iGPCRnet Conference Program

Day 1: Tuesday, November 30, 2021

14.00-14.20 : **Introduction**
Hosts: Ralf Jockers/ Martin Lohse/ Stephen Hill

Session 1: Structural dynamics of GPCR activation and signaling

Chairs: Andreas Bock and Chanjuan Xu

14.20-14.40 **Jianfeng Liu** (Huazhong University of Science and Technology, China)
Activation mechanism of neurotransmitter GPCR

14.40-15.00 **Fei Xu** (ShanghaiTech University, China)
A self-activated orphan GPCR

15.00-15.20 **Chris Tate** (MRC Laboratory of Molecular Biology, UK)
Structure and activation of a Class D GPCR

15.20-15.40 **Tracy Handel** (University of California, San Diego, USA)
Structure and activation mechanisms of G protein-coupled and atypical chemokine receptors

15.40-15.55 **Discussion**

5 min break

16.00-16.20 **Flash presentations 1**

Fabrizio Fierro *(Hebrew University of Jerusalem) Calcium is involved in satiety/hunger regulation mechanism through binding to the melanocortin receptor 4*

Zoe Köck *(Goethe University Frankfurt) Cryo-EM studies of a cell-free synthesized GPCR/G-protein complex*

Vaithish Velazhahan *(MRC Laboratory of Molecular Biology and University of Cambridge) A distinct activation mechanism in GPCR dimers: Insights from cryo-EM snapshots of the entire Class D1 GPCR activation pathway*

Florence Miller *(Berthold) Berthold objective: provide you powerful, repeatable & robust instruments*

10 min break

Day 1: Tuesday, November 30, 2021

Session 2: Computational biology in GPCR research

Chairs: Nicolas Floquet and Marcel Bermudez

16.30-16.50 **Masha Niv** (The Hebrew University, Israel)
Molecular match-making for taste GPCRs

16.50-17.10 **Patrick Barth** (Swiss Federal Institute of Technology, Switzerland)
Uncovering and reprogramming GPCR signaling by computational design

17.10-17.30 **Peter Kolb** (Philipps-University Marburg, Germany)
The pocketome of GPCRs reveals previously untargeted allosteric sites

17.30-17.50 **Xavier Deupi** (Paul Scherrer Institute, Switzerland)
Structural basis of the activation of the CC chemokine receptor 5 by a chemokine agonist

17.50-18.05 **Discussion**

5 min break

18.10-18.30 **Flash presentations 2**

Jiafei Mao *(Goethe University of Frankfurt am Main) Allosteric modulators explore the structural diversity of a peptide ligand on human badykinin 1 receptor*

Albert J. Kooistra *(University of Copenhagen)*
An online GPCR structure analysis platform

Alexandre Cabayé *(Université de Paris - UMR 8601 - CNRS)*
Towards a better understanding of the activation mechanism of the mGlu receptors

Marin Boutonnet *(Institut de Génomique Fonctionnelle)*
mGlu5 receptor function depends on membrane voltage

18.40-19.30 **Keynote Lecture 1**: **Jin Zhang** (University of California, San Diego, US)

Illuminating the Biochemical Activity Architecture of the Cell

Chair: Martin Lohse

Day 2: Wednesday, December 1, 2021

Session 3: Compartmentalisation of GPCR signaling

Chairs: Andreas Bock and Charlotte Kayser

14.00-14.20 **Viacheslav Nikolaev** (University Medical Center Hamburg, Germany)
Nanodomains of cardiac beta-adrenoceptor/cAMP signalling visualised by live cell imaging

14.20-14.40 **Manuela Zaccolo** (University of Oxford, UK)
Mapping beta-adrenergic nanodomains in the heart using proteomics

14.40-15.00 **Meritxell Canals** (University of Nottingham, UK)
Modulation of the mu-opioid receptor signalling

15.00-15.20 **John Scott** (University of Washington, Seattle, USA)
Exploring and exploiting the spatial constraints of cAMP signaling

15.20-15.35 **Discussion**

5 min break

15.40-16.00 **Flash presentations 3**

Romy Thomas *(Max-Delbrücl-Centrum Berlin) Improved FRET-based biosensors to monitor GPCR signaling in nanodomains*

Raghavender Reddy *(Gopireddy University Of California) Compartmentalized cAMP signaling in arterial myocytes*

Owen Underwood *(University of Nottingham) Use of intramolecular biosensors to monitor mu-opioid receptor-induced conformational changes of β-arrestins*

Bruno Tillier *(Synthelis) Cell-free expression services for your GPCR based projects*

10 min break

Session 4: GPCR Physiology and Pathology

Chairs: Françoise Bachelerie and Charlotte Kayser

16.10-16.30 **Aylin Hanyaloglu** (Imperial College London, UK)
Endosomal programming of gonadotropin hormone receptor signalling; new pharmacological targets and models of disease

16.30-16.50 **Torsten Schöneberg** (University of Leipzig, Germany)
GPCR dysfunctions as causes of human pathologies

16.50-17.10 **Julie Perroy** (Institut de Génomique Fonctionnelle, FR)
Restoring glutamate receptosome dynamics at synapses rescues autism-like deficits in Shank3-deficient mice

17.10-17.30 **Paul Insel** (University of California San Diego, USA)
GPCRs as novel therapeutic targets in cancers

17.30-17.45 **Discussion**

5 min break

17.50-18.10 **Flash presentations 4**

Itziar Muneta Arrate *(University of the Basque Country) Increased serotonin 5HT2A receptor constitutive activity on Gαi1- but not Gαq/11-proteins in human prefrontal cortex of subjects with schizophrenia*

Marta Lagana *(INSERM) The CXCR4 CHEMOKINE RECEPTOR IN HOST CONTROL OVER VIRAL LIFE CYCLE*

Abigail Walker *(Imperial College London) Targeting GPCR crosstalk in pregnant myometrium*

Leonie Alten *(Twist Bioscience)* *Writing the future of GPCRs*

10 min break

18.20-19.10 **Keynote Lecture 2: Jean-Philippe Pin**
(Institut de Génomique Fonctionnelle, FR)
Structure and dynamics of the asymmetric activation of class C GPCR dimers

Chair: Stephen Hill

Day 3: Thursday, December 2, 2021

Session 5: Up and coming technologies to target GPCRs

Chairs: Laura Kilpatrick and Laura Lemel

14.00-14.20 **Carl White** (University of Western Australia, Australia)
CRISPR-tagging of chemokine receptors with Nanoluciferase to probe endogenous receptor function

14.20-14.40 **Eleonora Comeo** (University of Nottingham, UK)
Illuminating the Life of the Adenosine Receptors: Development and Application of Fluorescent Probes for the Study of the Adenosine A1 Receptor in Living Cells

14.40-15.00 **Mark Wheatley** (Coventry University, UK)
GPCR-Lipid Particles: the shape of things to come

15.00-15.20 **Mikel Garcia-Marcos** (Boston University, USA)
Versatile optical biosensors of heterotrimeric G protein activity

15.20-15.35 **Discussion**

5 min break

15.40-16.00 **Flash presentations 5**

Xavier Gómez-Santacana *(IQAC-CSIC)* *A modular ligand-directed approach to label endogenous aminergic GPCRs in live cells*

Silvia Panarello *(IQAC-CSIC / IGF)* *Development of selective photoswitchable PAMs for the metabotropic glutamate receptor subtype 1*

Labani Nedjma *(Institut Cochin/INSERM)* *Identification of melatonin-like activities in plant extracts*

Vincent Corvest (*Calixar)* *New NAMs by AS-MS of Native A2A Adenosine Receptor*

10 min break

Session 6: GPCR crosstalk with microbe

Chair: Françoise Bachelerie

16.10-16.30 **Tim Lämmermann** (Max Planck Institute of Immunobiology and Epigenetics, Germany)
GPCR desensitization self-limits neutrophil swarms and control bacterial containment

16.30-16.50 **Martine Smit** (Vrije Universiteit Amsterdam, Netherlands)
Modulating viral GPCR function by nanobodies

16.50-17.10 **Nathalie Vergnolle** (INSERM U1220, FR)
Thrombin at the crossroads of GPCR and microbiota signalling

17.10-17.30 **Stefano Marullo** (Institut Cochin, FR)
Meningococcal/beta2 adrenergic interactions

17.30-17.45 **Discussion**

5 min break

17.50-18.10 **Flash presentations 6**

Katarina Nemec *(Max Delbrück Center for Molecular Medicine)*
Functional modulation of PTH1R activation and signaling by RAMP2

Lisa Maria Martin *(Max Delbrück Center for Molecular Medicine)*
Modulation of GPCR signaling by POPDC proteins, a novel class of cAMP effectors

Gage Stuttgen *(Medical College of Wisconsin)* *Novel Roles of FFAR4 in Macrophage Foam Cell Formation*

Yuanxu CUI *(Institut cochin/INSERM)* *Heterodimerization of melatonin and cannabinoid type 1 receptors*

10 min break

18.20-19.10 **Keynote Lecture 3: Stefan Offermanns**
(Max Planck Institute for Heart and Lung Research, Germany)

Novel GPCR functions in the cardiovascular and metabolic system

Chair: Ralf Jockers

Day 4: Friday, December 3, 2021

Session 7: Frizzled and adhesion GPCRs

Chairs: Laura Kilpatrick and Denis Servent

14.00-14.20 **Ines Liebscher** (University of Leipzig, Germany)
Translating the force – pharmacology and physiology of mechano-sensing adhesion GPCRs

14.20-14.40 **JinPeng Sun** (Shandong University School of Medicine, China)
Structural basis of recognizing steroid hormones by aGPCRs

14.40-15.00 **Hannes Schihada** (Karolinska Institute, Sweden)
Dissecting WNT-induced Frizzled activation and signalling with conformational sensors

15.00-15.20 **Xianhua Piao** (University of California San Francisco, USA)
GPCR-dependent Microglia Function in Development

15.20-15.35 **Discussion**

5 min break

15.40-16.00 **Flash presentations 7**

Gloria Somalo Barranco *(Institut Cochin U1016)* *Design, synthesis and validation of photo-activable tools for melatonin receptors*

Anne-Cécile Van Baelen *(CEA Saclay/JOLIOT/DRF/SIMOS/LPEM)* *Characterization of innovative animal toxins for the functional study of angiotensin receptors*

Fatih Yergöz *(Charité – Universitätsmedizin Berlin)* *Modulation of opioid receptor function by mediators of tissue injury and inflammation*

Florian Mignot *(PROMEGA)* *Bringing light to GPCR assays*

10 *min break*

Session 8: GPCRs and drug development

Chairs: Julie Sanchez and Denis Servent

16.10-16.30 **Mark Soave** (University of Nottingham, UK)
Monitoring Allosteric Interactions at CXCR4 using NanoBiT-conjugated Nanobodies

16.30-17.00 **Sadashiva S Karnik** (Cleveland Clinic Lerner College of Medicine, USA)
A Novel Allosteric Pocket in the Major Receptor for Angiotensin II

17.00-17.20 **Christa Müller** (University of Bonn, Germany)
Development of novel ligands for purinergic G protein-coupled receptors

17.20-17.40 **Eric Trinquet** (Perkin Elmer, France)
Innovative Solutions to investigate GPCR function & signaling

17.40-18.00 **Discussion**

10 min break

18.10-19.00 **Keynote Lecture 4: Laura Bohn** (The Scripps Research Institute, USA)

Imparting biased agonism and allostery at the mu opioid receptor to improve the therapeutic index

Chair: Phillipe Marin

19.00-19.20 **Awards & Conclusions**

Chairs: Ralf Jockers & Xavier Iturrioz

End of Conference

The i-GPCRnet meeting organizing committee

Françoise Bachelerie (Université Paris-Saclay, France)
Denis Servent (Université Paris-Saclay, France)
Andreas Bock (MDC Berlin, Germany)
Charlotte Kayser (MDC Berlin, Germany)
Laura Kilpatrick (University of Nottingham, UK)
Laura Lemel (University of Nottingham, UK)
Julie Sanchez (University of Nottingham, UK)
Chanjuan Xu (Huazhong University of Science and Technology, China)
Pin Yi (Huazhong University of Science and Technology, China)

Abstracts of the Flash Presentations

Flash Presentations Session 1

1.1 Calcium is involved in satiety/hunger regulation mechanism through binding to the melanocortin receptor 4

Fierro F. a*, Israeli H. b,c, Degtjarik O. b, Ben-Zvi D. c, McCormick P. d, Shalev-Benami M. b, Niv M.Y. a
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40% of the adult population is considered overweight or obese, with ~6 to 8% of early-onset severe obesity cases due to loss-of-function mutations on a single protein, the melanocortin receptor 4 (MC4R). MC4R is indeed involved in the regulation of hunger and satiety mechanisms and represents a primary target for drugs like Setmelanotide, a cyclic peptide recently approved for the treatment of obesity. Our recent work shed light on the MC4R activation mechanism by revealing the Cryo-EM structure in complex with Setmelanotide. The presence of a Ca2+ ion within the binding site was confirmed by classical molecular dynamics, with extracellular Ca2+ ions reaching the ion binding site after a few MD steps. In the same study, the Ca2+ role has been shown to be essential for the agonist/antagonist dualism necessary for the hunger/satiety regulation mechanism. In addition, density-guided MD simulations helped to clarify the protein/ligand/Ca2+ interactions, paving the way for the development of new potent and more selective drugs.

1.2 Cryo-EM studies of a cell-free synthesized GPCR/G-protein complex

Zoe Köck
Utz Ermel
Daniel Hilger
Achilleas Frangakis
Volker Dötsch
Frank Bernhard

The open accessibility of cell-free expression reactions allows adjusting reaction conditions according to the individual requirements of a synthesized protein. Furthermore, the expression of GPCRs in presence of supplied preformed nanodiscs enables their direct co-translational insertion into nanodisc membranes. Any contact with detergent is avoided and the strategy can provide fast access to non-modified or even labeled GPCR samples in native lipid environments. Key parameters such as ion and template DNA concentration, nanodisc size and composition as well as the presence of stabilizing compounds can have a strong impact on the resulting GPCR quality. Systematic optimization of these compounds generate cell-free reaction protocols specific for each target and finally result in accumulation of µM concentrations of functionally folded GPCR. We demonstrate the production of a full-length beta-1 adrenergic receptor inserted in defined nanodisc membranes in a customized cell-free expression system. The GPCR was produced as complex with heterotrimeric G proteins and analyzed by cryo-electron microscopy. We present the first 3D density map of a cell-free generated GPCR/G-protein complex co-translationally inserted in nanodiscs.

1.3 A distinct activation mechanism in GPCR dimers: Insights from cryo-EM snapshots of the entire Class D1 GPCR activation pathway

Vaithish Velazhahan and Christopher G. Tate

MRC Laboratory of Molecular Biology, Francis Crick Avenue, Cambridge, CB2 0QH, UK

G protein-coupled receptors (GPCRs) are divided phylogenetically into six classes, A-F. Over 600 structures of monomeric GPCRs have been determined and their mechanism of activation and coupling to a G protein has been well understood. However, other than the special case of Class C GPCRs that exist as obligate dimers due to the extracellular Venus flytrap domains, how canonical GPCRs that lack such domains form and function as dimers has been poorly characterized. We recently determined the structure of the Class D1 GPCR dimer Ste2 from S. cerevisiae which provided the first high-resolution evidence to understand how transmembrane-mediated GPCR dimers could form and showed that two G proteins could simultaneously couple to a GPCR dimer. To understand how Ste2 gets activated, we have developed new methods and have now determined four additional cryo-EM structures of Ste2 in multiple states along the activation pathway. These structures reveal a new activation mechanism that is different from all other GPCRs studied thus far. Upon agonist binding, there is a 6 Å outward movement of the extracellular end of helix H6, followed by a 20 Å outward movement of H7 that unblocks the G protein coupling site, with a 12 Å inward movement of H6 to form the G protein binding site. The Ste2 dimer has thus evolved a fundamentally different mechanism to configure the movement of H6 and H7 upon agonist binding to allow G protein coupling and provides the first model for how interactions at the dimer interface can alter during receptor activation, which could have implications for understanding signalling in other GPCR dimers. Furthermore, the high-resolution structures provide a template for the design of novel drugs against the widely conserved fungal GPCRs, which could be used to facilitate treatment of several intractable fungal diseases.

Flash Presentations Session 2

2.5 Allosteric modulators explore the structural diversity of a peptide ligand on human badykinin 1 receptor

Jiafei Mao
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Aditya Patra
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Georg Kuenze
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Institute of Biophysical Chemistry and Centre for Biomolecular Magnetic Resonance, Goethe University Frankfurt, Frankfurt am Main, Germany
Hartmut Michel
Department of Molecular Membrane Biology, Max Planck Institute of Biophysics, Frankfurt am Main, Germany

Peptide GPCRs are highly attractive and yet underexplored drug targets. Due to the intrinsic structural polymorphism of peptide ligands, these GPCRs may show more complex structural behaviors compared to GPCRs for small endogenous ligands. At the moment, besides the peptides, small-molecule allosteric modulators for peptide GPCRs are also extensively pursued, which could potentially resolve the selectivity and bioavailability issues. Here we focus on two related questions on peptide GPCRs, namely 1. does allosteric modulators crosstalk structurally with peptide ligands and 2. could peptide ligands show structural diversity in different pharmacological states on the same receptor. To address these questions, we have chosen human bradykinin 1 receptor (B1R), a class A GPCR, as our target and have monitored the conformation of its peptide ligand (des-arginine kallidin, DAKD) bound to B1R in the presence of various of ACE1 inhibitors as the allosteric modulators. To this end we have combined molecular docking, sensitivity-enhanced solid-state NMR spectroscopy and radioactive ligand binding assays. Our docking experiments suggest that structurally related ACE1 inhibitors could target B1R via distinct binding configurations. In response to these different allosteric ligand binding modes, the structure of the orthosteric DAKD peptide binding site is altered as shown by the bound peptide conformation changes mapped by NMR spectroscopy. Interestingly, the DAKD peptide could adopt distinct conformations in the presence of different allosteric modulators. These structural data align well with the changes of peptide binding affinities induced by allosteric modulators. The conformational diversity unmasked by this study may also explain the drastic conformational differences in different functional states as well as the partially tolerated peptide sequence diversity. Our findings could enlighten new pharmacological opportunities on this structurally complex peptide GPCR.

2.6 An online GPCR structure analysis platform

Albert J. Kooistra - Department of Drug Design and Pharmacology, University of Copenhagen, Denmark
Christian Munk - Department of Drug Design and Pharmacology, University of Copenhagen, Denmark
Alexander S. Hauser - Department of Drug Design and Pharmacology, University of Copenhagen, Denmark
David E. Gloriam - Department of Drug Design and Pharmacology, University of Copenhagen, Denmark

We present an online, interactive platform for comparative analysis of all available G protein-coupled receptor structures while correlating to functional data. The comprehensive platform encompasses structure similarity, secondary structure, protein backbone packing and movement, residue-residue contact networks, amino acid properties and prospective design of experimental mutagenesis studies. This lets any researcher tap the potential of sophisticated structural analyses enabling a plethora of basic and applied research studies. The platform is available to all at https://gpcrdb.org as part of the GPCRdb.

2.7 Towards a better understanding of the activation mechanism of the mGlu receptors

Alexandre Cabayé (ab), Francine Acher (a), Anne Goupil-Lamy (b), Hugues-Olivier Bertrand (b)

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Simulating the activation motions of large protein receptors can be challenging. Indeed, it is known that mGlu receptors' activation motion occurs on a millisecond time-scale [1], when actual computer simulations can produce a few hundred nanoseconds up to few microseconds.
To overcome this issue, we investigated a technic called targeted Molecular Dynamics (tMD) [2]. A tMD consists in applying steering forces to a subset of atoms in order to reduce linearly the distance between the initial and a final conformation. Therefore, the starting and the final states are required in order to be able to run tMD simulations.
Moreover, we tackled the problem of modelling for the first time, a full-length model of mGlu2 receptor, based on the recently published structure of mGlu5 receptor [3]. The mGlu2 receptor belongs to class C G-protein coupled receptor family, characterized by a large bilobate extracellular domain, named Venus Flytrap Domain (VFD). They function as mandatory dimers. We chose to build active and resting dimers, both bound to a well-known agonist, 3Me-CCG [4].
As all available structures for the transmembrane domain were in an inactive state, so it was for our models. However, this was not an issue, as our goal was to simulate the large transition motions between one conformation to the other. By achieving this we showed that depending on the starting conformation, the path followed for the transition is different, which could help for targeting a specific conformation for therapeutic purpose.

References
[1] P. Rondard, J. P. Pin. (2015). Curr. Opin. Pharmacol 20:95–106.
[2] J. Schlitter, M.Engels, P. Krüger. (1994). J. Mol. Graph 12 (2):84–89.
[3] A. Koehl, H. Hu, et al. (2019). Nature 566 (7742):79–84.
[4] I. Collado, C. Pedregal, et al. (2002). J. Med. Chem. 45 (17):3619–3629.

2.8 mGlu5 receptor function depends on membrane voltage

Marin Boutonnet*, Yan Chastagnier*, Nathalie Bouquier* , Solène Lappeman* and Julie Perroy*
* : Institut de Génomique Fonctionnelle, Centre National de la Recherche Scientique : UMR5203.

Objectives

G-Protein Coupled Receptors (GPCR) are seven transmembrane domain receptors found in a large variety of organs where they transduce extracellular signals such as hormones or neurotransmitters. In neurons they are exposed to wide membrane voltage variations recently shown to be an important direct modulator of some GPCRs activity but still remains unwell characterized. Metabotropic glutamate receptor 5 (mGlu5) is a class C GPCR localized on the glutamatergic post-synapse where it modulates synaptic transmission and plasticity. We investigated the sensitivity of mGlu5 to the neuronal membrane voltage.

Methods

We worked on Human Embryonic Kidney 293T (HEK 293T) cell lines stably expressing human mGlu5. We used a FRET-based sensor to report conformational changes (activation / inactivation) of mGlu5 triggered by agonist and antagonists following membrane depolarization by K+. Moreover, thanks to a fluorescent calcium sensor and microscopy imaging, we studied mGlu5 induced calcium release.

Results

We show that membrane depolarization potentiates the inactive-like conformation of mGlu5 triggered by LY341495 antagonist. Consistently, mGlu5 agonist-induced intracellular calcium increase was impaired by the depolarization of the cells. This global inhibition is the result of a reduced number of cells responding to the stimulation combined with a decreased mGlu5-induced calcium spiking activity in cells responding to the stimulation. Put together, these results reflect a disturbance of the mGlu5 functionality following depolarization.

Conclusion

Our findings support a role of membrane voltage on mGlu5 pharmacological activation and signaling in HEK 293T. This singular property of mGlu5 is of interest for glutamatergic synaptic transmission understanding.

Flash Presentations Session 3

3.9 Improved FRET-based biosensors to monitor GPCR signaling in nanodomains

Romy Thomas, Charlotte Kayser, Katarina Nemec, Lisa M. Martin, Martin J. Lohse and Andreas Bock

The ubiquitous second messenger cAMP regulates a vast range of physiological processes such as cardiac function by transmitting extracellular stimuli triggered by GPCRs to specific intracellular effector proteins. To achieve such specificity, we have recently shown that cAMP signaling is confined into nanometer-sized compartments in intact cells [1].
In order to precisely monitor the subtle cAMP changes inside such small nanodomains in real time and in intact cells, we set out to develop FRET-based cAMP biosensors with high dynamic ranges and fast kinetics. Based on a universally-taggable, positive cAMP switch, comprising a split cAMP-binding domain (CNBD), i.e. CUTie [2], we here report cloning and characterization of a family of improved FRET-based cAMP biosensors.
We found that using different combinations of several cyan and yellow fluorescent proteins within the same scaffold results in sensors with varying dynamic ranges, kinetics and affinities for cAMP. From our small library, we selected one sensor with a dynamic range that exceeds existing sensors by 2-3 fold, allowing to detect even subtle changes in cellular cAMP levels down to single molecules of cAMP.
We demonstrate that targeting this sensor to GPCRs and downstream cAMP effector proteins facilitates monitoring spatiotemporal signaling of the entire GPCR/cAMP signaling axis at the nanometer scale. In conclusion, we present a state-of-the-art toolbox to monitor receptor downstream signaling in cellular nanodomains.

References:

[1] Bock, A & Annibale, P et al. Optical Mapping of cAMP Signaling at the Nanometer Scale. Cell. 6, 182 (2020).
[2] Surdo, NC et al. FRET biosensor uncovers cAMP nano-domains at beta-adrenergic targets that dictate precise tuning of cardiac contractility. Nat. commun. 8, 15031 (2017).

3.10 Compartmentalized cAMP signaling in arterial myocytes

Gopireddy R Reddy1, Victor A. Flores1, Kent Sasse2, Sean M. Ward3, Manuela Zaccolo4, Yang K. Xiang1, Madeline Nieves-Cintron1, Manuel F. Navedo1

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Cyclic AMP (cAMP) is an important physiological second messenger that plays a central role in arterial myocyte excitability. While it is well-known that cAMP production by different Gs protein coupled receptors (GsPCRs) is confined to discrete subcellular compartments in many excitable cells, it is unclear if and how this spatial organization occurs in arterial myocytes that wrap around blood vessels. We hypothesized that ligand-induced cAMP signals are compartmentalized in arterial myocytes. Here, live-cell imaging with new targeted cAMP FRET-based (CUTie) biosensors was used to visualize cAMP signals in male and female human and mouse arterial myocytes. We found that receptor-mediated cAMP production was compartmentalized within the sarcoplasmic reticulum (SR) region of male, but not female arterial myocytes. PLA imaging suggested SR is closely associated with phosphodiesterase 4 (PDE4) and further analysis revealed that basal PDE4D expression was significantly higher in male arterial myocytes, which may contribute to spatially restricted subcellular cAMP signaling. Intriguingly, PDE4 activity was found to modulate SR Ca2+ load, as well as receptor-mediated vasorelaxation and blood flow (BF) in male arteries. These findings established a key role for PDE4 in regulating unique sexually dimorphic cAMP compartmentalization in arterial myocytes.

3.11 Use of intramolecular biosensors to monitor mu-opioid receptor-induced conformational changes of β-arrestins

Owen Underwood, 1,2, Raphael S Haider, 3, J Robert Lane, 1,2, Stephen J Briddon, 1,2 Carsten Hoffmann, 3, Meritxell Canals, 1,2.

1: Division of Physiology, Pharmacology and Neuroscience, School of Life Sciences, University of Nottingham, UK 2: Centre of Membrane Proteins and Receptors (COMPARE), University of Birmingham and University of Nottingham, UK. 3: Institute for Molecular Cell Biology, Jena University Hospital, Germany.

Mechanisms by which opioids cause limiting side effects such as respiratory depression are still cause for debate. β-arrestin2 (βarr2) has been suggested to participate in signal transduction of these effects, however recent evidence suggests otherwise. However, it is clear that different opioid agonists display a broad range of efficacies for recruiting βarr2 and receptor internalisation. βarr2 conformation upon receptor activation has a strong influence on its signalling capabilities. Here, we investigated the ability of mu-opioid receptor (MOR) agonists to induce conformational changes of βarr2 and βarr1 using newly developed intramolecular biosensors.

Intramolecular bioluminescence resonance energy transfer (BRET) βarr1 and βarr2 biosensors were developed in the Hoffmann lab[2] in which, βarr is tagged with a C-terminal nanoluciferase (NLuc) and a Fluorescein Arsenical Hairpin (FlAsH) moiety in defined positions within the βarr sequence.

HEK293T cells were transfected with βarr-FlAsH-NLuc constructs alongside MOR and GRK2. Cells were FlAsH-labelled[2], and treated with furimazine (5 µM) and opioid ligands (1 µM, 10 µM) . Bioluminescence and fluorescence were read at 475±30 nm and 535±30 nm respectively, using a PHERAstar FSX, 10 minutes post-ligand addition. Data were exported through MARS and analysed in GraphPad Prism V8. Net BRET ratio was calculated by subtracting measured BRET emission of F0, a βarr-NLuc construct with no FlAsH binding motif.

Transfected cells exhibited a luminescent signal that was localised in the cytoplasm, highlighted by clearly defined nuclei, confirming βarr-FlAsH-NLuc sensors are expressed and retain the expected localisation. Intramolecular BRET measurements suggest ligand-dependent conformational changes of βarr2, however βarr1 changes are only observed weakly in F5.

The βarr-FlAsH-NLuc sensors are expressed and exported to the cytoplasm. Our data suggest that MOR induces conformational changes in βarr2 following activation with ligands of different pharmacological profiles, however, can only weakly displace the βarr1 C-tail.

Flash Presentations Session 4

4.1 Increased serotonin 5HT2A receptor constitutive activity on Gαi1- but not Gαq/11-proteins in human prefrontal cortex of subjects with schizophrenia

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Centro de investigación Biomédica en Red de Salud Mental CIBERSAM, Spain

In animal and cellular models, activation of Gαi1-proteins by serotonin 5-HT2A receptor (5-HT2AR) agonist drugs represents a molecular fingerprint of hallucinogenic properties. On the other hand, supersensitive 5-HT2AR signalling through Gαi1-, but not Gαq/11–proteins, has been observed in schizophrenia. If this supersensitivity is consequence of altered constitutive activity of 5-HT2ARs in schizophrenia, is still unknown. Currently, inverse agonists of 5-HT2ARs are available, which allows the analysis of constitutive activity conditions. In this context, volinanserin has been described as selective 5-HT2AR inverse agonist on the Gαi1-protein-mediated pro-hallucinogenic pathway and for the canonical Gαq/11-protein-mediated pathway, in human prefrontal cortex.

[35S]GTPγS binding assays associated to immunoprecipitation with specific antibodies for Gαi1- and Gαq/11-proteins were carried out with volinanserin (10-9-10-5 M). The selectivity of volinanserin on 5-HT2ARs was measured by using the antagonist MDL-11,939 (10 μM). Post-mortem frontal cortex samples from 23 subjects with schizophrenia and their matched controls were analysed.

Volinanserin induced a higher inhibitory effect on 5-HT2AR coupling to Gαi1-proteins in schizophrenia subjects (Imax=20±2%) than in controls (Imax=14±1%) (p=0.0004). In contrast, no differences in the inhibitory effects of volinanserin were observed on the canonical Gαq/11-protein signalling pathway of 5-HT2ARs. Volinanserin activities on both Gαi1- and Gαq/11-proteins were sensitive to MDL-11,939, confirming the role of 5-HT2ARs.

These findings demonstrate an enhanced constitutive activity of 5-HT2ARs through the pro-hallucinogenic pathway in prefrontal cortex of subjects with schizophrenia. The constitutive 5-HT2AR hyperactivity could underlay the vulnerability to psychotic symptoms and suggest the relevance of the inverse agonism on 5-HT2ARs as new anti-psychotic pharmacological strategy.

4.15 The CXCR4 CHEMOKINE RECEPTOR IN HOST CONTROL OVER VIRAL LIFE CYCLE

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Background: CXCR4 is the G Protein-Coupled Receptor for the CXCL12 chemokine. Rare cases of CXCR4 dysfunction manifesting as gain-of-function and impaired desensitization are responsible for WHIM syndrome (WS). WHIM is characterized by panleukopenia and lesions induced by human papillomavirus (HPV). HPV (>400 types), mostly mucosal ones, are pathogens responsible for benign (warts) and cancerous lesions (5% of cancers) and remain a public health problem in the absence of treatment. More recently and surprisingly, HPVs were found to be the major eukaryotic viruses of the healthy skin viroma, indicating that they are asymptomatic when replicating, unlike cancerous lesions. Works in patients (NIH) and in mouse models of WS and HPV-induced cancer have shown the benefits of chronic administration of Plerixafor, a specific CXCR4 inhibitor. In the absence of specific treatment, a WHIM patient with cutaneous warts and perianal lesion, which progressed to squamous cell carcinoma (SCC), was treated with plerixafor (provided for compassionate use by Genzyme-Sanofi) for six months. We followed the evolution of SCC along with that of HPV skin viroma, which had not been done before, and monitored circulating leukocytes.

Results and perspectives: During the treatment, HPV types of patient's skin viroma remained stable but their loads were increased and SCC was cured. We interpret these findings as a rescue of viral replication. This is substantiated by the resolution of the SCC that occurred in a context where the number of circulating leukocyte is increased by plerixafor with a marked benefit on plasmacytoid cells that are regulated by CXCR4 and control the HPV life cycle. These findings highlight the role of CXCR4 as a gatekeeper of the HPV life cycle in epithelial and immune cells. They also reveal the carcinogenic potential of cutaneous HPV, hypothesis that will be investigated in 3D skin organoids, a unique model of HPV replication.

4.16 Targeting GPCR crosstalk in pregnant myometrium

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Preterm birth affects 15 million babies worldwide each year and is associated with significant morbidity and mortality. Despite this, no tocolytic strategy has been able to significantly lengthen gestation without severe fetal/maternal side effects. In the human pregnant myometrium, the prostaglandin E2 receptor, EP2, is a dually coupled G protein-coupled receptor (Gαs and Gαq/11), enabling it to mediate both myometrial quiescence (anti-labour) and pro-inflammatory pathways (pro-labour). In samples taken from women in labour EP2-mediated cAMP signalling was significantly decreased with an enhanced ability to increase levels of pro-labour/pro-inflammatory pathway, COX-2, which became sensitive to Gαi inhibitor pertussis toxin (PTX) in labouring samples. Oxytocin receptor (OTR) signalling via Gαi/o and Gαq/11 is critical for labour and in vitro pre-treatments of OT demonstrated functional crosstalk between EP2 and OTR leading to the signalling changes observed in labouring samples. EP2 and OTR's ability to dimerize was measured via proximity ligation assay, bioluminescence energy transfer and super-resolution photo-activated localisation microscopy (PALM). This revealed constitutive association between EP2 and OTR and increased frequency of heteromers, and rearrangement of EP2-OTR heterotetramers, following OT-stimulation. Predictions of EP2/OTR heteromeric complexes via molecular modeling showed substantial alignment with PD-PALM images and showed that the likely TM interfaces between EP2 and OTR heterotetramers include occlusion of EP2-TM6 when OTR is activated. This suggests that pre-treatment with OT favors the formation of 3EP2:1OTR* tetramers whose preferred architecture tends to bias towards OTR-mediated Gai-signaling. Two highly selective EP2 agonists, PGN9856i and AH13205, were investigated alongside butaprost for crosstalk with OTR and only PGN9856i increased anti-labour signals but was not able to increase Gαi/o or Gαq/11 pro-labour pathways, before or after OT treatment. Furthermore, pre-treatment with PGN9856i decreased OTR's ability to increase intracellular Ca2+ and PGE2. PGN9856i preferentially activates EP2-mediated anti-labour signalling, and may antagonise OTR, thus may represent a novel therapeutic strategy for pre-term labour management.

Flash Presentations Session 5

5.18 A modular ligand-directed approach to label endogenous aminergic GPCRs in live cells

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In the last two decades, new technologies based on luminescence have been developed to monitor the organization, signalling or ligand binding of G Protein-Coupled Receptors. These technologies rely on the overexpression of genetically modified (and/or fluorescently tagged) receptors of interest. However, there is an increasing interest in developping approaches to conjugate chemical labels to specific residues of native GPCRs, despite of the low reactivity and the high abundance of such residues.

Ligand directed approaches, may offer a solution to this problem. Such approaches consist in molecular probes that include a selective ligand moiety and a reactive moiety. Upon ligand binding, the labelling is directed to a nucleophilic amino acid in the vicinity of the binding pocket. However, this approach requires the use of non-nucleophilic ligand moieties, which is particularly difficult since many GPCR ligands contain amines or other nucleophilic functional groups.

In the present work, we developed an innovative ligand-directed toolbox based on a novel approach. The later uses molecular modules to build fluorescent ligand-directed probes to label an archetypical aminergic GPCR (D1R). Our molecular probes can be readily prepared before the labelling reaction from two molecular modules: an activated electrophilic linker which includes a fluorescent dye and a GPCR ligand that may include nucleophilic groups. Thanks to a fast and specific chemical reaction, the nucleophilic ligand can barely react with the activated linker before it is bound to the native target GPCR and the labelling reaction occurs. Subsequently, the ligand will unbind the GPCR pocket, leaving the receptor fluorescently labelled and fully functional.

This novel labelling approach allowed us to label endogenous D1 receptor both in transfected cells and primary cultures of neurons and will pave the way to develop new reagents and assays to monitor endogenous GPCRs distribution and activity in their native environment.

5.19 Development of selective photoswitchable PAMs for the metabotropic glutamate receptor subtype 1

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Keywords: G Protein-Coupled Receptor, mGlu1, Positive Allosteric Modulator, Photo-pharmacology, Photochromic ligands, Structure- activity relationship (SAR), Selectivity.

Positive allosteric modulators (PAMs) for metabotropic glutamate receptor 1 (mGlu1) have been postulated to treat neuropsychiatric diseases associated to mGlu1 dysfunction. Besides, obtaining a reversible and efficient spatiotemporal control of mGlu1 activity would be therapeutically advantageous. Photopharmacology may provide a solution on this topic, since it is based on the use of light and photoswitchable ligands to modulate a protein activity. This approach offers new perspectives for drug discovery and promises a better drug action control reducing side effects to unattained levels. We have focused on developing photoswitchable PAMs in order to precisely switch on/off the activity of the mGlu1 receptor with light. Replacing the phtalimide moiety of a known mGlu1 PAM [1] with a N=N bond led to azobenzene candidates. Subsequent in vitro assays revealed that Photoglurax-2 is a mGlu1 PAM ligand in the dark, whereas it loses activity under 380 nm light. It derived from the equipotent mGlu1/4 PAM Photoglurax-1 converted into a highly selective mGlu1 PAM [2]. The reversible photocontrol of mGlu1 activity may be advantageous to study the pharmacological and physiological implications of mGlu1 with an unprecedented precision, which may lead to unexpected findings in neuro-science.

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5.20 Identification of melatonin-like activities in plant extracts

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Melatonin (N-acetyl-5-methoxytryptamine) is a neurohormone known to regulate circadian rhythms and sleep in humans. The entrainment effect of melatonin to the natural day-night cycle and its direct effect on sleep homeostasis help to induce sleep by shortening its latency. This occurs through the activation of melatonin MT1 and MT2 receptors belonging to the G protein-coupled receptor family. Ramelteon and Tasimelteon are approved drugs for sleep improvement. The precise signaling pathways involved in sleep regulation are not fully understood. Traditional medicine uses plant extracts for a long time as therapeutic agents including the treatment of sleep-related disturbances. Here, we tested the hypothesis whether plant extracts, known to have beneficial properties on sleep and calming down the nervous system, act on melatonin receptor functions.

We tested the capacity of plant extracts to compete 2-[125I]-iodomelatonin for binding to the recombinant human MT1 and MT2 receptors expressed in HEK293 cells and to activate typical melatonin receptor signaling pathways (inhibition of cAMP production, activation ERK1/2, recruitment of beta-arrestin2). Several active plant extracts were identified that were active in at least one experimental protocol.

In conclusion, we identified several plant extracts that modulate melatonin receptor function in in-vitro assays. It will be interesting to test the effect of these extracts on sleep in animal models.

5.21 New NAMs by AS-MS of Native A2A Adenosine Receptor

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A collaborative project between CALIXAR, EDELRIS and the INSTITUT COCHIN leaded to the discovery of new negative allosteric modulators targeting the A2A adenosine receptor by Affinity Selection - Mass Spectrometry.
CALIXAR developed the production of the full-length, wild-type and functional adenosine receptor using their patented detergents to improve the solubilization and stabilization of the protein.
The fully native GPCR was used by EDELRIS for small molecules screening by AS-MS using their proprietary compounds library to identify 5 potential hits.
The functional evaluation, conducted by Ralf Jockers'group at the INSTITUT COCHIN using a cell-based assay showed an antagonistic effect for 2 of the 5 selected molecules and suggested that the both compounds could be negative allosteric modulators.

Flash Presentations Session 6

6.22 Functional modulation of PTH1R activation and signaling by RAMP2

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Receptor-activity-modifying proteins (RAMPs) are ubiquitously expressed membrane proteins that associate with different G protein-coupled receptors (GPCRs) including the parathyroid hormone 1 receptor (PTH1R), a class B GPCR and an important modulator of mineral ion homeostasis and bone metabolism. However, it is unknown whether and how RAMP proteins may affect PTH1R function.
Using different optical biosensors to measure the activation of PTH1R and its downstream signalling, we describe here that RAMP2 acts as a specific allosteric modulator of PTH1R, shifting PTH1R to a unique pre-activated state that permits faster activation in a ligand-specific manner. Moreover, RAMP2 modulates PTH1R downstream signalling in an agonist-dependent manner, most notably increasing the PTH-mediated Gi3 signalling sensitivity. Additionally, RAMP2 increases both PTH- and PTHrP-triggered β-arrestin2 recruitment to PTH1R.
These data uncover a critical role of RAMPs in the activation and signalling of a GPCR that may provide a new venue for highly specific modulation of GPCR function and advanced drug design.
The ubiquitous second messenger cAMP regulates a vast range of physiological processes such as cardiac function by transmitting extracellular stimuli triggered by GPCRs to specific intracellular effector proteins. To achieve such specificity, we have recently shown that cAMP signaling is confined into nanometer-sized compartments in intact cells [1].
In order to precisely monitor the subtle cAMP changes inside such small nanodomains in real time and in intact cells, we set out to develop FRET-based cAMP biosensors with high dynamic ranges and fast kinetics. Based on a universally-taggable, positive cAMP switch, comprising a split cAMP-binding domain (CNBD), i.e. CUTie [2], we here report cloning and characterization of a family of improved FRET-based cAMP biosensors.
We found that using different combinations of several cyan and yellow fluorescent proteins within the same scaffold results in sensors with varying dynamic ranges, kinetics and affinities for cAMP. From our small library, we selected one sensor with a dynamic range that exceeds

existing sensors by 2-3 fold, allowing to detect even subtle changes in cellular cAMP levels down to single molecules of cAMP.
We demonstrate that targeting this sensor to GPCRs and downstream cAMP effector proteins facilitates monitoring spatiotemporal signaling of the entire GPCR/cAMP signaling axis at the nanometer scale. In conclusion, we present a state-of-the-art toolbox to monitor receptor downstream signaling in cellular nanodomains.

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6.23 Modulation of GPCR signaling by POPDC proteins, a novel class of cAMP effectors

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The popeye domain-containig protein (POPDC) family consists of three subtypes: POPDC1, POPDC2 and POPDC3. These transmembrane proteins are mainly expressed in heart- and skeletal muscle cells and share a highly conserved C-terminal “Popeye Domain”, containing a cAMP-binding site. Intriguingly, it shares almost no sequence homologies with cyclic nucleotide binding domains (CNBDs) of other cAMP effector proteins. Therefore, the mode of cAMP binding is still unclear, as is the function of POPDC in GPCR signaling. However, studies have shown that POPDC downregulation leads to cardiac arrhythmia and heart failure in animal models, and to cardiac arrhythmia and muscular dystrophy in patients. Our goal was to monitor cAMP levels in the POPDC1-domain following ß-adrenergic stimulation, and to investigate a potential interaction between POPDC1 and PDE4. Moreover, we used the CNBD to develop a new FRET-based cAMP sensor.

6.24 Novel Roles of FFAR4 in Macrophage Foam Cell Formation

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Free fatty acid receptor 4 (FFAR4), also known as G-protein coupled receptor 120 (GPR120), is a long-chain unsaturated fatty acid receptor expressed in adipocytes, endothelial cells, and macrophages. Activation of FFAR4 helps maintain metabolic homeostasis by regulating adipogenesis, insulin sensitivity, and inflammation. While FFAR4 is best known for its role its role in preventing obesity and diabetes, recent studies have demonstrated that FFAR4 may also play an important role in the development of atherosclerosis and cardiovascular disease (CVD). Given FFAR4's importance in anti-inflammatory signaling and high expression levels in macrophages, we designed experiments to test the hypothesis that FFAR4 prevents the development of atherosclerosis by reversing macrophage foam cell formation, a hallmark of early atherogenesis. In these studies, we isolated peritoneal macrophages from wild-type C57/BL6J mice and incubated them with oxidized low-density lipoprotein (oxLDL) to generate foam cells. We then investigated the effects of FFAR4 activation by GW9508 (a synthetic agonist) on lipid accumulation, cytokine secretion, and cholesterol efflux. Activation of FFAR4 by GW9508 decreased macrophage secretion of pro-inflammatory cytokines. We also found that activation of FFAR4 with GW9508 reduced lipid accumulation in macrophages as observed by decreased Oil Red O staining and reduced cellular cholesterol content. Additionally, activation of FFAR4 by GW9508 increased [3H]-cholesterol efflux to high-density lipoprotein (HDL). Interestingly, the increased efflux was accompanied by decreased scavenger receptor CD36 expression (that mediates oxLDL uptake) and increased expression of ATP binding cassette transporters, ABCA1 and ABCG1 (that mediate cholesterol efflux). Taken together, our results support an exciting and novel role for FFAR4 in the reversal of foam cell formation, and could emerge this receptor as a new target for treating CVD by preventing accumulation of atherosclerotic plaque.

6.25 Heterodimerization of melatonin and cannabinoid type 1 receptors

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Membrane receptors belonging to the G protein-coupled receptor (GPCR) family form the largest family of proteins in the human genome with more than 800 members. Until recently GPCR functions were thought to occur only at the plasma membrane upon binding of their cognate ligand. However, evidences show that many functional GPCRs are found in intracellular compartments opening new direction of research to understand their roles in a cellular context. Among these intracellular compartments mitochondria are the latest organelle in which some GPCRs were identified. Melatonin receptor type 1 (MT1) and cannabinoid receptor type 1 (CB1) were identified in mouse neuronal mitochondria where they were shown to exert an inhibitory action on cytochrome c release (MT1) or on the respiratory chain (CB1). Using several techniques my current results describe a new cross-talk between MT1 and CB1 receptors. Confocal analysis of immunofluorescence experiments of cells coexpressing both receptors showed a high degree of colocalization. A combination of coimmunoprecipitation experiments performed on extracts of transfected HEK293T or HeLa cell lines and immunodetection of receptors by Western-blot revealed that MT1 and CB1 receptors can physically interact to form heterodimers in the absence of ligand. Heterodimer formation in mitochondria was also confirmed by Proximity Ligation Assay (PLA) experiments and colocalization with the mitochondrial marker TOMM20. Activation of MT1 and CB1 in mitochondrial preparations from mouse brain modulates mitochondrial respiration with synergistic effects at submaximal ligand concentrations. Taken together, my results indicate a novel intra-mitochondrial cross-talk of melatonin and endocannabinoids.

Flash Presentations Session 7

7.26 Design, synthesis and validation of photo-activable tools for melatonin receptors

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Melatonin (MLT) is a hormone that regulates physiological functions such as sleep and biological rhythms. These effects are mainly mediated by the activation of MT1 and MT2. MLT receptors are potential therapeutic targets, however, the mechanism by which they affect physiology is unclear. There is an interest in developing tools targeting MLT receptors. One interesting approach is to design ligands with photo-activable properties, as light modulates physiological systems with spatiotemporal precision, low toxicity and safety.

Here, we describe the synthesis and validation of photo-activable MLT receptor ligands, including photoswitches and caged molecules. Caged compounds are light-sensitive probes that covalently attach a photo-labile group into a biomolecule, rendering it inactive. Light triggers a photolytic reaction that releases the active molecule at the site of action. We synthesized four caged compounds, which release MLT with high yields upon illumination. Prior to uncaging, they displayed an important decrease in binding affinity for MT1 and MT2, and their potencies were correspondingly reduced. After illumination, the compounds showed excellent apparent pKi and EC50 values, in agreement with an efficient MLT release. Once validated the methodology, we applied these tools to study mitochondrial MT1 receptors.

Photoswitches are molecules that reversibly isomerize between two isomeric forms upon illumination, leading to molecular changes that are translated into different biological effects. We synthesized two compounds that photo-isomerize between trans and cis forms after applying suitable light conditions. Interestingly, trans-MCS-0468 was inactive on ERK and cAMP pathways for MT1, while cis-enriched form displayed an agonistic effect on both pathways. Interestingly, MCS-0468 did not induce β-arrestin2 recruitment on MT1, behaving as a biased cis-on ligand for G protein-mediated pathways on MT1.

In conclusion, we have developed the first photo-activable ligands for MLT receptors, presented as potential tools to study the mechanism of action of MT1 and MT2 and their implication in physiology.

7.27 Characterization of innovative animal toxins for the functional study of angiotensin receptors

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An overactivation of the renin angiotensin system (RAS) with increase in angiotensin II level is the main cause of arterial hypertension. AT1 receptor antagonists are goal standard for the treatment of this pathology. Nevertheless, other RAS related GPCRs (AT2, Mas and MrgD) are also involved in the regulation of blood pressure but are under exploited due to the lack of knowledge of their physiological properties. Furthermore, the development of biased ligand activating only certain intracellular signalling pathways is in full swing. The discovery of new ligands with original functions would be of great interest to better understand these receptors. Toxins from venomous animals are reticulated peptides that display antagonist, agonist or allosteric functions on GPCRs and because of their high target specificity and selectivity, they constitute a large reservoir of unexploited promising ligands.
The screening of a 800 toxins library was performed on the AT2 receptor by the mean of a radioligand binding assay and it highlighted three original toxins which originate from different organisms, with different structural profiles. These toxins display a micromolar affinity on AT2 and AT1 receptor and so far, pharmacological trials are more advanced on this receptor. A-CTX-cMila is a small cyclic toxin from the cone snail Conus miliaris, demonstrating a competitive antagonism on AT1 receptor with micromolar affinity, blocking both G-protein and b-arrestin pathways. A-CTX-MilVa, from the same cone snail, seems to be an AT1 agonist activating Gq protein pathway. Finally, A-TRTX-Por1a from the spider Poecilotheria ornata appears to display an agonist activity on AT1. These 3 toxins with their interesting structural and pharmacological profiles are the first toxins active on the RAS and may represent promising tools for the study of angiotensin receptors. More investigations are needed to fully characterize these toxins, especially on AT2. This work highlights a new family of GPCRs targeted by toxins.

7.28 Modulation of opioid receptor function by mediators of tissue injury and inflammation

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Opioid receptors are involved in modulation of painful tissue inflammation, wound healing and regeneration. We aim to identify inflammation-specific regulatory mechanisms of the mu-opioid receptor (MOR). We study binding, G-protein activation and downstream signaling pathways of MOR modulated by extracellular reactive oxygen species (H2O2) in vitro. Advancing such knowledge could lead to the development of novel compounds that target opioid receptors in injured tissue to promote tissue healing/regeneration and pain inhibition.

Flash Presentations in the Poster Room

Evolutionary History of Histamine Receptors: Early Vertebrate Origin and Expansion of the H3-H4 Subtypes

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Histamine receptors belonging to the superfamily of G protein-coupled receptors (GPCRs) mediate the diverse biological effects of biogenic histamine. They are classified into four phylogenetically distinct subtypes H1-H4, each with a different binding affinity for histamine and divergent downstream signaling pathways. Here we present the evolutionary history of the histamine receptors using a phylogenetic approach complemented with comparative genomics analyses of the sequences, gene structures, and synteny of gene neighborhoods. The data indicate the earliest emergence of histamine-mediated GPCR signaling by a H2 in a prebilaterian ancestor. The analyses support a revised classification of the vertebrate H3-H4 receptor subtypes. We demonstrate the presence of the H4 across vertebrates, contradicting the currently held notion that H4 is restricted to mammals. These non- mammalian vertebrate H4 orthologs have been mistaken for H3. We also identify the presence of a new H3 subtype (H3B), distinct from the canonical H3 (H3A), and propose that the H3A, H3B, and H4 likely emerged from a H3 progenitor through the 1R/2R whole genome duplications in an ancestor of the vertebrates. It is apparent that the ability of the H1, H2, and H3-4 to bind histamine was acquired convergently. We identified genomic signatures suggesting that the H1 and H3-H4 shared a last common ancestor with the muscarinic receptor in a bilaterian predecessor whereas, the H2 and the α-adrenoreceptor shared a progenitor in a prebilaterian ancestor. Furthermore, site-specific analysis of the vertebrate subtypes revealed potential residues that may account for the functional divergence between them.

Characterization of a Truncated Vasopressin V2 receptor in renal cancer: A novel target?

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Vasopressin V2 receptor (V2R) is a GPCR responsible to the antidiuretic effect in kidneys by activation of Gs/cAMP pathway. V2R is also implicated in proliferative pathologies such as cancers. We have shown that V2R is abnormally expressed in human renal cancer cells and antagonists of the receptor as Mambaquaretin, a toxin from green mamba venom, inhibit cell proliferation.
In addition to the Wild Type V2R, we characterized the presence of a splice variant in which the receptor is truncated and lacks the seventh transmembrane domain. The effect of this truncated form is poorly known but studies realized on V2R of Rat demonstrated a dominant-negative effect of truncated form on the Wild Type V2R by heterodimerization.
In order to understand the role of this truncated V2R in renal cancer, we evaluated its impact on the Wild Type form by transfection in CHO cells. We found that truncated form significantly inhibits the surface expression of Wild Type receptor and also cAMP production stimulated by vasopressin. The characterization of molecular mechanisms associated with this truncated V2R could lead to new therapeutic strategies for renal cancer.

Effects of the pharmacological chaperone UM0130866 on the cell-surface expression and signalling profile of melanocortin receptor type 4 (MC4R) variants associated with severe early-onset obesity

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Melanocortin type 4 receptor (MC4R) is a G-protein coupled receptor (GPCR) which central activation increases energy expenditure and decreases appetite. Individuals bearing a MC4R loss-of-function mutation can develop severe early-onset obesity. Many of those mutated receptors are misfolded and retained inside the cell, unable to reach their site of action. Pharmacological chaperones (PC) are membrane-permeable chemicals that can selectively promote proper folding and subsequent targeting of a GPCR, thus having the potential to rescue the function of mutants. Our goal is to develop MC4R PC that would promote agonist-induced activation toward signaling pathways important for weight loss in order to improve treatment efficiency and to reduce adverse effects. Here, we report that stimulation of the wild-type MC4R can strongly activate Gαs, Gα15, Gαz, and weakly activate both β-arrestins signaling pathways. The MC4R PC UM0130866 can restore the defective expression of the intracellularly retained and obesity-associated R165W MC4R variant, as well as its Gαs and Gα15 signaling pathways. Those results support our hypothesis that PC can rescue signalling pathways in a biased way, but more PC need to be tested to confirm.

AT1 receptor S-nitrosation : molecular and functional consequences

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The Angiotensin II type 1 receptor (AT1) has a key role in the regulation of the cerebrovascular system and in cerebrovascular pathologies like stroke. The renin-angiotensin system and the AT1 receptor are targeted by several classes of therapeutics, however, despite numerous beneficial effects in preclinical experiments, angiotensin receptor blockers (ARBs) in clinical trials failed to produce these beneficial effects in the acute phase of stroke. A better understanding of AT1 signaling and its mechanisms could lead to new therapeutic strategies to target the detrimental effects of AT1 activation.

Our previous works has shown on isolated cerebral arteries that pretreatment with Nitric oxide (NO) donors prevent the AT1-induced vasoconstriction involved in the deleterious effects of stroke. My thesis project aims to test the hypothesis that NO acts on AT1 by direct S-nitrosation (covalent bond between NO and a cysteine residue) promoting the stabilization of the alternative active conformation of AT1 known to block its Gq/contractile pathway without affecting its internalization nor its potential beneficial β-arrestin pathway (Delaitre et al., 2021).

Using cellular in vitro models and ex vivo models (isolated cerebral arteries), we will determine the impact of NO donors and S-nitrosation on AT1 signaling and internalization. In order to track AT1 internalization in tissues, new AT1-specific fluorescent probes have been synthetized and characterized.

Using cells overexpressing AT1, we found that our new fluorescent agonist act as a full agonist (Gq and β-arrestin activations; EC50= 822nM for calcium mobilization) but with a decrease in activity compared to angiotensin II (EC50= 15 nM). On isolated cerebral arteries, it induces vasoconstriction.

A new fluorescent antagonist shows an antagonistic power (pA2= 9.8) equivalent to that of its non-fluorescent compound (pA2= 8.2). Its pharmacological properties on cerebral arteries remains to be studied.

Our experiments validate these two probes and they will now enable to assess the impact of NO on internalization of endogenous AT1, consecutive to β-arrestin activation.

The orphan GPCR GPR50 and its involvement in the development of obesity/type2 diabetes

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Obesity is related to a misbalance between energy intake and expenditure. Thermogenic adipocytes in the subcutaneous white adipose tissue (WAT) possess a therapeutically attractive energy-expending capacity, namely a process called “browning”, while the alteration of this process can lead to the development of obesity and type 2 diabetes. This mechanism is conventionally induced by cold or by ligand-dependent activation of b3-adrenergic G protein-coupled receptor (GPCR). Here, we uncover an alternative paradigm of GPCR-mediated thermogenesis of white fat through the orphan receptor of the melatonin receptor family, GPR50. We show that GPR50 is expressed in subcutaneous WAT and isolated adipocytes and that the intracellular domain of GPR50 (ICD) can be cleaved and directly regulates the transcriptional induction of thermogenic genes conferring the browning capacity of GPR50. The process of “WAT browning” in GPR50-KO mice is impaired, while the rescued expression of the human GPR50 gene in KO animals or of the ICD in GPR50-KO adipocytes can restore the loss of the browning process subsequent to the absence of the receptor. In accordance with an impaired browning function, GPR50-KO mice are more obese and glucose intolerant than control littermates when fed a high fat diet. Taken together, our findings reveal a noncanonical mechanism for controlling WAT thermogenic activity through an original functional mechanism of GPCR signalling.

Carfentanil is an arrestin-biased agonist at the μ opioid receptor

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Introduction:
Fentanyl is a highly potent synthetic μ opioid receptor (MOPr) agonist with many structural analogues (referred to as fentanyls) including the extremely potent carfentanil. Here we investigated the signalling bias of carfentanil and other fentanyls.

Methods:
Agonist-induced G protein activation or Arrestin-3 recruitment was measured using bioluminescence energy transfer (BRET). Agonist-induced internalisation of MOPr was measured using a post-labelling enzyme linked immunosorbent assay (ELISA).
Whole cell voltage-clamped electrophysiological recordings were taken of carfentanil- and fentanyl-evoked GIRK currents in locus coeruleus (LC) neurones of male Wistar rats (4 weeks old).
One-way ANOVA with Tukey's multiple comparisons was carried out in Graphpad Prism 9.0.
Results:
The rank order of potency for agonists in the G protein activation and arrestin-3 recruitment BRET assays was carfentanil > sufentanil > fentanyl > DAMGO = alfentanil > morphine. Carfentanil produced a higher maximum response than DAMGO and the other fentanyls for arrestin recruitment and was also the only agonist that was more potent for arrestin recruitment compared to G protein activation (EC50: 3.6 ± 0.9 nM and 8.2 ± 1.1 nM respectively). Carfentanil displayed potent internalisation of MOPr compared to fentanyl and DAMGO (EC50: 0.8 ± 0.3 nM, 135 ± 32 nM and 982 ± 264 nM respectively).Bias relative to DAMGO was determined using ΔΔLogTR analysis, for G protein activation, arrestin recruitment and MOPr internalisation. Carfentanil was the only drug significantly different from DAMGO, in both the arrestin and internalisation direction when compared to G protein activation ($p<0.001$ and $p<0.005$ respectively). Carfentanil and fentanyl both induced desensitization of MOPr-mediated GIRK currents in LC neurones, the 10 minutes post-peak desensitisation of carfentanil was greater than that of fentanyl ($p=0.01$). This carfentanil desensitisation was blocked by compound101, a GRK inhibitor.
Conclusions:
Carfentanil, unlike fentanyl itself, is an arrestin-biased agonist. The functional consequences of carfentanil's arrestin bias appear to be highly potent internalisation of MOPr and increased desensitisation of the receptor.

The Orphan Class A GPCR 68 is Involved in the regulation of the hypothalamic melanocortin system

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Obesity has emerged as a worldwide public health problem. G-protein coupled receptors (GPCRs) are potential candidates for obesity drug therapy as some of these receptors may be involved in the regulation of the hypothalamic melanocortin system controlling food behavior and energy homeostasis by integrating satiety and adiposity signals. The consumption of a high fat diet (HFD) impairs the function of POMC-expressing neurons resulting in whole body energy imbalance. We hypothesized that GPCR68 and GPCR162 are activated in response to saturated fat acids in the hypothalamus, playing a role in the regulation of the melanocortin system. In order to identify orphan GPCRs, we performed a search in the GPCR database (https://gpcrdb.org/) that resulted in the identification of 81 class A orphan receptors. Then, we checked in the human database (https://www.genecards.org/) the orphan receptor mRNA expression in the hypothalamus. We analyzed the expression and modulation of GPCR68 and 162 in the hypothalamus of mice fed a chow or HFD for during 1, 3, 7, 14 and 28 days. The hypothalami were harvested for gene expression analysis. Both GPCRs were expressed in the hypothalamus, but only gpr162 was not modulated by HFD. The gpr68 was significantly increased in the hypothalamus of mice fed a HFD for 7, 14 and 28 days. Finally, the intracerebroventricular (ICV) treatment with positive and negative allosteric modulator of GPR68 activated hypothalamic neurons and the bilateral microinjection directly into the arcuate nucleus modulated anorexigenic neuropeptides. So far, these data suggest that the GPCR68 may act as a constitutively active receptor in response of saturated fatty acids, the treatment with negative allosteric modulator activated hypothalamic anorexigenic neurons contributing to the control of energy homeostasis and important new target in diet-induced obesity in hypothalamus.

Identification of an orphan GPCR, GPR151, as a target of BPA exposure and a potential actor of the GnRH network

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Bisphenol A (BPA) is a ubiquitous endocrine disruptor chemical that has been shown to alter pubertal timing. Recently, we demonstrated that an early postnatal exposure to BPA disturbed the neuroendocrine sexual maturation in female rat through altered GABAergic neurotransmission. A dose of 25 ng/kg/d induced a slowing down of GnRH secretion frequency while acceleration of GnRH secretion was measured after a dose of 5 mg/kg/d at PND 20. The results of an RNAsequencing analysis of mediobasal hypothalamus RNAs revealed that several genes were affected by these two doses of BPA (Franssen et al, 2016). Now, we show that GPR151 is the gene most affected and in an opposite way by the 2 doses since mRNA levels increase after the low dose of BPA and decrease after the high dose. We describe for the first time that this orphan GPCR sharing sequence homology with GPR54 is expressed in the fibers of GnRH neurons in the median eminence of pubertal and adult female rats. In this region, GPR151 mRNA expression increases throughout postnatal development. Because β-arrestin is responsible for GPCR internalization after GPCR activation by a ligand, we analysed the capacity of several neuropeptides (kisspeptin, galanin and RFRP3) to activate the receptor and induce the recruitment of β-arrestin 2 using a β-arrestin recruitment assay. None of these peptides induced this recruitment but we identified a high constitutive activity for GPR151.Using a GnRH cell line (GnV3 cells), that expresses GPR151, we highlight a potential role for this receptor in the mechanisms of GnRH release. The overexpression of GPR151 in GnV3 cells leads to an increase of GnRH release from these cells.
In conclusion, early postnatal exposure to BPA altered the onset of puberty in female rats through disruption of the GnRH release. This effect could involve changes in expression of a potential new regulator of the GnRH network, GPR151.

Molecular Investigation of Cell Free Expressed Neuropeptide Y4 Receptor

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The neuropeptide Y multiligand/multireceptor system consists of four receptor subtypes (neuropeptide Y1 receptor (Y1R), Y2R, Y4R and Y5R) and the three peptide ligands neuropeptide Y (NPY), peptide YY (PYY) and pancreatic polypeptide (PP). The Y4R with its high affinity binding ligand PP is involved in the transduction of anorexigenic signals making the receptor a potential target for the development of drugs against obesity. Detailed structural information about the Y4R are necessary for specific targeting. Therefore, the receptor is expressed and analysis by using photo-crosslinking and mass spectrometry could be performed. As an expression system we use a continuous exchange cell-free system based on E. coli BL21 S30 extract. A coupled in vitro transcription and translation is performed and the receptor is expressed as precipitate. During a subsequent reconstitution step, the receptor is incorporated into bicelles. Cys-deficient receptor variants were generated and expressed since the cysteine residues might cause folding problems and led to inactive receptor. For structural investigations, a photo-crosslinking of the ligand and the identification of the binding pocket by using mass spectrometry is suitable. Peptides containing a photoactive amino acid for photo-crosslinking were successfully synthesized and their activity was confirmed by using a cell-based assay. The establishment of this system contributes to the investigation of the molecular interactions of PP binding to the Y4R.

The G protein database, GproteinDb

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Two-thirds of signaling substances, several sensory stimuli and over one-third of drugs act via receptors coupling to G proteins. Here, we present an online platform for G protein research with reference data and tools for analysis, visualization and design of scientific studies across disciplines and areas. This platform may help translate new pharmacological, structural and genomic data into insights on G protein signaling vital for human physiology and medicine. The G protein database is accessible at https://gproteindb.org

Gene therapy with PDE2A improves cardiac function and limits ventricular arrhythmias in a mouse model of heart failure

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The chronic elevation of catecholamines in heart failure (HF) promotes cardiac remodeling and pump dysfunction via the activation of adrenergic receptors (AR). Various cyclic nucleotide phosphodiesterases (PDEs) finely tune ß-AR responses by degrading and compartmentalizing cAMP. Among these, PDE2A is upregulated in HF. Because its overexpression limits hypertrophy and PDE2A-transgenic mice are protected against catecholamine-induced arrhythmias, we sought to evaluate the cardioprotective effect of a cardiac gene therapy with PDE2A in a HF mouse model. C57BL/6N male mice were injected with serotype 9 adeno-associated viruses (AAV9, 10^12 vp/mouse) encoding for PDE2A, or luciferase (LUC) and 2 weeks later, were implanted with osmotic minipumps infusing NaCl or isoprenaline (Iso) and phenylephrine (IP, 30 mg/kg/day each) during 2 weeks. In LUC mice, echocardiography unveiled that IP treatment increased left ventricular weight over body weight ratio by 35±5% (p=0.0001) and decreased ejection fraction by 29±2% (p<0.0001). Both parameters were improved by PDE2A overexpression (p=0.039 and p=0.0003, respectively). To further characterize cardiac dysfunction, isolated ventricular myocytes were loaded with 1 µM Fura-2AM and stimulated at 1 Hz to record calcium transients (CaT) and sarcomere shortening (SS). In myocytes from NaCl-LUC mice, ß-AR stimulation (3 nM Iso) increased SS by 538% and CaT by 78% (p<0.0001). These effects were blunted in cells from IP-treated animals (SS: p=0.0003, CaT: p<0.0001 vs NaCl) but preserved in mice injected with AAV9-PDE2A (SS: p=0.044, CaT: p=0.0027 vs IP). Polymorphic ventricular tachycardia were triggered by ventricular pacing after Iso (1.5 mg/kg) and atropine (1 mg/kg) injection in LUC mice treated with IP (p=0.016) but not in animals treated with AAV9-PDE2A. These results reveal that gene therapy with PDE2A limits cardiac hypertrophy and dysfunction induced by catecholamines as well as ventricular arrhythmias.

Evolutionary information helps understand distinctive features of the angiotensin II receptors AT1 and AT2 in amniota

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In vertebrates, the octopeptide angiotensin II (AngII) is an important in vivo regulator of the cardiovascular system. It acts mainly through two G protein-coupled receptors, AT1 and AT2. To better understand distinctive features of these receptors, we carried out a phylogenetic analysis that revealed a mirror evolution of AT1 and AT2 split into two clades, separating fish from terrestrian receptors. It also revealed that hallmark mutations occurred at, or near, the sodium binding site in both AT1 and AT2. Electrostatics computations and MD simulations support maintained sodium binding to human AT1 with slow ingress from the extracellular side and an electrostatic component of the binding free energy around -3kT, to be compared to around -2kT for human AT2 and the δ opioid receptor. Comparison of the sodium binding modes in wild type and mutated AT1 and AT2 from humans and eels indicates that the allosteric control by sodium in both AT1 and AT2 evolved during the transition from fish to amniota. The unusual S7.46N mutation in AT1 is mirrored by a L3.36M mutation in AT2. In the presence of sodium, the N7.46 pattern in amniota AT1 stabilizes the inward orientation of N3.35 in the apo receptor, which is mandatory for biased signalling. The M3.36 pattern in amniota AT2 favours the outward orientation of N3.35 and the receptor promiscuity. Both mutations should contribute to the split of the renin-angiotensin system into the classical (AngII/AT1) and counter-regulatory (Ang1-7/AT2, Mas) arms in amniota.

Specific Targeting of Cancer Cells using the CMKLR1 Receptor and Chemerin 9

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Chemerin is a small chemotactic protein and a modulator of the innate immune system that mainly operates by activating the GPCR chemokine-like receptor 1 (CMKLR1). Its bioactivity is controlled by proteolytic cleavage of the C-terminus [1]. A C-terminal nonapeptide named chemerin 9 (C9) was developed and has been to activate the CMKLR1 with similar potency as wild type chemerin [2]. Recently, the overexpression of the CMKLR1 receptor on different tumors was described, offering a potential entry point for targeted delivery of chemotherapeutics [3].
In this study, we evaluate small synthetic peptides based on chemerin 9 that are biologically active and stable in blood plasma. First studies using bioluminescence resonance energy transfer (BRET) show efficient recruitment of arrestin to the CMKLR1 receptor after stimulation with chemerin 9 and its cyclic derivatives. In addition, we are able to confirm the peptide uptake into model cell lines using fluorescencently labeled variants of the peptides. Furthermore, we were able to design a shuttling system with the small molecule methotrexate, a chemotherapeutic agent, attached to cyclic chemerin 9. We demonstrate the efficiency and specificity of the peptide shuttling system with different cell types.
These data demonstrate that our peptide is suitable for specific delivery of toxic small molecule cargoes into cells and it represents an important step towards targeting CMKLR1 in cancer therapy to reduce side effects by unspecific drug uptake [4].

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Identification of the epitope of a nanobody for the mGlu2 receptor by molecular modelling

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Schizophrenia is a mental disorder that affects approximately 1% of the worldwide population. The pathophysiology behind this disorder is complex, hence it was proven to be related to the deficiency of glutamate signaling in the brain. Amongst glutamate targets, the metabotropic glutamate receptors (mGluRs) are interesting targets for the treatment of neurological diseases. Due to the highly conserved orthosteric binding site of mGluR subtypes, scientists developed an alternative approach to target mGluRs using camelid nanobodies as allosteric modulators. A nanobody is a small size antibody fragment made of the heavy chain-only. It usually recognizes conformational epitopes and thus it can behave as a positive allosteric modulator that potentiates the effect of an orthosteric ligand. Its unique properties made it an interesting therapeutic agent. Here, we report the epitope identification of the nanobody Nb2 on the receptor mGlu2 that binds both active and inactive conformations of mGlu2. We used molecular modeling techniques like docking and molecular dynamics. Among 17 common poses resulting from the docking of Nb2 to the active and inactive conformations of mGlu2, only three were selected and are currently being evaluated experimentally.

Cryo–electron microscopy structure of the antidiuretic hormone arginine-vasopressin V2 receptor signaling complex

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The antidiuretic hormone arginine-vasopressin (AVP) forms a signaling complex with the V2 receptor (V2R) and the Gs protein, promoting kidney water reabsorption. Molecular mechanisms underlying activation of this critical G protein– coupled receptor (GPCR) signaling system are still unknown. To fill this gap of knowledge, we report here the cryo–electron microscopy structure of the AVP-V2R-Gs complex. Single-particle analysis revealed the presence of three different states. The two best maps were combined with computational and nuclear magnetic resonance spectroscopy con- straints to reconstruct two structures of the ternary complex. These structures differ in AVP and Gs binding modes. They reveal an original receptor-Gs interface in which the G?s subunit penetrates deep into the active V2R. The structures help to explain how V2R R137H or R137L/C variants can lead to two severe genetic diseases. Our study provides import- ant structural insights into the function of this clinically relevant GPCR signaling complex.

The atypical chemokine receptor ACKR3/CXCR7 is a broad-spectrum scavenger for opioid peptides and the target of the natural analgesic conolidine

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Endogenous opioid peptides and prescription opioid drugs modulate pain, anxiety and stress by activating opioid receptors, currently classified into four subtypes (mu, delta kappa and nociceptin receptors). Here we demonstrate that ACKR3/CXCR7, hitherto known as an atypical scavenger receptor for chemokines, is a broad-spectrum scavenger of opioid peptides. Phylogenetically, ACKR3 is intermediate between chemokine and opioid receptors and is present in various brain regions together with classical opioid receptors. Functionally, ACKR3 is a scavenger receptor for a wide variety of opioid peptides, especially enkephalins and dynorphins, reducing their availability for the classical opioid receptors. ACKR3 is not modulated by prescription opioids such as morphine, fentanyl or naloxone, but we show that a new ACKR3-selective subnanomolar competitor peptide, LIH383, and conolidine, a natural analgesic used in traditional Chinese medicine, can restrain ACKR3‘s negative regulatory function on opioid peptides in vitro and in rat brain and potentiate their activity towards classical receptors. Altogether, our results reveal that ACKR3 is an atypical opioid receptor (AOR) with cross-family ligand selectivity, which may open alternative therapeutic avenues for opioid-related disorders.

Intracellular GPCR signaling involved in chronic pain

Alan Hegron, Nigel Bunnett

Background: Chronic pain is a major unmet medical problem. While 30% of drugs on market target G-protein coupled receptors (GPCRs), very few act on GPCRs involved in pain. The mechanisms that underlie the transition from acute (physiological) to chronic (pathological) pain are poorly understood and current therapies are inadequate. Proteases, substance P (SP) or calcitonin gene related peptide (CGRP) are released during injury and activate their corresponding receptors, Protease activated receptor 2 (PAR2), Neurokinin 1 receptor (NK1R) or Calcitonin receptor-like receptor (CLR) leading to specific intracellular signaling pathways involved in hyperexcitability and nociception. When activated, these GPCRs internalize and continue to signal from endosomes. Although, PAR2, NK1R and CLR have been implicated in chronic pain, extensive characterizations and the development of new tools to fight chronic pain are in need.

Hypothesis: Specific endosomal signaling pathways activated by PAR2, NK1R and CLR are responsible for chronic pain.

Methods: Our primary aim was to functionally characterize these GPCRs. We took advantage of mini-G proteins (mGs) recognizing active conformations of GPCRs to characterize PAR2, NK1R and CLR at the cell surface and in endosomes using a bioluminescence resonance energy transfer (BRET) assay monitoring recruitment of mGs and βarrestin-2 to cytoplasmic membrane or early endosomes. Our second aim was to develop and characterize inhibitors encapsulated in nanoparticles specifically targeting GPCRs in endosomes where pH is low.
Results: Results obtained by the functional characterization indicate different signaling signatures depending on the GPCR. Also, blocking endocytosis inhibited signaling from endosomes.
Conclusion: Characterization of GPCR signaling from different cell compartments will help develop new tools, like nanoparticles, to stop chronic pain with less side effects than actual treatments.

5-HT2AR/AMPAR Heteroreceptor Complex As A Mediator Of LSD Action

Etienne Billard, Terry Hébert

BACKGROUND.

The administration of psychedelic compounds in controlled clinical settings leads to antidepressant, anxiolytic effects, as well as changes in brain activity and connectivity. Lysergic acid diethylamide (LSD) is a potent biased agonists of the G protein-coupled receptor (GPCR) serotonin 2A (5-HT2AR). LSD also modulates the glutamatergic system, especially through the receptor AMPA (AMPAR). LSD enhances social behavior and has anxiolytic effects in rodents by promoting synaptic plasticity via an AMPAR-dependent mechanisms, and in layer V pyramidal neurons psychedelics are able to induce phosphorylation of AMPAR GluA2 subunit at Ser880 and promote its internalization. As several GPCRs (dopamine D2 and β2-adrenergic receptors) can form supramolecular complexes with AMPAR, the main hypothesis of this project is that LSD-mediated AMPAR activation can occur via an oligomeric AMPAR/5-HT2AR complex.

Methods

To demonstrate physical interactions between AMPAR and 5-HT2AR we used co-immunoprecipitation and proximity ligation assays in HEK-293 cells. To characterise the unique signalling fingerprint of this heteroreceptor complex we used BRET-based biosensors.

Results

We have demonstrated that 5-HT2AR and AMPAR can be co-immunoprecipitated, thus being part of a signalling complex. Proximity ligation assays confirmed such physical interaction and proved that 5-HT2AR/AMPAR complex is present at the plasma membrane. Cotransfection of AMPAR with 5-HT2AR did not impact Gq activation following treatment with either 5-HT or LSD, but affected both potency and efficacy for β-arrestin2 activation, demonstrating the impact of AMPAR in biasing 5-HT2AR signaling through lateral allostery.

Conclusion

Our in vitro data support the existence of a 5-HT2AR/AMPAR hetero-oligomers and suggests further research in primary neuronal culture and in vivo is required. We believe that the 5-HT2AR/AMPAR could be regarded as a new therapeutic target for treating mental illness.

Evidence for two modes of binding of the negative allosteric modulator, SB269,652, to the dopamine D2 receptor

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SB269,652 has been described as the first negative allosteric modulator (NAM) of the dopamine D2 receptor D2R, but the binding mode and allosteric mechanism of action of this ligand remain incompletely understood. SB269,652 comprises an orthosteric, primary pharmacophore and a secondary (or allosteric) pharmacophore, joined by a hydrophilic cyclohexyl linker and is known to form corresponding interactions with the orthosteric binding site (OBS) and the secondary binding pocket (SBP) in D2R.

In initial studies, we observed a surprisingly low potency of SB269,652 to antagonize G protein-coupled inward rectifier potassium channels (GIRK) activation induced by dopamine and decided to perform a more detailed investigation of the interaction between these two D2R ligands. The evidence thus gathered suggest an induced-fit mode of action, whereby SB269,652 inhibitory potency is increased by 6-fold upon preincubation, as compared to simultaneous application of both ligands. Mutagenesis experiments implicated both a conserved serine, S1935.42, in the OBS, and E952.65, previously reported to be crucial for the allosteric properties of SB269,652, in this induced-fit binding mode. Unexpectedly, alanine mutation of W100 in extracellular loop 1 (ECL1), which has recently been observed to prevent induced-fit insurmountable antagonism of bivalent D2R ligands, increased the effect of preincubation on SB269,652 antagonistic potency.

The present findings extend previous knowledge about how SB269,652 competes with the native agonist, dopamine, at the D2R, and may be useful for the development of novel D2R ligands, such as antipsychotic drug candidates.

Inactivation of the ACKR3 by a small-molecule inverse agonist

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The atypical chemokine receptor type 3 (ACKR3) is frequently explored as a drug target for the treatment of various diseases, like cancer and cardiovascular disease. Until recently, all characterized small-molecule-ligands that bind the ACKR3 were agonists and it was shown that the ACKR3 had a high propensity for activation when compared to other chemokine receptors.[1,2,3]

We identified an ACKR3 antagonist in the patent literature, which is an analog of the clinical candidate ACT-1004-1239 of Idorsia.[1] The antagonist (VUF16840) was synthesized and its effect on the ACKR3 was characterized. VUF16840 is confirmed to antagonize chemokine induced receptor activation. It is additionally shown that this ligand stabilizes the inactive conformation of the ACKR3, as is evident from a dose-dependent inhibition of constitutive receptor signalling. Despite the distinct intrinsic activity of VUF16840 at the ACKR3, site-directed mutagenesis studies show a partial overlap in receptor binding interactions between VUF16840 and a small-molecule agonist. It is finally shown that VUF16840 has selectivity for the ACKR3 over all other human chemokine receptors. Some off-target activity was observed for the CCR3, which is the only other chemokine receptor that is modulated by VUF16840 at a concentration of 1 μM.

We therefore suggest that VUF16840 constitutes a valuable tool compound for probing the utility of ACKR3 as a drug-target. Moreover, the pharmacological characterization of ACKR3 binding site might facilitate further design of new agonists and antagonists.

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