtos, IKur, and IKs. The new mouse AP models have Markov models for all major ion currents. We then designed two hypothetical IKtof specific blockers: a closed state blocker and an open channel blocker using Markov formulations, and studied the effects of these distinct blocking mechanisms in prolonging APD and changing restitution.
Results: First, we calculated the steady state APD-cycle length (CL) relationships of the endocardial and epicardial cell models under control and in the presence of each hypothetical drug at two doses. The simulations showed that: 1) the effect in prolonging APD caused by both drugs are CL dependent. 2) At the same CL, the two drugs had significantly different effects in prolonging APD. 3) The effect in prolonging APD caused by both drugs differed dramatically between early, middle, and late repolarization. We then tested the S1S2 restitution properties of the models in control and various drug conditions. The results again showed complicated effects depending on the drug binding mechanism, the S1S2 interval, and the stage of repolarization.
Discussion: This study shows the potential of Markov models of state-dependent drug binding in producing complex effects of a drug in changing APD and restitution. Future studies will aim to build similar models of real drugs and analyze their effect in changing heart rhythm. (Supported in part by NIH HL093631; HL062465; HL088058)

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- Sponsor candidates for Student membership only.
- Not sponsor a paper for a non-member at a Society meeting.
- Not vote in Society elections.
- Not hold an elected office in the Society.

Postdoctoral Members (Dues $75) have all the benefits of Regular members.

Student Members (Dues $30) have all the benefits of Regular members except they:

- Pay no dues their first year.
- Pay only $30 annual dues thereafter. Undergraduate Student members pay no dues and get their first graduate year free.
- Must have their papers at Society meetings sponsored by a member.
- May not vote in Society elections nor hold an elected office in the Society.

Application Instructions

Submit the completed Application for Membership form or use the online application form on the ASPET web site at **www.aspet.org/membership/apply**. Submit a current curriculum vitae including bibliography for Regular and Affiliate Membership.

Sponsor Statements: Submit a statement of qualifications of the applicant from one Regular/Retired/Postdoctoral Member of ASPET for Regular Membership, Affiliate Membership and Student Membership (Affiliate Members may also sponsor student applicants). In addition to the statement certifying that the applicant is qualified for ASPET membership, sponsors should provide their own current address, phone, fax, and email. It is the responsibility of the applicant to insure that these documents are submitted to the ASPET office.

American Society for Pharmacology and Experimental Therapeutics
9650 Rockville Pike, Bethesda, MD 20814-3995 USA
Phone: 301-634-7060 ♦ Fax: 301-634-7061 ♦ www.aspet.org

Membership Application

Please Complete All Sections:

Section 1: Application Details

Application for:

❑ **Regular Membership**

❑ **Affiliate Membership**

❑ **Postdoctoral Membership – Date of Graduation:** _____

❑ **Graduate Student – Expected Date of Graduation:** _____

❑ **Undergraduate Student - Year:** ❑ **Fr** ❑**Soph** ❑**Jr** ❑**Sr**

Section 2: Source

How did you hear about ASPET:

❑ **Meeting** _____

❑ **ASPET Journal** _____

❑ **Mentor** _____

❑ **Website** _____

❑ **Other** _____

Section 3: Personal Information

Name:

Institution:

Mailing Address:

Telephone:

Fax:

Email:

Section 4: Optional Demographics (Not Required)

Date of Birth: _____

Sex: ❑ Female ❑ Male

Ethnicity:

❑ Asian

❑ Black or African American

❑ American Indian or Alaskan Native

❑ Hispanic or Latino

❑ Native Hawaiian or Pacific Islander

❑ White

❑ Other: _____

The information in this section will be used by ASPET to collate statistics and will be kept private. Completion of this section is voluntary.

Section 5: Sponsor (Must be an ASPET Member)

Name and email of your sponsor:

Please have your sponsor send us a brief letter or e-mail outlining your qualifications for Membership in ASPET to the Membership Coordinator , Robert Phipps, (rphipps@aspet.org).

Section 6: Division Selection

Divisions: *Division membership is a benefit of ASPET membership and there is no additional charge to belong to a division. It is highly recommended that you join a division so that you may take full advantage of Society participation. Joining a division allows you to participate in creating the scientific program for the annual meeting, network with people in your field at mixers and divisional programs, and receive special notices and newsletters about items and activities of interest in your field. Be sure to pick a division!*

Indicate primary (1) and as many secondary (X) divisions to which you wish to belong:

___Division for Behavioral Pharmacology
___Division for Cardiovascular Pharmacology
___Division for Drug Discovery, Development & Regulatory Affairs
___Division for Drug Metabolism
___Division for Integrative Systems, Translational & Clinical Pharmacology
___Division for Molecular Pharmacology
___Division for Neuropharmacology
___Division for Pharmacology Education
___Division for Toxicology

Section 7: Curriculum Vitae

Regular, Affiliate, and Graduate Student applicants: Please send your *Curriculum Vitae* (including bibliography) by email to the Membership Coordinator, Robert Phipps, (rphipps@aspet.org).

Undergraduate Student Applicants Only:

Current Education :

Expected Degree & Date: School: City/State/Country: Major Field:

Applications are reviewed on a rolling basis. Please DO NOT submit payment with your application.
Upon membership approval, you will be sent a dues statement and welcome package.
Student Membership is FREE for the first year.

Call or e-mail the ASPET Membership Department for additional information: 301-634-7135 / rphipps@aspet.org.