The 18th International Symposium on Chromaffin Cell Biology

17-21 August, 2015 |
Pullman Reef Hotel Casino | Cairns |
Queensland | Australia

ISCCB 2015

Convenors:
Frederic Meunier | University of Queensland
Damien Keating | Flinders University

Image: Tourism and Events Queensland

ISN International Society for Neurochemistry
Flinders University
SANI South Australian Neuroscience Institute
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The University Of Queensland AUSTRALIA | QBI Queensland Brain Institute

University of Western Sydney
The University of Newcastle Australia
Children's Medical Research Institute

http://isccb2015.mtci.com.au/

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The convenors and committee of ISCCB 2015 are pleased to welcome international and national presenters and delegates, and our generous sponsors Thank you for your presentations, attendance and support.

Convenors: Professor Frederic Meunier, the University of Queensland
A/Professor Damien Keating, Flinders University, South Australia

ISCCB 2015 Local Organising Committee

Dr Stephen Bunn, Otago University, Dunedin, NZ
Professor Jens Coorssen, University of Western Sydney, AUS
A/Professor Phillip Dickson, University of Newcastle, AUS
Professor Peter Dunkley, University of Newcastle, AUS
Dr. Will Hughes, Garvan Medical Research Institute, AUS
Professor Christina Mitchell, Monash University, AUS
Professor Phillip Robinson, CMRI, AUS
Professor John Rostas, University of Newcastle, AUS

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ACADEMIC, CORPORATE AND SOCIETY SPONSORS

We are extremely grateful to our academic and company Sponsors for their generous support of the meeting: *Major Sponsors*: **ISN**; **Flinders University**; **QBI** - Queensland Brain Institute, the University of Queensland; **SANI** - South Australian Neuroscience Institute. *Gold Sponsor*: **Abcam** plc. *Session Sponsor*: **npi electronic** GmbH. *Sponsors and Exhibitors:* **The University of Western Sydney**; **The University of Newcastle**; **CMRI** Children's Medical Research Institute, the University of Sydney; **LEICA Microsystems**; **IMB**, The Institute for Molecular Bioscience, the University of Queensland; **Monash University**.

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Monash University

CONVENORS' WELCOME

On behalf of the organising committee, it is our great pleasure to welcome you to the 18th meeting of the International Society for Chromaffin Cell Biology - **ISCCB 2015** - in Cairns, Queensland Australia.

As you will see from the scientific program, we have attracted many high calibre scientists from across the globe focused on an array of research areas primarily around the chromaffin cell and cell signalling. Thank you to the Committee for tireless work, assistance and support in convening ISCCB 2015 in Australia. Our thanks to the Symposium Chairs who have invited their speakers and organised their sessions to produce the scientific program we have before us to stimulate and engage our interest, and develop on-going research collaborations. This meeting has a strong tradition of having an intimate and friendly environment amongst friends old and new, and for providing a platform for some of the most exciting developments seen in the field.

We are extremely grateful to our academic and company Sponsors for their generous support of the meeting: *Major Sponsors*: **ISN** - The International Neurochemistry Society; **Flinders University**, South Australia; **QBI** - Queensland Brain Institute, the University of Queensland; **SANI** - South Australian Neuroscience Institute. *Gold Sponsor*: **Abcam** plc. *Session Sponsor*: **npi electronic** GmbH. *Sponsors and Exhibitors*: **The University of Western Sydney**; **The University of Newcastle**; **CMRI** Children's Medical Research Institute, the University of Sydney; **LEICA Microsystems**; **IMB**, The Institute for Molecular Bioscience, the University of Queensland; **Monash University**.

Our meeting is not only an avenue for established scientists to showcase their most recent research, but also an opportunity for emerging research leaders to present their work and for us to support their professional development. With this in mind we are pleased to announce that a large number of competitive travel awards have been provided to many junior attendees who will be given the opportunity to present their work at this conference.

Over 15 different symposia cover a diverse set of themes. In addition to this we will be holding our famed *Wine and Cheese session* on the Tuesday night, including a series of short talks from junior researchers and sponsors.

Of special note is that two of our symposia will be held in honour of our late friends **Daniel O'Connor** and **Hans-Herman Gerdes**, who have left us far too early. These symposia are an opportunity for their peers to both celebrate their research and to acknowledge their legacy.

Social events include the Welcome Reception and Conference Dinner. Pre-booked excursions to either the World Heritage listed Great Barrier Reef or to the Cape Tribulation/Daintree Rainforest are scheduled on Thursday and will give you a glimpse of the wonders that Australia has to offer. The Reef tour operator will be at the registration desk early Monday for 90 minutes or so, if you have any questions.

We are very excited to welcome you to the **ISCCB 2015**. We sincerely hope you enjoy both the fantastic science and great company that will be on offer while here in beautiful Cairns.

Fred Meunier, convenor

Damien Keating, convenor

GENERAL INFORMATION

ISCCB

The International Symposium on Chromaffin Cell Biology - ISCCB - has been held every two years since 1982 (please see the history of our meeting http://isccb2015.mtci.com.au/ISCCB2015_history.htm) and is held this year in association with the **International Society for Neurochemistry (ISN) Biennial 2015**.

CAIRNS AND SURROUNDS

Cairns is the gateway to tropical North Queensland and Australia's Great Barrier Reef. Once a sleepy sugar-milling town, it's now a destination for major international meetings, sailing, diving and snorkelling. Tjapukai Aboriginal Cultural Park has displays of indigenous Aboriginal and Torres Strait Islander groups. Cairns Esplanade, lined in bars and restaurants, has a saltwater swimming lagoon.

The Great Barrier Reef is an astonishingly beautiful experience, snorkeling or diving. Charming Palm Cove and Port Douglas North of Cairns, are tropical, beachside villages offering relaxation and a variety of restaurants. The wonders of the Great Barrier Reef and Daintree Rainforest are there to rejuvenate and inspire. Serene waterfalls, towering trees and crater lakes contrast against a tapestry of farms along selfdrive trails to the region's villages and fascinating markets. The exotic tropical flavours, and unique wildlife and outdoor pursuits make the Tablelands and Kuranda one of the many must-do's within the region.

ACCOMMODATION

Two conference hotels: **Pullman Reef Hotel Casino** and **Pullman Cairns International Hotel**. The hotels are literally opposite each other. Rooms booked are single/double or twin-share. Your credit card is "swiped" on check-in for incidentals during your stay. All incidentals are charged to your room account and paid on check-out. Check-in is 2pm and check-out 11am. If twin-sharing, please check out together to assist Reception staff.

Pullman Reef Hotel Casino is the main conference hotel. Four restaurants, four bars, live entertainment, a boutique casino nightclub, and a unique roof-top Rainforest Dome.

Pullman Reef Hotel Casino
35-41 Wharf Street
Cairns 4870
Queensland, AUSTRALIA
Telephone: (+61) 7 4030 8888

Pullman Cairns International Hotel
17 Abbott Street
Cairns 4870
Queensland, AUSTRALIA
Telephone: (+61) 7 4031 1300

ATM/BANKING

There are ATMs in the foyer of both hotels and in the Casino. Full banking facilities in Cairns.

CONFERENCE ROOM

The Conference Room is Michaelmas Cay Room 1. Notepads and pens are placed on display tables in the room.

DATA PROJECTION

Please take your talk on a stick to the AV Desk where there is an IBM compatible laptop. You may also use your own computer. Mac users, please bring all connections.

DRESS CODE

Cairns is a laid-back, regional City. The weather will be a pleasant maximum of 25ºC, minimum of 21ºC: http://www.weatherzone.com.au/qld/nth-cst-andtableland/cairns
Extended 14-day forecast:
http://www.timeanddate.com/weather/australia/cairns/ext

For the conference: neat clothing and footwear, suitable for sitting in air-conditioned plenary sessions. Poolside Conference Dinner: Something with a little bit of sparkle? Don't forget swimmers, sunscreen, hat, comfortable shoes...

DRIVING

Australia drives on the lefthand side of the road. Major hire car companies have desks at the Airport. If you are driving, take special note of safety instructions and road suitability for your vehicle. Some roads are 4-wheel drive only.

EATING OUT

There are excellent restaurants on-site: Tamarind - fine dining; Café China Restaurant - authentic Peking and Hong Kong Cuisine, Yum Cha and Seafood; La Vinoteca... In your conference bag, there is a list of dining options around Cairns where you will receive a discount on presentation of the voucher.

ELECTRICITY

Australia uses 240V mains electricity and use 'three point' power sockets. The standard plug used within Australia is Type I (http://www.worldstandards.eu/electricity/plugs-and-sockets/). Universal adapters may be available from the hotel or electrical shops in Cairns. To find out more about plug types, pleas visit Electricity World Standards http://users.telenet.be/worldstandards/electricity.htm#plugs_i

EXCURSIONS

Thursday is excursion day. If you have not yet booked and paid for yours, check online using the links from the conference website, check with Concierge at your hotel.

Reef tour: walk to Reef Fleet Terminal, located at the southern end of the Esplanade, this is the primary departure point for trips to the Great Barrier Reef. You should know your assembly and departure tmes. You can hire wetsuits and lycra suits on the boat. Even when you are snorkelling, it is advisable to be sunsafe and wear a lycra suit to protect from the sun. Water clarity was to 15m today. You are in for a treat...

Cape Tribulation/Daintree Tour: Your pick-up point and time were noted with your registration. For those staying in the Pullman Reef Hotel Casino, your pick-up point is across at the Pullman Cairns International Hotel @6:50am on the Thursday. The days have been spectacular up at Cape Trib and in the Daintree.

EXHIBITION

Thank you to our generous sponsors and valued Exhibitors for their support for the meeting. The Exhibition is housed with the Posters and refreshments in Urchins 1. Morning and Afternoon teas and lunch are served here, along with the evening Poster sessions and Wine and Cheese. Set-up is from 9am on Monday 17th August and pack-down is from the end of afternoon tea on the 19th. Couriers should be organized to collect before 5pm on Wednesday.

GST AND TIPPING

A Government Goods and Services tax (GST) of 10% is applied to all goods and services and is included in the advertised price. It is usual – although not obligatory - to tip in restaurants. Also up to 10%.

INTERNET

For Club Accor members (you may join at the desk!) you have free WiFi in your room. The Lobby has free 30 minutes/day WiFi. Limited WiFi in the conference area. Please use sparingly. There are also internet cafés in town. Ask at the Concierge Desk.

NAME BADGES

Name badges must be worn at all times; entry to the Exhibition area, social functions and scientific sessions is by name badge only. Accompanying persons who have paid for Social functions will be issued wth a name badge.

PARKING

For those hiring cars, there is parking in designated parking areas at the hotel, or a public parking station under the hotels – entrance from Abbott Street. Enquire with Concierge for valet and self-parking rates.

POSTERS

Bring your Poster as early as possible on Monday to Urchins1. Posters are numbered in the Program; place your poster on your numbered board. Velcro dots are provided. A major Poster session is that night with the Welcome Reception. Posters are displayed all day, and viewed at the breaks and over lunchtimes. Posters should be removed by the close of Afternoon Tea on Friday.

AIRPORT TAXIS

Taxis are the main means of local transport. A fare to/from the Airport is around $25. Book taxis through Concierge at your hotel. Cairns Airport facilities noted here. http://www.cairnsairport.com.au

REFRESHMENTS

Refreshments are served as per the printed program in Urchins 1. Notified dietary requirements are catered for. Please advise now if you have a food allergy etc.

REGISTRATION DESK

Registration opens from 10am on Monday; and from 08:00 Tuesday, Wednesday and Friday. The registration desk is located in Urchins I, in the Exhibition area. Your Program and conference satchel are available from the registration desk from 10am Monday. If you have booked something extra and need to make payment please do so with online credit card payment at the Registration Desk. You can also book with Quicksilver for any remaining excursions to the Reef.

SAFETY

Don't swim on beaches around Cairns. Look for the warning signs (open crocodile jaws and jellyfish stingers). The North has crocodiles around the estuaries and inland rivers. Box jelly fish are in the ocean and signs and precautions should be strongly adhered to. Take all necessary precautions as advised for the area that you are visiting. When swimming in Australia, swim at beaches patrolled by lifesavers, swim between the flags and check beach / river safety signs. http://www.cairns.qld.gov.au/region/tourist-information

Wear sunscreen, sun hat and appropriate swimwear. Carry water. http://www.cairns.qld.gov.au/facilities-sport-leisure

SCIENTIFIC PROGRAM

Superb! The program book contains the running schedule, abstracts, author index and the conference attendee address list. Welcome Reception and Conference Dinner, teas and lunches are as per this schedule and are included in full registration. Wine and Cheese - 2 parallel sessions. 10 minutes each scientific presentations; 5 minutes each for Company talks. Sessions are held in Urchins 4 and in Michaelmas Cay. Refreshments around the Posters, follow the presentations. Beer, wine and cheeses - then cash bar.

SMOKING

All public venues, restaurants and most hotels are non-smoking in Australia. Hotel Rooms are non-smoking. Smoking is permitted outdoors in designated areas.

SOCIAL FUNCTIONS

The Welcome Reception with substantial canapés is held in Urchins I, in the Pullman Reef Hotel Casino on Monday 17th August, 2015. Included in full registration fee. Substantial cocktails, beer, wine and juice - followed by cash bar.

Wine and Cheese evening - refreshments follow the talks. Cheese platters, beer, wine, juice - followed by cash bar.

Conference Dinner: Wednesday 19th August, 2015, on the Pool Deck. Included in full registration fee. Additional tickets for accompanying persons may be paid for through the Registration desk.

VENUE

Michaelmas Cay Room is the conference room. Refreshments, Posters and Exhibitors are in Urchins 1 and Arlington Bar. The Wine and Cheese Parallel sessions are held in Michaelmas Cay and Urchins 4.

SECRETARIAT

Ros Barrett-Lennard,
Magic Touch Consultancies,
PO Box 717, Caringbah, Sydney, NSW 1495,
AUSTRALIA.

For family or emergency contact, please use my mobile (cell phone) It is always turned on
+61 (0)419 688 581

MAJOR SPONSOR OF ISCCB 2015

The International Society for Neurochemistry (ISN) has a proud history dating back to its establishment in 1965 and publishes the Journal of Neurochemistry. ISN as the sole international society focused on neurochemistry strives to promote all relevant aspects of molecular and cellular neuroscience.

The International Society for Neurochemistry (ISN) is a nonprofit membership organization of scientists and physicians who are active in the field of neurochemistr , cell and molecular neuroscience or related areas and aims:

- To facilitate the worldwide advancement of neurochemistry and related neuroscience disciplines
- To foster the education and development of neuroscientists, particularly of young and emerging investigators
- To disseminate information about neurochemistry and neurochemists' activities throughout the world

ISN Activities

- ISN creates avenues for sharing great science in an expanding field
- Holds the ISN Biennial meeting usually in August, attracting more than 1500 attendees from around the globe including outstanding plenary speakers & lectures
- Publishes The Journal of Neurochemistry (JNC), one of the leading sources for research into all aspects of neuroscience, with a particular focus on molecular and cellular aspects of the nervous system, the pathogenesis of neurological disorders and the development of disease specific biomarker
- ISN supports the neuroscience community, connecting people around the globe and across specialties.

About ISN Membership

Join the only international society focused on neurochemistry and striving to promote all relevant aspects of molecular and cellular neuroscience. http://neurochemistry.org/membership/aboutmembership.html

TOP REASONS FOR BEING A MEMBER

Be part of the ISN community, connecting people around the globe and across specialties and benefit from

- Reduced congress registration fees
- Opportunity to apply for grants & awards
- Online access to journal
  - »» Impact factor 4.337
  - »» Online access to all content of the Journal
  - »» Listing/access to member directory
  - »» Search for members including by key words
- Newsletter
  - »» Twice a year
  - »» Society news & email announcement
- Right to vote at ISN annual business meetings and by email ballot
- Reduced $1000 open access JNC publication fee

Categories and Fees

Full Members	- Initial FIRST two years**	$0	**Student members**	- Initial FIRST two years**	$0
	- One year membership*	$60		- One year membership*	$25
	- Two year membership*	$95		- Two year membership*	$25
Free Members	- Emeritus membership	$0			
	- Honorary membership	$0			

** Membership is valid for one calendar year (Jan 1st - Dec 31st)*

*** Initial FIRST two years :*

Membership is complimentary for the initial FIRST two years, ie: from Jan 1st of the year of the application until the Dec 31st of the following year; (eg: from Jan 1st 2015 to Dec 31st 2016). After the initial first two years fees will apply (as per the above)

Student Membership: if an applicant has only recently begun research in neurochemistry, cell and molecular neuroscience or related fields and is in the process of attaining a doctoral degree with a reasonable prospect of continuous activity in these fields or has held a postdoctoral position for less than 3 years they may be eligible for a reduced membership fee of $25

6:10 PM - 7:40 PM Posters 1: Welcome Reception, Trade and Posters

Urchins 1

9 The Chromogranin A-derived pyroglutaminated serpinin induces negative inotropism in teleost and amphibian hearts

Bruno Tota[1], Sandra Imbrogno[1], Rosa Mazza[1], Chiara Pugliese[1], Mariacristina Filice[1], Tommaso Angelone[1], Peng Loh[2] and Maria Cerra[1]

1 Dept. of Cell Biology, University of Calabria, Ponte Bucci , Arcavacata di Rende, Cosenza, Italy 87030, Italy; 2 Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Md 20892, USA

10 Chromogranin A and its peptide Catestatin play crucial roles in skeletal muscle function in endurance exercise and muscle fiber type-specific glucose metabolism

Sushil Mahata[1], Teresa Pasqua[1], Angshuman Biswas[2], Suvajit Sen[3], Biswa Choudhury[2] and Gautam Bandyopadhyay[2]

1Medicine, VA San Diego Healthcare System & 2University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0732, USA

11 The anti-hypertensive peptide Catestatin Improves hepatic insulin sensitivity by alleviating endoplasmic reticulum stress and improving mitochondrial function

Sumana Mahata[1], Sushil Mahata[1], Ennio Avolio[3], Teresa Pasqua[3], Nilima Biswas[3], Angshuman Biswas[3] and Gautam Bandyopadhyay[3]

California Institute of Technology, 1200 East California Blvd, Pasadena, CA 91125, USA; 2VA San Diego Healthcare System, San Diego, California, USA; 3Department of Medicine, University of California, San Diego, La Jolla, California, USA

12 Chromogranin A deficiency decreases secretory vesicle core formation, catecholamine storage, and energy metabolism in the adrenal medulla

Sumana Mahata[1], Teresa Pasqua[1], Gautam Bandyopadhyay[2], Angshuman Biswas[2], Guy Perkins[3], Amiya Sinha-Hikim[4], Lee Eiden[5] and Sushil Mahata[6]

California Institute of Technology, 1200 East California Blvd, Pasadena, CA 91125, USA; 2Department of Medicine, University of California, San Diego, La Jolla, California, USA; 3National Center for Microscopy and Imaging Research, San Diego, La Jolla, California, USA; 4Charles Drew University of Medicine and Science, Los Angeles, USA; 5Section on Molecular Neuroscience, NIMH-IRP, Bethesda, Maryland, USA; 6VA San Diego Healthcare System, San Diego, California, USA

13 A new calcium independent releasable pool in mouse chromaffin cells

Jose Moya-Diaz[1] and Fernando Marengo[1]

1. IFIByNE (CONICET). Departamento de Fisiología, Biología Molecular y Celular. Facultad de Ciencias Exactas y Naturales. Universidad de Buenos Aires. Pabellon II. Ciudad Universitaria, Buenos Aires, Buenos Aires 1428, Argentina.

14 Biogenesis of large dense core vesicles in adrenal chromaffin cells of newborn mice

Ekta Dembla, Elmar Krause, Jens Rettig and Ute Becherer

Kirrbergerstrasse, Bldg. 48, Homburg/Saar, Saarland 66421, Germany

15 Small molecules demonstrate the role of dynamin as a bi-directional regulator of the exocytosis fusion pore and vesicle release.

Jade Jackson[1], Andreas Papadopulos[2], Frederic Meunier[2], Adam McCluskey[3], Phillip Robinson[4] and Damien Keating[1]

1Discipline of Human Physiology, Centre for Neuroscience, Flinders University, Adelaide, Australia; 2Clem Jones Centre for Ageing Dementia Research, Queensland Brain Institute, The University of Queensland, Brisbane, Queensland, Australia; 3Centre for Chemical Biology and Chemistry, School of Environmental and Life Sciences, The University of Newcastle, Callaghan, New South Wales, Australia; 4Children's Medical Research Institute, University of Sydney, Westmead, New South Wales, Australia

16 Antidiabetic effects of flavonoids from Sophora flavescens EtOAc extract in Type 2 diabetic KK-Ay mice

Ping Zhao, Xinzhou Yang, Jing Yang and Yang Ye

College of Pharmacy, South-Central University for Nationalities, 182 Min-Zu Road, Wuhan, China, Wuhan, HuBei 430074, China

17 Imaging Mass Spectrometry Reveals the Chemistry in Chemically Fixed Adrenal Cells Prepared for Transmission Electron Microscopy Analysis

Jelena Lovric[1,2], Per Malmberg[1,2], Bengt Johansson[3], Johanna Höög[4], John Fletcher[1,2] and Andrew Ewing[1,2,5]

1. Department of Chemistry and Chemical Engineering, Chalmers University of Technology, Kemivagen 10, Gothenburg,412 96, Sweden; 2.National Center for Imaging Mass Spectrometry, Chalmers University of Technology andGothenburg University, SE-412 96, Gothenburg, Sweden 3.Electron Microscopy Unit, Institute of Biomedicine, University of Gothenburg, SE-405 30, Gothenburg, Sweden; 4.Department of Internal Medicine, Institute of Medicine, University of Gothenburg, SE-413 45, Gothenburg, Sweden; 5.Department of Chemistry and Molecular Biology, University of Gothenburg, SE-405 30, Gothenburg, Sweden

18 DID NOT ATTEND

19 DID NOT ATTEND

20 Transferrin coupled liposomes for brain targeting of 5-Florouracil

Mani Bhargava[1], Saurabh Bhargava[1], Rajul Jain[3] and Vishal Bhargava[3]

1 ICFAI University; 2 Manav Bharti University; 3 KRV Hospitals

21 Cellular and Molecular mechanisms of hypersecretion in pheochromocytomas

Pauline Croisé[1], Sébastien Houy[1], Joel Lanoix[2], Valérie Calco[1], Laurent Brunaud[3], Eustache Paramithiotis[2], Daniel Chelsky[2], Petra Tryoen-Toth[1], Stéphane Ory[1] and Stéphane Gasman[1]

1-CNRS UPR 3212-INCI, 5 rue Blaise Pascal, Strasbourg, 67084, France; 2-Caprion Proteome, Inc, Montreal, Canada; 3-Hospital Nancy-Brabois (CHU), Nancy, France

22 Characterizing enterochromaffin cell nutrient sensing capabilities in mouse colon and duodenum

Alyce Martin[1,2], Amanda Lumsden[1,2] and Damien Keating[1,2]

1. Centre for Neuroscience & Department of Human Physiology, Flinders University, Adelaide, Australia; 2. Nutrition and Metabolism Theme, South Australian Health and Medical Research Institute (SAHMRI), Adelaide, Australia

23 The effects of single and recurrent insulin induced hypoglycaemia on tyrosine hydroxylase phosphorylation in the rat brain and adrenal gland

Manjula Senthilkumaran[1], Michaela Johnson[1] and Larisa Bobrovskaya[1]
School of Pharmacy and Medical Sciences, University of South Australia, Frome road, Adelaide, South Australia 5000, Australia

24 PKA, AMPK, PGC-1alpha and NRF-1 couple cAMP-dependent antioxidant response and mitochondriogenesis during neuroendocrine differentiation

Isabelle Lihrmann[1], Houssni Abid[1], Dorthe Cartier[1], Abdallah Hamieh[1], Anne-Marie Bellan[2], Christine Bucharles[1], Destiny-Love Manecka[1], Jérôme LePrince[1], Sahil Adriouch[3] and Youssef Anouar[1]
1- U982 Inserm - IRIB -Faculty of Sciences, University of Rouen, 76821 Mont-Saint-Aignan, France; 2 - CNRS UMR 7286, CRN2M, Aix-Marseille, France; 3- U905 Inserm - IRIB - Faculty of Medicine, University of Rouen, 76000 Rouen, France

25 Tight mitochondrial control of calcium and exocytotic signals in chromaffin cells at embryonic life

Juan-Fernando Padín[1,2], Stefan Vestring[1,2], Jose-Carlos Fernández-Morales[1,2], Iago Méndez-López[1,2], Diego Castro-Musial[1,2,5], Antonio Miguel G. de Diego[1,2] and Antonio G. García[1,2,3]
1) Instituto Teófilo Hernando; 2) Departamento de Farmacología y Terapéutica, Facultad de Medicina, Universidad Autónoma de Madrid, 28029 Madrid, Spain. 3) Servicio de Farmacología Clínica, Instituto de Investigación Sanitaria, Hospital Universitario de la Princesa, UAM, Madrid, Spain. 4) Medizinische Fakultät Carl Gustav Carus, Technische Universität Dresden,Germany. 5) Department of Pharmacology, Federal University of São Paulo (UNIFESP), São Paulo, SP, Brazil.

26 Depressed excitability and ion currents linked to slow exocytotic fusion pore in chromaffin cells of the SOD1G93A mouse model of amyotrophic lateral sclerosis

Juan-Alberto Arranz-Tagarro[1,2], Enrique Calvo-Gallardo[1,2], Ricardo de Pascual[1,2], José-Carlos Fernández-Morales[1], Marcos Maroto[1], Carmen Nanclares[1,2], Luis Gandía[1,2], Antonio de Diego[1], Juan-Fernando Padín[1,2] and Antonio García[1,2,3,4]
1Instituto Fundación Teófilo Hernando, 2Departamento de Farmacología, Facultad de Medicina, Universidad Autónoma de Madrid, Madrid, Spain; 3Servicio de Farmacología Clínica, 4Instituto de Investigación Sanitaria, Hospital Universitario de La Princesa, Madrid, Spain

27 Two models for in vivo study of exocytosis by intracellular intravital microscopy

Andrius Masedunskas[1], Mark Appaduray[1], Christine Lucas[2], Annthony Kee[2], Peter Gunning[1] and Edna Hardeman[2]
1 Oncology Research Unit & 2 Neuromuscular and Regenerative Medicine Unit, School of Medical Sciences, UNSW, Sydney, Australia

98 Biogenesis of large dense core vesicles in adrenal chromaffin cells of newborn mice

Ekta Dembla, Elmar Krause, Jens Rettig and Ute Becherer
Department of Physiology, Cellular Neurophysiology, Saarland University, Building 59, Homburg, Saar 66421, Germany

Monday, August 17, 2015

9:00 AM **Exhibition Bump-in**

Urchins

10:00 AM **Registration OPEN**

Urchins 1

1:00 PM - 1:10 PM **Conference Opening: Guest of Honor**

Michaelmas Cay Room

Chair: Fred Meunier, Damien Keating

1:10 PM - 1:40 PM **A day on the Reef**

Michaelmas Cay Room

Chair: Fred Meunier, Damien Keating

1:10 PM 1 Sex and Violence, Seeing, Signals and Stomatopods: A day on the Reef

Justin Marshall
Sensory Neurobiology Group, Queensland Brain Institute, University of Queensland, St Lucia, Brisbane, QLD 4072, Australia

1:40 PM - 2:25 PM **Plenary 1: Reinhard Jahn**

Michaelmas Cay Room

Chair: Fred Meunier

1:40 PM 2 Calcium-dependent exocytosis - how are the SNAREs controlled?

Reinhard Jahn
Department of Neurobiology, Max-Planck-Institute for Biophysical Chemistry, Goettingen, Am Fassberg 11, Goettingen, Niedersachsen 37077, Germany

2:25 PM - 3:25 PM **Exhibition, Tea and Posters**

Urchins 1

3:25 PM - 5:20 PM **Session 2: Lipids in a chromaffin granule's journey: from biosynthesis to fusion**

Michaelmas Cay Room

Chair: Nicolas Vitale, Christina Mitchell

3:25 PM 3 Chromogranin A / Phosphatidic Acid interaction is crucial to the biogenesis of secretory granules

Maité Montero-Hadjadje[1], Ophélie Carmon[1], Charlène Delestre-Delacour[1], Tam Tahouly[2], Laetitia Fouillen[3], Sophie Bernard[4], Stéphane Alexandre[5], Nicolas Vitale[2] and Youssef Anouar[1]
1- Inserm U982, University of Rouen, Mont-Saint-Aignan, France; 2- CNRS UPR 3212, INCI, Strasbourg, France; 3- CNRS UMR 5200, Metabolome Platform, Bordeaux, France; 4- PRIMACEN, Mont-Saint-Aignan, France; 5- CNRS UMR 6270, PBS, Mont-Saint-Aignan, France.

3:50 PM **4** **Resident CAPS (aka CADPS) on dense-core vesicles docks and primes vesicles for fusion**

Thomas Martin, Greg Kabachinski, D. Michelle Kielar-Grevstad, Xingmin Zhang and Declan James

Department of Biochemistry, University of Wisconsin, 433 Babcock Drive, Madison, WI 53706, USA

4:15 PM **5** **Involvement of different phospholipase D1 generated phosphatidic acid species during regulated exocytosis**

M Ammar, N Kassas, P Costé de Bagneaux and Nicolas Vitale

INCI, CNRS UPR-3212, 5 rue Blaise Pascal, Strasbourg, Strasbourg 67084, France

4:40 PM **6** **Uncovering a role for saturated free fatty acids in neuroexocytosis and memory**

Vinod Narayana[1,2], Francois Windels[1,2], Robert Sullivan[1,2], Nicolas Vitale[3], Pankaj Sah[1,2], David Kvaskoff[2,4] and Frederic Meunier[1,2]

1. Clem Jones Centre for Ageing Dementia Research, 2. Queensland Brain Institute, Brisbane, 3. Institut des Neurosciences Cellulaires et Intégratives (INCI), UPR-3212 Centre National de la Recherche Scientifique and Université de Strasbourg. 4. University of Queensland Centre for Clinical Research, The University of Queensland, Australia.

5:05 PM **7** **The Ca^{2+} sensor Doc2B controls Munc13-1 during GqPCR-mediated potentiation of exocytosis**

Claudia Bauer[1], Robert Woolley[1] and Elizabeth Seward[1]

Biomedical Science, University of Sheffield, Western Bank, Sheffield, S Yorkshire S10 2TN, UK

5:20 PM - 5:30 PM **BioBreak**

5:30 PM - 6:15 PM **Plenary 2: Peter Dunkley**

Michaelmas Cay Room

Chair: Phillip Robinson

5:25 PM **8** **Control of Tyrosine Hydroxylase Activity: Responses to inflammation stress over time**

Peter Dunkley[1], Lin Kooi Ong[1], Luba Sominsky[2], Deborah Hodgson[2] and Phillip Dickson[1]

1School of Biomedical Sciences and Pharmacy, 2School of Psychology. The University of Newcastle, University Drive, Callaghan, Newcastle, NSW 2308, Australia

6:15 PM - 7:40 PM **Welcome Reception, Trade and Posters**

Urchins 1

7:40 PM **Dinner at Leisure**

check vouchers in your satchel / see website / Concierge for suggested restaurants

END OF MONDAY'S PROGRAM

Tuesday, August 18, 2015

8:20 AM - 8:30 AM **Housekeeping**

Michaelmas Cay Room

Chair: Damien Keating

8:30 AM - 10:25 AM **Session 4: Exo-endocytosis coupling in neurons and neuroendocrine cells**

Michaelmas Cay Room

Chair: Stephane Gasman

8:30 AM **28** **Diffusional spread and confinement of newly exocytosed synaptic vesicle proteins**

Volker Haucke
Molecular Pharmacology and Cell Biology, Leibniz Institute for Molecular Pharmacology, Robert-Roessle-Str. 10, Berlin, D-13125, Germany

8:55 AM **29** **Membrane lipid reorganization and Rho GTPases signaling regulate exo-endocytosis coupling of large dense core vesicles in chromaffin cells**

Sébastien Houy, Catherine Estay-Ahumada, Pauline Croisé, Valérie Calco, Anne-Marie Haeberlé, Yannick Bailly, Sylvette Chasserot-Golaz, Marie-France Bader, Stéphane Ory and Stéphane Gasman
CNRS UPR 3212-INCI, 5 rue Blaise Pascal, Strasbourg, 67084, France

9:20 AM **30** **Imaging vesicle exo- and endocytosis in chromaffin cells**

Ling-Gang Wu
NINDS, NIH, 35 Convent Dr., Bethesda, MD 20892, USA

9:45 AM **31** **Dynamin Rings, Regulation and Allostery: Sensing the oligomerisation signal**

Phillip Robinson, Michaela Collett, Sai Krishnan and Adam McCluskey
Cell Signalling Unit, Children's Medical Research Institute, The University of Sydney, 214 Hawkesbury Road, Westmead, Sydney, NSW 2145, Australia

10:10 AM **32** **Rapid recovery of exocytosis after action potential-like stimulation in mouse chromaffin cells is coupled to a fast endocytotic process**

José Moya Díaz, Yanina Álvarez, Mauricio Montenegro, Verónica Belingheri and Fernando Marengo
IFIByNE (CONICET). Departamento de Fisiología,Biología Molecular y Celular, Facultad de Ciencias Exactas y Naturales - Universidad de Buenos Aires, Ciudad Universitaria. Pabellón 2. 2do piso, Buenos Aires, CABA 1428, Argentina.

10:25 AM - 11:00 AM **Coffee, Posters and Exhibition**

Urchins 1

11:00 AM - 1:05 PM Session 5: Cell signalling in other cell types: What can we learn?

Michaelmas Cay Room

Chair: Damien Keating

11:00 AM 33 How the exocytotic fusion pore opens and closes?

Jernej Jorgačevski[1,2], Nina Vardjan[1,2], Ana Calejo[2], Alenka Guček[2], Boštjan Rituper[2], Marko Kreft[1,2,3] and Robert Zorec[1,2]

1) Celica Biomedical, Tehnološki Park, Ljubljana, Slovenia EU; 2) LN-MCP, Institute of Pathophysiology, Univerza v Ljubljani, Medicinska fakulteta, Zaloška cesta 4, Ljubljana, Slovenia EU; 3) Biotechnical Faculty, Univerza v Ljubljani, Ljubljana, Slovenia EU

11:25 AM 34 Mechanisms controlling insulin granule exocytosis in health and disease

Peter Thorn

School of Biomedical Sciences, University of Queensland, St Lucia, Brisbane, QLD 4072, Australia

11:50 AM 35 Enterochromaffin cells display distinct release kinetics

Damien Keating

Human Physiology and Centre for Neuroscience, Flinders University, Adelaide, SA 5000, Australia

12:15 PM 36 The other synapse: calcium-dependent exocytosis in the immune system

Jens Rettig

Department of Physiology, Saarland University, Kirrberger Strasse, Homburg, 66421, Germany

12:40 PM 37 Calpains cleave dysferlin to release a synaptotagmin-like module for the calcium-dependent exocytosis of membrane repair

Greg Redpath[1,2], Natalie Woolger[1,2], Angela Lek[1,2], Ann-Katrin Piper[1,2], Frances Lemckert[1,2], Peter Greer[4], Kathryn North[3] and Sandra Cooper[1,2]

1. Institute for Neuroscience and Muscle Research, The Children's Hospital at Westmead, The University of Sydney, Locked Bag 4001, Westmead, Sydney, NSW 2145, Australia; 2. Discipline of Paediatrics and Child Health, The University of Sydney, NSW 2006, Australia. ; 3. Murdoch Children's Research Institute, The Royal Children's Hospital, Department of Paediatrics, Faculty of Medicine, Dentistry and Health Sciences. University of Melbourne, VIC 3010, Australia; 4. Department of Pathology and Molecular Medicine, Queen's University, Division of Cancer Biology and Genetics, Queen's Cancer Research Institute, Kingston, Ontario K7L 3N6, Canada

1:05 PM - 2:05 PM Lunch, Posters and Exhibition

Urchins 1

2:05 PM - 3:25 PM Session 6: Chromogranin/secretogranin peptides

Michaelmas Cay Room

Chair: Bruno Tota

2:05 PM 38 Cardiac interaction and signalling between full length Chromogranin A and Tumor Necrosis Factor alpha

Bruno Tota[1], Teresa Pasqua[1], Angelo Corti[2], Mariacarmela Cerra[1] and Tommaso Angelone[1]
1 Dept of Biology, Ecology, and E.S., University of Calabria, Rende (CS), Italy; 2 Tumor Biology and Vascular Targeting Unit, Division of Molecular Oncology, San Raffaele Scientific Institute, Milan, Italy.

2:30 PM 39 Human genetic variants of the chromogranin A-derived dysglycemic peptide Pancreastatin: implications for cardiometabolic disorders

Nitish Mahapatra
Department of Biotechnology, Indian Institute of Technology Madras, Alumni Avenue, Chennai, Tamil Nadu 600036, India

2:55 PM 40 Secretoneurin gene therapy

Rudolf Kirchmair
Internal Medicine III, Medical University Innsbruck, Anichstr. 35, Innsbruck, Tyrol 6020, Austria

3:20 PM Open discussion

3:25 PM - 4:05 PM Tea, Posters and Exhibition

Urchins 1

4:05 PM - 6:00 PM Session 7: Cytoskeletal mechanisms linked to chromaffin cell function

Michaelmas Cay Room

Chair: Luis Guttierrez

4:05 PM 41 Measurement and cytoskeletal regulation of open and closed exocytosis

Andrew Ewing
Chemistry and Chemical Engineering, Chalmers University, Kemivagen 10, Gothenburg, VG 41296, Sweden

4:30 PM 42 An Acto-Myosin II Constricting Ring Initiates the Fission of Activity-Dependent Bulk Endosomes in Neurosecretory Cells

Rachel Gormal, Tam Nguyen, Sally Martin, Andreas Papadopulos and Frederic Meunier
The Clem Jones Centre for Ageing Dementia Research, Queensland Brain Institute, The University of Queensland, Brisbane 4072, Queensland, Australia

4:55 PM 43 F-actin organization and the fate of vesicles and organelles.

José Villanueva, Yolanda Gimenez-Molina, Salvador Viniegra and Luis Gutiérrez
Instituto de Neurociencias de Alicante, CSIC-Universidad Miguel Hernandez, Cra de Valencia s/n, Sant Joan delacant, Alicante, Alicante 03550, Spain

continued, over

5:20 PM **44** **Activity-driven relaxation of the cortical actomyosin II network synchronizes Munc18-1-dependent neurosecretory vesicle docking**

Andreas Papadopulos[1], Guillermo Gomez[1], Sally Martin[1], Jade Jackson[3], Rachel Gormal[1], Damien Keating[3], Alpha Yap[2] and Frederic Meunier[1]

1 The Clem Jones Centre for Ageing Dementia Research, Queensland Brain Institute, The University Of Queensland, Building 79, Upland Rd, St Lucia, St Lucia, QLD 4072, Australia; 2 Division of Molecular Cell Biology Institute for Molecular Bioscience, The University of Queensland, Brisbane, Queensland 4072, Australia. 3 Molecular and Cellular Neuroscience Laboratory, Department of Human Physiology, Flinders University, Adelaide, South Australia, Australia

5:45 PM **45** **Annexin A2 bundles actin filaments to promote secretory granule docking and fusion**

Marion Gabel[1], Franck Delavoie[1], Cathy Royer[3], Valérie Demais[3], Yannick Bailly[1], Nicolas Vitale[1], Marie-France Bader[1] and Sylvette Chasserot-Golaz[1]

1- INCI UPR 3212 CNRS, 5 Rue Blaise Pascal, Strasbourg, FRANCE 67084; 2- UMR5099 CNRS-Université de Toulouse III, 118 route de Narbonne, Toulouse, France 31000; 3-Plateforme Imagerie In Vitro, Neuropôle de Strasbourg 5 Rue Blaise Pascal, Strasbourg, France 67084

6:00 PM - 7:45 PM **Dinner at Leisure**

1 3/4 hrs for Dinner - suggest restaurants downstairs in the hotel

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8:00 PM - 10:30 PM **Session 8: Wine and Cheese Symposium (Parallel sessions)**

Posters and Exhibition, with Wine & Cheeses, followed by cash bar

Michaelmas Cay / Urchins 1 and Arlington Bar

8:00 PM - 9:00 PM **Session 8.i: Neurotransmitter release**

MIchaelmas Cay Room

Chair: Luis Guttierrez

The nature of chomaffin cell cytoskeleton and art

Luis Gutiérrez

Instituto de Neurociencias de Alicante, CSIC-Universidad Miguel Hernandez, Cra de Valencia s/n, Sant Joan delacant, Alicante, Alicante 03550, Spain

46 **Huntingtin-associated protein 1 (HAP1) is a Synapsin-binding protein and regulates vesicle exocytosis and endocytosis**

Kim Mackenzie, Amanda Lumsden, Feng Guo, Michael Duffield, Timothy Chataway, Xin-Fu Zhou and Damien Keating

Human Physiology, Flinders University, Sturt Road, Bedford Park, Adelaide, SA 5042, Australia

47 **Unconventional Research demands flexible tools**

Anthony Swinscoe

Leica Microsystems

48 **Does depolarization-induced reprogramming of the presynaptic phosphoproteome mediate changes in neurotransmitter release?**

Kasper Engholm-Keller[1,2], Phillip Robinson[1,2] and Mark Graham[1]

1 Children's Medical Research Institute, Westmead, NSW, Australia; 2 Department of Biochemistry and Molecular Biology, University of Southern Denmark, Odense, Denmark

49 Effect of external high osmolality on vesicular neurotransmitter content in chromaffin cells

Hoda Fathali, Soodabeh Majdi, Jelena Lovric, Ann-Sofie Cans and Andrew Ewing
Chemistry and Chemical Engineering, Chalmers Tekniska Högskola AB, Maskingränd 2, Gothenburg , Sweden 41296, Sweden

50 The Effect of Excited Fluorophore on Vesicle Fusion at the Surface of the Electrode

Neda Najafinobar[1], Jelena Lovric[1], Johan Dunevall[1], Hoda Fathali[1], Andrew Ewing[1,2] and Ann Sofie Cans[1]
1Department of Chemical and Biological Engineering, Chalmers University of Technology, Kemivägen 10, SE-41296 Gothenburg, Sweden; 2 Department of Chemistry and Molecular Biology, University of Gothenburg, 412 96 Gothenburg, Sweden

51 Dissecting phosphoregulation of exocytosis: assessing the roles of kinases in late steps of the exocytotic pathway

Prabhodh Abbineni, Deepti Dabral and Jens Coorssen
Department of Molecular Physiology, and Molecular Medicine Research Group, School of Medicine, University of Western Sydney, Building 30, Goldsmith Avenue, Campbelltown, Sydney, NSW 2560, Australia

8:00 PM - 9:00 PM Session 8.ii: Neurotransmitter in diseases

Urchins 4

Chair: Reinhard Jahn

52 Augmented catecholamine release from chromaffin cells of diabetic and hypertensive rats

Iago Méndez-López[1,2], Juan A. Arranz-Tagarro[1,2], Juan F. Padín[1,2], Diego C. Musial[4], Ghilherme H. Bomfim[4], Regiane Miranda-Ferreira[4], Aron Jurkiewicz[4], Neide H. Jurkiewicz[4] and Antonio García García[1,2,3]
1- Instituto Teófilo Hernando de I+D del Medicamento, Madrid, Spain; 2- Dep. de Farmacología y Terapéutica, Fac. Medicina, Universidad Autónoma de Madrid. Madrid, Spain; 3- Instituto de Investigación Sanitaria, Servicio de Farmacología Clínica, Hospital Universitario de La Princesa, Madrid, Spain; 4- Dep. Farmacología, Universidade Federal de São Paulo, São Paulo, Brazil

47 Unconventional Research demands flexible tools

Scott Merrington, LEICA Microsystems
Leica Microsystems

54 Sorting Nexin 27 links PTHR Signalling to the Retromer for Postnatal Bone Growth

Audrey Chan[1], Thomas Clairfeuille[1], Euphemie Landao[1], Genevieve Kinna[2], PeiYing Ng[1], Lishen Loo[3], Rohan Teasdale[2], Wanjin Hong[3], Brett Collins[2] and Nathan Pavlos[1]
*1. School of Surgery,The University of Western Australia, Crawley, Western Australia 6009; 2. Institute for Molecular Bioscience, The University of Queensland, St Lucia, Queensland 4072; 3. Institute of Molecular and Cell Biology, A*STAR, Proteos Building, Singapore 138673*

55 An in vivo examination of IL-6 actions in the mouse adrenal gland

Danielle Tranter and Stephen Bunn
Department of Anatomy, University of Otago, Dunedin, Otago, New Zealand

56 **Why (some) old men get hypertension: testosterone and a receptor tyrosine kinase EphB6 in concert regulate catecholamine synthesis and release in chromaffin cells, hence modulating blood pressure**

Yujia Wang[1], Jonathan Ledoux[1], Hongyu Luo[1] and Jiangping Wu[1]
1CHUM Research Center and 2Montreal Heart Institute, University of Montreal

57 **Persistently active Rab3a, and a chimaeric protein containing its amino-terminal portion, prevent the late stages of sperm exocytosis by stabilizing open fusion pores**

Matías Bustos[1], María Quevedo[1], Carlos Roggero[2], Ornella Lucchesi[1], Luis Mayorga[1] and Claudia Tomes[1]
Morpho physiology, IHEM-CONICET, Cuyo National University, Centro Universitario , Mendoza, Mendoza 5500, Argentina

Wine and Cheeses - *Arlington Bar and Urchins 1*

END OF TUESDAY'S PROGRAM

NOTES

Wednesday, August 19, 2015

8:20 AM - 8:30 AM **Housekeeping**

Michaelmas Cay Room

Chair: Fred Meunier

8:30 AM - 10:25 AM **Session 9: Biophysics**

Chairs: Michael Kozlov and Andreas Papadopulos

Michaelmas Cay Room

8:30 AM **58** **Fusion pore opening**

Manfred Lindau[1,2], Ying Zhao[1,2], Qinghua Fang[2], Satyan Sharma[2] and Shailendra Rathore[1]
1 School of Applied and Engineering Physics, Cornell University, 272 Clark Hall, Ithaca, NY 14850, United States; 2 Laboratory for Nanoscale Cell Biology, Max-Planck-Institute for Biophysical Chemistry, D-37077 Goettingen, Germany

8:55 AM **59** **What is the docked state of a release-ready vesicle?**

Jens Coorssen
Molecular Physiology, School of Medicine, University of Western Sydney, Locked Bag 1797, Penrith, NSW 1797, AUSTRALIA

9:20 AM **60** **Modeling Mechanisms Shaping Endoplasmic Reticulum**

Tom Shemesh[2], Tom Rapoport[2] and Michael Kozlov[1]
1Tel Aviv University, Sackler School of Medicine, Tel Aviv, Israel, 69978, Israel; 2Technicon, Biology, Haifa, Israel; Biology, Haifa, Israel, 3Harvard University, Medical School, Boston, MA, USA

9:45 AM **61** **Pituitary Adenylate Cyclase-Activating Peptide (PACAP) excitation of the adrenal medulla.**

Corey Smith[1]
Physiology and Biophysics, Case Western Reserve University, 2109 Adelbert Road, SOM Rm E521, Cleveland, OH 44106, USA

10:10 AM **62** **Multiple mechanisms for vesicle content release in the post-fusion phase of surfactant exocytosis**

Pika Miklavc, Manfred Frick, Konstantin Ehinger, Paul Dietl, Nadine Kittelberger and Markus Breunig
Institute of General Physiology, Ulm University, Albert-Einstein-Allee 11, Ulm, Baden-Wuerttemberg 89081, Germany

10:25 AM - 11:00 AM **Coffee, Posters and Exhibition**

Urchins 1

11:00 AM - 12:40 PM Session 10: Dan O'Connor Memorial Session

Michaelmas Cay Room

Chairs: Youssef Anouar and Sushil Mahata

11:00 AM 63 Cell-specific function of chromogranin A in dense core vesicle biogenesis and core formation in adrenomedullary chromaffin and pancreatic beta cells

Sushil Mahata[1], Sumana Mahata[1], Teresa Pasqua[2], Angshuman Biswas[2], Guy Perkins[4], Amiya Sinha-Hikim[5] and Lee Eiden[6]

Medicine, VA San Diego Healthcare System & University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0732, USA

11:20 AM 64 Coupling of oxidative stress control and energy supply during PC12 cell differentiation

Youssef Anouar[1], Housni Abid[1], Dorthe Cartier[1], Abdallah Hamieh[1], Anne-Marie François-Bellan[2], Christine Bucharles[1], Destiny-Love Manecka[1], Jerome Leprince[1], Sahil Adriouch[3] and Isabelle Lihrmann[1]

1, Inserm, U982, Institute for Research and Innovation in Biomedicine, University of Rouen, Normandy University, Mont-Saint-Aignan, France; 2, CNRS UMR 7286, CRN2M, Aix-Marseille University, Marseille, France ; 3, Inserm U905, Institute for Research and Innovation in Biomedicine, Rouen, France

11:40 AM 65 Interleukin-6 interaction with the chromaffin cell

Stephen Bunn[1]

Centre for Neuroendocrinology and Dept of Anatomy, University of Otago, PO Box 913, Dunedin, Otago 9001, New Zealand

12:00 PM 66 PACAP at the adrenomedullary synapse, and in the brain: a master regulator of the stress response

Lee Eiden

SMN, LCMR, NIMH-IRP, 9000 Rockville Pike, Bethesda, Maryland 20892, USA

12:20 PM 67 ATP in secretory vesicles, Why?

Judith Estevez-Herrera[1], J. David Machado[1], Marta Pardo[1,2], Ayoze Santana[1], Natalia Dominguez[1,3] and Ricardo Borges[1,2]

1-Pharmacology, University of La Laguna, Facultad de Medicina, La Laguna, Tenerife E38071, Spain; 2-IUBO, University of La Laguna, La Laguna, Tenerife E38071, Spain.; 3- Present address: Center for Neurogenomics and Cognitive Research (CNCR) Amsterdam. The Neerderlands.

12:40 PM - 12:50 PM GROUP PHOTO

Arlington Bar Steps

12:50 PM - 1:40 PM Lunch with Posters and Exhibition

Urchins 1

1:40 PM - 3:10 PM Session 11: Young Investigators

Michaelmas Cay Room

Chair: Phil Dickson

1:40 PM 68 First identification of a human mutation in synaptotagmin1 reveals perturbation of synaptic vesicle cycling

Sarah Gordon[1,3], Kate Baker[1,3], Michael Cousin[1] and Lucy Raymond[2]

1. Centre for Integrative Physiology, University of Edinburgh, Edinburgh, UK; 2. Department of Medical Genetics, Cambridge Institute for Medical Research, University of Cambridge, Cambridge, UK; 3. Florey Institute of Neuroscience and Mental Health, University of Melbourne, 30 Royal Parade, Parkville, Melbourne, Vic 3052, Australia

2:00 PM 69 Phosphoproteomic profiling of activity-dependent presynaptic signalling

Kasper Engholm-Keller, Phillip Robinson and Mark Graham

Children's Medical Research Institute, 214 Hawkesbury Road, Westmead, NSW 2145, Australia

2:20 PM 70 Munc13s in LDCV docking and priming

Kwun-nok Man[1], Cordelia Imig[1], Alexander Walter[2], Paulo Pinheiro[3], David Stevens[4], Jens Rettig[4], Jakob Sørensen[3], Benjamin Cooper[1], Nils Brose[1] and Sonja Wojcik[1]

1 Molecular Neurobiology, Max Planck Institute for Experimental Medicine, Hermann-Rein-Str. 3, D-37075 Goettingen, Germany; 2 Leibniz Institute for Molecular Pharmacology, D-13125 Berlin, Germany; 3 Department of Neuroscience and Pharmacology, Faculty of Health and Medical Sciences and Lundbeck Foundation Center for Biomembranes in Nanomedicine, University of Copenhagen, 2200N Copenhagen, Denmark; 4 Department of Physiology, Saarland University, D-66421 Homburg, Saar, Germany

2:40 PM 71 Molecular mechanism of LDCV dead-end docking in mouse chromaffin cells

Ekta Dembla[1], Dieter Bruns[1], Jens Rettig[1] and Ute Becherer[1]

1.Department of Physiology, Cellular Neurophysiology, Saarland University, Building 59, Homburg, Saar 66421, Germany; 2.Department of Physiology, Molecular Neurophysiology, Saarland University, Building 59, Homburg, Saar 66421, Germany

3:00 PM 72 Flexibility of v-SNARE transmembrane domain regulates vesicular exocytosis

Madhurima Dhara[1], Antonio Yarzagaray[1], Yvonne Schwarz[1], Ralf Mohrmann[1] and Dieter Bruns[1]

1. Department of Physiology, University of Saarland, Kirrberger Str. 8, Building No. 59, Homburg, Saarland 66421, Germany

3:10 PM - 3:45 PM Tea, Posters and Exhibition

Urchins 1

3:45 PM - 4:25 PM Plenary 3: Hans-Herman Gerdes Memorial Lecture

Michaelmas Cay Room

Chair: Ricardo Borges

3:45 PM 73 Gerdes-Memorial: A Sympathetic Drive of Locomotion

Ruediger Rudolf[1,2,3]

1 Interdisciplinary Center for Neurosciences, University of Heidelberg, Germany; 2 Institute of Molecular and Cell Biology, Mannheim University of Applied Sciences, Germany; 2 Institute of Toxicology and Genetic, Karlsruhe Institute of Technology, Germany

4:25 PM - 4:35 PM **BioBreak**

4:35 PM - 6:10 PM **Session 13: Chromogranin / secretogranin proteins in health & diseases**

Michaelmas Cay Room

Chair: Sushil Mahata , Angelo Corti

4:35 PM 74 Regulation of tumor growth by circulating chromogranin A

Flavio Curnis[1], Mimma Bianco[1], Anna Gasparri[1], Alice Dallatomasina[1], Barbara Colombo[1], Angelina Sacchi[1], Martina Fiocchi[1], Ricardo Borges[2], Sushil Mahata[3] and Angelo Corti[1]

1) Tumor Biology and Vascular Targeting Unit, Division of Experimental Oncology, San Raffaele Scientific Institute, Milan; 2) Pharmacology Unit, Medical School, La Laguna University, Tenerife, Spain; 3) VA San Diego Healthcare System and University of California, San Diego, USA

4:55 PM 75 Novel roles for Chromogranin A peptide catestatin in cardiac metabolism and physiology

Sanjib Senapati[1], Jan Schilling[2,3], Teresa Pasqua[2], Venkat R. Chirasani[1], Nilima Biswas[2], Ennio Avolio[2], Hongqiang Cheng[4], Suvajit Sen,[5] Alice E. Zemljic-Harpf[2,3], John P. Headrick[6], Heidi N. Fridolfsson,[2,3] Adam Kassan,[2,3] Kevin Yei,7 Sumana Mahata,[7], Gautam Bandyopadhyay[2], David Roth[2,3], Nicholas Webster[2,3], Hemal Patel[2,3] and Sushil Mahata[2,3]*

1Department of Biotechnology, Indian Institute of Technology Madras, Chennai, India, 2University of California, San Diego, La Jolla, CA, USA, 3VA San Diego Healthcare System, 3350 La Jolla Village Drive, San Diego, CA, USA, 4Department of Pathology and Pathophysiology, Zhejiang University School of Medicine, Hangzhou, China, 5David Geffen School of Medicine, University of California, Los Angeles, 6Heart Foundation Research Centre, Griffith Health Institute, Australia, 7California Institute of Technology, Pasadena, CA.

5:15 PM 76 Localization and function of serpinin peptides

Peng Loh

NIH, 49, Convent Drive, Bldg 49, Rm 6C10, Bethesda, MD 20892, USA

5:35 PM 77 Combined measurement of granin and granin-derived peptides improves the diagnosis of pheochromocytoma

Laurent Yon[1,2,3], Johann Guillemot[1,2,3], Marlène Guérin[1,2,3], Maité Montéro-Hadjadje[1,2,3], Jérôme Leprince[1,2,3], Hervé Lefebvre[1,2,3,5], Marc Klein[6], Mihaela Muresan[7] and Youssef Anouar[1,2,3]

1 INSERM Unit 982, Mont-Saint-Aignan, France; 2 Normandie University, France; 3 Rouen University, Laboratory of Neuronal and Neuroendocrine Differentiation and Communication, Mont-Saint-Aignan, France; 4 Laboratory of Biochemical Neuroendocrinology, Clinical Research Institute of Montreal, Montreal, Quebec, Canada; 5 Department of Endocrinology, Diabetes and Metabolic Diseases, Rouen University Hospital, Bois-Guillaume, France; 6 Department of Endocrinology, Hôpital de Brabois, University of Nancy, Nancy, France; 7 Unit of Endocrinology, Hôpital Notre-Dame de Bon Secours, Metz, France

5:55 PM 78 Chromogranin A deficiency decreases secretory vesicle core formation, catecholamine storage, and energy metabolism in the adrenal medulla

Sumana Mahata[1], Teresa Pasqua[1], Gautam Bandyopadhyay[2], Angshuman Biswas[2], Guy Perkins[3], Amiya Sinha-Hikim[4], Lee Eiden[5] and Sushil Mahata[6,1]

1California Institute of Technology, Pasadena, California, USA; 2Department of Medicine, University of California, San Diego, La Jolla, California, USA; 3National Center for Microscopy and Imaging Research, San Diego, La Jolla, California, USA; 4Charles Drew University of Medicine and Science, Los Angeles, USA; 5Section on Molecular Neuroscience, NIMH-IRP, Bethesda, Maryland, USA; 6VA San Diego Healthcare System, San Diego, California, USA

6:10 PM - 6:15 PM **BioBreak**

6:15 PM - 7:15 PM **Plenary 4: Erwin Neher**

Michaelmas Cay Room

Chair: Corey Smith

6:10 PM **79** **Catecholamine and Glutamate Release: A Comparison**

Erwin Neher
Max Planck Institute for Biophysical Chemistry, Goettingen, Am Fassberg 11, Goettingen, Lower Saxony 37077, Germany

7:10 PM - 7:30 PM **Pre-dinner drinks**

7:30 PM - 11:00 PM **Conference Dinner**

Pool Deck

END OF WEDNESDAY'S PROGRAM

NOTES

Thursday, August 20, 2015

EXCURSION DAY! Great Barrier Reef - Diving, Snorkelling; Daintree Rain forest

Friday, August 21, 2015

8:20 AM - 8:30 AM **Housekeeping**

Michaelmas Cay Room

Chair: Damien Keating

8:30 AM - 10:35 AM **Session 15: Mechanisms of SNARE-mediated exocytosis**

Chair: Brett Collins

8:30 AM **80** **Elucidating the molecular mechanisms of neurotransmitter release and its regulation**
Joseph Rizo
Biophysics, UT Southwestern Medical Center, 6000 Harry Hines Boulevard, Dallas, TX 75390, USA

8:55 AM **81** **Elucidating the role of syntaxin-1 N-peptide in neurotransmitter release in PC12 cells and C. elegans**
Seungmee Park and Shuzo Sugita
University of Toronto; University Health Network, 60 Leonard Avenue, 7KD-419, Toronto, Toronto, ONTARIO M5T2S8, Canada

8:55 AM **82** **Assembly of the secretory machinery during insulin granule docking**
Sebastian Barg and Nikhil Gandasi
Medical Cell Biology, Uppsala University, BMC 571, Uppsala, - 75123, Sweden

9:45 AM **83** **DOC2B translocates in a diffusion-like and in a PIP2 dependent manner to the plasma membrane**
Lirin Michaeli, Irit Gottfried and Uri Ashery
Department of Neurobiology, Life Sciences Faculty, The Sagol School of Neuroscience, Tel aviv University, Tel Aviv, Israel

10:10 AM **84** **Structure-function studies of Munc18 in vesicle exocytosis**
Sally Martin[1], Andreas Papadoulos[1], Ye Chai[1], Nancy Malintan[1], Jennifer Martin[2], Frederic Meunier[1] and Brett Collins[2]
1. Clem Jones Centre for Ageing Dementia Research, Queensland Brain Institute, The University of Queensland, Brisbane, QLD 4072, Australia; 2. Institute for Molecular Bioscience, University of Queensland, Carmody Rd, St Lucia, QLD 4171, Australia

10:35 AM - 11:05 AM Coffee, Posters

Urchins 1

11:05 AM - 1:05 PM Session 16: From the pre- to the post-synapse

Michaelmas Cay Room

Chair: Rudiger Rudolf

11:05 AM 85 The Morphological and Molecular Nature of Synaptic Vesicle Priming at Presynaptic Active Zones

Nils Brose

Molecular Neurobiology, Max Planck Institute of Experimental Medicine, Hermann-Rein-Strasse 3, Goettingen, Lower Saxony 37075, Germany

11:30 AM 86 Visualization of AMPA Receptor Synaptic Plasticity In Vivo

Richard Huganir

The Solomon H. Snyder Department of Neuroscience, Johns Hopkins University , 725 North Wolfe Street, Baltimore, MD 21205, USA

11:55 AM 87 Role of Bassoon in the regulation of neurotransmitter release

Carolina Montenegro-Venegas[1], Eneko Pina[2], Claudia Marini[1], Eckart Gundelfinger[1], Vesna Lazarevic[2],and Anna Fejtová[2]

1 AG Chemical Synapses, Department of Neurochemistry and Molecular Biology; 2 RG Presynaptic Plasticity, Leibniz Institute for Neurobiology, Magdeburg, Germany

12:120 PM 88 Phosphorylation of Synaptic Vesicle Protein 2A at Thr84 Controls the Specific Retrieval of Synaptotagmin-1

Sarah Gordon[1], Ning Zhang[1], Maximilian Fritsch[2], Noor Esoof[2], David Campbell[2], Thomas Macartney[2], Mark Peggie[2], Daan van Aalten[2], Dario Alessi [2] and Michael Cousin[1]

1) Centre for Integrative Physiology, University of Edinburgh, 15 George Square, Edniburgh, Lothian EH10 6QQ, Scotland; 2) Medical Research Council Protein Phosphorylation and Ubiquitylation Unit, College of Life Sciences, University of Dundee, Dundee DD1 5EH, Scotland

12:45 PM 89 Neurotransmission alterations related to the progression of Alzheimer´s disease in 3xTg-AD transgenic mice

Carmen Nanclares[1], Inés Colmena[1], Andrea Crespo-Castrillo[1], Enrique Calvo-Gallardo[1], Juan Alberto Arranz-Tagarro[1], Antonio G. García[1] and Luis Gandía[1]

1 Instituto Teófilo Hernando, Departamento de Farmacologia y Terapeutica, Facultad de Medicina, Universidad Autonoma de Madrid. C/Arzobispo Morcillo, 4, Madrid, 28029, Spain.

1:05 PM - 2:00 PM Lunch

Urchins 1

2:00 PM - 4:00 PM Session 17: Biosensing and Signal processing

Michaelmas Cay Room

Chair: Manfred Lindau

2:00 PM 90 Detection and estimation of amperometric spike parameters using matched filtering algorithms

Supriya Balaji Ramachandran[1,2] and Kevin Gillis[1,2]
1Department of Bioengineering, 2Dalton Cardiovascular Research Center, University of Missouri, Columbia, 134 Research Park Dr, Columbia, MO 65211, USA

2:25 PM 91 The function of SNARE membrane anchors

Meyer Jackson
Department of Neuroscience, University of Wisconsin, 1111 Highland Ave, Madison, Madison, Wisconsin 53705, USA

2:50 PM 92 TBA

Tao Xu
Institute of Biophysics, CAS, 15 Datun Road, Chaoyang District, Beijing, Beijing 100101, China

3:15 PM 93 Complexin: small but capable

Dieter Bruns
Medical Faculty, CIPMM, Molecular Neurophysiology, University of Saarland, Kirrbergerstr. 100, Homburg, Saarland 66421, Germany

3:40 PM 94 Studies of neurosecretion by remote control of exocytosis and endocytosis with light

Pau Gorostiza[1,2,3]
(1) Institute for Bioengineering of Catalonia, Baldiri Reixac 15-21, Barcelona, Barcelona 08028, Spain; (2) Catalan Institution for Research and Advanced Studies (ICREA); (3) Networking Center of Biomedical Research in Biomaterials, Bioengineering and Nanomedicine (CIBER-BBN)

4:00 PM - 5:35 PM Session 18 and Plenary V: Matthijs Verhage

Michaelmas Cay Room

Chair: Fred Meunier

4:00 PM 95 Regulation of phosphoinositide signaling

Christina Mitchell, Jennifer Dyson, Michele Davies, Rajendra Gurung and Lisa Ooms
1Department of Biochemistry and Molecular Biology, Monash University, Wellington Road, Clayton, Melbourne, Victoria 3800, Australia

4:25 PM 96 Regulating synaptic strength across the dendritic tree

Mathieu Letellier[1], Thomas Chater[1], Yun Kyun Park[2] and Yukiko Goda[2]
1. Université Bordeaux, Institut Interdisciplinaire de Neurosciences, UMR5297, Bordeaux, FRANCE; 2. RIKEN Brain Science Institute, 2-1 Hirosawa, Wako-shi, Saitama 351-0198, JAPAN

4:50 PM 97 Trafficking, docking and fusion of secretory vesicles

Matthijs Verhage
Functional Genomics, VU University Medical Center (VUmc) Amsterdam, De Boelelaan 1085, Amsterdam, n/a 1081HV, The Netherlands

5:35 PM - 5:45 PM Conference Close, Awards and Prizes

1 SEX AND VIOLENCE, SEEING, SIGNALS AND STOMATOPODS: A DAY ON THE REEF

Justin Marshall
Sensory Neurobiology Group, Queensland Brain Institute, University of Queensland, St Lucia, Brisbane, QLD 4072, Australia

Watch the magic unfold on the screen!

2 CALCIUM-DEPENDENT EXOCYTOSIS - HOW ARE THE SNARES CONTROLLED?

Reinhard Jahn
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Abstract not available at time of going to print

3 CHROMOGRANIN A / PHOSPHATIDIC ACID INTERACTION IS CRUCIAL TO THE BIOGENESIS OF SECRETORY GRANULES

Maité Montero-Hadjadje[1], Ophélie Carmon[1], Charlène Delestre-Delacour[1], Tam Tahouly[2], Laetitia Fouillen[3], Sophie Bernard[4], Stéphane Alexandre[5], Nicolas Vitale[2] and Youssef Anouar[1]

1- Inserm U982, University of Rouen, Mont-Saint-Aignan, France; 2- CNRS UPR 3212, INCI, Strasbourg, France; 3- CNRS UMR 5200, Metabolome Platform, Bordeaux, France; 4- PRIMACEN, Mont-Saint-Aignan, France; 5- CNRS UMR 6270, PBS, Mont-Saint-Aignan, France.

In neuroendocrine cells, dense core secretory granules (DCSG) bud from the TGN membrane after the formation of neurohormone aggregates including soluble glycoproteins called chromogranins. Since chromogranin A (CgA) acts as an on/off switch regulating the formation of DCSG, we investigated the interaction between CgA and membrane lipids to decipher the molecular mechanisms mediating this process. Using lipid-protein overlay assays, we observed that recombinant CgA specifically binds to phosphatidic acid (PA), a phospholipid that plays a crucial role in DCSG formation and membrane curvature. The quantitative and comparative analysis by LC-MS/MS of the membrane lipidome of purified CgA-containing granules and the Golgi apparatus revealed an enrichment of PA in the granule membrane, and the predominance of PA36:1, PA38:2 and PA40:6 species. Moreover, using a pull-down assay with liposomes enriched with various phospholipids including phosphatidylserine, phosphatidylcholine or distinct PA species, we have shown that CgA from CgA-expressing COS7 cell lysates, specifically interacts with the predominant PA species identified by our lipidomic study. Furthermore, we observed using confocal microscopy that the alteration of PA production by pharmacological drugs that inhibit enzymes involved in PA synthesis (phospholipase D or DAG kinase) provokes the retention of CgA in the Golgi apparatus, associated to a significant decrease in the number of CgA granules. Preliminary analyses by electron microscopy of the ultrastructure of these inhibitor-treated cells revealed a significant swelling of the Golgi apparatus, suggesting neurohormone retention in this compartment due to an alteration of DCSG budding in the absence of PA. Altogether, these data suggest that the budding of DCSG and the subsequent neurohormone secretion are regulated by CgA interaction with PA at the level of the TGN membrane. We postulate that CgA interaction with PA at the level of the TGN membrane is at the origin of microdomain formation that could govern the TGN membrane curvature and/or the recruitment of cytosolic proteins involved in the DCSG trafficking crucial to neurohormone secretion

This work is supported by INSERM, the University of Rouen, the Conseil Régional de Haute-Normandie and the Ministère de l'Enseignement Supérieur et de la Recherche.

4 RESIDENT CAPS (AKA CADPS) ON DENSE-CORE VESICLES DOCKS AND PRIMES VESICLES FOR FUSION

Thomas Martin, Greg Kabachinski, D. Michelle Kielar-Grevstad, Xingmin Zhang and Declan James

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The Ca^{2+}-dependent exocytosis of dense-core vesicles in neuroendocrine cells requires a priming step during which SNARE protein complexes assemble. CAPS (aka CADPS) is one of several factors required for vesicle priming, however, the localization and dynamics of CAPS at sites of exocytosis in live cells has not been determined. We imaged CAPS before, during, and after single vesicle fusion events in PC12 cells by TIRF microscopy. CAPS was present in clusters of ~9 molecules near the plasma membrane that corresponded to docked/tethered vesicles, and it was also a resident on cytoplasmic vesicles. CAPS accompanied vesicles to the plasma membrane and was present at all exocytic events but dissociated during exocytosis at high [Ca^{2+}]. The knockdown of CAPS by shRNA eliminated the VAMP-2-dependent docking and evoked exocytosis of fusion-competent vesicles. CAPS residence on vesicles was essential because a CAPS(deltaC135) protein that did not localize to vesicles failed to rescue vesicle docking and evoked exocytosis in CAPS-depleted cells. Our results indicate that dense-core vesicles carry the resident priming factor CAPS to sites of exocytosis where CAPS promotes vesicle docking and priming likely by initiating SNARE complex assembly.

5 INVOLVEMENT OF DIFFERENT PHOSPHOLIPASE D1 GENERATED PHOSPHATIDIC ACID SPECIES DURING REGULATED EXOCYTOSIS

M Ammar, N Kassas, P Costé de Bagneaux and Nicolas Vitale
INCI, CNRS UPR-3212, 5 rue Blaise Pascal, Strasbourg, Strasbourg 67084, France

Exocytosis of neurotransmitters and hormones occurs through the fusion of secretory vesicles with the plasma membrane. This highly regulated process involves key proteins such as SNAREs but most likely specific lipid rearrangements are also occurring at the site of membrane fusion. Phospholipases D (PLD1/2) have recently emerged as promoters of membrane fusion in various exocytotic events potentially by providing fusogenic cone-shaped phosphatidic acid (PA) and/or by recruiting key proteins. In agreement with this view overexpression and silencing approaches have suggested that PLD1 plays a positive role in secretory granule exocytosis. Using novel pharmacological and genetic approaches we now strengthen this model. Electronic microscopy analysis revealed a significant reduction in the number of secretory granules morphologically docked after PLD1 knockout. Furthermore amperometric recordings from mice PLD1-/- chromaffin cells in culture suggest that PA produced by PLD1 favors docking/priming and a late step in membrane fusion. Using a molecular probe for PA, we also show that the fusogenic lipid accumulates at the plasma membrane facing chromaffin granules that appeared morphologically docked at the electronic microscopy level. Using a lipidomic approach combined to subcellular fractionation we found that the composition in the different forms of PA varied between the plasma membrane and the secretory granule membrane. While no significant variation in the levels of the different PA species was found between resting and stimulated secretory granules, the levels of few PA species were dramatically increased at the plasma membrane after stimulation. Finally a reconstitution assay indicated that different forms of PA may be involved in different steps of the exocytotic process.

6 UNCOVERING A ROLE FOR SATURATED FREE FATTY ACIDS IN NEUROEXOCYTOSIS AND MEMORY

Vinod Narayana[1,2], Francois Windels[1,2], Robert Sullivan[1,2], Nicolas Vitale[3], Pankaj Sah[1,2], David Kvaskoff[2,4] and Frederic Meunier[1,2]
1. Clem Jones Centre for Ageing Dementia Research, 2. Queensland Brain Institute, Brisbane, 3. Institut des Neurosciences Cellulaires et Intégratives (INCI), UPR-3212 Centre National de la Recherche Scientifique and Université de Strasbourg. 4. University of Queensland Centre for Clinical Research, The University of Queensland, Australia.

The role of free fatty acids in neuroexocytosis has long been discussed focussing mainly on arachidonic acid production. We here designed a iTRAQ-like multiplex method to detect changes in free fatty acid production occurring during secretagogue stimulation. The picomolar sensitivity of our assay has allowed us to venture in minute area of the brain and subcellular fractions. We uncovered a wide ranges of changes suggestive of a major role for saturated free fatty acids in neuroexocytosis and memory acquisition.

7 THE CA^{2+} SENSOR DOC2B CONTROLS MUNC13-1 DURING GQPCR-MEDIATED POTENTIATION OF EXOCYTOSIS

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Potentiation of exocytosis following Gq-protein-coupled receptor (GqPCR)-activation requires phospholipase C (PLC)-mediated production of diacylglycerol (DAG) as well as inositol-1,4,5-trisphosphate (IP3)-mediated Ca^{2+} release from stores. DAG regulates the vesicle priming protein Munc13-1 but the role and sensor for the agonist-induced Ca2+ signal is unknown.
Here we identify Doc2B as a Ca^{2+} sensor for agonist-induced Ca^{2+} signals. Using live cell imaging in PC12 and adrenal chromaffin cells we show that GqPCR activation caused a simultaneous, transient translocation of Munc13-1 and Doc2B to the plasma membrane. Munc13-1 translocation required PLC-mediated DAG production and binding of DAG to Munc13-1 whereas translocation of Doc2B relied on Ca^{2+} release from stores but not on DAG or Munc13-1. High-resolution capacitance measurements combined with voltage-clamp recordings in bovine chromaffin cells revealed that binding of Doc2B to Munc13-1 is however, required for agonist-dependent potentiation of stimulus-coupled exocytosis. Thus our data support a model in which Doc2B and Munc13-1 act as co-incident detectors for GqPCRs-generated signals, which following recruitment to the plasma membrane interact directly to increase priming of vesicles whose exocytosis is tightly coupled to the activation of neuronal voltage-gated calcium channels.

8 CONTROL OF TYROSINE HYDROXYLASE ACTIVITY: RESPONSES TO INFLAMMATION STRESS OVER TIME

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Tyrosine Hydroxylase (TH) is the rate limiting enzyme in the synthesis of the catecholamines, dopamine, noradrenaline and adrenaline. It is present in the adrenal medulla, the sympathetic nervous system and the brain. TH activity is dependent on the catecholamine and TH protein levels in the cell, as well as the extent of TH phosphorylation, especially at serine 40 and serine 31. TH activity is generally increased in response to a range of stressors, depending on the cell type, by increasing TH phosphorylation during the first hour and if the stress is sufficient by increasing TH protein in the first 72 hours. Most studies have not investigated the effects of stress on TH activity beyond this period. Lipopolysaccharide (LPS) has been used to induce inflammation stress and here we have investigated the effects of LPS in a developmental model and in a Parkinson's disease associated model. We first used of two low doses of LPS (each 0.1 mg/Kg IP) in rats on days 3 and 5 after birth and investigated TH activity, TH protein and TH phosphorylation for up to 3 months in the adrenal medulla and in brain catecholaminergic neurons. With this low dose LPS model we found that adrenal medulla TH was activated by TH phosphorylation at 4h and 24h after the second LPS administration. Adrenal medulla TH continued to be activated by TH phosphorylation, without changes in TH protein synthesis, into adolescence (50 days) and adulthood (85 days). In the adult brain TH activity was substantially increased in the Loceus Coeruleus (LC), due to an increase in both TH phosphorylation and TH protein, while the Substantia Nigra (SN) was not altered. These results indicate that neonatal inflammation produces long-term developmental changes in neuro-endocrine regulation in both the adrenal medulla and LC.

We then used one high dose of LPS (2mg/Kg IP) in 8 week old rats and found that there were substantial changes in TH activity 1 day after injection, with decreases in the adrenal medulla and increases in the SN and the adjacent Ventral Tegmental Area (VTA). After this the adrenal medulla and the VTA returned to normal for the next 6 months. However, there was a substantial increase in TH activity in the SN at 7 and 28 days that could not be explained by the small increases in TH protein or changes in TH phosphorylation. After returning to normal levels of TH activity at 85 days, there was a second increase in TH activity in the SN observed at 168 days which was due entirely to an increase in TH phosphorylation. TH protein levels had decreased to below normal due to the degeneration of the SN neurons. We have found similar responses in the SN to neurodegeneration in Parkinson's disease post-mortem tissue. These results indicate that after the first day of inflammatory responses to LPS the SN, but not the adjacent VTA, dopaminergic neurons respond by substantially increasing TH activity. This would lead to the production of excess dopamine which would facilitate neurodegeneration by increasing the risk of oxidative damage to the cells. The selective long-term effect of LPS on SN versus VTA neurons is consistent with their relative susceptibility to degeneration in PD (SN >>VTA).

9 THE CHROMOGRANIN A-DERIVED PYROGLUTAMINATED SERPININ INDUCES NEGATIVE INOTROPISM IN TELEOST AND AMPHIBIAN HEARTS

Bruno Tota[1], Sandra Imbrogno[1], Rosa Mazza[1], Chiara Pugliese[1], Mariacristina Filice[1], Tommaso Angelone[1], Peng Loh[2] and Maria Cerra[1]

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Chromogranin A (CgA) is an acidic protein stored in secretory granules of endocrine cells and neurons together with hormones and neuropeptides. Proteolytic cleavage in the highly conserved C terminus of CgA generates a 2.9 kDa peptide named serpinin (Ala26Leu) that can be modified at its N terminus to form a pyroglutamate residue (pGlu23Leu). Ala26Leu and pGlu23Leu have been recently proposed as novel cardiac modulators. On the Langendorff perfused rat heart, Ala26Leu and pGlu23Leu increased contractility and relaxation. Both peptides act through a β1-adrenergic receptor (β1AR)/adenylate cyclase/cAMP/Protein Kinase A (PKA) pathway, indicating a β-adrenergic-like action (Tota et al., FASEB J. 26: 2888-98, 2012).

In a comparative perspective, we explored whether Ala26Leu and pGlu23Leu could also affect myocardial contractility in two poikilotherm vertebrate species, such as the goldfish (Carassius auratus) and the frog (Rana esculenta).

Using working heart preparations, we observed that pGlu23Leu causes a reduction of stroke volume (SV) in both species tested, while treatment with Ala26Leu reduces contractility in the frog heart, being ineffective in the goldfish heart. In both species, pGlu23Leu activates the Akt-Nitric Oxide Synthase (NOS)-Nitric Oxide (NO)-cGMP signal transduction pathway, involving Endothelin-1 (ET-1) B receptors (ETBR) in frog and β3-ARs in goldfish. The pGlu23Leu signaling through cGMP was confirmed by the increased cGMP concentrations revealed by ELISA, whereas the role of Akt and NOS in pGlu23Leu-induced cardio-depression was supported by western blotting analysis.

In conclusion, this is the first report showing that both in teleosts and amphibians pGlu23Leu inhibits mechanical cardiac performance, supporting the evolutionary importance of the peptide in the control of the cardiac function of vertebrates.

10 CHROMOGRANIN A AND ITS PEPTIDE CATESTATIN PLAY CRUCIAL ROLES IN SKELETAL MUSCLE FUNCTION IN ENDURANCE EXERCISE AND MUSCLE FIBER TYPE-SPECIFIC GLUCOSE METABOLISM

Sushil Mahata[1], Teresa Pasqua[1], Angshuman Biswas[2], Suvajit Sen[3], Biswa Choudhury[2] and Gautam Bandyopadhyay[2]

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Background. Type 2 diabetes (T2D), which affects millions of people worldwide, arises from a combination of genetic susceptibility and environmental factors including physical inactivity and poor nutrition. Lack of physical activity is considered a major risk factor for the development of insulin resistance and T2D, and regular physical exercise can delay or prevent the onset of this disease. Chromogranin A (CgA) is co-stored and co-released with catecholamines from adrenal medulla and post-ganglionic sympathetic axons. The extracellular functions of CgA include the generation of bioactive peptides, such as the insulin-regulatory hormone pancreastatin; vasodilator and cardioprotective vasostatin; anti-hypertensive, anti-adrenergic, anti-obesity, pro-angiogenic and cardioprotective catestatin and pro-adrenergic serpinin. The intracellular function of CgA includes the initiation and regulation of dense core vesicle biogenesis and sequestration of hormones in neuroendocrine cells. Here, we report novel post-synaptic and systemic effects of CgA and catestatin on muscle function and glucose metabolism.

Methods and Results. We found that in vivo and in situ skeletal muscle functions are impaired in Chromogranin A knockout (Chga-KO) mice as shown by reduction of maximal running speed, time-to-fatigue (endurance) during treadmill running and reduced generation of maximal force in in situ gastrocnemius (GAS) complex. We have previously shown that Chga-KO mice displayed heightened hepatic insulin sensitivity but insulin resistance in muscle. Here, we show impairment of muscle performance in Chga-KO mice in endurance test. Skeletal muscle is composed of multiple myofibers that differ in their metabolic and contractile properties, including oxidative slow-twitch (type I; soleus), mixed oxidative-glycolytic fast-twitch (type IIa; GAS) and glycolytic fast-twitch (type IIb; EDL or extensor digitorum longus) myofibers. Exercise stimulates multiple signaling pathways and enzyme activities, including MAP kinase and AMPK pathways. Some of the signaling pathways overlap with downstream insulin signaling pathway involving Akt and Akt substrates, AS160 and TB1C1D1, all leading to Glut4 translocation. We found fiber specific shift in metabolic and signaling pattern leading to metabolic defects in oxidative soleus muscle in Chga-KO mice as evidenced by low TBC1D1, p38 kinase and JNK signaling in glycolytic EDL muscle. As a result, glucose uptake in Chga-KO muscle was low which was improved by activation of AMPK though endurance exercises. But muscle performance in Chga-KO mice in endurance test was poor because of a deficiency in p38 kinase signaling in soleus muscle, which was improved by CST supplementation and consequent improvement in muscle function. Since muscle glycogen serves as the primary fuel source during prolonged moderate-to-high intensity exercise, we have measured glycogen content by a highly sensitive HPAEC-PAD (High Performance Anion Exchange Chromatography with Pulsed Amperometric Detector) method (detection limit: 10 ng/ml) (Fig. 5A-C). We also assessed glycogenesis by incorporation of 3H-glucose into glycogen. Endurance exercise caused dramatic depletion (70-90%) of muscle glycogen. We hypothesize that in the absence of CST, exercise-induced AMPK signaling will carry out normal glucose uptake but would not compensate for impaired oxidative metabolism and muscle performance.

Conclusion. We conclude that CgA and its peptide catestatin play a pivotal role in skeletal muscle function by maintaining the integrity of the downstream signaling pathway shared by both insulin and exercise-induced stimuli and fiber type specific glucose metabolism

11 THE ANTI-HYPERTENSIVE PEPTIDE CATESTATIN IMPROVES HEPATIC INSULIN SENSITIVITY BY ALLEVIATING ENDOPLASMIC RETICULUM STRESS AND IMPROVING MITOCHONDRIAL FUNCTION

Sumana Mahata[1], Sushil Mahata[1], Ennio Avolio[3], Teresa Pasqua[3], Nilima Biswas[3], Angshuman Biswas[3] and Gautam Bandyopadhyay[3]

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Background. The endoplasmic reticulum (ER) is the main site of protein and lipid synthesis, membrane biogenesis, and cellular calcium storage. Perturbation of ER homeostasis (excessive protein synthesis; the accumulation of unfolded or misfolded proteins in the ER lumen) leads to ER stress and the activation of the unfolded protein response (UPR). The main role of UPR is to increase the capacity of protein folding and to decrease unfolded protein load. Chronic activation of ER stress has been implicated in the development of insulin resistance and type 2 diabetes (T2DM). Improvement in ER function has been shown to restore glucose homeostasis in mouse models as well as in obese individuals and people with T2DM. Mitochondrial dysfunction has also been associated with insulin resistance and T2DM. Here we report that the anti-hypertensive peptide CST acts as an insulin-sensitizing peptide in diet (high-fat)-induced obese (DIO) mice.

Methods and Results. Insulin resistance was created by feeding 60% high fat diet for 3 months to C57BL/6 mice. Peripheral administration of CST (5 µg/g BW, twice daily, IP) for 15 days improved insulin sensitivity in DIO and in obese and diabetic db/db mice, as assessed by glucose and insulin tolerance tests (decreased area under the curve) and insulin signaling (increased phospho-AKT signaling). Ultrastructural studies of DIO livers by Transmission Electron Microscopy (TEM) revealed dilation of endoplasmic reticulum (ER) lumen (61.65±3.17 nm) as compared to normal chow diet (NCD) fed mice (22.71±0.84 nm), suggesting ER stress. This stress was reversed by chronic treatments (15 days) with CST (ER lumen: DIO: 61.65+3.17 nm; DIO+CST: 16.12±1.66 nm; DIO+ROSI: 17.09±1.76 nm; one-way ANOVA: $p<0.0001$). Consistent with EM findings we found decreased phosphorylation of canonical UPR signaling molecules eIF2α (PERK arm of the UPR signaling leading to suppression of protein synthesis and apoptosis) and IRE1α (only ER stress sensor present in all eukaryotes and therefore reflects the most ancient and most conserved branch of the UPR. This arm of the UPR leads to increased expression of chaperone protein and lipid synthesis enzymes) and decreased expression of ER chaperone protein GRP78/BiP after chronic CST treatment. It should be pointed out that the chaperoning aspect of ER stress serves as a double-edged knife, which needs to be precisely regulated; too little or too much can both be dangerous. The above findings implicate that CST alleviates ER stress. DIO mice also showed fewer mitochondria, with broken cristae and decreased cristae surface area as compared to NCD fed mice. Chronic treatment of DIO mice with CST or ROSI reversed both mitochondrial numerical density (mitochondria number: DIO: 20.55±1.54; DIO+CST: 28.5±1.42; ROSI: 29.86±2.22; one-way ANOVA: $p<0.006$) and cristae surface area (cristae surface area: DIO: 1.65±0.12 µm; DIO+CST: 2.28±0.068 µm; DIO+ROSI: 2.84±0.186 µm; one-way ANOVA: $p<0.0001$). Using streptavidin-agarose affinity chromatography with biotinylated CST pull down followed by analysis with LC-tandem MS/MS mass spectrometry in mouse liver homogenates, we have identified CST binding with mitochondrial enzyme carbamoyl phosphate synthase (CPS-1), which converts ammonia, bicarbonate, and ATP into carbamoyl phosphate, which are the initial steps in the urea cycle. In this reaction process, CPS-1 generates 2 molecules of ADP, the substrate for ATP synthase in mitochondria. Thus, in addition to CPS-1's role in nitrogen homeostasis, it contributes to ADP build up inside mitochondria thereby regulating mitochondrial respiration. It will be groundbreaking to establish that the insulin sensitizing effects of CST involves mitochondrial CPS-1.

Conclusion. We conclude that CST improves insulin sensitivity by reversing ER stress and improving mitochondrial function. Given the anti-hypertensive, cardioprotective, anti-obesity and insulin-sensitizing effects of CST, we believe that CST offers dual therapeutic potential that can be developed to reduce hypertension and improve insulin sensitivity in diabetic patients at the same time.

12 CHROMOGRANIN A DEFICIENCY DECREASES SECRETORY VESICLE CORE FORMATION, CATECHOLAMINE STORAGE, AND ENERGY METABOLISM IN THE ADRENAL MEDULLA

Sumana Mahata[1], Teresa Pasqua[1], Gautam Bandyopadhyay[2], Angshuman Biswas[2], Guy Perkins[3], Amiya Sinha-Hikim[4], Lee Eiden[5] and Sushil Mahata[6]

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Background. Chromogranin A is a prohormone and granulogenic factor in neuroendocrine tissues with a regulated secretory pathway. CgA-deficient mice are dysregulated in catecholamine-driven diurnal blood pressure control and other parameters of metabolic and cardiovascular performance that depend on catecholamine (CA) secretion. The impact of CgA depletion on secretory granule formation has been previously demonstrated in cell culture. However, no studies have been performed to link the structural effects of CgA deficiency with secretory performance and cell metabolism in the chromaffin cells of the adrenal medulla in vivo.

Methods. Transmission Electron Microscopy (TEM) was used to evaluate structural changes of subcellular organelles. Adrenal CA contents were measured by HPLC. Colorimetric and radioactive methods were utilized to determine glycogen content and glycogenesis, respectively. In vivo metabolic physiology of substrate (glucose and fatty acid) uptake and utilization relied on radioactive methods.

Results. CAs were decreased in the adrenal gland of Chga-KO mice, consistent with impaired CA storage and smaller DC. TEM of the adrenal medulla revealed NE-storing vesicles as osmiophilic with intensely electron dense granules, and smaller, moderately electron-dense E-storing vesicles. Decreased DCV numbers in both NE and E cells of Chga-KO mice indicates a role of CgA in DCV biogenesis. Ultrastructural analyses revealed three vesicle types in Chga-KO mice: (i) normal DCV, (ii) decreased DCV with swelled halo (SDCV), and (iii) swollen empty vesicles without DCs (SEV). DCV diameter in Chga-KO mice is smaller (100-200 nm) than in WT mice (200-350 nm). Both volume density and vesicles number per μm_2 were significantly lower in Chga-KO mice. Chga-KO mice display an ~47% increase in DCV versus DC, implying vesicle swelling due to increased osmotically active free CAs. Increased glucose uptake and its utilization for glycogenesis suggest cellular stress within Chga-KO chromaffin cells. Despite increased fatty acid uptake, oxidation of fatty acid was reduced in Chga-KO mice, possibly associated with altered mitochondrial structure (dilated and shorter cristae) with consequent decrease in mitochondrial function.

Conclusion. Secretory vesicle formation and biogenic amine storage are profoundly altered in NE and E chromaffin cells of the AM in CgA-deficient mice. Lack of CgA alters cellular metabolism including glucose utilization, suggesting that in addition to enabling regulated CA secretion from the chromaffin cell, Cg also functions to coordinate metabolic function and 'secretory stress' in the adrenomedullary chromaffin cell

13 A NEW CALCIUM INDEPENDENT RELEASABLE POOL IN MOUSE CHROMAFFIN CELLS

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A brief depolatization resembling an action potential applied on mouse chromaffin cells in conditions of complete inhibition of Ca^{2+} currents (ICa^{+2}) induced a moderate increase in capacitance. To study this phenomenon more systematically we applied square depolarizations (from -80 to +10 mV) of variable durations in presence of (i) 0 mM external nominal Ca^{2+} or (ii) 5 mM Ca^{2+} + 100 µM Cd+2. In both conditions we measured an increase in capacitance that saturated at 17±2 and 14±1 fF respectively at 100 ms pulse duration, in the absence of measurable ICa+2 and cytosolic Ca^{2+} transients. To buffer any contaminant Ca^{2+} that might enter to the cell or be released from internal stores we made experiments in 0 extracellular Ca^{2+} + 4 mM intracellular BAPTA, obtaining again a significant exocytosis (14±2 fF) in response to 100 ms depolarizations. The calcium release blocker 2-APB or a pretreatment with the SERCA inhibitor thapsigargin were both unable to block this exocytosis process (15±2fF and 14±2 fF, respectively). Moreover, this Ca^{2+}-independent exocytosis process followed a sigmoid dependence with membrane potential, reaching the 50% of the saturating value at approximately -30 mV. When this vesicle pool was completely depleted by application of a 100 ms depolarization, it recovered with a time constant of 1.04±0.18 s. In agreement with this result, synchronous exocytosis at 0 extracellular Ca^{2+} did not decrease noticeably at low frequency stimulation (0.2-0.5 Hz), but the application of trains at higher frequencies (2-5 Hz) induced a pronounced decrease in this parameter. These results suggest the existence of a Ca^{2+} independent, but membrane potential dependent, mechanism of secretion in chromaffin cells that would be relevant at low frequencies.

14 BIOGENESIS OF LARGE DENSE CORE VESICLES IN ADRENAL CHROMAFFIN CELLS OF NEWBORN MICE

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Chromaffin cells play an important role in stress induced responses by secreting catecholamines and peptides in a Ca^{2+}- dependent manner. The hormones are stored in large dense core vesicles (LDCVs) and, although chromaffin cells became a model system to study fast Ca^{2+}-dependent exocytosis, much less is known about LDCVs biogenesis and recycling. It has been shown that LDCVs are generated at the level of the trans-Golgi network (TGN) under the control of chromogranins. However, the sorting of associated and integral membrane components to LDCVs is not well understood.

In order to follow the biogenesis of LDCVs in chromaffin cells, we transfected the chromaffin cells with NPY-mCherry that is specifically localized to the LDCVs and fixed the cells with increasing delay. To determine its subcellular localization we co-stained the cells with various markers. Further, we investigated the association of LDCV's membrane components (vSNAREs and Synaptotagmin1) with LDCVs and studied their endocytic and recycling pathway.

We found that LDCVs appear to be retained at the Golgi network for about 1 hour before moving to the reserve pool of vesicles indicating a maturation step that might involve sorting of different vesicular membrane proteins to the LDCVs . This hypothesis was tested by colocalization studies with Synaptobrevin 2 and Cellubrevin immunolabelling in NPY-mCherry expressing cells. Surprisingly, both vSNAREs seems to get associated with mature LDCVs at late stage in the biogenesis indicating that vesicular proteins are transferred to mature LDCVs via the fusion of precursor vesicles. The question then was whether the LDCV's membrane components that are associated with new LDCVs are newly synthesized or recycled proteins. To address this question we are studying the endocytosis and recycling of Synaptotagmin 1.

15 SMALL MOLECULES DEMONSTRATE THE ROLE OF DYNAMIN AS A BI-DIRECTIONAL REGULATOR OF THE EXOCYTOSIS FUSION PORE AND VESICLE RELEASE.

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Hormones and neurotransmitters are stored in specialised vesicles and released from excitable cells through exocytosis. During vesicle fusion with the plasma membrane, a transient fusion pore is created that enables transmitter release. The protein dynamin is known to regulate fusion pore expansion (FPE). The mechanism is unknown, but requires its oligomerisation-stimulated GTPase activity. We used a palette of small molecule dynamin modulators to reveal bi-directional regulation of FPE by dynamin and vesicle release in chromaffin cells. The dynamin inhibitors Dynole 34-2 and Dyngo 4a and the dynamin activator Ryngo 1-23 reduced or increased catecholamine released from single vesicles, respectively. Total internal reflection fluorescence (TIRF) microscopy demonstrated that dynamin stimulation with Ryngo 1-23 reduced the number of neuropeptide Y (NPY) kiss-and-run events, but not full fusion events, and slowed full fusion release kinetics. Amperometric stand-alone foot signals, representing transient kiss-and-run events, were less frequent but were of longer duration, similarly to full amperometric spikes and pre-spike foot signals. These effects are not due to alterations in vesicle size. Ryngo 1-23 action was blocked by inhibitors of actin polymerisation or myosin II. Therefore, we demonstrate using a novel pharmacological approach that dynamin not only controls FPE during exocytosis, but is a bi-directional modulator of the fusion pore that increases or decreases the amount released from a vesicle during exocytosis if it is activated or inhibited, respectively. As such, dynamin has the ability to exquisitely fine-tune transmitter release

16 ANTIDIABETIC EFFECTS OF FLAVONOIDS FROM SOPHORA FLAVESCENS ETOAC EXTRACT IN TYPE 2 DIABETIC KK-AY MICE

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Ethnopharmacological relevance: Bitter and cold Chinese medicines have been long used for the treatment for diabetes mellitus (DM) for thousands of years in China. The roots of Sophora flavescens Ait., one of bitter and cold Chinese medicines commonly used to remove lung heat have been used to counteract DM and exerted good clinical effects for diabetic patients in some folk hospitals in Fujian province, P.R. China. However, the corresponding active principles and antidiabetic mechanism of this Traditional Chinese Medicine remain unclear. Therefore, in this study, we aim at chemical profiling of the active principles, validating the potential antidiabetic effects of the active EtOAc extract (SF-EtOAc) in vitro and in vivo, and elucidating its probable antidiabetic mechanism as well as evaluating its acute oral toxicity.

Materials and methods: An off-line semipreparative LC-NMR and LC-UV-ESIMS protocol was developed to determine the chemical principles of the active EtOAc extract rapidly and unambiguously. The effect of SF-EtOAc on the GLUT4 translocation in L6 myotubes was examined. T2DM KKAy mice were induced by high fat diet. SF-EtOAc was orally administration at the dose of 30, 60 and 120 mg/kg/d, for 21 days. Metformin was used as positive control. Body weight, plasma glucose, oral glucose tolerance test, serum insulin and blood-lipid indexes were measured. Phosphorylation of the AMP-activated protein kinase (AMPK) expression in liver was measured.

Results: We found that SF-EtOAc significantly improved oral glucose tolerance, increased serum HDL-C and reduced body weight, blood glucose and other related blood-lipid indexes. Mechanistically, SF-EtOAc elevated phosphorylation of AMP-activated protein kinase (AMPK) and stimulated membrane translocation of glucose transporter type 4 (GLUT4). Moreover, it was unveiled that oral median lethal dose (LD50) of SF-EtOAc was more than 7500 mg/kg, suggesting that SF-EtOAc was practically non-toxic for mice.

Conclusions: SF-EtOAc improves glucose tolerance, reduces hyperglycemia and resumes insulin levels, at least in part, by activating GLUT4 translocation which may be modulated by AMPK pathway. According to the results of the present study, SF-EtOAc possesses a potent antidiabetic activity and could be used as a safe remedy for the treatment of diabetes.

17 IMAGING MASS SPECTROMETRY REVEALS THE CHEMISTRY IN CHEMICALLY FIXED ADRENAL CELLS PREPARED FOR TRANSMISSION ELECTRON MICROSCOPY ANALYSIS

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Cell biology has been substantially studied with electron microscopy and transmission electron microscopy (TEM) is playing a key role in furthering our understanding of cell architecture and relating it to cellular function. However, due to the ultrahigh vacuum environment in the TEM instrument, biological materials have to be fixed. The most commonly applied sample preparation has been chemical fixation involving several complex processes. Understanding these chemical processes and the advantages and disadvantages of complicated fixation procedures can greatly improve the reliability of the imaging results

In this work, we have employed time-of-flight secondary ion mass spectrometry (ToF-SIMS) to reveal the chemistry in chemically fixed bovine adrenal cells prepared for TEM. ToF-SIMS has the ability to analyze most of the elements from the periodic table in parallel with great sensitivity and high mass and lateral resolution. This study aims to correlate the cellular information obtained from well-established electron microscopy procedures and chemical information extracted with ToF-SIMS and to provide insights into the selectivity properties of specific fixatives and stains towards biomolecules present in adrenal cells. Here, we performed chemical imaging with TOF.SIMS V instrument equipped with a bismuth liquid metal ion gun. Fixed adrenal cell sections with a thickness of 500 nm were analyzed in negative ion mode by probing the sample with Bi_{3+} primary ion beam and high spatial resolution chemical maps were obtained. The characteristic ion fragments associated with employed stains, fixation agents, and biomolecules have been detected and their localization in several cell compartments has been observed. Interestingly, there is a possibility for ToF-SIMS to allow differentiation between chromaffin and cortex adrenal cells based on different chemistry present in adrenal cells. In the future, these findings can lead to improvement of fixation procedures for specific scientific questions not only in TEM imaging, but in imaging techniques in general. Thus, we suggest the application of ToF-SIMS in advancing high-resolution imaging from the perspective of better sample preservation and simpler imaging approaches as well.

18 DID NOT ATTEND

19 DID NOT ATTEND

20 TRANSFERRIN COUPLED LIPOSOMES FOR BRAIN TARGETING OF 5-FLOROURACIL

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The drug delivery to the brain has been particularly challenging because of the presence of blood brain barrier (BBB). Therefore, active targeting to the brain is crucial for the effective treatment of brain disease.

The objective of this study is to achieve enhanced delivery of 5-fluorouracil (5-FU) to brain through transferrin coupled liposomes via receptor mediated endocytosis.

5-FU was radiolabelled with 99mTc-DTPA. Liposomes were prepared by cast film method and the surface was coupled with transferrin in presence of EDC as a chemical crosslinker and characterized for particle size, shape, entrapment efficiency & in-vitro drug release. In-vitro cytotoxicity assay was performed with various CNS cell lines. In-vivo percent brain uptake of 99mTc-DTPA labelled 5-FU was determined.

The optimized ratio exhibited a particle size of 194-214nm with maximum entrapment efficiency of 37.60 & 33.06% for uncoupled and coupled liposomes. The in-vitro drug release studies shows 74.8% drug release in 24h from uncoupled liposomes which was decreased to 66.7% on coupling of liposomes with transferrin. In-vitro cytotoxicity studies show 80% reduction with IMR-32 & SK-NS-H cell lines. Biodistribution studies show the enhanced delivery of drug to brain.

The brain uptake of transferrin-coupled liposomes was found to be approximately 17 and 10 times higher as compared to plain drug and uncoupled liposomal formulations respectively. Therefore, the transferrin coupled liposomes as a drug delivery transport vector can be used for the transport of drug molecules across the BBB. Such systems would be useful in the treatment brain tumor and neurological diseases.

21 CELLULAR AND MOLECULAR MECHANISMS OF HYPERSECRETION IN PHEOCHROMOCYTOMAS

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Neuroendocrine tumors (NETs) are neoplasms arising from hormone/peptide-secreting cells. Although NETs are heterogeneous, a common critical feature is the dysfunction of the secretory activity leading to hypersecretion. Pheochromocytomas are NETs that arise from chromaffin cells of the adrenal medulla, which are characterized by an excess of catecholamine secretion, leading to hypertension, cardiomyopathy and high risk of stroke. Although this aspect is well known by clinicians, it has never been explored at the cellular and mole¬cular level. Here, we have analyzed the aberrant secretion of catecholamine at a single cell level by applying carbon fiber amperometry technique on human pheochromocytoma resection. We have observed a drastic increase of exocyto tic events in tumoral cells comparing to non tumoral chromaffin cells from the same patient. These data demonstrate that hypersecretion is a direct consequence of a deregulation of the secretagogue induced secretion and not simply a mass effect due to the proliferation of tumoral cells. According to their widely accepted involvement in tumorigenesis and their important function in neuroendocrine secretion, Rho GTPases and their regulator pathways appear as good candidates to be involved in secretion defect and/or development of pheochromocytoma. Our results show a decrease of the GTPases Rac1 and Cdc42 activities in human pheochromocytoma compared to non-tumoral tissue. Moreover, by investigating protein expression changes in tumor through a mass spectrometry approach, we have demonstrated that ARHGEF1 and FARP1, two guanine nucleotide exchange factors that activate Rho GTPases are down-regulated. We then confirmed by in vitro experiment in PC12 cells that down-regulation of ARHGEF1 and FARP1 triggers the inactivation of Rac1 and Cdc42, respectively.
Altogether, our results demonstrate a deregulation of the secretory activity at a cellular level, and an alteration of the Rho GTPase pathways in pheochromocytoma.

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22 CHARACTERIZING ENTEROCHROMAFFIN CELL NUTRIENT SENSING CAPABILITIES IN MOUSE COLON AND DUODENUM

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INTRODUCTION: Enterochromaffin (EC) cells are a specialised type of enteroendocrine cell that are located in the gastrointestinal (GI) mucosa. The major function of this cell type is to synthesise and secrete almost all (90-95%) of total body serotonin (5-hydroxytryptamine, 5-HT), via the rate-limiting enzyme TPH1. The release of 5-HT by EC cells has a multitude of functions, including but not limited to platelet adhesion, liver regeneration, osteoregulation and modulation of both gastrointestinal motility and glucose homeostasis. Ingestion of dietary nutrients, such as sugars and free fatty acids, has been associated with increased 5-HT release. Despite this knowledge, the mechanisms by which EC cells sense their nutrient environment remain unknown. The aim of this study was to investigate the nutrient sensing profile of EC cells in the colon and duodenum, and determine if sugars cause 5-HT release.

METHOD: EC cells were isolated from sections of mouse colon and duodenum, using a Percoll® density gradient (Raghupathi et al., 2013). Pure EC cell cultures were used for RNA extraction and real-time PCR. Cells were also stimulated with glucose, fructose and sucrose, and cell activation measured by observing calcium (Ca^{2+}) flux by flow cytometry, along with 5-HT release measured by ELISA.

RESULTS: For the first time, pure EC cell cultures from mouse colon and duodenum have been obtained. Compared to colonic cells, duodenal EC cells (n = 3-7) have higher expression of the nutrient sensors Ffar3, Glut5, Glut2 as well as Tph1. Glut1 ($p < 0.05$), Ffar2 ($p < 0.05$) and Ffar4 ($p < 0.0001$) are more highly expressed in colonic EC cells than in duodenal, indicating that EC cell nutrient sensing may differ throughout the GI tract. In colonic, but not duodenal EC cells, stimulation with high glucose caused an increase in intracellular Ca^{2+} and 5-HT release. High (300mM) fructose stimulation also increased 5-HT release ($p < 0.05$ vs control) in colonic EC cells, while the osmotic control, 300mM sucrose, had no effect. This adds to data from guinea-pig colon in which we used amperometry to demonstrate glucose-induced 5-HT release in tissue sections and an increase in quantal release (spike area) in single cell studies.

CONCLUSION: This study demonstrates that EC cells can be isolated from different regions of the mouse GI tract, and that nutrient sensing mechanisms within the gut may be location-dependent. EC cells sense and respond to glucose and fructose at concentrations consistent with luminal nutrient ingestion. By understanding the nutrient sensing mechanisms present in EC cells, we gain significant insight into how di ferent nutrients, by augmenting 5-HT release, can affect a variety of physiological functions.

REFERENCE: Rahgupathi et al., 2013, J Physiol, 591(23), 5959-5975.

23 THE EFFECTS OF SINGLE AND RECURRENT INSULIN INDUCED HYPOGLYCAEMIA ON TYROSINE HYDROXYLASE PHOSPHORYLATION IN THE RAT BRAIN AND ADRENAL GLAND

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Recurrent hypoglycaemia is an unavoidable side effect of intensive insulin therapy leading to diminished plasma adrenaline defence response to subsequent hypoglycaemic episodes. Our study investigated the effects of single and recurrent insulin induced hypoglycaemia on tyrosine hydroxylase (TH) phosphorylation and protein (as a measure of TH activation and catecholamine biosynthesis) in the adrenal gland and certain catecholaminergic brain regions. Male SD rats either received daily saline (control group) or insulin i.p. injections (10U/kg) (recurrent hypoglycaemia group) on 3 consecutive days; or saline i.p. injections on days 1 and 2 and insulin i.p. injection (10U/kg) on day 3 (single hypoglycaemia group) (n=5-8 per group). Rats were euthanised at 60 min after the last injection on day 3. Plasma adrenaline (measured by ELISA) was significantly increased in response to single hypoglycaemia; this response was diminished by 41% ($p<0.01$) in response to recurrent hypoglycaemia. TH phosphorylation at Ser40, Ser31, Ser19 and TH protein was analysed by western blotting. pSer31TH was significantly increased in the adrenal gland (4-5 fold; $p<0.0001$), C1 neurons (1.5-2 fold; $p<0.01$), substantia nigra (2 fold; $p<0.01$), ventral tegmental area (VTA, 2 fold; $p<0.01$) and nucleus accumbens (NAc, 1.3 fold; $p<0.05$) in response to single hypoglycaemia relative to controls; these responses were diminished in response to recurrent hypoglycaemia only in VTA and NAc. Total TH was significantly increased in response to recurrent hypoglycaemia compared to controls only in the adrenal gland (1.7 fold; $p<0.01$). pSer40TH was not significa tly altered in response to either single or recurrent hypoglycaemia in any tissue. The increased pSer31TH in the adrenal gland with no changes in pSer40TH suggests that pSer31TH alone can increase TH activity. The increased pSer31TH in the brain suggests that catecholamines might have been released in response to insulin induced hypoglycaemia. The main effect of recurrent hypoglycaemia to reduce adrenaline secretion might occur at the level of adrenal gland affecting its ability to release catecholamines.

24 PKA, AMPK, PGC-1ALPHA AND NRF-1 COUPLE CAMP-DEPENDENT ANTIOXIDANT RESPONSE AND MITOCHONDRIOGENESIS DURING NEUROENDOCRINE DIFFERENTIATION

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The mechanisms connecting tolerance to oxidative stress and energy supply to promote neuritogenesis and survival in neuroendocrine cells are largely unknown. We showed here that PACAP- and cAMP-induced PC12 cell differentiation is accompanied by the expression of a novel selenoprotein, named selenoprotein T (SelT), which exerts an essential role in neuritogenesis and tolerance against oxidative stress. By combining SelT promoter studies, RNA interference and ChIP analysis, we demonstrated that nuclear respiratory factor 1 (NRF-1), a key transcription factor regulating mitochondrial biogenesis, is crucial for SelT gene regulation by the cAMP/PKA pathway in PC12 cells. In addition, we showed that peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α), a potent NRF-1 coactivator involved in the transcriptional integration of mitochondriogenesis, is also required for cAMP/PKA-induced SelT gene transcription. Combined pharmacology, immunoblotting and knockdown experiments revealed that two critical kinases, LKB1 and AMP protein kinase (AMPK) operate downstream of PKA in order to promote PGC-1α expression. Concurrently, using mitochondrial fluorescent tracking and determination of the ratio of mitochondrial to nuclear DNA, we found that PACAP and cAMP induced mitochondriogenesis in differentiating PC12 cells through a PKA/AMPK-dependent pathway. Taken together, these results show that a PKA/LKB1/AMPK/PGC-1/NRF-1 pathway might couple tolerance to oxidative stress and mitochondrial biogenesis in response to cAMP elevation during neuroendocrine differentiation.

25 TIGHT MITOCHONDRIAL CONTROL OF CALCIUM AND EXOCYTOTIC SIGNALS IN CHROMAFFIN CELLS AT EMBRYONIC LIFE

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Calcium buffering by mitochondria plays a relevant physiological function in the regulation of Ca^{2+} and exocytotic signals in mature chromaffin cells (CCs) from various adult mammals. Whether a similar or different role of mitochondrial Ca2+ buffering is present in immature CCs at early life, has not been explored. Here we present a comparative study in rat embryonic CCs and rat mother CCs, of various physiological parameters that are known to be affected by mitochondrial Ca^{2+} buffering during cell activation. We found that the clearance of cytosolic Ca^{2+} transients ($[Ca^{2+}]_c$) elicited by high K^+ was 7-fold faster in embryo CCs with respect mother CCs. This strongly suggests that at embryonic life, mitochondria play a more significant role in the clearance of $[Ca^{2+}]_c$ loads, with respect adult life. Consistent with this view are the following results concerning the transient suppression of mitochondrial Ca^{2+} buffering by protonophore FCCP, in embryonic CCs with respect mother CCs: (i) faster and greater inactivation of inward calcium currents; (ii) higher K^+-elicited $[Ca^{2+}]_c$ transients with 25-fold faster clearance; (iii) higher increase of basal catecholamine release; (iv) higher potentiation of K^+-evoked secretion. These pronounced differences could be explained by two additional features (embryo versus mother CCs): a) slower recovery of mitochondrial resting membrane potential after the application of a transient FCCP pulse; and b) greater relative density of mitochondria in the cytosol. This tighter control by mitochondria of Ca^{2+} and exocytotic signals may find an explanation in the physiological and pathophysiological context of survival at early life: at this stage CCs behave as oxygen sensors ant their mitochondria are highly sensitive to the frequent hypoxic stress frequently occurring at embryonic life, at delivery, and at neonatal life. Under these conditions, mitochondria will lose their potent Ca^{2+} buffering capacity to secure a healthy life-saving and Ca^{2+}-dependent catecholamine surge. Understanding of regulation by mitochondria of the exocytotic release of catecholamine may provide pathogenic and therapy clues for the prevention and treatment of the infant sudden death syndrome.

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26 DEPRESSED EXCITABILITY AND ION CURRENTS LINKED TO SLOW EXOCYTOTIC FUSION PORE IN CHROMAFFIN CELLS OF THE SOD1G93A MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Altered synaptic transmission with excess glutamate release has been implicated in the loss of motoneurons occurring in amyotrophic lateral sclerosis (ALS). Hyperexcitability or hypoexcitability of motoneurons from mice carrying the ALS mutation SOD1G93A (mSOD1) has also been reported. Here we have investigated the excitability, the ion currents, and the kinetics of the exocytotic fusion pore in chromaffin cells from postnatal day 90 to postnatal day 130 mSOD1 mice, when motor deficits are already established. With respect to wild-type (WT), mSOD1 chromaffin cells had a decrease in the following parameters: 95% in spontaneous action potentials, 70% in nicotinic current for acetylcholine (ACh), 35% in Na+ current, 40% in Ca2+-dependent K^+ current, and 53% in voltage-dependent K^+ current. Ca^{2+} current was increased by 37%, but the ACh-evoked elevation of cytosolic Ca^{2+} was unchanged. Single exocytotic spike events triggered by ACh had the following differences (mSOD1 vs. WT): 36% lower rise rate, 60% higher decay time, 51% higher half-width, 13% lower amplitude, and 61% higher quantal size. The expression of the α3-subtype of nicotinic receptors and proteins of the exocytotic machinery was unchanged in the brain and adrenal medulla of mSOD1, with respect to WT mice. A slower fusion pore opening, expansion, and closure are characteristics likely linked to the pronounced reduction in cell excitability and in the ion currents driving action potentials in mSOD1, compared with WT chromaffin cells. These drastic changes in cell excitability, ion currents, and exocytotic fusion pore suggest that ALS is a systemic disease that at later evolution stages affects excitable neurosecretory cells other than motoneurons.

Keywords: amyotrophic lateral sclerosis; fusion pore; chromaffin cells; exocytosis; ion channel currents; cell excitability

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27 TWO MODELS FOR IN VIVO STUDY OF EXOCYTOSIS BY INTRACELLULAR INTRAVITAL MICROSCOPY

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We established an intravital microscopy-based system to study actin cytoskeleton dynamics in secretory granules during regulated exocytosis in salivary glands of living mice. Using transgenic mouse models that express selected fluorescently labeled molecules, we discovered several key aspects of exocytosis that were not seen in ex vivo models. Here we present data suggesting that tropomyosins (Tpms), which form co-polymers along the length of actin filaments, modulate the actomyosin scaffold post-fusion, and thus control the completion phase exocytosis. We found that upon beta-2 adrenergic agonist stimulation were recruited the Tpm4.2 and Tpm3.1 onto the fused secretory granules together with F-actin, but with different kinetics to that of bulk filaments. Genetic ablation of Tpm3.1 or exposure to an anti-Tm compound altered the kinetics of granule exocytosis but did not prevent the completion of granule exocytosis. More recently, we developed an intracellular intravital microscopy approach to quantitate GLUT4 trafficking events in vivo to investigate the role of the actin cytoskeleton in GLUT4 exocytosis in skeletal muscle of living mice. We employed novel dual colour GLUT4 probe comprised of pH-sensitive pHluorin inserted into the first exofacial loop of GLUT4 and tdTomato at the C-terminus. This allows the simultaneous tracking of GLUT4 fusion events (bursts of green pHluorin signal) and GLUT4 vesicle movement (red tdTomato signal). We electroporate mouse skeletal muscle with rGLUTpHluor and detected for the first time GLUT4 vesicle fusion events in live anaesthetised animals. We recorded GLUT4 fusion events both at the T-tubules and sarcolemma that increased in frequency after insulin stimulation. To our knowledge this is the first report to capture sub-diffraction-sized vesicle fusion events in a living mammal and may serve as a powerful approach to study exocytosis in mouse models.

28 DIFFUSIONAL SPREAD AND CONFINEMENT OF NEWLY EXOCYTOSED SYNAPTIC VESICLE PROTEINS

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Neurotransmission relies on the calcium-triggered exocytic fusion of non-peptide neurotransmitter-containing small synaptic vesicles (SVs) with the presynaptic membrane at active zones (AZs) followed by compensatory endocytic retrieval of SV membranes. While the mechanisms of SV exocytosis and endocytosis have been studied in some detail little is known about the surface mobility and diffusional behavior of newly exocytosed SV proteins within the presynaptic terminal. Here, we studied the diffusional fate of newly exocytosed SV proteins in hippocampal neurons by high-resolution time-lapse imaging. Newly exocytosed SV proteins rapidly dispersed within the first seconds post-fusion until confined within the presynaptic bouton. Rapid diffusional spread and confinement was followed by slow reclustering of SV proteins at the periactive endocytic zone. Confinement within the presynaptic bouton was mediated in part by SV protein association with the clathrin-based endocytic machinery to limit diffusional spread of newly exocytosed SV proteins. These data suggest that diffusion and axonal escape of newly exocytosed vesicle proteins are counteracted by the clathrin-based endocytic machinery together with a presynaptic diffusion barrier.

29 MEMBRANE LIPID REORGANIZATION AND RHO GTPASES SIGNALING REGULATE EXO-ENDOCYTOSIS COUPLING OF LARGE DENSE CORE VESICLES IN CHROMAFFIN CELLS

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In chromaffin cells, release of catecholamines and hormones/neuropeptides occurs through calcium-regulated exocytosis of large-dense core vesicles. To allow secretory vesicle recycling and maintain membrane homeostasis, exocytosis is followed by compensatory membrane uptake. How chromaffin cells coordinate exocytosis with compensatory endocytosis remains poorly understood. Here, we focused on Rho-GTPases signaling and lipid remodeling, two key processes of large dense core granules trafficking in neuroendocrine cells.
Oligophrenin-1 (OPHN1), a Rho-GTPase activating protein (Rho-GAP) containing a Bin-Amphiphysin-Rvs (BAR) domain and involved in X-linked mental retardation, has been shown to control synaptic vesicle endocytosis. Using carbon fiber amperometry, we found that exocytosis is impaired at the stage of membrane fusion and that compensatory endocytosis is severely inhibited in chromaffin cells isolated from Ophn1 knockout mice. Experiments performed with ectopically expressed OPHN1 mutants indicate that OPHN1 requires its Rho-GAP domain and RhoA inactivation to control fusion pore dynamics whereas the BAR domain implicates OPHN1 in granule membrane recapture after exocytosis.
Reagarding lipid remodeling, we have shown that secretory granule exocytosis is accompanied by the redistribution of phosphatidylserine (PS) leading to the disruption of plasma membrane asymmetry. We demonstrated that PS translocation occurred at the vicinity of the secretory granule fusion sites and was dependent on Phospholipid Scramblase-1 (PLSCR-1). Remarkably, secretory granule membrane recapture after exocytosis was impaired in mouse chromaffin cells knocked out for PLSCR1 demonstrating that PLSCR1-dependent lipid rearrangement is critical for compensatory endocytosis.
Altogether, these data demonstrate for the first time that OPHN1 and PLSCR1 are bi-functional protein able to couple, through distinct mechanisms, exocytosis with compensatory endocytosis in adrenal chromaffin cells

30 IMAGING VESICLE EXO- AND ENDOCYTOSIS IN CHROMAFFIN CELLS

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Exo- and endocytosis are fundamental cellular processes. When a vesicle fuses with the plasma membrane, an Ω-shaped membrane profile is formed, which either closes its pore rapidly to limit vesicular content release and membrane protein delivery (kiss-and-run), or merges with the plasma membrane (Ω-profile merging) to promote complete release and membrane protein delivery. The merge is followed by classical endocytosis involving membrane invagination and fission. Although these exo- and endocytosis processes are generally accepted, they have not been visualized in live cells. Here we attempted to image these processes in live neuroendocrine chromaffin cells. With confocal and STED imaging techniques, we found that the fusion-generated Ω-profile merges with the plasma membrane via shrinking the Ω-profile instead of the generally assumed fusion pore dilation (full-collapse fusion). We are now studying the molecular mechanisms mediating the shrinking of the Ω-profile. We also found that kiss-and-run is rather different from the traditional definition. Instead of rapidly closing the fusion pore to form a vesicle the same as the exocytosed one, the fusion-generated Ω-profile can enlarge or shrink to some extent, then close its pope at various times after stimulation, resulting in the generation of different sizes of vesicles. Calcium influx triggers a dynamin-dependent fusion pore closure, which is the main driving force for mediating whole-cell endocytosis. These results suggest modification of the definition, roles, and the underlying mechanisms of full-collapse fusion and kiss-and-ru

31 DYNAMIN RINGS, REGULATION AND ALLOSTERY: SENSING THE OLIGOMERISATION SIGNAL

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The classical dynamins, dynI, II and III, are characterised by their ability to oligomerise to large assemblies with elevated GTPase activity. They mediate clathrin-mediated endocytosis (CME) and have CME-independent roles in fusion pore expansion and cytokinesis. They share a common G domain, bundle signal element (BSE) and middle domain (MiD). The maximum GTPase activity is stimulated through a concerted structural rearrangement that brings pairs of G domains face-to-face. They have two signalling input domains: the proline rich domain (PRD) binds different SH3 domain proteins at different sites and stimulates ring oligomerisation and mid-level activity; while their pleckstrin homology domains (PHDs) detect specific vesicle neck lipids to stimulate helical assembly and high-activity for fission. Oligomerisation is thought to account for activity stimulation, yet it is unclear how the enzyme distinguishes multiple oligomerisation signals.
We report three studies showing how dynamin is regulated by differential signal inputs via the PRD or the PHD to produce structurally distinct oligomerised assembly states, via the MiD. Within the 4-helix bundle of the MiD dynI and II have an alternatively spliced α2 helix, producing a or b versions of each. We show that this helix mediates assembly of a variety of dynamin oligomers with different activity states. These studies reveal the existence of multiple dynamin oligomerisation conformations within rings or helices. We also report on a specific allosteric signalling pathway within dynamin, which suggests a mechanism whereby the energy from GTP hydrolysis at the G domain may be transmitted through the MiD to mediate membrane fission by the PH domain

32 RAPID RECOVERY OF EXOCYTOSIS AFTER ACTION POTENTIAL-LIKE STIMULATION IN MOUSE CHROMAFFIN CELLS IS COUPLED TO A FAST ENDOCYTOTIC PROCESS

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The immediately releasable pool (IRP) is a group of vesicles that is selectively released by short length stimuli, and consequently might be responsible of chromaffin cell secretion at basal action potential frequency. However, the process of replenishment after IRP depression is too slow (t=7.5±1.1 s) to allow a sustainable exocytosis even at low frequencies. Using membrane capacitance measurements on mouse chromaffin cells, we analyzed the process of exocytosis recovery after the application of an action potential like stimulus (APIs). The exocytosis triggered by APIs (ETAP) represents a fraction of IRP (11±2 fF), and recovered with a time constant of 0.73±0.11 s, what is fast enough to maintain synchronous exocytosis at 0.2-0.5 Hz stimulation. We investigated the possible mechanisms involved in rapid ETAP recovery. First, we found that the depletion of the ready releasable pool (RRP) significantly delayed the recovery of ETAP. Since we regularly observed a fast endocytosis (t=0.71±0.21 s) after ETAP, we also studied the possibility that this process might be involved in ETAP recovery. When we inhibited fast endocytosis with dynasore, nitrendipine, or using an anti-dynamin monoclonal antibody, ETAP recovery was delayed respect to the control condition. The application of the same antibody also provoked the progressive inhibition of synchronous exocytosis during low frequency APIs stimulation. Therefore, we conclude that vesicle mobilization from RRP and fast endocytosis are both involved in rapid ETAP recovery.

33 HOW THE EXOCYTOTIC FUSION PORE OPENS AND CLOSES?

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Regulated exocytosis is a multistage process involving a merger between the vesicle and the plasma membranes, leading to the formation of a fusion pore, a channel, through which secretions are released from the vesicle to the cell exterior. A stimulus may influence the pore by either dilating it completely (full-fusion exocytosis) or mediating a reversible closure (transient exocytosis). In neurons, these transitions are short-lived and not accessible for experimentation. However, in some neuroendocrine cells and astrocytes, initial fusion pores may reopen several hundred times, indicating their stability. Moreover, these pores are too narrow to pass luminal molecules to the extracellular space, but their diameter can dilate upon stimulation. To explain the stability of the initial narrow fusion pores, anisotropic membrane constituents with non-axisymmetrical shape were proposed to accumulate in the fusion pore membrane. Although the nature of these is unclear, they may consist of lipids and proteins, including SNAREs, which may facilitate and regulate the pre- and post-fusional stages of exocytosis.

34 MECHANISMS CONTROLLING INSULIN GRANULE EXOCYTOSIS IN HEALTH AND DISEASE

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The environment of the islets of Langerhans strongly influences the control of insulin secretion. Compared to single beta cells, islets secrete less insulin at low glucose concentrations and much more at higher glucose stimuli[1]. To understand the control of insulin secretion in the islets we have developed methods that record the secretory activity of individual beta cells within intact islets.

Our work has quantified the glucose dose dependence in the islet as both recruitment of cells and enhanced responses within the cell[2]. In the db/db model of type 2 diabetes we have shown, using similar methods, that loss of responding cells is the major contributor to the reduced secretion[3]. Using 3D imaging we have shown that insulin granule exocytosis is targeted towards the islet blood vessels and that this is likely to be regulated by localisation of scaffold proteins to form a synaptic-like structure at the vascular face of the beta cells[4].

We conclude that arrangement of beta cells within the islets has a strong influence on secretory function

References

1. Halban PA, Wollheim CB, Blondel B et al.(1982). Endocrinology 111: 86-94.
2. Low JT, Mitchell JM, Do OH et al.(2013). Diabetologia 56: 2629-2637.
3. Do O, H, Low JT, Gaisano HY, Thorn P.(2014). Diabetologia 57: 1400-1409.
4. Low JT, Zavortink M, Mitchell JM, et al. (2014) Diabetologia 57: 1655-1663

35 ENTEROCHROMAFFIN CELLS DISPLAY DISTINCT RELEASE KINETICS

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Enteroendocrine cells collectively constitute the largest endocrine tissue in the body. These cells are scattered amongst the gastrointestinal (GI) epithelium and make-up about 1% of all cells lining the GI tract. They consist of an array of different cell types, each containing specific hormone markers. It is unsurprising that these endocrine cells serve a multitude of physiological functions but are difficult to study in isolation. We have focused our recent studies on the largest population of these cells, the serotonin (5-HT) containing enterochromaffin (EC) cells. EC cells produce ~95% of the body's 5-HT and this circulating 5-HT is vital for a multitude of bodily functions including enteric motility, bone mass, liver regeneration, fat mass and glucose homeostasis. Despite the importance of EC cells no studies have investigated the physiological function of single primary EC cells. We have developed a method of rapid primary culture of guinea pig, mouse and human EC cells, allowing analysis of single EC cell function using electrophysiology, electrochemistry, Ca^{2+} imaging, immunocytochemistry and 3D modelling. We find that EC cells release 5-HT from single vesicles in response to an array of stimuli, that secretion involves Ca^{2+} entry via plasma membrane Ca^{2+} channels and that EC cells release 60-100 times less 5-HT per fusion event compared to chromaffin cells. We will further describe some of the cellular mechanisms that regulate EC cell 5ht exocytosis.

36 THE OTHER SYNAPSE: CALCIUM-DEPENDENT EXOCYTOSIS IN THE IMMUNE SYSTEM

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Ca^{2+}-dependent exocytosis of signalling substances is one of the most important tasks of any cell in our body. The most heavily studied exocytic event takes place at synapses between neurons where neurotransmitters are released from synaptic vesicles. However, the molecular mechanism of neurotransmitter release is difficult to study due to technical reasons like synapse size and speed.
Cytotoxic T lymphocytes (CTLs) are part of the adaptive immune system and kill target cells by formation of an immunological synapse (IS) followed by the directed release of toxic substances from lytic granules. Interestingly, a number of proteins like Munc13, Munc18 or syntaxin, which have been shown to be involved in neurotransmitter release, are instrumental for lytic granule release as well.
We have investigated the molecular mechanism of IS formation and function in primary CTLs from mouse and human. Knockout/knockdown approaches have been combined with high-resolution fluorescence microscopy, electron microscopy and functional assays to elucidate the contribution of several key proteins. In addition, molecular states preceding LG fusion could be resolved by total internal reflection fluorescence microscopy (TIRFM) in combination with whole-cell patch-clamp recordings. I will present the latest findings from our lab which identify parts of the molecular machinery that is required for sequential fusion events occuring at the IS.

37 CALPAINS CLEAVE DYSFERLIN TO RELEASE A SYNAPTOTAGMIN-LIKE MODULE FOR THE CALCIUM-DEPENDENT EXOCYTOSIS OF MEMBRANE REPAIR

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The ability to repair a plasma membrane injury is an intrinsic property of almost all eukaryotic cells. Membrane repair is calcium-dependent, and thought to involve the aggregation and fusion of vesicles at the site of injury, forming a "patch" to seal ruptures in the cell membrane, with mechanistic parallels with synaptic exocytosis.
Dysferlin belongs to an ancient family of vesicle fusion proteins, with the unique feature of seven tandem C2 domains, calcium-regulated lipid binding domains. Mutations within the Dysf gene cause a form of inherited muscular dystrophy, and dysferlin-deficient muscle fibres are unable to reseal an acute plasma membrane injury. Thus it is proposed that dysferlin mediates the vesicle fusion of membrane repair.
We show that with membrane injury, activated calpains cleave dysferlin to release a C-terminal module we termed mini-dysferlinC72. Our results suggest it is the cleaved mini-dysferlinC72 that is specifically recruited and undergoes calcium-dependent vesicle fusion at injury sites. Calpain cleavage of dysferlin is mediated by the ubiquitous calpains, via a cleavage motif encoded by an alternately spliced exon, exon 40a.
Importantly, we reveal other members of the ferlin family are also cleaved enzymatically to release similar C-terminal modules, bearing two C2 domains and transmembrane domain, with structural similarity to the classical mediators of synaptic vesicle fusion, the synaptotagmins. Our results suggest that calpain-cleavage of ferlins presents an ancestral means to release synaptotagmin-like effector modules for vesicle fusion where and when calcium signaling demands, and for dysferlin, this includes the calcium-activated vesicle fusion of membrane repair.

38 CARDIAC INTERACTION AND SIGNALLING BETWEEN FULL LENGTH CHROMOGRANIN A AND TUMOR NECROSIS FACTOR ALPHA

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Elevated levels of Chromogranin A (CgA) are present in the blood of patients with chronic heart failure and represent an independent prognostic indicator of mortality. Increased CgA blood levels prevent vascular leakage induced by Tumor Necrosis Factor-alpha (TNF), a major cytokine directly implicated in the heart failure humoral/neuroendocrine scenario. However, its role remains to be elucidated. In this context, we hypothesize that the sympatho-chromaffin CgA may contribute to link the neuroendocrine system to TNF-alpha-mediated cardiac modulation. Hence, following the evidence of the CgA-induced cardiac modulation previously demonstrated by us, we investigated the putative interactions between TNF alpha/TNF alpha Receptors-(Rs) system activation and the CgA- induced effects on cardiac performance. To this aim, we administered mouse TNF-alpha and mouse TNF-alpha plus human CgA on isolated and perfused Langendorff rat hearts. Results indicated that TNF-alpha increased myocardial contractility (positive inotropism) at 1 pM and 25 pM, as demonstrated by a significant increase of systolic left ventricular pressure (LVP), accompanied by increased coronary pressure (CP), signifi ant at 25 pM. Decreased contractility (negative inotropism) was observed from 1 nM to 50 nM together with a coronary vasodilation significant at 4 and 20 nM. These effects mimic those of CgA administrated alone at similar doses on the isolated heart. Co-administration experiments showed that the negative inotropic effect and the coronary dilation observed in the presence of CgA alone were not modified by 4 pM TNF-alpha which, on the other hand, at 25 pM abolished the CgA-elicited negative inotropism without modifying CP. On the contrary, the positive inotropic effect and the increased CP induced by TNF-alpha alone were abolished by the contemporary perfusion with CgA. In addition, dose-response curves performed with anti-TNFR1 (from 1 pM to 50 nM) showed a significant negative inotropic effect at 25 and 50 pM at the highest concentration tested (4-50 nM). While CP was not modified by low concentrations, a significant vasodilation was observed from 1 nM to 20 nM TNF alpha. In conclusion, for the first time, our data provide evidence that these two signal molecules implicated in important homeostatic processes may be able to interact at cardiac level, being thus putative effectors of the adaptive and maladaptive responses of the diseased heart.

39 HUMAN GENETIC VARIANTS OF THE CHROMOGRANIN A-DERIVED DYSGLYCEMIC PEPTIDE PANCREASTATIN: IMPLICATIONS FOR CARDIOMETABOLIC DISORDERS

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Chromogranin A (CHGA), ubiquitously expressed in secretory cells of the endocrine/neuroendocrine system, has recently emerged as an important regulator in cardiovascular disease states. CHGA acts as a prohormone giving rise to bioactive peptides including pancreastatin [PST=human CHGA250-301]. Studies over the years established the anti-insulin or “dysglycemic” nature of the PST peptide. However, systematic analyses of genetic variants of PST in different human populations have not been carried out. Re-sequencing of the PST region in ~3700 Indian subjects revealed the occurrence of a rare and novel variant Glu287Lys (PST-287K) and two common variants: Gly297Ser (PST-297S) and Arg253Trp (PST-253W). Association analysis of the Gly297Ser variation with cardiovascular/metabolic diseases states displayed significantly higher levels of plasma glucose (fasting/post-prandial/random), insulin resistance, HbA1c, triglycerides, total cholesterol, LDL-cholesterol, diastolic blood pressure and catecholamines in Gly/Ser subjects than wild-type Gly/Gly individuals. PST-287K and PST-297S peptides increased endogenous expression of gluconeogenic genes, inhibited insulin-stimulated glucose uptake more effectively and caused increased catecholamine secretion than PST-WT. Both in silico (molecular modeling) and in vitro (CD spectroscopy) experiments showed that PST-287K/PST-297S contained higher alpha helical contents than PST-WT. The PST Gly297Ser variant (estimated to occur in ~300 million people worldwide) appeared to increase the risk for cardiovascular/metabolic diseases. The higher potency of PST-297S for various relevant cellular processes provides mechanistic basis for this finding in vivo

40 SECRETONEURIN GENE THERAPY

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Secretogranin-II, a protein of neuro-endocrine storage versicles gives rise to the biologically active peptide secretoneurin. Beside effects on the nervous and the inflammatory system secretoneurin also acts on endothelial cells and induces the growth of new blood vessels, a process called angiogenesis. In order to test potential therapeutic effects of secretoneurin in animal models of myocardial and hindlimb ischemia we generated a plasmid secretoneurin gene therapy vector. In the hindlimb ischemia model secretoneurin gene therapy improved blood perfusion and reduced clinical outcome parameters like limb necosis or amputation. Secretoneurin induced angiogenesis in this model but also stimulated vasculogenesis, the generation of blood vessels by bone marrow-derived endothelial cells. We also could show that secretoneurin stimulates nitric oxide production and observed effects in-vivo and on endothelial cells in-vitro were dependent on nitric oxide.

We also used the rat myocardial infarction model and observed positive effects by secretoneurin gene therapy like improvement of left ventricular function (as determined by echocardiography and invasively by catheter measurements) and reduction of fibrosis. Also in this model secretoneurin stimulated angiogenesis and arteriogenesis in the infarct border zone. In this work we could demonstrate, as a novel mode of action, that receptors of important angiogenic factors like vascular endothelial growth factor, insulin-like growth factor-1 or fibroblast growth factor were stimulated by secretoneurin. As shown for vascular endothelial growth factor we could show that secretoneurin stimulates the binding of this growth factor to its low affinity binding sites neuropillin and heparan-sulfate proteoglycans

In summary, we could show that secretoneurin induces angiogenesis and gene therapy with this substance improves outcome in animal models of hindlimb and myocardial ischemia. The robust effect of secretoneurin observed in these studies might be explained by stimulation of different potent angiogenic growth factor receptors.

41 MEASUREMENT AND CYTOSKELETAL REGULATION OF OPEN AND CLOSED EXOCYTOSIS

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Electrochemical cytometry is a new method we have developed to separate nanometer vesicles, lyse them on an electrode surface, and amperometrically detect the active contents of each vesicle in a high throughput manner. We have developed a method of electrochemical cytometry to rapidly measure the total content of single neurotransmitter vesicles and we compare this to the levels released during exocytosis. The vesicles appear to adsorb onto the electrode surface and sequentially spread out over the electrode surface trapping their contents against the electrode. These contents are then oxidized and a peak results for each vesicle that bursts. We have also been able to accomplish this type of cytometry in the cytoplasm of living PC12 and adrenal cells. Comparison of the contents of these biological vesicles to the release of catecholamine from single cells supports the concept that only a fraction of transmitter is released during exocytosis.
Considering open and closed exocytosis, we then examined the cytoskeletal factors that affect this process and find that dynamin is part of the mechanism to push open the pore formed following SNARE activation and that actin is part of the mechanism to regulate pore closing.
The ERC, Swedish VR, and the Knut and Alice Wallenberg Foundation supported this work.

42 AN ACTO-MYOSIN II CONSTRICTING RING INITIATES THE FISSION OF ACTIVITY-DEPENDENT BULK ENDOSOMES IN NEUROSECRETORY CELLS

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Activity-dependent bulk endocytosis allows neurons to internalize large portions of the plasma membrane in response to stimulation. However, whether this critical type of compensatory endocytosis is unique to neurons or also occurs in other excitable cells is currently unknown. Here we used fluorescent 70 kDa dextran to demonstrate that secretagogue-induced bulk endocytosis also occurs in bovine chromaffin cells. The relatively large size of the bulk endosomes found in this model allowed us to investigate how the neck of the budding endosomes constricts to allow efficient recruitment of the fission machinery. Using time-lapse imaging of Lifeact–GFP-transfected chromaffin cells in combination with fluorescent 70 kDa dextran, we detected acto-myosin II rings surrounding dextran-positive budding endosomes. Importantly, these rings were transient and contracted before disappearing, suggesting that they might be involved in restricting the size of the budding endosome neck. Based on the complete recovery of dextran fluorescence after photobleaching, we demonstrated that the actin ring-associated budding endosomes were still connected with the extracellular fluid. In contrast, no such recovery was observed following the constriction and disappearance of the actin rings, suggesting that these structures were pinched-off endosomes. Finally, we showed that the rings were initiated by a circular array of phosphatidylinositol(4,5)bisphosphate microdomains, and that their constriction was sensitive to both myosin II and dynamin inhibition. The acto-myosin II rings therefore play a key role in constricting the neck of budding bulk endosomes before dynamin-dependent fission from the plasma membrane of neurosecretory cells

43 F-ACTIN ORGANIZATION AND THE FATE OF VESICLES AND ORGANELLES.

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The cortical F-actin cytoskeleton of chromaffin cells has an intricate organization formed by multiple polygonal cages leaving narrow spaces that entrap secretory granules in the proximity of active sites. We wonder if the distribution of other organelles such as mitochondria and the ER relates with the cytoskeleton. Using confocal fluorescent microscopy and electron microscopy we have found that indeed there is a cortical population of mitochondria with different morphological properties when compared to the perinuclear one. Instead such distribution was not found for the ER. The distribution of mitochondria might be the consequence of organelle transport experiencing increased restriction in the cell cortex.
Interestingly, the distributions of chromaffin granules and mitochondria are different in cultured cells when compared to that present in cells forming part of the adrenal gland. Moreover, this difference seems to relate with a clear change in the distribution of the F-actin cytoskeleton that appear to be widely distributed throughout the cytoplasm of the chromaffin cells forming part of the adrenomedullary tissue whereas it accumulated in the cortical zone in isolated and cultured cells.

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44 ACTIVITY-DRIVEN RELAXATION OF THE CORTICAL ACTOMYOSIN II NETWORK SYNCHRONIZES MUNC18-1-DEPENDENT NEUROSECRETORY VESICLE DOCKING

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In neurosecretory cells, secretory vesicles (SVs) undergo Ca2þ-dependent fusion with the plasma membrane to release neurotransmitters. How SVs cross the dense mesh of the cortical actin network to reach the plasma membrane remains unclear. Here we reveal that, in bovine chromaffin cells, SVs embedded in the cortical actin network undergo a highly synchronized transition towards the plasma membrane and Munc18-1-dependent docking in response to secretagogues. This movement coincides with a translocation of the cortical actin network in the same direction. Both effects are abolished by the knockdown or the pharmacological inhibition of myosin II, suggesting changes in actomyosin-generated forces across the cell cortex. Indeed, we report a reduction in cortical actin network tension elicited on secretagogue stimulation that is sensitive to myosin II inhibition. We reveal that the cortical actin network acts as a 'casting net' that undergoes activity-dependent relaxation, thereby driving tethered SVs towards the plasma membrane where they undergo Munc18-1- dependent docking.

45 ANNEXIN A2 BUNDLES ACTIN FILAMENTS TO PROMOTE SECRETORY GRANULE DOCKING AND FUSION

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Annexin A2, a calcium-, actin- and lipid-binding protein involved in exocytosis, mediates the formation of lipid microdomains required for the structural and spatial organisation of fusion sites at the plasma membrane. To understand how annexin A2 promotes this membrane remodelling, the involvement of cortical actin filaments in lipid domain organisation was investigated. 3D electron tomography showed that cortical actin bundled by annexin A2 connected docked secretory granules to the plasma membrane and contributed to the formation of GM1-enriched lipid microdomains at the exocytotic sites in chromaffin cells. When an annexin A2 mutant with impaired actin filament-bundling activity was expressed, the formation of plasma membrane lipid microdomains and the number of docked granules were decreased and the fusion kinetics were slower, whereas the pharmacological activation of the intrinsic actin-bundling activity of endogenous annexin A2 had the opposite effects. Thus, annexin A2-induced actin bundling is essential for generating active exocytotic sites. Our results reveal that AnxA2 and the actin cytoskeleton are essential partners to provide lipid platforms for granule docking and fusion, and challenge the classical role depicted for the cortical actin cytoskeleton in calcium-dependent exocytosis.

46 HUNTINGTIN-ASSOCIATED PROTEIN 1 (HAP1) IS A SYNAPSIN-BINDING PROTEIN AND REGULATES VESICLE EXOCYTOSIS AND ENDOCYTOSIS

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Subcellular localisation and protein interaction data indicate that Huntingtin-associated protein 1 (HAP1) may be important in vesicle trafficking and cell signalling. However, no physiological evidence exists to verify this possibility. We measured exocytosis using carbon-fibre amperometry on chromaffin cells cultured from HAP1?/?, and $HAP1^{+/+}$ mice. Levels of exocytosis in $HAP1^{+/+}$ (102.2 ± 10.2 exocytotic events, n=29) cells are significantly greater than in $HAP1^{-/-}$ cells (60.4 ± 7.1, n=35; $p<0.01$). Capacitance measurements indicate the size of the ready releasable pool is smaller in $HAP1^{-/-}$ cells and EM analysis demonstrates this is due to the reduced number of vesicles docked with the plasma membrane. In cortical neurons, FM dye analysis illustrates a defect in vesicle release and endocytosis. Using a proteomics approach, we identified that HAP1 binds Synapsin 1 in neurons and that vesicles containing Synapsin-1 are specifically mislocalised in $HAP1^{-/-}$ neurons. FRAP analysis of neurons transfected with Synapsin-EGFP demonstrate that the trafficking of Synapsin vesicles is impaired in $HAP1^{-/-}$ neurons. This has functional outcomes given that the amount of glutamate released from $HAP1^{-/-}$ cortical brain slices was significantly less (30 ± 5 nM/mg protein, n=4 animals) compared to $HAP1^{+/+}$ (60 ± 6, n=4 animals, $p<0.01$). Our study reports a novel role of HAP1 as a regulator of neurotransmitter release by influencing the rate of exocytosis, the size of the RRP and that it may do this via binding to the major vesicle protein, Synapsin-1.

47 UNCONVENTIONAL RESEARCH DEMANDS FLEXIBLE TOOLS

Anthony Swinscoe and Scott Merrington
Leica Microsystems

Leica Microsystems has introduced a new microscope range which offers flexibility and modularity to complemen research using various imaging techniques. This presentation will outline this system. *Also see Stand*

48 DOES DEPOLARIZATION-INDUCED REPROGRAMMING OF THE PRESYNAPTIC PHOSPHOPROTEOME MEDIATE CHANGES IN NEUROTRANSMITTER RELEASE?

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Learning and memory result from synaptic plasticity - the ability of synaptic transmission to be modulated over time in response to stimuli. Compared to the more well-studied post-synaptic changes, pre-synaptic change in neurotransmitter release (exocytosis) from synaptic vesicles (SVs) is a mechanistically less well-defined form of synaptic plasticity. It is known that the phosphorylation of several exocytic proteins affects transmitter release. Exocytosis is followed by synaptic vesicle endocytosis which is initiated by rapid dephosphorylation of endocytic proteins upon depolarisation. Phosphorylation levels of a few key presynaptic proteins have thus been shown to play an essential role in neurotransmitter release and synaptic vesicle regeneration. However, changes in pre-synaptic protein phosphorylation in response to depolarization and recovery has previously not been characterised globally, in spite of the highly likely role of phosphorylation in pre-synaptic plasticity. We have used quantitative mass spectrometry-based phosphoproteomics to study protein phosphorylation in isolated rat nerve terminals (synaptosomes) that were at rest, depolarised for 10 s using KCl stimulation, or depolarised and allowed to recover for up to 15 min after depolarisation. The quantitative results show a prolonged, widespread reprogramming of the presynaptic phosphoproteome, with an overrepresentation of exo- and endocytic proteins displaying long-term (15 min.) altered phosphorylation levels after high intensity depolarization. Kinase-substrate analysis indicated long-term per-synaptic effects of specific kinases after high, but not low intensity depolarisation. Additionally, key phosphatase regulatory proteins showed differential phosphorylation upon depolarisation/recovery, offering another potential mechanism for the global phosphoproteome reprogramming. We therefore hypothesise that the initial short depolarisation changes the potential for subsequent neurotransmitter release on a longer time-scale via global changes in the presynaptic phosphoproteome. Exo- and endocytosis assays from rat synaptosomes as well as electrophysiological experiments in mechanically dissociated as well as cultured hippocampal neurons are currently being performed to elucidate the functional effects of the global phosphorylation changes.

49 EFFECT OF EXTERNAL HIGH OSMOLALITY ON VESICULAR NEUROTRANSMITTER CONTENT IN CHROMAFFIN CELLS

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Previous studies on chromaffin cells at hypertonic condition have demonstrated a decrease in the frequency of exocytosis events and to cause less number of molecules to be released from each single exocytosis event.
Here, in this study we have applied a newly developed amperometric technique to directly quantify the neurotransmitter content of vesicles in in situ in the cell cytoplasm. A flame etched elongated conical carbon fiber microelectrode with an average size of 100 nanometer tip was inserted into the cytoplasm of chromaffin cells and was used to electrochemically quantify the total content of electroactive neurotransmitters from individual vesicles. The method benefits from vesicles encountering the electrode surface inside the living cell, where the vesicles rupture upon adsorption and release the vesicle content at electrode surface, which is is recorded as single amperometric spikes. The intracellular neurotransmitter quantification was compared to the amount of neurotransmitter released during exocytosis events. The data from these measurements demonstrate that the effect of hypertonic solution on vesicular content is already affected at prior stage of exocytosis. This suggests that the osmotic stress to these cells also directly reduces the vesicle neurotransmitter content, which results in a reduction in neurotransmitter release during exocytosis.

50 THE EFFECT OF EXCITED FLUOROPHORE ON VESICLE FUSION AT THE SURFACE OF THE ELECTRODE

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Electrochemical methods can be used to detect neurotransmitter molecules in vesicles or liposomes by electrochemically oxidizing transmitters upon vesicular release. Previous work in quantifying the neurotransmitter content of single secretory vesicles has been performed using a technique called electrochemical cytometry. This technique involves separation of vesicles or liposomes using capillary electrophoresis followed by amperometric detection of the content as vesicles exit the capillary and lyse onto the surface of a carbon fiber microelectrode. In our lab we have recently developed a simpler technique with the same aim. It involves lysis of vesicles onto an electrode surface without need for a preceding electrophoretic separation step. We apply the new cytometry technique to study the effect of excited fluorophores on the fusion properties of chromaffin vesicles at the electrode surface

Here, we probe the vesicle content of chromaffin cells and their fusibility onto a disk-shaped carbon fiber microelectrode. We specifically probe the effect of placing a fluorophore in the vesicle membrane. When a vesicle collides with the electrode surface, the vesicle ruptures over the surface and its content is trapped against the electrode. Vesicular content is oxidized and the resulting peak from each vesicle impact is used to quantify the amount of neurotransmitters present in granules. The amperometric peaks initially rise quickly and decay at a slower rate. It can be explained by the membrane pore formation that causes an initial rapid transfer of vesicle material to the electrode surface and is most likely followed by a subsequent membrane distention of the vesicle over the surface thus slowing the release kinetics. The frequency of the recorded amperometric spikes in each experiment has been used to probe the fusibility of vesicle as a function of fluorophore concentration in the membrane. Chromaffin granules were incubated with different concentration of fluorescent-labeled phospholipids before each experiment. The fluorescent probes we have used are N- (lissamine Rhodamine B sultonyl) dipalmitoylphosphatidylethanolamine (Rh-DOPE) and/or N-[7-Nitrobenz-2-oxa-1,3-diazol-4-yl]psychosine sulfate (NBD-PS). The study of vesicle fusion was carried out before and after subjection of samples to excitation wavelength of red (570nm) for Rh-DOPE and blue (490nm) light for NBD-PS.

A significant increase in the number and frequency of vesicle fusion onto the surface of the electrode is observed when the fluorophore in the membrane is excited. Our findings show that only the combination of fluorescently labeled vesicles and applied excitation light increases the frequency of vesicle fusion and thus excited fluorescent-labeled phospholipids can change the properties of the membrane and facilitate vesicle fusibility.

51 DISSECTING PHOSPHOREGULATION OF EXOCYTOSIS: ASSESSING THE ROLES OF KINASES IN LATE STEPS OF THE EXOCYTOTIC PATHWAY

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Small molecule inhibitors of kinases have been extensively used to assess the roles of phosphorylation in regulated exocytosis, implicating protein (de)phosphorylation in the modulation of all stages (i.e. trafficking, docking, priming, and fusion of secretory vesicles) [1, 2]. Several proteins involved in regulated exocytosis are known to be phosphorylated by distinct classes of kinases [3]; however, the exact stage(s) at which these proteins act (via phosphorylation related functions) has not been fully characterised. We use fully primed, release-ready cortical vesicles (CV) isolated from sea urchin oocytes, coupled with a pharmacological approach, to study the influence of phosphorylation on the Ca^{2+}-triggered steps of exocytosis in vitro [4-6]. Isolated CV undergo docking and fusion reactions in the absence of cytosolic factors; the CV membrane thus contains the minimal essential components of the fundamental Ca^{2+}-triggered fusion mechanism.

Bioinformatic analysis confirms the high degree of identity and similarity between human and urchin kinase sequences; functional domains, in particular, show a high degree of conservation (e.g. PKC, alpha type: 83.6% similarity, 71.9% identity; ATP-binding site: 93.4% similarity, 80.3% identity). Seven small molecules known to inhibit the activity of six classes of protein kinases (protein kinase C, casein kinase 2, rho kinase, Abl/Src kinase, and Ca^{2+} Calmodulin kinase), three classes of lipid kinases, and five small molecules that act as broad-spectrum kinase and phosphatase inhibitors were screened using a high throughput, plate-reader-based kinetic assay. In the absence of cytosolic factors, inhibition of protein and lipid kinases/phosphatases had no effect on the rate or extent of CV fusion triggered by high [Ca2+]free. Thus, in primed, release-ready vesicles, phosphorylation either does not influence the Ca^{2+}-triggered steps leading to membrane fusion, or critical proteins are already stably phosphorylated at key sites. We think the latter less likely considering the CV isolation process and the well-established similarity between CV-CV and CV-plasma membrane fusion [7, 8]. Nonetheless, specific assays of fusion pore dynamics will be required to confirm whether or not there is a direct influence of protein phosphorylation on the process of membrane merger. In contrast, preliminary evidence indicates that in the presence of cytosolic factors, inhibition of specific lipid kinases has opposing effects on the initial rate of CV fusion. Taken together, these results raise questions concerning the role(s) of phosphoproteins in the late steps of Ca^{2+}-triggered exocytosis, but suggest that very specific lipid classes may have key roles

References

1. Morgan, A., et al., Biochemical Society Transactions, 2005. 33, p. 1341-1344.
2. Turner, K.M., R.D. Burgoyne, and A. Morgan, Trends in neurosciences, 1999. 22, p. 459-64.
3. Collins, M.O., et al., Journal of Biological Chemistry, 2005. 280, p. 5972-5982.
4. Rogasevskaia, T.P. and J.R. Coorssen, J Chem Biol, 2011. 4, p. 117-36.
5. Abbineni, P.S., J.E. Hibbert, and J.R. Coorssen, The Biological Bulletin, 2013. 224, p. 200-17.
6. Coorssen, J.R., et al., J Cell Biol, 1998. 143, p. 1845-57.
7. Churchward, M.A., et al., J Cell Sci, 2005. 118, p. 4833-48.
8. Furber, K.L., D.M. Brandman, and J.R. Coorssen, J Chem Biol, 2009. 2, p. 27-37.

52 AUGMENTED CATECHOLAMINE RELEASE FROM CHROMAFFIN CELLS OF DIABETIC AND HYPERTENSIVE RATS

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Diabetic and hypertensive patients have enhanced activities of their sympathetic nervous system as well as their renin-angiotensin axis. In hypertensive patients and in genetic spontaneously hypertensive rats (SHR), increased sympathetic activity translates into enhanced circulating levels of catecholamine released from adrenal medullary chromaffin cells challenged with depolarising stimuli. Whether chromaffin cells from diabetic rats exhibit similar augmented secretory responses is unknown. Here we present a comparative study in chromaffin cells from control Wistar Kyoto rats (WKY), rats made diabetic with streptozotocin (STZ), and SHR. With respect WKY cells, STZ and SHR cells exhibited the following statistically significant differences in the various parameters studies: (i) smaller whole-cell Ca^{2+} current amplitude; (ii) higher angiotensin II-elicited cytosolic Ca2+ elevations ($[Ca^{2+}]_c$); (iii) higher angiotensin II-elicited quantal catecholamine release; (iv) higher K^+-elicited catecholamine release; (v) lower ATP content; and (vi) higher systolic blood pressure. These parallel changes indicate the existence of common features in diabetes and hypertension as far as augmented $[Ca^{2+}]_c$ transient and catecholamine release were concerned. Those enhanced responses could be related with lower mitochondrial bioenergetics, as indicated by decreased ATP levels and impairment of mitochondrial Ca^{2+} buffering capacity, as suggested by higher $[Ca^{2+}]_c$ transients in STZ and SHR chromaffin cells, with respect WKY cells. It will be of interest to make diabetic with STZ the hypertensive rats to explore a possible interaction and/or additivity of the parameters here studied. This could reveal new clues to treat more effectively the hypertensive diabetic patient.

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53 DID NOT ATTEND

54 SORTING NEXIN 27 LINKS PTHR SIGNALLING TO THE RETROMER FOR POSTNATAL BONE GROWTH

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Long viewed as an inert structure that is necessary for mobility, bone is now widely appreciated as a metabolically active organ that is frequently the target of endocrine hormones such as the parathyroid hormone (PTH). During postnatal life, PTH regulates blood mineral ion homeostasis and bone development via its receptor, PTHR, a class B GPCR. Following stimulation, PTHR is internalised into endosomes where it elicits cAMP-associated signalling. Termination of cAMP-signalling from PTHR has been shown to require recruitment of the retromer sorting complex; however the molecular basis remains unclear. Here we show that sorting nexin 27 (SNX27), a PDZ-domain containing endosomal protein, functions as a cargo adapter to link PTHR to the retromer complex for PTH-signal termination. By coimmunoprecipitation and live cell microscopy we demonstrate that SNX27 and PTHR for an endocytic complex in response to PTH stimulation. Accordingly, SNX27 directs PTHR trafficking to the retromer-associated endosome-plasma membrane recycling pathway. SNX27-PTHR binding occurs via a PDZ-domain interaction which is stabilised and enhanced in the presence of the retromer subunit VPS26. At the atomic level, the SNX27-PDZ cavity serves as a scaffolding module that simultaneously wires the C-terminal PDZ consensus motif, ETVM, of PTHR and VPS26 through mutually exclusive contacts. Depletion of the SNX27-retromer augments PTH-signalling and misdirects PTHR trafficking into lysosomes resulting in reduced PTHR expression at the cell surface. Similar disturbances in PTH-signalling are observed in osteoblasts lacking SNX27 which are dysfunctional and contribute to the severe bone deficits manifest in postnatal SNX27-deficeint mice. Our findings provide a molecular basis to integrate PTHR signal activation and retromer-mediated signal termination and unveil a previously unrecognised function for SNX27 in the growth and maintenance of the postnatal skeleton.

55 AN IN VIVO EXAMINATION OF IL-6 ACTIONS IN THE MOUSE ADRENAL GLAND

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Bi-directional interactions between the immune system and the adrenal gland have been implicated in various pathologies. This relationship may be mediated in part by pro-inflammatory cytokines, such as tumour necrosis factor, interleukin-1 and interleukin-6 (IL-6). Previous research in our lab has demonstrated an increase in the phosphorylation of Signal Transducer and Activator of Transcription 3 (STAT3) in isolated bovine and murine chromaffin cells following incubation with IL-6. The aim of the current study was to determine whether murine chromaffin cells are responsive to IL-6 in vivo. Male mice were administered 1µg IL-6 by i.p injection, and were deeply anaesthetised and perfused with 4% paraformaldehyde 10, 30 or 60 minutes later. To investigate the effects of endogenous cytokines on adrenal function, an additional group of male mice were treated with 10 µg/kg lipopolysaccharide (LPS) for 3h before perfusion. The adrenal glands were removed, and 10µm sections cut and processed for immunohistochemistry. IL-6 treatment resulted in an increase in pSTAT3 immunoreactivity in the adrenal medulla compared to saline only controls after 10 min, with the response becoming less apparent at later time points. However, the staining did not show the distribution expected for chromaffin cells. Dual-label immunofluoresc nce using tyrosine hydroxylase as a marker of chromaffin cells confirme that the response was largely in non-chromaffin cells, with very few TH positive cells co-expressing pSTAT3. In contrast, the LPS treated adrenal sections demonstrated a dramatic increase in nuclear pSTAT3 within chromaffin cells. Further experiments are underway using LPS alongside an IL-6 blocking antibody to determine whether this increase in pSTAT3 can be attributed to IL-6

56 WHY (SOME) OLD MEN GET HYPERTENSION: TESTOSTERONE AND A RECEPTOR TYROSINE KINASE EPHB6 IN CONCERT REGULATE CATECHOLAMINE SYNTHESIS AND RELEASE IN CHROMAFFIN CELLS, HENCE MODULATING BLOOD PRESSURE

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We have found that a receptor tyrosine kinase EphB6 is crucial in regulating catecholamine release and synthesis in a sex hormone-dependent way. In male EphB6 KO mice, the catecholamine secretion is reduced, although they are normotensive, due to the balancing effects between the reduced ambient catecholamine secretion and increased vascular tone, which is also caused by EphB6 deletion in vascular smooth muscle cells. However, after castration, these KO mice have increased catecholamine release accompanied by hypertension. The adrenal glands and medulla of KO mice are of normal size, and the KO and WT AGCCs (adrenal gland chromaffin cells) are similar in size. At the molecular level, AGCCs from male EphB6 KO males show decreased catecholamine release and content, and such decreases revert to normal levels after castration. Male EphB6 KO AGCCs manifest reduced Ca^{2+} influx upon acetylcholine (Ach) stimulation, but the reduction is nullified after castration. The L-type Ca^{2+} channel current is insignificant in mouse WT or KO AGCCs; the T-type Ca^{2+} current is actually increased in KO AGCCs. EphB6 KO AGCCs present augmented BK channel currents, and such augmentation returns to normal levels after castration, while the BK current in WT AGCCs is not affected by castration. This has raised an intriguing possibility that the increased BK current augments membrane potential in Ach-stimulated KO AGCCs, and causes the T-type Ca^{2+} channel to close prematurely, resulting in a lower Ca^{2+} influx. The primary cause of such altered ion channel functions seems to be in AchR, which EphB6 physically interacts with and regulates. Moreover, EphB6 also affects catecholamine synthesis via several transcription factors such as Egr1 and JunB, again in a testosterone-dependent way.

These finding explain, at lease for a subpopulation of males, why ageing is accompanied by blood pressure increase, possibly due to a mutation in the EphB6 gene accompanied by hypogonadism. Such a 2-hit event leads to increased ambient catecholamine release in the subpopulation who needs a low catecholamine level to compensate for their increased arterial vascular tone. A large scale genotyping project assessing the mutations in EphB6 gene in hypogonadic hypertensive male patients is in progress.

57 PERSISTENTLY ACTIVE RAB3A, AND A CHIMAERIC PROTEIN CONTAINING ITS AMINO-TERMINAL PORTION, PREVENT THE LATE STAGES OF SPERM EXOCYTOSIS BY STABILIZING OPEN FUSION PORES

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Sperm contain a single, large dense-core secretory granule (the acrosome) whose contents are secreted at fertilization by a special regulated exocytosis termed the acrosome reaction (AR). Minutes after the arrival of the triggering signal, the acrosomal and plasma membranes dock at multiple sites and fusion pores open at the contact points. It was assumed that immediately afterwards fusion pores dilated spontaneously, originating tubules and vesicles that are shed, together with the acrosomal contents in the vicinity of the egg. The AR relies on the same fusion molecules as all other secretory cells; one such molecule is the small GTPase Rab3A. We have conducted a deep biochemical characterization of Rab3A's role in secretion by scrutinizing the exocytotic response of streptolysin O-permeabilized human sperm to the acute application of a number of Rab3A-containing constructs. Full length, geranylgeranylated and active Rab3A elicits human sperm exocytosis per se. With Rab3A/Rab22A chimeric proteins we demonstrated that the carboxy-terminus domain of the Rab3A molecule was necessary and sufficient to promote exocytosis whereas its amino-terminus prevented calcium-triggered secretion. Interestingly, full length Rab3A halted secretion when added after the docking of the acrosome to the plasma membrane. This effect depended on Rab3A's inability to hydrolyze GTP. Under these conditions, SNARE proteins were engaged in botulinum toxin B-resistant- and ?-SNAP/NSF-sensitive complexes; thus, we hypothesized that they had reached a post-fusion cis configuration. Because a dye applied to the medium entered the acrosome, we deduced that there was a connection between the intravesicular and extracellular compartments, most likely through open fusion pores. Yet, exocytosis was inhibited. Thus, we propose that inhibitory Rabs interfere with the vesiculation of membranes and release of the acrosomal contents after the opening of fusion pores.

58 FUSION PORE OPENING

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The SNARE (Soluble NSF Attachment protein REceptor) complex, which in mammalian neurosecretory cells is composed of the proteins synaptobrevin 2 (also called VAMP2), syntaxin and SNAP-25, plays a key role in vesicle fusion. However, in spite of an enormous body of research it is still not known how these proteins open the fusion pore that connects the vesicular lumen to the extracellular space, allowing release of the vesicular contents. Protein conformational changes can be detected using constructs incorporating fluorophores suitable for Fluorescence Resonance Energy Transfer (FRET). In individual chromaffin cells, we tracked conformational changes in SNAP25 by total internal reflection FRET microscopy while exocytotic catecholamine release from single vesicles was simultaneously recorded using a microfabricated electrochemical detector array. A local rapid and transient FRET change occurred precisely where individual vesicles released catecholamine. To overcome the low time resolution of the imaging frames needed to collect sufficient signal intensity, a method named event correlation microscopy was developed, which revealed that the FRET change was abrupt and preceded the opening of an exocytotic fusion pore by ~90 ms. A similar delay was reported between release of a hydrophobic fluorescent vesicular probe and catecholamine release measured as an amperometric spike (Liu and Gillis 2012 Biophys.J. 102, p318a). Based on synaptobrevin 2 transmembrane domain mutations and molecular dynamics simulations the hypothesis that in neurosecretory cells, fusion pore formation is directly accomplished by a conformational change in the SNARE complex via movement of the transmembrane domains will be discussed.

59 WHAT IS THE DOCKED STATE OF A RELEASE-READY VESICLE?

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What does it mean for a vesicle to be docked, Ca^{2+} sensitive, and release-ready? How does that state then promote and support subsequent membrane merger (i.e. fusion)? How do the spectrum of known (and perhaps unknown) molecular components mediate and/or modulate these steps? Based on some conflicting observations, we have taken an alternative approach to these questions. Some studies have been interpreted to indicate that release-ready vesicles are stably hemi-fused to the plasma membrane (PM); this is said to explain the speed of Ca^{2+}-triggered fusion [1,2]. However, such stable hemi-fusion is energetically 'expensive' and not consistently seen by electron microscopy [3,4]. If stable hemi-fusion is the physiological pre-release state of docked vesicles then the well-established urchin egg model, with ~15,000 release-ready cortical vesicles (CV) and highly conserved proteins associated with exocytosis [5], is an ideal system with which to test the hypothesis.

We have used reagents that 'break' the hemi-fusion diaphragm in an attempt to drive full CV fusion. Molecules such as chlorpromazine (CPZ) preferentially partition into the inner leaflet of membranes, inducing curvature and thus pores in arrested hemi-fusion diaphragms; these are standards for analysing viral fusion states [6]. If release-ready CV in intact eggs are hemi-fused, such reagents should induce full envelope elevation (FEE; complete fusion of all CV) within a time somewhat comparable to that of Ca^{2+}-triggered fusion (i.e. minimal timescale of seconds). Preliminary experiments show that only a very small fraction of CV fuse, in a seemingly random fashion, even after >6 min exposure of eggs to CPZ; even after 10 min there is no evidence of FEE. We interpret the data to indicate that release-ready CV are not stably hemi-fused but rather undergo transient, reversible hemi-fusion — that we 'capture' with CPZ to cause full fusion of some CV. Our working hypothesis is that fully docked, primed, release-ready vesicles are far more dynamic than previously thought. It seems the mechanism is 'set' such that increasing $[Ca^{2+}]$free then 'trips a switch' enabling membrane merger. We are using a number of techniques, and looking at both proteins and lipids, to test this working hypothesis and identify key players in the triggering and membrane merger steps.

References

1. Zampighi, G.A., et al., Conical electron tomography of a chemical synapse: vesicles docked to the active zone are hemi-fused, Biophys. J., 2006. 91: p. 2910-2918.
2. Wong, J.L., D.E. Koppel, A.E. Cowan, and G.M. Wessel, Membrane hemifusion is a stable intermediate of exocytosis, Dev. Cell, 2007. 12: p. 653-659.
3. Parsons, T., J.R. Coorssen, H. Horstmann, and W. Almers, Docked granules, the exocytic burst, and the need for ATP hydrolysis in endocrine cells, Neuron, 1995. 15, p. 1085-1096.
4. Nagwaney, S., et al., Macromolecular connections of active zone material to docked synaptic vesicles and presynaptic membrane at neuromuscular junctions of mouse, J Comp Neurol, 2009. 513, p. 457-468.
5. Abbineni, P.S., J.E. Hibbert, and J.R. Coorssen, Critical role of cortical vesicles in dissecting regulated exocytosis: overview of insights into fundamental molecular mechanisms. The Biological Bulletin, 2013. 224, p. 200-17.
6. Zavorotinskaya T, Qian Z, Franks J, Albritton LM., A point mutation in the binding subunit of a retroviral envelope protein arrests virus entry at hemifusion. J Virol., 2004. 78, p. 473-481.

60 MODELING MECHANISMS SHAPING ENDOPLASMIC RETICULUM

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The peripheral endoplasmic reticulum (ER) forms different morphologies composed of tubules and sheets. Proteins such as the reticulons shape the ER by stabilizing the high membrane curvature in cross-sections of tubules and sheet edges. We describe a theoretical model that explains virtually all observed ER morphologies. The model is based on two types of curvature-stabilizing proteins that either generate straight or negatively curved edge lines (R- and S- type proteins). Dependent on the concentrations of R- and S-type proteins, membrane morphologies can be generated that consist of tubules, sheets, sheet fenestrations, and sheet stacks with helicoidal connections. We propose that reticulons 4a/b are representatives of R-type proteins that favor tubules and outer edges of sheets. Lunapark is an example of S-type proteins that promote junctions between tubules and sheets. In a tubular ER network, lunapark stabilizes three-way junctions, i.e. small triangular sheets with concave edges. The model agrees with experimental observations and explains how curvature-stabilizing proteins determine ER morphology.

61 PITUITARY ADENYLATE CYCLASE-ACTIVATING PEPTIDE (PACAP) EXCITATION OF THE ADRENAL MEDULLA.

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Neuroendocrine adrenal medullary chromaffin cells receive synaptic excitation through the sympathetic splanchnic nerve to elicit catecholamine release into the circulation. Under basal sympathetic tone, splanchnic-released acetylcholine evokes chromaffin cells to fire action potentials, leading to synchronous phasic catecholamine release. Under elevated splanchnic firing, experienced under the sympatho-adrenal stress response, chromaffin cells undergo desensitization to cholinergic excitation. Yet, stress evokes a persistent and elevated adrenal catecholamine release. This sustained stress-evoked release has been shown to depend on splanchnic release of a peptide transmitter, Pituitary Adenylate Cyclase-Activating Peptide (PACAP). PACAP stimulates catecholamine release through a signaling pathway that is mechanistically independent of cholinergic excitation. We will examine key characteristics of PACAP-dependent adrenal excitation.

62 MULTIPLE MECHANISMS FOR VESICLE CONTENT RELEASE IN THE POST-FUSION PHASE OF SURFACTANT EXOCYTOSIS

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In neuroendocrine cells and neurons, where low molecular weight solutes can diffuse from secretory vesicles after fusion pore formation, the amount of secreted material is determined by the number of fused vesicles. In contrast, in cells secreting poorly soluble material the molecular mechanisms for vesicle content release during the post-fusion stage of exocytosis can regulate the amount and sometimes the composition of secreted material. Alveolar type II (ATII) cells in the lungs secrete hydrophobic lipo-protein surfactant (surface-active agent), which does not readily diffuse from the vesicle after fusion. By imaging single vesicle fusion events with high-resolution microscopy in isolated ATII cells we could demonstrate that at least two mechanisms are employed for surfactant extrusion from the vesicle during the post-fusion phase of exocytosis. Ca^{2+} influx through the vesicular P2X4 channels after fusion pore formation enables faster dilation of the fusion pore, whereas the compression of the actin coat, which forms exclusively on the fused surfactant-containing vesicles, provides the force for active surfactant extrusion. We could recently demonstrate that myosin II inhibition slowed down actin coat compression without completely abolishing it. Our data suggest that force generated from actin filament depolymerization by cofilin and subsequent cross-linking by β-actinin complements myosin II activity. Here we focus on the role of class I myosins for surfactant extrusion. Myosin I binds membranes to actin cytoskeleton and may connect actin coat on fused vesicles to the vesicle membrane and thereby affect vesicle compression. We could show that several members of the myosin I family are recruited to fused secretory vesicles, however myosin Ic isoform appears to be the one relevant for actin coat compression. These findings suggest that an elaborate cellular machinery is necessary for vesicle content release during ATII cell exocytosis.

63 CELL-SPECIFIC FUNCTION OF CHROMOGRANIN A IN DENSE CORE VESICLE BIOGENESIS AND CORE FORMATION IN ADRENOMEDULLARY CHROMAFFIN AND PANCREATIC BETA CELLS

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Background. Chromogranin A (CgA), an index member of the chromogranin/secretogranin protein family has been firmly implicated in the initiation and regulation of dense core vesicle (DCV) biogenesis and sequestration of hormones in neuroendocrine cells. The granulogenic effects of CgA have been shown in PC12 cells and in fibroblasts in culture, and in adrenal medulla in vivo in CgA knockout and knockdown mice. However, the structural effects of CgA deficiency in vivo have yet to be functionally linked to regulation of vesicular biogenesis and its quanta in chromaffin and beta cells in vivo.
Methods. Transmission Electron Microscopy (TEM) was used to evaluate structural changes of subcellular organelles. Adrenal catecholamine (CA) contents were measured by HPLC. The commercially available ELISA kit was employed to determine plasma insulin levels.
Results. CAs were decreased in the adrenal medulla (AM) of Chga-KO mice, consistent with impaired CA storage and smaller dense core (DC), and plasma insulin levels were also low compared to WT mice. TEM of the adrenal medulla revealed NE-storing vesicles as osmiophilic with intensely electron dense granules, and smaller, moderately electron-dense E-storing vesicles. Pancreatic insulin granules appeared with an electron dense core, with an intragranular halo by TEM. Decreased DCV numbers were observed in both NE and E cells of Chga-KO mice while, surprisingly, insulin vesicles per μm_2 were more numerous in pancreatic beta cells of Chga-KO, compared to wild-type mice Ultrastructural analyses revealed three vesicle types in chromaffin cells of Chga-KO mice: (i) normal DCV, (ii) decreased DCV with swelled halo, and (iii) swollen empty vesicles without DCs. Beta cells contained a mixture of immature and mature secretory granules. DC area was smaller in both chromaffin and beta cells of Chga-KO mice than in their WT counterparts, indicating a positive role of CgA in regulation of vesicular quanta in both cell types
Conclusion. Secretory granule size is decreased in NE and E chromaffin cells of the AM, and in beta cells of the pancreas, in CgA-deficient mice. Vesicle biogenesis, on the other hand, is decreased in AM and increased in endocrine pancreas by CgA deficienc . That CgA defici ncy leads to a decrease in secretory quanta in both endocrine tissues points to a primary role for CgA both vesiculogenesis and granulogenesis in endocrine tissue in which it is the main secretory component, and a primary role for Chga in granulogenesis, but not vesiculogenesis, in endocrine tissue in which the main secretory component is not CgA.

64 COUPLING OF OXIDATIVE STRESS CONTROL AND ENERGY SUPPLY DURING PC12 CELL DIFFERENTIATION

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The mechanisms connecting tolerance to oxidative stress and energy supply to promote neuritogenesis and survival in neuroendocrine cells are largely unknown. We showed here that PACAP- and cAMP-induced PC12 cell differentiation is accompanied by the expression of a novel antioxidant selenoprotein, named selenoprotein T (SelT), which exerts an essential role in neuritogenesis and tolerance against oxidative stress. By combining SelT promoter studies, RNA interference and ChIP analysis, we demonstrated that nuclear respiratory factor 1 (NRF-1), a key transcription factor regulating mitochondrial biogenesis, is crucial for SelT gene regulation by the cAMP/PKA pathway in PC12 cells. In addition, we showed that peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α), a potent NRF-1 coactivator involved in the transcriptional integration of mitochondriogenesis, is also required for cAMP/PKA-induced SelT gene transcription. Combined pharmacology, immunoblotting and knockdown experiments revealed that two critical kinases, LKB1 and AMP protein kinase (AMPK) operate downstream of PKA in order to promote PGC-1α expression in PC12 cells. Concurrently, using mitochondrial fluorescent tracking and determination of the ratio of mitochondrial to nuclear DNA, we found that PACAP and cAMP induced mitochondriogenesis in differentiating PC12 cells through a PKA/AMPK-dependent pathway. Taken together, these results show that a PKA/LKB1/AMPK/PGC-1/NRF-1 pathway couple tolerance to oxidative stress and mitochondrial biogenesis in response to cAMP elevation during neuroendocrine differentiation.

65 INTERLEUKIN-6 INTERACTION WITH THE CHROMAFFIN CELL

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Adrenal medullary chromaffin cells are subject to multiple regulatory influences including neuronal and hormonal inputs. In order to investigate a possible interaction between the stress and immune systems we have examined the effect of a number of cytokines including interleukin-6 (IL6) on chromaffin cell function. IL6 had a complex interaction with the secretory response. Acutely it suppressed nicotine-induced catecholamine release, while chronically it appeared to reduce nicotinic receptor desensitization thus enhancing release. Treatment with IL-6 resulted in a rapid (5 min), transient increase in the phosphorylation of ERK1/2 and slower, more sustained increase in the tyrosine phosphorylation of STAT3 (15-60 min). Coincident with the rise in IL6-mediated ERK activation there was a selective increase in the ser-31 (but not ser-19 or ser-40) phosphorylation of tyrosine hydroxylase. Interestingly, this response was accompanied by a small rise in catecholamine synthesis (approx. 150% basal). ERK activity also appeared to "cross talk" to the STAT3 signalling pathway by increasing STAT3 serine but not tyrosine phosphorylation. Microarray and qPCR analysis revealed that prolonged exposure to IL-6 (24 h) resulted in extensive changes to chromaffin cell gene expression. In the context of the above noted IL-6 mediated increase in tyrosine hydroxylase activity it was interesting to observe a small rise (2-fold) in tyrosine hydroxylase mRNA levels. As expected, chronic IL-6 exposure promoted an elevation in SOCS3 mRNA but there was also a marked (4-6 fold) increase in mRNA for a number of neuropeptides including galanin, vasointestinal peptide, parathyroid hormone-related protein and gastrin releasing peptide. These data suggest there is a complex, and potentially important, interaction between IL6 and the adrenal chromaffin cell. In order to assess the physiological significance of these findings we have recently examined this interaction in vivo using the mouse model. These latter data will be presented in a poster format.

66 PACAP AT THE ADRENOMEDULLARY SYNAPSE, AND IN THE BRAIN: A MASTER REGULATOR OF THE STRESS RESPONSE

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Release of the neuropeptide PACAP at the adrenomedullary synapse causes PAC1 receptor-dependent catecholamine release from chromaffin cells in response to systemic and psychogenic stressors. In the brain, PACAPergic transmission is required to activate the hypothalamo-pituitary-adrenocortical (HPA) axis in response to psychogenic, but not systemic, stressors. Chronic psychogenic stress (social defeat, restraint) results in non-habituating corticosterone (CORT) elevation and development of depressive and anxious behaviors (decreased social interaction, hypophagia/ weight loss, increased forced swim immobility), all of which are attenuated in PACAP-deficient mice. These effects of chronic psychogenic stress are phenocopied in PAC1-deficient mice. The site(s) of action of PACAP in brain may include synapses in hippocampus (contextual fear conditioning), extended amygdala (anxiety), and hypothalamus (CRH gene activation). How PACAP activation of PAC1 receptors at these sites is affected by catecholamine and CORT release from the adrenal gland, will help focus treatments for affective disorders driven by acute (traumatic) or chronic psychogenic stressors.

67 ATP IN SECRETORY VESICLES, WHY?

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Chromaffin granules are similar organelles to the large dense core vesicles (LDCV) found in several neuroendocrine cells and neurons. An intriguing question is to figure how all the vesicular cargo substances can be maintained at that high concentration without cause the osmotic lysis of vesicles. Chromogranins have described as essential components for concentrating amines in LDCV. However, although chromogranins contribute largely to the package of vesicular solutes, even in their complete absence, the catecholamine cargo results just halved (Diaz-Vera et al, 2012), being this resulting concentration even too high to avoid the osmotic swelling of LDCV. It is become clear that other factors are also contributed to the passive concentration of solutes. ATP is highly concentrated in all secretory vesicles regardless the cell type, transmitter or animal species (Borges, 2013). In LDCV of chromaffin cell ATP is accumulated up to 100-300 mM, this seems to participate in the concentration of amines as was shown in vitro (Kopel & Westhead, 1982). As cells cannot be deprived of ATP, our experimental approach is acting on its expression using siRNA on vesicle nucleotide carrier –the VNUT–. Depletion of vesicular ATP results in a drastic reduction in the catecholamine content, which is more evident in the newly synthesized vesicles. Also, after reducing the expression of VNUT, the frequency of exocytotic events is also reduced.
These data are the first demonstration of intravesicular role of ATP as chelating agent in living cells allowing the concentration of all ATP co-transmitters.

-Díaz-Vera, JM. et al (2012) FASEB J. 26, 430-438.
-Borges, R. (2013) Purinergic Sig. 9, 5-6.
-Kopell, WN. & Westhead, EW. (1982) J Biol Chem. 257, 5707-5710.

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68 FIRST IDENTIFICATION OF A HUMAN MUTATION IN SYNAPTOTAGMIN1 REVEALS PERTURBATION OF SYNAPTIC VESICLE CYCLING

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The integral synaptic vesicle (SV) protein synaptotagmin-1 (SYT1) is the calcium sensor responsible for mediating fast synchronous neurotransmitter release, and additionally regulates the kinetics of endocytosis. Despite the key role for SYT1 in presynaptic function, there has been no confirmati n to date that this gene and the molecular events it controls are essential for human neurodevelopment, because no individual with a pathogenic SYT1 variant has been identified. We describe the first known human condition associated with a rare variant in SYT1. Clinical features are an early-onset dyskinetic movement disorder, severe motor delay, and profound cognitive impairment. Rat SYT1 containing the equivalent human mutation was expressed in mouse primary hippocampal cultures to determine the impact of this mutation on SV recycling dynamics. Mutant SYT1 slowed SV fusion kinetics, in agreement with the published role for I368T in calcium-dependent membrane penetration. Interestingly, expression of this SYT1 variant also altered the kinetics of SV endocytosis. The clinical features, electrophysiological phenotype and in vitro neuronal phenotype associated with this dominant negative variant in SYT1 highlight presynaptic mechanisms essential for human motor control and cognitive development.

69 PHOSPHOPROTEOMIC PROFILING OF ACTIVITY-DEPENDENT PRESYNAPTIC SIGNALLING

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The functions of many presynaptic proteins are regulated by rapid phosphorylation or dephosphorylation following depolarisation of the presynaptic nerve terminals. However, the extent of signalling is not well defined except for a small group of proteins. It is known that some endocytic proteins are rapidly dephosphorylated. Also, a small number of phosphoproteins involved in regulating synaptic vesicles are linked to synaptic plasticity. Thus, a major function of activity-dependent phospho-signalling may be to regulate multiple aspects of the synaptic vesicle cycle, i.e. the biogenesis, localisation and fusion of neurotransmitter filled vesicles. In turn, this regulates the amount of neurotransmitter released, which is a property of presynaptic plasticity. The metabolic labelling of isolated nerve terminals with 32P has provided a view of how the presynaptic protein machinery engages with activity-dependent signalling, particularly for the endocytic protein dynamin 1 and the synaptic vesicle tethering protein synapsin 1. We have used chemical depolarisation of isolated presynaptic nerve terminals to profile presynaptic signalling and compared this to 32P metabolic labelling. We have shown that the type and level of stimulus determines the presynaptic response and have identified regulators of the synaptic vesicle cycle and other biological processes as targets for activity dependent phospho-signalling.

70 MUNC13S IN LDCV DOCKING AND PRIMING

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The molecular requirements of large dense core vesicle (LDCV) exocytosis in adrenal chromaffin cells are thought to be similar to those of synaptic vesicle (SV) exocytosis in neurons. However, a role for mammalian uncoordinated 13 (Munc13) proteins, essential regulators of SV docking and priming, has only been inferred in chromaffin cells based on overexpression studies. We have characterized LDCV exocytosis in chromaffin cells from mice deficient in each of the neuronal Munc13s, i.e. Munc13-1, Munc13-2, Munc13-3, and Baiap3. Loss of ubMunc13-2 leads to a dramatic reduction of LDCV exocytosis and catecholamine release. Munc13 isoforms differ in their ability to promote LDCV priming, with ubMunc13-2 and Munc13-1 conferring distinct release kinetics. However, in contrast to synapses lacking Munc13-1 and Munc13-2, chromaffin cells of this genotype do not exhibit an LDCV docking defect, even when analyzed at high resolution with 3D electron tomography, which indicates that the molecular steps of SV and LDCV docking are distinct.

71 MOLECULAR MECHANISM OF LDCV DEAD-END DOCKING IN MOUSE CHROMAFFIN CELLS

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Docking of LDCVs at the plasma membrane involves the interaction of vesicle-bound protein with a plasma membrane bound acceptor complex consisting of Syntaxin and SNAP-25. Recently, we uncovered an alternative unproductive docking mechanism. We showed that even under massive stimulation of secretion about 15% of LDCVs stayed tethered to the plasma membrane (Hugo et al.; 2013). A first molecular characterization showed that these LDCVs were docked to an unproductive t-SNARE acceptor complex composed of 2 Syntaxin and 1 SNAP-25 (Hugo et al., 2013). Our goal is now to identify the molecular link between the vesicle and the unproductive t-SNARE acceptor complex and to further characterize it. To do so we are using our well established combination of patch-clamp electrophysiology and TIRF-microscopy in conjunction with KO and rescue experiments. We found that vSNAREs do not seem to be involved, whereas Synaptotagmin variants appear to play distinctive roles in this process.

72 FLEXIBILITY OF V-SNARE TRANSMEMBRANE DOMAIN REGULATES VESICULAR EXOCYTOSIS

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Regulated exocytosis is defined as the Ca^{2+}-triggered fusion of vesicles with the plasma membrane, enabling the release of cargo molecules. Pioneering studies in past have established SNARE proteins as the molecular engines that overcome the energy barrier of membrane fusion via protein-protein as well as protein-lipid interactions. Here we experimentally address the nature of the interplay between synaptobrevin II (SybII) transmembrane domain (TMD) and surrounding phospholipids, which fine tunes the fusion energetics and determines neurotransmitter release.

Using a combination of photolytic 'uncaging' of intracellular Ca^{2+} with membrane capacitance measurement in chromaffin cells, we demonstrate that reduced flexibility of the SybII TMD severely impairs Ca^{2+} triggered exocytosis, whereas mutants which show enhanced or similar flexibility compared to wildtype SybII, can fully rescue secretion.

Analyses of single amperometric spikes in combination with simultaneous membrane capacitance measurements reveal that reduced flexibility of the SybII TMD slows the kinetics of neurotransmitter discharge from single vesicle and hinders the fusion pore jitter. In contrast, TMD mutants with higher flexibility speed up the kinetics of single vesicle fusion and enhance the fusion pore dynamics.

Thus our results demonstrate that SNARE TMDs play an active role in the fusion process that goes beyond simple anchoring of the protein. Specificall , we show that flexibility of TMD determines the magnitude of Ca2+ triggered exocytosis and kinetics of cargo discharge from single vesicles.

73 GERDES-MEMORIAL: A SYMPATHETIC DRIVE OF LOCOMOTION

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This contribution commemorates our colleague, mentor, and friend, Hans-Hermann Gerdes, who passed away in August 2013 at the age of only sixty years. Hans-Hermann was a meticulous and careful scientist yet always open to new ideas – even if these contradicted current thinking. His delightful personality and pleasant way to discuss scientific concepts deeply ingrained him in several communities. His major contributions were in the fields of Granin biology, the use of GFP to study neurosecretion, and the discovery of intercellular Tunnelling Nanotubes. Hans-Hermann was an excellent and responsible mentor, always keeping the advancement of his disciples in focus. As one of his former students, this contributor introduces novel data on vertebrate neuromuscular junctions of skeletal muscle. These paradigm chemical synapses, which are necessary for generating skeletal muscle movements, have been considered as exclusively cholinergic. Recent clinical data show that sympathicomimetics are successful in the treatment of neuromuscular transmission disorders, but how they operate in this context remains unclear. Our studies reveal that in mice skeletal muscles neuromuscular junctions normally receive dual innervation by cholinergic motor neurons and noradrenergic sympathetic neurons. The latter activate postsynaptic beta2-adrenoceptors and cAMP production and function to maintain neuromuscular synapse homeostasis. Deterioration of neuromuscular junctions is similar upon sympathectomy and in neuromuscular transmission disorder phenotypes and is corrected in both cases by sympathicomimetics. These findings provide new insights into the organization of the neuromuscular synapse and may have clinical relevance beyond neuromuscular transmission disorders, as synaptic failure has been implicated in various processes such as motor neuron disease, muscle atrophy and ageing.

74 REGULATION OF TUMOR GROWTH BY CIRCULATING CHROMOGRANIN A

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Chromogranin A (CgA), a protein released in circulation by the neuroendocrine system, is present in variable amounts in the blood of normal subjects and cancer patients. We have previously reported that circulating CgA may have a role as a regulator of the endothelial barrier function in tumors, with important implications for tumor growth, metastatic dissemination and drug penetration in tumor tissue. We have recently found that physiological levels of circulating CgA can also contribute to regulate angiogenesis. Based on these notions we investigated whether circulating CgA has a regulatory function in non-neuroendocrine tumor progression. We found that pathophysiologically relevant concentrations of circulating full-length CgA can reduce tumor growth in murine models of fibrosarcomas, mammary adenocarcinomas, and primary and metastatic melanomas, with biphasic (i.e. hormetic) dose-response curves. Mechanistic studies showed that CgA could reduce, in a hormetic manner, tumor vessel density and blood flo . Angiogenesis assays and structure-function studies showed that the C-terminal region of CgA contains the structural determinants of the hormetic mechanism, and that cleavage of this region causes a marked loss of anti-angiogenic and anti-tumor potency. In vitro studies on endothelial cells showed that CgA could regulate, with biphasic dose-response curves, the size of endoplasmic-reticulum (ER) lumen and number of ER-bound ribosomes, as well as the production of protease nexin-1, a serine protease inhibitor endowed of anti-angiogenic activity. Neutralization of protease nexin-1 with specific antibodies inhibited the anti-tumor activity of CgA. These results suggest that circulating CgA can regulate, in a hormetic manner, tumor angiogenesis and blood flow (and consequently tumor growth) and that induction of protease nexin-1 is crucial mechanism for its activity. Changes in CgA blood levels and/or fragmentation might affect disease progression in patients with non-neuroendocrine tumors.

75 NOVEL ROLES FOR CHROMOGRANIN A PEPTIDE CATESTATIN IN CARDIAC METABOLISM AND PHYSIOLOGY

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More than 50 million people suffer from cardiometabolic syndrome in the US. Catestatin (CST), a peptide derived form chromogranin A acts as an antihypertensive, cardioprotective, pro-angiogenic, and insulin-sensitizing peptide. The mechanistic role of CST in cardioprotection and substrate utilization under normal and stressed conditions is not well understood. We hypothesized that CST is a key regulator of cardiac metabolism and stress adaptation. We investigated wild-type (WT) and CST KO mice to evaluate cardiac function and metabolism. We utilized in vivo physiology, substrate metabolism, gene array, electron microscopy, affinity binding and molecular dynamics simulations to illustrate the possible mechanisms underlying CST regulation of cardioprotection. CST-KO mice display hypertension and are hyperadrenergic. Comparison of injuries after ischemia-reperfusion and ischemic preconditioning showed that CST-KO mice had diminished ischemic tolerance and could not be protected. Fatty acid uptake and incomplete fatty acid oxidation was higher in left ventricles of CST-KO mice. Ultrastructural studies revealed decreased sarcomere length and altered mitochondrial morphology in CST-KO mice. Phospho AMPK as well as insulin- stimulated phosphorylation of Akt and GSK-3β were decreased in CST-KO hearts. Additionally, we used proteomics and molecular simulations to identify CST as a binding partner for ATP synthase and describe possible binding sites as well as potential conformational changes within the ATP-synthase complex upon CST binding. Taken together, we show that KO of CST leads to decreased ischemic tolerance and loss of cardioprotective signaling and this appears to be dependent on altered structure of the contractile apparatus and impaired energy utilization by mitochondria.

76 Localization and function of serpinin peptides

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Serpinins are derived from processing of Chromogranin A at the C-terminus. Three forms have been found: Serpinin, p-Glu-serpinin and Serpinin-RRG. We have analysed the expression of serpinin peptides in the central nervous system and adrenal gland. Based on HPLC analysis, serpinin and p-Glu serpinin have been found in the adrenal gland, retina olfactory system and heart. In addition, we have found p-Glu serpinin immunostaining in Substantia Nigra, Nucleus Centrum-Parafacicularis Thalami and Nucleus Mammillaris. Cell biological localization studies suggest that these peptides are in nerve terminals and they may function as neurotransmitters or neuromodulators. We have demonstrated that serpinin regulates granule biogenesis in endocrine cells by induction of protease nexin-1 transcription via a cAMP-Sp1-dependent signaling pathway. Furthermore p-Glu-serpinin appears to be a neuroprotectant and a cardioprotectant acting through stimulation of ERK and AKT pathways to increase BCL2, a prosurvival protein. Our studies also suggest that p-glu serpinin action in the heart involves binding to β1-adrenergic receptors to activate adenylate Cyclase/cAMP/PKA signaling pathway to mediate changes in calcium channel activity and heart muscle contraction /relaxation.

77 COMBINED MEASUREMENT OF GRANIN AND GRANIN-DERIVED PEPTIDES IMPROVES THE DIAGNOSIS OF PHEOCHROMOCYTOMA

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Granins and their derived peptides are valuable circulating biological markers of neuroendocrine tumors. The aim of the present study was to investigate the clinical usefulness of the CgA-derived peptide WE-14, and the relevance of combining its plasma measurement with that of CgA and EM66 test assays for the diagnosis of pheochromocytoma. In patients with pheochromocytoma, plasma WE-14 levels were 5.4-fold higher than those of healthy volunteers, and returned to normal values after surgical resection of the tumor. Determination of plasma CgA and EM66 concentrations in the same series of patients revealed that the test assays for these markers had an overall 84% diagnostic sensitivity, identical to that determined for WE-14. However, WE-14 measurement improved the diagnostic sensitivity when combined with the results of CgA or EM66 assays. In addition, combination of the results of the three assays increased the sensitivity to 95% for the diagnosis of pheochromocytoma. In fact, the combination of WE-14 and CgA or EM66 test assays achieved 100% sensitivity for the diagnosis of paragangliomas and sporadic or malignant pheochromocytomas if taken separately to account for the heterogeneity of the tumor. These data indicate that WE-14 is produced in pheochromocytoma and secreted into the general circulation, and that elevated plasma WE-14 levels are correlated with the occurrence of this chromaffin cell tumor. In addition, in association with other biological markers, such as CgA and/or EM66, WE-14 measurement systematically improves the diagnostic sensitivity for pheochromocytoma. These findings strengthen the notion that granin-processing products should be considered as complementary tools for the diagnosis of neuroendocrine tumors.

78 CHROMOGRANIN A DEFICIENCY DECREASES SECRETORY VESICLE CORE FORMATION, CATECHOLAMINE STORAGE, AND ENERGY METABOLISM IN THE ADRENAL MEDULLA

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Background. Chromogranin A is a prohormone and granulogenic factor in neuroendocrine tissues with a regulated secretory pathway. CgA-deficient mice are dysregulated in catecholamine-driven diurnal blood pressure control and other parameters of metabolic and cardiovascular performance that depend on catecholamine (CA) secretion. The impact of CgA depletion on secretory granule formation has been previously demonstrated in cell culture. However, no studies have been performed to link the structural effects of CgA deficiency with secretory performance and cell metabolism in the chromaffin cells of the adrenal medulla in vivo

Methods. Transmission Electron Microscopy (TEM) was used to evaluate structural changes of subcellular organelles. Adrenal CA contents were measured by HPLC. Colorimetric and radioactive methods were utilized to determine glycogen content and glycogenesis, respectively. In vivo metabolic physiology of substrate (glucose and fatty acid) uptake and utilization relied on radioactive methods.

Results. CAs were decreased in the adrenal gland of Chga-KO mice, consistent with impaired CA storage and smaller DC. TEM of the adrenal medulla revealed NE-storing vesicles as osmiophilic with intensely electron dense granules, and smaller, moderately electron-dense E-storing vesicles. Decreased DCV numbers in both NE and E cells of Chga-KO mice indicates a role of CgA in DCV biogenesis. Ultrastructural analyses revealed three vesicle types in Chga-KO mice: (i) normal DCV, (ii) decreased DCV with swelled halo (SDCV), and (iii) swollen empty vesicles without DCs (SEV). DCV diameter in Chga-KO mice is smaller (100-200 nm) than in WT mice (200-350 nm). Both volume density and vesicles number per μm_2 were significantly lower in Chga-KO mice. Chga-KO mice display an ~47% increase in DCV versus DC, implying vesicle swelling due to increased osmotically active free CAs. Increased glucose uptake and its utilization for glycogenesis suggest cellular stress within Chga-KO chromaffin cells. Despite increased fatty acid uptake, oxidation of fatty acid was reduced in Chga-KO mice, possibly associated with altered mitochondrial structure (dilated and shorter cristae) with consequent decrease in mitochondrial function.

Conclusion. Secretory vesicle formation and biogenic amine storage are profoundly altered in NE and E chromaffin cells of the AM in CgA-deficient mice. Lack of CgA alters cellular metabolism including glucose utilization, suggesting that in addition to enabling regulated CA secretion from the chromaffin cell, CgA also functions to coordinate metabolic function and 'secretory stress' in the adrenomedullary chromaffin cell

79 CATECHOLAMINE AND GLUTAMATE RELEASE: A COMPARISON

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The core machinery of membrane fusion seems to be quite similar for hormone and neurotransmitter release. However the latter, in particular glutamate release at nerve terminals, have been trimmed during evolution for speed in a dual sense: i) speed in the release process itself and speed in vesicle recycling during sustained activity. I will shortly review our (older) data on chromaffin cells and then discuss neuronal specializations at a synapse, which is exceptional for its speed and its accessability for biophysical investigation: The Calyx of Held.
Among the special features of the Calyx of Held, which I will discuss, are: i) A heterogeneous population of readily-releasable vesicles ii) fast recruitment of vesicles to a readily-releasable pool and iii) a limitation of that recruitment by a refractory period, which is lengthened, if components of the endocytotic machinery are perturbed.

80 ELUCIDATING THE MOLECULAR MECHANISMS OF NEUROTRANSMITTER RELEASE AND ITS REGULATION

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We use structural biology and reconstitution approaches in combination with functional studies performed in collaboration with Thomas Sudhof and Christian Rosenmund to elucidate the mechanism of neurotransmitter release. Results from multiple groups including ours have led to a model whereby: i) the SNAREs syntaxin-1, synaptobrevin and SNAP-25 form SNARE complexes that bridge the vesicle and plasma membranes to catalyze membrane fusion; ii) NSF/SNAPs disassemble SNARE complexes; iii) Munc18-1 binds to a self-inhibited 'closed' conformation of syntaxin-1 and, together with Munc13, orchestrates SNARE-complex assembly; iv) synaptotagmin-1 acts as the Ca2+ sensor that triggers fast release; and v) complexins play dual, active and inhibitory roles in a tight interplay with synaptotagmin-1. Key to understand the essential functions of Munc18-1 and Munc13 were our reconstitution experiments showing that the efficient fusion observed previously between syntaxin-1/SNAP-25-liposomes and synaptobrevin-synaptotagmin-1-liposomes is abrogated by NSF/α-SNAP because they disassemble these syntaxin-1/SNAP-25 complexes, and then fusion requires Munc18-1 and Munc13 because they mediate SNARE complex assembly in an NSF/SNAP-resistant manner.

Recent progress in two areas will be presented. First, using nuclear magnetic resonance experiments that measure lanthanide-induced pseudocontact shifts, we have elucidated the major synaptotagmin-1-SNARE complex binding mode in solution. The dynamic structure revealed by our data supports a model whereby, upon Ca^{2+} influx, synaptotagmin-1 releases the inhibition caused by complexin and cooperates with the SNAREs to bring the synaptic vesicle and plasma membranes together to induce fast membrane fusion. Second, in recent reconstitution experiments we have uncovered a tight interplay between the different domains of Munc13 that correlates with their functions in neurotransmitter release and presynaptic plasticity, including a Ca^{2+}-independent function in docking, a crucial role for the MUN domain in SNARE complex assembly, a key function for the C2C domain in release, and modulatory roles for the C1 and C2B domains that depend on DAG and on Ca^{2+}-PIP2, respectively.

81 ELUCIDATING THE ROLE OF SYNTAXIN-1 N-PEPTIDE IN NEUROTRANSMITTER RELEASE IN PC12 CELLS AND C. ELEGANS

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Syntaxin-1 is the central SNARE protein for neuronal exocytosis. It interacts with Munc18-1 through its cytoplasmic domains including the N-terminal peptide (N-peptide). Here, we investigate the role of the N-peptide binding in two conformational states ("closed" vs. "open") of syntaxin-1 using PC12 cells and C. elegans. Our preliminary results reveal a striking interplay between the syntaxin-1 N-peptide and conformational state of the protein. We propose that, instead of playing an essential role in the SNARE-mediated exocytosis, the N-peptide plays a critical role in intracellular trafficking of syntaxin-1, which is dependent on the conformational state of this protein

82 ASSEMBLY OF THE SECRETORY MACHINERY DURING INSULIN GRANULE DOCKING

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The assembly of the secretory machinery at the plasma membrane is a poorly understood prerequisite for regulated exocytosis. For example, it is not known whether the required proteins are preassembled at the release site or instead are recruited and assembled after vesicle docking. Current models propose that docking of the vesicle occurs through binding to either raft-like clusters of SNARE proteins or to structural proteins such as RIM1, in both cases implying at least partial assembly of the secretory machinery prior to docking. However, direct evidence for this is lacking. Using high resolution live cell microscopy we showed recently that the transition from a loosely tethered to the stably docked state occurs within seconds after vesicle arrival by recruitment of syntaxin and munc18, and that recruitment of several other proteins was further delayed. Here we extend on this work and present quantification for several exocytosis proteins (syntaxin, SNAP25, munc18, munc13, rab3 and 27, rabphilin, granuphilin, RIM1, CaV1.2; tagged with EGFP) at the insulin granule release site during docking, priming and exocytosis. We find that the Rab3 interacting protein RIM1 and Rabphilin were enriched at docking sites prior to vesicle tethering and docking. A slow increase in RIM1 fluorescence was seen during granule maturation into the releasable pool (priming), suggesting roles for RIM1 in both docking and priming. None of the other proteins were present before granule arrival, but these were instead recruited during docking or even later during priming. Granules that successfully docked carried Rab3 and Rabphilin, whereas those that only temporarily tethered did not. In contrast, Rab27 and its effector Granuphilin were present on both types of granules. We conclude that sites enriched in RIM1 at the plasma membrane may facilitate docking by weakly tethering the incoming granule through interaction with rab3/rabphilin. Successful docking requires acute clustering of syntaxin/munc18, and we propose that this cluster then nucleates assembly of the exocytosis machinery.

83 DOC2B TRANSLOCATES IN A DIFFUSION-LIKE AND IN A PIP2 DEPENDENT MANNER TO THE PLASMA MEMBRANE

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DOC2B is a high affinity Ca2+ sensor, which translocates from the cytosol to the plasma membrane (PM) upon Ca^{2+} elevation and regulates exocytosis by promoting priming and fusion. This protein contains a Munc13 interacting domain (MID) in its N-terminal and two tandem C2 domains in its C-terminal. These C2 domains are Ca^{2+} binding motifs, which bind phospholipids (PL) and soluble NSF attachment receptor proteins (SNAREs) in a Ca^{2+} dependent manner. In this work we investigated the mechanism of translocation and PM targeting of DOC2B. We found that low temperature, depletion of ATP or disruptions to the cytoskeleton did not alter the translocation kinetics measured in Total Internal Reflection Fluorescence (TIRF) microscopy in PC12 cells. This implies that the translocation is an energetically independent, diffusion-like process. Using a combination of structural and biochemical tools we demonstrated that only the C2B domain of DOC2B bind Ca^{2+} in physiological concentrations. Live imaging of PC12 cells strengthened these findings by demonstrating that the C2B is the minimal sequence responsible for DOC2B Ca^{2+} dependent translocation, although to a lesser extent then the tandem C2AB, suggesting that the C2A enhances the interaction of DOC2B with the PM. DOC2B might be targeted to phospholipids or to SNARE proteins at the PM. Altering PM composition in live cells and examination of DOC2B mutants allowed us to determine that PIP2 is crucial for DOC2B PM translocation while SNARE proteins as well as other phosphoinositides are not. PI(4,5)P2 depletion from the PM or mutation that disrupts PIP2 binding abolish DOC2B translocation. In addition, PIP2 synthesis in cellular membranes that do not normally include this phosphoinositide recruits DOC2B to these membranes suggesting a role for PIP2 as a targeting DOC2B. This work provides novel information about DOC2B mode of translocation and PM targeting.

84 STRUCTURE-FUNCTION STUDIES OF MUNC18 IN VESICLE EXOCYTOSIS

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Munc18-1 is a critical component of the core machinery controlling neurosecretory vesicle exocytosis. In particular it combines with the SNARE protein synatixin-1 to regulate syntaxin-1 stability, trafficking and availability for SNARE complex mediated vesicle fusion, and also interacts with the SNARE complex to promote the vesicle fusion process. We have recently shown that a region of Munc18-1 within the domain3a can undergo conformational changes that are important for Munc18-1 to switch between its roles in syntaxin-1 chaperoning and membrane fusion. We have also found that a mutation in Munc18-1 C180Y causing early infantile epileptic encephalopathy (EIEE) perturbs Munc18-1 function by promoting ubiquitin-mediated turnover, also suggesting a role for Ub-mediated turnover of Munc18-1 protein in it normal physiology. This mutation can also lead to formation of large co-aggregates of Munc18-1(C180Y) with wild-type Munc18-1, and also with α-synuclein. Here we discuss this work in the context of Munc18-1's antagonistic roles in syntaxin binding and synaptic vesicle secretion.

85 THE MORPHOLOGICAL AND MOLECULAR NATURE OF SYNAPTIC VESICLE PRIMING AT PRESYNAPTIC ACTIVE ZONES

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Synaptic vesicle docking, priming, and fusion at active zones are orchestrated by a complex molecular machinery. We employed hippocampal organotypic slice cultures from mice lacking key presynaptic proteins, cryofixation, and three-dimensional electron tomography to study the mechanism of synaptic vesicle docking in the same experimental setting, with high precision, and in a near-native state. We dissected previously indistinguishable, sequential steps in synaptic vesicle active zone recruitment (tethering) and membrane attachment (docking) and found that vesicle docking requires Munc13/CAPS family priming proteins and all three neuronal SNAREs, but not Synaptotagmin-1 or Complexins. Our data indicate that membrane-attached vesicles comprise the readily releasable pool of fusion-competent vesicles and that synaptic vesicle docking, priming, and trans-SNARE complex assembly are the respective morphological, functional, and molecular manifestations of the same process, which operates downstream of vesicle tethering by active zone components.

86 VISUALIZATION OF AMPA RECEPTOR SYNAPTIC PLASTICITY IN VIVO

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Regulation of AMPA receptor (AMPAR) membrane trafficking plays a critical role in synaptic plasticity and learning and memory. However, how AMPAR trafficking occurs in vivo remains elusive. We have recently developed techniques to image AMPA receptor trafficking in vivo in awake and behaving mice. Using in vivo two-photon microscopy in the somatosensory barrel cortex, we found that acute whisker stimulation leads to significant increases in the expression of surface AMPA receptor GluA1 subunit (sGluA1) in both synaptic spines and dendritic shafts and only small changes in spine size and no changes in spine turnover. Interestingly, initial spine properties bias changes in spine sGluA1 content and spine size. The sensory stimulated increase in spine sGluA1 is NMDA receptor dependent and long lasting similar to major forms of synaptic plasticity in the brain. We are now imaging AMPA receptor trafficking during motor learning and sensory learning tasks. Our findings shed light on the complexity of AMPAR membrane trafficking and experience-dependent remodeling of synaptic strength in vivo.

87 ROLE OF BASSOON IN THE REGULATION OF NEUROTRANSMITTER RELEASE

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Neurotransmitter release from presynaptic boutons is crucial for accurate signal transfer at chemical synapses. It takes place at the active zone (AZ), a region of the presynaptic plasma membrane characterized by the presence of an electron-dense structure called the cytomatrix at the active zone (CAZ). Multidomain scaffold proteins (RIM, RIM-BP, Munc-13, CAST/ELKS, Liprins α, Piccolo and Bassoon), are the main constituents of this cytoskeletal matrix, which functionaly and spatially organizers of synaptic vesicle exo- and endocytosis[1]. The role of Bassoon in presynaptic function has been investigated in several studies[2-4], which suggest that this protein does not have an essential role in synapse formation but rather contributes to the plasticity of neurotransmitter release. In order to understand the mechanism of this Bassoon function, we characterized presynaptic composition and function in primary hippocampal neurons derived from Bsn knock out mice. We observed decrease in the synaptic abundance of most CAZ proteins in Bassoon-lacking synapses and in line with our previous studies deffects in synaptic vesicle release. To disect this phenotype we used the synaptophysin-pHluorin-based reporter technique allowing investigation of release characteristics and analysis of synaptic vesicle pools. We found that ready-releasable pool and recycling pool were reduced and proportion of resting vesicles that do not participate in release was increased in the absence of Bassoon. Using phamacological intervention we identified involvement of CDK5- and PKA-dependent singaling in this proces. Moreover, we tested involvement of UPS-dependent protein degradation, which was recently shown to be target of regulation by Bassoon and its homologue Piccolo[5]. Together our study provides new inside into mechanistical understaning of Bassoon function at presynapse.

1. Gundelfinger ED, Fejtova A (2012) Molecular organization and plasticity of the cytomatrix at the active zone. Curr Opin Neurobiol 22:423-430.
2. Altrock W, Dieck S , Sokolov M, Meyer A, Sigler A, Brakebush C ,Fässler R, Richter K, Boeckers T, Potschka H, Brandt C, Löscher W,Grimberg D, Dresbach T, Helpelmann A, Hassan H, Balschun D, Frey J, Brandstätter H ,Garner C, Rosenmund C and Eckart Gundelfinger (2003) Functional Inactivation of a fraction of excitatory synapses in mice deficient for the active zone bassoon. Neuron 37: 787-800
3. Hallermann S, Fejtova A, Schmidt H, Weyhersmüller A, Silver A , Gundelfinger ED and Eilers J(2010) Bassoon speeds vesicle reloading at a central excitatory synapse. Neuron 68:710-723.
4. Davydova D, Marini C, King C, Klueva J, Bischof F, Romorini S, Montenegro-Venegas C, Heine M, Schneider R, Schroder MS, Altrock WD, Henneberger C, Rusakov DA, Gundelfinger ED, Fejtova A (2014) Bassoon specifically controls presynaptic P/Q-type Ca2+ channels via RIM-binding protein. Neuron 82:181-194
5. Waites CL, Leal-Ortiz SA, Okerlund N, Dalke H, Fejtova A, Altrock WD, Gundelfinger ED, Garner CC (2013) Bassoon and Piccolo maintain synapse integrity by regulating protein ubiquitination and degradation. EMBO J 32:954-969.

88 PHOSPHORYLATION OF SYNAPTIC VESICLE PROTEIN 2A AT THR84 CONTROLS THE SPECIFIC RETRIEVAL OF SYNAPTOTAGMIN-1

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Synaptic vesicle protein 2A (SV2A) has proposed roles in both SV trafficking and neurotransmitter release, however little is known regarding how SV2A function is regulated. We demonstrate that phosphorylation of SV2A by Casein kinase 1 family members triggers binding to the C2B domain of human synaptotagmin-1 (SYT1), the calcium sensor for fast synchronous neurotransmitter release. Phosphorylation of Thr84 on SV2A is essential for this interaction. SV2A phosphorylated on Thr84 binds to a pocket on the surface of the SYT1 C2B domain formed by three conserved Lys residues. Phosphorylation of SV2A at Thr84 had no effect on its own targeting to SVs or retrieval kinetics during endocytosis; however, it did control the retrieval of SYT1. Mutant SYT1 that cannot bind SV2A showed increased plasma membrane localisation and altered retrieval during endocytosis when expressed in primary hippocampal neuronal cultures. Importantly, knockdown of SV2A, or rescue with a phosphorylation-null Thr84 SV2A mutant, also caused increased surface stranding of SYT1 and specifically altered SYT1 retrieval during endocytosis. We propose that SV2A is a phospho-dependent chaperone required for the specific retrieval of SYT1 during SV endocytosis

89 NEUROTRANSMISSION ALTERATIONS RELATED TO THE PROGRESSION OF ALZHEIMER´S DISEASE IN 3XTG-AD TRANSGENIC MICE

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The dramatic rise in life expectancy during the 20th century has led to an increase in the elderly population, which in turn results in an enhancement in the prevalence of neurodegenerative disorders. Among these, Alzheimer´s disease (AD) has emerged as the most common form of dementia. The pathology is characterized by a marked atrophy of the cerebral cortex, the formation of senile plaques composed by the accumulation of beta-amyloid (βA) protein, and the appearance of neurofibrillary tangles, constituted by hyperphosphorylated tau protein. It has been reported that although initially the clinical features of AD correlates well with a deficit of cholinergic neurotransmission, behavioural changes that appear in the advanced stages of the disease comprises the involvement of other neurotransmitter systems. These alterations in neurotransmission processes could be correlated with changes in the synthesis, storage or release of neurotransmitter.

In this study we have used a triple transgenic murine model of AD (3xTg-AD). This animal model contains mutations in the gene encoding the amyloid precursor protein (βAPPSwe), presenilin-1 (PS1M146V) and tauP301L, which determines a progressive development of both plaques and tangles, in an age- and region-dependent manner that mimics the development of the disease on alzheimer's patients.
We propose to study here the last steps of the exocytotic neurotransmitter release in chromaffin cells of 3xTg-AD mice using the amperometric technique, and possible changes that may occur with the progressive development of AD, comparing the characteristics of the exocytotic events present in mice of different ages. The release of catecholamines was induced applying the physiological agonist acetylcholine (ACh) or by means of a depolarizing solution enriched in K^+.

We have found significant changes in the exocytosis of catecholamines that occur in mice of 6 and more than 12 months of age, where the pathology is already established and consolidated, respectively, when compared with prepathologic and cognitively unimpaired mice (2 months). These changes show an increase of the amperometric spikes during the development of the disease, both in response to ACh and K^+, although the quantal catecholamine content of each spike is lower. Kinetic analysis of secretory spikes shows that as the disease progresses amperometric spikes are faster and shorter in duration. Quite similar results have been observed in the pre-spike foot, a parameter indicating the fusion pore formation.

These preliminary data indicate the existence of alterations in the neurosecretory process in chromaffin cell of 3xTg-AD mice, which could form the basis of the various neurotransmitter deficits that occur with the progression of AD.

90 DETECTION AND ESTIMATION OF AMPEROMETRIC SPIKE PARAMETERS USING MATCHED FILTERING ALGORITHMS

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Electrochemical microelectrodes located immediately adjacent to the cell surface can detect spikes of amperometric current during exocytosis as the transmitter released from a single vesicle is oxidized on the electrode surface. Automated techniques are needed both to detect spikes that vary considerably in amplitude and time course and then estimate spike parameters that quantify the amount and time course of transmitter release. We have extended a Matched Filter detection algorithm that scans the data set with a library of prototype spike templates while performing a least-squares fit to determine the amplitude and standard error. The ratio of the fit amplitude to the standard error constitutes a criterion score that is assigned for each time point and for each template. A spike is detected when the criterion score exceeds a threshold and the highest-scoring template is identified. The search for the next spike commences only after the score falls below a second, lower threshold in order to reduce false positives. Receiver Operating Characteristic plots demonstrate that the algorithm detects 94% of manually identified spikes with a false-positive rate of 1%, and performs significantly better than several commercial and “freeware” spike-detecting applications. Subsequently, the time, amplitude, offset and best-fit templates are used as seed values for non-linear curve fitting of each spike with a function consisting of an exponential rise and one or two exponential decays. The resulting fits are then used to estimate parameters of interest such as spike area and spike width at half maximum.
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91 THE FUNCTION OF SNARE MEMBRANE ANCHORS

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The synaptic SNAREs syntaxin and synaptobrevin each have a single transmembrane domain (TMD) at their C-terminus. These domains have been hypothesized to form the initial fusion pore during Ca2+-triggered exocytosis, and their association has been hypothesized to contribute to the driving force of the final step of SNARE zipping during membrane fusion. Synaptobrevin with its TMD replaced by various lipidation motifs showed little if any support for membrane fusion in chromaffin cells, synapses, or liposome fusion. Mutations in the synaptobrevin TMD reduced or increased catecholamine flux through the initial fusion pore in chromaffin cells, with effects depending on side chain size and charge. The structure of the fusion pore implied by these results involves alternating faces of the TMD and a constriction in the fusion pore through the vesicle membrane near the cytoplasmic face. Mutations in the synaptobrevin TMD that perturb lipid packing alter the stability of the initial fusion pore. The pattern of kinetic perturbation varies in a periodic manner as the perturbation moves between the inner and outer leaflets of the lipid bilayer. Increases and decreases in fusion pore stability induced by these packing defects suggest that they alter spontaneous membrane curvature, and that perturbations actually influence the stability of the downstream lipidic fusion pore state formed by expansion of an initial proteinaceous fusion pore.

92 TBA

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Abstract not available at time of going to print

93 COMPLEXIN: SMALL BUT CAPABLE

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Regulated exocytosis is a specialized process allowing fast secretion of the vesicular content in response to a triggering Ca^{2+} stimulus. While it is a well accepted notion that exocytosis is mediated by SNARE proteins, the action and potential interplay of SNARE-regulating proteins like ComplexinII (CpxII) and SynaptotagminI (SytI) (both of which bind directly to SNAREs) have remained unclear. Using a combination of photolytic 'uncaging' of intracellular Ca^{2+} with membrane capacitance measurement in chromaffin cells, we demonstrate that C-terminal domain of CpxII prevents premature exocytosis at submicromolar Ca^{2+} concentration and thereby increases the primed vesicle pool. In addition, N-terminal domain of CpxII shortens the secretory delay and accelerates the kinetics of Ca^{2+} triggered exocytosis by increasing the Ca^{2+} affinity of synchronous exocytosis. Therefore, combined actions of two distinct CpxII domains provide two independent but synergistic functions to enhance synchronous secretion.
Analysis of single amperometric spike in combination with simultaneous membrane capacitance measurement reveals that CpxII C-terminus attenuates fluctuations of the early fusion pore and slows its expansion, but is functionally antagonized by SytI at the moment of fusion pore opening, enabling rapid transmitter discharge from single vesicles. Thus, our results illustrate how crucial properties of CpxII, SytI and their functional interplay regulate SNARE action to meet the speed requirement of regulated synchronized exocytosis.

94 STUDIES OF NEUROSECRETION BY REMOTE CONTROL OF EXOCYTOSIS AND ENDOCYTOSIS WITH LIGHT

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Optogenetics and optopharmacology allow to remotely controlling the activity of proteins, cells and entire organisms with light. These tools are revolutionizing research in biology with their high selectivity and spatiotemporal resolution. We have developed methods to photomanipulate the fundamental processes of secretion, exocytosis and endocytosis, in a way that is non-invasive and orthogonal to pharmacological and electrophysiological techniques.

Optical control of calcium-regulated exocytosis is achieved by exploiting the calcium permeability of the light-gated channel LiGluR in order to reversibly manipulate cytosolic calcium concentration (1). Light-gated exocytic events in bovine chromaffin cells expressing LiGluR can be detected by amperometry or by whole-cell patch-clamp to quantify membrane capacitance and calcium influx. Secretion of catecholamines can be adjusted between zero and several Hz by changing the wavelength of illumination between violet and green colors. The differences in secretion efficacy found between the activation of LiGluR and native voltage-gated calcium channels (VGCCs) will be discussed. LiGluR activation directly and reversibly increases the intracellular calcium concentration and allows controlling calcium-regulated exocytosis without the need of applying depolarizing solutions or voltage clamping in chromaffin cells. In addition, LiGluR can be activated by pulsed infrared light (two-photon absorption) (2). This enables deeper penetration in tissue and more focalized stimuli than violet light, leading to the activation of neurons and astrocytes with cellular and subcellular resolution.

On the other hand, control of endocytosis with light is achieved with inhibitory peptides of the clathrin pathway modified with a photoisomerizable crosslinker in order to manipulate their structure and binding affinit . These "traffic light peptides" (3, 4) act as stop and go signals for membrane traffic in living cells, and can be used to dissect the role of clathrin-mediated endocytosis in receptor internalization and in cell growth, division, and differentiation, with subcellular resolution.

(1) Izquierdo-Serra M, Trauner D, Llobet A, Gorostiza P. Biochimica et Biophysica Acta 2013; 1830:2853-60.
(2) Izquierdo-Serra M, Gascón-Moya M, Hirtz JJ, Pittolo S, Poskanzer KE, Ferrer È, Alibés R, Busqué F, Yuste R, Hernando J, Gorostiza P. Journal of The American Chemical Society. 2014; 136:8693-701.
(3) Nevola L, Martín-Quirós A, Eckelt K, Camarero N, Tosi S, Llobet A, Giralt E, Gorostiza P. Angewandte Chemie International Edition 2013; 52:7704-8.
(4) Martín-Quirós A, Nevola L, Eckelt K, Madurga S, Gorostiza P, Giralt E. Chemistry and Biology 2015; 22:31-7.

95 REGULATION OF PHOSPHOINOSITIDE SIGNALING

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Phosphoinositides are membrane bound signaling molecules that interact with a plethora of effector proteins to regulate vesicular trafficking, metabolism, actin dynamics, cell proliferation and survival. The generation and turnover of phosphoinositide signals on specific membranes is achieved by the activity of both phosphoinositide kinases and phosphatases, which phosphorylate and dephosphorylate respectively phosphates from the inositol head group of phosphoinositide signaling molecules, and thereby phosphoinositides regulate the recruitment of effector proteins to specific subcellular membranes. Phosphoinositide 3-kinase (PI3K) generates the signaling molecule, PtdIns(3,4,5)P3, which can be dephosphorylated by the inositol polyphosphate 5-phosphatases to generate PtdIns(3,4)P2 which is in turn dephosphorylated at the plasma membrane and on early endosomes by 4-phosphatases to generate PtdIns(3)P. There are ten mammalian 5-phosphatases that degrade PtdIns(3,4,5)P3 and/or PtdIns(4,5)P2 and recently the loss of function of several of these enzymes has been implicated in aberrant vesicular trafficking, embryonic development and developmental syndromes. Genetic mutations in the 5-phosphatase, INPP5E, are causative of the ciliopathy syndromes of Joubert and MORM, which are associated with mental retardation, abnormal neuronal development, polydactyly and other abnormalities. Deletion of murine Inpp5e causes mid-gestation lethality with ciliopathy phenotypes including neural tube defects, exencephaly, polydactyly and polycystic kidneys providing an ideal model to examine its role in ciliopathies. The molecular mechanisms by which 5-phosphatases, by degrading membrane bound phosphoinositide signaling molecules, in turn regulate embryonic and postnatal development will be explored.

96 REGULATING SYNAPTIC STRENGTH ACROSS THE DENDRITIC TREE

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Dendrite is a neuronal structure specialized for receiving and processing information through its many synapses. How incoming activity modifies synaptic strength distribution across the dendrite is fundamental to understanding brain function although much remains unclear. Using a combination of electrophysiology and imaging approaches in simple circuits, we have studied the relationship between neighbouring synapses in single postsynaptic neurons. We provide evidence for a novel cellular mechanism in balancing presynaptic strengths of convergent synaptic connections that target the same dendritic tree.

97 TRAFFICKING, DOCKING AND FUSION OF SECRETORY VESICLES

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Most cells contain a variety of transport vesicles traveling to different destinations. Formation of SNARE complexes between vesicle and target membrane is an essential aspect of the final fusion reaction in most intracellular routes and the regulation of SNARE-complex formation helps tuning intercellular communication. We study the molecular mechanisms that orchestrate trafficking, docking and secretion of secretory vesicles using molecular genetic tools, life cell imaging, electrophysiology, evanescent field microscopy (TIRFM) and electron microscopy in chromaffin cells and neurons. We have characterized the minimal machinery that docks secretory vesicles at the target membrane and conclude that the protein Munc18-1 renders syntaxin-1/SNAP-25 acceptor complexes at the target membrane receptive for vesicular synaptotagmin-1 to dock secretory vesicles. Furthermore, we have identified a new family of C2-domain containing proteins, the Doc2 family, to drive spontaneous fusion events in neurons and synchronizes release from chromaffin cells. Finally, we have characterized trafficking and recruitment of secretory vesicles in neurons using GFP- and superecliptic pHluorin-tagged cargo. In this talk, I will present the main data that led to these conclusions and present new data on the role of additional genes, modulation by protein phosphorylation and cytoskeletal components to regulate vesicle docking at the target, the availability of fusion-competent vesicles and activity dependent adaptations in these processes.

98 BIOGENESIS OF LARGE DENSE CORE VESICLES IN ADRENAL CHROMAFFIN CELLS OF NEWBORN MICE

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Chromaffin cells play an important role in stress induced responses by secreting catecholamines and peptides in a Ca^{2+}- dependent manner. The hormones are stored in large dense core vesicles (LDCVs) and, although chromaffin cells became a model system to study fast Ca^{2+}-dependent exocytosis, much less is known about LDCVs biogenesis and recycling. It has been shown that LDCVs are generated at the level of the trans-Golgi network (TGN) under the control of chromogranins. However, the sorting of associated and integral membrane components to LDCVs is not well understood.

In order to follow the biogenesis of LDCVs in chromaffin cells, we transfected the chromaffin cells with NPY-mCherry that is specifically localized to the LDCVs and fixed the cells with increasing delay. To determine its subcellular localization we co-stained the cells with various markers. Further, we investigated the association of LDCV's membrane components (vSNAREs and Synaptotagmin1) with LDCVs and studied their endocytic and recycling pathway.

We found that LDCVs appear to be retained at the Golgi network for about 1 hour before moving to the reserve pool of vesicles indicating a maturation step that might involve sorting of different vesicular membrane proteins to the LDCVs . This hypothesis was tested by colocalization studies with Synaptobrevin 2 and Cellubrevin immunolabelling in NPY-mCherry expressing cells. Surprisingly, both vSNAREs seems to get associated with mature LDCVs at late stage in the biogenesis indicating that vesicular proteins are transferred to mature LDCVs via the fusion of precursor vesicles. The question then was whether the LDCV's membrane components that are associated with new LDCVs are newly synthesized or recycled proteins. To address this question we are studying the endocytosis and recycling of Synaptotagmin 1.

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