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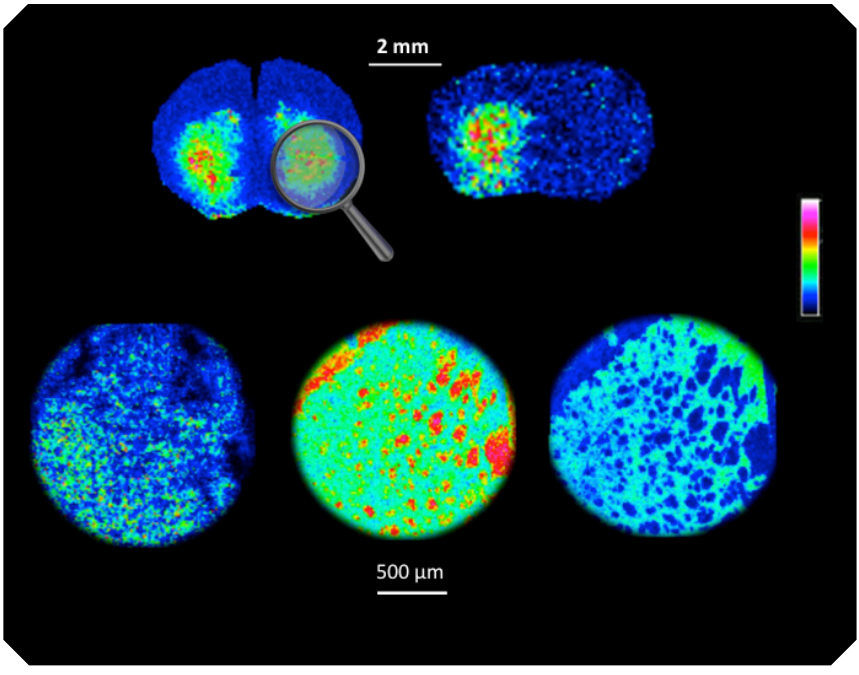
Farmbio 2013/32

ANNUAL REPORT 2012

Department of Pharmaceutical Biosciences



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Department of Pharmaceutical Biosciences

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Layout: Louise Engström
Front page illustration: Medical Mass Spectrometry

"Matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) imaging of the neurotransmitter dopamine directly in brain tissue sections (control-top left, and unilaterally 6-OHDA-lesioned mouse-top right) acquired with spatial resolution of 100 µm. The highest concentration of dopamine is detected in the striatal region of the brain, while its signal is completely absent in the lesioned side of the brain. High-resolution MALDI-MS images of a selected area of the striatal brain structure show distribution of acetylcholine (bottom left) and lipids with m/z 779.2 (mid bottom) and m/z 592.3 (bottom right) with a spatial resolution close to cellular level (15 µm)."

Introduction

This annual report highlights the research activities in pharmaceutical biosciences during 2012. The research covers expanding areas such as pharmacometric modeling and simulations used in drug development as well as pharmaceutical proteomics and bioinformatics. In addition, there is significant focus on pharmacodynamics, pharmacokinetics, drug dependency, drug metabolism and drug-induced adverse effects. Some research groups are developing tools directly applicable to the pharmaceutical industry, while others are studying drug dependency and environmental contaminants with important societal implications. This annual report also shows that the research groups continue to have worldwide international collaboration within their research areas. In addition, research underpins all our teaching and the achievements during 2012 form a strong foundation for the future education and training.

A fast-changing environment

The Swedish pharmaceutical area has changed dramatically during the last recent years. A new structure among the pharmaceutical companies is emerging with more small-scale contract research organisations (CROs) in biotechnology and diagnostics and less international big pharmaceutical companies. There is also an extensive transformation of the Swedish pharmacy market. Private pharmacies have been established and frequently merged into various chains. In addition, there is an increasing interest to recruit clinical pharmacists in hospitals and health care centers in order to prevent drug-related problems. All these rapid external changes will influence the department and we aim to keep developing our research and education to respond to the *changing* needs.

Research

Pharmaceutical bioscience research has many interfaces with medicine and medicinal chemistry, and the research groups at the department participate in a number of interdisciplinary research programmes and EU projects. For example, in 2012 the Swedish research councils FORMAS and FAS funded projects on epigenetic and genetic approach to psychiatric disorders in children and adolescents and the role of genetic factors on alcohol-induced effects on cognitive ability. The Molecular neuropsychopharmacology research group is the academic lead for these projects together with research groups at the University of Sydney and at King's College, London. The project aims to study if alterations in DNA methylation induced by adverse effects in the young human brain are critical for the genetically regulated gene expression variations that underlie psychiatric disorders. Another example is a project on doping and game addiction funded by the Swedish research council FORMAS to the Drug dependence research group. This grant makes it possible for the research group to increase the understanding of brain mechanisms of relevance for the aetiology of addiction and to develop strategies for relevant treatments.

A VR-funded project within the Pharmaceutical Bioinformatics group is focusing on the development of a national infrastructure for standardizing distributed chemical safety predictions and has partners from academia, government, and industry in collaboration with the European framework OpenTox. A final example is the SafeSciMET network which is funded by grants from the Innovative Medicines Initiative (EU). This is a pan-European education and training network, providing master's level courses in safety sciences for medicines. The toxicology/

drug safety research group is responsible for the development and running of the student office and communication. In addition, a course on Pharmaco-/toxicokinetics and pharmaco-/toxicodynamics is offered within SafeSciMET by the Translational PKPD research group.

The present annual report includes scientific reports from all research groups at the department. Novel scientific results are continuously published in peer-reviewed, international scientific journals. A full account of those publications, as well as books and PhD theses are available at DiVA – the Uppsala University Academic Archive On-line (http://uu.diva-portal.org). More than 1000 peer-reviewed publications from 2001 to December 2012 have been registered in DiVA. In 2012, 128 new peer-reviewed scientific publications were registered in this database. Some of them are freely available as pre-prints in the academic on-line archive.

The department is running two regular research seminar series: the Bioscience series and the Pharmacometrics/PKPD series. External and internal investigators and PhD students present current research. All department members and master degree students are welcome to participate in these seminar series.

Our external website (www.farmbio.uu.se) is continuously updated in order to present the current research. Links to the most recent publications can always be found as well as lists of scientific publications. In addition, there are automatically updated links to all PhD theses in DiVA – the academic on-line archive. Traffic statistics show that the website has an increasing numbers of international visitors per month.

Education

Together with the other departments at the Faculty of Pharmacy we offer professional training leading to Bachelor and Master in Science degrees in Pharmacy. The national education authority is continuously evaluating the quality of the higher education in Sweden mainly based on the assessments of the students' independent degree projects. In 2012, the overall evaluation of the 3-year programme for Bachelor of Science Degree in Pharmacy and Clinical Pharmacy indicated that the quality should be improved and that the faculty should put more focus on the students' independent projects. The study directors and teachers of the department started revising the structure and introduction to the degree projects. In addition, the thesis instructions were revised in order to ensure quality improvements so that the BSci in Pharmacy will remain attractive for potential students.

Furthermore, discussions and preparations for a major revision of the curricula of both the BSci and MSci degree programmes in Pharmacy commenced. This is a major undertaking that will continue for a number of years.

The relationship between education and research is strongly emphasized within the department and specialized courses on the advanced level are offered by most research groups. The courses cover for instance neuropharmacology, drug-dependency, pharmacokinetics, clinical pharmacy and pharmacotherapy, gene regulation, adverse drug reactions and pharmacovigilance. In addition, a web-based course in pharmaceutical bioinformatics attracts many foreign students. The department website is continuously updated in order to present courses on the advanced level for prospective students. All research groups also offer master degree projects during which the students are actively involved in the current research work at the department under supervision of an experienced researcher.

All research groups are also heavily involved in the research and training programmes leading to a PhD degree.

Societal interactions

There are numerous collaborations with national and international pharmaceutical companies and other parts of the society. The aim is to carry out research from the pharmaceutical bioscience area and transfer new knowledge and skills to the society. For example, the Drug dependence research group has successfully initiated and organized an Uppsala University Center (U-FOLD) to deal with issues related to drug addiction. U-FOLD provides a platform for researchers, practitioners and caretakers who contribute their unique competence and experience related to drug addiction. In this network all competence at the university in relation to addiction to abusing substances but also to various behaviors (e.g. pathological gambling) are collected. This includes aspects of prevention, control, and treatment of addiction, as well as judicial and cultural aspects of addiction. The sharing of new findings within preventative action, treatment and legal action is made more efficient by the expanded network, which is aimed to include contacts with researchers and actors in the whole region.

New academic staff

The staff is key to our research and teaching success and the development of the department. In 2012, Dr Elisabet Nielsen was recruited and appointed as senior lecturer in clinical pharmacy. We are looking forward to working together with her.

Organisational structure

The department is an integral part of the Faculty of Pharmacy and is organised on the basis of the core activities in research and teaching, and the collected support activities (i.e. management, finance/staff and education administration, IT and infrastructure). The Board of the department is responsible for major decisions and policies within the framework of the Disciplinary Domain of Medicine and Pharmacy.

Financial review

The basic funding for education and research is provided by the government. The peer-reviewed scientific publications, the PhD degrees and the external grants provide the basis for the allocation of the basic funding of research. The research groups also compete for external funding from national and international research councils and pharmaceutical companies, and the research group budgets are often supplemented by external fundings. The skills and enthusiasm of the scientific staff are of fundamental importance for the external funding and the revenues from research councils, EU, foundations and pharmaceutical companies are vital for the research activities at the department. The teaching load of some the scientific staff is, however, often too high resulting in limited time for the acquisition of external funding.

The number of students that are registered and pass the examinations provide the basis for the allocation of the basic governmental funding of education. The administrative support activities are funded by an annual percentile overhead on salaries and operating costs.

A short overview of the income and expenditure of 2012 is given below.

Income 2012 (kSEK)	
Research and graduate education - government	33 082
Research grants – research councils	28 054
Research – commissioned	4 901
Education – basic and advanced level - government	34 024
Education – commissioned	1 000

Expenditure 2012 (kSEK)	
Staff costs	61 998
Operating expenses etc.	16 998
Premises	12 630
University/faculty support activities	11 412
Library	2 389
Depreciation	2 126

Future developments

The years ahead promise many changes in terms of research and education. A number of our professors will retire in the next few years. Drug development is multidisciplinary and needs expertise from many research areas as well as unique and original projects. New funding should strengthen particularly strong research, but also introduce new areas of research that can attract funding from national and international research councils, the EU and pharmaceutical drug companies.

Uppsala June 30, 2013

Eva Brittebo

List of Contents

Organisation

Chairman
Eva Brittebo

Deputy chairman
Mats Karlsson

Department board
Eva Brittebo, *chairman*
Marianne Danersund, *secretary*
Mats Karlsson, *teacher representative*
Margareta Hammarlund-Udenaes, *teacher representative*
Ernst Oliw, *teacher representative*
Björn Hellman, *teacher representative*
Ann-Marie Falk, *teacher representative*
Mathias Hallberg, *teacher representative, deputy*
Andrew Hooker *teacher representative, deputy*
Raili Engdahl, *technical/administrative representative*
Agneta Hortlund, *technical/administrative representative*
Marina Rönngren, *technical/administrative representative, deputy*
Patrik Källback, *graduate student representative*
Åsa Johansson, *graduate student representative*
Erika Brolin, *graduate student representative, deputy*
Sebastian Axelsson, *student representative*
Linh Nguyen, *student representative, deputy*

Professors
Georgy Bakalkin
Sven Björkman
Eva Brittebo
Lennart Dencker
Margareta Hammarlund-Udenaes
Björn Hellman
Mats Karlsson
Matti Lang *leave of absence*
Fred Nyberg
Ingrid Nylander
Ernst Oliw
Jarl Wikberg
Kjell Wikvall

Professor emeritus
Lennart Paalzow

Adjunct professors
Bengt RG Danielsson
Staffan Eksborg
Anders Grahnén
Niclas Jonsson
Nils Gunnar Lindquist

Senior lecturers
Per Andrén, *docent*
Jörgen Bengtsson*
Lena Bergström, *docent*
Agneta Freijs
Lena Friberg, *docent*
Mathias Hallberg, *docent*
Ronnie Hansson
Andrew Hooker
Ulrika Simonsson, *docent*
Anne-Lie Svensson
Elisabet Nielsen
Anders Grahnén*
Henrik Alm*

Assistant professors
Malin Andersson
Maria Norlin, *docent*
Erika Roman, *docent*

Postdocs, researchers and PhD students
Listed in the Scientific reports

Junior lecturers

Ann-Marie Falk
Lena Klarén
Anna-Karin Lidehäll*
Emma Lundkvist
Jonna Lübcke
Maria Swartling
Matts Balgård*
Maria Ellgren*
Åsa Johansson*
Srebrenka Dobric*

Directors of undergraduate studies

Lena Bergström
Jörgen Bengtsson
Ann-Marie Falk
Mathias Hallberg
Björn Hellman
Lena Klarén
Jonna Lübcke
Ingrid Nylander
Anna-Karin Lidehäll
Jarl Wikberg
Kjell Wikvall

Technical and administrative staff

Mikaela Andersson
Ulrica Bergström
Annika Bokström
Marianne Danersund
Agneta Hortlund
Johanna Svensson
Magnus Jansson
Marina Rönngren
Karin Tjäder
Yvonne Wiessing*
Kjell Åkerlund
Birgitta Hellsing*
Malin Gadeborg*
Elisabeth Jonsson

Laboratory staff

Jessica Dunhall
Raili Engdahl
Britt Jansson
Lena Norgren

Safety officers

Marianne Danersund
Raili Engdahl
Ronnie Hansson
Lena Norgren
Henrik Wadensten
Sviatlana Yahorava
Kjell Åkerlund

The work environment group

Eva Brittebo, *chairman*
Raili Engdahl
Ernst Oliw
Marina Rönngren
Patrik Källback***

Working group on graduate studies

Margareta Hammarlund-Udenaes, *chairman*
Anna Carlsson***
Patrik Källback***
Maria Norlin

Working group on equal opportunity

Elin Svensson, *chairman*
Johanna Svensson
Jonna Lübcke
Marina Rönngren*
Ronnie Hansson, *adjunct*
Sebastian Axelsson**

Gender equality representative

Elin Svensson

* *temporary position*
** *student representative*
*** *graduate student representative*

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Course List

List of courses on basic and advanced levels

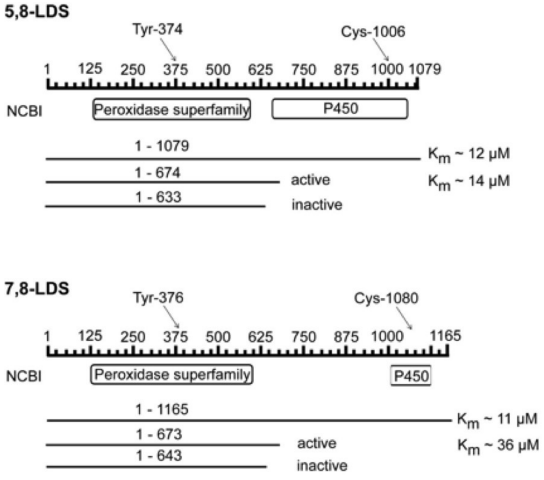
Abuse and Addiction, 7,5 c
Advanced Pharmacotherapy B, 7.5 c
Advanced Pharmacotherapy Second cycle, 7.5 c
Adverse Drug Reactions and Pharmacovigilance Second cycle, 7.5 c
Analytical Toxicology Second cycle, 30 c
Applied Pharmacotherapy, Pharmacokinetics and Therapeutics Second cycle, 15c
Biochemistry 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 Pharmacokinetics and Pharmacodynamics C, 7.5 c
Clinical Pharmacy C, 7.5 c
Degree Project in Drug Discovery and Development Second cycle, 30 c
Degree Project in Drug Management Second cycle, 30 c
Degree Project in Pharmaceutical Biochemistry Second cycle, 30 c
Degree Project in Pharmaceutical Biochemistry First cycle, 15 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 C First cycle, 15 c
Degree Project in Pharmacokinetics C First cycle, 30 c
Degree project in Pharmacokinetics D Second cycle, 30 c
Degree Project in Pharmacology First cycle, 15 c
Degree Project in Pharmacotherapy C First cycle, 15 c
Degree Project in Pharmacotherapy C First cycle, 30 c
Degree Project in Pharmacotherapy D Second cycle, 30 c
Degree Project in Toxicology First cycle, 15 c
Degree Project, Toxicology D, 30 c
Drug Dependence Mechanisms, Prevention of Cannabis Abuse (Contract education) 7,5 c
Drug Development and Drug Usage First cycle, 7.5 c
Drug Management Second cycle, 7.5 c
Drugs and Dependence, Advanced Course C Second cycle, 7.5 c
Drugs and the Elderly B, 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 First cycle, 7.5 c
Evidence Based Clinical Pharmaceutical Methods Second cycle, 12 c
Models for Biological Systems C, 7.5 c
Models for Biological Systems Second cycle, 7.5 c
Molecular Mechanisms for Enzymatic Activation Second cycle, 7.5 c
Molecular Pharmacology First cycle, 7.5 c
Neuropharmacology Second cycle, 7.5 c
Pharmaceutical Biochemistry First cycle, 9 c
Pharmaceutical Biochemistry and Cell Biology First cycle
Biochemistry and Cell Biology A, 7.5 c
Pharmaceutical Bioinformatics Second cycle, 7.5 c
Pharmacokinetics B, 7.5 c
Pharmacokinetics B, 3 c
Pharmacokinetics First cycle, 7.5 c

Pharmacokinetics First cycle, 3 c
Pharmacokinetics First cycle, 7.5 c
Pharmacokinetics and Statistics First cycle, 9 c
Pharmacology First cycle, 15 c
Pharmacology First cycle, 16.5 c
Pharmacology for engineering students 7,5 c
Pharmacotherapy B, 7.5 c
Pharmacotherapy First cycle, 7.5 c
Pharmacotherapy in Self-Treatment First cycle, 9 c
Research Project in Clinical Pharmacy Second cycle, 15 c
Toxicology B First cycle, 7.5 c
Toxicology for Engineering Students Second cycle, 7.5 c
Toxicology, Advanced Course D Second cycle, 30 c
Toxicology, Drug Metabolism and Safety Assessment First cycle, 4.5 c
Toxicology, Drug Metabolism and Safety Assessment First cycle, 7.5 c
Toxicology, Intermediate Course C Second cycle, 15 c
Veterinary Pharmacology Second cycle, 7.5 c

Scientific Reports

Biochemical Pharmacology

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



Ernst H. Oliw

The two LDS enzymes have one dioxygenase domain with homology to the peroxidase superfamily and one hydroperoxide isomerase domain with homology to the P450 superfamily.

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.

Bio-activation 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) Cytochrome 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).

(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 G*äumannomyces graminis*, an important pathogen of wheat, and its structurally similar lipoxygenases of *Magnaporthe oryzae*, and *Aspergillus fumigatus*.

(iii) DOX-CYP fusion proteins, linoleate diol synthases (LDS): LDS and other fungal enzymes oxidize oleic and linoleic acids to a series of vicinal diols (5,8-dihydroxy-, 7,8-dihydroxy-, and 8,11-dihydroxyoctadecadienoic acids) and hydroperoxides (8-hydroperoxy- and 10-hydroperoxylinoleic acid), which likely function as sporulation hormones in *A. nidulans*. The gene deletion of 7,8-LDS of *M. grisea* and characterization of diol synthase of *Pseudomonas aeruginosa*, and the unqiue 7,10-diol synthase of *Pseudomonas aeruginosa* with a related oxygenation mechanism, are described in three papers in *The Journal of Biological Chemistry*. Another novel discovery is the 9*R*-dioygenase and allene oxide synthase of *A. terreus*, the 9S-Mn-lipoxygenase of M. salvinii, and the catalytic confergence of Fe- and Mn-lipoxygenases.

Members of the group during 2012

Ernst H. Oliw, MD PhD, Professor
Johan Bylund, PhD
Fredrik Jernerén, PhD student
Inga Hoffmann, PhD student
Anneli Wennman, PhD student

Publications 2010-2012

Nilsson, T., Ivanov, I.G., and Oliw, E.H. (2010) LC-MS/MS analysis of epoxyalcohols and epoxides of arachidonic acid and their oxygenation by recombinant CYP4F8 and CYP4F22, *Arch. Biochem. Biophys.* **494**, 64-71

Jernerén, F., Sesma, A., Francheschetti, M., Hamberg, M., and Oliw, E.H. (2010) Gene deletion of 7,8-linoleate diol synthase of the rice blast fungus. Studies on products, stereochemistry, reaction mechanisms and pathogenicity. *J. Biol. Chem.* **285**, 5308-5316

Jernerén, F., U. Garscha, I. Hoffmann, H. M., and E. H. Oliw (2010). Reaction mechanism of 5.8-linoleate diol synthase, 10R-dioxygenase and 8,11-hydroperoxide isomerase of *Aspergillus clavatus*. *Biochim. Biophys. Acta* **1801**: 503-7.

Jernerén, F., I. Hoffmann, and E. H. Oliw (2010). Linoleate 9R-dioxygenase and allene oxide synthase activities of Aspergillus terreus. *Arch. Biochem. Biophys.* **495**: 67-73.

Martínez, E., Hamberg, M., Busquets, M, Díaz, P, Manresa, A., and Oliw, E.H. (2010) Biochemical characterization of the oxygenation of unsaturated fatty acids by the dioxygenase and hydroperoxide isomerase of Pseudomonas aeruginosa 42A2, *J. Biol. Chem.* **285**, 9339-9345.

Nilsson, T., Martínez, E., Manresa, A., and Oliw, E.H. (2010) Liquid chromatography/tandem mass spectrometric analysis of 7,10-dihydroxyoctadecenoic acid, its isotopomers, and other 7,10-dihydroxy fatty acids formed by Pseudomonas aeruginosa 42A2. *Rapid Commun. Mass Spectrom.*, **24**, 777-83.

Hoffmann, I., F. Jerneren, U. Garscha, and E. H. Oliw. (2010). Expression of 5,8-LDS of Aspergillus fumigatus and its dioxygenase domain. A comparison with

7,8-LDS, 10-dioxygenase, and cyclooxygenase. Arch. Biochem. Biophys., **506**, 216-222

Oliw, E. H., F. Jernerén, I. Hoffmann, M. Sahlin, and U. Garscha. (2011). Manganese lipoxygenase oxidizes bis allylic hydroperoxides and octadecenoic acids by different mechanisms. *Biochim. Biophys. Acta*: **1811**, 138-147.

Oliw EH, Wennman A, Hoffmann I, Garscha U, Hamberg M, Jernerén F. (2011). Stereoselective oxidation of regioisomeric octadecenoic acids by fatty acid dioxygenases. *J. Lipid Res.* **52**: 1995-2004.

Palmieri-Thiers C, Alberti JC, Canaan S, Brunini V, Gambotti C, Tomi F, Oliw EH, Berti L, Maury J. (2011). Identification of putative residues involved in the accessibility of the substrate-binding site of lipoxygenase by site-directed mutagenesis studies. Arch. Biochem. Biophys. 509: 82-89.

Oliw EH, Wennman A, Hoffmann I, Garscha U, Hamberg M, Jernerén F. (2011). Stereoselective oxidation of regioisomeric octadecenoic acids by fatty acid dioxygenases. *J Lipid Res* **52**: 1995-2004.

Jernerén, F., F. Eng, M. Hamberg, and E. H. Oliw. (2012). Linolenate 9R-dioxygenase and allene oxide synthase activities of Lasiodiplodia theobromae. *Lipids* **47**: 65-73.

Jernerén, F., and E. H. Oliw. (2012). The fatty acid 8,11-diol synthase of Aspergillus fumigatus is inhibited by imidazole derivatives and unrelated to PpoB. *Lipids* **47**: 707-17

Wennman, A., F. Jernerén, M. Hamberg, E.H. Oliw (2012) Catalytic convergence of Mn- and Fe-lipoxygenases by replacement of a single amino acid, *J. Biol. Chem.*, **287**: 31757-31765.

Hoffmann,I., M. Hamberg, R. Lindh, E.H. Oliw, Novel insights into cyclooxygenases, linoleate diol synthases, and lipoxygenases from deuterium kinetic isotope effects and oxidation of substrate analogs (2012) *Bichim. Biophys. Acta*, **1821**: 1508-1517.

Agencies that support the work/Funding 2012

The Swedish Research Council Medicine

Projects

Novel transformations of polyunsaturated fatty acids and eicosanoids.

Ernst Oliw, Johan Bylund

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.

Characterization of heme-containing fatty acid dioxygenases and hydroperoxide isomerases of human and plant pathogens

Inga Hoffmann, 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 grisea, and destroys ~25% of rice crops worldwide. Aspergillus and M. grisea 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. 5,8-LDS and 7,8-LDS are DOX-CYP fusion proteins, as outlined in the figure above.

Characterization of the reaction mechanism of allene oxide synthase, linoleate diol synthase, and metal ligands of manganese-lipoxygenase

Anneli Wennman, Margareta Sahlin, Ernst Oliw

Our aim is to study the reaction mechanisms of allene oxide synthase by EPR. The Mn-LOX uniquely transforms hydroperoxides to peroxyl radicals. We have now established that this occurs by proton coupled electron transfer from the hydroperoxide anion to the catalytic metal.

Characterization of allene oxide synthase and 9R-dioxygenase of Aspergillus terreus.

Inga Hoffmann, Ernst Oliw.

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. We are now trying to clone and express these enzymes, and the allene oxide synthase was recently identified.

Biological Research on Drug Dependence

Biological Research on Drug Dependence

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

Fred Nyberg and Mathias Hallberg

Members of the group during 2012

Fred Nyberg, Professor
Mathias Hallberg, PhD Associate Professor
Dan Henrohn, PhD Student
Jenny Johansson, PhD Student
Alfhild Grönbladh, PhD, Student
Anna Carlsson PhD student
Erika Brolin, PhD student
Myron Zaluha, Project Leader

Publications 2010-2012

Rossbach UL, Le Grevès M, Nyberg F, Zhou Q, Le Grevès P. Acute 19-nortestosterone transiently suppresses hippocampal MAPK pathway and the phosphorylation of the NMDA receptor. Mol Cell mmiuhuihbEndocrinol. 2010 Jan 15;314(1):143-9.

Carlsson A, Ohsawa M, Hallberg M, Nyberg F, Kamei J. Substance P(1-7) induces antihyperalgesia in diabetic mice through a mechanism involving the naloxone-sensitive sigma receptors. Eur J Pharmacol. 2010 Jan 25;626(2-3):250-5.

Myrelid A, Bergman S, Elfvik Strömberg M, Jonsson B, Nyberg F, Gustafsson J, Annerén G. Late effects of early growth hormone treatment in Down syndrome. Acta Paediatr. 2010 May;99(5):763-9.

Fransson R, Botros M, Sköld C, Nyberg F, Lindeberg G, Hallberg M, Sandström A. Discovery of dipeptides with high affinity to the specific binding site for substance P1-7. J Med Chem. 2010 Mar 25;53(6):2383-9

Mahalingam AK, Wan Y, Murugaiah AM, Wallinder C, Wu X, Plouffe B, Botros M, Nyberg F, Hallberg A, Gallo-Payet N, Alterman M. Selective angiotensin II AT(2) receptor agonists with reduced CYP 450 inhibition. Bioorg Med Chem. 2010 Jun 15;18(12):4570-90.

Dabo F, Nyberg F, Qin Zhou, Sundström-Poromaa I, Akerud H. Plasma levels of beta-endorphin during pregnancy and use of labor analgesia. Reprod Sci. 2010 Aug;17(8):742-7.

Ghasemzadeh N, Wilhelmsen TW, Nyberg F, Hjertèn S. Precautions to improve the accuracy of quantitative determinations of biomarkers in clinical diagnostics. Electrophoresis. 2010 Aug;31(16):2722-9.

Skårberg K, Nyberg F, Engström I. Is there an association between the use of anabolic-androgenic steroids and criminality? Eur Addict Res. 2010;16(4):213-9

Johansson T, Elfverson M, Zhou Q, Nyberg F. Allosteric modulation of the NMDA receptor by neurosteroids in rat brain and the impact of long term morphine

administration. Biochem Biophys Res Commun. 2010 Oct 29;401(4):504-8.
Dabo F, Grönbladh A, Nyberg F, Sundström-Poromaa I, Akerud H. Different SNP combinations in the GCH1 gene and use of labor analgesia. Mol Pain. 2010 Jul 15;6:41.
Bakalkin G, Watanabe H, Jezierska J, Depoorter C, Verschuuren-Bemelmans C, Bazov I, Artemenko KA, Yakovleva T, Dooijes D, Van de Warrenburg BP, Zubarev RA, Kremer B, Knapp PE, Hauser KF, Wijmenga C, Nyberg F, Sinke RJ, Verbeek DS. Prodynorphin mutations cause the neurodegenerative disorder spinocerebellar ataxia type 23. Am J Hum Genet. 2010 Nov 12;87(5):593-603.
Andersson H, Demaegdt H, Vauquelin G, Lindeberg G, Karlén A, Hallberg M, Erdélyi M, Hallberg A. (2010) Disulfide cyclized tripeptide analogues of angiotensin IV as potent and selective inhibitors of insulin-regulated aminopeptidase (IRAP). J Med Chem. 53:8059-71.
Andersson H, Demaegdt H, Johnsson A, Vauquelin G, Lindeberg G, Hallberg M, Erdelyi M, Karlen A, Hallberg A. Potent macrocyclic inhibitors of insulin-regulated aminopeptidase (IRAP) by olefin ring-closing metathesis. J Med Chem. 2011 Jun 9;54(11):3779-92.
Elfverson M, Johansson T, Zhou Q, Le Grevès P, Nyberg F. Chronic administration of the anabolic androgenic steroid nandrolone alters neurosteroid action at the sigma-1 receptor but not at the sigma-2 or NMDA receptors. Neuropharmacology. 2011 Dec;61(7):1172-81. Epub 2011 Jan 18.
Ghasemzadeh N, Rossbach UL, Johansson BM, Nyberg F. Application of artificial gel antibodies for investigating molecular polymorphisms of human pituitary growth hormone. Amino Acids. 2011 Apr;40(4):1249-55. Epub 2011 Feb 11.
Taqi MM, Bazov I, Watanabe H, Nyberg F, Yakovleva T, Bakalkin G. Prodynorphin promoter SNP associated with alcohol dependence forms noncanonical AP-1 binding site that may influence gene expression in human brain. Brain Res. 2011 Apr 18;1385:18-25.
Taqi MM, Bazov I, Watanabe H, Sheedy D, Harper C, Alkass K, Druid H, Wentzel P, Nyberg F, Yakovleva T, Bakalkin G. Prodynorphin CpG-SNPs associated with alcohol dependence: elevated methylation in the brain of human alcoholics. Addict Biol. 2011 Jul;16(3):499-509. doi: 10.1111/j.1369-1600.2011.00323.x. Epub 2011 Apr 26.
Kehr J, Ichinose F, Yoshitake S, Goiny M, Sievertsson T, Nyberg F, Yoshitake T.Mephedrone, compared with MDMA (ecstasy) and amphetamine, rapidly increases both dopamine and 5-HT levels in nucleus accumbens of awake rats. Br J Pharmacol. 2011 Dec;164(8):1949-58. doi: 10.1111/j.1476-5381.2011.01499.x.
Zhou Q, Carlsson A, Hallberg M, Nyberg F. Substance P N-terminal fragment SP(1-7) attenuates chronic morphine tolerance and affects dynorphin B and nociceptin in rats. Peptides. 2011 Aug;32(8):1661-5. Epub 2011 Jul 7
Bazov I, Kononenko O, Watanabe H, Kuntić V, Sarkisyan D, Taqi MM, Hussain MZ, Nyberg F, Yakovleva T, Bakalkin G. The endogenous opioid system in human alcoholics: molecular adaptations in brain areas involved in cognitive control of addiction. Addict Biol. 2011 Sep 28. doi: 10.1111/j.1369-1600.2011.00366.x. [Epub ahead of print]
Ohsawa M, Carlsson A, Asato M, Koizumi T, Nakanishi Y, Fransson R, Sandström A, Hallberg M, Nyberg F, Kamei J. The dipeptide Phe-Phe amide attenuates signs of hyperalgesia, allodynia and nociception in diabetic mice using a mechanism involving the sigma receptor system. Mol Pain. 2011;7:85
Ohsawa M, Carlsson A, Asato M, Koizumi T, Nakanishi Y, Fransson R, Sandström A, Hallberg M, Nyberg F, Kamei J.The effect of substance P1-7 amide on nociceptive threshold in diabetic mice. Peptides. 2011 32(1):93-8.
Ali MA, Adem A, Chandranath IS, Benedict S, Pathan JY, Nagelkerke N, Nyberg F, Lewis LK, Yandle TG, Nicholls GM, Frampton CM, Kazzam E. Responses to dehydration in the one-humped camel and effects of blocking the renin-

angiotensin system. PLoS One. 2012;7(5):e37299.
Enhamre E, Carlsson A, Grönbladh A, Watanabe H, Hallberg M, Nyberg F. (2012) The expression of growth hormone receptor gene transcript in the prefrontal cortex is affected in male mice with diabetes-induced learning impairments. Neurosci Lett. 523:82-6.
Grönbladh A, Johansson J, Nyberg F, Hallberg M. Recombinant Human Growth Hormone Affects the Density and Functionality of GABA(B) Receptors in the Male Rat Brain. Neuroendocrinology. 2012 Aug 25. [Epub ahead of print]
Heddini U, Bohm-Starke N, Grönbladh A, Nyberg F, Nilsson KW, Johannesson U. GCH1-polymorphism and pain sensitivity among women with provoked vestibulodynia. Mol Pain. 2012 Sep 12;8:68.
Isaksson J, Nilsson KW, Nyberg F, Hogmark A, Lindblad F. J Psychiatr Res. Cortisol levels in children with attention-deficit/hyperactivity disorder. 2012 Nov;46(11):1398-405.
Murugaiah AM, Wu X, Wallinder C, Mahalingam AK, Wan Y, Sköld C, Botros M, Guimond MO, Joshi A, Nyberg F, Gallo-Payet N, Hallberg A, Alterman M. From the first selective non-peptide AT(2) receptor agonist to structurally related antagonists. J Med Chem. 2012 Mar 8;55(5):2265-78.
Pettersson FD, Grönbladh A, Nyberg F, Sundström-Poromaa I, Åkerud H. The A118G single-nucleotide polymorphism of human μ-opioid receptor gene and use of labor analgesia. Reprod Sci. 2012 Sep;19(9):962-7.
Watanabe H, Mizoguchi H, Verbeek DS, Kuzmin A, Nyberg F, Krishtal O, Sakurada S, Bakalkin G. Non-opioid nociceptive activity of human dynorphin mutants that cause neurodegenerative disorder spinocerebellar ataxia type 23. Peptides. 2012 Jun;35(2):306-10.

Reviews 2010-2012

Nyberg F, Hallberg M. Localization of neuropeptides by radioimmunoassay. Methods Mol Biol. 2011;789:191-201.
Hallberg M. Impact of anabolic androgenic steroids on neuropeptide systems. Mini Rev Med Chem. 2011 May;11(5):399-408. Review.
Nyberg F, Hallberg M. (2012) Interactions between opioids and anabolic androgenic steroids: implications for the development of addictive behavior. Int Rev Neurobiol. 102:189-206.
Nyberg F, Hallberg M. (2012) Cognition-Enhancing Peptides and Peptidomimetics. In: Neuropeptides in Neuroprotection and Neurogeneration. Eds. Nyberg F, Chapter 15, pp 271-298. CRC Press, New York.
Hallberg M, Nyberg F. (2012) Growth hormone receptors in the brain and their potential as therapeutic targets in central nervous system disorders. The Open Endocrinology Journal. 6:(Suppl 1) 27-33.

Edited book 2012

Fred Nyberg (ed.): Neuropeptides in Neuroprotection and Neurogeneration. 2012; CRC Press, New York.

Dissertations 2012

Johansson, Jenny
Then Impact of growth Hormone and Gamma-Hydroxybutyrate (GHB) on Systems related to Cognition Digital COmprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy, ISSN 1651-6192; 168

http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-185631

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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 staff members 2012

Fred Nyberg: Member of the Governmental Advisory Board for Addictive drugs (ANT-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 Executive committee for the International Narcotics Research Conference (INRC) from 2006 to the present.
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. Member of Editorial Board of Scientific journals: Peptides, Open J Endocrinology (Editor in Chief), Pharmacology-on-line, J Musc Skel. Pain .
Mathias Hallberg: Curr Protein Pept Sci. and The Open Biochemistry Journal
Fred Nyberg: PI at the Uppsala Berzelii Technology Center for Neurodiagnostics; PI at the Linne project Impact of Religion: Challenges for Society, Law and Democracy; PI at the FAS supported project on alcohol effects on cognitive functions.
Swedish Research Council/Medicin for peptidergic mechanism in the development of drug dependence.

Projects

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.

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 behavioral assays.

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.

Studies on atypical opioid peptides (endomorphins, hemorphins and casomorphins) in relation to behaviour and mechanisms for their release.

Studies on synthetic compounds acting on angiotensin receptors.

Receptor assays specific for the AT1, AT2 and AT4 receptors are used to guide synthesis and design of peptide and non-peptide analogues. Compounds with high affinity and selectivity are further studied with regard to agonist activity in functional assay *in vitro* or *in vivo*.

Molecular Neuropsychopharmacology

Georgy Bakalkin

The endogenous opioid systems comprise the κ, δ, μ and nociceptin receptors and their respective endogenous ligands, the dynorphins, enkephalins, endorphins and nociceptin. These systems modulate cognitive, emotional, reward and pain processing and are implicated in a wide range of physiological responses, for example, stress. Opioid gene expression is altered in the brain of patients with mental disorders and genetic variants in the promoters of these genes are associated with, for example, alcohol dependence. Our general aim is to characterize the opioid systems at the molecular and cellular levels and to elucidate the role of molecular alterations in these systems in mental and pain disorders. The main focus is on epigenetic and transcriptional control of the gene encoding dynorphins, prodynorphin (Pdyn).

Members of the group during 2012

Georgy Bakalkin, PhD, Professor
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Hiroyuki Watanabe, PhD, Research scientist
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Publications 2010-2012

Karpyak VM, Winham SJ, Preuss UW, Zill P, Cunningham JM, Walker DL, Lewis KA, Geske JR, Colby CL, Abulseoud OA, Hall-Flavin DK, Loukianova LL, Schneekloth TD, Frye MA, Bazov I, Heit JA, Bakalkin G, Mrazek DA, Biernacka JM. Association of the PDYN gene with alcohol dependence and the propensity to drink in negative emotional states. *Int J Neuropsychopharmacol*. 2012 Oct 29:1-11. [Epub ahead of print]

Taqi MM, Wärmländer SK, Yamskova O, Madani F, Bazov I, Luo J, Zubarev R, Verbeek D, Gräslund A, Bakalkin G. Conformation effects of CpG methylation on single-stranded DNA oligonucleotides: analysis of the opioid peptide dynorphin-coding sequences. *PLoS One*. 2012;7(6):e39605. doi: 10.1371/journal.pone.0039605. Epub 2012 Jun 29.

Watanabe H, Mizoguchi H, Verbeek DS, Kuzmin A, Nyberg F, Krishtal O, Sakurada S, Bakalkin G. Non-opioid nociceptive activity of human dynorphin mutants that cause neurodegenerative disorder spinocerebellar ataxia type 23. *Peptides*. 2012 Jun;35(2):306-10. doi: 10.1016/j.peptides.2012.04.006. Epub 2012 Apr 17.

Hussain ZM, Fitting S, Watanabe H, Usynin I, Yakovleva T, Knapp PE, Scheff SW, Hauser KF, Bakalkin G. Lateralized response of dynorphin a peptide levels after traumatic brain injury. *J Neurotrauma*. 2012 Jun 10;29(9):1785-93. doi:

10.1089/neu.2011.2286. Epub 2012 May 21.

Kuzmin A, Liljequist S, Meis J, Chefer V, Shippenberg T, Bakalkin G. Repeated moderate-dose ethanol bouts impair cognitive function in Wistar rats. *Addict Biol*. 2012 Jan;17(1):132-40. doi: 10.1111/j.1369-1600.2010.00224.x. Epub 2011 Oct 26.

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.

Bazov I, Kononenko O, Watanabe H, Kuntić V, Sarkisyan D, Taqi MM, Hussain MZ, Nyberg F, Yakovleva T, Bakalkin G. The endogenous opioid system in human alcoholics: molecular adaptations in brain areas involved in cognitive control of addiction. *Addict Biol*. 2011 Sep 28. doi: 10.1111/j.1369-1600.2011.00366.x. [Epub ahead of print]

Vukojević V, Gräslund A, Bakalkin G. Fluorescence imaging with single-molecule sensitivity and fluorescence correlation spectroscopy of cell-penetrating neuropeptides. *Methods Mol Biol*. 2011;789:147-70.

Dong L, Bilbao A, Laucht M, Henriksson R, Yakovleva T, Ridinger M, Desrivieres S, Clarke TK, Lourdusamy A, Smolka MN, Cichon S, Blomeyer D, Treutlein J, Perreau-Lenz S, Witt S, Leonardi-Essmann F, Wodarz N, Zill P, Soyka M, Albrecht U, Rietschel M, Lathrop M, Bakalkin G, Spanagel R, Schumann G. Effects of the circadian rhythm gene period 1 (per1) on psychosocial stress-induced alcohol drinking. *Am J Psychiatry*. 2011 Oct;168(10):1090-8. Epub 2011 Aug 9.

Madani F, Taqi MM, Wärmländer SK, Verbeek DS, Bakalkin G, Gräslund A. Perturbations of model membranes induced by pathogenic dynorphin A mutants causing neurodegeneration in human brain. *Biochem Biophys Res Commun*. 2011 Jul 22;411(1):111-4. Epub 2011 Jun 25.

Taqi MM, Bazov I, Watanabe H, Sheedy D, Harper C, Alkass K, Druid H, Wentzel P, Nyberg F, Yakovleva T, Bakalkin G. Prodynorphin CpG-SNPs associated with alcohol dependence: elevated methylation in the brain of human alcoholics. *Addict Biol*. 2011 Jul;16(3):499-509. doi: 10.1111/j.1369-1600.2011.00323.x. Epub 2011 Apr 26.

Schumann G, Coin LJ, Lourdusamy A, Charoen P, Berger KH, Stacey D, Desrivières S, Aliev FA, Khan AA, Amin N, Aulchenko YS, Bakalkin G, Bakker SJ, Balkau B, Beulens JW, Bilbao A, de Boer RA, Beury D, Bots ML, Breetvelt EJ, Cauchi S, Cavalcanti-Proença C, Chambers JC, Clarke TK, Dahmen N, de Geus EJ, Dick D, Ducci F, Easton A, Edenberg HJ, Esko T, Fernández-Medarde A, Foroud T, Freimer NB, Girault JA, Grobbee DE, Guarrera S, Gudbjartsson DF, Hartikainen AL, Heath AC, Hesselbrock V, Hofman A, Hottenga JJ, Isohanni MK, Kaprio J, Khaw KT, Kuehnel B, Laitinen J, Lobbens S, Luan J, Mangino M, Maroteaux M, Matullo G, McCarthy MI, Mueller C, Navis G, Numans ME, Núñez A, Nyholt DR, Onland-Moret CN, Oostra BA, O'Reilly PF, Palkovits M, Penninx BW, Polidoro S, Pouta A, Prokopenko I, Ricceri F, Santos E, Smit JH, Soranzo N, Song K, Sovio U, Stumvoll M, Surakk I, Thorgeirsson TE, Thorsteinsdottir U, Troakes C, Tyrfingsson T, Tönjes A, Uiterwaal CS, Uitterlinden AG, van der Harst P, van der Schouw YT, Staehlin O, Vogelzangs N, Vollenweider P, Waeber G, Wareham NJ, Waterworth DM, Whitfield JB, Wichmann EH, Willemsen G, Witteman JC, Yuan X, Zhai G, Zhao JH, Zhang W, Martin NG, Metspalu A, Doering A, Scott J, Spector TD, Loos RJ, Boomsma DI, Mooser V, Peltonen L, Stefansson K, van Duijn CM, Vineis P, Sommer WH, Kooner JS, Spanagel R, Heberlein UA, Jarvelin MR, Elliott P. Genome-wide association and genetic functional studies identify autism susceptibility candidate 2 gene (AUTS2) in the regulation of alcohol consumption. *Proc Natl Acad Sci U S A*. 2011 Apr 26;108(17):7119-24. Epub 2011 Apr 6.

Taqi MM, Bazov I, Watanabe H, Nyberg F, Yakovleva T, Bakalkin G. Prodynorphin

promoter SNP associated with alcohol dependence forms noncanonical AP-1 binding site that may influence gene expression in human brain. *Brain Res*. 2011 Apr 18;1385:18-25. Epub 2011 Feb 19.

Chefer V, Meis J, Wang G, Kuzmin A, Bakalkin G, Shippenberg T. Repeated exposure to moderate doses of ethanol augments hippocampal glutamate neurotransmission by increasing release. *Addict Biol*. 2010 Dec 23. doi: 10.1111/j.1369-1600.2010.00272.x. [Epub ahead of print].

Bakalkin G, Watanabe H, Jezierska J, Depoorter C, Verschuuren-Bemelmans C, Bazov I, Artemenko KA, Yakovleva T, Dooijes D, Van de Warrenburg BP, Zubarev RA, Kremer B, Knapp PE, Hauser KF, Wijmenga C, Nyberg F, Sinke RJ, Verbeek DS. Prodynorphin mutations cause the neurodegenerative disorder spinocerebellar ataxia type 23. *Am J Hum Genet*. 2010, 87: 593-603.

Reviews 2010-2012

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.

Walker BM, Valdez GR, McLaughlin JP, Bakalkin G. Targeting dynorphin/kappa opioid receptor systems to treat alcohol abuse and dependence. *Alcohol*. 2012 Jun;46(4):359-70. doi: 10.1016/j.alcohol.2011.10.006. Epub 2012 Mar 27. Review.

Jin Z, Bazov I, Kononenko O, Korpi ER, Bakalkin G, Birnir B. Selective Changes of GABA(A) Channel Subunit mRNAs in the Hippocampus and Orbitofrontal Cortex but not in Prefrontal Cortex of Human Alcoholics. *Front Cell Neurosci*. 2011;5:30. doi: 10.3389/fncel.2011.00030. Epub 2012 Jan 3.

Yakovleva T, Bazov I, Watanabe H, Hauser KF, Bakalkin G. Transcriptional control of maladaptive and protective responses in alcoholics: a role of the NF-κB system. *Brain Behav Immun*. 2011 Jun;25 Suppl 1:S29-38.

Dissertations 2012

Malik Mumtaz Taqi
Mechanisms of Prodynorphin Gene Dysregulation in the Brain of Human Alcoholics.
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Other commitments/assignments of staff members 2012

Editor in Addiction Biology journal

Projects

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.

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.

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, three out of four 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.

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

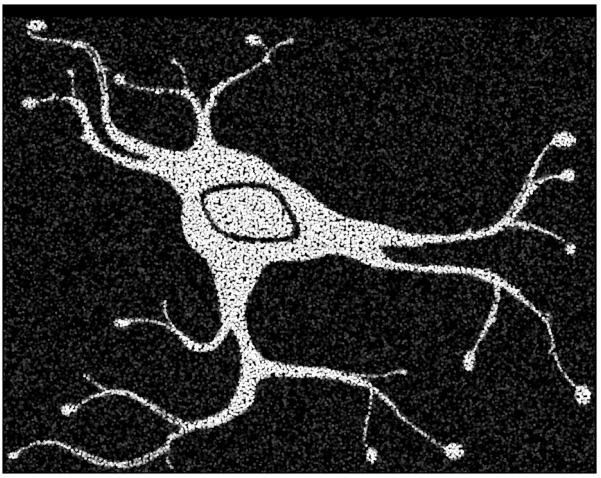
Chronic pain including neuropathic pain is an extremely disabling condition with the enormous cost for society and affected individuals and loss in work productivity. This pain is resistant to standard treatment protocols and thus represents a significant unmet medical and social need. We in collaboration 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.

Developmental toxicology

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



A stylised neuronal cell

Lennart Dencker

Toxicology is generally seen as being divided into three main branches: mechanistic toxicology (how toxicants exert their effects in models - in vitro and in vivo – and humans), predictive toxicology (how the data from in silico, in vitro and/or in vivo models is to be extrapolated) and regulatory toxicology (the societal implementation of toxicological knowledge from the mechanistic and predictive branches). Our research spans the mechanistic and predictive branches with a particular focus on developmental toxicology and neurotoxicity. Developmental toxicology is one the most challenging and complex areas in predictive toxicology as the developmental period of organisms is much more sensitive to toxicants than the adult period, the effects span from more obvious morphological defects to potentially irreversible subclinical behavioral alterations, and the effects tend to be an amalgam of the dosage and chemical properties of the toxicant(s), species, the genetic and epigenetic inheritance, sex, and the maternal health condition (such as nutritional status, maternal diabetes, epilepsy). Organismal development is furthermore characterized by prenatal and postnatal periods of particular sensitivity to environmental perturbations (medical drugs, drugs of abuse and environmental chemicals), and the effects (severity, type, reversibility/irreversibility) of toxicants may differ markedly depending on when the exposure occurs. Our more specific interests follow three lines of inquiry: 1) predictive models for developmental toxicity during prenatal organogenesis (especially developmental cardiotoxicity), 2) mechanistic and predictive models for developmental neurotoxicity (DNT) focused on a synaptogenesis sensitivity period during postnatal brain development and 3) the mechanistic basis of the resilience that allows DNT and certain types of adult neurotoxicity (with sub-cytotoxic neurotoxic effects) to become propagated over time (evident as irregular motoric and cognitive behaviors).

Members of the group during 2012

Lennart Dencker, Professor
Michael Stigson, PhD, Researcher

Henrik Alm, PhD Researcher
Birger Scholz, PhD, Researcher
Mats Nilsson, PhD student
Raili Engdahl, Technician
Lena Norgren, Technician

Publications 2010-2012

Kultima K, Jergil M, Salter H, Gustafson AL, Dencker L, Stigson M. Early transcriptional responses in mouse embryos as basis for selection of molecular markers predictive of valproic acid teratogenicity. *Reprod Toxicol*. 2010; 30:457-68.

Nilsson MF, Danielsson C, Sköld AC, Johansson A, Blomgren B, Wilson J, Khan KM, Bengtsson E, Kultima K, Webster WS, Danielsson BR. Improved methodology for identifying the teratogenic potential in early drug development of hERG channel blocking drugs. *Reprod Toxicol* 2010; 29: 156-63.

Abela D, Ritchie H, Ababneh D, Gavin C, Nilsson MF, Khan MK, Carlsson K, Webster WS. The effects of drugs with ion channel-blocking activity on the early embryonic rat heart. *Birth Def Res Dev B Reprod Toxicol*. 2010; 89:429-40.

Scholz B, Alm H, Mattsson A, Nilsson A, Kultima K, Savitski MM, Fälth M, Sköld K, Brunström B, Andren PE, Dencker L. Neuropeptidomic analysis of the embryonic Japanese quail diencephalon. *BMC Dev Biol*. 2010; 10:30.

Scholz B, Sköld K, Kultima K, Fernandez C, Waldemarson S, Savitski MM, Söderquist M, Borén M, Stella R, Andrén P, Zubarev R, James P. Impact of temperature dependent sampling procedures in proteomics and peptidomics - a characterization of the liver and pancreas post mortem degradome. *Mol Cell Proteomics*. 2011; 10:M900229MCP200.

Karén J, Rodriguez A, Friman T, Dencker L, Sundberg C, Scholz B. Effects on the histone deacetylase inhibitor valproic acid on human pericytes in vitro. *PLoS One*. 2011; 6:e24954.

Jergil M, Forsberg M, Salter H, Stockling K, Gustafson AL, Dencker L, Stigson M. Short-time gene expression response to valproic acid and valproic acid analogs in mouse embryonic stem cells. *Toxicol Sci*. 2011; 121:328-42.

Muhammad Khalid Khan Niazi, Muhammad Talal Ibrahim, Mats F. Nilsson, Anna-Carin Sköld, Ling Guan, Ingela Nyström. Robust Signal Generation and Analysis of Rat Embryonic Heart Rate In Vitro Using Laplacian Eigenmaps and Empirical Mode Decomposition. *Lecture Notes in Computer Science 2011*, Vol 6855/2011: 523-530. DOI: 10.1007/978-3-362-23678-5_62

Reviews 2010-2012

Alm, H., Scholz B. *Developmental Neurotoxicity of PBDEs, Mechanisms and Implications*. NOVA publishing. 2010. ISBN: 978-1-61668-295-8

Supporting the work/Funding 2012

The Swedish Research Council (Medicine)
The Swedish Research Council Formas EU
Research and Innovation for Sustainable Growth (Vinnova)
The Swedish Association of the Pharmaceutical Industry
Swedish Fund for Research without Animal Experiments

Other commitments/assignments of staff members 2012

Lennart Dencker

ExCo member of an EU-project within IMI JU, planning a pan-European training programme in safety of medicines (see SafeSciMET.eu).

ExCo member of MRA, (http://www.medicinesacademy.org/index.php/Home/8/0/), a newly established industrial oriented medicines research education cooperation between Lund University, Technical University of Denmark, University of Copenhagen and Uppsala University.

Presiden of EUFEBS (http://www.eufeps.org/), an organization representing and serving the pharmaceutical sciences community/ies and innovative drug research in Europe.

Member of Toxikologiska Rådet, Kemikalieinspektionen.

Member of Läkemedelsnämnden, Läkemedelsverket

Projects

In vitro system development for prenatal cardiotoxicity

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. They can be reversible, but have occasionally detrimental morphological and functional downstream effects. We aim to improve the mechanistic understanding of teratogenic processes and develop improved in vitro methodologies in developmental toxicology. One project is to develop improved image analysis software for the characterization and scoring of rodent embryos undergoing organogenesis in whole embryo culture (WEC).

By combining image analysis with multivariate analysis to assess adverse effects of embryonic development *in vitro*, we believe that the objectivity and the sensitivity of the method will increase. One aspect of the image analysis software project is to develop better ways of analysing the developmental toxicity effect of drugs on heart rate in WEC conditions. For the purpose of standardization and to improve scoring and data handling in the assessment of WEC, we are also developing a controlled vocabulary in the form of a OBO and OWL compatible upper ontology (Phenosemantic ontology, PSO) together with a phenotypic domain ontology (Rodent Organogenesis and Toxicology Ontology, ROTO) that encompasses observable physiological and morphological endpoints during organogenesis (applicable both *in vivo* and *in vitro*).

Developmental neurotoxicity during the postnatal synaptogenic sensitivity period

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.

Developmental contingencies and DNT in vitro

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 how the developmental contingency of the original conditions (i.e. environmental experiences of the donor, age of cell isolation, brain region, sex, and species) influences regular and DNT properties of neural stem/progenitor cells (NSPCs) derived from prenatal and postnatal mice and rats. Neurite outgrowth and synaptogenesis is a fundamental event in postnatal brain development. We have previously shown, both in mouse in vivo and in primary CNS cell cultures in vitro, that PBDEs disrupt the normal expression of proteins necessary for neuritogenesis and synapse formation. These findings, together with the ability of certain PBDEs to accumulate in CNS cells, particularly in astrocytes, and to elicit stress in these cells has prompted us to investigate the effects of PBDEs on astrocyte-neuron interactions leading to altered neuritogenesis and synaptogenesis. NSPC cultures are neural cell mixes that – depending on their developmental contingency- are composed of different proportions of neurons, astrocytes and oligodendrocytes.

The analysis involves both functional genomic characterizations and the use of high content imaging (HCA) systems and is conducted with Kim Kultima at the department of Medical sciences, Uppsala University. Besides rodent NSph, the project will also use primary human NSph derived from human glioblastoma biopsies.

Stem cells for embryotoxicity testing

In addition, we use 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. Using the antiepileptic and teratogenic drug valproic acid (VPA), an histone deacetylase inhibitors (HDACi), together with some analogs of valproic acid, we try to visualize which categories of genes may be representative for the teratogenic action (such as neural tube defects) of VPA, and in addition find coherent responses, on the level of gene regulation, to these compounds in the two species. Presently, larger batteries of teratogenic and non-teratogenic compounds are tested with respect to their gene (de)regulation in murine embryonic stem cells.

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.

Mechanistic studies of CRABP1

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.

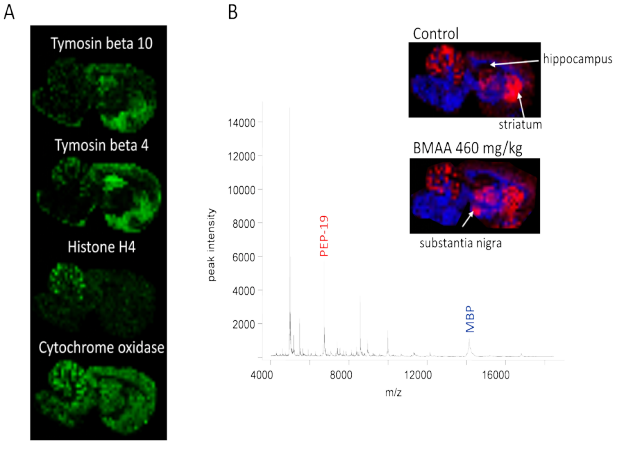
Resilience of memory traces

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. The project is conducted in collaboration with Dr Kerrie Thomas at Cardiff University, UK. An 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 and sample inactivation, post-mortem effects).

Bioactivation and Toxicity

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



Matrix-assisted laser desorption/ionization imaging mass spectrometry (Maldi-IMS) was used to reveal hundreds of small proteins in sagittal brain sections. The rats were treated with the neurotoxin BMAA (460 mg/kg) or vehicle on postnatal days 9–10 and evaluated at 23 weeks of age. Some brain areas such as the striatum and the hippocampus displayed significant BMAA-induced changes in peak intensities.

Eva Brittebo

The studies are directed towards characterization of toxicant-induced pertubations leading to cell damage in the brain and cardiovascular tissues. The aim is to reveal mechanisms of long-term cognitive impairments and neurodegeneration in the adult brain following neonatal exposure to neurotoxins as well as to elucidate the role of environmental contaminants in endothelial dysfunction. In addition, the delivery of therapeutic agents to the brain via nasal administration is being examined.

Members of the group during 2012

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Oskar Karlsson, PhD
Elena Piras, PhD Student
Lisa Ersson, PhD student

Publications 2010-2012

Karlsson O, Berg AL, Lindström AK, Hanrieder J, Arnerup G, Roman E, Bergquist J, Lindquist NG, Brittebo EB, Andersson M. Neonatal exposure to the cyanobacterial toxin BMAA induces changes in protein expression and neurodegeneration in adult hippocampus.Toxicol Sci. 2012; 130: 391-404

Fransson M, Piras E, Burman J, Nilsson B, Essand M, Lu B, Harris RA, Magnusson

PU, Brittebo E, Loskog AS. CAR/FoxP3-engineered T regulatory cells target the CNS and suppress EAE upon intranasal delivery. J Neuroinflammation. 2012; 9:112

Helmestam M, Andersson H, Stavreus-Evers A, Brittebo E, Olovsson M. Tamoxifen modulates cell migration and expression of angiogenesis-related genes in human endometrial endothelial cells. Am J Pathol. 2012;180: 2527-35

Andersson H, Brittebo E. Proangiogenic effects of environmentally relevant levels of bisphenol A in human primary endothelial cells. Arch Toxicol. 2012;86:465-74.

Karlsson O, Roman E, Berg AL, Brittebo EB. Early hippocampal cell death, and late learning and memory deficits in rats exposed to the environmental toxin BMAA (beta-N-methylamino-L-alanine) during the neonatal period. Behav Brain Res. 2011; 219: 310-20.

Andersson H, Garscha U, Brittebo E. Effects of PCB126 and 17-beta-oestradiol on endothelium-derived vasoactive factors in human endothelial cells. Toxicology. 2011; 285: 46-56.

Andersson H, Helmestam M, Zebrowska A, Olovsson M, Brittebo E. Tamoxifen-induced adduct formation and cell stress in human endometrial glands. Drug Metab Dispos. 2010; 38: 200-7.

Agencies that support the work/Funding 2012

The Research Council FORMAS

Other commitments/Assignments of staff members 2012

Head of the Department
Member of the Faculty of Pharmacy Committee

Projects

Bioactivation and toxicity of pollutants and drugs in vascular tissues

Helén Andersson and Eva Brittebo

The persistent industrial chemicals PCBs were banned in the 1970s but are still present in the environment and humans are mainly exposed through diet. Epidemiological and experimental studies suggest an association between elevated serum levels of co-planar PCBs and hypertension. Our data demonstrate that PCB126 increased the expression of the endothelial vasoconstriction factors COX-2 and reactive oxygen species and stimulated the formation of the COX-2-derived vasoconstrictor prostaglandin PGF_2 in blood vessel endothelial cells (HUVEC).

This indicates that PCB126 can modulate the expression and production of vasoconstriction factors in the human endothelium in a way that is characteristic for endothelial dysfunction related to human hypertension.

Bisphenol A (BPA) is widely used in the manufacturing of consumer products such as plastic food containers and food cans. Experimental studies suggest a relationship between exposure to BPA and changes in metabolic processes and reproductive organs. Also, epidemiological studies report an association between elevated exposure to BPA and cardiovascular disease and diabetes. Although alterations in the vascular endothelium are implicated in pathological conditions associated with BPA, little is known about the effects of BPA in the human

endothelium. This study aimed to investigate the effects of BPA on selected biomarkers of endothelial dysfunction, inflammation, and angiogenesis in human umbilical vein endothelial cells (HUVEC). The mRNA expression of biomarkers was assayed using qRT-PCR, and the production of nitric oxide and reactive oxygen species was determined. The effect of BPA on phosphorylated eNOS was examined using Western blot and immunofluorescence, and the endothelial tube formation assay was used to investigate in vitro angiogenesis. BPA (≤1 μM) 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.

In another project we have investigated the cell specific expression of drug metabolising CYP enzymes and the effects of the selective estrogen receptor modulator tamoxifen in the highly vascularised human endometrium and in primary human endometrial endothelial cells (HEEC). The breast cancer drug tamoxifen is the most widely used agent for treatment and prevention of oestrogen receptor positive breast cancer. However, the beneficial effects are compromised by an increased risk for endometrial polyps, hyperplasia, and cancer in the endometrium. We studied the distribution of tamoxifen metabolites and the expression of cell stress proteins in human endometrial biopsies exposed to tamoxifen ex vivo. Histological analysis of endometrial biopsies demonstrated that tamoxifen metabolites were covalently bound to glandular and surface epithelial cells and also that tamoxifen induced the expression of cell stress proteins in glandular and surface epithelium. In contrast, no covalent binding of tamoxifen or induction of cell stress proteins were observed in the blood vessel walls following exposure to tamoxifen. Analysis of tamoxifen metabolising enzymes revealed a constitutive expression of the tamoxifen-metabolising enzymes CYP1A1 and CYP1B1 in the endometrial blood vessel walls whereas CYP1B1/2A6/2B6/2C8/2D6/3A4 and SULT2A1 were expressed in the glandular and surface epithelia. The colocalization of tamoxifen adducts, expression of stress proteins and tamoxifen-metabolising enzymes in human glandular and surface epithelia suggest a local bioactivation of tamoxifen at these sites and that epithelial cells are early target sites for tamoxifen-induced cell stress in the human endometrium.

The effects of tamoxifen on cell migration and angiogenesis-related gene expression in HEECs has also been studied. The data suggest that tamoxifen changes the regulation of angiogenesis in the endometrium, likely by reducing angiogenic activity and that endometrial stromal cells regulate some of tamoxifen's effects in HEECs. These studies were performed in collaboration with Professor Matts Olovsson at the Department of Women's and Children's Health at Uppsala University.

Neurodegeneration following neonatal exposure to a cyanobacterial toxin

Oskar Karlsson, Nils Gunnar Lindquist and Eva Brittebo

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 ^{3}H-BMAA across the blood-brain barrier (BBB) with no specific localisation in discrete brain regions in adult mice. In neonatal mice, however, there was an efficient transport across the BBB and a selective uptake of ^{3}H-BMAA in discrete brain regions such as the hippocampus and striatum. Neonatal exposure to BMAA also gave rise to cognitive impairments such as reduced spatial learning and memory abilities in adulthood without any effects on recognition memory. In addition, neonatal rat

pups treated with a high dose BMAA showed early neuronal cell death in the hippocampus, retrosplenial and cingulate cortices. These brain areas are all important for cognitive function. Histopathological analysis identified major changes i.e., neuronal degeneration, cell loss, calcium deposits and astrogliosis in the hippocampus of adult animals following neonatal exposure. Lower doses of BMAA caused distinct impairments in learning and memory function without any acute morphological changes in the brain. However, MALDI imaging studies revealed that BMAA decreased the expression of proteins involved in energy metabolism and intracellular signalling in the adult hippocampus at a dose (150 mg/kg) that gave no histopathological lesions. Developmental exposure to a higher dose (460 mg/kg) also induced changes in the expression of S100β, histones, calcium and calmodulin-binding proteins as well as guanine nucleotide-binding proteins. Overall, these observations imply that the developing brain is particularly sensitive to BMAA. The corresponding period in humans, starts during the last trimester of pregnancy and continues the first few years after birth.

The risk posed by BMAA as a potential human neurotoxin merits further consideration, particularly if the proposed biomagnification in the food chain is confirmed. These studies were performed in collaboration with associate professor Erika Roman and assistant professor Malin Andersson at the Dept. of Pharmaceutical Biosciences and associate professor Anna-Lena Berg at AstraZenca, Södertälje.

Nasal transfer of therapeutic agents

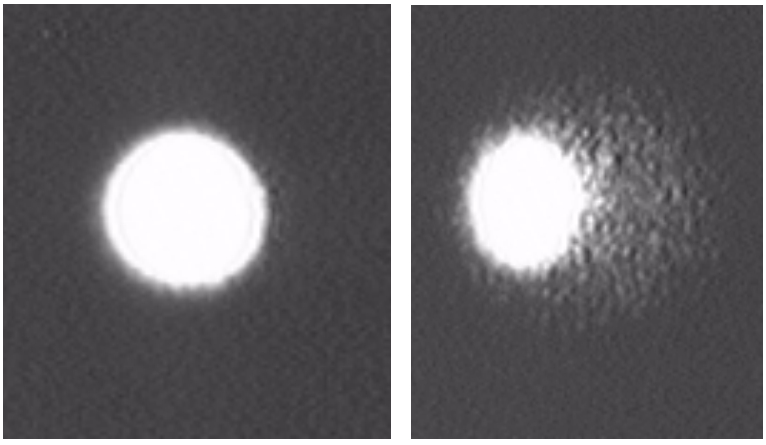
Elena Piras and Eva Brittebo

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. We have previously demonstrated a transfer of morphine and dopamine via the olfactory pathways to the brain following intranasal administration in rodents.

The transfer of other therapeutic agents to the brain is currently under study. We have demonstrated that nasal administration of genetically engineered cells such as CNS-targeting modified CD4+ T cells suppressed ongoing encephalomyelitis as demonstrated by reduced disease symptoms as well as decreased IL-12 mRNA in a mouse model (EAE) of multiple sclerosis. Immunohistochemical markers for myelination and reactive astrogliosis confirmed recovery in mice treated with engineered Tregs compared to controls. Symptom-free mice recovering from EAE were rechallenged with a second EAE-inducing inoculum but remained healthy, demonstrating the sustained effect of engineered Tregs. The studies were performed in collaboration with associate professor Angelica Loskog at the Dept. of Immunology, Genetics and Pathology at Uppsala University.

Genetic Toxicology

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



Cells showing different levels of DNA damage after 10 min of electrophoresis under alkaline conditions (the "comet" with a more prominent "tail" is a cell which has a higher level of DNA-strand breaks than the normal backgroundlevel of strand breaks)

Björn Hellman

When testing the potential DNA-damaging effects by pharmaceutical drugs and other chemicals, the test systems are generally based on experimental animals, bacteria or various kinds of transformed cells. For the safety evaluation it would be of advantage if healthy cells from humans could be used instead, since they have a normal and stable set of chromosomes. In case of using primary cultures of human lymphocytes, it is hardly possible to use a blood sample on more than one single testing occasion. This will often give varying results from different testing occasions, since new blood samples (in general from different donors) have to be taken all the time. Our extended-term cultures of lymphocytes allow one single blood sample to be used for up to 50 different experiments. We measure the DNA-damage with the so called Comet Assay, which is a relatively quick, simple and cheap method for evaluating DNA-strand breaks in individual cells. The major objective of our *in vitro* studies using the comet assay in various cell lines is to improve the risk assessment regarding exposures to genotoxic agents.

In another project, we are evaluating the genotoxic and anti-genotoxic effects of some plant extracts 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 is associated with a lower level of oxidative DNA damage in mononuclear leukocytes, we have also on-going studies on the effect of β-carotene on catechol-induced DNA damage in mouse lymphoma cells. In collaboration with colleagues from the Ångström Laboratory at Uppsala University, and FOI, Division of CBRN Defence and Security in Umeå, we are currently also evaluating cellular uptake and DNA damage in human lung cancer cells exposed to reactive nanoparticles of titanium dioxide using Raman microspectroscopy and

the Comet Assay.

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*. In the most recent project we are therefore also evaluating the effect of oxidative stress on the nucleotide pool and the integrity of DNA using various compounds previously shown to induce gene mutations, but only at rather high concentrations.

Members of the group during 2012

Björn Hellman, Professor
Rikard Åsgård, Ph.D. student
Lena Norgren, Laboratory assistant

Publications 2010-2012

Yeshak MY, Göransson U, Burman R, Hellman B (2012). Genotoxicity and cellular uptake of cyclotides: Evidence for multiple mode of action. *Mutat. Res.* 747: 176-181.

Demma J, Elseedi H, Engidawork E, Leta Aboye T, Göransson U, Hellman B. (2012) An in vitro study on the DNA damaging effects of phytochemicals partially isolated from an extract of Glinus lotoides. *Phytother. Res.*, in press.

Reviews 2010-2012

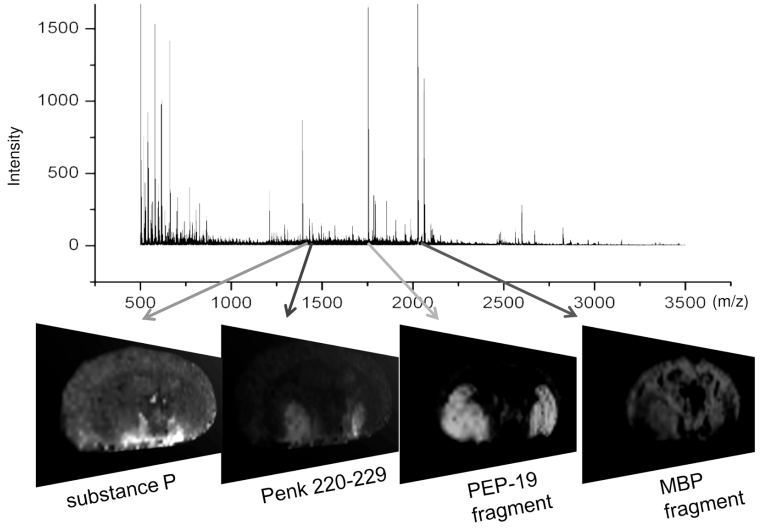
B. Hellman & K. Broberg. Genetiska förändringar som biologiska markörer för exponering, sjukdom och känslighet. I boken: Arbets- och miljömedicin; 3:e upplagan. Redaktörer: C. Edling, G. Nordberg, M. Albin & M. Nordberg, Studentlitteratur, Lund; 2010.

Other commitments/assignments of staff members 2012

Member of the local committee for scholarships at the Faculty of Pharmacy, Uppsala University
Member of the committee for undergraduate courses (GRUFF) at the Faculty of Pharmacy, Uppsala University
Deputy member of the ethical committee for animal experiments in Uppsala
Deputy member of "Docenturkommittén" at the Disciplinary Domain of Medicine and Pharmacy, Uppsala University

Neurotoxicology

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



Malin Andersson

We use MALDI –TOF imaging mass spectrometry (MALDI IMS) for the topographical elucidation of proteins, neuropeptides and neurotransmittors and their changing concentrations in brain during physiological and pathophysiological events. In particular we focus on Parkinson's disease which is characterized by degeneration of dopaminergic neurons and accompanied by a dramatic loss of DA 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. DA 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 (LID). Despite large efforts in the field of LID research, the difficulties we face today remain as how to dissociate the molecular changes that arise by the motor execution of LID 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 LID in an experimental model of Parkinson's disease.

Members of the group during 2012

Malin Andersson, Assistant professor
Madelene Svedin, Research assistant

Publications 2010-2012

Nilsson A, Fehniger TE, Gustavsson L, Andersson M, Kenne K, Marko-Varga G,

Andrén PE. (2010) Fine mapping the spatial distribution and concentration of unlabeled drugs within tissue micro-compartments using imaging mass spectrometry. PLoS One. 2010 Jul 14;5(7):e11411

Hanrieder J, Wicher G, Bergquist J, **Andersson M**, Fex-Svenningsen A. (2011). MALDI mass spectrometry based molecular phenotyping of CNS glial cells for prediction in mammalian brain tissue. Anal Bioanal Chem. 2011 Jul;401(1):135-47.

Ljungdahl A, Hanrieder J, Fälth M, Bergquist J, **Andersson M**. (2011). Imaging Mass Spectrometry Reveals Elevated Nigral Levels of Dynorphin Neuropeptides in L-DOPA-Induced Dyskinesia in Rat Model of Parkinson's Disease. PLoS One. 2011;6(9):e25653.

Hanrieder J, Ljungdahl A, Fälth M, Mammo SE, Bergquist J, **Andersson M**. (2011). L-DOPA-induced Dyskinesia is Associated with Regional Increase of Striatal Dynorphin Peptides as Elucidated by Imaging Mass Spectrometry. Mol Cell Proteomics. 2011 Oct;10(10):M111.009308.

H.S. Lindgren, D. Rylander, H. Iderberg, **M. Andersson,** S.S. O'Sullivan, D.R. Williams, A.J. Lees and M.A. Cenci (2011) Putaminal Upregulation of FosB/FosB-Like Immunoreactivity in Parkinson's Disease Patients with Dyskinesia. Journal of Parkinson's Disease 1(4): 347-357

Hanrieder J, Ljungdahl A, Andersson M. (2012). MALDI Imaging Mass Spectrometry of Neuropeptides in Parkinson's Disease. JOVE, J Vis Exp. 2012 Feb 14;(60). pii: 3445

Hanrieder J, Ekegren T, Andersson M, Bergquist J (2013) MALDI Imaging of Post Mortem Human Spinal Cord in Amyotrophic Lateral Sclerosis. J. Neurochem. J Neurochem. 2012 Sep 19. PMID: 22994484.

Karlsson O, Berg AL, Lindström AK, Arnerup G, Roman E, Bergquist J, Hanrieder J, Lindquist NG, Brittebo E, Andersson M. (2012) Neonatal exposure to the cyanobacterial toxin BMAA induces changes in protein expression, and neurodegeneration in adult hippocampus. Toxicol Sci. 2012 Aug 7. PubMed PMID: 22872059

Reviews 2010-2012

Andersson, M., Andren, P.A.., Caprioli, R.M. MALDI Imaging and Profiling Mass Spectrometry in Neuroproteomics. (Frontiers in neuroscience, Boca Raton, FL : CRC Press, c2010.)

Agencies that support the work/Funding 2012

Swedish Research Council
The Royal Swedish Academy of Sciences

Projects

Imaging Mass Spectrometry Study of Basal Ganglia Levels of Neuropeptides in L-DOPA-induced Dyskinesia in experimental Parkinson's Disease.

Anna Ljungdahl, Madelene Svedin, Kristen Burnum (PNLL), Jonas Bergquist, and Malin Andersson

We study 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).

Imaging MALDI mass spectrometry characterization of opioid peptides after a single dose cocaine or morphine.

Emma Gustafsson, Jonas Bergquist, Jan Rodriguez Parkitna and Ryszard Przewłocki (Polish Academy of Science) and Malin Andersson

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.

MALDI mass spectrometry based molecular phenotyping of CNS and PNS glial cells for prediction in mammalian brain tissue.

Jörg Hanrieder, Grzegorz Wicher, Karin Forsberg Nilsson, Jonas Bergquist, Åsa Fex-Svenningsen (SDU, Denmark) and Malin Andersson

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.

Assessment of brain neuropeptides and proteins after neonatal exposure to the environmental toxin BMAA using MALDI imaging mass spectrometry.

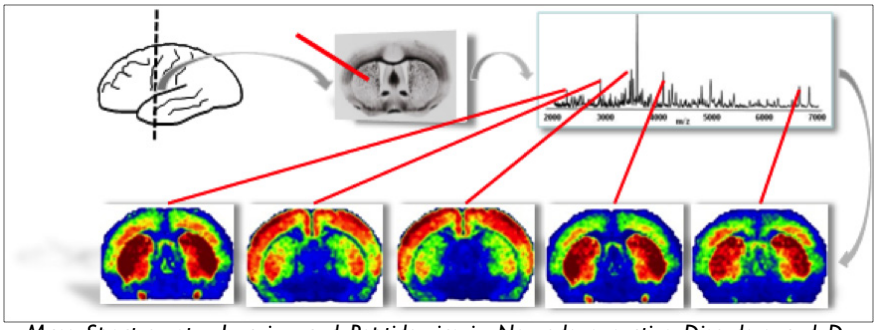
Oskar Karlsson, Eva Brittebo and Malin Andersson

Exposure to the nonprotein amino acid β-N-methylamino-L-alanine (BMAA) during the neonatal period can cause cognitive impairment in adult rats. BMAA is produced by various cyanobacteria and has been proposed to cause and/or contribute to the pathogenesis of several neurodegenerative diseases. This study searches for molecular correlates of changed cognitive function in several brain areas using MALDI IMS.

Medical Mass Spectrometry

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

Per Andrén



Mass Spectrometry Imaging and Peptidomics in Neurodegenerative Disorders and Drug Discovery.

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

Mass spectrometry Imaging (MSI) is a novel technique used to determine the spatial distribution of peptides, proteins, drugs and metabolites in biological tissue sections in situ. The technology allows analysis and visualization of endogenous proteins and peptides as well as drugs and its 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) (VR-M grant 2011-3170)**. The aim is to define neuropeptides and proteins that are differentially expressed in the basal ganglia complex of animals with striatal dopamine depletions, and to determine which of these neuropeptides and proteins are regulated by loss of dopamine signaling, as well as to investigate protein and peptide 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 (VR-NT grant 2010-5421) 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., two MALDI MS imaging instruments, Ultraflextreme and UltraFlex II (Bruker Daltonics) and two electrospray ionization mass spectrometers, LTQ (Thermo) and a high-resolution Q-Tof mass spectrometer (Maxis Impact, Bruker Daltonics).

Members of the group during 2012

Per Andrén, Assoc. Prof.
Anna Nilsson, researcher
Henrik Wadensten, researcher
Henrik Lodén, reseacher
Mohammadreza Shariatgorji, researcher
Cecilia Eriksson, post-doc
Richard Goodwin, postdoc
Sara Ståhl, post-doc
Patrik Källback, PhD student

Publications 2010-2012

Sundström I, Arts J, Westerlund D, Andren PE (2010) In vivo investigation of brain and systemic ketobemidone metabolism. Analyst. 135:405-413.

Alm H, Scholz B, Kultima K, Nilsson A, Andrén PE, Savitski MM, Bergman A, Stigson M, Fex-Svenningsen A, Dencker L (2010) In Vitro Neurotoxicity of PBDE-99: Immediate and Concentration-Dependent Effects on Protein Expression in Cerebral Cortex Cells. J Proteome Res. 9:1226-1235

Végvári A, Fehniger TE, Gustavsson L, Nilsson A, Andren PE, Kenne K, Nilsson J, Laurell T, Marko-Varga G (2010) Essential tactics of tissue preparation and matrix nano-spotting for successful compound imaging mass spectrometry. J Proteomics. 73:1270-1278.

Scholz B, Alm H, Mattsson A, Nilsson A, Kultima K, Savitski MM, Fälth M, Sköld K, Brunström B, Andren PE, Dencker L (2010) Neuropeptidomic analysis of the embryonic Japanese quail diencephalon. BMC Dev Biol. Mar 18;10:30.

Vintila S, Jonasson S, Wadensten H, Nilsson A, Andren PE, El-Shehawy R (2010) Proteomic profiling of the Baltic Sea cyanobacterium Nodularia spumigena strain AV1 during ammonium supplementation. J Proteomics. 73:1670-1699. Epub 2010 May 12.

Nilsson A, Fehniger TE, Gustavsson L, Andersson M, Kenne K, Marko-Varga G, Andren PE (2010) Fine mapping the spatial distribution and concentration of unlabeled drugs within tissue micro-compartments using imaging mass spectrometry. PLoS One. Jul 14;5(7):e11411.

Scholz B, Sköld K, Kultima K, Fernandez C, Waldemarson S, Savitski MM, Svensson M, Boren M, Stella R, Andren P, Zubarev R, James P (2011) Impact of temperature dependent sampling procedures in proteomics and peptidomics - A characterization of the liver and pancreas post mortem degradome. Mol Cell Proteomics Mol Cell Proteomics. 10: 10.1074/mcp.M900229-MCP200, 1–15. PMID: 20110281.

Colgrave ML, Xi L, Lehnert SA, Flatscher-Bader T, Wadensten H, Nilsson A, Andren PE, Wijffels G (2011) Neuropeptide profiling of the bovine hypothalamus: Thermal stabilization is an effective tool in inhibiting post-mortem degradation. Proteomics. 11:1264-76. doi: 10.1002/pmic.201000423.

Zhang X, Andren PE, Glennon RA, Svenningsson P (2011) Distribution, level, pharmacology, regulation and signalling of 5-HT(6) receptors in rats and marmosets with special reference to an experimental model of parkinsonism. J Comp Neurol. 519:1816-1827. doi: 10.1002/cne.22605.

Madeira A, Yang J, Zhang X, Vikeved E, Nilsson A, Andren PE, Svenningsson P (2011) Caveolin-1 interacts with alpha-synuclein and mediates toxic actions of cellular alpha-synuclein overexpression. Neurochem Int. 59:280-289. Epub 2011 Jun 13.

Goodwin RJ, Mackay CL, Nilsson A, Harrison D, Farde L, Andren PE, Iverson SL (2011) Qualitative and quantitative MALDI imaging of the positron emission tomography ligands raclopride (a D2 dopamine antagonist) and SCH 23390 (a D1 dopamine antagonist) in rat brain tissue sections using a solvent-free dry matrix application method. Anal Chem. 83:9694-9701.

Nilsson A, Stroth N, Zhang X, Qi H, Fälth M, Sköld K, Hoyer D, Andren PE, Svenningsson P (2012) Neuropeptidomics of mouse hypothalamus after imipramine treatment reveal somatostatin as a potential mediator of antidepressant effects. Neuropharmacology. 62:347-57.

Petruzziello F, Fouillen L, Wadensten H, Kretz R, Andren PE, Rainer G, Zhang X (2012) Extensive Characterization of Tupaia belangeri Neuropeptidome Using an Integrated Mass Spectrometric Approach. J Proteome Res. 11:886-896.

Goodwin RJ, Iverson SL, Andren PE (2012) The significance of ambient-temperature on pharmaceutical and endogenous compound abundance and distribution in tissues sections when analyzed by matrix-assisted laser desorption
/ionization mass spectrometry imaging Rapid Commun Mass Spectrom. 26:494-498. doi: 10.1002/rcm.6125.

Zhang X, Petruzziello F, Zani F, Fouillen L, Andren PE, Solinas G, Rainer G (2012) High identification rates of endogenous neuropeptides from mouse brain. J Proteome Res. 11:2819-2827.

Jones EA, van Zeijl RJ, Andren PE, Deelder AM, Wolters L, McDonnell LA (2012) High Speed Data Processing for Imaging MS-Based Molecular Histology Using Graphical Processing Units. J Am Soc Mass Spectrom. 23:745-752. Epub 2012 Feb 4.

Shariatgorji M, Källback P, Gustavsson L, Schintu N, Svenningsson P, Goodwin RJA, Andren PE (2012) Controlled-pH tissue clean-up protocol for signal enhancement of small molecule drugs analyzed by MALDI-MS imaging. Anal Chem.;84:4603-7.

Goodwin RJ, Nilsson A, Borg D, Langridge-Smith PR, Harrison DJ, Mackay CL, Iverson SL, Andren PE (2012) Conductive carbon tape used for support and mounting of both whole animal and fragile heat-treated tissue sections for MALDI MS imaging and quantitation. J Proteomics. 75:4912-4920. doi: 10.1016/j.jprot.2012.07.006.

Källback P, Shariatgorji M, Nilsson A, Andren PE (2012) Novel mass spectrometry imaging software assisting labeled normalization and quantitation of drugs and neuropeptides directly in tissue sections. J Proteomics. 75:4941-4951. doi: 10.1016/j.jprot.2012.07.034.

Shariatgorji M, Nilsson A, Goodwin RJ, Svenningsson P, Schintu N, Banka Z, Kladni L, Hasko T, Szabo A, Andren PE (2012) Deuterated matrix-assisted laser desorption ionization matrix uncovers masked mass spectrometry imaging signals of small molecules. Anal Chem. 84:7152-7157. doi: 10.1021/ac301498m.

Akhtar MN, Southey BR, Andren PE, Sweedler JV, Rodriguez-Zas SL. (2012) Evaluation of Database Search Programs for Accurate Detection of Neuropeptides in Tandem Mass Spectrometry Experiments. J Proteome Res. 11:6044-55. doi: 10.1021/pr3007123.

Nilsson A, Forngren B, Bjurström S, Goodwin RJ, Basmaci E, Gustafsson I,

Annas A, Hellgren D, Svanhagen A, Andren PE, Lindberg J (2012) In situ mass spectrometry imaging and ex vivo characterization of renal crystalline deposits induced in multiple preclinical drug toxicology studies. PLoS One. 2012;7(10):e47353. doi: 10.1371/journal.pone.0047353.

Reviews 2010-2012

Andersson M, Andren PE, Caprioli RM (2010) MALDI Imaging and Profiling Mass Spectrometry in Neuroproteomics. In: Frontiers in Neuroscience: Neuroproteomics (ed. Alzate O), Chapter 7. CRC Press, Taylor & Francis Group, Boca Raton, FL, USA, p 115-135, PMID: 21882452

Andren PE, Verhaert P, Svenningsson P (2010) Proteomics. In: Encyclopedia of Psychopharmacology (ed. Stolerman IP) 1st Ed., 1386 p. Springer-Verlag Berlin Heidelberg, p 1077-1083.

Madeira A, Vikeved E, Nilsson A, Sjögren B, Andren PE, Svenningsson P (2011) Identification of protein-protein interactions by surface plasmon resonance followed by mass spectrometry. Curr Protoc Protein Sci. 2011 S 65:Aug;Chapter 19: Unit19.21.

Corthals GL, Dunn M, James P, Gil C, Penque D, Albar JP, Andrén P, Rabilloud T, Marko-Varga G (2011) The transition of the European Proteomics Association into the future. J Proteomics. 75:18-22

McDonnell LA, Heeren RM, Andren PE, Stoeckli M, Corthals GL (2012) Going forward: Increasing the accessibility of imaging mass spectrometry. J Proteomics. 75:5113-5121. doi: 10.1016/j.jprot.2012.05.016.

McDonnell L, Andren PE, Corthals GL (2012) Imaging mass spectrometry: a user's guide to a new technique for biological and biomedical research. J Proteomics. 75:4881-4882. doi: 10.1016/j.jprot.2012.07.033.

Agencies that support the work/Funding 2012

The Swedish Research Council (VR), NT, MH and RFI (Per Andrén).
The Swedish Research Council (VR)-NT. Post-doc Position (Mohammadreza Shariatgorji)
The National Institute of Health (NIH)/the National Institute on Drug Abuse (NIDA) (Per Andrén).
VINNOVA. Japan Society for the Promotion of Science (JSPS) Joint Projects (Per Andrén).
VINNOVA. Marie Curie Chair (Cecilia Eriksson).
AstraZeneca, Global DMPK, Safety Assessment (Per Andrén)

Other commitments/assignments of staff members 2012

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
Peptidomics, Editorial Board
European Proteomics Association (EuPA), Chairman for Mass Spectrometry Imaging Initiative
Cooperation in Science and Technology (COST), Mass Spectrometry Imaging: New Tools for Healthcare Research Infrastructure, Member of the Management Committee

Awards and Appointments 2012

Richard Goodwin

AstraZeneca Innovative Medicines Global Science Awards - Post-Doc of the Year. Development of innovative of mass spectrometry imaging techniques for whole body and intact body tissue sections.

Projects

Neurochemical characterization of basal ganglia neuropeptides and proteins in levodopa-induced dyskinesia in experimental Parkinson's disease using Imaging Mass Spectrometry and Peptidomics (VR-MH grant 2011-3170)

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 and proteins that are differentially expressed in the basal ganglia complex of animals with striatal dopamine depletions, with and without LID.

Imaging Mass Spectrometry: Direct analysis of peptides, proteins and drugs in tissue sections (VR-NT grant 2010-5421)

Collaboration with Per Svenningsson, Karolinska Institutet, Liam McDonnell, Leiden University, the Nedtherlands.

The aim of this project is to create an approach to solve complex clinical problems by developing novel clinical tools utilizing mass spectrometry imaging directly on biological tissue sections. The mass spectrometry imaging technology is used to identify individual peptides and proteins and their 2-dimensional expression patterns in tissue sections as well as drugs and their metabolites in different animal disease models, but also in human tissues. We describe the development of new biomarkers and technologies in personalized medicine for marker-assisted diagnosis, prognosis and targeted therapies derived from an individual´s molecular profile. Conceptually, the project integrates in vivo disease models, biomarker discovery, personalized drugs which translate to clinic.

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 (NIDA grant R21 DA027548-01)

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.

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.

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 imaging mass spectrometry 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.

Characterization of drug-induced kidney toxicity using MS-Imaging

Collaboration with AstraZeneca

The project is aimed at developing a routine methodology around 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.

Characterization of PET-ligands using MS-Imaging

Collaboration with AstraZeneca, Mats Larhed, UU

The aim of the presented work is to optimize the technical platform and adequately apply the MS-imaging technique to improve our understanding of the distribution characteristics of PET-ligands and their metabolites in the brain. This is important since no other imaging methods can give spatial information on drug metabolites and this information will help in understanding the PK/PD modeling of such ligands. One project is in collaboration with AstraZeneca (novel PET-ligand characterization) and a second project is in collaboration with Prof. Mats Larhed.

Novel inactivation technology stabilizes the in vivo levels of proteins, peptides, phosphorylations, lipids in tissue samples

Collaboration with Per Svenningsson, Karolinska Institutet, Denator AB, Uppsala and Göteborg, Sweden

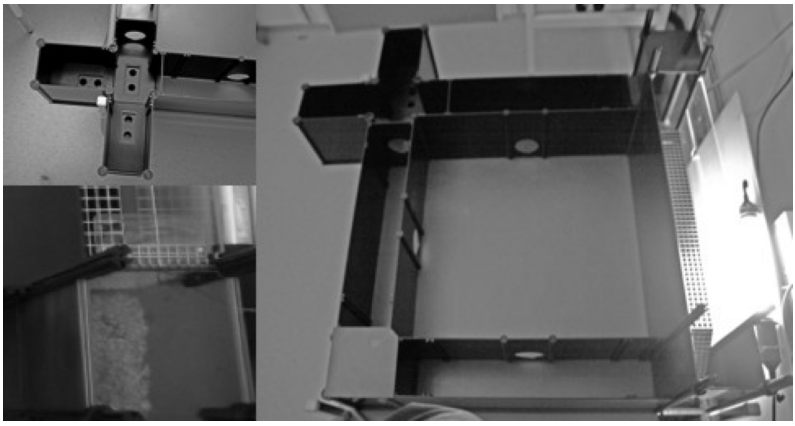
After tissue or blood sampling, proteases and other protein-modifying enzymes can rapidly change proteome composition. Subsequent analytical results reflect a mix of in vivo proteome and degradation products. Vital information about the 'pre-sampling' state may be destroyed or distorted, leading to variation 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, Addiction & Behaviour

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

Ingrid Nylander, Lena Bergström, Erika Roman and Anne-Lie Svensson



Development of addiction involves transition from controlled drug consumption to compulsive intake beyond control. Environmental factors, particular early in life, impinge on a set of genes and determine the individual propensity for addiction. We investigate how early-life experiences affect neuronal networks and epigenetic processes, and the association between behavioural phenotype and drug consumption. Mechanisms underlying risk are studied as well as protective factors that can counteract risk in a predisposed individual.

Research is devoted to studies on basic neurobiology, physiological and pathophysiological mechanisms in the brain, and neuropharmacology of relevance for disorders such as drug addiction, neurodegenerative diseases and mood disorders. During 2012, several new members have been recruited to the research group to assemble competences from relevant research areas and be able to address mechanisms and mediators in the individual propensity for addiction. We have also initiated collaboration with Drs E Comasco and L Oreland (Department of Neuroscience), 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.

Current projects include studies of neurobiological substrates for individual differences in addiction processes, especially vulnerability for risk consumption of alcohol and alcohol addiction, 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.

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 analyze effects on receptors, transmitters and mRNA in tissue samples and brain slices but also analysis of transmitter release and re-uptake patterns using an in vivo amperometric technique, Fast Analytical Sensing Technology (FAST).

Specific research activities within the group are described shortly under projects.

Members of the group during 2012

Ingrid Nylander, Professor
Lena Bergström, Associate Professor
Erika Roman, Associate Professor
Anne-Lie Svensson, Senior lecturer
Maria Ellgren, Junior lecturer
Lova Perup-Segerström, Researcher
Samuel Rowley, Post-doc
Loudin Daoura, PhD student
Shima Momeni, PhD student
Sara Palm, PhD student
Linnea Granholm, PhD student
Bengt J Meyerson, Professor Emeritus
Marita Berg, Technician
Martina Sjöström, Research assistant
Levar Youkhanis, Research assistant

Publications 2010-2012

Stanic D, Kuteeva E, Nylander I, Hökfelt T. Characterization of CGRP protein expression in "satellite-like" cells and dendritic arbours of the mouse olfactory bulb. *J Comp Neurol (2010) 518,6: 770-784*

Oreland S, Nylander I, Pickering C. Prolonged maternal separation decreases granule cell number in the dentate gyrus of 3-week-old male rats. *Int J Dev Neurosci (2010) 28: 139-144*

Oreland S, Gustafsson-Ericson L, Nylander I. Short- and long-term consequences of different early environmental conditions on central immunoreactive oxytocin and arginine vasopressin levels in male rats. *Neuropeptides (2010) 44:391-398*

Oreland S, Daoura L, Gustafsson-Ericsson L, Damberg M, Hytiä P, Oreland L, Nylander I. Does the transcription factor AP2b have an impact on the genetic and early environmental influence on ethanol consumption? *J Neural Transmission (2010) 117:1077-1081*

Daoura L, Hjalmarsson M, Oreland S, Nylander I, Roman E. Postpartum behavioral profiles in Wistar rats following maternal separation – altered exploration and

risk-assessment behavior in MS15 dams. *Frontiers Behav Neurosci (2010) 18,4: 37*

Palm S, Roman E*, Nylander I*. Differences in voluntary ethanol consumption in Wistar rats from five different suppliers. Alcohol (2011) 45:607-614, *shared senior authorship.

Berglund K, Roman E, Balldin J, Berggren U, Eriksson M, Gustavsson P, Fahlke C. Do men with excessive alcohol consumption and social stability have an addictive personality? Scand J Psychology (2011) 52:257-260

Karlsson O, Roman E, Berg AL, Brittebo E. Early hippocampal cell death, and late learning and memory deficits in rats exposed to the environmental toxin BMAA (β-N-methylamino-L-alanine) during the neonatal period. Behav Brain Res (2011) 219:310-320

Palm S, Hävermark Å, Meyerson BJ, Nylander I*, Roman E*. When is a Wistar a Wistar? Behavioral profiling of outbred Wistar rats from five different suppliers using the MCSF test. Appl Animal Behav Sci (2011) 135:128-137 *shared senior authorship.

Daoura L, Nylander I. The response to naltrexone in ethanol-drinking rats depends on early environmental experiences. *Pharmacol Biochem Behav (2011) 99:626-633*

Daoura L, Haaker J, Nylander I. Early environmental factors differentially affect voluntary ethanol consumption in adolescent and adult male rats. *Alcohol Clin Exp Res (2011) 35 (3) 506-515*

Oreland S, Raudkivi K, Oreland L, Harro J, Aborelius L, Nylander I. Ethanol-induced effects on the dopamine and serotonin systems in adult Wistar rats are dependent on early-life experiences. *Brain Res (2011) 1405:57-68*

Franck J, Nylander I (Eds.) Beroendemedicin, Studentlitteratur, 2011

Nylander I. Beroendemekanismer. In Beroendemedicin (Franck & Nylander, Eds.), Studentlitteratur, 2011

Roman E. Djurexperimentell metodik. In Beroendemedicin (Franck & Nylander, Eds.), Studentlitteratur, 2011

Svensson A, Nikotin. In Beroendemedicin (Franck & Nylander, Eds.), Studentlitteratur, 2011

Nylander I, Belöning och beroende. Effekter av missbruksdroger på hjärnan. In Handbok i missbrukspsykologi (Fahlke, Ed.), 2012

Roman E, Stewart RB, Bertholomey ML, Jensen, ML, Colombo G, Hyytiä P Badia-Elder N, Grahame NJ, Li TK, Lumeng L. Behavioral profiling of multiple pairs of rats selectively bred for high and low alcohol intake using the MCSF test. Addict Biol (2012) 1 33-46

Palm S, Roman E, Nylander I. Differences in basal and ethanol-induced levels of opioid peptides in Wistar rats from five different suppliers. Peptides (2012) 36 1-8

Karlsson O, Berg AL, Lindström AK, Arnerup G, Roman E, Bergquist J, Hanrieder J, Lindquist NG, Brittebo E, Anderson M. Neonatal exposure to the cyanobacterial toxin BMAA induces changes in protein expression, and neurodegeneration in adult hippocampus. Toxicol Sci (2012) 130 391-404

Reviews 2010-2012

Nylander I, Roman E. Neuropeptides as mediators of the early-life impact on the brain; implications for alcohol use disorders. Front Mol Neurosci (2012) 5 77

Agencies that support the work/Funding 2012

The European Foundation for Alcohol Research (PI Nylander, co-applicants

Roman and Svensson)
The Swedish Research Council (Nylander)
The Alcohol Research Council of the Swedish Alcohol Retailing Monopoly (two separate projects; Nylander and Roman)
Facias Foundation (three separate projects; Momeni and Roman)
Magnus Bergwalls Stiftelse (Roman)
Åhlén-stiftelsen (Bergström)
Gun och Bertil Stohnes stiftelse (Svensson)
Åhlén-stiftelsen (Svensson)
Åke Wibergs stiftelse (Roman)

Other commitments/assignments of staff members 2012

Lena Bergström

Member of the Academic Senate, Uppsala University

Ingrid Nylander
Executive member in the committee of the organization for International Narcotic Research Conference
Grant committee member, Alcohol Research Council of the Swedish Alcohol Retailing Monopoly
Grant committee member, KI
Member of the Uppsala University Quality committee
Chairman of the quality assurance group at the Disciplinary Domain of Medicine and Pharmacy
Member of the Faculty of Pharmacy committee

Erika Roman
International Adjunct Associate, Department of Psychology, Indiana University Purdue University at Indianapolis (IUPUI), Indianapolis, IN, USA
Review Editor, Frontiers in Addictive Disorders and Behavioral Dyscontrol
External mentor Salvatore Magara, Karolinska Institutet
Member of the board, The Society for Swedish Alcohol and Drug Research (SAD)
Member of the board of Uppsala University Laboratory Animal Resources (SUUF), Uppsala University
Member of the Postgraduate Programs Committee, Uppsala University
Member of the Uppsala Animal Ethical Committee
Approved Supervisor by the Swedish Board of Agriculture, Department of Pharmaceutical Biosciences
Uppsala University representative in the committee for Laboratory Animals, The Swedish Board of Agriculture
Member of the expert panel, Swedish Centre for Animal Welfare (SCAW)
One of three coordinators of Uppsala University Behavioral 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

The impact of early environmental experiences on endogenous opioids, alcohol consumption and alcohol-induced effects

Loudin Daoura, Sara Palm, Maria Ellgren, ***Ingrid Nylander***

We have previously shown that adverse experiences early in life cause long-term neurobiological and behavioural alterations. These changes may contribute to the increased vulnerability for drug addiction that is seen in the clinic, but also provide protection in a predisposed individual. A rodent maternal separation (MS) model is used to simulate different environmental settings in studies on mechanisms underlying protective and risk factors for excessive alcohol consumption. Rearing conditions are used that are associated with resilience (after short MS) or vulnerability (after longer MS) in terms of adult risk consumption. We have shown that central neuropeptides and monoamines may contribute to these differences. Rats reared in a stressful environment have signs of a dysfunctional opioid system with low basal opioid levels and enhanced response to alcohol in a voluntary alcohol consumption paradigm. They also respond differently to treatment with naltrexone; animals reared in a risk environment reduce their alcohol intake whereas other individuals do not respond. These results highlight the importance of the early environment in drug consumption, drug-induced affects and treatment outcome. The consequences of adolescent voluntary alcohol consumption on the brain and behaviour are currently examined. For example, we assess the effects of adolescent drug exposure on opioid networks, expression and methylation of genes involved in stress and reward processes and on alcohol consumption, motivation and relapse in adulthood.

In vivo and in vitro studies of drug-induced effects in the brain

Sara Palm, Sam Rowley, Anne-Lie Svensson, Martin Lundblad, ***Ingrid Nylander***

Fast Analytical Sensing Technology (FAST) has recently been established in the lab and is currently used for in vivo analysis of dopamine in the brain after various treatments. FAST is a chronoamperometric technique that enables in vivo electrochemical detection of transmitters 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 by the use of different electrodes; the brain damage is reduced to a minimum. In current studies, FAST is used to analyze effects of adolescent drug exposure on dopamine function.

Behavioural profiling of animals exposed to early environmental stress and adolescent alcohol consumption

Loudin Daoura, Sara Palm, ***Erika Roman, Ingrid Nylander***

Current experiments analyze the short- and long-term behavioural consequences of rearing in different environmental settings and of long-term alcohol consumption. In ongoing studies the behavioural effects of adolescent voluntary alcohol drinking are examined. The project comprises development of animal experimental models to assess maternal behaviour and interactions between the dam and offspring during different environmental conditions. 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.

Development and validation of the MCSF test

Erika Roman, *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. Ongoing work aims 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.

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.

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. Ongoing studies assess the animal's motivation for passing the risk area and reach a reinforcer 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.

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-taking behaviour and the association with voluntary alcohol intake, addiction processes and response to treatment. With regard to neurobiology, focus is on the cannabinoid 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 high risk-taking behaviour exerts one aspect of impulsive behaviour 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 mu 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.

Neuroprotective properties of cannabinoids against different toxic insults

Anne-Lie Svensson

The cannabinoid system is widespread in the central nervous system and is involved in many neurophysiological processes. Neurodegenerative disorders, such as Alzheimer´s disease (AD), is associated with excitotoxicity, oxidative stress and neuroinflammation. Cannabinoids have been demonstrated to affect the progression of neurodegeneration. In the AD brain, expression of microglial cannabinoid receptor (CB_1 and CB_2) is increased in areas with high load of amyloid-β, while the expression of CB_1 receptor in hippocampus is reduced. Furthermore, an upregulation of the endocannabinoid-metabolizing enzyme (FAAH) has been reported. These alterations in AD provide evidence that the endocannabinoid system may be involved in the progression of AD pathogenesis. In ongoing studies the neuroprotective properties of different cannabinoids against toxic insults are investigated in numerous cell types.

*Neurosteroids and Alzheimer's disease: Mechanistic studies of neuroprotection and amyloid-*b*-modulation*

Anne-Lie Svensson

Neurosteroids are endogenous modulators of neuronal functions responsible for many biological and pathophysiological effects. Normal aging is associated with several alterations in neurosteroid production and secretion. Decreases in neurosteroid levels might contribute to aging of the brain and loss of important nervous functions, such as memory. A plausible link between neurosteroids and neurodegenerative disorders, like Alzheimer´s disease (AD), has been discussed. AD is characterized pathologically by deposits of amyloid plaques in cortex and hippocampus. The principal component of amyloid plaques is the amyloid-β peptide, which is known to play a central role in the pathogenesis of AD, through the ability of amyloid-β monomers to aggregate and form protofibrils. The significance of neurosteroidogenesis in regulating neurodegenerative mechanisms is unknown. Accumulation of amyloid-β, induced by toxic events in cells might be able to reduce the synthesis of neuroprotective neuroteroids, thus favour/support neurodegenerative processes.

Translational Pharmacokinetics/ Pharmacodynamics

http://farmbio.uu.se/research/researchgroups/tPKPD/
***Picture**, black and white only. PDF, EPS or high resolution TIFF.*

Our research aims to improve the understanding of drug distribution and elimination in relation to drug effects, pharmacokinetics and pharmacodynamics (PKPD), in health and disease. It strives to translate preclinical data into clinical reality and thereby optimizing drug discovery and promote efficient treatment of CNS diseases. In particular, this includes experimental and clinical studies of CNS active drugs and their transport to the brain by focusing on the role of the blood-brain barrier and intra-brain distribution.

To study the rate and extent of drug distribution to the brain, a holistic approach is used for the information acquired from advanced techniques for in vivo and in vitro experiments. The information is acquired by developing and utilizing advanced in vivo, in vitro and in silico techniques. Pharmacokinetic and pharmacodynamic principles are also applied to the clinical use of drugs for other diseases, in order to design rational dosage regimens. Our work emphasizes the importance of bridging the expertise within academia and pharmaceutical industry in order to seek excellence in method development for better therapeutics development.

Members of the group during 2012

Sven Björkman, PhD, Professor
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Jessica Dunhall, Laboratory Assistant
Ulrika Gillespie, M Sc, PhD Student (PhD March 2012)
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Publications 2010-2012

Alenius M, Hammarlund-Udenaes M, Hartvig P and Lindstrom L Knowledge and insight in relation to functional remission in patients with long-term psychotic disorders. *Soc Psychiatry Psychiatr Epidemiol* **45**:523-529 (2010).

Björkman S, Blanchette VS, Fischer K, Oh M, Spotts G, Schroth P, Fritsch S, Patrone L, Ewenstein BW for the ADVATE Clinical Program Group, and Collins PW: Comparative pharmacokinetics of plasma- and albumin-free recombinant factor VIII in children and adults: the influence of blood sampling schedule on observed age-related differences and implications for dose tailoring. *J. Thrombos. Haemostas.* **8:** 730-736 (2010).

Björkman S: Limited blood sampling for pharmacokinetic dose tailoring of FVIII in the prophylactic treatment of haemophilia A. *Haemophilia* **16**: 597-605 (2010).

Collins PW, Björkman S, Fischer K, Blanchette V, Oh M, Schroth P, Fritsch S, Casey K, Spotts G and Ewenstein BW: Factor VIII requirement to maintain a target plasma level in the prophylactic treatment of severe haemophilia A: influences of variance in pharmacokinetics and treatment regimens. *J. Thrombos. Haemostas.* **8**: 269-275 (2010).

Friden M, Ljungqvist H, Middleton B, Bredberg U and Hammarlund-Udenaes M. Improved measurement of drug exposure in the brain using drug-specific correction for residual blood. *J Cereb Blood Flow Metab* **30**:150-161 (2010).

Hammarlund-Udenaes M. Active-site concentrations of chemicals - are they a better predictor of effect than plasma/organ/tissue concentrations? *Basic Clin Pharmacol Toxicol* **106**:215-220 (2010).

Björkman S: Evaluation of the TCIWorks Bayesian computer program for estimation of individual pharmacokinetics of FVIII. *Haemophilia* **17:** e239-e240 (2011).

Fischer K, Collins P, Björkman S, Blanchette V, Oh M, Fritsch S, Schroth P, Spotts G and Ewenstein B: Trends in bleeding patterns during prophylaxis for severe haemophilia: observations from a series of prospective clinical trials. *Haemophilia* **17**: 433-438 (2011).

Fridén M, Bergström F, Wan H, Rehngren M, Ahlin G, Hammarlund-Udenaes M, and Bredberg U. Measurement of unbound drug exposure in brain: modeling of pH partitioning explains diverging results between the brain slice and brain homogenate methods. *Drug Metab Dispos.* **39**:353-62 (2011).

Sadiq MW, Salehpour M, Forsgard N, Possnert G, and Hammarlund-Udenaes M: Morphine brain pharmacokinetics at very low concentrations studied with accelerator mass spectrometry and liquid chromatography-tandem mass spectrometry. *Drug Metab Dispos.* **39**:174-9 (2011).

Sadiq MW, Borgs A, Okura T, Shimomura K, Kato S, Deguchi Y, Jansson B, Björkman S, Terasaki T, and Hammarlund-Udenaes M: Diphenhydramine active uptake at the blood-brain barrier and its interaction with oxycodone in vitro and in vivo. *J Pharm Sci.* **100**:3912-23 (2011).

Alassaad A, Gillespie U, Bertilsson M, Melhus H, and Hammarlund-Udenaes M: Prescription and transcription errors in multidose-dispensed medications on discharge from hospital: an observational and interventional study. *J Eval Clin Pract.* Dec 29. doi: 10.1111/j.1365-2753.2011.01798.x. [Epub ahead of print] (2012).

Björkman S, Oh M, Spotts G, Schroth P, Fritsch S, Ewenstein BM, Casey K, Fischer K, Blanchette VS and Collins PW: Population pharmacokinetics of recombinant factor VIII – the relationships of pharmacokinetics to age and

body weight. *Blood* **119**: 612-618 (2012).
Björkman S, and Åhlén V: Population pharmacokinetics of plasma-derived factor IX in adult patients with haemophilia B: implications for dosing in prophylaxis. *Eur J Clin Pharmacol* **68**: 969-977 (2012)
Gillespie U, Morlin C, Hammarlund-Udenaes M, Hedstrom M. Perceived value of ward-based pharmacists from the perspective of physicians and nurses. International journal of clinical pharmacy **34**:127-135 (2012).
Lindqvist A, Jansson B, Hammarlund-Udenaes M Quantitative analysis of the opioid peptide DAMGO in rat plasma and microdialysis samples using liquid chromatography-tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci **900**:11-17 (2012).
Lindqvist A, Rip J, Gaillard PJ, Bjorkman S, Hammarlund-Udenaes M. Enhanced Brain Delivery of the Opioid Peptide DAMGO in Glutathione PEGylated Liposomes: A Microdialysis Study. Molecular Pharmaceutics, epub ahead of print (2012).
Lindvall K, Astermark J, Björkman S, Ljung R, Carlsson KS, Persson S and Berntorp E: Daily dosing prophylaxis for haemophilia: a randomized crossover pilot study evaluating feasibility and efficacy. *Haemophilia* **18**: 855-859 (2012)
Stevens J, Ploeger BA, Hammarlund-Udenaes M, Osswald G, van der Graaf PH, Danhof M, de Lange EC. Mechanism-based PK-PD model for the prolactin biological system response following an acute dopamine inhibition challenge: quantitative extrapolation to humans. Journal of pharmacokinetics and pharmacodynamics **39**:463-477 (2012).

Reviews and book chapters 2010-2012

Björkman S and Berntorp E: Pharmacokinetics. In CA Lee, EE Berntorp and WK Hoots: Textbook of Hemophilia, 2nd Edition, Blackwell, Oxford, pp 104-110 (2010).
Syvanen S and Hammarlund-Udenaes M. Using PET studies of P-gp function to elucidate mechanisms underlying the disposition of drugs. *Curr Top Med Chem* **10**:1799-1809 (2010).
Collins PW, Fischer K, Morfini M, Blanchette VS and Björkman S: Implications of coagulation factor VIII and IX pharmacokinetics in the prophylactic treatment of haemophilia. *Haemophilia* **17**:2-10 (2011).
Björkman S: A commentary on the differences in pharmacokinetics between recombinant and plasma-derived factor IX and their implications for dosing. *Haemophilia* **17**:179-184 (2011).
Hammarlund-Udenaes M. Microdialysis in CNS PKPD research: Unraveling unbound concentrations. In M Müller: Microdialysis in Drug Development, Springer, pp X - y (2012).

Dissertations 2012

Ulrika Gillespie. Effects of Clinical Pharmacists' Interventions: on Drug-Related Hospitalisation and Appropriateness of Prescribing in Elderly Patients. Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy, ISSN 1651-6192; 154,
http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-167343
Muhammad Waqas Sadiq. In Vivo Active Drug Uptake and Efflux at the Blood-Brain Barrier: With Focus on Drug Transport Interactions. Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy, ISSN 1651-6192; 165
http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-180824

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Other commitments/assignments of staff members 2012

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, vice Chair of the Gordon Conference on Barriers of the CNS 2012.

Sven Björkman: Member of the International Haemophilia Prophylaxis Study Group Pharmacokinetics Expert Working Group. Member of the International Society for Thrombosis and Haemostasis Factor VIII/Factor IX Subcommittee; working parties on pharmacokinetics of Factor VIII and Factor IX in clinical practice. Visiting Sabbatical Professor, Department for Modeling and Simulation, Novartis, Basel CH.

Projects

Concepts and method development regarding drug delivery to the brain

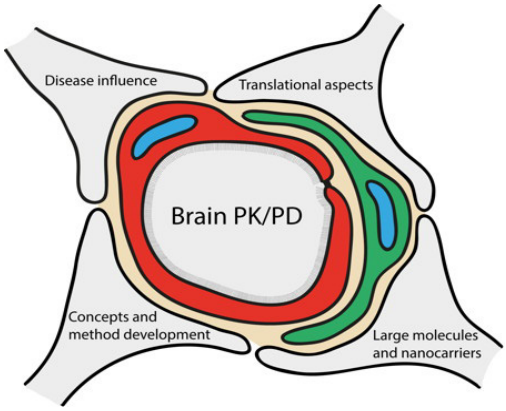


Figure: Research interests of the translational PKPD group, depicted in the neurovascular unit of the brain with an endothelial cell comprising the blood-brain barrier, a pericyte and astrocyte end feet.

Margareta Hammarlund-Udenaes, Irena Loryan, Annika Lindqvist, Xiaomei Chen in collaboration with prof. David Smith (University of Michigan), Drs Vikash Sinha, Achiel van Peer, Claire Mackie and Donald Heald (Janssen Pharmaceuticals).

The blood-brain barrier (BBB) is a functional and dynamic interface between blood and brain. It protects the brain but is also the major hindrance for the successful treatment of CNS disorders. Accumulating evidence indicates that the BBB and the neurovascular unit as a whole play an important role in disease etiology

and progression and may as such affect drug delivery to the brain. Our overall goal is to understand and develop the general principles of brain drug delivery in relation to functionality of the neurovascular unit.

The multidisciplinary approach entails development of advanced methodology for quantitative evaluation of the transport of small and large molecules across the BBB. The strategy is based on combination of high-throughput in vitro techniques, such as equilibrium dialysis and brain slices, with specialized in vivo pharmacokinetic studies using advance methods in particular microdialysis. Novel bioanalytical methods (i.e. LC-MS/MS) for determination of studied compounds are developed and validated, as well as advanced models for data analysis. Neuropharmacokinetic parameters such as unbound brain-to-plasma concentration ratio (Kp,uu,brain), unbound volume of distribution in brain (Vu,brain), permeability clearance into the brain (CLin), identified by means of systematic PK-PD analysis, are descriptors of BBB function and intracerebral distribution developed by the group.

The pharmacokinetic estimates are also used for mechanistic understanding of BBB transport systems particularly for verification of the quantitative role of the transporters, such as P-glycoprotein (P-gp), breast cancer resistance protein (BCRP), peptide transporter 2 (PEPT2), and their potential contribution to drug delivery to the brain. Moreover, transgenic and humanized preclinical models are used for evaluation of species differences in function of BBB transport systems and their influence on brain PK.

The developed holistic methodology is presently used as a tool for selection of candidate drugs in early drug discovery. Furthermore, projects focused on identification of desirable physicochemical properties for CNS penetration are aiming to facilitate discovery and development of novel neurotherapeutics. As a final point, integration of overall findings is directed towards development of physiologically based mathematical models of BBB transport.

Translational aspects in health and disease

Margareta Hammarlund-Udenaes, Sofia Gustafsson, Nebojsa Mihajlica in collaboration with Prof. Christer Betsholtz (Dept of Immunology, Genetics and Pathology, Uppsala University) and Dr. Stina Syvänen (Dept of Public Health and Caring Sciences).

Translational pharmacokinetics is the science of accurate conversion of pharmacokinetic data from experimental to clinical settings and constitutes a major issue in the development of new CNS pharmaceuticals. The interpretation and translation of neuropharmacokinetic data might be even more challenging during disease conditions, where disrupted integrity and function of the blood-brain barrier (BBB) is apparent. Recent findings even point towards dysfunctional BBB as being the cause of disease etiology and progression. As a result, dysfunction in BBB processes might result in altered brain pharmacokinetic profiles of CNS drugs as well as drugs acting in the periphery which normally have a very low brain penetrance. 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 drug distribution in the brain. Results from microdialysis and non-invasive imaging techniques will be integrated and used to address the current issues.

Biomolecular drugs and nanocarriers

Margareta Hammarlund-Udenaes, Annika Lindqvist, Thomas Nässtrom and Sven Björkman.

Neurological disorders such as Alzheimer's disease and stroke are becoming more common in our society, and therefore effective treatments are greatly

needed. 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, are crucial in order to succeed in the clinic. There are various innovative strategies to enhance the drug delivery to the brain. However, little is known about their mechanistic and therapeutic contribution. Emerging technologies like nanocarriers can facilitate the use of these large molecules as therapeutics agents. Our research focuses on the pharmacokinetics and quantitative benefits of using nanocarrier systems and their effect on the pharmacodynamic outcome. In vivo preclinical studies are preformed to estimate the modulation of penetration across the blood-brain barrier using targeted liposomes. For authentication of the principles, physiological based pharmacokinetics population modeling is applied.

Clinical pharmacokinetics of coagulation factors VIII and IX

Sven Björkman, with Erik Berntorp, Jan Astermark, Karin Lindvall (Malmö), Peter Collins (Cardiff), Kathelijn Fischer (Utrecht), and Victor Blanchette (Toronto).

The aim of the project is to optimize the prophylactic treatment of haemophilia with coagulation factors VIII and IX by the use of individually tailored dosing. Optimizing the dosing by means of clinical pharmacokinetic (PK) principles can potentially yield important benefits both from a purely medical as well as from an economical point of view. The project started in 1989 and has resulted in widespread international understanding of the importance and uses of PK in this particular field of disease management. The activity during 2012 included:

- Designing and applying limited blood sampling schedules for the dose tailoring of factor VIII and factor IX in clinical practice, with evaluation of computer software for Bayesian PK analysis.
- Evaluating the PK differences between various types of factor IX preparations and creating a population PK model for recombinant factor IX.
- Evaluating the PK differences between different coagulation factors in a physiological context.
- Disseminating knowledge of PK dose tailoring to physicians, at national and international meetings and courses and through writing of reviews and commentaries.

Clinical Pharmacy Research

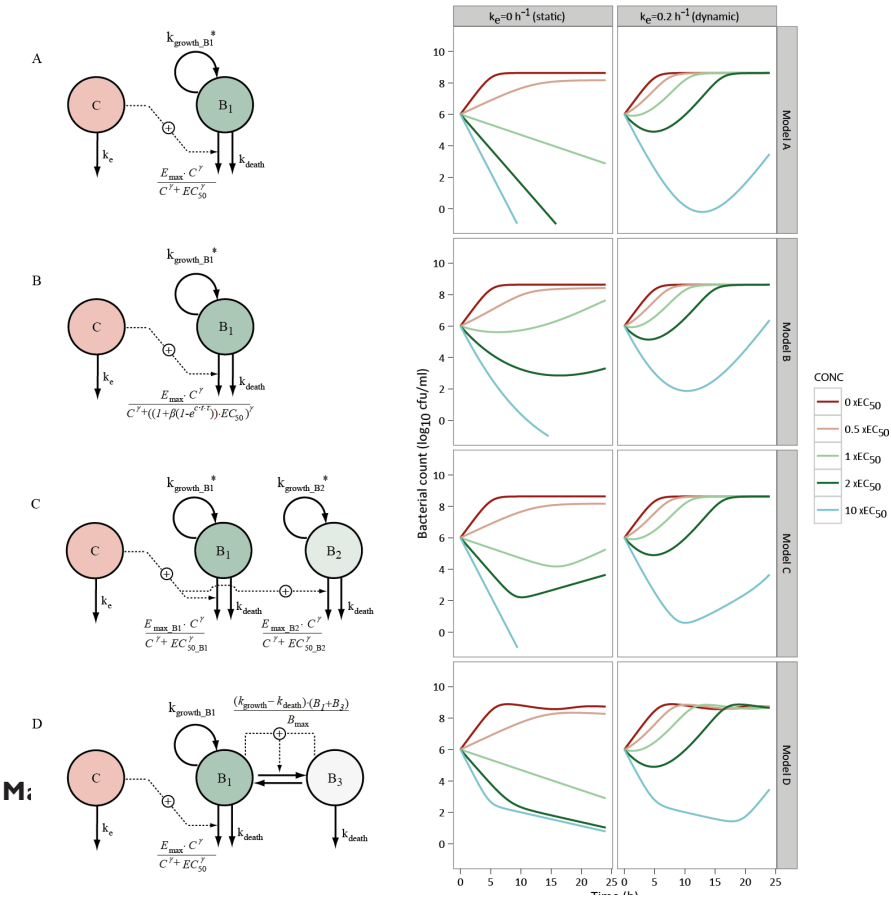
Margareta Hammarlund-Udenaes in collaboration with Anna Alassaad (PhD student), Prof Håkan Melhus (Clinical Pharmacology, Uppsala), Drs Claes Mörlin (Medicine, Uppsala) and Ulrika Gillespie (Akademiska Hospital, Uppsala).

We are interested in measuring the results of clinical pharmacist interventions in acute medical care, with a specific focus on readmissions of patient 80 years and older. The purpose of this research is to see if and if so, how 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 this area of work for pharmacists. A seminal paper was published in 2009 in Arch Intern Med, which received much attention in Sweden. Here we showed that clinical pharmacist intervention saved money and decreased the number of readmissions to hospital.

Pharmacometrics

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

Mats Karlsson



Schematic illustrations of four Pharmacokinetic-Pharmacodynamic (PKPD) model structures used to characterize antibacterial drug effects and emergence of resistance. To the right, model predictions of the change in bacterial counts over time following either static or dynamic drug exposure are given for each model.

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 2012

Mats O Karlsson, Professor
Lena Friberg, Senior Lecturer, Docent
Anders Grahnén, Adjunct Professor
Andrew Hooker, Senior Lecturer
Niclas Jonsson, Adjunct Professor, Docent
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Siv Jönsson, Researcher
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Ron Keizer, Post-doctoral fellow
Akash Khandelwal, Post-doctoral Fellow
Elke Krekels, Post-doctoral fellow
Ronald Niebecker, Post-doctoral fellow
Mirjam Trame, Post-doctoral Fellow
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Anders Burild, Visiting PhD student
Kanji Komatsu, Visiting Scientist
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Etienne Pigeolot, Visiting Scientist
Trine Rose, Visiting PhD student
Alexandre Sostelly, Visiting PhD student
Coen van Hasselt, Visiting PhD student
Marie Berglund, stipend
Ari Brekkan Viggosson, stipend
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Jesmin Permal, stipend

Publications 2010-12

Bizzotto, R., Zamuner, S., De Nicolao, G., Karlsson, M. O. & Gomeni, R. Multinomial logistic estimation of Markov-chain models for modeling sleep architecture in primary insomnia patients. J Pharmacokinet Pharmacodyn 37, 137-155, doi:10.1007/s10928-009-9148-2 (2010).

Björnsson, M. A., Norberg, A., Kalman, S., Karlsson, M. O. & Simonsson, U. S. A two-compartment effect site model describes the bispectral index after different rates of propofol infusion. J Pharmacokinet Pharmacodyn 37, 243-255, doi:10.1007/s10928-010-9157-1 (2010).

Dahl, S. G., Aarons, L., Gundert-Remy, U., Karlsson, M. O., Schneider, Y. J., Steimer, J. L. & Troconiz, I. F. Incorporating physiological and biochemical mechanisms into pharmacokinetic-pharmacodynamic models: a conceptual framework. Basic Clin Pharmacol Toxicol 106, 2-12, doi:10.1111/j.1742-7843.2009.00456.x (2010).

Delattre, I. K., Musuamba, F. T., Nyberg, J., Taccone, F. S., Laterre, P. F., Verbeeck, R. K., Jacobs, F. & Wallemacq, P. E. Population pharmacokinetic modeling and optimal sampling strategy for Bayesian estimation of amikacin exposure in critically ill septic patients. Ther Drug Monit 32, 749-756, doi:10.1097/FTD.0b013e3181f675c2 (2010).

Elsherbiny, D., Ren, Y., McIlleron, H., Maartens, G. & Simonsson, U. S. Population pharmacokinetics of lopinavir in combination with rifampicin based antitubercular treatment in HIV-infected South African children. Eur J Clin Pharmacol 66, 1017-1023, doi:10.1007/s00228-010-0847-9 (2010).

Ernest, C. S., 2nd, Hooker, A. C. & Karlsson, M. O. Methodological comparison of in vitro binding parameter estimation: sequential vs. simultaneous non-linear regression. Pharm Res 27, 866-877, doi:10.1007/s11095-010-0082-1 (2010).

Fanta, S., Jönsson, S., Karlsson, M. O., Niemi, M., Holmberg, C., Hoppu, K. & Backman, J. T. Long-term changes in cyclosporine pharmacokinetics

after renal transplantation in children: evidence for saturable presystemic metabolism and effect of NR1I2 polymorphism. J Clin Pharmacol 50, 581-597, doi:10.1177/0091270009348223 (2010).

Friberg, L. E., Sandstrom, M. & Karlsson, M. O. Scaling the time-course of myelosuppression from rats to patients with a semi-physiological model. Invest New Drugs 28, 744-753, doi:10.1007/s10637-009-9308-7 (2010).

Gupta, P., Friberg, L. E., Karlsson, M. O., Krishnaswami, S. & French, J. A semi-mechanistic model of CP-690,550-induced reduction in neutrophil counts in patients with rheumatoid arthritis. J Clin Pharmacol 50, 679-687, doi:10.1177/0091270009346060 (2010).

Hamberg, A. K., Wadelius, M., Lindh, J. D., Dahl, M. L., Padrini, R., Deloukas, P., Rane, A. & Jonsson, E. N. A pharmacometric model describing the relationship between warfarin dose and INR response with respect to variations in CYP2C9, VKORC1, and age. Clin Pharmacol Ther 87, 727-734, doi:10.1038/clpt.2010.37 (2010).

Hansson, E. K., Wallin, J. E., Lindman, H., Sandstrom, M., Karlsson, M. O. & Friberg, L. E. Limited inter-occasion variability in relation to inter-individual variability in chemotherapy-induced myelosuppression. Cancer Chemother Pharmacol 65, 839-848, doi:10.1007/s00280-009-1089-3 (2010).

Karlsson, K. E., Wilkins, J. J., Jonsson, F., Zingmark, P. H., Karlsson, M. O. & Jonsson, E. N. Modeling disease progression in acute stroke using clinical assessment scales. Aaps J 12, 683-691, doi:10.1208/s12248-010-9230-0 (2010).

Keizer, R. J., Gupta, A., Karlsson, M. O., Wanders, J., Jansen, M., Beijnen, J. H., Schellens, J. H. M. & Huitema, A. D. R. Evaluation of management of hypertension in response to the receptor tyrosine kinase inhibitor, E7080: a modeling and simulation approach. Ejc Suppl 8, 144-144 (2010).

Keizer, R. J., Gupta, A., Mac Gillavry, M. R., Jansen, M., Wanders, J., Beijnen, J. H., Schellens, J. H., Karlsson, M. O. & Huitema, A. D. A model of hypertension and proteinuria in cancer patients treated with the anti-angiogenic drug E7080. J Pharmacokinet Pharmacodyn 37, 347-363, doi:10.1007/s10928-010-9164-2 (2010).

Kumpulainen, E., Valitalo, P., Kokki, M., Lehtonen, M., Hooker, A., Ranta, V. P. & Kokki, H. Plasma and cerebrospinal fluid pharmacokinetics of flurbiprofen in children. Br J Clin Pharmacol 70, 557-566, doi:10.1111/j.1365-2125.2010.03720.x (2010).

Ma, G., Friberg, L. E., Movin-Osswald, G. & Karlsson, M. O. Comparison of the agonist-antagonist interaction model and the pool model for the effect of remoxipride on prolactin. Br J Clin Pharmacol 70, 815-824, doi:10.1111/j.1365-2125.2010.03758.x (2010).

Maloney, A., Schaddelee, M., Freijer, J., Krauwinkel, W., van Gelderen, M., Jacqmin, P. & Simonsson, U. S. An example of optimal phase II design for exposure response modelling. J Pharmacokinet Pharmacodyn 37, 475-491, doi:10.1007/s10928-010-9168-y (2010).

Petersson, K. J., Friberg, L. E. & Karlsson, M. O. Transforming parts of a differential equations system to difference equations as a method for run-time savings in NONMEM. J Pharmacokinet Pharmacodyn 37, 493-506, doi:10.1007/s10928-010-9169-x (2010).

Plan, E. L., Karlsson, K. E. & Karlsson, M. O. Approaches to simultaneous analysis of frequency and severity of symptoms. Clin Pharmacol Ther 88, 255-259, doi:10.1038/clpt.2010.118 (2010).

Ribbing, J., Hamren, B., Svensson, M. K. & Karlsson, M. O. A model for glucose, insulin, and beta-cell dynamics in subjects with insulin resistance

and patients with type 2 diabetes. J Clin Pharmacol 50, 861-872, doi:10.1177/0091270009349711 (2010).

Silber, H. E., Frey, N. & Karlsson, M. O. An integrated glucose-insulin model to describe oral glucose tolerance test data in healthy volunteers. J Clin Pharmacol 50, 246-256, doi:10.1177/0091270009341185 (2010).

Silber, H. E., Jauslin, P. M., Frey, N. & Karlsson, M. O. An integrated model for the glucose-insulin system. Basic Clin Pharmacol Toxicol 106, 189-194, doi:10.1111/j.1742-7843.2009.00510.x (2010).

Steimer, J. L., Dahl, S. G., De Alwis, D. P., Gundert-Remy, U., Karlsson, M. O., Martinkova, J., Aarons, L., Ahr, H. J., Clairambault, J., Freyer, G., Friberg, L. E., Kern, S. E., Kopp-Schneider, A., Ludwig, W. D., De Nicolao, G., Rocchetti, M. & Troconiz, I. F. Modelling the genesis and treatment of cancer: the potential role of physiologically based pharmacodynamics. Eur J Cancer 46, 21-32, doi:10.1016/j.ejca.2009.10.011 (2010).

Wallin, J. E., Friberg, L. E. & Karlsson, M. O. Model-based neutrophil-guided dose adaptation in chemotherapy: evaluation of predicted outcome with different types and amounts of information. Basic Clin Pharmacol Toxicol 106, 234-242, doi:10.1111/j.1742-7843.2009.00520.x (2010).'

van der Bol, J. M., Mathijssen, R. H., Creemers, G. J., Planting, A. S., Loos, W. J., Wiemer, E. A., Friberg, L. E., Verweij, J., Sparreboom, A. & de Jong, F. A. A CYP3A4 phenotype-based dosing algorithm for individualized treatment of irinotecan. Clin Cancer Res 16, 736-742, doi:10.1158/1078-0432.CCR-09-1526 (2010).

Wilmshurst, J. M., van der Walt, J. S., Ackermann, S., Karlsson, M. O. & Blockman, M. Rescue therapy with high-dose oral phenobarbitone loading for refractory status epilepticus. J Paediatr Child Health 46, 17-22, doi:10.1111/j.1440-1754.2009.01611.x (2010).

Zamuner, S., Di Iorio, V. L., Nyberg, J., Gunn, R. N., Cunningham, V. J., Gomeni, R. & Hooker, A. C. Adaptive-optimal design in PET occupancy studies. Clin Pharmacol Ther 87, 563-571, doi:10.1038/clpt.2010.9 (2010).

Zandvliet, A. S., Karlsson, M. O., Schellens, J. H., Copalu, W., Beijnen, J. H. & Huitema, A. D. Two-stage model-based clinical trial design to optimize phase I development of novel anticancer agents. Invest New Drugs 28, 61-75, doi:10.1007/s10637-008-9216-2 (2010).

Zvada, S. P., Van Der Walt, J. S., Smith, P. J., Fourie, P. B., Roscigno, G., Mitchison, D., Simonsson, U. S. & McIlleron, H. M. Effects of four different meal types on the population pharmacokinetics of single-dose rifapentine in healthy male volunteers. Antimicrob Agents Chemother 54, 3390-3394, doi:10.1128/AAC.00345-10 (2010).

Baverel, P. G., Savic, R. M. & Karlsson, M. O. Two bootstrapping routines for obtaining imprecision estimates for nonparametric parameter distributions in nonlinear mixed effects models. J Pharmacokinet Pharmacodyn 38, 63-82, doi:10.1007/s10928-010-9177-x (2011).

Bergmann, T. K., Brasch-Andersen, C., Green, H., Mirza, M., Pedersen, R. S., Nielsen, F., Skougaard, K., Wihl, J., Keldsen, N., Damkier, P., Friberg, L. E., Peterson, C., Vach, W., Karlsson, M. O. & Brosen, K. Impact of CYP2C8*3 on paclitaxel clearance: a population pharmacokinetic and pharmacogenomic

study in 93 patients with ovarian cancer. Pharmacogenomics J 11, 113-120, doi:10.1038/tpj.2010.19 (2011).

Bergstrand, M., Hooker, A. C., Wallin, J. E. & Karlsson, M. O. Prediction-corrected visual predictive checks for diagnosing nonlinear mixed-effects models. Aaps J 13, 143-151, doi:10.1208/s12248-011-9255-z (2011).

Bizzotto, R., Zamuner, S., Mezzalana, E., De Nicolao, G., Gomeni, R., Hooker, A. C. & Karlsson, M. O. Multinomial logistic functions in markov chain models of sleep architecture: internal and external validation and covariate analysis. Aaps J 13, 445-463, doi:10.1208/s12248-011-9287-4 (2011).

Björnsson, M. A. & Simonsson, U. S. Modelling of pain intensity and informative dropout in a dental pain model after naproxcinod, naproxen and placebo administration. Br J Clin Pharmacol 71, 899-906, doi:10.1111/j.1365-2125.2011.03924.x (2011).

Bogason, A., Quartino, A. L., Lafolie, P., Masquelier, M., Karlsson, M. O., Paul, C., Gruber, A. & Vitols, S. Inverse relationship between leukaemic cell burden and plasma concentrations of daunorubicin in patients with acute myeloid leukaemia. Br J Clin Pharmacol 71, 514-521, doi:10.1111/j.1365-2125.2010.03894.x (2011).

Fransson, M. N., Green, H., Litton, J. E. & Friberg, L. E. Influence of Cremophor EL and genetic polymorphisms on the pharmacokinetics of paclitaxel and its metabolites using a mechanism-based model. Drug Metab Dispos 39, 247-255, doi:10.1124/dmd.110.035394 (2011).

Gennemark, P., Danis, A., Nyberg, J., Hooker, A. C. & Tucker, W. Optimal design in population kinetic experiments by set-valued methods. Aaps J 13, 495-507, doi:10.1208/s12248-011-9291-8 (2011).

Jauslin, P. M., Frey, N. & Karlsson, M. O. Modeling of 24-hour glucose and insulin profiles of patients with type 2 diabetes. J Clin Pharmacol 51, 153-164, doi:10.1177/0091270010362536 (2011).

Jauslin, P. M., Karlsson, M. O. & Frey, N. Identification of the Mechanism of Action of a Glucokinase Activator From Oral Glucose Tolerance Test Data in Type 2 Diabetic Patients Based on an Integrated Glucose-Insulin Model. J Clin Pharmacol, doi:10.1177/0091270011422231 (2011).

Johansson, Å. M., Hill, N., Perisoglou, M., Whelan, J., Karlsson, M. O. & Standing, J. F. A population pharmacokinetic/pharmacodynamic model of methotrexate and mucositis scores in osteosarcoma. Ther Drug Monit 33, 711-718, doi:10.1097/FTD.0b013e31823615e1 (2011).

Jönsson, S., Davidse, A., Wilkins, J., Van der Walt, J. S., Simonsson, U. S., Karlsson, M. O., Smith, P. & McIlleron, H. Population pharmacokinetics of ethambutol in South African tuberculosis patients. Antimicrob Agents Chemother 55, 4230-4237, doi:10.1128/AAC.00274-11 (2011).

Karlsson, K. E., Plan, E. L. & Karlsson, M. O. Performance of three estimation methods in repeated time-to-event modeling. Aaps J 13, 83-91, doi:10.1208/s12248-010-9248-3 (2011).

Khandelwal, A., Harling, K., Jonsson, E. N., Hooker, A. C. & Karlsson, M. O. A fast method for testing covariates in population PK/PD Models. Aaps J 13, 464-472, doi:10.1208/s12248-011-9289-2 (2011).

Kivikko, M., Sundberg, S., Karlsson, M. O., Pohjanjousi, P. & Colucci, W. S. Acetylation status does not affect levosimendan's hemodynamic effects in

heart failure patients. Scand Cardiovasc J 45, 86-90, doi:10.3109/14017431.2010.540762 (2011).

Kjellsson, M. C., Ouellet, D., Corrigan, B. & Karlsson, M. O. Modeling sleep data for a new drug in development using markov mixed-effects models. Pharm Res 28, 2610-2627, doi:10.1007/s11095-011-0490-x (2011).

Nielsen, E. I., Cars, O. & Friberg, L. E. Pharmacokinetic/pharmacodynamic (PK/PD) indices of antibiotics predicted by a semimechanistic PKPD model: a step toward model-based dose optimization. Antimicrob Agents Chemother 55, 4619-4630, doi:10.1128/AAC.00182-11 (2011).

Nielsen, E. I., Cars, O. & Friberg, L. E. Predicting in vitro antibacterial efficacy across experimental designs with a semimechanistic pharmacokinetic-pharmacodynamic model. Antimicrob Agents Chemother 55, 1571-1579, doi:10.1128/AAC.01286-10 (2011).

Plan, E. L., Ma, G., Nagard, M., Jensen, J. & Karlsson, M. O. Transient lower esophageal sphincter relaxation pharmacokinetic-pharmacodynamic modeling: count model and repeated time-to-event model. J Pharmacol Exp Ther 339, 878-885, doi:10.1124/jpet.111.181636 (2011).

Roshammar, D., Simonsson, U. S., Ekvall, H., Flamholc, L., Ormaasen, V., Vesterbacka, J., Wallmark, E., Ashton, M. & Gisslen, M. Non-linear mixed effects modeling of antiretroviral drug response after administration of lopinavir, atazanavir and efavirenz containing regimens to treatment-naive HIV-1 infected patients. J Pharmacokinet Pharmacodyn 38, 727-742, doi:10.1007/s10928-011-9217-1 (2011).

Sjogren, E., Nyberg, J., Magnusson, M. O., Lennernas, H., Hooker, A. & Bredberg, U. Optimal experimental design for assessment of enzyme kinetics in a drug discovery screening environment. Drug Metab Dispos 39, 858-863, doi:10.1124/dmd.110.037309 (2011).

Soto, E., Keizer, R. J., Troconiz, I. F., Huitema, A. D., Beijnen, J. H., Schellens, J. H., Wanders, J., Cendros, J. M., Obach, R., Peraire, C., Friberg, L. E. & Karlsson, M. O. Predictive ability of a semi-mechanistic model for neutropenia in the development of novel anti-cancer agents: two case studies. Invest New Drugs 29, 984-995, doi:10.1007/s10637-010-9437-z (2011).

Trame, M. N., Bergstrand, M., Karlsson, M. O., Boos, J. & Hempel, G. Population pharmacokinetics of busulfan in children: increased evidence for body surface area and allometric body weight dosing of busulfan in children. Clin Cancer Res 17, 6867-6877, doi:10.1158/1078-0432.CCR-11-0074 (2011).

Wallin, J. E., Bergstrand, M., Wilczek, H. E., Nydert, P. S., Karlsson, M. O. & Staatz, C. E. Population pharmacokinetics of tacrolimus in pediatric liver transplantation: early posttransplantation clearance. Ther Drug Monit 33, 663-672, doi:10.1097/FTD.0b013e31823415cc (2011).

Westin, J., Nyholm, D., Palhagen, S., Willows, T., Groth, T., Dougherty, M. & Karlsson, M. O. A pharmacokinetic-pharmacodynamic model for duodenal levodopa infusion. Clin Neuropharmacol 34, 61-65, doi:10.1097/WNF.0b013e31820b570a (2011).

Wilkins, J. J., Langdon, G., McIlleron, H., Pillai, G., Smith, P. J. & Simonsson, U. S. Variability in the population pharmacokinetics of isoniazid in South

African tuberculosis patients. Br J Clin Pharmacol 72, 51-62, doi:10.1111/j.1365-2125.2011.03940.x (2011).

Ahn, J. E., Plan, E. L., Karlsson, M. O. & Miller, R. Modeling longitudinal daily seizure frequency data from pregabalin add-on treatment. J Clin Pharmacol 52, 880-892, doi:10.1177/0091270011407193 (2012).

Alskar, O., Korell, J. & Duffull, S. B. A pharmacokinetic model for the glycation of albumin. J Pharmacokinet Pharmacodyn 39, 273-282, doi:10.1007/s10928-012-9249-1 (2012).

Bender, B. C., Schaedeli-Stark, F., Koch, R., Joshi, A., Chu, Y. W., Rugo, H., Krop, I. E., Girish, S., Friberg, L. E. & Gupta, M. A population pharmacokinetic/pharmacodynamic model of thrombocytopenia characterizing the effect of trastuzumab emtansine (T-DM1) on platelet counts in patients with HER2-positive metastatic breast cancer. Cancer Chemother Pharmacol 70, 591-601, doi:10.1007/s00280-012-1934-7 (2012).

Bergstrand, M., Soderlind, E., Eriksson, U. G., Weitschies, W. & Karlsson, M. O. A semi-mechanistic modeling strategy to link in vitro and in vivo drug release for modified release formulations. Pharm Res 29, 695-706, doi:10.1007/s11095-011-0594-3 (2012).

Bergstrand, M., Soderlind, E., Eriksson, U. G., Weitschies, W. & Karlsson, M. O. A semi-mechanistic modeling strategy for characterization of regional absorption properties and prospective prediction of plasma concentrations following administration of new modified release formulations. Pharm Res 29, 574-584, doi:10.1007/s11095-011-0595-2 (2012).

de Graan, A. J., Lancaster, C. S., Obaidat, A., Hagenbuch, B., Elens, L., Friberg, L. E., de Bruijn, P., Hu, S., Gibson, A. A., Bruun, G. H., Corydon, T. J., Mikkelsen, T. S., Walker, A. L., Du, G., Loos, W. J., van Schaik, R. H., Baker, S. D., Mathijssen, R. H. & Sparreboom, A. Influence of polymorphic OATP1B-type carriers on the disposition of docetaxel. Clin Cancer Res 18, 4433-4440, doi:10.1158/1078-0432.CCR-12-0761 (2012).

de Graan, A. J., Loos, W. J., Friberg, L. E., Baker, S. D., van der Bol, J. M., van Doorn, L., Wiemer, E. A., van der Holt, B., Verweij, J. & Mathijssen, R. H. Influence of smoking on the pharmacokinetics and toxicity profiles of taxane therapy. Clin Cancer Res 18, 4425-4432, doi:10.1158/1078-0432.CCR-12-0728 (2012).

Delattre, M., Savic, R. M., Miller, R., Karlsson, M. O. & Lavielle, M. Analysis of exposure-response of CI-945 in patients with epilepsy: application of novel mixed hidden Markov modeling methodology. J Pharmacokinet Pharmacodyn 39, 263-271, doi:10.1007/s10928-012-9248-2 (2012).

Eechoute, K., Fransson, M. N., Reyners, A. K., de Jong, F. A., Sparreboom, A., van der Graaf, W. T., Friberg, L. E., Schiavon, G., Wiemer, E. A., Verweij, J., Loos, W. J., Mathijssen, R. H. & De Giorgi, U. A long-term prospective population pharmacokinetic study on imatinib plasma concentrations in GIST patients. Clin Cancer Res 18, 5780-5787, doi:10.1158/1078-0432.CCR-12-0490 (2012).

Friberg, L. E., Ravva, P., Karlsson, M. O. & Liu, P. Integrated population pharmacokinetic analysis of voriconazole in children, adolescents, and adults. Antimicrob Agents Chemother 56, 3032-3042, doi:10.1128/AAC.05761-11 (2012).

Hamren, B., Ohman, K. P., Svensson, M. K. & Karlsson, M. O. Pharmacokinetic-pharmacodynamic assessment of the interrelationships between tesaglitazar exposure and renal function in patients with type 2 diabetes mellitus. J Clin Pharmacol 52, 1317-1327, doi:10.1177/0091270011416937 (2012).

Hansson, E. K. & Friberg, L. E. The shape of the myelosuppression time profile is related to the probability of developing neutropenic fever in patients with

docetaxel-induced grade IV neutropenia. Cancer Chemother Pharmacol 69, 881-890, doi:10.1007/s00280-011-1769-7 (2012).

Henin, E., Bergstrand, M., Standing, J. F. & Karlsson, M. O. A mechanism-based approach for absorption modeling: the Gastro-Intestinal Transit Time (GITT) model. Aaps J 14, 155-163, doi:10.1208/s12248-012-9324-y (2012).

Hennig, S., Nyberg, J., Fanta, S., Backman, J. T., Hoppu, K., Hooker, A. C. & Karlsson, M. O. Application of the optimal design approach to improve a pretransplant drug dose finding design for ciclosporin. J Clin Pharmacol 52, 347-360, doi:10.1177/0091270010397731 (2012).

Jauslin, P. M., Karlsson, M. O. & Frey, N. Identification of the mechanism of action of a glucokinase activator from oral glucose tolerance test data in type 2 diabetic patients based on an integrated glucose-insulin model. J Clin Pharmacol 52, 1861-1871, doi:10.1177/0091270011422231 (2012).

Kagedal, M., Cselenyi, Z., Nyberg, S., Jonsson, S., Raboisson, P., Stenkrona, P., Hooker, A. C. & Karlsson, M. O. Non-linear mixed effects modelling of positron emission tomography data for simultaneous estimation of radioligand kinetics and occupancy in healthy volunteers. Neuroimage 61, 849-856, doi:10.1016/j.neuroimage.2012.02.085 (2012).

Kang, D., Bae, K. S., Houk, B. E., Savic, R. M. & Karlsson, M. O. Standard Error of Empirical Bayes Estimate in NONMEM(R) VI. Korean J Physiol Pharmacol 16, 97-106, doi:10.4196/kjpp.2012.16.2.97 (2012).

Karlsson, M. O. & Bergstrand, M. Letter to the editor regarding: "A reduction in between subject variability is not mandatory for selecting a new covariate". J Pharmacokinet Pharmacodyn 39, 725-726, doi:10.1007/s10928-012-9273-1 (2012).

Kjellsson, M. C., Via, L. E., Goh, A., Weiner, D., Low, K. M., Kern, S., Pillai, G., Barry, C. E., 3rd & Dartois, V. Pharmacokinetic evaluation of the penetration of antituberculosis agents in rabbit pulmonary lesions. Antimicrob Agents Chemother 56, 446-457, doi:10.1128/AAC.05208-11 (2012).

Krogh-Madsen, M., Bender, B., Jensen, M. K., Nielsen, O. J., Friberg, L. E. & Honore, P. H. Population pharmacokinetics of cytarabine, etoposide, and daunorubicin in the treatment for acute myeloid leukemia. Cancer Chemother Pharmacol 69, 1155-1163, doi:10.1007/s00280-011-1800-z (2012).

Lacroix, B. D., Friberg, L. E. & Karlsson, M. O. Evaluation of IPPSE, analternative method for sequential population PKPD analysis. J Pharmacokinet Pharmacodyn 39, 177-193, doi:10.1007/s10928-012-9240-x (2012).

Lledo-Garcia, R., Hennig, S., Nyberg, J., Hooker, A. C. & Karlsson, M. O. Ethically attractive dose-finding designs for drugs with a narrow therapeutic index. J Clin Pharmacol 52, 29-38, doi:10.1177/0091270010390041 (2012).

Lledo-Garcia, R., Kalicki, R. M., Uehlinger, D. E. & Karlsson, M. O. Modeling of red blood cell life-spans in hematologically normal populations. J Pharmacokinet Pharmacodyn 39, 453-462, doi:10.1007/s10928-012-9261-5 (2012).

Mohamed, A. F., Karaiskos, I., Plachouras, D., Karvanen, M., Pontikis, K., Jansson, B., Papadomichelakis, E., Antoniadou, A., Giamarellou, H., Armaganidis, A., Cars, O. & Friberg, L. E. Application of a loading dose of colistin methanesulfonate in critically ill patients: population pharmacokinetics, protein binding, and prediction of bacterial kill.

Antimicrob Agents Chemother 56, 4241-4249, doi:10.1128/AAC.06426-11 (2012).

Mohamed, A. F., Nielsen, E. I., Cars, O. & Friberg, L. E. Pharmacokinetic-pharmacodynamic model for gentamicin and its adaptive resistance with predictions of dosing schedules in newborn infants. Antimicrob Agents Chemother 56, 179-188, doi:10.1128/AAC.00694-11 (2012).

Nyberg, J., Hoglund, R., Bergstrand, M., Karlsson, M. O. & Hooker, A. C. Serial correlation in optimal design for nonlinear mixed effects models. J Pharmacokinet Pharmacodyn 39, 239-249, doi:10.1007/s10928-012-9245-5 (2012).

Nyberg, J., Ueckert, S., Stromberg, E. A., Hennig, S., Karlsson, M. O. & Hooker, A. C. PopED: an extended, parallelized, nonlinear mixed effects models optimal design tool. Comput Methods Programs Biomed 108, 789-805, doi:10.1016/j.cmpb.2012.05.005 (2012).

Pilla Reddy, V., Petersson, K. J., Suleiman, A. A., Vermeulen, A., Proost, J.-H. & Friberg, L. E. Pharmacokinetic–Pharmacodynamic Modeling of Severity Levels of Extrapyramidal Side Effects With Markov Elements. CPT: Pharmacomet Syst Pharmacol 1, doi:doi:10.1038/psp.2012.9 (2012).

Plan, E. L., Elshoff, J. P., Stockis, A., Sargentini-Maier, M. L. & Karlsson, M. O. Likert pain score modeling: a Markov integer model and an autoregressive continuous model. Clin Pharmacol Ther 91, 820-828, doi:10.1038/clpt.2011.301 (2012).

Plan, E. L., Maloney, A., Mentre, F., Karlsson, M. O. & Bertrand, J. Performance comparison of various maximum likelihood nonlinear mixed-effects estimation methods for dose-response models. Aaps J 14, 420-432, doi:10.1208/s12248-012-9349-2 (2012).

Quartino, A. L., Friberg, L. E. & Karlsson, M. O. A simultaneous analysis of the time-course of leukocytes and neutrophils following docetaxel administration using a semi-mechanistic myelosuppression model. Invest New Drugs 30, 833-845, doi:10.1007/s10637-010-9603-3 (2012).

Russu, A., Marostica, E., De Nicolao, G., Hooker, A. C., Poggesi, I., Gomeni, R. & Zamuner, S. Joint modeling of efficacy, dropout, and tolerability in flexible-dose trials: a case study in depression. Clin Pharmacol Ther 91, 863-871, doi:10.1038/clpt.2011.322 (2012).

Sauermann, R., Feurstein, T., Karch, R., Kjellsson, M. C., Jager, W., Bohmdorfer, M., Puspok, A., Langenberger, H., Wild, T., Winkler, S. & Zeitlinger, M. Abscess penetration of cefpirome: concentrations and simulated pharmacokinetic profiles in pus. Eur J Clin Pharmacol, doi:10.1007/s00228-012-1270-1 (2012).

Sauermann, R., Karch, R., Kjellsson, M. C., Feurstein, T., Puspok, A., Langenberger, H., Bohmdorfer, M., Jager, W. & Zeitlinger, M. Good penetration of moxifloxacin into human abscesses. Pharmacology 90, 146-150, doi:10.1159/000341550 (2012).

Smythe, W., Khandelwal, A., Merle, C., Rustomjee, R., Gninafon, M., Bocar Lo, M., Sow, O. B., Olliaro, P. L., Lienhardt, C., Horton, J., Smith, P., McIlleron, H. & Simonsson, U. S. A semimechanistic pharmacokinetic-enzyme turnover model for rifampin autoinduction in adult tuberculosis patients. Antimicrob Agents Chemother 56, 2091-2098, doi:10.1128/aac.05792-11 (2012).

Svensson, E., van der Walt, J. S., Barnes, K. I., Cohen, K., Kredo, T., Huitema, A., Nachega, J. B., Karlsson, M. O. & Denti, P. Integration of data from multiple sources for simultaneous modelling analysis: experience from nevirapine

population pharmacokinetics. Br J Clin Pharmacol 74, 465-476, doi:10.1111/j.1365-2125.2012.04205.x (2012).

Taneja, A., Nyberg, J., Danhof, M. & Della Pasqua, O. Optimised protocol design for the screening of analgesic compounds in neuropathic pain. J Pharmacokinet Pharmacodyn 39, 661-671, doi:10.1007/s10928-012-9277-x (2012).

Taneja, A., Nyberg, J., de Lange, E. C., Danhof, M. & Della Pasqua, O. Application of ED-optimality to screening experiments for analgesic compounds in an experimental model of neuropathic pain. J Pharmacokinet Pharmacodyn 39, 673-681, doi:10.1007/s10928-012-9278-9 (2012).

Tarning, J., Chotsiri, P., Jullien, V., Rijken, M. J., Bergstrand, M., Cammas, M., McGready, R., Singhasivanon, P., Day, N. P., White, N. J., Nosten, F. & Lindegardh, N. Population pharmacokinetic and pharmacodynamic modeling of amodiaquine and desethylamodiaquine in women with plasmodium vivax malaria during and after pregnancy. Antimicrob Agents Chemother 56, 5764-5773, doi:10.1128/AAC.01242-12 (2012).

Valitalo, P., Kumpulainen, E., Manner, M., Kokki, M., Lehtonen, M., Hooker, A. C., Ranta, V. P. & Kokki, H. Plasma and cerebrospinal fluid pharmacokinetics of naproxen in children. J Clin Pharmacol 52, 1516-1526, doi:10.1177/0091270011418658 (2012).

Viljoen, M., Karlsson, M. O., Meyers, T. M., Gous, H., Dandara, C. & Rheeders, M. Influence of CYP2B6 516G>T polymorphism and interoccasion variability (IOV) on the population pharmacokinetics of efavirenz in HIV-infected South African children. Eur J Clin Pharmacol 68, 339-347, doi:10.1007/s00228-011-1148-7 (2012).

Vong, C., Bergstrand, M., Nyberg, J. & Karlsson, M. O. Rapid sample size calculations for a defined likelihood ratio test-based power in mixed-effects models. Aaps J 14, 176-186, doi:10.1208/s12248-012-9327-8 (2012).

Xu, X. S., Yuan, M., Karlsson, M. O., Dunne, A., Nandy, P. & Vermeulen, A. Shrinkage in nonlinear mixed-effects population models: quantification, influencing factors, and impact. Aaps J 14, 927-936, doi:10.1208/s12248-012-9407-9 (2012).

Zhang, C., Denti, P., Decloedt, E., Maartens, G., Karlsson, M. O., Simonsson, U. S. & McIlleron, H. Model-based approach to dose optimization of lopinavir/ritonavir when co-administered with rifampicin. Br J Clin Pharmacol 73, 758-767, doi:10.1111/j.1365-2125.2011.04154.x (2012).

Zhang, C., Denti, P., van der Walt, J. S., Ren, Y., Smith, P., Karlsson, M. O. & McIlleron, H. Population pharmacokinetic model for adherence evaluation using lamivudine concentration monitoring. Ther Drug Monit 34, 481-484, doi:10.1097/FTD.0b013e31825c6067 (2012).

Zhang, C., McIlleron, H., Ren, Y., van der Walt, J. S., Karlsson, M. O., Simonsson, U. S. & Denti, P. Population pharmacokinetics of lopinavir and ritonavir in combination with rifampicin-based antitubercular treatment in HIV-infected children. Antivir Ther 17, 25-33, doi:10.3851/imp1915 (2012).

Zvada, S. P., Denti, P., Geldenhuys, H., Meredith, S., van As, D., Hatherill, M., Hanekom, W., Wiesner, L., Simonsson, U. S., Jindani, A., Harrison, T. & McIlleron, H. M. Moxifloxacin population pharmacokinetics in patients with pulmonary tuberculosis and the effect of intermittent high-dose rifapentine. Antimicrob Agents Chemother 56, 4471-4473, doi:10.1128/AAC.00404-12 (2012).

Dissertations 2012

Emma K Hansson. Pharmacometric Models for Biomarkers, Side Effects and Efficacy in Anticancer Drug Therapy. Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy, ISSN 1651-6192; 157. http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-17073

Klas Petersson. Population Pharmacodynamic Modeling and Methods for D2-receptor Antagonists. Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy, ISSN 1651-6192; 161. http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-172540

Alan Maloney. Optimal (Adaptive) Design and Estimation Performance in Pharmacometric Modelling. Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy, ISSN 1651-6192; 166. http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-182284

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Other commitments/assignments of staff members 2012

Lena Friberg, Organizing committee, chair scientific program, PAGE conference, Venice 2012 & Glasgow 2013
Lena Friberg, Deputy Editor-in-chief, CPT: Pharmacometrics & Systems Pharmacology
Andrew Hooker, Organizing committee, PODE conference
Andrew Hooker, Deputy Department Board Member
Siv Jönsson, Deputy Board Member, Swedish Academy of Pharmaceutical Sciences, Section for Pharmacokinetics and Drug Metabolism
Siv Jönsson, Organising committee, Rosenön Meeting 2013
Mats Karlsson, Deputy Head of Department
Mats Karlsson, Department Board Member
Mats Karlsson, Scientific committee, PAGE conference, Athens
Mats Karlsson, Organizing committee, WCoP conference, Seoul
Mats Karlsson, Scientific committee, ASCPT, Pharmacometric Section Committee member
Mats Karlsson, Editor Journal of Pharmacokinetics and Pharmacodynamics
Mats Karlsson, Editorial Board on Clin Pharmacol Ther, Eur J Pharm Sci, Basic Clin Pharmacol Toxicol, Adv Pharmacol Sci, CPT: Pharmacometrics & Systems Pharmacology
Maria Kjellsson, Organising committee, Rosenön Meeting 2013
Elisabet Nielsen, Board Member, Swedish Academy of Pharmaceutical Sciences, Section for Hospital Pharmacy
Ulrika Simonsson, Board Member, Swedish Academy of Pharmaceutical Sciences, Section for Pharmacokinetics and Drug Metabolism
Ulrika Simonsson, Board Member, CPTR Regulatory Science Consortium, Critical Path to TB Drug Regimens. Clinical Disease Progression Modeling Workgroup. http://cptrinitiative.org/.
Ulrika Simonsson, Board member of IF´s stiftelse, Swedish Academy of Pharmaceutical Sciences.

Projects

Methodological research

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 different randomization schemes for dose-finding trials. It was found that dose-randomized trials are more powerful to characterize 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 calculation 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. API-optimal designs, which take into account that the underlying system (model) is not known before the study takes place). 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 and 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.

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.

Diagnostic tools

Andrew Hooker, Mats Karlsson

A main problem for the complex pharmacometric models and data is to evaluate how well the models fit the data. Often standard errors of model parameter estimates based are used as a first step. However, numerical approximations must be made to determine these standard errors, and it is often not clear what the consequences of these approximations are. We are thus developing new ways to evaluate the standard errors of parameter estimates using computer intensive and resampling based methods. In addition we are developing new methods of evaluating model quality using for example simulation-based criteria. A range of new methods and tools have been developed and evaluated in our group.

Software development

Andrew Hooker, Mats Karlsson, Joakim Nyberg

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).

Pharmacodynamic modelling of discrete outcomes

Andrew Hooker, Mats Karlsson, Elodie Plan

For many diseases, the main outcome is considered discrete: stage category, symptom severity, number of events, or occurrence of events. In pharmacometrics, we distinguish non-ordered categorical models, ordered categorical models, count models, and repeated time-to-event models to handle this type of data. In the first component of 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 and resulted in good simulation properties and high power. The application of similar models on other clinical data is on-going.

The second component of this project concerns 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.

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.

Pharmacometric modelling of biologic medicinal products

Siv Jönsson, 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

Antibiotics

Lena Friberg, Mats Karlsson, Elisabet Nielsen

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 developed model structure has been shown to be applicable across drugs and bacteria strains, for both static and dynamic concentration experiments, and for different sizes of start inocula. The model has been extended to describe different types of resistance; the adaptive resistance development of gentamicin and colistin, pre-existing mutants resistant to ciprofloxacin in starting inocula and resistance mutants from clinical isolates of meropenem. For ciprofloxacin and *E.coli*, the model can be investigated for wild-type and 9 well-characterized mutants, in addition to 3 clinical isolates. The model can also be used to predict competition experiments of wild-type and mutants. There is limited knowledge on combination treatments of antibiotics and predictions from PKPD-models based on *in vitro* data are performed to guide in the selection process of potential drug combinations to test clinically. 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.

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 for CMS and colistin in different subpopulations show that the drug is typically underdosed and a loading dose of 6-9 MU has been shown to be applicable and of value. The non-linear protein binding of colistin has been quantified as well as unspecific binding to lab material. Whole-body Physiology-based Pharmacokinetic (WBPBK) models for CMS and colistin have been developed based on data from patients, healthy volunteers and several animal species. Such a model can be used to understand the time-courses of the antibiotics, and thereby the bacteria kill, in different tissues.

Infectious diseases

Martin Bergstrand, Mats Karlsson, Ulrika Simonsson

Plasmodium falciparum, the human immunodeficiency virus (HIV), and Mycobacterium tuberculosis 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, this positive development might be counteracted by a possible emerging resistance to these drugs. 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.

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 in 2030. The disease occurs when the body does not produce enough insulin or cannot effectively use the insulin produced, resulting in increased blood glucose levels

which in the extension leads to a multitude of conditions; e.g. cardio-vascular diseases (CVD). The aim with all treatment against diabetes is to bring the glucose level in blood down to the healthy levels. The success of a treatment is assess both on short and long term measurements; the most common biomarkers being fasting plasma glucose concentration (FPG) and the fraction glycosylated haemoglobin (HbA1c) for short and long term assessment respectively.

Short term clinical studies of diabetes vary greatly in designs. Different provocation studies are used to characterize the functionality of the glucose-insulin system in both healthy volunteers (HV) and type II diabetic (T2DM) patients, including clamping of glucose or insulin by variable rate infusions, intravenous bolus administration of glucose or insulin and oral administration of glucose solution or meals. We have developed integrated models with simultaneous analysis of glucose, insulin and/or HbA1c concentration-time profiles. These models, which include production, disposition and control (homeostatic) mechanisms of the system, have shown to be able to realistically simulate the outcome of short- and long-term trial designs at the raw data level, i.e. glucose, insulin and HbA1c concentrations. Current development of models for short term clinical studies involve characterizing the effect of incretin hormones on gastric emptying and insulin secretion, characterizing pre-hepatic insulin, mechanistic description of oral glucose absorption as well as inclusion of exogenous insulin for insulin treated patients.

Long term clinical trials in T2DM patients mainly focus on HbA1c. HbA1c is the fraction of the haemoglobin, in red blood cells, that has been glycosylated. This is a naturally occurring non-enzymatic reaction depending on the plasma glucose concentration; the higher the glucose concentration in plasma, the higher the fraction glycated haemoglobin. As the life-span of red blood cells ranges from 2 to 4 months, the HbA1c supplies a measurement of the glycaemic control during the past 2-4 months. We have developed a mathematical model establishing the mechanistic link between FPG and HbA1c, including aspects of production and elimination of red blood cells. This link has also been characterised for daily average glucose. In a complementary model, the relationship between insulin sensitivity, glucose production and disposition and changes in beta-cell mass has been quantified. All models can realistically simulate the outcome of clinical trials with respect to glucose, insulin and HbA1c. Models describing changes in insulin sensitivity as a function of weight change is under development as are models describing the disease progression from impaired insulin tolerance to diabetic.

The overall endpoint of most treatments against type 2 diabetes 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.

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. 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 neutrophils has been characterized based on generated data from a clinical study. IL-6 and AAG are also being evaluated for their predictive value in chemotherapy-induced neutropenia. The model has also been applied for other types of cells and indications, most recently on platelet counts from studies on an antibody drug conjugate, TDM-1. The PK of the different antibody components of TDM-1 has also been modelled.

Models that integrate biomarkers, efficacy and adverse events are of interest. As an example, we have performed an analysis of a large data base from four studies on sunitinib in gastrointestinal stromal cancer. The usefulness of angiogenetic biomarkers (VEGF,s-VEGFR-2, s-VEGFR-3 and s-KIT), as well as SUV measurements, to predict tumour response, toxicity and overall survival have been investigated. Longitudinal measurements were superior over fixed time point measurements. The models provide a framework for simulation that will be useful for understanding which biomarkers to measure and which patients benefit from a continuation of the therapy. A similar framework is currently being developed for axitinib in metastatic renal cell carcinoma.

Models for progressive diseases

Andrew Hooker, 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 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 a (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, and the potentially more informative ACR-N scale is being investigated.

The challenges in the development of new therapeutic agents for Alzheimer's Disease (AD) become apparent through the high number of failed late phase trials. Despite an increasing interest in biomarkers, cognition remains the primary

regulatory accepted clinical outcome. The most frequently used test, ADAS-cog, consists of a broad spectrum of tasks that test different components of cognition. The total ADAS-cog score is obtained by rating a subject's performance in each of the subtests and summing up the resulting subscores to yield an overall assessment. In turn, pharmacometric models traditionally describe Alzheimer's disease progression using this summary score. We explore and further develop an alternative approach, to model each subscore separately and link the model subcomponents to a common unobserved variable "cognitive disability". In psychometrics, this method is used to study the sensitivity of items in standardized educational tests, and the approach is referred to as item response theory (IRT).

Pharmacodynamic modelling in other disease areas

Lena Friberg, Andrew Hooker, Mats Karlsson, Ulrika Simonsson

Apart from the disease areas described above we are working on pharmacodynamic models for several other effects and adverse events. A mechanism-based agonist-antagonist interaction model for antipsychotic drug-induced prolactin elevations, developed by us, is investigated for its use in drug development. For a range of drugs the model can predict the full time-course of prolactin given system-related parameters (common for all drugs) and Ki-values determined *in vitro*. The relationship of prolactin to the disease state variable PANSS is being investigated as well as the possibility to apply prolactin data from rats to further increase the accuracy of the *in vitro*-patient prediction. Item response theory is applied to investigate whether analysis of the individual PANSS items can provide further information on the dose-concentration-effect relationship.

Pharmacokinetic-pharmacodynamic models in the therapeutic area of pain relief are investigated. The aim is to characterize the exposure-response relation of individual drugs as well as develop models for simulation of study design of future studies and drugs. (Repeated) Time to event modelling of drop out in depression and pre-clinical addiction studies have been described.

Pharmacodynamic models in some other disease areas are described in the section "Pharmacodynamic modelling of discrete outcomes".

Dose individualisation in transplantation

Siv Jönsson, Mats Karlsson

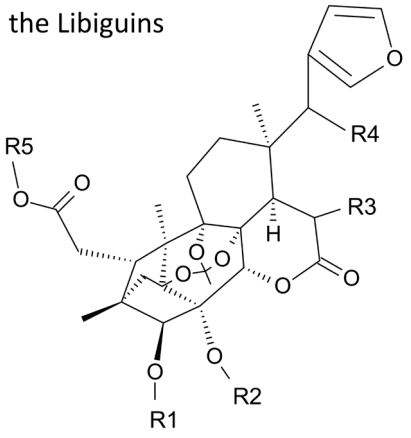
Cyclosporine is a commonly used drug in paediatric transplantation. Cyclosporine is characterised by a narrow therapeutic index with an increased risk of an acute rejection due to low systemic exposure and conversely high systemic exposure may lead to e.g. renal dysfunction and other toxicities. Therefore, plasma concentrations are monitored to optimise treatment for each individual. However, evidence that monitoring has an effect on the rate of acute rejection and leads to impaired renal function is sparse. Furthermore, the optimal target concentrations and monitoring strategies has not been established.

For the last 20 years, virtually all renally transplanted children in Finland have been monitored for their plasma drug concentrations by the Clinical Pharmacology group at the University Hospital in Helsinki resulting in a unique data base. In collaboration with this group, and including also other therapy information from these patients, the ultimate goal is to improve the individualised dosing by exploring and establishing the therapeutic window for cyclosporine. Until now, we have characterized determinants of variability in pharmacokinetics for this population before transplantation and over time after transplantation. Furthermore, the pre-

transplantation test procedure has been optimized with respect to convenience and information content. Relationships between plasma drug concentration and biomarkers/clinical endpoints are explored to allow better decision criteria for dose adjustments.

Pharmaceutical Bioinformatics

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



Jarl Wikberg

During the year the Bioclipse workbench (www.bioclipse.net) was extended to support the statistical programming language R for integrated analysis. The Bioclipse Decision Support features were extended with more models for computational drug safety, such as models for HeRG and PGP. A project in collaboration with AstraZeneca for the prediction of secondary pharmacology continues and has shown very good preliminary results.

An approach for large-scale proteochemometric modeling of cytochrome P450 (CYP) inhibition was further developed and implemented under Bioclipse Decision Support and made publicly available at www.cyp450model.org, allowing the prediction of the CYP inhibition capacity of random chemicals.

A VR-funded planning project for the development of an e-infrastructure for chemical safety predictions was initiated, and will continue until end of 2013. This involves strategic planning, investigation of requirements and use cases as well as international collaboration with the FP7 projects OpenTox (www.opentox.org) and ToxBank (www.toxbank.org). Collaboration has also been established with the IMI-project Open PHACTS (www.openphacts.org). A key aspect of the e-infrastructure is the inclusion of omics data in chemical safety predictions to allow for mechanistic interpretations.

Proteochemometrics was further utilized to develop a low-molecular weight inhibitory peptide for Dengue virus NS2B/NS3 proteases and molecular docking studies showed it's likely binding mode to the NS2B/NS3 proteases. Extensive studies were undertaken to develop methods for 3D modeling of melanocortin receptor structures using homology modeling and molecular dynamics simulations, and to merge 3D-modelling/ligand docking approaches with proteochemometrics. The project is well underway, and very promising to improved ligand design and understanding of molecular recognition processes.

The libiguin project also continued. A series of libiguins A-H were produced semi-synthetically and evaluated for their highly potent and powerful ability to stimulate of sexual behavior. An [^{11}C]-labelled PET probe of the libiguins was developed and characterized biologically. Studies were continued to understand the molecular basis of the powerful actions of the libiguins. Further natural

products were isolated from different meliace and determined to their structure. These studies included the discovery of a novel protolimonoid, as well as other novel natural compounds, as well as novel semi-synthetic compounds, with highly interesting novel pharmacological properties.

Members of the group during 2012

Jarl Wikberg, Professor
Sviatlana Yahorava, PhD, Post Doc
Maris Lapins, PhD, Researcher
Ola Spjuth, PhD, Researcher
Jonathan Alvarsson, PhD student
Martin Eklund, PhD, Researcher
Arvid Berg, Programmer
Muhammad Junaid, PhD student
Iryna Shutava, PhD, Post Doc
Aleh Yahorau, Technician
Valentin Georgiev, PhD, Programmer
Polina Georgieva, PhD
Klas Jönsson, Programmer
Chanin Nantasenamat, PhD, Post Doc

Publications 2010-2012

Spjuth O, Carlsson L, Alvarsson J, Georgiev V, Willighagen E, Eklund M: Open source drug discovery with bioclipse. Curr Top Med Chem. 2012;12(18):1980-6.

Hardy B, Apic G, Carthew P, Clark D, Cook D, Dix I, Escher S, Hastings J, Heard DJ, Jeliazkova N, Judson P, Matis-Mitchell S, Mitic D, Myatt G, Shah I, Spjuth O, Tcheremenskaia O, Toldo L, Watson D, White A, Yang C. Food for thought ... A toxicology ontology roadmap. ALTEX. 2012;29(2):129-37.

Williams AJ, Ekins S, Spjuth O, Willighagen EL: Accessing, using, and creating chemical property databases for computational toxicology modeling. Methods Mol Biol. 2012;929:221-41.

Andersen GN, Andersen M, Nagaeva O, Wikberg JES, Mincheva-Nilsson L: Dermal melanocortin receptor rebound in diffuse systemic sclerosis after anti-TGFβ1 antibody therapy. Scand J Immunol. 2012 Nov;76(5):478-82. doi: 10.1111/j.1365-3083.2012.02757.x.

Junaid M, Chalayut C, Sehgelmeble Torrejon A, Angsuthanasombat C, Shutava I, Lapins M, Wikberg JE, Katzenmeier G: Enzymatic analysis of recombinant Japanese encephalitis virus NS2B(H)-NS3pro protease with fluorogenic model peptide substrates. PLoS One. 2012;7(5):e36872. doi: 10.1371/journal.pone.0036872. Epub 2012 May 15.

Fossen T, Rasoanaivo P, Manjovelo CS, Raharinjato FH, Yahorava S, Yahorau A, Wikberg JES: A new protolimonoid from Capuronianthus mahafalensis. Fitoterapia. 2012 Jul;83(5):901-6. doi: 10.1016/j.fitote.2012.03.023. Epub 2012 Apr 1.

Alvarsson J, Andersson C, Spjuth O, Larsson R, Wikberg JES. Brunn: an open source laboratory information system for microplates with a graphical plate layout design process. BMC Bioinformatics. 2011 May 20;12:179.

Spjuth O, Eklund M, Lapins M, Junaid M, Wikberg JES. Services for prediction of drug susceptibility for HIV proteases and reverse transcriptases at the HIV

drug research centre. Bioinformatics. 2011 Jun 15;27(12):1719-20. Epub 2011 Apr 14.
Willighagen EL, Alvarsson J, Andersson A, Eklund M, Lampa S, Lapins M, Spjuth O, Wikberg JES. Linking the Resource Description Framework to cheminformatics and proteochemometrics. J Biomed Semantics. 2011 Mar 7;2 Suppl 1:S6.
O'Boyle NM, Guha R, Willighagen EL, Adams SE, Alvarsson J, Bradley JC, Filippov IV, Hanson RM, Hanwell MD, Hutchison GR, James CA, Jeliazkova N, Lang AS, Langner KM, Lonie DC, Lowe DM, Pansanel J, Pavlov D, Spjuth O, Steinbeck C, Tenderholt AL, Theisen KJ, Murray-Rust P. Open Data, Open Source and Open Standards in chemistry: The Blue Obelisk five years on. J Cheminform. 2011 Oct 14;3(1):37.
Spjuth O, Eklund M, Ahlberg Helgee E, Boyer S, Carlsson L. Integrated decision support for assessing chemical liabilities. J Chem Inf Model. 2011 Aug 22;51(8):1840-7. Epub 2011 Aug 5.
Salaemae, W., Junaid. M., Angsuthanasombat, C., Katzenmeier, G. (2010) Structure-guided mutagenesis of active site residues in the dengue virus two-component protease NS2B-NS3. J. Biomed. Sci, 17(1): 68.
Willighagen EL, Jeliazkova N, Hardy B, Grafström RC, Spjuth O. Computational toxicology using the OpenTox application programming interface and Bioclipse. BMC Res Notes. 2011 Nov 10;4:487.

Reviews 2010-2012

Hardy B, Apic G, Carthew P, Clark D, Cook D, Dix I, Escher S, Hastings J, Heard DJ, Jeliazkova N, Judson P, Matis-Mitchell S, Mitic D, Myatt G, Shah I, Spjuth O, Tcheremenskaia O, Toldo L, Watson D, White A, Yang C. ALTEX. 2012;29(2):139-56. Review.
Wikberg, JES, Eklund, M, Willighagen, EL, Spjuth, O, Lapins, M, Engkvist, O, Alvarsson, J. Introduction to Pharmaceutical Bioinformatics, Oakleaf Academic, 2011. ISBN: 978-91-979403-0-6.

Dissertations 2012

Junaid, M:
Studies of Retroviral Reverse transcriptase and Flaviviral Protease Enzymes as Antiviral Drug Targets. Applications in Antiviral Drug Discovery & Therapy. Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy, ISSN 1651-6192; 163.
http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-173504

Agencies that support the work/Funding 2012

The Swedish Research Council
AstraZeneca
Swedish Institute

Other commitments/assignments of staff members 2012

Ola Spjuth, Deputy Director, UPPMAX

Projects

Pharmacology of the libiguins

Jarl Wikberg et al.

Studies on the mechanisms of action for the effects of libiguins on sexual behavior; in part a collaboration with Philippe Rasoanaivo, IMRA, Antananarivo, Madagascar, Gunnar Antoni, PET-centre, Uppsala University and Aigars Jirgensons, IOS, Riga, Latvia.

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.

Proteochemometrics

Jarl Wikberg et al.

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

The Bioclipse Workbench

Ola Spjuth et al.

Development of the Bioclipse workbench for e-Science. Main focus is on drug discovery, safety assessment, and predictive modeling, but other developments include plugins for Next-Generation Sequencing in collaboration with SciLifeLab and UPPMAX.

Predictive toxicology

Ola Spjuth et al.

Studies on predictive modeling in toxicology, mainly drug safety. Collaboration with the EU FP7 project OpenTox, AstraZeneca R&D, Karolinska Institutet, and the National Food Agency.

Prediction of metabolic sites

Ola Spjuth et al.

Studies on predictions of site-of-metabolism, manifested in the MetaPrint2D method. Collaboration with AstraZeneca R&D and University of Copenhagen.

Steroid P450

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

Maria Norlin and Kjell Wikvall

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 2012

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

Publications 2010-2012

Lundqvist, J., Norlin, M., and Wikvall, K. 1a,25-Dihydroxyvitamin D_3 affects hormone production and expression of steroidogenic enzymes in human adrenocortical NCI-H295R cells. *Biochim Biophys Acta – Mol Cell Biol Lipids, 1801, 1056-1062 (2010)*

Pettersson, H., Lundqvist, J., and Norlin, M. Effects of CYP7B1-mediated catalysis on estrogen receptor activation. *Biochimica et Biophysica Acta – Mol Cell Biol Lipids 1801, 1090-1097 (2010)*

Norlin M, Pettersson H, Tang W, and Wikvall K. Androgen receptor-mediated regulation of the anti-atherogenic enzyme CYP27A1 involves the JNK/c-jun pathway. *Arch Biochem Biophys. 506, 236-241 (2011)*

Lundqvist J, Norlin M, and Wikvall K. 1α,25-Dihydroxyvitamin D_3 exerts tissue-specific effects on estrogen and androgen metabolism. *Biochim Biophys Acta – Mol Cell Biol Lipids*, 1811, 263-270 (2011)

Fex Svenningsen Å, Wicher G, Lundqvist J, Pettersson H, Corell M, and Norlin M. Effects on DHEA levels by estrogen in rat astrocytes and CNS co-cultures via the regulation of CYP7B1- mediated metabolism. *Neurochem. Int. 58, 620-624 (2011)*

Lundqvist J, and Norlin M. Effects of CYP7B1-related steroids on androgen receptor activation in different cell lines. *Biochim Biophys Acta - Mol Cell Biol Lipids, 1821, 973-979 (2012)*

Lundqvist J, Wikvall K, and Norlin M. Vitamin D-mediated regulation of CYP21A2 transcription - a novel mechanism for vitamin D action. *Biochim Biophys Acta - General Subjects, 1820, 1553-1559 (2012)*
Emanuelsson I and Norlin M. Protective effects of 27- and 24-hydroxycholesterol against staurosporine-induced cell death in undifferentiated neuroblastoma SH-SY5Y cells. *Neurosci. Lett. 525, 44-48 (2012)*

Reviews 2010-2012

Norlin, M. and Wikvall, K . CYP7A1 (cytochrome P450, family 7, subfamily A, polypeptide 1). Atlas Genet Cytogenet Oncol
Haematol. January 2010. URL : http://AtlasGeneticsOncology.org/Genes/CYP7A1ID40254ch8q12.html
Norlin M and Wikvall K. Tissue-specific regulation of sex hormone biosynthesis and metabolism: novel aspects on hormonal signalling and maintenance of cellular steroid levels in "Sex Hormones", Ed. R.K. Dubey, ISBN 978-953-307-856-4. InTech, Rijeka, Croatia, 2012.

Other commitments/assignments of staff members 2012

Kjell Wikvall
Chair of the Scholarships committee for the Faculty of Pharmacy.
Member of the Scholarships committee for Uppsala University.

Projects

Vitamin D-mediated effects on cellular function: effects on steroidogenesis and sex hormone metabolism

Johan Lundqvist, Kjell Wikvall and Maria Norlin

Vitamin D deficiency, which affects many physiological functions, is associated with increased risks of prostate and breast cancers. Our studies, aimed to investigate the cellular functions of vitamin D in various parts of the body, have revealed vitamin D-mediated effects on the production of steroid hormones and regulation of the expression of crucial steroidogenic enzymes in several cell types. Steroid hormones play important roles in the pathogenesis of prostate cancer and breast cancer. Recent data from our laboratory show that the active vitamin D hormone, 1,25-dihydroxyvitamin D_3, exerts tissue-specific effects on production and metabolism of sex hormones, providing important information for further research in the fields of prostate and breast cancer. Our findings, indicating regulation of intracellular levels of androgens and estrogens by vitamin D, open new possibilities in prevention and treatment of hormone-dependent cancer. 1,25-Dihydroxyvitamin D_3 and analogs exert anti-proliferative and pro-differentiative effects and are therefore considered as potential anti cancer agents in development of future drug therapy.

Functions and regulation of neurosteroids and steroid-metabolizing enzymes in CNS cells.

Ida Emanuelsson, Mokhtar Almokhtar and Maria Norlin

Steroids produced locally in the nervous system, such as DHEA

(dehydroepiandrosterone), pregnenolone or 27-hydroxycholesterol, have been termed neurosteroids and are considered of particular importance for brain development and function. Vitamin D has recently been suggested as a neurosteroid and is believed to play a role in psychiatric as well as neurodegenerative disease, e g Parkinson's disease. However, the mechanisms behind the effects of neurosteroids and the regulation of neurosteroid levels remain unclear. Our studies using cultured cells and animal studies, are directed to understand the processes of enzymatic actions and gene regulation that affect cellular steroid levels and steroid hormone signaling in the brain. Regulators of interest include endogenous compounds as well as drugs e g SERM (selective estrogen receptor modulators). We have reported that CYP7B1-mediated 7-hydroxylation of DHEA in primary cultures of rat astrocytes and co-cultures of CNS cells is strongly suppressed by estrogens. Our recent data indicate that oxysterols, such as 27-hydroxycholesterol, may have neuroprotective effects.

Enzymatic regulation of nuclear receptor activation with special focus on sex hormones

Hanna Pettersson, Johan Lundqvist and Maria Norlin

Estrogenic and androgenic steroids, including estradiol, testosterone and many more, have numerous important functions in human tissues. These hormones, endogenously produced or provided in therapy, affect e g bone formation, brain development and function, cardiovascular and immune systems and cellular growth and viability. Most of the physiological effects of androgens and estrogens are mediated via the androgen receptor (AR) and the estrogen receptors ERα and β, which are present in numerous tissues. This project, using cell models derived from several hormone target tissues, includes studies on the mechanisms for sex hormone signalling and how enzymatic conversion of steroids regulate nuclear receptor activation.

Mechanisms behind adverse drug effects on bone health: roles of gene regulation in vitamin D homeostasis

Christine Wegler, Kjell Wikvall and Maria Norlin

Glucocorticoids and antiretroviral drugs are examples of drugs known to result in high incidence of low bone mineral density. Currently, glucocorticoid-induced osteoporosis is the most common cause of osteoporosis in adults aged 20–45 years, as well as the most common cause of iatrogenic osteoporosis. High incidence of low bone mineral density, vitamin D deficiency and osteomalacia is a concern in HIV treatment with antiretroviral drugs such as efavirenz. The etiology and underlying mechanisms behind these disorders remain largely unknown. The project includes studies on effects of hormones, drugs and drug candidates on vitamin D-related genes in cell models. The enzymatic activation of vitamin D is well known to be essential for normal bone health. In this project we use bone cell lines and primary human osteoblasts in collaboration with clinicians at Uppsala Academic Hospital. The aim is to obtain more information on regulation of vitamin D levels in bone cells and to characterize the molecular background of adverse drug effects that influence bone health.

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