UPPSALA
UNIVERSITET

Annual Report 2014

Department of Pharmaceutical Biosciences

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The scientific reports were written by the different research groups.

Introduction

This annual report highlights the research activities in the department of pharmaceutical biosciences during 2014. The research areas cover many different areas, including research on basic mechanisms of drug action, drug dependency and adverse health effects, as well as studies on drug metabolism, pharmacokinetics and pharmacodynamics. In addition, there is also a significant focus on pharmaceutical bioinformatics and proteomics, as well as pharmacometric modeling and simulations used in drug development. Some research groups are developing tools directly applicable to the pharmaceutical industry, while others are studying issues such as drug dependency and environmental contaminants with important socioeconomically implications. This report confirms that the research groups continue to have national and international collaborations within their research areas. Another important activity is the teaching we promote, and most of our junior lecturers, senior lecturers, and professors, and also some of our researchers, are heavily engaged in our education on various programs (focusing on pharmacy students). Most of our education is research-based and it is our aim to ensure that there is a synergy between teaching and research. Besides providing professional pharmacy education, the department also offers graduate pharmacy students specialization in clinical pharmacy.

Funding

All research activities require funding from national and international research councils, pharmaceutical companies, and the government. For example, in 2014 major grants were obtained from various national and international research councils such as the Swedish Foundation for Strategic Research (VR and FORMAS), EU Innovative Medicines Initiative, Research and Innovation for Sustainable Growth (VINNOVA), the Swedish Association of the Pharmaceutical Industry (LIF), Swedish Fund for Research without Animal Experiments, the National Institute on Drug Abuse (NIDA), Kjell and Märta Beijers Foundation, Carl Tryggers Foundation, Science for Life Laboratory (SciLife), Swedish Council for Working Life and Social Research, Swedish Insitute, Uppsala University, the Alcohol Research Council of the Swedish Alcohol Retailing Monopoly, Berzeli Centre for Biotechnological Research, the Research Council of Swedish Criminal Care, the Swedish Governmental Agency for Innovation Systems, the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning, Genentech, EU FP7 Health, Innovative Medicines Initiative (IMI), Swedish Cancer Society, the National Board of Health and Welfare, National Institute of Health (NIH), AFA Försäkring. Many projects also have financial support from pharmaceutical companies such as AstraZeneca, Janssen Pharmaceuticals, Novartis, Pfizer and Roche.

Organisation and financial review

The department is organised on the basis of our main core activities, i.e., research, teaching and the collected support activities (i.e., management, finance/staff administration, education administration and infrastructure). All support activities are funded by a percentile overhead on salaries and operating costs. The government provides the basic funding for teaching and research. The peer-reviewed scientific publications, PhD examinations and the external grants provide the foundation for any decisions concerning the allocation of university funding of research. The revenues from research councils, EU, foundations and pharmaceutical companies are very important to increase the research activities at the department. Below follows a short summary of incomes and expenditures 2014.

Major incomes 2014	*(kSEK)*
Research and graduate education- government	35 690
Research grants – research councils	35 174
Research – commissioned	12 914
Education – basic and advanced level –government	39 489

Major expenditures 2014	*(kSEK)*
Staff costs	66 988
Operating expenses etc.	12 726
Premises	13 696
University/faculty support activities	13 155
Library	2 473
Depreciation	2 690
Travels	3 384

Core facilities and other engagements

Apart from being involved in regular research and teaching activities, our department also host two major core facilities, NRMSI and UUBF. We are also involved in the platform UPPNEX hosted by the SciLifeLab. The department is also engaged in "the third mission", mainly by hosting and leading the activities of U-FOLD. In addition, the department of Pharmaceutical Biosciences is also one of the participants in the EU-supported education project SafeSciMet, involving both the Academy and Pharmaceutical Industry in several European countries, and we are also one of the participants in SweTox, a national resource centre for academic interdisciplinary collaboration within toxicology sciences.

The National Resource for Mass Spectrometry Imaging (NRMSI) was founded at the Uppsala University in 2010 with funds provided by the Swedish Research Council-Research Infrastructure in 2010 and Uppsala University-Infrastructure in 2013. The mission of the resource (previously named NCMSI) is to accelerate the adoption of

MALDI (matrix-assisted laser desorption ionization)-MSI technology in the biological and medical research community. NRMSI provides access to advanced technologies and promotes interactions between scientists who are experts in these technologies and researchers conducting focused biological investigations. Such collaborations provide new insights into the targeted biological problems and a better understanding of health and disease at the molecular level. Another important activity of the NRMSI is training, informing scientists about the capabilities and limitations of the technology, good practices, and to established methods and protocols. NRMSI uses state-of-the art mass spectrometry technologies and cutting edge research to help projects in e.g., drug discovery and development, neuroscience, oncology and pathology applications. NRMSI is located at Dr. Per Andrén´s Medical Mass Spectrometry research group at the Dept. of Pharmaceutical Biosciences.

Uppsala University Behavioral Facility (UUBF) was established in 2011 through strategic funding from the Faculty of Medicine and Pharmacy. UUBF functions as a non-profit core facility that administers and organizes experimental behavioral testing of mice, rats and fish. UUBF has a large variety of behavioral tests and can assist with everything from providing the experimental apparatus to performing the complete experiment. The aim of UUBF is to optimize access to well validated tests for research groups at Uppsala University as well as for external researchers, and promote the further development of high quality behavioral tests. UUBF provides equipment and professional assistance for behavioral experiments as well as data analysis and interpretation. At the core facility, several tests for motor behavior, learning and memory tests, sensorimotor tests and cognition can be run. UUBF also provides specific software programs for behavioral tracking and recording, and advanced statistical analyses including multivariate data analysis. The primary contact persons at our department are: Associate Professor Erika Roman and Dr. Sara Ekmark-Lewén. E-mail: uubf@farmbio.uu.se. Homepage: http://www.farmbio.uu.se/Corefacility/uubf/

The Bioinformatics Compute and Storage facility (UPPNEX) at Science for Life Laboratory (SciLifeLab) is headed from the Department of Pharmaceutical Biosciences. The facility provides high-performance computing and storage resources, maintain relevant bioinformatics software and data (e.g. reference genomes), and offer educational courses and user support. The facility had over 500 active projects during 2014 with computational resources are hosted at UPPMAX - Uppsala University's resource for high-performance computing and a node in the Swedish National Infrastructure for Computing (SNIC). The facility had 4.5 FTE during 2014 and is funded by SciLifeLab and BILS. The primary contact person at our department is: Associate Professor Ola Spjuth, Manager of the Bioinformatics Compute and Storage facility and Co-Director of UPPMAX. E-mail: ola.spjuth@farmbio.uu.se Homepage: http://www.scilifelab.se/facilities/uppnex/.

U-FOLD is a forum for research on addiction to medical products and illegal drugs. This network is the link between research, prevention and treatment activities in Uppsala regarding different types of addiction. The network brings broad experience and different perspectives and the major aim of the activities within U-FOLD is to contribute to greater understanding and a common approach regarding the underlying chain of events leading to addiction. Within the network we can create new methods to

prevent drug addiction, including preventive measures, care and legal actions. U-FOLD gather today twenty participants from academia, government, and nonprofit organizations. U-FOLD has lowered the threshold for new appointments and flow of ideas with clear practical effects. During 2014, the first education in cannabis prevention was completed, and the project on children in abusive environments initiated by Regionförbundet and U-FOLD, received further support. Also, a pilot study on prevention strategies against pathological gambling supported by the Public Health Agency, Sweden, was initiated during 2014. In addition, leading research groups related to U-FOLD received grants from Swedish Research Council in the highly competitive area of addiction diseases. A number of meetings and seminars have been arranged by U-FOLD, most of them focused on hot topics related to the area of addiction. During 2014, U-FOLD also participated at several mational radio- and TV-programmes. More information (still only in Swedish) can be found on http://www.ufold.uu.se

SafeSciMET is a European education and training network, developing and establishing a comprehensive education programme in safety sciences for medicines. Teachers at 15 European universities and experts from the pharmaceutical industry and regulatory authorities have together developed 20 courses in the area. The courses are especially designed to support scientists and professionals in medicines research and development and address the needs in small and large pharmaceutical companies, regulatory authorities, academic institutes and health care. The courses in safety sciences are clustered within five domains and each domain deals with one or more specialized topics and contains from two to six individual courses. Professor Lennart Dencker was among those that initiated and developed this education and training programme. He was responsible for the Student Office that manages the students, the SafeSciMET website and the web-based administration/registration tool. After a number of years in this project he left during the autumn 2014 due to retirement. His efforts were essential and much appreciated within the consortium. Professor Eva Brittebo will now carry on and take responsibility for the Student Office. The progress in the SafeSciMET project has put new demands on website and the Executive committee therefore decided that a new attractive website should be developed for all kind of devices. During the end of 2014 the development of the public website started. The focus of the new website will be on external information and the presentation of courses will be directed towards prospective students and staff from pharmaceutical companies.

The Swedish toxicology sciences research center (Swetox) is based on a consortium of eleven Swedish universities. Swetox was established as a national resource for academic interdisciplinary collaboration within toxicology sciences in 2013 and it promotes research, education and societal interactions related to chemicals, health and environment. A Swetox hub at Södertälje has been established as a research centre in modern laboratory facilities for safety assessment of pharmaceuticals. These facilities were closed-down by AstraZeneca in 2012. A vice chancellor council and a board govern the Swetox consortium. The board consists of representatives from all consortium universities and Professor Eva Brittebo is the ordinary representative from Uppsala University.

Future development

The years ahead promise many changes in terms of research and education and one can only hope that we will be successful in our attempts to get our different types of activities financially supported so that we can keep the high level of research and teaching also in the future. What we do know is that a couple of professors, teachers and members of our technical and administrative staff retired during 2013/2014, and more staff members will retire during 2015/2016. It is indeed a real challenge to find equally good replacements, but at the same time, it will also provide many possibilities for renewal. New professors under recruitment are one professor in pharmacology, and one professor in pharmaceutical cell biology, and they will hopefully have joined us and started up their research activities during 2015.

Uppsala May 25, 2015
Björn Hellman

List of Contents

Organisation 2014

Chairman
Björn Hellman

Deputy chairman
Mats Karlsson

Department board
Björn Hellman, *chairman*
Mats Karlsson*, teacher representative*
Margareta Hammarlund-Udenaes*, teacher representative*
Maria Kjellsson*, teacher representative*
Siv Jönsson, *teacher representative*
Jörgen Bengtsson, *teacher representative*
Mathias Hallberg, *teacher representative*
Lena Bergström, *teacher representative, deputy*
Karin Tjäder*, technical/administrative representative*
Elisabeth Jonsson *technical/administrative representative*
Patrik Källback, *graduate student representative*
Ida Netterberg*, graduate student representative, deputy*
Björn Clausson, *student representative*
Desirée Legetth, *student representative, deputy*
Marina Rönngren*, personnel coordinator, adjunct*
Agneta Hortlund, *economical coordinator*, *adjunct*
Mikaela Andersson, *secretary, adjunct* (until February 2014)
Stina Silander, *secretatary, adjunct* (from February 2014)

Professors
Georgy Bakalkin
Eva Brittebo
Lena Friberg (from December 2014)
Margareta Hammarlund-Udenaes
Björn Hellman
Mats Karlsson
Ingrid Nylander
Ernst Oliw
Jarl Wikberg
Kjell Wikvall

Professor emeriti
Lennart Paalzow
Lennart Dencker

Senior Professor
Fred Nyberg

Adjunct Professors
Jan Kehr
Markus Friden

Senior lecturers
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Jörgen Bengtsson*
Lena Bergström, *Associate professor*
Agneta Freijs
Lena Friberg, *Associate professor* (until December 2014)
Mathias Hallberg, *Associate professor*
Ronnie Hansson
Andrew Hooker
Ulrika Simonsson, *Associate professor*
Anne-Lie Svensson
Erika Roman, *Associate professor*
Elisabet Nielsen
Henrik Alm* (until September 2014)
Ola Spjuth (from January 2015)

Postdocs, Researchers and PhD students
Listed in the scientific reports

Junior lecturers
Ann-Marie Falk
Lena Klarén
Anna-Karin Lidehäll*
Emma Lundkvist
Maria Swartling
Maria Ellgren*
Åsa Johansson*
Srebrenka Dobric*
Oskar Karlsson*
Loudin Daoura*

Directors of undergraduate studies
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Jörgen Bengtsson
Eva Brittebo
Ann-Marie Falk
Mathias Hallberg
Björn Hellman
Lena Klarén
Elisabet Nielsen
Ingrid Nylander
Anna-Karin Lidehäll
Jarl Wikberg
Kjell Wikvall

Laboratory staff
Jessica Dunhall
Raili Engdahl (until June 2014)
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Lena Norgren

Technical and administrative staff
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Agneta Hortlund
Johanna Svensson
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Karin Tjäder
Karin Elg*
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Arvid Berg
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Valentin Georgiev*
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Hans Lindén*
Johanna Elnersson*
Anna Iatsyshyna*
Wei Sun*
Rikard Nordgren
Samuel Lampa*

Safety officers
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Raili Engdahl (until June 2014)
Ronnie Hansson
Lena Norgren
Henrik Wadensten
Sviatlana Yahorava
Kjell Åkerlund

The work environment group
Björn Hellman, *chairman*
Raili Engdahl
Ernst Oliw
Marina Rönngren
Patrik Källback

Working group on post-graduate studies
Margareta Hammarlund-Udenaes, *chairman*
Anna Carlsson
Patrik Källback
Maria Norlin

Working group on equal opportunities
Elin Svensson, *chairman and gender equality representative*
Johanna Svensson
Mattias Hallberg
Marina Rönngren*
Ronnie Hansson, *adjunct*
Sebastian Axelsson

**Temporary position*

Scientific Reports 2014

Biochemical Pharmacology

Ernst H. Oliw

http://farmbio.uu.se/research/researchgroups/biokemfarm/

Arachidonic acid and a few other polyunsaturated fatty acids are bioactivated in humans by enzymatic oxygenation to prostaglandins, leukotrienes, epoxides (EETs) and other local hormones, which contribute to fever, pain, inflammation and cancer development, and to regulation of physiological processes. Common drugs such as aspirin, acetaminophen (paracetamol, APAP) and ibuprofen inhibit biosynthesis of prostaglandins and reduce symptoms of disease, but may also cause side effects related to their actions. Other drugs are based leukotriene receptor antagonists (e.g., montelukast), which are used for treatment of bronchial asthma.

Bioactivation of polyunsaturated fatty acids also occur in plants and fungi where oxygenation of linoleic and linolenic acids is important for the plant-pathogen interaction and for fungal reproduction and pathogenicity. The goal of our research is to investigate the mechanism of oxygenation and bioactivation of fatty acids and to determine their biological function.

We investigate mainly three groups of enzymes: (i) cytochromes P450, (ii) lipoxygenases, and (iii) DOX-CYP fusion proteins with dioxygenase (DOX) and cytochrome P450 (CYP) domains. These enzyme classes occur in man but also in important fungal pathogens, e.g., *Aspergillus fumigatus* causing farmer´s lung disease and *Magnaporthe oryzae*, causing rice blast disease and destruction of 25% of the rice crop of Japan. Our goal is to understand how the enzymes work in order to understand their physiological functions.

(i) DOX-CYP fusion proteins: The DOX-CYP family consists of at least six subfamilies. LDS oxidize oleic and linoleic acids to a series of vicinal diols (e.g., 5,8-dihydroxy-, 7,8-dihydroxyoctadecadienoic acids) and hydroperoxides (e.g., 8-hydroperoxy- and 10-hydroperoxylinoleic acidd), which likely function as sporulation hormones. Recent discoveries are the 9*R*-dioygenase and allene oxide synthase of *Aspergillus terreus* and *A. niger*, and the 9*S*-dioxygenase-allene oxide synthases of *Fusarium oxysporum,* and the 10*R*-dioxygenase-epoxy alcohol synthase of *M. oryzae.*

(ii) Lipoxygenases: All lipoxygenases contain a catalytic metal, iron in humans and plants. We focus our basic research on the first described manganese-lipoxygenases, which are important for *Gäumannomyces graminis*, an important pathogen of wheat, and its structurally similar lipoxygenases of *Magnaporthe salvinii* and *M. oryzae*, and the catalytic convergence of Fe- and Mn-lipoxygenases by a single amino acid substitution.

(iii) Cytochromes P450: In humans, the prostaglandin endoperoxide, PGH_2, can be transformed by cytochromes P450 to thromboxanes, prostacyclin and to 19-hydroxy-PGH_2, the precursor of 19-hydroxy-PGE_2. The latter is the main prostaglandin of human seminal fluid and occurs in high concentration in human semen, where it is formed by CYP4F8 of the seminal vesicles. In other tissues, CYP4F8 is a prominent ω3 oxygenase and recently implicated in prostate cancer development. CYP4F8 and CYP4F22 are also expressed in skin and we investigate their oxygenation of fatty acids in biosynthesis of the skin water barrier. We also investigate the reaction mechanism of allene oxide synthase and compare it with prostacyclin synthase (CYP8A1).

Members of the group during 2014

Ernst H. Oliw, MD, PhD, Professor
Inga Hoffmann, PhD
Saeid Karkehabadi, PhD
Anneli Wennman, PhD student
Marie Ottosson, research assistant

Publications 2012 – 2014

1. Hoffmann, I., Hamberg, M., Lindh, R., and Oliw, E. H. (2012) Novel insights into cyclooxygenases, linoleate diol synthases, and lipoxygenases from deuterium kinetic isotope effects and oxidation of substrate analogs. Biochim. Biophys. Acta 1821, 1508-1517
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6. Hoffmann, I., and Oliw, E. H. (2013) 7,8- and 5,8-Linoleate diol synthases support the heterolytic scission of oxygen-oxygen bonds by different amide residues. Arch. Biochem. Biophys. 539, 87-91
7. Hoffmann, I., and Oliw, E. H. (2013) Discovery of a linoleate 9S-dioxygenase and an allene oxide synthase in a fusion protein of Fusarium oxysporum. J. Lipid Res. 54, 3471-3480
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Agencies that supported the work/Funding 2014

The Swedish Research Council Medicine.

Projects

(I) *Characterization of heme-containing fatty acid dioxygenases and P450 fusion enzymes of human and plant pathogens (Linda Sooman, Margareta Sahlin, Ernst Oliw)*

Fungi are severe pathogens of man and can be devastating for important crops. Aspergillus causes farmer´s lung disease and invasive aspergillosis of immunocompromized patients. Rice blast disease is caused by *Magnaporthe oryzae*, and destroys ~25% of rice crops worldwide. Aspergillus and *M. oryzae* contain cyclooxygenase-related enzymes, diol synthases and dioxygenases, which transform linoleic acid

into hydroperoxides and dihydroxy fatty acids. Our aim is to characterize the enzymes by enzyme expression, gene targeting and by studies on their biological importance.

(i) Characterization of 9R- and 9S-dioxygenases linked to allene oxide synthases of the Aspergillus and Fusarium complexes*:* The quest for genes with homology to 7,8-LDS led us to investigate *A. terreus*, where we found a novel *9R*-dioxygenase linked to an allene oxide synthase (JBC, 2013). This enzyme is also present in *A. niger*, and homologues with 9S-dioxygenase-allene oxide synthase activities are found in the Fusarium complex. We are now investigating the reaction mechanisms.

(ii) Charaterization of a novel subfamily with epoxy alcohol synthase activity: Our most recent discovery is the 10R-dioxygenase-epoxy alcohol synthase of M. oryzae, and we are now investigating the catalytic mechanism.

(iii) EPR analysis of the protein radicals of CYP-DOX fusion enzymes. The Tyr residues of recombinant dioxygenases with a catalytic Tyr radical will be labeled with deuterium to conclusively identify that a Tyr radical is formed during catalysis.

(II) *Characterization of the reaction mechanism and 3D structures of manganese-lipoxygenases (Anneli Wennman, Saeid Karkehabadi, Ernst Oliw)*

Our aim is to study the reaction mechanisms of lipoxygenases and to determine their 3D structures. We will focus on lipoxygenases from different sources based in recent discovery are novel lipoxygenases of *M. oryazae* and additional fungi.

(III) *Novel transformations of eicosanoids (Ernst Oliw).*

Arachidonic acid can be oxygenated to biologically important mediators of fever, pain and inflammation, viz. prostaglandins, leukotrienes and epoxyeicosatrienoic acids (EETs). We focus on the oxygenation of arachidonic acid and eicosanoids by cytochrome P450 4 family enzymes: CYP4F8 (prostaglandin H 19-hydroxylase) and two orphan enzymes, CYP4F22 and CYP4V2. Mutations of two latter have been implicated in retinal and skin diseases and we are now expressing these enzymes in yeasts in order to characterize these enzymes. We report oxygenation of epoxides and epoxyalcohols by CYP4F8 and CYP4F22.

Drug Safety and Toxicology

Eva Brittebo

http://farmbio.uu.se/research/researchgroups/dst/

This research group studies mechanisms of adverse effects of environmental contaminants as well as pharmaceuticals and traditionally used medicinal plants, by the use of various *in vitro* and *in vivo* models. The major objective of the studies is to improve safety predictions based on mechanistic understanding of potential adverse effects and to improve the risk assessment regarding exposures to toxic agents. The research is focusing on the following research areas: Bioactivation and toxicity, Genetic toxicology, Neurotoxicology and Developmental toxicology.

The studies on bioactivation and toxicity are directed towards characterization of toxicant-induced perturbations leading to cell damage in the cardiovascular tissues and to elucidate the role of environmental contaminants in endothelial dysfunction. The studies on genetic toxicology are based on the Comet Assay for evaluating DNA-strand breaks in individual cells. An important issue in the safety evaluation of drugs is if there are threshold doses or not for agents found to be genotoxic *in vitro* or *in vivo*. The studies on neurotoxicology aim to reveal mechanisms of long-term cognitive impairments and neurodegeneration in the adult brain following neonatal exposure to neurotoxins. Furthermore, the focus is on a troublesome adverse effect (dyskinesia) of L-DOPA pharmacotherapy for patients with Parkinson disease. The neurotoxicology studies are based on MALDI –TOF imaging mass spectrometry (MALDI IMS) that is used for the topographical elucidation of proteins, neuropeptides and neurotransmittors and their changing concentrations in brain during physiological and pathophysiological events. In addition, the direct delivery of therapeutic agents to the brain via nasal administration is being examined. Finally, developmental toxicology is one the most challenging areas in predictive toxicology as the developmental period of organisms is much more sensitive to toxicants than the adult period. Some of these studies are directed towards the development of in vitro system for prenatal cardiotoxicity. Other studies aim to elucidate mechanisms of developmental neurotoxicity during the postnatal synaptogenic sensitivity period as well as mechanistic studies of CRABP1 - a transporters of vitamin A. Finally, 3R-oriented developmental toxicology studies using short-term toxicogenomic responses in pluripotent embryonic stem cells are used to predict developmental toxicity *in vitro*.

Members of the group during 2014

Eva Brittebo, Professor
Björn Hellman, Professor
Lennart Dencker, Professor emeritus
Malin Andersson, Associate Professor
Henrik Alm, PhD post-doc (on leave)
Oskar Karlsson, PhD, post-doc
Mats Nilsson, PhD Researcher
Birger Scholz, PhD, Researcher
Michael Stigson, PhD, Researcher
Lisa Ersson, PhD student
Elena Piras, PhD student (on leave)
Rikard Åsgård, PhD student
Hans Lindén, senior project coordinator
Lena Norgren, Technician
Raili Engdahl, Technician

Publications 2012 – 2014

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7. Karlsson O, Berg AL, Hanrieder J, Arnerup G, Lindström AK, Brittebo EB.Intracellular fibril formation, calcification, and enrichment of chaperones, cytoskeletal, and intermediate filament proteins in the adult hippocampus CA1 following neonatal exposure to the nonprotein amino acid BMAA. Arch Toxicol. Epub 2014 May 9.
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14. Hanrieder J., Ekegren T., Andersson M., Bergquist J. (2013) MALDI Imaging of Post Mortem Human Spinal Cord in Amyotrophic Lateral Sclerosis. J Neurochem. 2013;124(5):695-707.
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Dissertations 2014

1. Rikard Åsgård. *Effects of antioxidants and pro-oxidants on oxidative stress and DNA damage using the comet assay. Studies on blood cells from type 2 diabetes subjects and mouse lymphoma cells.* 2014. Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy, 185. ISSN 1651-6192.

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Other commitments/assignments of group members 2014

Eva Brittebo: Member of the working group "Goals and strategies for Uppsala University". Member of the Quality Advisory Board on issues of Uppsala University quality management. Study director in toxicology. Member of the Executive Committee for the SafeSciMET education and training programme in safety sciences for medicines. Member of the Swetox Consortium Board.
Björn Hellman: Head of Department; Member of the local committee for scholarships at the Faculty of Pharmacy; Study director in toxicology.
Lennart Dencker: Member of the Executive Committee for the SafeSciMET education and training programme in safety sciences for medicines.

Projects

(*I) Toxicity of pollutants and pharmaceuticals in vascular tissues (Eva Brittebo, Helén Andersson, in collaboration with Malin Helmeskog, Matts Olovsson, Dept Women's and Children's Health, Uppsala University)*

Bisphenol A (BPA) is widely used in the manufacturing of consumer products such as plastic food containers and food cans. Epidemiological studies report an association between elevated exposure to BPA and cardiovascular disease and diabetes. Our data demonstrate that BPA increased the mRNA expression of the proangiogenic genes VEGFR-2, VEGF-A, eNOS, and Cx43 and increased the production of nitric oxide in HUVEC. Furthermore, BPA increased the expression of phosphorylated eNOS and endothelial tube formation in HUVEC. These studies demonstrate that environmentally relevant levels of BPA have direct proangiogenic effects on human primary endothelial cells in vitro suggesting that the human endothelium may be an important target for BPA.The selective estrogen receptor modulator tamoxifen is the most widely used agent for treatment and prevention of oestrogen receptor positive breast cancer. The adverse effects of tamoxifen include vaginal endometrial bleeding, endometrial hyperplasia, and cancer, conditions associated with angiogenesis. We have examined the effects of tamoxifen on cell migration and angiogenesis-related gene expression in human endometrial endothelial cells (HEECs). The data suggest that tamoxifen changes the regulation of angiogenesis in the endometrium, most likely by reducing angiogenic activity and that endometrial stromal cells regulate some of tamoxifen's effects in HEECs.

(II) Neurodegeneration and intracellular fibril formation following neonatal exposure to a cyanobacterial toxin (Eva Brittebo, Oskar Karlsson, Lisa Ersson, Malin Andersson, Per Andrén in collaboration with Marie Andersson and Ingvar Brandt, Dept Environmental Toxicology, Uppsala university; Mikael Enskog and Curt Petterson, Dept of Medicinal Chemistry Uppsala University; Anna-Lena Berg, AstraZenca, Södertälje; Jörg Hanrieder, University of Gothenburg, and Leopold Ilag, Liying Jiang, Stockholm University)

BMAA (beta-N-methylamino-L-alanine) is a neurotoxic amino acid that is produced by cyanobacteria present in terrestrial and aquatic environments. This neurotoxin has been suggested to contribute to a particular neurodegenerative disease in humans. Our studies demonstrated a poor transfer of radiolabelled BMAA across the blood-brain barrier (BBB) with no specific localisation in discrete brain regions in adult mice. In neonatal mice, however, there is an efficient transport across the BBB and a selective uptake of radioactivity in the hippocampus and striatum. Neonatal exposure to BMAA also induces cognitive impairments such as reduced spatial learning and memory abilities in adulthood. In addition, neonatal rat pups treated with a high dose BMAA show acute neuronal cell death in the hippocampus, retrosplenial and cingulate cortices. These brain areas are all important for cognitive function. Histopathological and ultrastructural analysis identified intracellular fibril formation, neuronal degeneration, cell loss, calcium deposits and astrogliosis in the hippocampus of adult animals following a short-term neonatal exposure. Lower doses of BMAA cause distinct impairments in learning and memory function in adult animals following neonatal exposure without acute morphological changes in the brain.

Proteomic analysis of the adult hippocampus demonstrated an enrichment of chaperones, cytoskeletal and intermediate filament proteins, and proteins involved in the antioxidant defense system in the damaged area. Several of the most enriched proteins (plectin, glial fibrillar acidic protein, vimentin, Hsp 27, and ubiquitin) are known to form complex astrocytic inclusions, so-called Rosenthal fibers, in the neurodegenerative disorder Alexander disease. Moreover, neonatal exposure to BMAA results in increased protein ubiquitination in the adult hippocampus indicating that BMAA may induce protein aggregation. MALDI-TOF imaging mass spectrometry demonstrated that developmental exposure to BMAA induces changes in the expression of S100β, histones, calcium and calmodulin-binding proteins as well as guanine nucleotide-binding proteins in the damaged hippocampus. In addition, time-of-flight secondary ion mass spectrometry based imaging revealed BMAA-induced localization of phosphatidylcholine lipids in the damaged hippocampus. In addition, mass spectrometric quantification of the relative levels of endogenous neuropeptides in the neonatal striatum revealed that 25 peptides from 13 neuropeptide precursors were significantly changed. In another study we characterized changes of major intermediary metabolites in serum following neonatal exposure to BMAA using a non-targeted metabolomic approach. NMR data indicated that d-glucose, lactate, 3-hydroxybutyrate, creatine and acetate, were changed in serum of BMAA-treated neonatal rats. These metabolites are associated with changes in energy metabolism and amino acid metabolism.

Autoradiographic imaging of ^{14}C-labelled BMAA demonstrated a distinct uptake of radioactivity that was retained following acid extraction in tissues with a high rate of cell turnover and/or protein synthesis. Ultra-high performance liquid chromatography-tandem mass spectrometry analysis demonstrated a dose-dependent increase of protein-associated BMAA in neonatal rat tissues. The level of protein-associated BMAA in the liver was more than 10 times higher than that in brain regions not fully protected by the blood-brain barrier which may be due to the higher rate of protein synthesis in the liver. The association of BMAA to rat proteins suggests that BMAA may be misincorporated into proteins. However, protein-associated BMAA seems to be cleared over time, as none of the samples from adult rats have any detectable free or protein-associated BMAA. Another study suggests that, secretion of radiolabelled BMAA into milk is an elimination pathway in lactating mothers. Following secretion of radiolabelled L-BMAA into milk, the levels of radioactivity in the brains of the suckling neonatal mice significantly exceeded the levels in the maternal brains. *In vitro* studies using the mouse mammary epithelial HC11 cell line confirmed a more efficient influx and efflux of L-BMAA than of D-BMAA in cells, suggesting enantiomer-selective transport. Competition experiments with other amino acids suggest that the amino acid transporters LAT1 and LAT2 may contribute to the transport of L-BMAA into milk.

(III) Nasal transfer of therapeutic agents (Eva Brittebo, Elena Piras, in collaboration with Moa Fransson and Angelica Loskog, Dept. of Immunology, Genetics and Pathology, Uppsala University)

The nasal olfactory pathway is a potential route of delivery of therapeutic agents that do not easily pass the blood-brain barrier. The olfactory neurons have direct contact with the external environment via dendrites in the nasal mucus and with the brain via axons that reach the olfactory bulb without synaptic connections. The olfactory transfer of therapeutic agents into the brain is a novel principle for drug delivery. Intranasal administration of CNS-targeting modified CD4+ T cells significantly reduced disease symptoms as well as decreased IL-12 mRNA in a mouse model of brain inflammation - experimental autoimmune encephalomyelitis (EAE). Immunohistochemical markers for myelination and reactive astrogliosis confirmed recovery in mice treated with engineered Tregs. Furthermore, Treg-treated symptom-free mice recovering from EAE were rechallenged with a second EAE-inducing inoculum but remained healthy, demonstrating a sustained effect of engineered Tregs. In another study, intranasal administration of engineered mesenchymal stromal cells (MSCs) expressing a CNS-targeting receptor significantly reduced disease symptoms of EAE. Mice treated with CNS-targeting MSCs were resistant to further EAE induction whereas non-targeted MSCs did not give such persistent effects. This demonstrates that intranasal delivery of central nervous system-retargeted human mesenchymal stromal cells can prolong treatment efficacy in EAE.

(IV) Evaluation of genotoxic/antigenotoxic effects of dietary anti-oxidants, traditionally used medicinal plants and other compounds of naturally origin (Björn Hellman Rikard Åsgård, Jasem A Shemaili, Lena Norgren, in collaboration with Ulf Göransson, Dept Medicinal Chemistry, Uppsala University, Abdu Adem, Thomas Edward Adrian,UAE University Al Ain)

In this project, we have mainly been evaluating the genotoxic and anti-genotoxic effects of some plants used in traditional medicine in Ethiopia and other countries, and in these studies we also include fractions of extracts and/or pure compounds from extracts. Following up clinical studies showing that intake of β-carotene and other antioxidants from the diet was found to be associated with a lower level of oxidative DNA damage in mononuclear leukocytes, and we have also recently published a study on the effect of β-carotene on catechol-induced DNA damage in mouse lymphoma cells. Our most recent study on compounds of natural origin is a study on Frondoside A (a triterpenoid glycoside isolated from the Atlantic sea cucumber *Cucumaria frondosa*) which was found to enhance the anti-cancer effects of gemcitabine, a finding, which in the future may turn out to be of clinical benefit for patients with pancreatic cancer.

(V) Evaluation of DNA damaging effects of nanoparticles in vitro (Björn Hellman, in collaboration with Lars Österlund, Barbro Ekstrand Hammarström, Linnea Ahlinder Ångström Laboratory, FOI, Umeå*)*

In this project we have evaluated cellular and nuclear uptake and DNA damage in human lung cancer cells exposed to reactive nanoparticles of titanium dioxide using Raman microspectroscopy and the Comet Assay. The results clearly indicated a nuclear uptake of the nanoparticles, and that a direct interaction between reactive TiO_2 nanoparticles and DNA cannot be ruled out as one of the mechanisms leading to an increased level of DNA damage in cells that had been exposed to a rather low concentration of particles.

(VI) Evaluation of the potential genotoxicity of drinking water (Björn Hellman, Lena Norgren in collaboration with Agneta Oskarsson and Karin Winberg, Swedish Agricultural University)

In our most recent project evaluating the potential genotoxicity of water samples intended to be used as drinking water, we have introduced HepG2-cells as an additional cell line to be used in our tests. The latter cells are liver cells of human origin, commonly used when screening for toxicological profiles of xenobiotics *in vitro*. The main objective of the drinking water project (which we only are a small part of) is to develop methods for assessment of hazardous chemicals in drinking water by integrating chemical analysis and in vitro toxicity testing. Read more about the “SafeDrink project” on http://www.slu.se/en/departments/aquatic-sciences-assessment/research/safedrink/

(VII) Imaging Mass Spectrometry study of basal ganglia levels of neuropeptides in L-DOPA-induced dyskinesia in experimental Parkinson's disease (Malin Andersson in collaboration with Anna Ljungdahl, Madelene Svedin, Kristen Burnum (PNLL), Jonas Bergquist, Uppsala University)

In this project we study Parkinson's disease (PD) which is characterized by degeneration of dopaminergic neurons and accompanied by a dramatic loss of dopamine in the striatum, particularly in the putamen. About 1% of the population over 65 years suffers from PD and the cardinal symptoms that include akinesia, bradykinesia, muscle rigidity, and tremor. Dopamine replacement therapy with L-DOPA is currently the most effective pharmacotherapy for patients with PD, however with PD disease progression and long-term L-DOPA treatment complications occur in many patients. The main complications are troublesome motor complications such as "wearing off" fluctuations and L-DOPA-induced dyskinesia. Despite large efforts in the field of dyskinesia research, the difficulties we face today remain as how to dissociate the molecular changes that arise by the motor execution of dyskinesia from causative changes that induce or predispose to dyskinesias. Our group studies the brain structures of the basal ganglia for molecular correlates of the incidence and severity of dyskinesia in an experimental model of Parkinson's disease. We focus on neuropeptides and proteins involved in the development of L-DOPA-induced dyskinesias in experimental Parkinson's disease. Here we use MALDI-TOF imaging mass spectrometry (IMS) for unbiased assessment and topographical elucidation of neuropeptides and proteins in the basal ganglia of high and low dyskinetic animals. MALDI-IMS has the unique advantage of high sensitivity, high molecular specificity, and the detection of hundreds of molecular species in a single tissue section. Indeed, several dynorphins and enkephalins have been detected in these studies, including dynorphin B, dynorphin A(1-8), alpha-neoendorphin, MetEnkRF, MetEnkRGL, PEnk (198-209, 219-229).

(VIII) Imaging MALDI mass spectrometry characterization of opioid peptides after a single dose cocaine or morphine (Malin Andersson in collaboration with Emma Gustafsson, Jonas Bergquist, Jan Rodriguez Parkitna and Ryszard Przewłocki (Polish Academy of Science)

Drugs of abuse causes rapid changes in neurotransmission, for example release followed by synthesis of opioid peptides in different nuclei of the basal ganglia. In this study we examine the localization and time course of opioid peptides after an acute dose of either cocaine or morphine.

(IX) MALDI mass spectrometry based molecular phenotyping of CNS and PNS glial cells for prediction in mammalian brain tissue (Malin Andersson in collaboration with Jörg Hanrieder, Grzegorz Wicher, Karin Forsberg Nilsson, Ping Sui, Jonas Bergquist, Åsa Fex-Svenningsen SDU, Denmark)

These are several similar studies that examine the use of differential protein expression profiling of mammalian neural cells by means of MALDI TOF MS. MALDI MS profiling analysis is rapid, sensitive, robust and specific for large biomolecules in complex matrices. A new straightforward methodology was developed for direct characterization of rodent CNS glial cells using MALDI MS based intact cell mass spectrometry. Molecular phenotyping enables the characterization of cell growth stages, (stem) cell differentiation as well as probing cellular responses towards different stimulations. On-going work includes profiling of glioblastoma multiforme-derived cell lines for the prediction of cancer severity to facilitate diagnosis.

(X) Assessment of neuropeptides and proteins rat spinal cord (Malin Andersson in collaboration with Ping Sui, Jonas Bergquist, Georgy Bakalkin Uppsala University)

This study searches for regional localization of neuropeptides in different domains of the spinal cord using MALDI IMS.

(XI) In vitro system development for prenatal cardiotoxicity (Mats Nilsson in collaboration with Helen Ritchie, William S. Webster, University of Sydney, Australia)

The embryo is not protected from pharmaceuticals and environmental pollutants. Intended and unintended pharmacological effects of drugs are often exerted in the conceptus as well. Various therapeutic drugs may affect the functioning of the early embryonic heart resulting in periods of bradycardia, arrhythmia and/or heart block. Such an effect would result in periods of hypoxia in the embryo and may result in spontaneous abortion, fetal growth retardation or in rare cases birth defects. In this project we examine the effect on rat embryonic cardiac function *in vitro* of pharmaceutical drugs with various ion channel-blocking properties.

(XII) Developmental neurotoxicity during the postnatal synaptogenic sensitivity period (Birger Scholz, Henrik Alm)

Brain development includes key neurodevelopmental prenatal and postnatal stages where environmental stimuli (such as neurotoxicants) can be particular efficient in inducing long lasting changes in neurodevelopmental trajectories. During the first two to three postnatal weeks of rodent life, there is a synaptogenic sensitivity period, corresponding to the first ~2-3 years of human life. DNT exposure within (but not outside) this period commonly leads to potentially irreversible alterations in rodent adult brain function. Substances as chemically diverse as metals, environmental chemicals (PCBs, Bisphenol A and polybrominated flame retardants (PBDEs)) and medical drugs (GABA type A agonists and NMDA antagonists) lead all to similar long lasting behavioral effects in animals when exposed during this sensitivity period. An important challenge is therefore to identify the molecular basis of the disruptions that lead to irreversible changes in adult behaviour and the nature of how the effects from such dissimilar substances can converge into similar phenotypes. This research project aims at identifying how neurotoxicants (both environmental toxicants and medical drugs) induce both structural and epigenomic changes during the sensitivity period (postnatal day 10 in rodents) and how these effects become propagated to adult age (4 months). We have so far focused on Polybrominated diphenyl ether (PBDE), Bisphenol A and Ketamine induced effects on neural DNA methylation, gene expression and neurosignalling (peptidomic) changes in the cortex and hippocampus.

(XIII) Developmental contingencies and DNT in vitro (Birger Scholz)

The issue of in-vitro extrapolation to in-vivo conditions is of great importance in predictive toxicology considering how resource-intensive animal studies are. Unfortunately, the majority of todays *in silico* and in vitro assays suffer from weak predictive power for more complex toxicological endpoints. The mechanistic information from the postnatal sensitivity project is to be applied to the development of a range of more representative and specific DNT in vitro tests. To this end, this project investigates if and under what in-vitro conditions the observed developmental sensitivity period specific gene regulation changes can be replicated in cell lines (immortalized somatic neural cell lines and teratocarcinoma derived cell lines). In order to make the *in vitro* tests as relevant as possible for possible future hazard identification, they are conducted with and optimized for High Content Analysis/High Content Screening.

(XIV) Stem cells for embryotoxicity testing (Michael Stigson)

We are using the information from embryos (cultured in vitro, or exposed in vivo), and apply it on mouse and human embryonic stem (ES) cells, to develop mechanism-based in vitro cell test systems to reveal the teratogenic potential of substances. For the purpose to screen in vitro for teratogenic action on specific developmental processes, we use differentiation of murine ES cells along a verity of lineages under the influence of teratogenic compounds. To further extend the usefulness, and facilitate the implementation of murine ES cells in HTS of developmental toxicity assays, we have adopted these cells to culture conditions free of animal products, such as serum and feeder cells.

(XV) Mechanistic studies of CRABP1 (Birger Scholz)

Retinoic Acid (RA) and derivatives thereof are currently used therapeutically to treat relatively common diseases such as cystic acne and psoriasis. Neural-crest cells and tissues developed from them are among the organs and tissues most often malformed in new-borns exposed to RA during pregnancy. RA is also known to be important during postnatal synaptogenesis. We and others have previously shown that the same tissues and cells that accumulate radioactively labelled RA and its analogues also express high levels of the protein CRABP1. However, CRABP1 is one of the most important intracellular transporters of vitamin A and is believed to regulate normal as well as teratogenic activation of nuclear receptors for vitamin A. The exact relation between RA and CRABP1 with regard to developmental toxicity is currently unknown. Using CRABP1 knockout mice, this project aims at studying the involvement of CRABP1 in retinoid induced developmental toxicity and brain development using behavioural and neuropeptidomics studies.

(XVI) Resilience of memory traces (Birger Scholz in collaboration with Kerrie Thomas, Cardiff University, UK)

Numerous developmental neurotoxic agents (environmental chemicals and medical drugs) are able to influence different aspects of adult cognitive functions in animals, including learning and memory. Life experiences, during postnatal age or later, also influence these cognitive processes. It is generally unclear how previous experiences (neurotoxic or otherwise) develop into resilient states that are difficult to reverse/change. One of the more common methods for studying hippocampus-dependent learning and memory processes is the use of contextual fear conditioning where rodents are exposed with startling stimuli (unconditional stimuli, US) within a given context (contextual stimuli, CS) and then continue to connect the CS with the US (i.e. they have created fear memories). The project aims at using a functional genomic approach together with a more targeted brain-region specific infusion to study the basic mechanisms of how experience dependent resilience is related to the basic memory processes of consolidation, reconsolidation and extinction. Previous and on-going studies have found that the strength of these memory processes can be manipulated on a molecular epigenetic level by HDACi in a context and experience specific manner and that the hippocampal reconsolidation and extinction processes are markedly distinct. The reconsolidation process is for instance heavily dependent on certain classes of cytokines whereas the extinction process involves the specific regulation of protease-mediated activation of neurotrophins and other proteins in the neural extracellular matrix. A additional side project related to this and other projects involving the use of proteomic and peptidomic (endogenous small proteins and peptides) methodologies is the investigation of issues surrounding in vivo and in vitro sample handling dependent sample quality and sample degradation (such as choice of sample inactivation techniques, time between sampling

Medical Mass Spectrometry

Per Andrén

http://farmbio.uu.se/research/researchgroups/mms

The research group focus on new approaches in mass spectrometry (MS), i.e. matrix-assisted laser desorption ionization MS imaging ((MALDI-MSI) of biological tissue sections, and peptidomics, the comprehensive study of endogenous peptides.

Mass spectrometry imaging is a novel technique used to determine the spatial distribution of molecular species in biological tissue sections *in situ*. The technology allows analysis and visualization of endogenous proteins, peptides and neurotransmitters, as well as drugs and their metabolites, in their native biochemical states within the same tissue section with high molecular specificity. Molecular images are created by rasterizing over the sample while collecting MS or tandem MS (MS/MS) spectra from every position at a chosen spatial resolution. The localization pattern from individual molecular species present on the tissue surface can then be extracted and positioned on the original histological image with the abundances represented by a concentration dependent color scale.

Peptidomics involves the comprehensive analysis of the endogenous peptide content of a certain cell, organ, body fluid, or organism. It complements molecular biology approaches in its ability to characterize the processing of translation products, including changes in expression or posttranslational modifications (PTMs) of peptides and small proteins. By comparing the proteins and peptides in samples of diseased tissue with those in normal tissue, differential expression patterns can be detected that may lead to the identification of novel biomarkers.

The objective of our research is to utilize MSI and peptidomics approaches to study neurochemical processes in Parkinson's disease (PD) and specifically L-Dopa-induced dyskinesias (LID). The aim is to define neuropeptides, proteins and neurotransmitters that are differentially expressed in the basal ganglia complex of animals with striatal dopamine depletions, and to determine which of these molecules are regulated by loss of dopamine signaling, as well as to investigate their expression patterns in subjects with and without LID symptoms.

Understanding the relationship between pharmacokinetics and pharmacodynamics is crucial in the development of effective drug therapies. Current technologies only provide information on the total amount of drug in the whole tissue with no possibility to address micro-environmental localization of the compound or metabolic derivatives. The application of MALDI MSI in drug discovery studies provide a new tool to accurately measure local tissue concentrations of drug/drug metabolite in context with efficacy achieved with the targeted biology or in the context of safety.

Our laboratory is equipped with the latest separation and MS technologies, i.e., three MALDI-MSI instruments; two Ultraflextreme TOF/TOF (Bruker Daltonics) and one MALDI/ESI Q-TOF Synapt G2si (Water Corp.), and two electrospray ionization mass spectrometers, LTQ (Thermo Scientific) and a high-resolution Q-TOF mass spectrometer (Maxis Impact, Bruker Daltonics).

Members of the group during 2014

Per Andrén, Associate Prof.
Anna Nilsson, Researcher
Henrik Wadensten, Researcher
Henrik Lodén, Researcher
Mohammadreza Shariatgorji, Researcher
Patrik Källback, PhD student
Theodosia Vallianatou, PhD student

Publications 2012-2014.

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Agencies that supported the work/Funding 2014

The Swedish Research Council (VR); NT (2014–6215), MH (2008–5597, 2013–3105) and RFI (2009-6050); the National Institute on Drug Abuse (NIDA) (grant R21 DA027548-01); VINNOVA; Japan Society for the Promotion of Science (JSPS) Joint Projects; AstraZeneca Global DMPK, Safety Assessment;; FP7-PEOPLE-2013- Marie Curie Initial Training Networks (ITN); Uppsala University – Infrastructure.

Other commitments/assignments of group members 2014

Per Andrén: Swedish Academy of Pharmaceutical Sciences, Section for Drug Analysis (member of the board); Swedish Proteomics Society (member of the board); Journal of Proteomics (editorial board); European Proteomics Association (EuPA) Open Proteomics (editorial board), Peptidomics (editorial board); EuPA, Chairman for mass spectrometry imaging initiative; Cooperation in Science and Technology (COST) Action; Mass Spectrometry Imaging (member of the management committee).

Projects

(I) Functional neuroproteomic changes associated with L–Dopa–induced dyskinesia in Parkinson´s disease (Collaboration with Per Svenningsson, Karolinska Institutet, Alan Crossman, University of Manchester, UK, Erwan Bezard, Univ. of Bordeaux 2, France)

The main objective of the present research is to study neurochemical processes in Parkinson disease and specifically L-Dopa-induced dyskinesias (LID). No treatment exists yet for the management of LID, a debilitating complication of L-dopa therapy for PD. The aim is to define neuropeptides, proteins, neurotransmitters and endogenous metabolites that are differentially expressed in the basal ganglia complex of animals with striatal dopamine depletions, with and without LID. Project supported by VR-MH grant 2013–3105.

(II) Mass Spectrometry Imaging in Drug Discovery – Targeting drugs, metabolites, peptides and neurotransmitters

The project utilizes the full potential of matrix-assisted laser desorption ionization mass spectrometry imaging (MALDI-MSI) to provide state-of-the-art molecular tools aiding drug discovery and development. General objectives include development of MALDI-MSI in the following health research and drug discovery areas, a) ADME, high-resolution imaging the spatial distribution and absolute concentration of unlabeled drugs within tissue micro-compartments, b) Safety Toxicology, monitoring of molecular toxicology in organs (kidney), c) Blood-Brain
Barrier (BBB), determine BBB profile for drug candidates in the early stages of the drug development, d) Neuroscience, absolute quantitation of neurotransmitters directly in tissue sections, e) msIQuant, MSI software development for quantitation of drugs, metabolites and endogenous compounds directly in tissue sections. The application of these methods will provide new tools aiding pharmaceutical development, as well as for clinical and experimental model systems. Project supported by VR-NT grant 2014–6215.

(III) Integration of resources and studies to elucidate neuropeptide signaling (Collaboration with Dr. J. Sweedler and S. Rodrigues-Zas, University of Illinois Urbana-Champaign, IL, USA)

The aim is to develop a public and comprehensive neuropeptide resource much needed by the research community by collectively analyzing proteomic and transcriptomic experiments to augment the understanding of extracellular signaling peptides both at the fundamental neuroscience as well as the applied substance abuse levels. To accomplish these objectives, we integrate complementary peptide repositories and develop tools to assemble and effectively query a comprehensive and public resource of experimental and in silico predictions; mine this resource to perform secondary and joint analysis of available high proteomic experiments; and perform integrated analysis of proteomic and transcriptomic experiments. The overarching strategy is to integrate complementary information across databases, experiments and platforms to provide a unique and comprehensive understanding of the dynamic neuropeptide complement. The outcome of this project will be resources, tools and information that will fill critical gaps in the knowledge on intercellular signaling systems. Project supported by NIDA grant R21 DA027548-01.

(IV) Identification and functional characterization of protein-protein interactions in cerebrospinal fluid and brain tissue from Parkinson's disease (PD) patients and experimental PD models (Collaboration with Per Svenningsson, Karolinska Institutet)

Using surface plasmon resonance technique (Biacore 3000) coupled to mass spectrometry technology, new protein partners of α-synuclein and parkin have been captured and identified in cerebrospinal fluid or post-mortem human tissue from PD patients. In addition to using native α-synuclein and parkin, mutated forms of these proteins seen in familiar forms of PD will be immobilized on the sensor chip and used as baits. Project supported by VR-MH grant 2013–3105 and 2011-4722.

(V) Fine mapping the spatial distribution and concentration of unlabeled drugs within tissue micro-compartments using imaging mass spectrometry (Collaboration with AstraZeneca)

In respiratory inhalation drug discovery projects, one key objective is to optimize retention of compound in the lung and consequently achieve duration of effect. Current technologies only provide information on the total amount of compound in the whole lung with no possibility to address micro-environmental localization of the compound or metabolic derivatives. The application of MALDI-MSI in such studies would provide a new tool to accurately measure local tissue concentrations of drug/drug metabolite in context with efficacy achieved with the targeted biology or in the context of safety. Such technology could provide new and important information.

(VI) Characterization of drug-induced kidney toxicity using MALDI-MSI (Collaboration with AstraZeneca)

The project is aimed at developing a routine methodology for the use of MALDI imaging in problem solving of toxicity questions. It will include preparation of tissue material, interpretation of the data generated and structure elucidation of new analytes.

(VII) Novel inactivation technology stabilizes the in vivo levels of proteins, peptides, phosphorylations, drugs in tissue samples (Collaboration with Denator AB, Uppsala and Göteborg, Sweden)

After tissue or blood sampling, proteases and other protein-modifying enzymes can rapidly change proteome composition. Subsequent analytical results reflect a mix of in vivo proteome and degradation products. Vital information about the 'pre-sampling' state may be destroyed or distorted, leading to variation between samples or even erroneous conclusions. Enzyme inactivation and standardization of sample handling can address this problem. Here a novel tissue stabilization system is used to halt degradation. After treatment samples are analyzed with downstream techniques such as western blotting or mass spectrometry.

Neuropharmacology and Biological Research on Addiction

Ingrid Nylander

The research group Neuropharmacology and Biological Research on Addiction have three different research areas focusing on the role of basic neurology, and physiological and pathophysiologial mechanisms leading to addiction and neurodegenerative diseases. The research group is divided into the three following subgroups:

- Neuropharmacology, Addiction and Behavior
- Biological Research on Drug Dependence
- Molecular Neuropsychopharmacology

Neuropharmacology, Addiction and Behaviour

Ingrid Nylander, Lena Bergström, Lova Segerström, Erika Roman, Anne-Lie Svensson

http://farmbio.uu.se/research/researchgroups/nab

The research in the group is devoted to studies on basic neurobiology, physiological and pathophysiological mechanisms in the brain, and neuropharmacology of relevance for substance use disorder and neurodegenerative diseases. Current projects include studies of neurobiological substrates for individual differences in addiction processes, especially vulnerability for risk consumption of alcohol and alcohol use disorder, and responses to drugs used in treatment of addiction. Alcohol addiction is a complex trait and the phenotype related to vulnerability for dependence is based on the interaction of multiple genes and environmental factors. Of particular interest for us is the impact of early environmental factors, such as rearing environment and the consequences of adolescent drug intake, and the association between behavioural characteristics, such as risk taking behaviour, and later drug consumption. It is hypothesized that disruption of early developmental processes in transmitter networks either by rearing factors or drug intake early in life, causes long-term changes in brain function and behaviour that, in turn, affects alcohol consumption later in life. We continue to collaborate with Drs E Comasco, L Oreland (Department of Neuroscience) and K Nilsson (Centre for Clinical Research, Västerås) in projects that include investigation of how epigenetic processes are involved in long-term consequences of exposure to various early-life environmental factors.

Another line of research investigates the role of cannabinoids and neurosteroids for neurogenesis and for interactive processes that are ongoing in neurodegenerative disorders, like Alzheimer's disease (AD). Since AD is associated with excitotoxicity, oxidative stress and neuroinflammation, the research line emphasis on neuroprotective properties of cannabinoids and neurosteroids against different toxic insults in *in vitro* cell models.

In the projects, experimental models are used in combination with extensive evaluation of neurobiological and behavioural consequences of different early environmental conditions. A number of behavioural models within the field of neuroscience and neuropharmacology are employed including tests for assessment of neonatal development, exploratory behaviour, locomotor activity, anxiety-like behaviour, self-administration, learning and memory and a multivariate test arena (the multivariate concentric square field™, MCSF) and utilizes multivariate data analysis approaches. Neurobiological assessment includes methods to analyse effects on receptors, transmitters and mRNA in tissue samples and brain slices but also analysis of transmitter release and re-uptake patterns using *in vivo* chronoamperometric analysis using

Fast Analytical Sensing Technology (FAST). Specific research activities within the group are described shortly under projects.

Members of the group during 2014

Ingrid Nylander, Professor
Lena Bergström, Associate Professor, Senior Lecturer
Erika Roman, Associate Professor, Senior Lecturer
Anne-Lie Svensson, Senior lecturer
Maria Ellgren, Junior lecturer
Lova Perup-Segerström, Researcher
Samuel Rowley, Post-doc
Shima Momeni, PhD student
Sara Palm, PhD student
Linnea Granholm, PhD student
Stina Lundberg, PhD student
Bengt J Meyerson, Professor Emeritus
Marita Berg, Technician
Jenny Gustavsson, Research assistant

Publications 2012-2014

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1. Sara Palm *Early Environment, Adolescent Alcohol Drinking and Neurobiological Responses to Drugs*, 2014. Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy, 190. ISSN 1651-6192.

Agencies that supported the work/Funding 2014

The Swedish Research Council (Nylander); The Alcohol Research Council of the Swedish Alcohol Retailing Monopoly (two separate projects; Nylander and Roman); Gun och Bertil Stohnes stiftelse (Svensson); Stiftelsen för Gamla Tjänarinnor (Svensson)

Other commitments/assignments of group members 2014

Lena Bergström: Member of the Academic Senate, Uppsala University
Ingrid Nylander: Grant committee member Alcohol Research Council of the Swedish Alcohol Retailing Monopoly; Grant committee member ALF, KI; Member of the Uppsala University Quality committee; Chairman, the quality assurance group at the Disciplinary Domain of Medicine and Pharmacy; Member of the Faculty of Pharmacy committee; vice chairman, the recruitment committee Faculty of Pharmacy; chairman, board of undergraduate education Faculty of Pharmacy; vice dean, Disciplinary Domain of Medicine and Pharmacy
Erika Roman: International Adjunct Associate, Department of Psychology, Indiana University, USA; Review Editor: Frontiers in Addictive Disorders and Behavioural Dyscontrol; External mentor Salvatore Magara, KI; Member of the board: The Society for Swedish Alcohol and Drug Research; Member of the board of Uppsala University Laboratory Animal Resources; Member of the Animal Welfare Body, Uppsala University; Member of the Postgraduate Programs Committee (KUF), Uppsala University; Approved Supervisor by the Swedish Board of Agriculture; Representative in the National Committee, the Swedish Board of Agriculture; Member of the expert panel, Swedish Centre for Animal Welfare; One of three coordinators of Uppsala University Behavioural Facility (UUBF), Uppsala University
Anne-Lie Svensson: Member of the committee for undergraduate education (GRUFF) at the Faculty of Pharmacy, Uppsala University; Member of the gender equality committee at the Disciplinary Domain of Medicine and Pharmacy, Uppsala University.

Projects

(I) The impact of early life environment on endogenous opioids, monoamines and the stress axis (Sara Palm, Linnea Granholm, Stina Lundberg, Lova Perup-Segerström, Maria Ellgren, ***Ingrid Nylander****)*

In parallel experiments we study long-term effects of early life adversity and of drug exposure.
Early life adversity: We have previously shown that adverse experiences early in life cause long-term neurobiological and behavioural alterations. Currently, we study the mechanisms underlying long-term consequences of early life stress. In collaboration with Dr E Comasco, we assess the effects of early-life stress on expression and methylation of genes involved in regulation of stress and reward. A rodent maternal separation (MS) model is used to simulate different environmental settings. Rearing conditions are used that are associated with resilience (after short MS) or vulnerability (after longer MS) in terms of adult risk consumption.
Adolescent drug intake: We study the long-term effects of adolescent drug exposure on opioid networks and on alcohol consumption or self-administration of amphetamine in adulthood. The impact of social deprivation in single housing drinking paradigms has been evaluated. Social deprivation affected opioid peptides and the drinking paradigm was further developed to avoid confounding by single housing. Effects of voluntary alcohol consumption on opioid peptides were for the first time described. Currently we examine the effects of adolescent alcohol and also the combined alcohol and nicotine exposure on opioid networks.

(II) In vivo and in vitro studies of drug-induced effects in the brain (Sara Palm, Sam Rowley, Anne-Lie Svensson, Martin Lundblad, ***Ingrid Nylander****)*

Chronoamperometry with Fast Analytical Sensing Technology (FAST) is currently used for *in vivo* analysis of dopamine in the brain. This technique enables *in vivo* electrochemical detection of dopamine in anaesthetized or awake animals. Microelectrodes are used to measure electrochemically active substances like dopamine. FAST offers unique advantages as compared to *in vivo* microdialysis: high temporal resolution and close spatial orientation enables a second-by-second analysis instead of several minutes, high sensitivity allows measurement of resting levels, high specificity and the microelectrodes cause minimal brain damage. We analyse effects of exposure to early life stress or adolescent alcohol exposure on dopamine dynamics and have so far shown differences in the dorsal striatum, a brain area involved in transition from controlled to compulsive drug taking behaviour. It is also used to correlate *in vivo* dopamine dynamics with behaviour, e.g. risk taking or risk assessment.

(III) Behavioural profiling of animals exposed to early environmental stress and adolescent alcohol consumption (Sara Palm, Linnea Granholm, Stina Lundberg, ***Erika Roman, Ingrid Nylander****)*

Current experiments analyse the short- and long-term behavioural consequences of rearing in different environmental settings and of long-term alcohol consumption. The project comprises development of animal experimental models to assess maternal behaviour, interactions between the dam and offspring and play behaviour. In addition, the MCSF test is employed to examine behavioural profiles after different rearing conditions and before and after adolescent alcohol consumption. Individual behavioural profiling in animals subjected to various rearing conditions and alcohol early in life in controlled experimental groups enables distinction between rearing-induced and drug-induced consequences for behaviour later in life. It is also examined whether and how altered behaviour relate to vulnerability for drug addiction.

*(IV) Tissue stabilization as a means to avoid post-sampling degradation of peptides (Jenny Gustavsson, Lova Segerström****, Ingrid Nylander****)*

Post-mortem metabolism is a major concern in the analysis of opioid peptides in biological samples and we have used the bench top Stabilizor T1 instrument (Denator AB, Gothenburg, Sweden) for heat stabilization of tissue samples to establish a procedure for rapid and efficient enzyme inactivation. We were able to optimize the measurements of opioid peptides in the brain and achieve detection of peptide levels more similar to the *in vivo* concentration with a reliable, reproducible and easy to use procedure to avoid peptide metabolism in the handling of fresh tissues.

*(V) Development and validation of the MCSF test (**Erika Roman,** Stina Lundberg, **Bengt J Meyerson)***

The multivariate concentric square field™ (MCSF) test is a technique with an ethoexperimental approach that in one and the same test situation includes a variety of physical situations meaning risk, safety, exploratory incentives etc. that permit measurements of general activity, exploration, approach/avoidance in open versus sheltered areas and the motivation for seeking reinforcers. The purpose of the multivariate design is to gather information that taken together should illustrate a personality profile including how this profile is altered under various forms of challenges. The MCSF test is under continuous development. On-going work aims to i) study the impact of pharmacological substances on behavioural profiles, and ii) develop plug-in units for studies of motivated behaviours and expanding the use of the MCSF for studies of learning and memory.

*(VI) Behavioural profiling of selectively bred alcohol-preferring and alcohol-avoiding rodent lines (**Erika Roman**, Robert Stewart, Nicholas Grahame, Tiebing Liang, Giancarlo Colombo, Petri Hyytiä, Lawrence, Lumeng)*

Genetic aspects of alcohol use disorders have been modelled using rats and mice selectively bred for extremes of alcohol preference and voluntary alcohol intake. Several different lines of rodents have been selectively bred for high and low oral alcohol preference and intake. These lines show similar alcohol drinking phenotypes but have different genetic and environmental backgrounds and may therefore display diverse behavioural traits as seen in human alcohol dependent individuals. This project involves behavioural characterization of selectively bred alcohol preferring and non-preferring rat and mouse lines as well as congenic rat lines using the MCSF test. Moreover, the response to natural reinforcing stimuli is characterized in selectively bred alcohol preferring and non-preferring rat lines. This extensive behavioural characterization enables a deeper understanding of behavioural traits of importance for understanding of alcohol use disorders and related addiction processes.

*(VII) Ethoexperimental studies of appetitive and consummatory mechanisms related to natural rewarding stimuli and drugs of abuse (**Erika Roman**, **Bengt J Meyerson**)*

The project aims at exploring basic mechanisms of reinforcing stimuli with special focus on differentiating appetite for seeking reinforcers such as food, sexual activity and drugs of abuse from consummatory behaviours. On-going studies assess the animal's motivation for passing the risk area and reach reinforcement by increasing the resistance of passing. The association between natural rewards, such as sexual activity and food intake, and drugs of abuse, i.e. alcohol, is subject for examination. The hypothesis is that reward motivated behaviours are different in animals with different voluntary intake of drugs of abuse.

*(VIII) The role of individual differences in drug-seeking and drug-intake behaviour and associated neurobiological effects of relevance to vulnerability for addiction (Shima Momeni,, **Lena Bergström**, **Erika Roman**)*

We use experimental methods to examine the neurobiological basis for individual differences in risk-related behaviours and the association between voluntary alcohol intake, addiction processes and response to treatment. With regard to neurobiology, focus is on the cannabinoid, opioid and dopamine systems. A multivariate behavioural approach with an ethological foundation that incorporates several aspects of the behavioural repertoire and evolutionary conserved behaviours is used. The hypothesis is that risk-related behaviours are of importance for liability for excessive alcohol intake and also affects the response to drug treatment. The impact of individual differences in risk-taking behaviour on voluntary alcohol intake and CB1 and opioid receptor density is currently investigated. We also study the effects of alcohol on FAAH and MAGL enzyme activity, i.e. enzymes metabolizing the endogenous cannabinoids anandamide and 2-AG.

*(IX) Neurosteroids and Alzheimer's disease: Mechanistic studies of neuroprotection and amyloid-β-modulation (**Anne-Lie Svensson**)*

Neurosteroids are endogenous modulators of neuronal functions responsible for many biological and pathophysiological effects. Plausible links between neurosteroids and neurodegenerative disorders, like Alzheimer´s disease (AD), are suggested. Accumulation of amyloid-b induced by toxic events in cells might be able to reduce the synthesis of neuroprotective neurosteroids, thus favour/support neurodegenerative processes. In ongoing studies the neuroprotective properties of neurosteroids and their metabolites, against amyloid-b-induced toxicity are investigated. Since neurosteroids most likely affects

neuronal and glial cells differently, their effects on amyloid-b-induced toxicity are also investigated in numerous cell types.

*(X) Neuroprotective properties of endocannabinoids against different toxic insults (**Anne-Lie Svensson**)*

The endocannabinoid system is widespread in the central nervous system and involved in many neurophysiological processes. Neurodegenerative disorders, such as Alzheimer´s disease (AD), are associated with excitotoxicity, oxidative stress and neuroinflammation. Endoannabinoids have been demonstrated to affect the progression of neurodegeneration. In ongoing studies the neuroprotective properties of different endocannabinoids against toxic insults are investigated in numerous cell types.

Biological Research on Drug Dependence

Mathias Hallberg and Fred Nyberg

http://farmbio.uu.se/research/researchgroups/brdd/biolbero

The first objective of our research is to improve the understanding of brain mechanisms of relevance for the etiology of drug addiction and to develop strategies for relevant treatment. Studies of drug effects on brain circuits involved in reward and dependence are combined with attempts to develop peptidomimetics reducing the expression of opioid withdrawal, an important aspect in the treatment of opioid addiction. Studies are also aimed to find strategies to repair drug-induced brain damages, i.e. using peptidomimetics or growth factors counteracting apoptosis and stimulating neurogenesis. As animal models do not reflect all complexity of addiction current research also involves epigenetic adaptations and regulation of plastic neuropeptide genes in human brain. The second objective of our research is a) to discover new classes of analgesics with potential use in the treatment of neuropathic pain and b) to discover unique cognitive enhancers. These two subprojects are derived from findings in the tachykinin and renin-angiotensin systems.

Members of the group during 2014

Mathias Hallberg, PhD Associate Professor
Fred Nyberg, Senior Professor
Alfhild Grönbladh, PhD
Anna Carlsson PhD student
Erika Brolin, PhD student
Erik Nylander, PhD student
Shanti Diwakarla, Postdoc
Anna Lesniack, Postdoc
Johanna Elnersson, Project assistant
Myron Zaluha, Project leader

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7. Fred Nyberg (ed.): Neuropeptides in Neuroprotection and Neurogeneration. 2012; CRC Press, New York.
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Agencies that supported the work/Funding 2014

Swedish Research Council - Medicine and Health; Kjell and Märta Beijers Foundation; Carl Tryggers Foundation; Science for Life Laboratory (SciLifeLab); Swedish Council for Working; Life and Social Research; Precision Science System; Swedish Foundation for Strategic Research; Berzelii Centre for Biotechnological Research; Swedish Institute, Visby Program; Disciplinary Domain of Medicine and Pharmacy; The Alcohol Research Council of the Swedish Alcohol Retailing Monopoly; The Research Council of Swedish Criminal Care.

Other commitments/assignments of group members 2014

Fred Nyberg: Member of the Governmental Advisory Board for Addictive drugs (ANDT-S Advisory Board). Member of the Uppsala University Center for Studies of the Religion in the Society since 2006 and the National Center for Mens Violence against women since 2006. Member of the Board for the Medical Committee of the Swedish Criminal Care. Member of the Board of the Alcohol Research Council of the Swedish Alcohol Retailing Monopoly
Member of the Board of The Research Council of the Swedish Criminal Care.
President of the International Narcotics Research Conference (INRC) from 2014-2017.
Member of Editorial Board of Scientific journals (Fred Nyberg): Peptides, Open J Endocrinology (Editor in Chief), Pharmacology-on-line, J Musc Skel. Pain. PI at the Uppsala Berzelii Technology Center for Neurodiagnostics (100 milj, SEK 2006-2015); PI at the Linne project Impact of Religion: Challenges for Society, Law and Democracy (50 milj. SEK 2008-2017); PI at the FAS supported project on alcohol effects on cognitive functions (12 milj. 6 years). Swedish Research Council/Medicin and health for peptidergic mechanism in the development of drug dependence: 2.4 milj SEK for 2012-2014 and 3 milj SEK for 2015-2017.
Mathias Hallberg: Vice Chairman of the Board for Education (GRUFF), Faculty of Pharmacy, Member of the Steering Committee/Reference group for the development of the Language Workshop, Uppsala University, Vice Chairman of the Student Recruitment Committee, Faculty of Pharmacy. Referee: Curr Protein Pept Sci. and The Open Biochemistry Journal. Research grants from Kjell och Märta Beijers Stiftelse, 1 000 000 SEK/year 2014-2018, Research grants from Carl Tryggers Stiftelse, 295 000 SEK 2014 and 295 000 SEK 2015, PI with research support from SciLifeLab, DDD platform; AT2R antagonists for neuropathic pain.

Projects

(I) Studies on neuropeptides, neurohormones and steroids in relation to opioid sensitivity and chronic pain (including animal experimental models, in vitro cell cultures and clinical studies). In clinical studies the project directs to assessment of genetic polymorphism in various transmitter systems combined with quantification of neuropeptide levels in various body fluids. Peptides chosen for analysis includes the tachykinins and opioid peptides. In animal studies models for nociceptive, neuropathic pain are chosen.

(II) Studies on neuropeptides, neurohormones and steroids (in particular anabolic androgenic steroids = AAS) in relation to drug dependence (including experimental animal models, in vitro cell cultures and clinical studies). In clinical studies the project directs to assessment of genetic polymorphism in various transmitter systems combined with quantification of neuropeptide levels in various body fluids. Peptides chosen for analysis includes the tachykinins and opioid peptides. In animal studies models to investigate opiate tolerance and withdrawal and drug self-administration (in collaboration with other laboratories) are used. Endogenous peptides with high potency to attenuate withdrawal reactions have been identified and serve as basis for design and synthesis of peptides and non-peptides that may be further developed to act as drugs in the treatment of opiate addiction. In studies of effects of AAS on the brain neurochemical technologies (radioimmunoassays, autoradiograhy, Western blot, etc.) are combined with various behavioural assays.

(III) Studies on the functions of growth hormone (GH) and prolactin (PRL) and their receptors in the central nervous system (including experimental animal models, in vitro cell cultures and clinical studies). Receptors for GH have been identified in the brain in areas of relevance for many of the known effects of GH on the central nervous system (CNS). Beneficial effects of GH on cognitive functions are recorded by the assessment of memory and cognition using the Water maze in conjunction with various neurobiological techniques.

(IV) Studies on synthetic compounds acting on the angiotensin II AT2 receptor and the angiotensin IV receptor, insulin-regulated aminopeptidase. Receptor assays animal models are used to guide synthesis and design of peptide and non-peptide analogues. The objective is a) to discover new classes of analgesics with potential use in the treatment of neuropathic pain and b) to discover unique cognitive enhancers.

These two subprojects are derived from findings in the tackykinin and renin-angiotensin systems (RAS) that we have studied for a long period. The substance P metabolite substance P (1-7) alleviates pain and drug-like substances mimicking substance P (1-7) have been discovered that powerfully alleviate pain in animal models. In parallel selective drug-like angiotensin II AT2 receptor antagonists are made as potential analgesics.

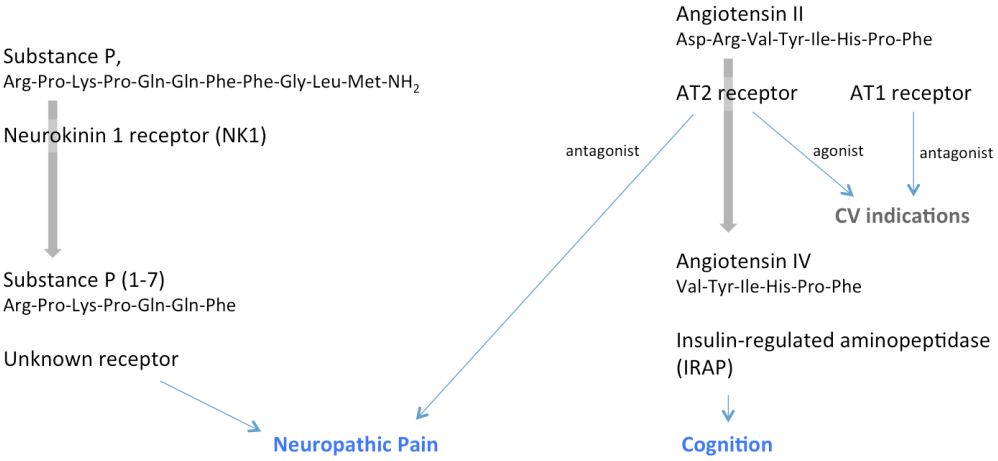

Figure: Degradation of bioactive neuropeptides may lead to fragments with different biological activities as compared to the parent peptide (Hallberg, 2014 Med Res Rev).

Angiotensin IV (angiotensin 3-8), a fragment derived from the hypertensive angiotensin II, improves memory after i.c.v. injection in rats. Potent drug-like compounds that mimic the effects of angiotensin IV and e.g. increase dendritic spine density in primary hippocampal neuronal cultures and that display neuroprotective effects have been identified. The density and morphological architecture of dendritic spines correlate with parameters associated with cognition. These compounds, aimed at serving as potential cognitive enhancers inhibit the proposed molecular target for angiotensin IV, insulin-regulated aminopeptidase (IRAP). At present considerable research efforts are devoted to these drug discovery programs that now involve both national and international collaborations.

Molecular Neuropsychopharmacology

Georgy Bakalkin

http://farmbio.uu.se/research/researchgroups/brdd/molneuropsyfarm

The main goal for the research group on Molecular Neuropsychopharmacology is to increase the understanding of molecular and epigenetic mechanisms underlying the development of alcoholism and substance addiction, and to develop treatment strategies. Identification of biomarkers and new therapeutic targets for the treatment of developmental psychiatric disorders is also in focus. Specific research activities within the group are described under projects.

Members of the group during 2014

Georgy Bakalkin, PhD, Professor
Tatiana Yakovleva, PhD, Senior scientist
Hiroyuki Watanabe, PhD, Research scientist
Daniil Sarkisyan, PhD, Research scientist
Qin Zhou, PhD, Research scientist
Lada Stålhandske, PhD, Research scientist
Ashot Pahlevanyan, PhD, System administrator
Wei Sun, Ph.D., Postdoctoral scientist
Xing Wu Zhou, PhD, Postdoctoral scientist
Igor Bazov, PhD student
Olga Kononenko, PhD student

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20. Ahmed AS, Li J, Erlandsson-Harris H, Stark A, Bakalkin G, Ahmed M. Suppression of pain and joint destruction by inhibition of the proteasome system in experimental osteoarthritis. Pain. 2012 Jan;153(1):18-26. doi: 10.1016/j.pain.2011.08.001. Epub 2011 Oct 22.

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1. Sirohi S, Bakalkin G, Walker BM. Alcohol-induced plasticity in the dynorphin/kappa-opioid receptor system. Front Mol Neurosci. 2012;5:95. doi: 10.3389/fnmol.2012.00095. Epub 2012 Sep 27.
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Agencies that supported the work/Funding 2014

The Swedish Council for Working Life and Social Research; The Swedish Governmental Agency for Innovation Systems; The Swedish Institute (Visby grant for collaboration with Ukraine); The Swedish Research Council; The Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning.

Other commitments/assignments of group members 2014

Georgy Bakalkin: Editor in Addiction Biology journal

Projects

(I) Integrated genetic and epigenetic approach to developmental psychiatric disorders: analysis of human blood and brain

Environmental stimuli influence the developmental trajectories of neural circuits from birth through adolescence. Exposure to harmful environmental stimuli during these developmental stages may result in increased vulnerability to psychiatric disorders. These effects are suggested to be partly dependent on genotype and mediated by epigenetic mechanisms. We aim to identify biomarkers and new therapeutic targets for the treatment of developmental psychiatric disorders, primarily alcohol dependence. We will perform genome-wide analysis of DNA methylation in blood from 2000 adolescents part of the IMAGEN study on factors that influence mental health in adolescents (http://www.imagen-europe.com/en/the-imagen-study.php), and of genotype, DNA methylation and gene expression in brain from circa 400

controls at different developmental stages and adult alcohol dependents. Loci associated with phenotypic traits relevant to alcohol dependence, DNA methylation and gene expression in the IMAGEN sample and diagnosis, DNA methylation and gene expression in the brain sample will be considered candidate biomarkers for alcohol dependence. Mechanisms underlying these associations will be considered candidate therapeutic targets for the treatment of alcohol dependence.

(II) Alcohol binge drinking – induced cognitive impairment in health and working life: genetic vulnerability and novel pharmacotherapy

Alcohol binge drinking patterns and excessive alcohol consumption have severe effects on public health and working life in Sweden and its Nordic neighbors. Delayed impairment of cognitive capabilities and executive functions are the most deteriorating consequence of heavy drinking. We examine the novel pathophysiological mechanism of this impairment, and develop clinically manageable strategies to identify subpopulations at risk and effective pharmacotherapy to treat alcohol-produced cognitive impairment. According to the mechanism, cycles of alcohol intoxication and withdrawal impair cognition through dysregulations in neurotransmission that is controlled by the endogenous opioid systems (EOS) including kappa-opioid receptor (KOPr) and its ligands dynorphins, prodynorphin (PDYN) products. 1) We examine whether genetic variants of the KOPr/*OPRK1* and *PDYN* genes contribute to alcohol use, abuse and dependence, and to alcohol-induced cognitive impairments. Population-based cohorts of young adult monozygotic and dizygotic twin pairs and office workers/social drinkers are analysed. 2) By conducting human post-mortem studies, we are identifing and characterizing dysregulations in the opioid and glutamate neurotransmitter systems, potential targets for pharmacological interference, in human heavy alcohol drinkers; and analyze underlying epigenetic mechanism. We focus on epigenetic modifications that may cause long-lasting behavioral alterations. 3) We plan to use animals models to mimic binge alcohol - induced impairment of cognitive functions and evaluate if these detrimental effects are reversed by available and novel pharmacological means. Characterization of the novel mechanism of alcohol binge drinking - induced cognitive impairment will open new possibilities for identification of subpopulations at risk, and for therapeutic intervention with opioid or glutamate antagonists.

(III) Pathogenic mechanisms of human neuropeptide mutations: implications for regulation of the anti-reward system

Much of our knowledge about functions of genes and proteins in human brain has come from analysis of rare human and mouse mutations. Such mutations represent entry points into novel signaling pathways and molecular mechanisms. In collaboration with Dr. Dineke Verbeek, Dept. Genetics, Univ. Med. Center Groningen, The Netherlands, we have identified missense mutations in the human prodynorphin gene (*PDYN*) coding for a precursor to opioid peptides dynorphins (Bakalkin et al., 2010). These mutations cause profound neurodegeneration in the cerebrum and cerebellum underlying the development of spinocerebellar ataxia type 23, a dominantly inherited neurodegenerative disorder. Remarkably, seven out of eight mutations are located in dynorphins which also have non-opioid neurodegenerative activities. This is the first finding on neuropeptide mutations underlying human neuropathology. The best-established function of the dynorphins in the brain is regulation of the anti-reward system by inducing dysphoria that counterbalances positive hedonic effects of endorphins and enkephalins, and addictive substances. Analysis of PDYN mutations could uncover novel molecular and cellular mechanisms of anti-reward - reward regulation that are generally unknown. We focus on two mechanisms. First, the mutations may impair correct folding of PDYN molecules in the endoplasmic reticulum, resulting in PDYN aggregation, cellular stress and the unfolded protein response. Long-lasting activation of the unfolded protein response by mutant PDYNs, or by wild-type - PDYN excessively produced under pathological conditions such as substance addiction, may lead to neuronal dysfunction, atrophy and neurodegeneration. Second, *PDYN* mutations may enhance non-opioid pathogenic activities of dynorphins. Our previous analysis suggests that dynorphins may mediate communications between neurons through non-receptor excitatory mechanism. This mechanism may be engaged in pathogenic effects of upregulated wild-type - dynorphins and mutant peptides. Our general goal is to understand pathogenic mechanisms of PDYN mutations and, in the following studies to evaluate whether wild-type - PDYN and dynorphins engage these mechanisms to regulate reward circuits under normal conditions and in substance addiction when these neuropeptides are excessively produced. We explore pathogenic mechanisms underlying actions of wild-type- and mutant-PDYN in cellular and in vitro biochemical/ biophysical studies, and are planning to use in vivo transgenic mice expressing human wild-type- or pathogenic mutant-PDYN that have been produced by Dr. Verbeek. Generalized pathological changes including cerebral cortical and subcortical atrophy, and agenesis of corpus callosum in patients caring PDYN mutations emphasize the fundamental role of neuropeptides in brain functions, and propose that the essential molecular mechanisms are affected; the phenomenon that requires detailed investigation.

Dissection of these mechanisms is critically important for understanding of reward – anti-reward, substance addiction, depression and chronic pain, all neuropathological conditions in which dynorphins play a critical role, as well as for the neuropeptide and neurodegeneration fields in general because of the novelty of mechanisms identified.

(IV) Pharmacotherapy of chronic pain. A novel approach that targets the ubiquitin-proteasome system

Chronic pain including neuropathic pain is an extremely disabling condition with the enormous cost for society and affected individuals and loss in work productivity. This pain is resistant to standard treatment protocols and thus represents a significant unmet medical and social need. We in collaboration with Prof. Frank Porreca group (Dept. Pharmacol., University of Arizona, USA) discovered a critical role of the ubiquitin-proteasome system (UPS), the specialized system for protein degradation, in the development and maintenance of neuropathic pain. This study provides experimental background for novel molecular concept that states that the development and maintenance of neuropathic pain critically depends on regulated protein degradation. We also demonstrated strong pain-killing effects of the UPS inhibitors. This is an especially promising possibility, because proteasome inhibitor velcade (bortezomib) has been recently approved in the US and Europe for the treatment of cancer. We now focus on the selection of the most potent and safe UPS inhibitors for further medical applications, and on molecular and cellular mechanisms of chronic pain. Actions of the UPS inhibitors are apparently mediated through pronociceptive sensory neuropeptides including dynorphins and CGRP.

Pharmaceutical Bioinformatics

Jarl Wikberg and Ola Spjuth

http://farmbio.uu.se/research/researchgroups/pb

During the year, a project funded by AstraZeneca on prediction of target profiles based on structural properties resulted in a new computational method and an open source chemical fingerprint that performed on par with the best fingerprints available. A follow-up study to determine parameters for model building resulted in a parameter "sweet-spot" that enables robust and accurate models to be built with less effort. New methods for visualization of predictive models in chemical structures were developed and integrated into the Bioclipse workbench, and work on conformal prediction to enable prediction intervals were continued. Studies in predictive metabolism were advanced, and the XMetDB open access database for xenobiotic metabolism reached beta-status.

Studies on automation of bioinformatics analysis on high-performance computing resources were started, and a project for evaluating predictive modeling on cloud computing was completed. A project in translational bioinformatics is aiming to bridge the gap between academia and hospitals for interpreting data from next-generation sequencing. Pilot projects were established, including analysis of mutations in BCR-ABL1 for chronic myelogenous leukemia (CML) based on long-read amplicon sequencing. A second pilot concerns bioinformatics analysis on whole-genome sequences of multi-drug resistant bacteria for providing decision-aid in clinical management.

The three-dimensional structures of the melanocortin receptors MC_1, MC_3, MC_4 and MC_5Rs, were modeled using agonist agonist-bound human adenosine A_{2A} receptor (A_{2A}R) as template. The models were relaxed using extensive molecular dynamics simulations, which resulted in the hitherto most accurate models of melanocortin receptors in the agonist bound state. The models where used to dock our earlier determined crystal structure of the MC_4R agonist THIQ into the MC-receptor models using molecular dynamics. This revealed that the THIQ MC_4R specificity was mainly due to charge and shape differences in the receptor cavities plus some less favorable amino acid interactions in the other MC-receptors.

During the year a novel synthesis method allowing the production of libiguins in large quantities was published as well as a study concerning the remarkable potency of the libiguins in enhancing sexual activity in male rodents.

Members of the group during 2014

Jarl Wikberg, Professor
Ola Spjuth, PhD, Assistant Professor
Maris Lapins, PhD, Researcher
Wesley Schaal, PhD, Researcher
Iryna Shutava, PhD, Post-Doc
Jonathan Alvarsson, PhD student
Samuel Lampa, PhD student
Martin Dahlö, PhD student on
Arvid Berg, Software engineer
Valentin Georgiev, PhD, Software engineer
Aleh Yahorau, Technician
Polina Georgieva, PhD
Martin Eklund, PhD, Researcher
Marco Capuccini and Staffan Andersson, Masters students

Publications 2012-2014

1. Alvarsson, J., Eklund, M., Andersson, C., Carlsson, L., Spjuth, O., & Wikberg, J. E. (2014). Benchmarking Study of Parameter Variation When Using Signature Fingerprints Together with Support Vector Machines. Journal of chemical information and modeling, 54(11), 3211-3217.
2. Alvarsson, J., Eklund, M., Engkvist, O., Spjuth, O., Carlsson, L., Wikberg, J. E., & Noeske, T. (2014). Ligand-based target prediction with signature fingerprints. Journal of chemical information and modeling, 54(10), 2647-2653.
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Other commitments/assignments of group members 2014

Ola Spjuth: Co-Director, UPPMAX High Performance Computing center. Manager, Bioinformatics Compute and Storage Facility at Science for Life Laboratory.

Projects

(I) Pharmacology of the libiguins (Jarl Wikberg et al.)
Studies on the mechanisms of action for the effects of libiguins on sexual behaviour; in part a collaboration with Philippe Rasoanaivo, IMRA, Antananarivo, Madagascar, Gunnar Antoni, PET-centre, Uppsala University and Aigars Jirgensons, IOS, Riga, Latvia.

(II) Isolation, structural determination and pharmacology of novel natural and semi-synthetic compounds (Jarl Wikberg et al.)
Studies devoted to the isolation, structural determination of novel natural compounds and semi-synthetic derivatives therefrom; collaborations with Torgils Fossen, Centre for Pharmacy, Department of Chemistry University of Bergen, Bergen, Norway, Philippe Rasoanaivo, IMRA, Antananarivo, Madagascar, and Aigars Jirgensons, IOS, Riga, Latvia.

(III) Proteochemometrics (Jarl Wikberg et al.)
Studies directed to further development of proteochemometics: Large scale proteochemometrics and integration of 3D-molecular modelling with proteochemometric modelling. Wet laboratory experimentations in relation to development of antivirals directed towards dengue and Japanese encephalitis viruses; in part a collaboration Mahidol University, Salaya, Thailand.

(IV) The Bioclipse Workbench (Ola Spjuth et al.)
Development of the Bioclipse workbench for e-Science. Main focus is on consumption and visualization of predictive models in drug discovery applications. Collaboration with Lars Carlsson at AstraZeneca R&D.

(V) Automated large-scale predictive modeling (Ola Spjuth et al.)
Studies on predictive modeling applied to large pharmaceutical data sets, requiring high-performance computing and cloud computing infrastructures. Collaboration with Åke Edlund at KTH Royal Institute of Technology.

(VI) Predictive toxicology and site-of-metabolism (Ola Spjuth et al.)
Studies on informatics and predictive modeling in toxicology and site-of-metabolism. Collaboration with Lars Carlsson at AstraZeneca R&D, Egon Willighagen at Maastricht University NL, Nina Jeliazkova at Ideaconsult BG, Roland Grafström at Karolinska Institutet and the OpenTox consortium (www.opentox.org).

(VII) Translational Bioinformatics (Ola Spjuth et al.)
Studies devoted to translating bioinformatics-based analysis of long-read amplicon sequencing data to clinical diagnostics with applications in chronic myelogenous leukemia (CML) and multi-drug resistant bacteria. Collaboration with Åsa Melhus at Department of Medical Sciences and Uppsala Academic Hospital, Adam Ameur at National Genomics Infrastructure, Lucia Cavelier at Department of Genetics and Pathology, and Johan Rung at Clinical Diagnostics, Science for LifeLaboratory, Uppsala University.

Pharmacometrics

Mats Karlsson

http://farmbio.uu.se/research/researchgroups/pharmacometrics

Pharmacometric research focuses on nonlinear mixed effects (”population”) models. Such models describe data, generally the response-time profiles observed in a clinical trial, by a basic model, accounting for the general structure of the underlying system, and a set of hierarchical variability components, accounting for variability between subjects, within subjects over time and remaining between observation variability. Research at the pharmacometrics group can be divided into four main areas. First, development and evaluation of methods for efficient and robust model building. This involves development of estimation algorithms, methods for model diagnosis and sequential procedures for model building. The result of the research, when applicable, is made available as free software. Second, so-called platform models are being developed for the use in specific therapeutic areas or for particular therapeutic/pharmacological principles. Such a model may involve the time-course of a biomarker or a system of biomarkers during normal, diseased and/or provoked situations. The third research area concerns utilization of the developed models for the purpose of designing studies, deciding upon dosing strategies and other developmental decisions. Fourth, we also do analyses of dose-concentration-response data from trials to understand therapies with existing drugs with the aim of allowing improved therapy.

Members of the group during 2014

Mats O Karlsson, Professor
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Ulrika Simonsson, Senior Lecturer, Docent
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Publications 2012-2014

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Reviews 2012-2014

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Dissertations 2014

1. Johansson ÅM. *Methodology for Handling Missing Data in Nonlinear Mixed Effects Modelling*, 2014. Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy, 189. ISSN 1651-6192
2. Kågedal M. *Nonlinear Mixed Effects Methods for Improved Estimation of Receptor Occupancy in PET Studies*, 2014. Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy, 188. ISSN 1651-6192
3. Ueckert S. *Novel Pharmacometric Methods for Design and Analysis of Disease Progression Studies,* 2014. Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy, 184. ISSN 1651-6192
4. Vong C. *Model-Based Optimization of Clinical Trial Designs*, 2014. Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy, 192. ISSN 1651-6192

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Other commitments/assignments of group members 2014

Lena Friberg: Deputy Editor-in-chief, CPT: Pharmacometrics & Systems Pharmacology; Organizing committee, chair scientific program, PAGE conference, Alicante 2014 and Crete 2015; Executive committee, WCoP 2016 conference; Scientific committee, 1st international workshop on dose optimization strategies for targeted drugs, Amsterdam 2015; Faculty of Pharmacy: Board member

Andrew Hooker: Organizing committee, PODE conference

Siv Jönsson: Department Board Member; Board Member: Swedish Academy of Pharmaceutical Sciences, Section for Pharmacokinetics and Drug Metabolism

Mats Karlsson: Deputy Head of Department; Department Board Member; Equal Opportunities Board Member; Editor Journal of Pharmacokinetics and Pharmacodynamics; Editorial Board on Clin Pharmacol Ther, Eur J Pharm Sci, Basic Clin Pharmacol Toxicol, CPT: Pharmacometrics & Systems Pharmacology

Maria Kjellsson: Department Board Member, Board Member: Swedish Academy of Pharmaceutical Sciences, Section for Pharmacokinetics and Drug Metabolism

Elisabet Nielsen: Executive Committee member, EPASG, ESCMID PK/PD Study Group
Ulrika Simonsson: Board Member: Swedish Academy of Pharmaceutical Sciences, Section for Pharmacokinetics and Drug Metabolism; Board Member: CPTR Regulatory Science Consortium, Critical Path to TB Drug Regimens. Clinical Disease Progression Modeling Workgroup. http://cptrinitiative.org/; Board member of IF´s stiftelse, Swedish Academy of Pharmaceutical Sciences.

Projects

Methodological research

(I) Optimal design/Clinical trial design (Martin Bergstrand, Andrew Hooker, Kristin Karlsson, Mats Karlsson, Joakim Nyberg)

There are two principle ways in which models can be used to evaluate and optimize clinical and pre-clinical experiments. The first is by simulation of a set of proposed designs from a model (or set of models), followed by evaluation of those resulting data sets using a metric of interest. The simulations, repeated many times with different random seeds, provide information about the expected results of various different designs (for example, measures of the precision and bias of parameter estimates, or the power to detect a drug effect of a specific size). With this methodology we have investigated, for example, differences in randomization schemes for dose-finding trials where it was found that dose-randomized trials are more powerful in characterizing the underlying relation compared to concentration-randomized trials. This increase in power can be achieved with, in most instances, a similar or lower number of observed side effects.

The second way of evaluating and optimizing trial designs is through the use of optimal experimental design methodologies. These methods often rely on calculations of an Information Matrix (e.g. the Fisher Information Matrix), which characterizes the information content of any possible design. Each design evaluation is much quicker than clinical trial simulation, thus one can investigate the landscape of possible designs (within constraints) potentially available for an experiment, and even optimize a design based on this information. We have developed methods and software (PopED) that utilize these methods with both local and global design criteria (e.g. E-family optimal designs, which take into account the underlying uncertainty in a pharmacometric model description of a biological system. Additionally, while optimal design has previously focused on optimization of sampling times in an experiment, we have extended the methodology to apply to other aspects of trial designs, such as the dose administered or the length of run-in, treatment and wash-out phases of an experiment. Further, we have extended optimal design methodology to optimize a study for power, as opposed to the traditional optimization based on model parameter estimation uncertainty.

The two methods of evaluating and optimizing trial designs can be combined to evaluate and explore adaptive optimal designs. In these types of trial designs, interim analyses are used to update models used to describe the system being investigated and then to use this information to re-optimize the next cohort of patients coming into a study. With combined simulation/optimization one can explore the adaptation and optimization rules one will use in an adaptive trial. We are currently developing such a tool (MBAOD), and are investigating the use of such designs in, for example, pedatric bridging studies.

(II) Model building and parameter estimation (Andrew Hooker, Mats Karlsson)

Pharmacometric models are based on (patho-)physiological and pharmacological knowledge. The complexity and heterogeneity of biological data makes the knowledge about, and development of, statistical data analysis methods a central part of this scientific field. There are many benefits to using pharmacometric models in the analysis of data from clinical trials, for example the ability to handle sparse data and to integrate different types of observations into one model; however, these models are complex and intrinsically non-linear which presents technical challenges in model building and estimation.

One main challenge is to reduce the time it takes to develop these models. With complex, non-linear models and data from a clinical trial that can have thousands of data points from hundreds of patients with multiple response variables, computer runtimes become non-ignorable. Generally, run-times can be divided into short (minutes), intermediate (hours to days) and long (days to months). The number of runs in a complete analysis tends to range between 30 and many hundred. We are investigating the implementation and automation of important modelling tasks through the use of new algorithms developed in our research group. Additionally, we are developing new methods of model building and new algorithm development that can shorten run times and the number of steps needed in the model building process.

Other areas of active research include the influence on parameter estimates of single observations and rational and statistically correct algorithms for adding explanatory variables, .i.e. covariates, to the models.

(III) Diagnostic tools (Andrew Hooker, Mats Karlsson, Siv Jönsson)

A main problem for complex pharmacometric models and data is to evaluate how well the models fit the data. For the assessment of an adequate description of data, methods of evaluations can be based on model predictions, residuals, simulations from the model, simulations followed by evaluation and simulations followed by full re-estimation. We are developing diagnostic tools based on all of these principles and for both continuous and categorical type data. Knowing about model and parameter uncertainty is often crucial in model-informed decision-making. We are developing methods for diagnosing existing methods of uncertainty estimates as well as developing new methods for both model and parameter uncertainty and how these can be applied in decision making.

(IV) Software development (Andrew Hooker, Mats Karlsson, Kajsa Harling, Rikard Nordgren)

One integral part of all of our research activities is the implementation of the methods developed in freely available software to facilitate a wider and consistent use of the new algorithms. Software developed by the group is PopED (http://poped.sf.net), PsN (http://psn.sf.net) and Xpose (http://xpose.sf.net).

(V) Pharmacodynamic modelling of discrete outcomes (Andrew Hooker, Mats Karlsson, Elodie Plan)

For many diseases, the main outcome is of discrete nature: stage category, symptom severity, number of events, or occurrence of events. In pharmacometrics, we distinguish non-ordered categorical data, ordered categorical data, count data, and (repeated) time-to-event data. Models handling this type of data are based on probabilities.

In this project we aim to describe disease progression and treatment exposure-response, and to develop new models for simulations of future studies. The time course of sleep stages and its relation to placebo and drug effects has been analysed using Markov models in patients with insomnia. Pain scores rated on a Likert scale by neuropathic patients have been modelled by including features for under-dispersion and serial correlation. Daily numbers of seizures have been used in the investigation of over-dispersion and Markov patterns in count data. Simultaneous characterization of drug effect on severity and time to acid reflux events has been made possible by a new type (repeated time-to-categorical event) of model and resulted in good simulation properties and high power to detect a drug effect. Parametric time-to-event models have been compared to semi-parametric Cox proportional hazard models implemented in NONMEM and methodology to simulate large scale unbiased (repeated) time-to-event data has been developed.

In parallel we also investigate the performance of available estimation methods with discrete models. We have pointed out the fact that the Laplacian estimation method in NONMEM and NLMIXED results in biased parameter in situations with non-even distributions of the response categories. In another study the Laplace method produced accurate parameter estimation for Poisson models, with or without Markov elements and mixture distribution, whereas we identified a small bias in the random effect of zero-inflated Poisson, generalized Poisson and negative binomial models. The performance of the SAEM and

importance sampling have been shown to be generally higher than Laplace in repeated time-to-events models where the frequency of individuals with events was low, while at high frequencies all methods were equal in performance. We have also conducted a study investigating all methods available in NONMEM version 7 for all types of discrete models, where we highlighted the overall fast and robust results obtained with Laplace.

Currently we are interested in handling the within-individual variability for discrete models. More specifically we adapted methods to handle inter-occasion variability in count models. Since count data are often collected on a regular basis (e.g. daily) in clinical trials, the time and length of potential occasions is not predefined but can be estimated through dynamic implementations. Alternatively the noise present in these types of records can be characterised using stochastic differential equations. Additionally we study ways to characterize underlying discrete processes with the implementation of hidden Markov models (HMM) in NONMEM. When there are suspected transient or definite transitions from an unobserved state to another one (low to high epileptic activity for example), these models permit to estimate the transition probability, on which time and drug effects can be investigated. Finally the individual sequences can be retrieved post-hoc via a Viterbi algorithm.

(V) Mechanism-based pharmacokinetic models (Martin Bergstrand, Mats Karlsson)

Clinical pharmacokinetic experiments typically measures drug concentrations in plasma only. As a consequence, pharmacokinetic models, used in drug development, aim to describe observations of drug concentration in plasma with minimum model complexity. Such models have limited capacity for extrapolations and to predict concentration-time profiles in tissues and organs. Also, mechanistic insight about drug disposition dependence of factors related to individual organs and tissues may not be possible to incorporate in a fully satisfactory manner. Physiologically-based pharmacokinetic (PBPK) models, which have a structure based on anatomy, can provide predictions in tissues and organs. However, because of their complexity, such models are traditionally not used for analysing clinical data. We have demonstrated that PBPK model parameters can be estimated based on clinical observations and are currently investigating the possibilities for improving such a combined “bottom-up and top down approach”. We have showed that for a relatively simple PBPK model such analyses can become feasible by using informative prior information about physiology and drug-related parameters. To further improve such an approach we are combining information about (co-)variability in organ and tissue properties from a data base representing physiological values for about 30000 subjects, tissue composition models and models for relating drug molecular properties and *in vitro* data to expected behaviour in tissues and organs.

A mechanism based approach has also been applied to better understand the processes involved in oral absorption. Several factors influencing oral absorption varies along the GI tract, e.g. pH, active influx and efflux transporters, gut wall metabolism. Models characterizing gastro intestinal transit and absorption properties along the GI region can be especially useful in the case of modified release formulations and/or substances with slow dissolution rate. Mechanism based models have been used for prospective population predictions of plasma concentrations based on *in vitro* dissolution data.

(VI) Pharmacometric modelling of biologic medicinal products (Siv Jönsson, Andrew Hooker, Mats Karlsson)

Biological medicinal products are becoming an important contributor in the treatment of many diseases, e.g. multiple sclerosis, rheumatoid arthritis, cancer, psoriasis. Characterization of biologics benefit from pharmacometric modelling, since they exhibit complex disposition characteristics, quite different to the processes and pathways utilized for small molecules, e.g. monoclonal antibodies exhibit target mediated drug disposition (TMDD).

Available TMDD models describe the formation of one complex (a dimer), but in reality further complexes may be formed (trimers, hexamers, etc), as described for IgE and omalizumab. We aim to explore and develop alternative TMDD models for the interaction between a target and drug, taking into account formation of different complexes. Furthermore, to explore study design options for studies in different stages of drug development, optimal design methodology is applied to TMDD models.

Applied research/Disease areas

(I) Antibiotics (Lena Friberg, Mats Karlsson, Elisabet Nielsen)

Antibiotics are considered one of the greatest discoveries of modern therapeutic medicine and have turned previously fatal diseases into treatable minor illnesses. Today, treatment failures due to multidrug-resistant bacteria are becoming more frequently observed. The evolution of resistance is a natural phenomenon; however, the use and misuse of antibiotics will accelerate this phenomenon. We aim to advance the understanding of pharmacokinetic-pharmacodynamic relationships for antibiotics of value for improving dosing recommendations and minimizing resistance development.

Today, dosing regimens are typically selected based on PK/PD indices that discard information on dynamic changes in the drug-bacteria interaction. Mechanism-based models describing time-kill curves from *in vitro* experiments form the basis for the modelling. The models have shown to be applicable across drugs and bacteria strains (including clinical isolates), for both static and dynamic concentration experiments, for different sizes of start inocula, for mixtures of wild-type and resistant bacteria and for drug combinations, and for predicting predicting competition experiments of wild-type and mutants. Optimal experimental design techniques are applied to find experimental protocols that increase the efficiency of both static and dynamic time-kill curve experiments and in clinical studies of colistin.

For colistin and meropenem, PKPD models based on *in vitro* data have been able to successfully predict previously determined pre-clinical *in vivo* PK/PD indices. Simulations have shown the PK/PD indices to be sensitive to experimental design, misspecification of the MIC, and differences in PK characteristics. The use of a mechanism-based PKPD modelling approach in dose selection has been suggested for increased robustness and extrapolation potential, especially for special patient populations.

Colistin has regained interest in recent years as a promising drug to overcome antibiotic drug resistance. With an in-house developed LC-MS-MS method we can quantify colistin and its prodrug CMS in both clinical plasma samples and in samples from *in vitro* experiments. Developed pharmacokinetic models have been applied to suggest loading doses of 6-12 MU. Whole-body Physiology-based Pharmacokinetic (WBPBK) models for CMS, colistin and ciprofloxacin have been developed based on data from various sources, including patients, healthy volunteers and several animal species. Such a model can be used to understand the time-courses of the antibiotics, and thereby the bacterial killing, in different tissues.

(II) Infectious diseases (Martin Bergstrand, Mats Karlsson, Ulrika Simonsson)

Plasmodium falciparum, the human immunodeficiency virus (HIV), and Mycobacterium tuberculosis (TB) are three devastating pathogens in tropical areas. Due to the geographical overlap of malaria, HIV and TB prevalence, the diseases are likely to co-exist in a great number of individuals. For these individuals, there is an obvious need for concomitant use of antimalarial, antiretroviral and antitubercular drugs. Drug-drug interactions may result from concurrent administration of drugs leading to diminished therapeutic efficacy of or increased toxicity from one or more of the administered drugs. Drug-drug interactions are an important concern in the management of patients with HIV because of the large number of antiretroviral drugs and other drugs that are required by these patients for the management of co-morbidities and opportunistic infections. Combination therapy has also been introduced in the management of malaria and TB, to overcome drug resistance. The exposure response relationships are not fully understood for the management of malaria and TB whereas there is more knowledge in the field of HIV. Better understanding of the exposure response relationships in malaria and TB is urgently needed. Our research focus also in suggesting dosing recommendations for children based on scaling from adult data or evaluation of pediatric data. Limited information is available in the literature on drug-drug interactions between the artemisinin antimalarial drugs and other drugs such as antiretrovirals or antitubercular drugs; consequently, the extent of such interactions is not fully known.

Activities of CYP enzymes and consequently drug-drug interactions occurring due to their inhibition or induction can be studied by using probe drugs. The pharmacokinetics of probes and drugs under investigation can be described by mathematical models in order to characterise and quantify the interaction.

We have developed enzyme turnover models to describe the time course of induction of different CYP450 enzymes by different artemisinin derivatives. One focus of the work has been to compare the potential for drug-drug interactions among the artemisinin drugs to choose a derivative that is suitable for combination therapy from a drug-drug interaction perspective.

One commonly used antitubercular drug is rifampicin which is known to induce CYP450 enzymes. Our work has involved quantitative analysis by modelling the pharmacokinetics of other drugs metabolised by these enzymes and which are used in HIV treatment. The need for potential dose adjustment has been evaluated for example nevirapine and lopinavir. For example, the population pharmacokinetics of nevirapine in HIV-infected patients taking nevirapine-based antiretroviral therapy in the presence and absence of the antitubercular drug rifampicin has been evaluated.

Pharmacokinetic drug-drug interactions can possibly be compensated for by dose adjustment of the target drug. The developed nevirapine model was used for simulations of different doses of nevirapine which revealed that increasing the dose of nevirapine to 300 mg twice daily elevated nevirapine concentrations above sub-therapeutic levels in most patients, with minimum exposure above the recommended maximum concentration. We have also investigated the population pharmacokinetics of lopinavir in TB/HIV co-infected children taking lopinavir/ritonavir in a ratio of 1:1 in the presence of the antitubercular drug rifampicin, with that of lopinavir in HIV-infected children taking lopinavir/ritonavir in a ratio of 4:1. Increasing the ritonavir dose in the TB/HIV co-infected children resulted in model predicted lopinavir trough concentrations above the recommended minimum therapeutic concentration.

Malaria was estimated to cause 800,000 deaths and 225 million cases worldwide in 2010. The mortality has recently been decreasing and is expected to decrease further due to more widespread use of effective treatment with drugs from the artemisinin class. However, a possible emerging resistance to these drugs might counteract this positive development. Drug resistance has appeared repeatedly within the area of malaria chemotherapy and drastically hampered our ability to fight the disease. It has been hypothesised that such development could have been avoided and or delayed with a better treatment regimen. We are conducting research with the aim of optimising anti-malarial treatment regimens with regards to both short and long-term outcome. Pharmacometric models have been used for translational simulations of expected treatment outcome in vulnerable populations such as children and pregnant and to optimize the treatment regimen.

(III) Type 2 Diabetes (Mats Karlsson, Maria Kjellsson)

Diabetes is a chronic disease, affecting more than 220 million people worldwide and the “diabetic epidemic” is projected to affect 366 million people by 2030, of which more than 90% will suffer from type 2 diabetes (T2D). This disease occurs when the body does not effectively respond to insulin and is unable to produce enough insulin to account for the inefficient use of insulin. This results in elevated blood glucose levels which is toxic and lead to complications; e.g. cardio-vascular diseases (CVD). The aim with most anti-diabetic treatment is to bring glucose levels down to healthy levels. Diagnosis of diabetes is mainly based on fasting plasma glucose (FPG) but also on glycosylated haemoglobin (HbA1c). The success of treatments is assessed on both FPG and HbA1c but also on dynamic glucose after provocation studies.

Provocation studies are used to characterize the functionality of the glucose-insulin system and could vary greatly in design from clamping of glucose or insulin by variable rate infusions, graded glucose infusions, intravenous bolus administration of glucose or insulin and oral administration of glucose solution or meals. We have developed several integrated models with simultaneous analysis of dynamic glucose and insulin after such provocations. These models, which include production, disposition and control (homeostatic) mechanisms of the system, have shown to realistically simulate outcomes of short dynamic glucose provocations at the raw data level. These models have been develop to describe both healthy subjects and patients with T2D as well as for the effect of incretin hormones on gastric emptying and insulin secretion, characterizing pre-hepatic insulin, mechanisms of oral glucose absorption as well as inclusion of exogenous insulin for insulin treated patients. We are focusing our current research on

including mechanism of glucagon release and glucagon effects on glucose and insulin as well as a fully mechanistic whole-body integrated glucose homeostasis model. As diabetes is a progressing disease we are also exploring the possibility of describing how glucose and insulin after provocation studies change over time for a pre-diabetic population.

Long-term clinical trials in T2D patients mainly focus on HbA1c. HbA1c is the fraction of the haemoglobin, in red blood cells (RBC), that has been glycosylated. This is a naturally occurring reaction depending on the amount of glucose in plasma; the higher the glucose concentration, the higher the HbA1c. As the life-span of RBCs ranges from 2 to 4 months, the HbA1c supplies a measurement of the sustained glycaemic control. Several models have been developed in-house to describe the relationship between HbA1c and FPG or mean plasma glucose (MPG) either using an empirical description or a mechanistic approach using knowledge about RBC life-span. Also models acknowledging the mechanism of insulin sensitivity, glucose production and disposition and changes in beta-cell mass or function in relation to weight loss has been developed. Currently we are investigating in what conditions which models perform the best in terms of power to detect drug effects and accuracy in estimates.

The overall endpoint of most anti-diabetic treatments is to lower the risk of long-term complications, such as CVD, retinopathy and chronic kidney disease. Long term studies commonly involve assessments of the risk of CVD in relation to elevated levels of HbA1c or FPG. We are developing parametric risk models, using registry data, quantifying the relationship between CVD and time-varying covariates such as HbA1c and other predictors of CVD, i.e. blood pressure, blood lipids, etc.

All models have been developed for the purpose of being used to quantify changes in the system following interventions (drug administration, diet changes, etc) and associate these changes with known or hypothesized mechanisms of impact of the system. Further the models are intended as tools for hypothesis generation regarding single or combined interventions as well as clinical trial design optimization.

(IV) Oncology (Lena Friberg, Mats Karlsson)

Within the oncology area, we are working on PK and PKPD models describing the time-courses of biomarkers drug-induced toxicity, tumour size measurements (SLD, sum of longest diameter), tumor activity measurements (SUV, standard uptake value) and overall survival. The models are aimed to be mechanism-based and be applicable across different drugs, and thereby valuable in development of new and existing drugs, including individualization of the therapy. By integrating information of different variables in to a modelling framework the variables' relations and predictive value can be tested, and a better overview of both desired and adverse effects from a changed dosing regimen can be obtained. The models can also be used to explore different concepts of study design in oncology. This type of modelling framework, including biomarkers, side-effects, tumor response and survival, has been developed for sunitinib in gastrointestinal stromal tumors and is being developed for axinitib in renal cell carcinoma. Different metrics of tumor size, both constant and time-varying, as well as one dimension (diameter) vs. three-dimensional (volume), is being compared for predicting overall survival.

Projects are on-going around extensions and applications of a semi-physiological model that can describe neutropenia for numerous anticancer drugs. As an example, the interaction between G-CSF and neutrophil, as well as the time-courses of IL-6 and CRP after initiation of chemotherapy, has been characterized based on generated data from a clinical study. In addition, the predictive value of frequent measurements of neutrophils, that would be possible with a device for home measurements, is being explored based on simulations from the semi-physiological model. The time-courses of thrombocytopenia have been characterized for abexinostat and TDM-1 using the semi-physiological model as basis. For TDM-1, an integrated model that in addition to platelets includes the liver enzymes ASAT and ALAT is being developed, where the driver for the toxicity is being explored.

Patient Reported Outcomes (PRO) is an increasingly used component for comparison of drug treatments during oncology drug development. Item Response Theory has shown to be a promising methodology to analyse and interpret these type of data where questions are grouped into different categories.

(V) Progressive disorders (Lena Friberg, Andrew Hooker, Mats Karlsson, Elodie Plan, Ulrika Simonsson)

Progressive neurological disorders such as Alzheimer's Disease and Parkinsons Disease represent challenges in many respects. For characterising drug effects, the slow progression may make it difficult to assess in what aspects the treatment impacts the disease, in a disease-modifying or symptomatic manner. How to best design and analyse trials in these diseases, but also diseases with other progression pattern such as Multiple Sclerosis and Schizophrenia and the subject of such evaluations. Further, the severity of most of these diseases are followed over time using clinical scoring scales which are formed from tests or questions probing individual aspects of the disease. Rather than using the overall clinical scale, we are developing methodology and models based on item response theory (IRT). In these each item (i.e. question or test) is modelled individually as a latent variable of the disease-related disability.

(VI) Immunological disorders (Lena Friberg, Mats Karlsson)

Multiple Sclerosis is both a complex and chronic neurological disease of the CNS. The natural course of MS is slow and difficult to monitor clinically. The overall aim of this project is to establish the first population, data driven, MS disease progression model in order to construct a mathematical modelling platform where the interplay between the majority of relevant aspects of the disease, such as time course of disability progression, relapse rate dynamics, time course of the imaging data, time course of lymphocytes and population characteristics are incorporated. Model building involves sequential development of (i) separate models for all components of interest (disease progression, relapse rate dynamics, MRI dynamics and lymphocytes including CD4+, CD8+) and (ii) the covariate model, which explore the underlying patient factors influencing within- and between-subject variability in treatment response. The final anticipated model shall enable simultaneous characterization of the interplay between relapse rate dynamics, total CD4+ and CD8+ lymphocytes and their ratio, and MRI readout dynamics in the evolution of disease and, most importantly, link the time-course of MRI and clinical outcome (relapse rate and disability) and lymphocyte data.

In rheumatoid arthritis a range of variables of the disease are summarized into a clinical endpoint for evaluation of drug response - the dichotomous ACR20 score. Integrated longitudinal transition models with dropout are useful for understanding the outcome of different dosing schedules and by expanding such models to also include the more stringent ACR70 criteria more information can be preserved. To increase the information on the concentration-effect relationship in the available data, a longitudinal transition model describing the probability of ACR20, ACR50 and ACR70 responses have been developed.

(VII) Dose individualisation in hemophilia (Siv Jönsson, Elisabet Nielsen)

Hemophilia is a group of hereditary genetic disorders impairing blood coagulation. Hemophilia A and B refer to the coagulation factor VIII and IX deficiency, respectively, whereof hemophilia A is the more common although rare: in Sweden 14 of 100 000 boys and men have hemophilia, whereof 80 % has hemophilia A. By substitution therapy with the coagulation factors, on-demand or prophylactically, the disease status can be controlled. In prophylactic treatment of factor VIII and IX, pharmacokinetic (PK) tailored dose individualization, i.e. the individual dose is based on Bayesian estimation using pharmacokinetic information and a population PK model, is promoted and appears to be a good approach for reducing the total doses administered.

We are involved in one project where the feasibility of PK tailored dosing for prophylactic FVIII treatment is evaluated in routine clinical practice. Our contribution in the project refers to the Bayesian estimation of the dose and in the future re-development of previously existing population PK models. Furthermore, for factor IX we have re-developed a population PK model and, based on the model, explored alternative clinical practically sampling schedules to be used in PK tailoring.

Steroid P450

Maria Norlin & Kjell Wikvall

http://farmbio.uu.se/research/researchgroups/steroidp450

Our research is focused on the properties and regulation of cytochrome P450-mediated enzymatic processes involving steroids. Steroids and steroid-related genes and enzymes, producing biologically active metabolites, are vital for human physiology. Many steroids or steroid-related compounds are used as drugs. We apply our expertise in biochemistry and molecular biology to obtain knowledge of relevance for drug discovery and development and drug management. In particular we focus on steroids. We study metabolic conversions, gene regulation and cellular effects of steroids.

Primary missions and goals are to find new drug targets, understand the mechanisms for effects of steroids and steroid-related enzymes, compare effects of endogenous molecules and drugs, examine effects of drugs and drug candidates on steroid-related processes and study molecular mechanisms behind adverse drug effects. Our research activities, focusing on basic research, provide important information that increases the understanding of mechanisms for steroid action. This should improve drug management (e.g. counteract/avoid adverse side-effects) and can be used for development of novel, better drugs.

Members of the group during 2014

Kjell Wikvall, MD, PhD, Professor
Maria Norlin, PhD, Associate Professor
Ida Emanuelsson, PhD student
Christine Wegler, PhD student
Mokhtar Almokhtar, PhD student
Ahmad Zayny, PhD student

Publications 2012-2014

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Other commitments/assignments of staff members 2014

Kjell Wikvall: Chair of the Scholarships committee for the Faculty of Pharmacy; Member of the Scholarships committee for Uppsala University; Study director in Pharmaceutical Biochemistry
Maria Norlin: Senior member of the Departmental Committee for PhD education (FUG).

Projects

(I) Functions of steroids and steroid-metabolizing enzymes for hormonal signalling and cellular viability

This research concerns steroids involved in hormonal signalling, sex hormone biosynthesis and brain function. The studies are focused on physiological and pharmacological control of steroid levels, effects of metabolic events and regulation of gene expression. The project concerns endogenous steroids, steroid drugs and drugs affecting steroid hormone receptors such as SERMs (selective estrogen receptor modulators) and vitamin D analogues.

The studies include mechanisms of importance for estrogenic and androgenic signalling. Some examples of steroids of interest are pregnenolone and dehydroepiandrosterone (DHEA). These steroids are well-known as precursors for androgens and estrogens but also play roles in brain function and in connection with cell growth and viability, e g in neurodegenerative processes. Tissue-specific metabolism of DHEA leads to a number of metabolites with differential effects on cell functions. Enzymes that regulate the concentration of neuroactive steroids in the brain may be future targets for therapy of importance for abnormal cell growth, immune function or in neurodegenerative conditions. Some of our current studies involve enzymes and genes of importance for the levels of neurosteroids such as DHEA and pregnenolone in neurons and glial cells. Regulation of hormone metabolism in the nervous system by endogenous steroids and pharmaceutical compounds is also studied.

Steroids may affect growth and differentiation in several tissues. Thus properties of steroids may be of interest in a wide range of normal and disease conditions, e g in neuroprotection or cancer therapy. We study effects of steroids such as enzymatically formed oxysterols (cholesterol derivatives), hormones and vitamin D-like compounds on cellular survival and growth. These studies particularly focus on cells of the central nervous system.

(II) Bioactivation and metabolism of vitamin D including vitamin D-mediated effects on cellular function

This research is focused on enzymes and genes of importance for vitamin D bioactivation, metabolism and function. Effects on these processes by endogenous and pharmacological compounds are studied. Many drugs result in adverse side effects, including increased risk of bone disease. We study the mechanisms by which anti-inflammatory, anti-viral and anti-epileptic drugs affects cells, with particular focus on the bone and the central nervous system. Vitamin D is needed for regulation of calcium levels in the body and vitamin D deficiency leads to skeletal diseases such as rickets in children and osteomalacia/osteoporosis in adults. The biologically active form, 1,25-dihydroxyvitamin D, is formed through metabolic activation. The activated form of vitamin D blocks cell division and increases cell differentiation. Vitamin D analogues (synthetic compounds with vitamin D-like effects) are used in the treatment of psoriasis and are of interest in development of new cancer therapy. In addition, epidemiological data during recent years have indicated that vitamin D may have many more targets than previously known. Altered vitamin D levels in plasma have been linked to a number of different conditions including neuropsychiatric and neurodegenerative disease. For these reasons, it is important to obtain more knowledge about the enzymes and genes in activation and metabolism of vitamin D and the roles of the formed metabolites. Part of this project is focused on cellular effects of vitamin D and vitamin D analogues in order to explore previously unknown functions of these compounds.

Translational Pharmacokinetics/Pharmacodynamics

Margareta Hammarlund-Udenaes

http://farmbio.uu.se/research/researchgroups/tPKPD

Our research focuses on understanding the fundamental and functional aspects of pharmacokinetics-pharmacodynamics (PKPD) in health and disease. Within the area of translational PKPD we address conversion of pharmacokinetic data from the preclinical to the clinical setting. This is strongly needed for optimizing drug discovery processes and promoting efficient treatment of CNS diseases. We are interested in brain drug delivery in relation to transport processes of both small and large molecules across the blood brain barrier (BBB), where a quantitative role of nanocarriers is specifically studied. Using the concepts and methodologies developed for the brain we are also exploring drug distribution in the lung. This is important for the design and evaluation of lung targeted drugs which may be administered by inhalation. For this purpose, methods are being developed to help analyzing the pharmacologically relevant unbound concentrations in brain and other tissues. Our work also emphasizes the importance of bridging the expertise within academia and pharmaceutical industry in order to seek excellence in method development for better therapeutics treating CNS and lung disorders.

Members of the group during 2014

Xiomei Chen, PhD student (guest from University of Michigan, USA)
Jessica Dunhall, Laboratory Assistant
Sofia Gustafsson, MSc in Biomedicine, PhD student
Margareta Hammarlund-Udenaes, PhD, Professor
Britt Jansson, Laboratory Engineer
Annika Lindqvist, MSc in Pharmacy, PhD student
Irena Loryan, MD, PhD, Researcher
Erik Melander, MSc in Pharmacy, PhD student
Nebojsa Mihajlica, MSc in Pharmacy, PhD student
Maryam Payan, PhD student (guest from University of Teheran, Jan - May)

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Discussion articles and blogs 2014

Booklet about "Understanding brain drug delivery":
http://www.adjacentgovernment.co.uk/wp-content/uploads/2014/01/Uppsala-Pharma-ebook-web.pdf
Blog about drug development within the CNS area:
http://www.pharmaphorum.com/articles/why-look-for-the-key-where-you-lost-it-when-you-can-look-for-it-where-there-is-more-light

Agencies that supported the work/Funding 2014

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Other commitments/assignments of staff members 2014

Margareta Hammarlund-Udenaes: Associate Editor of the Journal Pharmaceutical Research, Editorial Advisory Board member of the Journal of Pharmaceutical Sciences and of Fluids and Barriers of the CNS, Member of the Executive Committee of the European Federation of Pharmaceutical Sciences with responsibility regarding Education and Training. Vice Chair of the Research Education Committee of the area of Medicine and Pharmacy.

Projects

(I) Concepts and method development regarding drug delivery to the brain (Irena Loryan and Margareta Hammarlund-Udenaes)

Drug transport across the BBB and cellular barriers with subcellular distribution in the brain parenchyma are key processes of interest. Moreover, brain pharmacokinetic processes are also investigated at the level of its regions. In order to enhance the mechanistic understanding of brain target-site pharmacokinetics, several advanced methods are being developed by the group.

Neuropharmacokinetic parameters such as unbound brain-to-plasma concentration ratio (Kp, uu, brain), unbound volume of distribution in brain (Vu,brain), and permeability clearance into the brain (CLin), are descriptors of BBB function and intracerebral distribution established by the group. Kp, uu, cell is another unique parameter characterizing the extent of cellular barrier transport. These parameters are identified by means of systematic PKPD analyses.For quantitative evaluation of BBB transport we are using cerebral microdialysis, recognized as the "gold standard" for measurement of unbound drug concentration in the brain. An alternative strategy is based on combination of high-throughput in vitro techniques, such as brain homogenate equilibrium dialysis and brain slices.

(II) Translational aspects of brain drug distribution in health and disease (Sofia Gustafsson, Irena Loryan and Margareta Hammarlund-Udenaes in collaboration with Stina Syvänen and Martin Ingelsson, Dept Public Health and Caring Sci.)

The interpretation and translation of neuropharmacokinetic data might be even more challenging during disease conditions, where a disrupted integrity and function of the BBB is apparent.

Recent findings even point towards dysfunctional BBB as being the cause of neurodegenerative disease etiology and progression. As a result, dysfunction in BBB processes might lead to altered brain pharmacokinetics of CNS drugs as well as peripherally acting drugs, which normally have a very low brain penetrance, resulting in unpredicted CNS effect or side effect profiles. Moreover, CNS disorder pathology usually affects certain areas of the brain which might result in regional differences of brain drug distribution and binding.

Hence, our research aims to investigate differences in drug distribution to and within the brain as well as drug binding in separate brain regions both in health and disease, preclinically and clinically. By combining and comparing in vitro and in vivo experiments with clinical studies the current project strives to increase the understanding of pharmacokinetics and disease implication on brain drug distribution. Results from in vitro techniques as well as microdialysis and non-invasive imaging techniques, such as positron emission tomography (PET), is integrated and used to address the current issues.

(III) The role of pericytes for brain drug distribution (Nebojsa Mihajlica, Margareta Hammarlund-Udenaes in collaboration with Christer Betsholtz, Dept Immunol, Genet and Pathol).

Endothelial cells of the BBB represent a key component of the neurovascular unit (NVU), which also includes other types of cells such as pericytes, astrocytes, vascular smooth muscle cells, microglia and neurons.

Although the anatomical relationship between pericytes and endothelial cells suggests their close interaction, the relative contribution of the pericytes is still not sufficiently elucidated. Interaction between cells within the NVU is essential for the normal functioning of the central nervous system, whilst impairments in their communication can result in the development of many pathological conditions.

The principal aim of this project is to improve our understanding of the mechanisms of the NVU regarding the drug distribution into brain parenchyma, with special emphasis on the contribution of pericytes. Hence, better understanding of pericyte role in BBB regulation and drug distribution into the CNS has a great potential for the improvement of current pharmacotherapy of neurodegenerative diseases and other pathological conditions associated with BBB impairment.

(IV) *Biomolecular drugs and nanocarriers (Annika Lindkvist, Erik Melander and Margareta Hammarlund-Udenaes in collaboration with Drs Pieter Gaillard and Jaap Rip, to-BBB, and Ulf Göransson, Div Pharmacognosy, UU)*.

Peptides and proteins play a crucial role in the regulation of brain activity in health and disease conditions. They are therefore promising candidates in the development of new neurotherapeutics. Understanding the use of large molecules and their interaction with the barriers of the CNS is crucial in order to succeed in the clinic.

One promising group of peptides is the cyclic peptides originating from different plant families. These peptides exhibit exceptional stability due to a cyclic cysteine knot forming disulfide bonds. Their cyclic nature makes them less susceptible to degradation by proteases, causing a great interest from a drug development perspective.

Our research focuses on the pharmacokinetics of brain delivery of peptides and the possible quantitative benefits of using nanocarrier systems, including their effect on the pharmacodynamic outcome. In vivo preclinical studies are performed to estimate the modulation of penetration across the BBB using targeted liposomes. For authentication of the principles, physiologically based pharmacokinetics population modeling is applied.

(V) Optimizing brain penetration of drug candidates (Irena Loryan and Margareta Hammarlund-Udenaes)

The combinatory mapping approach developed by our group can be used as a BBB screening toolbox for selection of candidate drugs in early drug discovery by pharmaceutical companies.

In addition, a present project is focused on identification of desirable physicochemical properties for CNS penetration using computational models, aiming to facilitate discovery and development of novel neurotherapeutics.

Integration of overall findings is directed towards the development of physiologically based mathematical models of brain drug disposition.

(VI) Drug distribution in the lung (Erica Bäckström, Elin Boger (Marie Curie program IMPACT, University of Warwick), Markus Fridén (AstraZeneca) and Margareta Hammarlund-Udenaes)

This is a new project started in 2013 in collaboration with AstraZeneca. The treatment of asthma and chronic obstructive pulmonary diseases (COPD) was revolutionized by the introduction of inhaled corticosteroids (ICS) and bronchodilators such as beta adrenergics and anti-muscarinics. For these drug classes topical delivery by inhalation has provided an efficient means of overcoming systemically mediated side-effects, that previously limited the dosing and therapeutic response.

Despite the historical success of inhalation medicines and the significant share (10 %) of the global drug market, there is very little known about the fundamental prerequisites for a drug molecule to be retained in and exert a localized effect in the lung when administered by inhalation.

Our research aims to study basic mechanisms of drug distribution in the lung which include non-specific tissue binding, lysosomal trapping and carrier-mediated membrane transport as well as the profound influence of blood perfusion. We are employing a lung slice methodology, equilibrium dialysis of lung tissue and in vivo methodologies to study the extent of drug distribution in the lung and the absorption half-life of inhaled drugs.

As an indicator of the unbound and pharmacologically active drug concentration we are collaborating with industrial and academic partners on measurement of target occupancy in the lung after inhalation using mass-spectrometry or positron emission tomography (PET). Along the same lines we are also developing physiologically based pharmacokinetic (PBPK) models to better explain and predict the possible advantage of the inhaled route of drug delivery.

(VII) Clinical Pharmacy Research (Johanna Sulku, Anna Alassaad, Elisabet Nielsen and Margareta Hammarlund-Udenaes in collaboration with Håkan Melhus, Dept Med Sci, Ulrika Gillespie, Uppsala Univ Hospital, and Hirsh Koyi, Gävle)

We are interested in evaluating the impact of clinical pharmacist interventions in medical care.

The purpose of this research is to see whether, and in what way the contributions of clinical pharmacy services in the hospital ward changes patient treatment and status. This research area is new in Sweden and important for the development of clinical pharmacy in this country. A seminal paper was published in 2009 in Arch Intern Med, which received much attention. Here we showed that clinical pharmacist intervention reduced costs and decreased the number of readmissions to hospital. The research is further oriented towards appropriate use of medicines in chronic obstructive lung disease.

Undergraduate Teaching 2014

Most of the teachers were involved in the continued work with revision of the pharmacy programme, now with focus on areas of renewal and development of more focused skill training during the course of the programme. Lena Bergström and Ann-Marie Falk led the work with an improved programme structure for training of skills, such as oral and written communication and lab skills, and also for better professional training in the new programme. Anne-Lie Svensson and Jörgen Bengtsson led the project with half-time assessment, improved feed-back and examination of students in their bachelor project. The project was evaluated and received positive feed-back from both students and supervisors.

The major part of the undergraduate teaching is within the two Pharmacy programmes. During 2014 the extent of undergraduate teaching was 461 *hst* (full-time equivalents) and that represents 45% of the total number of hst within the Faculty of Pharmacy. In addition, the department is involved in teaching at the Master of Science in Chemical Engineering with specialization in drugs and the Biomedical programme. Students attending internet-based courses during 2014 comprised 83 hst.

Pharmaceutical Biosciences comprises a number of courses: Drug development and Drug usage, Drug metabolism and safety, Gene technology, Immunology, Infection Biology, Microbiology, Molecular Biology, Pharmaceutical Biochemistry, Pharmaceutical bioinformatics, Pharmacology, Pharmacokinetics, Pharmacotherapy, Physiology, Toxicology. A mix of traditional teaching and problem-based learning with lectures, laboratory sessions, seminars, workshops and computer sessions characterizes the teaching. In addition, the teachers are involved in interdisciplinary training in laboratory practice, communication skills and professional development.

Teaching at the basic level

The main teaching at the basic level is within the Bachelor of Science in Pharmacy programme that comprises three years studies (180 hp) and the first years of the Master of Science in Pharmacy programme, see below. Completed studies at the Bachelor programme provide the necessary theoretical and practical competence that is required to apply for a pharmacist license as *receptarie*. The teachers also instruct in undergraduate projects (15 hp) at the basic level. These projects are individual and they are examined by an oral presentation and a written report. During 2014, the teachers within the department supervised 86% (30 students) out of the total number of undergraduate projects within the Bachelor of Science in Pharmacy programme.
Some basic level courses are open for other students than pharmacy students and attract both students at other programmes at Uppsala University or other universities and also professionals.

Teaching at the advanced level

The department gives courses at the Master of Science in Pharmacy programme that comprises five years studies (300 hp). Completed studies at the programmes provide the necessary theoretical and practical competence that is required to apply for a pharmacist license as *apotekare*. The teachers instruct in undergraduate projects (30 hp) at the advanced level. These projects are individual and are examined by an oral presentation

and a written report. The majority of the projects are laboratory-based and involve the student in ongoing research projects. During 2014, the teachers supervised 79% (57 students) of the total number of undergraduate projects within the Master of Science in Pharmacy programme.

The teachers lead and teach a number of elective courses and single subject courses at the advanced level. These courses mirror research profiles within the department, such as Bioinformatics, Clinical pharmacy, Drug metabolism and safety, Drug addiction and Pharmacokinetics. The courses attract a large number of students, not only pharmacy students but also other students and professionals showing the proper prerequisites. During 2014, 77% (310 students) of the students within the Master of Science in Pharmacy programme and 76% (84 students) of the students within the Bachelor of Science in Pharmacy programme participated in elective courses given by the department.

The teachers also contribute to the teaching within several master programs within the Faculty of Pharmacy, Drug development, Drug management and safety and Clinical Pharmacy, and at master programmes at the Faculty of Medicine, Infection biology and Forensic Science.

Uppsala 2015-05-05

Ingrid Nylander

Course List 2014

List of courses on basic and advanced (second cycle) levels

Abuse and Addiction, 7,5 c
Acute Intoxications and Clinical Toxicology Second cycle, 7.5 c
Advanced Pharmacotherapy Second cycle, 7.5 c
Adverse Drug Reactions and Pharmacovigilance Second cycle, 7.5 c
Analytical Toxicology Second cycle, 30 c
Applied Pharmaceutical Bioinformatics Second cycle, 5 c
Applied Pharmaceutical Structural Bioinformatics Second cycle, 5 c
Applied Pharmacotherapy, Pharmacokinetics and Therapeutics Second cycle, 15 c
Biochemistry of Gene Regulation Second cycle, 7.5 c
Clinical Attachment and Service Development Second cycle, 18 c
Clinical Drug Trials with Applied Biostatistics Second cycle, 7.5 c
Clinical Pharmacokinetics and Pharmacodynamics Second cycle, 7.5 c
Clinical Pharmacy, 7.5 c
Degree Project in Drug Discovery and Development Second cycle, 30 c
Degree Project in Drug Management, 15 c
Degree Project in Drug Management Second cycle, 30 c
Degree Project in Pharmaceutical Biochemistry, 15 c
Degree Project in Pharmaceutical Biochemistry Second cycle, 30 c
Degree Project in Pharmaceutical Bioinformatics Second cycle, 30 c
Degree Project in Pharmaceutical Bioscience Second cycle, 20 c
Degree project in Pharmaceutical Pharmacology Second cycle, 30 c
Degree project in Pharmacokinetics, 15 c
Degree Project in Pharmacokinetics Secnd cycle, 30 c
Degree project in Pharmacokinetics D Second cycle, 30 c
Degree Project in Pharmacology, 15 c
Degree Project in Pharmacotherapy, 15 c
Degree Project in Pharmacotherapy Second cycle, 30 c
Degree Project in Pharmacotherapy D Second cycle, 30 c
Degree Project in Toxicology, 15 c
Degree Project, Toxicology D Second cycle, 30 c
Drug Dependence Mechanisms, Prevention of Cannabis Abuse (Contract education) 7,5 c
Drug Development and Drug Usage, 7.5 c
Drug Management Second cycle, 7.5 c
Drugs and Dependence, Advanced Course Second cycle, 7.5 c
Drugs and the Elderly Second cycle, 7.5 c
Embryotoxicology, Advanced Course D Second cycle, 7.5 c
Embryotoxicology, Intermediate Course B, 7.5 c
Evidence Based Clinical Pharmaceutical Methods Second cycle, 12 c
Models for Biological Systems Second cycle, 7.5 c
Molecular Biology with Focus on Drug Therapy, 7.5c
Molecular Mechanisms for Enzymatic Activation Second cycle, 7.5 c
Molecular Pharmacology, 7.5 c
Neuropharmacology Second cycle, 7.5 c
Pharmaceutical Biochemistry, 9 c
Pharmaceutical Biochemistry and Cell Biology, 7.5 c
Pharmaceutical Bioinformatics Second cycle, 7.5 c
Pharmaceutical Bioinformatics (Contract Education), 7.5c
Pharmaceutical Molecular Biology with Bioinformatics, 7.5c
Pharmacokinetics, 3 c
Pharmacokinetics, 7.5 c
Pharmacokinetics and Statistics, 9 c
Pharmacology, 15 c
Pharmacology, 16.5 c

Pharmacology for engineering students, 7.5 c
Pharmacotherapy, 7.5 c
Pharmacotherapy in Self-Treatment, 9 c
Preclinical Safety Assessment and Pharmacovigilance, 7.5c
Research Project in Clinical Pharmacy Second cycle, 15 c
Toxicology B, 7.5 c
Toxicology for Engineering Students, 7.5 c
Toxicology, Advanced Course D Second cycle, 30 c
Toxicology, Drug Metabolism and Safety Assessment, 4.5 c
Toxicology, Drug Metabolism and Safety Assessment, 7.5 c
Toxicology, Intermediate Course C Second cycle, 15 c
Veterinary Pharmacology Second cycle, 7.5 c

Research Education 2014

The Department has a high priority for research training. The aim is to prepare the PhD students to become independent researchers able to make significant contributions to academia and/or pharmaceutical industry, and to take on highly qualified professional tasks. The Department has two weekly seminar series for PhD students and young scientists – one series on pharmacokinetics/pharmacometrics and one series on basic biomedical research. Here, the PhD students can improve their oral presentation skills and their ability to discuss research, which is an important goal within the scientific training.

The 4-year PhD program consists of research work and a number of courses, with a total course requirement of 30 points (one semester full-time). Most PhD students take more courses than that. The PhD thesis is a doctoral thesis consisting of separately published articles with a comprehensive introductory summary. A PhD thesis typically contains 4 – 5 scientific papers, of which at least half are published in scientific journals at the time of the public thesis defense. Besides the research activities, the PhD students participate in seminars and at international conferences, of which one presentation at an international conference is obligatory.

Many PhD students also perform important tasks in undergraduate teaching, comprising around 20 % of their time, making the time for a PhD exam be around 5 years in total. The teaching assignments give the students a broader knowledge base than their own thesis area. It also gives them leadership and communication skills that are of importance in further professional life. PhD students may also tutor master students in experimental research work. At the end of 2014 there were 54 PhD students registered, plus 7 PhD students who defended their theses during the year.

The Research Education Group at the Department actively structures the application procedure for PhD student positions, especially by contributing to the process of establishing new positions and selecting new PhD students. This group consists of two representatives from teachers and one PhD student. The group through the Chair also follows the training by requesting yearly follow up document from each PhD student – supervisor where issues regarding coursework and communication between student and supervisor can be evaluated. The Chair is also a member of the Research Education Committee of the area of Medicine and Pharmacy, hereby connecting to central decision making.

Uppsala 2015-04-27

Margareta Hammarlund-Udenaes

Awards 2014

1. Eva Brittebo, Appointed Uppsala University's Rudbeck Medal
2. Lennart Dencker, Appointed Uppsala University's Gustaf Adolf Medal
3. Mats Karlsson, Appointed the Peter Coates Lecturer at Pharmacokinetics UK (PKUK)
4. Mats Karlsson, Appointed The Sheiner-Beal Award by The American Society for Clinical Pharmacology and Therapeutics (ASCPT)