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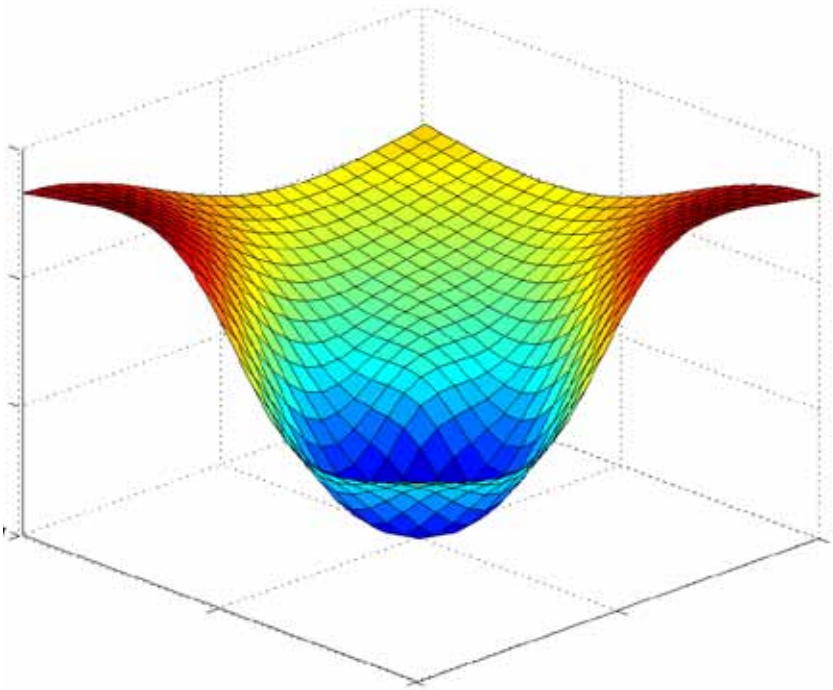
Farmbio 2012/41, 5:9

# ANNUAL REPORT 2011

## Department of Pharmaceutical Biosciences



UPPSALA
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Farmbio 2012/41, 5:9

# ANNUAL REPORT 2011

## Department of Pharmaceutical Biosciences

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Editing committee: Eva Brittebo, Marianne Danersund, Annika Bokström, Andrew Hooker and Lena Bergström
Layout: Magnus Jansson
Front page illustration: Joakim Nyberg, Pharmacometrics

*"The information derived from a clinical drug trial given different measurement times for two samples of drug concentration in plasma for that trial. Figures like this allow optimization of information content in clinical drug trials. Information is computed using the determinant of the Fisher Information Matrix. For more information see: Nyberg J, Karlsson MO, and Hooker AC. Simultaneous optimal experimental design on dose and sample times. Journal of pharmacokinetics and pharmacodynamics. 36(2):125-45, 2009."*

# Introduction

This annual report highlights a selected range of the activities at the department. We focus the research on basic mechanisms of drug action, drug dependency and adverse effects, as well as studies on drug metabolism and pharmacokinetics and pharmacodynamics. In addition, there is a significant focus on pharmaceutical bioinformatics and proteomics, as well as modelling and simulations used in drug development. Several research groups are developing tools directly applicable to the pharmaceutical industry, while others are studying issues such as drug dependency and environmental contaminants with important societal implications. This report confirms that the research groups continue to have worldwide international collaboration within their research areas. Research underpins all our teaching and the achievements over the past year form a strong foundation for education and training.

### *International peer review*

An overall evaluation of research at Uppsala University, called "Quality and Renewal", was carried out in 2011. The objective of the project was to identify strong areas of research and successful constellations within the university. Moreover, it aimed to identify emerging science and promising initiatives for future research. The evaluation was carried out by panels with international experts from many countries. The panels made a one-week visit to the site in May 2011 when they met faculty staff and PhD students for interviews, presentations, and laboratory visits. Written background material was provided by the department. It contained an extensive self-assessment, a number of quantified quality indicators reflecting international and national recognition, as well as facts and figures concerning research publications, research exams, finance, etc.

The international panel was impressed with the breadth and depth of scientific capabilities and the ambition of the various research groups at the department. They considered the research to be conducted at a high quality level within each thematic research group, with all major instrumentation available in modern lab facilities. Some major leaders in their respective fields were rated as "top quality" in the department. The Pharmacometrics group was considered to be worldwide recognized as the leading program for training pharmacometricians providing new methods and software for study design and analysis, developing novel models and strategies for data analysis. The research groups covering Neuropharmacology and drug addiction were considered to have a major strength in the integrated molecular, epigenetic, behavioural, tolerance and addiction studies conducted on substances of abuse. Finally, the Proteomics group was considered a significant asset, creating a core pharmaceutical resource in biomedical applications of imaging mass spectrometry.

### *Funding*

All research activities require funding from national and international research councils, pharmaceutical companies and the government. For example, in 2011 a EU-funded project within Innovative Medicines Initiative involving 24 partner organisations started with the Pharmacometrics research group as academic lead. This project aims to develop a drug/disease model library and framework. In addition, The Swedish Council FORMAS allocated funding for studies on a non-protein amino acid which is a potential human neurotoxin produced by cyanobacteria detected in terrestrial and aquatic environments. This grant makes it possible for the Drug Safety and Toxicology research group to clarify mechanisms of actions for the neurodegenerative changes and cognitive impairments that are observed in adult animals following neonatal exposure to this toxin.

*New academic staff*

The staff is the key to our research and teaching success and the development of the department. During 2011 the following academic staff members joined the department: Lena Friberg was appointed senior lecturer and Lena Klarén was appointed junior lecturer. In addition, Elisabet Nielsen, Maria Kjellsson and Anna Nilsson were appointed researchers in the Pharmacometrics and Medical Masspectrometry groups, respectively. We are looking forward to working together with them.

*Scientific output*

Most research councils and foundations require that research findings financed by them must be published by Open Access. The department's research results are always published in peer-reviewed, international scientific journals and a full account of those publications, as well as books and all 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 2011 have been registered in DiVA. In 2011, 106 new peer-reviewed scientific publications were registered in the database. Some of them are freely available as preprints in the academic on-line archive. Links to the most recent publications can always be found on our website.

*www.farmbio.uu.se*

The new website with extensive information about our research and education was launched a year ago. It is continuously updated in order to present current research and teaching activities. Furthermore, lists of recent scientific publications, personalised lists of scientific publications, as well as PhD theses are automatically updated from DiVA. Traffic statistics show that the new website has an increasing numbers of unique visitors per month.

*Organisation and financial review*

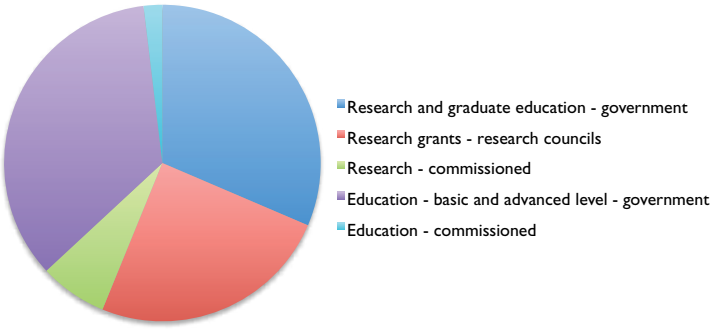
The department is organised on the basis of core activity i.e. research, teaching and the collected support activities (i.e. management, finance/staff administration, education administration and infrastructure). All support activities are funded by a percentile overhead on salaries and operating costs.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

A short summary of the income and expenditure of 2011 is given below.

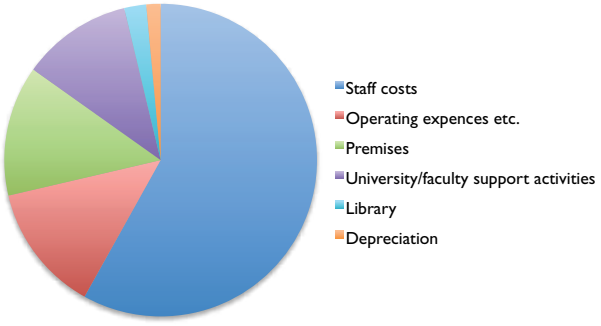
| *Income 2011 (kSEK)* | |
|---|---|
| Research and graduate education - government | 29 964 |
| Research grants – research councils | 23 581 |
| Research – commissioned | 6 581 |
| Education – basic and advanced level - government | 33 363 |
| Education – commissioned | 1 817 |

| *Expenditure 2011 (kSEK)* | |
|---|---|
| Staff costs | 55 240 |
| Operating expenses etc. | 12 605 |
| Premises | 12 821 |
| University/faculty support activities | 10 851 |
| Library | 2 167 |
| Depreciation | 1 401 |

## Income




## Expenditure




*Future development*

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 good research, but also introduce new areas of research that can attract funding from national research councils, the EU and drug companies.

Uppsala March 28, 2012

*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*
Angelica Quartino, *graduate student representative*
Åsa Johansson, *graduate student representative*
Alfhild Grönblad, *graduate student representative, deputy*
Erik Melander *student representative*
Sam Jabbar, *student representative, deputy*
Katrina Dee, *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

*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*

*Directors of undergraduate studies*

Lena Bergström
Jörgen Bengtsson
Sven Björkman
Ann-Marie Falk
Mathias Hallberg
Björn Hellman
Lena Klarén
Emma Lundkvist
Jonna Lübcke
Ingrid Nylander
Anne-Lie Svensson
Maria Swartling
Jarl Wikberg
Kjell Wikvall

*Working group on graduate studies*

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

*Working group on gender equality and other policy issues*

Elin Svensson, *chairman*
Johanna Svensson
Ulrika Simonsson
Ronnie Hansson, *adjunct*
Katrina Dee

*Gender equality representative*

Anne-Lie Svensson

*Technical and administrative staff*

Agneta Bergström
Ulrica Bergström
Annika Bokström
Marianne Danersund
Agneta Hortlund
Johanna Svensson
Magnus Jansson
Marina Rönngren
Johanna Svensson
Karin Tjäder
Yvonne Wiessing*
Kjell Åkerlund

*Laboratory staff*

Marita Berg
Jessica Dunhall
Raili Engdahl
Britt Jansson
Britt-Marie Johansson
Lena Norgren

*Safety officers*

Marianne Danersund
Raili Engdahl
Ronnie Hansson
Britt-Marie Johansson
Lena Norgren
Henrik Wadensten
Sviatlana Yahorava
Kjell Åkerlund

*The work enviroment group*

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

* *temporary position*

# Address

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# Awards and Appointments

- Fred Nyberg Appointed as Visiting Prof.at Hoshi University, Tokyo (Japan) in 2009; Fred Nyberg received Uppsala University Gustaf Adlof's Medal in Gold in 2011. Fred Nyberg recived The Swedish CAN prize for drug research in 2011.

# Undergraduate Teaching

During 2011 the teachers have devoted extra time to development of examination, with special focus on goal-directed examination, and also on training of the students communication skills. This work is part of the quality enhancement programme at the University, Creative Development of University Education (KrUUt/CrED). The CrED initiative comprises a number of enhancement themes that have been identified for focused developmental work during 2010-2012 to optimize the quality within educational activities.

The major part of the undergraduate teaching is within the two Pharmacy programmes. During 2011 the extent of undergraduate teaching was 434 *hst* (full-time equivalents) and that represents 41% of the total number of hst within the Faculty of Pharmacy. In addition, the department is involved in teaching at the Master of Science in Chemical Engineering with specialization in drugs and the Biomedical programme. Students attending internet-based courses steadily increase and during 2011 these courses comprised 87 hst.

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

### *Teaching at the basic level*

The main teaching is within the Bachelor of Science in Pharmacy programme that comprises three years studies (180 hp). Completed studies at the programme provide the necessary theoretical and practical competence that is required to apply for a pharmacist license as *receptarie*. The teachers also instruct in undergraduate projects (15 hp) at the basic level. These projects are individual and they are examined by an oral presentation and a written report. During 2011, the teachers within the department supervised 76% (38 students) out of the total number of undergraduate projects within the Bachelor of Science in Pharmacy programme.

### *Teaching at the advanced level*

The department gives courses at the Master of Science in Pharmacy programme that comprises five years studies (300 hp). Completed studies at the programmes provide the necessary theoretical and practical competence that is required to apply for a pharmacist license as *apotekare*. The teachers instruct in undergraduate projects (30 hp) at the advanced level. These projects are individual and are examined by an oral presentation and a written report. The projects are laboratory-based and involve the student in ongoing research projects. During 2011, the teachers supervised 62% (82 students) of the total number of undergraduate projects within the Master of Science in Pharmacy programme.

The teachers lead and teach a number of elective courses and single subject courses at the advanced level. These courses mirror research profiles within the department, such as Bioinformatics, Clinical pharmacy, Drug metabolism and safety, Drug addiction and Pharmacokinetics. The courses attract a large number of students, not only pharmacy students but also other students showing the proper prerequisites. During 2011, 79% (348 out of 440 students) of the students within the Master of Science in Pharmacy programme and 65% (65 out of 100 students) of the students within the Bachelor of Science in Pharmacy programme participated

in courses given by the department.

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

Uppsala April 2012

*Ingrid Nylander*

# Course List

## List of courses on basic and advanced levels

Advanced Course in Toxicology C, 15 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, 15 c
Biochemistry of Gene Regulation Second cycle, 7.5 c
Clinical Attachment and Service Development Second cycle, 18 c
Clinical Drug Trials with Applied Biostatistics Second cycle, 7.5 c
Clinical Drug Trials with Applied Biostatistics C, 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
Clinical Pharmacy Second cycle, 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 Drug Usage Second cycle, 15 c
Degree Project in Drug usage Second cycle, 15 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 Development and Drug Usage First cycle, 7.5 c
Drug Management Second cycle, 7.5 c
Drugs and Dependence C, 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
Evidence-Based Treatment with Cognitive Approach (Contract Education) First cycle, 15 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
Molecular Pharmacology First cycle, 7.5 c
Molecular pharmacology and applied toxicology First cycle, 7.5 c
Neuropharmacology C, 7.5 c

Neuropharmacology Second cycle, 7.5 c
Pharmaceutical Biochemistry First cycle, 9 c
Pharmaceutical Biochemistry and Cell Biology First cycle, 7.5 c
Pharmaceutical Biochemistry and Cell Biology A, 7.5 c
Pharmaceutical Bioinformatics C, 7.5 c
Pharmaceutical Bioinformatics Second cycle, 7.5 c
Pharmaco/toxicokinetics and pharmaco/toxicodynamics Second cycle, 3 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 First cycle, 7.5 c
Pharmacokinetics and Statistics First cycle, 9 c
Pharmacology First cycle, 15 c
Pharmacology First cycle, 16.5 c
Pharmacology First cycle, 15 c
Pharmacology First cycle, 15 c
Pharmacology and Medicinal Chemistry First cycle, 15 c
Pharmacotherapy B, 7.5 c
Pharmacotherapy First cycle, 7.5 c
Pharmacotherapy in Self-Treatment First cycle, 9 c
Physiology and Molecular Cell Biology First cycle, 10 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 C Second cycle, 30 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

# Research Education

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

The 4-year program is made up of research work and a number of courses, with a total course requirement of 30 points (one semester full-time). Most PhD students fulfill more than this requirement. The PhD thesis is *a doctoral thesis consisting of separately published articles with an introductory comprehensive summary*. A PhD thesis is typically comprised of around 4 – 5 scientific papers, of which at least half are published in scientific journals at the time of the public thesis defense. Besides the research activities, the PhD students participate in seminars and at international conferences. Apart from research, many PhD students perform important tasks in undergraduate teaching, comprising around 20 % of their time, making the time for a PhD exam be around 5 years. The teaching assignments give the students experience of a broader knowledge base than their own thesis area. It also gives them leadership and communication skills that are of importance in further professional life. PhD students may also tutor master students in experimental research work. During 2011 there were on average 54 PhD students registered. Thirteen PhD students defended their theses and 9 new PhD students were registered.

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

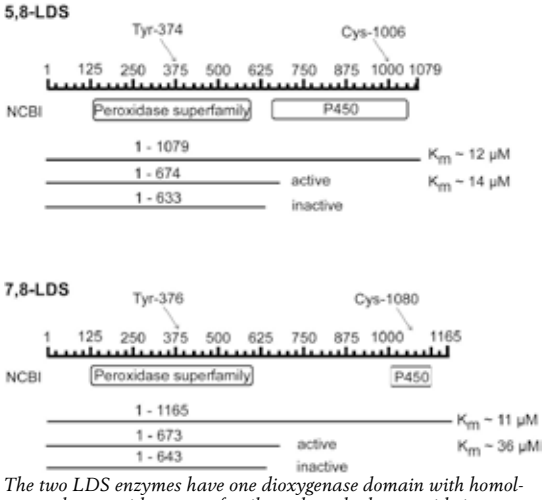
Uppsala March 2012

*Margareta Hammarlund-Udenaes*
*Chair of the Research Education Group*

# Scientific Reports

# Biochemical Pharmacology

*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.

**(ii) Lipoxygenases:** All lipoxygenases contain a catalytic metal, iron in humans and plants. We focus our basic research on the first described manganese-lipoxygenases, which are important for *Gäumannomyces graminis*, an important pathogen of wheat, and its structurally similar lipoxygenases of *Magnaporthe 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* (publications # 2, 5, and 8). Another novel discovery is the 9*R*-dioygenase and allene oxide synthase of *A. terreus* (publication # 6).

### *Members of the group during 2011*

Ernst H. Oliw, MD PhD, Professor
Erica Johansson, administrative assistant (diseased 2011-10-01)
Fredrik Jernerén, PhD student
Inga Hoffmann, PhD student
Anneli Wennman, PhD student

### *Publications 2009-2011*


1. Palmieri-Thiers, C., Canaan, S., Brunini, V., Lorenzi, V., Tomi, F., Desseyn, JL., Garscha, U., Oliw, E.H., Bert,i L., Maury, J. (2009) A lipoxygenase with dual positional specificity is expressed in olives (Olea europaea L.) during ripening. *Biochim. Biophys. Acta*, **1791**, 339-346.
2. Garscha, U. and Oliw, E. H. (2008) Leucine/Valine Residues Direct Oxygenation of Linoleic Acid by (10R)- and (8R)-Dioxygenases. EXPRESSION AND SITE-DIRECTED MUTAGENESIS OF (10R)-DIOXYGENASE WITH EPOXYALCOHOL SYNTHASE ACTIVITY, *J. Biol. Chem.* **284**, 13755-13765.
3. Pettersson, H, Lundqvist, J, Oliw, E, Norlin, M. (2009) CYP7B1-mediated metabolism of 5alpha-androstane-3alpha,17beta-diol (3alpha-Adiol): A novel pathway for potential regulation of the cellular levels of androgens and neurosteroids. *Biochim. Biophys. Acta* **1791**, 1206-2115.
4. 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
5. 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
6. 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.
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9. 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.
10. 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
11. 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.
12. 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.

*Dissertations 2011*

1. Fredrik Jernerén
   *Novel Fatty Acid Dioxygenases of Human and Plant Pathogenic Fungi: Studies by Gene Deletion and Expression*.
   Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy, ISSN 1651-6192; 135
   http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-143065

*Agencies that support the work/Funding 2011*

The Swedish Research Council Medicine

## Projects

*Novel transformations of polyunsaturated fatty acids and eicosanoids.*

*Ernst Oliw, Johan Bylund, Tomas Nilsson*
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.
Publication # 4.

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

*Fredrik Jernerén, Inga Hoffmann, 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.
Publications # 2, 6, 9, 10, and 12.

*Gene deletion of 7,8-LDS in Magnaporthe oryzae*

*Fredrik Jernerén, Ane Sesma, Ernst Oliw*
The lipidomics of *M. oryzae* has been studied with identification and deletion of the 7,8-LDS gene and discovery of the unique oxylipins formed by a strain with constitutive PKA activity.
Publication # 5.

*Characterization of the reaction mechanism and metal ligands of manganese-lipoxygenase*

*Fredrik Jernerén, Anneli Wennman, Ulrike Garscha, Margareta Sahlin, Ernst Oliw*
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.
Publications #10, 11

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

*Fredrik Jernerén, Mats Hamberg, 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.
Publication # 7.

*Characterization of lipoxygenases and allene oxide synthase and 9R-dioxygenase of Lasiodiplodia theobromae*

*Fredrik Jernerén, Filip Eng, Mats Hamberg, Ernst Oliw*
Jasmonic acid is produced by Lasiodiplodia theobromae and we have investigated its biosynthesis and found a novel lipoxygenase.
Publication # 13.

# Biological Research on Drug Dependence

## Biological Research on Drug Dependence

*Fred Nyberg and Mathias Hallberg*

*Members of the group during 2011*

Fred Nyberg, Professor
Mathias Hallberg, PhD Associate Professor
Qin Zhou, PhD, Researcher
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Dan Henrohn, PhD Student
Jenny Johansson, PhD Student
Alfhild Grönbladh, PhD, Student
Anna Carlsson PhD student
Erika Enhamre, PhD student
Britt-Marie Johansson, Technician

*Publications 2009-2011*


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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.
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22. 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.
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and nociceptin in rats. Peptides. 2011 Aug;32(8):1661-5. Epub 2011 Jul 7
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27. 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.

*Reviews 2009-2011*

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2. Hallberg M. (2009) Targeting insulin-regulated aminopeptidase/AT4 receptor for cognitive disorders. Drug News Perspect. 22:133-139.
3. Nyberg F, Hallberg M. Localization of neuropeptides by radioimmunoassay. Methods Mol Biol. 2011;789:191-201.
4. Hallberg M. Impact of anabolic androgenic steroids on neuropeptide systems. Mini Rev Med Chem. 2011 May;11(5):399-408. Review.

*Agencies that support the work/Funding 2011*

Swedish Research Council Medicine
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*

Fred Nyberg: Director of Research Issues at the Swedish National Drug Policy Coordinator 2002-2007,. 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 Editorial Board of Scientific journals (Fred Nyberg): 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
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

Fred Nyberg: PI at the Uppsala Berzelii Technology Center for Neurodiagnostics (100 milj, SEK 2006-2015); PI at the Linne project Impact of Religion: Challenges for Society, Law and Democracy (50 milj. SEK 2008-2017); PI at the FAS supported project on alcohol effects on cognitive functions (12 milj. 6 years).
Swedish Research Council/Medicin for peptidergic mechanism in the develop-

ment of drug dependence: 1.95 mil, SEK 2009-2011 (2009 additional grant 2.3 milj SEK for 2009) and 2.4 milj SEK for 20012-2014.

## Projects

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

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

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

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

(V) 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 include opioid receptors and their endogenous ligands - opioid peptides dynorphins, enkephalins, and endorphins. These systems are critical for regulation of pain processing, modulation of reward induced by intake of addictive substances and stress-induced behavioral responses. Expression of the opioid genes is altered in the brain of drug abusers and psychiatric patients, and allelic variations in promoters of these genes are associated with cocaine abuse, epilepsy and affective disorders. Our general aim is to characterize the opioid systems at the molecular and cellular levels and to elucidate the role of molecular changes in these systems in addictive, pain, and psychiatric disorders. The focus is on the regulation of the prodynorphin gene transcription by epigenetic mechanisms including DNA methylation and chromatin modifications.

*Members of the group during 2011*

Georgy Bakalkin, PhD, Professor
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Igor Bazov, PhD student
Hiroyuki Watanabe, PhD, Postdoctoral scientist
Richard Henriksson, PhD student
Malik Mumtaz Taqi, PhD student
Muhammad Zubair Hussain, PhD student
Olga Kononenko, PhD student
Olga Yamskova, PhD, Postdoctoral scientist

*Publications 2009-2011*


1. 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]
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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.

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16. Kuzmin A, Jerlhag E, Liljequist S, Engel J. Effects of subunit selective nACh receptors on operant ethanol self-administration and relapse-like ethanol-drinking behavior. *Psychopharmacology (Berl)*. 2009, 203, 99-108.

*Reviews 2009-2011*

1. 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.
2. Tan-No K, Takahashi H, Nakagawasai O, Niijima F, Sakurada S, Bakalkin G, Terenius L, Tadano T. Nociceptive behavior induced by the endogenous opioid peptides dynorphins in uninjured mice: evidence with intrathecal N-ethylmaleimide inhibiting dynorphin degradation. *Int Rev Neurobiol*. 2009, 85: 191-205.

*Dissertations 2011*

1. Malik Mumtaz Taqi
*Mechanisms of Prodynorphin Gene Dysregulation in the Brain of Human Alcoholics.*
Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy, ISSN 1651-6192; 147
http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-158235

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The Swedish Research Council
The Swedish Council for Working Life and Social Research
The Swedish Institute (Visby grant for collaboration with Ukraine)

*Other commitments/assignments of staff members*

Editor in Addiction Biology journal

## Projects

*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.

# Drug Safety and Toxicology

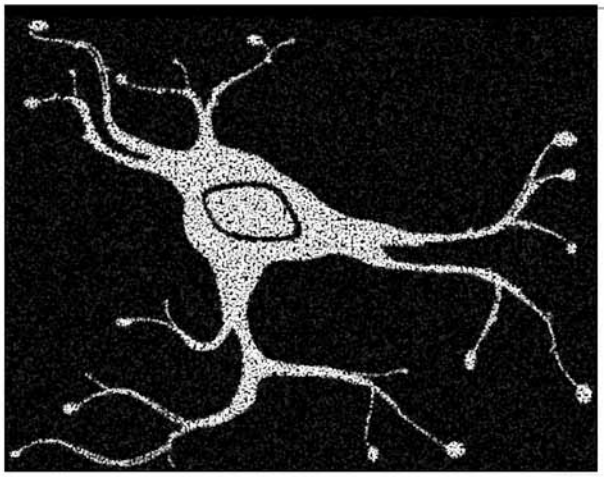
We are studying mechanisms of adverse effects of environmental contaminants, commercial and novel pharmaceuticals, by the use of various in vitro and in vivo models. The goal is to understand the causative effects at a morphological, biochemical, protein and gene regulatory level.

The research is focused on the following areas:

- Developmental toxicology (L Dencker)
- Bioactivation and toxicity (E Brittebo)
- Genetic toxicology (B Hellman)
- Neurotoxicology (M Andersson)

## Developmental toxicology

*Lennart Dencker*



*Members of the group during 2011*

Lennart Dencker, Professor
Bengt R Danielsson, Adjunct Professor
Michael Stigson, PhD, Researcher
Henrik Alm, PhD Researcher
Birger Scholz, PhD, Researcher
Måns Jergil, PhD, Researcher
Mats Nilsson, PhD student
Raili Engdahl, Technician
Lena Norgren, Technician

*Publications 2009-2011*


1. Alm, H., Scholz, B., Kultima, K., Savitski, MM., Nilsson A, Bergman Å, Andren, PE, Dencker, L., and Stigson, M.. In vitro neurotoxicity of PBDE-99: immediate and concentration-dependent effects on protein expression in cerebral cortex cells. *J. Proteome Res*. 2009; 9:1226-35.
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3. Kultima K, Nilsson A, Scholz B, Rossbach UL, Fälth M, Andrén PE. Development and evaluation of normalization methods for label-free relative quantification of endogenous peptides. *Mol Cell Proteomics*. 2009; 8:2285-95
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6. 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.

7. 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.
8. 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.
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10. 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.
11. 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 2009-2011*

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

*Supporting the work/Funding 2011*

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*

*Lennart Dencker*
Vice Chairman, Domain of Medicine and Pharmacy at Uppsala University -2011
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

*Culturing embryos and image analysis*

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). This is performed in collaboration with Professor Ewert Bengtsson at the Centre of Image Analysis, Uppsala University. 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 analyzing the developmental toxicity effect of drugs on heart rate in WEC conditions.

To improve scoring and data handling in the assessment of WEC, we are also developing and anatomical ontology and controlled vocabulary (Rodent Organogenesis Toxicology Ontology, ROTO) that incorporates early stages of possible malformation phenotypes observed during organogenesis (*in vivo* and *in vitro*). These approaches will, when appropriate, be combined with our molecular biology data (functional genomics) and enable an improved monitoring of the "symphony" and its different tunes concerted by the collected expression of genes and proteins governing embryonic development and brain maturation.

*Stem cells for teratogenicity 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 inhibitor (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, a larger battery of teratogenic and non-teratogenic compounds are tested with respect to their gene (de)regulation in murine embryonic stem cells, a project carried out in collaboration with AstraZeneca.. 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. A third cell type, mesenchymal stem cell-like pericytes isolated from human full term placentas are also being evaluated for cellular and molecular VPA effects. The later project was performed in the context of a EU FP6 project (ReproTect) and in collaboration with Dr Christian Sundberg at IMBIM, Uppsala University and ended during 2009. We are at the same time exploring the global epigenomic effects of VPA:s HDACi capacity in collaboration with Professor Claes Wadelius at Rudbeck laboratory. Here, we have used chromatin immunoprecipitation (ChIP) on chip (ChIP-chip) to study histone modification changes in the model system (human hepatoma cell lines) used by Wadelius group as a precursor for later studies in a more embryonic context. These studies have shown us that VPA as an HDAC inhibitor has unsuspected complex genome wide effects outside the prediction of the literature so far by removing large regions of histone acetylation instead of promoting it. This project was ended during 2009.

*Astrocyte-Neuron Interactions in Developmental Neurotoxicity*

This project examines the extent to which astrocyte-neuron interactions influence normal brain development, including the way in which developmental neurotoxicants, such as Polybrominated diphenyl ethers (PBDEs), can hamper the cross-talk between astrocytes and neurons during brain development. Neurite outgrowth is a fundamental event in brain development, and astrocyte-neuron interactions play a key role in this process, with astrocytes releasing factors that promote neurite outgrowth. 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. An new but integral part of this project is to look up-stream of these effects and investigate the effects of PBDEs on fate determination in neural stem/progenitor cells (NSPCs) from fetal rats. In the presence of fibroblast growth factor-2 (FGF-2), the NSPCs maintain the cellular multipotency. When these factors are withdrawn, NSPCs gradually lose their expression of the NSPC marker nestin, and form mature CNS neurons, astrocytes, and oligodendrocytes. Using this set-up combined with PBDE exposure, these studies aim to evaluate whether select PBDEs can alter NSPC differentiation and maturation.

*Epigenomic analysis of neurodevelopmental toxicity mechanisms*

It is well established by now that the neonatal brain growth spurt period in rodents is sensitive to environmental influences such as exogenous disturbances. This early exposure commonly leads to potentially irreversible alterations in adult brain function. An important challenge is to identify the molecular basis of the disruptions that lead to irreversible changes in adult behavior. We believe that there is a strong epigenetic component involved in the transference of early effects to late phenotypes. This research project aims at identifying structural and stable epigenomic changes during brain development from neonatal age (PND10) to adult age (4 months), with or without exposure to exogenous chemicals. From a toxicological perspective we have so far focused on PBDE and Ketamine induced effects on DNA methylation.

*Mechanistic studies of CRABP1 and the relation to normal development of the embryo*

Retinoic Acid (RA) and derivatives therof 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 newborns exposed to RA during pregnancy. We and others have previously shown that the same tissues and cells that accumulate radioactively labeled RA and its analogues also express high levels of the protein CRABP1. The high expression of CRABP1 in the cells sensitive to the teratogenic effects of RA resulted in an idea that this protein was a part of the mechanism with which RA induces malformations in these structures. One theory was that the protein binds RA intracellularly, and thereby regulates the activation of nuclear receptors.

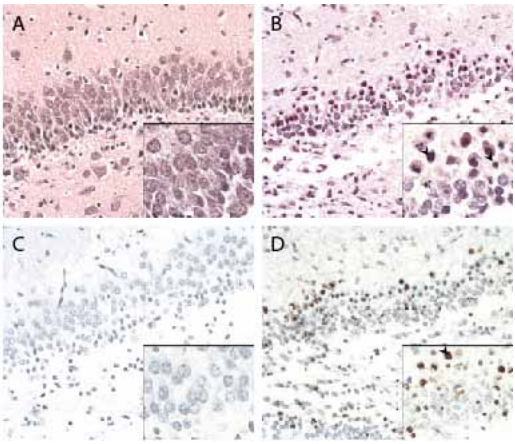
It was therefore somewhat surprising that mice not expressing this protein showed the same sensitivity to RA-induced teratogenicity as mice expressing the protein. This suggests that the higher sensitivity of CRABP1-expressing cells is independent of CRABP1 expression. However, CRABP1 is the most important intracellular transporter of Vitamin A and surely interacts with several other proteins to regulate normal as well as teratogenic activation of neuclear receptors for Vitamin A. Which proteins and how they interact with CRABP1 is currently unknown. Using CRABP1 knockout mice, this project aims at characterizing potential interaction partners and their involvement in malformations induced by retionoids.

*Other projects*

Members of the group have been involved in projects carried out on the BMMS in collaboration with groups in France and Great Britain (funded by the Michael J Fox foundation). This has resulted in publications on L-Dopa induced dyskinesia in a non-human primate parkinsonian model, were we report the proteomic changes in the striatum induced by both neurotoxin induced Parkinsonism (PD) and the effects of de-novo L-Dopa treatment and long-term treatment leading to dyskinesia. Our data points to a before now unprecedented and long term impact of the first de-novo L-Dopa dose in PD individuals. There are also ongoing studies that look closer of the impact of sample handling and the postmortem interval time on protein sample quality and tissue specific degradomes. The later is of clear importance for interpreting proteomics and biomarker data and the establishment of sample handling procedures in clinical biobanks.

## Bioactivation and Toxicity

*Eva Brittebo*




*Detail from the hippocampus of BMAA-treated neonatal rats. The control rat (A, C) shows normal cellularity whereas the BMAA-treated rat (B,D) displays marked cellular loss and apoptosis.*

The studies are directed towards characterisation of toxicant-induced changes 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 human endothelial dysfunction. In addition, the delivery of therapeutic agents to the brain via nasal administration is being examined.

### *Members of the group during 2011*

Eva Brittebo, Professor
Nils Gunnar Lindquist, Adjunct Professor
Helén Andersson, PhD Student
Oskar Karlsson, PhD Student
Elena Piras, PhD Student

### *Publications 2009-2011*

1. 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.
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4. Karlsson O, Berg C, Brittebo EB, Lindquist NG. Retention of the cyanobacterial neurotoxin beta-N-methylamino-l-alanine in melanin and neuromelanin-containing cells--a possible link between Parkinson-dementia complex and pigmentary retinopathy. Pigment Cell Melanoma Res. 2009;22:120-30.
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6. Andersson H, Piras E, Demma J, Hellman B, Brittebo E. Low levels of the air pollutant 1-nitropyrene induce DNA damage, increased levels of reactive oxygen species and endoplasmic reticulum stress in human endothelial cells. Toxicology. 2009;262:57-64.
7. Karlsson O, Lindquist NG, Brittebo EB, Roman E. Selective brain uptake and behavioral effects of the cyanobacterial toxin BMAA (beta-N-methyl-amino-L-alanine) following neonatal administration to rodents. Toxicol Sci. 2009;109:286-95.

*Dissertations 2011*

1. Oskar Karlsson
   *Distribution and Long-term Effects of the Environmental Neurotoxin β-N-methylamino-L-alanine (BMAA) : Brain changes and behavioral impairments following developmental exposure.*
   Uppsala: Acta Universitatis Upsaliensis; 2010. Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy, 134.
   http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-140785
2. Helén Andersson
   *Experimental Studies of Endocrine Disrupting Compounds in Vascular Cells and Tissues.*
   Uppsala: Acta Universitatis Upsaliensis; 2011. Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy, 150.
   http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-160662

*Agencies that support the work/Funding 2011*

The Research Council FORMAS

*Other commitments/Assignments of staff members*

Head of the Department
Member of the panel at the Norwegian Research Council ranking grant applications in Environment, genetics and health
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. Another environmental pollutant, 1-nitropyrene, is one of the most abundant organic compounds in diesel exhaust and is likely to contribute to the harmful effects of air pollutants in the cardiovascular system. Our results reveal that exposure to 1-nitropyrene resulted in DNA damage and cell stress in HUVEC, as indicated by increased DNA fragmentation, increased production of reactive oxygen species and elevated expression of cell stress proteins. The results also suggest that the effects of 1-nitropyrene on HUVEC were caused by metabolites formed by nitroreduction.

In another project we have investigated the cell specific expression of drug metabolising CYP enzymes and the effects of tamoxifen in the highly vascularised hu-

man 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. 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 non-protein amino acid that is produced by cyanobacteria present in terrestrial and aquatic environments including temperate aquatic ecosystems. 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 also identified major changes 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. 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*

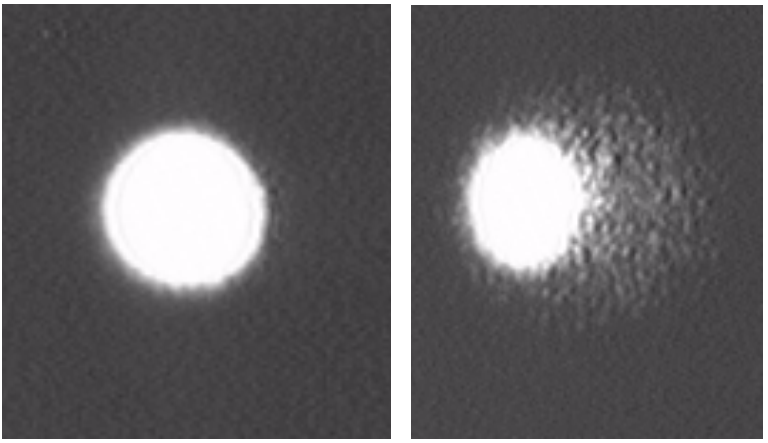
The nasal olfactory pathway is a potential route of delivery of therapeutic agents which do not easily pass the blood-brain barrier. The olfactory neurons have direct contact with the external environment via dendrites in the nasal mucus and with the brain via axons that reach the olfactory bulb without 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. Recent studies have demonstrated that nasal administration of genetically engineered cells such as T regulatory cells or multipotent mesenchymal stromal cells reduced CNS inflammation and behavioural changes in mice with EAE – a mouse model of multiple sclerosis. These studies were performed in collaboration with associate professor Angelica Loskog at the Dept. of Immunology, Genetics and Pathology at Uppsala University.

## Genetic Toxicology

*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, supported by SIDA/SAREC, we are evaluating the genotoxic and antigentoxic effects of some plant extracts used in traditional medicine in Ethiopia, and in these studies we also include fractions of extracts and/or pure compounds from extracts. One example of this is our recent study on plumbagin (Demma et al., 2009), a naphtoquinone present in the roots of Plumbago zeylanica, a traditionally used medicinal plant that has been reported to have many beneficial effects but also many side effects. The potential genotoxicity and antigenotoxicity of plumbagin was evaluated in mouse lymphoma L5178Y cells. Without affecting the cell viability, plumbagin itself induced significant DNA damage at concentrations as low as 0.25 ng/ml. When the cells were exposed to non-DNA damaging concentrations of plumbagin, together with NQNO (known to interact with DNA in many different ways) or catechol (known to induce oxidative DNA damage), plumbagin was found to significantly reduce the catechol-induced DNA damage, but to be without protective effect against the NQNO-induced damage. These findings provides further support for the idea that plumbagin may act as an antioxidative agent at low non-DNA damaging concentrations.

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 2011*

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

*Publications 2009-2011*

1. L. J. K. Durling, L. Busk & B. Hellman. Evaluation of the DNA damaging effect of the heat-induced food toxicant 5-hydroxymethylfurfural (HMF) in various cell lines with different activities of sulfotransferases. *Food. Chem. Toxicol*, 47(2009)880-884.
2. J. Demma, E. Engidawork & B. Hellman. Potential genotoxicity of plant extracts used in Ethiopian traditional medicine. *J. Ethnopharmacol.*, 122(2009)136-142.
3. J. Demma, K. Hallberg & B.Hellman. Genotoxicity of plumbagin and its effects on catechol and NQNO-induced DNA damage in mouse lymphoma cells. *Toxicology In Vitro*, 23(2009)266-271.
4. H. Andersson, E. Piras, J. Demma, B. Hellman & E. Brittebo. Low levels of the air pollutant 1-nitropyrene induce DNA damage, increased levels of reactive oxygen species and emdoplasmatic reticulum stress in human endothelial cells. *Toxicology*, 262(2009)57-64.

*Reviews 2009-2011*

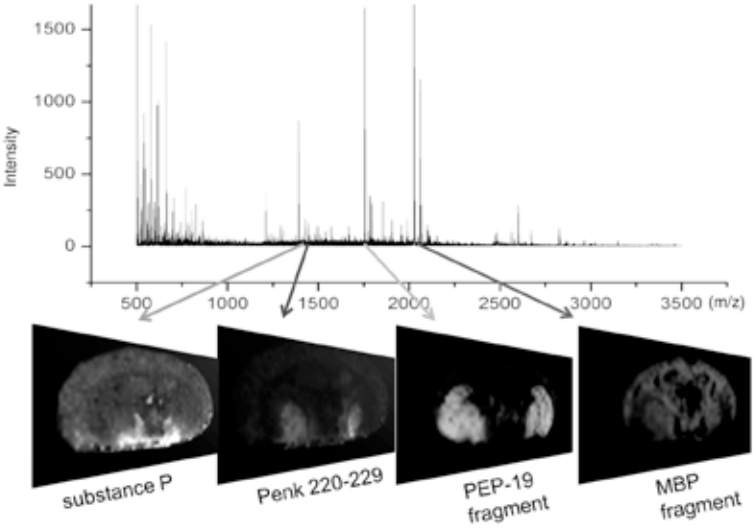
1. B. Hellman & K. Broberg. Genetiska förändringar som biologiska markörer för exponering, sjukdom och känslighet. I boken: Arbets- och miljömedicin; 3:e upplagan. Redaktörer: C. Edling, G. Nordberg, M. Albin & M. Nordberg, Studentlitteratur, Lund; 2010.

*Other commitments/assignments of staff members*

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
Study director in toxicology

## Neurotoxicology

*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 2011*

Malin Andersson, Assistant professor
Jörg Hanrieder, post-doc

### *Publications 2009-2011*

1. Svensson. M., Borén, M., Sköld, K., Fälth, M., Sjögren, B., Andersson, M., Svenningsson, P., Andrén, P. (2009) Heat stabilization of the tissue proteome: a new technology for improved proteomics. J Proteome Res. 2009 Jan 21
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*Agencies that support the work/Funding 2011*

Swedish Research Council, Disciplinary Domain of Medicine and Pharmacy
The Royal Swedish Academy of Sciences
The Royal Swedish Academy of Sciences
Astrid och Gustaf Kaleens fond
Åke Wibergs Stiftelse
Parkinsonfonden

*Other commitments/assignments of staff members*

Director of studies for the Biomedicine Program (180 hp)

## Projects

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

*Anna Karlsson, Jörg Hanrieder, Maria Fälth, Jonas Bergquist, and Malin Andersson*
In collaboration with Professor Jonas Bergquist (Dept. Analytical Chemistry, UU) and Maria Fälth (PhD, German Cancer Research Center, Heidelberg, Germany) we study neuropeptides involved in the development of L-DOPA-induced dyskinesias in experimental Parkinson's disease. For example, Prodynorphin mRNA levels correlate well with the severity of dyskinesia in animal models of Parkinson's disease; however the identities of the actual neuroactive opioid effectors in their target basal ganglia output structures has remained unknown. Here we use MALDI-TOF imaging mass spectrometry (IMS) for unbiased assessment and topographical elucidation of dynorphins and prodynorphin-derived peptides 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 could be detected in these studies, including dynorphin B, dynorphin A(1-8), alpha-neoendorphin, MetEnkRF, MetEnkRGL, PEnk (198-209, 219-229).

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

*Jörg Hanrieder, Grzegorz Wicher, Jonas Bergquist,* Åsa Fex-Svenningsen *and Malin Andersson*

In collaboration with Ass. Prof. Åsa Fex-Svenningsen (University of Southern Denmark, Odense, Denmark) and Prof. Jonas Bergquist (Dept. Analytical Chemistry, UU).

This study examines the use of differential protein expression profiling of mammalian neural cells using direct analysis by means of MALDI TOF MS. MALDI MS 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.

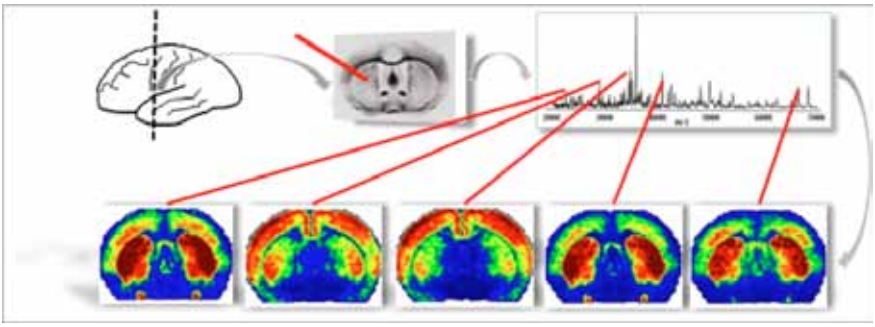
*Assessment of brain neuropeptides and proteins after neonatal exposure to the environmental toxin BMAA using MALDI imaging mass spectrometry.*

*Oskar Karlsson, Eva Brittebo and Malin Andersson*

In collaboration with Oskar Karlsson (PhD) and Prof. Eva Brittebo (Drug Safety and Toxicology, UU), and Prof. Jonas Bergquist (Dept. Analytical Chemistry, UU). Exposure to the nonprotein amino acid βN-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

*Per Andrén*



*Imaging Mass Spectrometry 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 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 IMS 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 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 (one Q-Tof mass spectrometer (Waters), one LTQ mass spectrometer (Thermo), one

Tof-Tof mass spectrometer (Bruker). In addition, VR-KFI (grant 2009-6050) has recently funded two mass spectrometers (Ultraflextreme and Maxis Impact, Bruker Daltonics), specifically for the MS imaging applications.

*Members of the group during 2011*

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

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2. 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.

*Agencies that support the work/Funding 2011*

The Swedish Research Council (VR), NT.
The Swedish Research Council (VR)-NT. Post-doc Position (Mohammadreza Shariatgorji)
The National Institute of Health (NIH)/the National Institute on Drug Abuse (NIDA).
VINNOVA. Japan Society for the Promotion of Science (JSPS) Joint Projects 2011.
VINNOVA. Marie Curie Chair (Cecilia Eriksson).

AstraZeneca, Global DMPK, Safety Assessment (GSA)
Denator AB, Göteborg, Sweden.

*Other commitments/assignments of staff members*

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), Member of the Board General Council, Chairman for Imaging Mass Spectrometry Initiative
Cooperation in Science and Technology (COST), Mass Spectrometry Imaging: New Tools for Healthcare Research Infrastructure Member of the Board

## Projects

*Neurochemical characterization of basal ganglia neuropeptides and proteins in levodopa-induced dyskinesia in experimental Parkinson's disease using Imaging Mass Spectrometry and Peptidomics*

*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.

*Integration of resources and studies to elucidate neuropeptide signaling.*

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

*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.

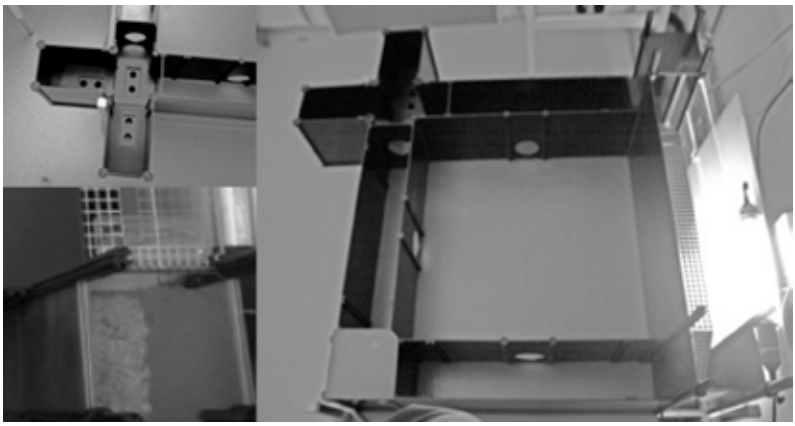
*Novel inactivation technology stabilizes the in vivo levels of proteins, peptides, phosphorylations, lipids in tissue samples*

*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

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



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. Recent development includes the successful establishment of a number of behavioral models within the field of neuroscience and neuropharmacology and this work, led by Erika Roman, particularly involves ethoexperimental models and methods using a multivariate approach.

The concept of ethoexperimental studies of behaviour promotes the advantage of integrating ethology and experimental psychology. With this approach, the aim is to use test conditions and procedures that are based on the circumstances and challenges the animal meets under natural conditions. The behaviour laboratory is under continuous development with regard to tests and techniques and is used by a number of groups within the Faculty of Medicine and Pharmacy. Besides the listed projects, advice is given and collaborations are established in projects related to behavioural neuroscience. The laboratory comprises ethologically founded tests, including tests for assessment of neonatal development, exploratory behaviour, locomotor activity, anxiety-like behaviour, learning and memory and a multivariate test arena (the multivariate concentric square field™, MCSF) and utilizes multivariate statistical approaches.

The MCSF test (illustrated in the figure) is designed to include opportunity for exploration, risk assessment, risk taking, shelter seeking and approach and avoidance behaviour in rodents. The guiding principle of the MCSF test is that it is unprejudiced, i.e. the test is not designed to measure a particular mental condition. Instead the test situation involves a free choice of different environmental settings and items that provide the opportunity to assess essential features of the animal's mentality. In this way a behavioural profile is generated in one and the same test session. The MCSF arena is also useful in studies of reward motivated behaviours and learning and memory.

Specific research activities within the group are described shortly under projects. Current projects include studies of neurobiological substrates for individual differences in addiction processes, especially vulnerability for risk consumption of alcohol, 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. It is hypothesized that disruption of early developmental processes in transmitter

networks either by rearing factors or drug intake early in life, cause long-term changes in behavior that, in turn, affects alcohol consumption later in life. Animal experimental models in combination with extensive evaluation of neurobiological and behavioral consequences of different early environmental conditions are used in the projects.

Another line of research investigates the link between neurosteroids and neurodegenerative disorders, like Alzheimer´s disease (AD). The role of neurosteroids for neurogenesis and for interactive processes that is ongoing in neurodegenerative disorders is studied, with emphasis on neuroprotective properties of neurosteroids against amyloid-b-induced toxicity.

*Members of the group during 2011*

Ingrid Nylander, Professor
Lena Bergström, Associate Professor
Erika Roman, Associate Professor
Anne-Lie Svensson, Lecturer
Loudin Daoura, PhD student
Shima Momeni, PhD student
Sara Palm, PhD student
Linnea Granholm, Research Assistant
Bengt J Meyerson, Professor Emeritus
Marita Berg, Technician

*Publications 2009-2011*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13. 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*
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25. Svensson A, Nikotin. In Beroendemedicin (Franck & Nylander, Eds.), Studentlitteratur, 2011
26. Nylander I, contributing author in "Steget före" – forskare om hur framtidens vuxna mår idag. Pocketerad populärvetenskap, Vetenskapsrådet, 2009

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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; Daoura, Palm and Roman)
Magnus Bergwalls Stiftelse (Bergström)
Åhlén-stiftelsen (Bergström)
Lars Hiertas Minnesfond (Bergström)
Gun och Bertil Stohnes stiftelse (Svensson)
Åhlén-stiftelsen (Svensson)

*Other commitments/assignments of staff members*

*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
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

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## Projects

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

*Loudin Daoura, Sara Palm, Stefan Sclussmann,* ***Ingrid Nylander***
We have previously shown that adverse experiences early in life cause long-term neurobiological and behavioral alterations. These changes may contribute to the increased vulnerability for drug addiction that is seen in the clinic. The environment may also provide protection, for instance 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. Rat pups are separated from the caregiver short

or prolonged periods during the first postnatal weeks. Shorter MS are similar to natural conditions where the mother regularly leaves the litter for shorter periods of time. Prolonged MS interfere with early social interactions and are used to simulate an emotional stressful environment for the rat pups and/or the mother. These rearing conditions are associated with resilience (after short MS) and 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. They have low basal opioid levels and enhanced response to alcohol in a voluntary alcohol consumption paradigm in addition to other characteristics relating to high alcohol preference. 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. However, free access to alcohol during adolescence resulted in high alcohol intake regardless of rearing conditions. The consequences of adolescent voluntary alcohol consumption on neuropeptides in individuals subjected to different rearing conditions are currently examined.

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

*Sara Palm, 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. FAST is not suitable for analysis of peptides and for that purpose in vivo microdialysis will be used to measure peptides in the extracellular fluid and relate to the assessments of dopamine.

### *Behavioral 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 behavioral consequences of rearing in different environmental settings and of long-term alcohol consumption. In ongoing studies the behavioral effects of adolescent voluntary alcohol drinking are examined. The project comprises development of animal experimental models to assess maternal behavior, neonatal adolescent and adult behavior. In addition, behavioral profiling using the MCSF test is employed to examine the phenotype of different rat strains as complement to the assessment of voluntary alcohol consumption and neurobiological analysis. With various behavioral tests the effects are analyzed at different ages. Individual behavioral 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 behavior later in life. It is also examined whether and how altered behavior 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, ii) develop plug-in units for studies of motivated behaviors and expanding the use of the MCSF for studies of learning and memory, and iii) at developing an automatic tracking and scoring system.

### *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 modeled 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 behavioral traits as seen in human alcohol dependent individuals. This project involves behavioral 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 behavior 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 behavior 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 behavioral approach with an ethological foundation that incorporates several aspects of the behavioral repertoire and evolutionary conserved behaviors is used. The hypothesis is that high risk-taking behavior exerts one aspect of impulsive behavior of importance for liability for excessive alcohol intake and also affects the response to drug treatment.

### *Neurosteroids and Alzheimers´s disease: Mechanistic studies of neuroprotection and amyloid-β-modulation*

*Linnea Granholm,* ***Anne-Lie Svensson***

Neurosteroids are produced in brain in the presence of steroidogenic enzymes. Specific neurosteroids are endogenous modulators of neuronal functions responsible for many biological and pathophysiological effects. Some neurosteroids might have important roles in cognitive functions. 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. However, the mechanisms of their mode of action at cellular and molecular level are not well understood. 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. Amyloid-β has been implicated in cell death during the course of AD and exerts toxic effects on neurons both *in vivo* and *in vitro*. An important goal of the therapeutic strategies of AD is to identify compounds able to prevent Aβ formation, aggregation and thereby prevent protofibril formation. 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. The aim of this project is to more in depth further study neuroprotective properties of neurosteroids and their metabolites, against amyloid-β-induced toxicity, as well as the underlying molecular mechanism(s), with focus on neurogenesis and apoptosis.

# Pharmacokinetics/Pharmacodynamics

*Margareta Hammarlund-Udenaes and Sven Björkman*

Our research aims to improve the understanding of drug distribution and elimination in relation to drug effects. 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. Our work emphasizes the importance of bridging fundamental academic principles and pharmaceutical industry expertise to seek excellence in method development and translational research for better therapeutic agents curing CNS disorders. Pharmacokinetic and pharmacodynamic principles are also applied to the clinical use of drugs, in order to design rational dosage regimens.

*Members of the group during 2011*

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*Agencies that support the work/Funding 2011*

Janssen Pharmaceuticals
Swedish Research Council

*Other commitments/assignments of staff members*

Margareta Hammarlund-Udenaes: Associate Editor of Pharmaceutical Research, EAB member of Journal of Pharmaceutical Sciences and of Fluids and Barriers of the CNS, incoming Chair of the Gordon Conference on Barriers of the CNS in 2014.

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.

## Projects

*Blood-brain barrier transport of drugs – mechanisms and methods*

*Margareta Hammarlund-Udenaes, Sven Björkman, Annika Borgs, Sofia Gustafsson, Muhammad Waqas Sadiq, with collaborators Markus Fridén and Ulf Bredberg (AstraZeneca), Yoshiharu Deguchi (Teikyou Univ, Japan), Tetsuya Terasaki (Tohoku Univ, Japan), Mehran Salehpour and Göran Possnert (Uppsala Univ Angstrom lab), Pieter Gaillard and Jaap Rip (to-BBB, the Netherlands).*

Our research is focused on understanding how the blood-brain barrier (BBB) functions regarding drug transport to the brain in health and disease, and to optimize methods to measure brain penetration of drugs, including translational aspects to humans. The research focuses on unbound drug concentrations and is important for the drug industry that has problems in finding good drug candidates for brain diseases, partly due to a lack of understanding of which parameters to optimize for. We have contributed significantly to define key parameters that describe BBB transport of unbound drugs. The thesis work of Markus Fridén, defended in

October of 2010, was instrumental in the development of rapid methods for this purpose. Positron Emission Tomography (PET) is used as a way of understanding BBB transport of drugs from a translational perspective. We are also studying active uptake across the BBB of drugs like oxycodone and diphenhydramine, and their interactions. The transporter responsible for this uptake is investigated in research collaboration with researchers in Japan. A specific area of interest is studying the pharmacokinetic role of nanoparticles in possible improvement of peptide transport to the brain. Publications during 2011 have centered on in vitro methods for estimating blood-brain barrier transport and brain distribution parameters in early discovery, and of drug interaction studies at the BBB.

### *Clinical Pharmacy Research*

*Margareta Hammarlund-Udenaes and Ulrika Gillespie, with collaborators Håkan Melhus (Clinical Pharmacology, Uppsala), Claes Mörlin (Medicine, 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.

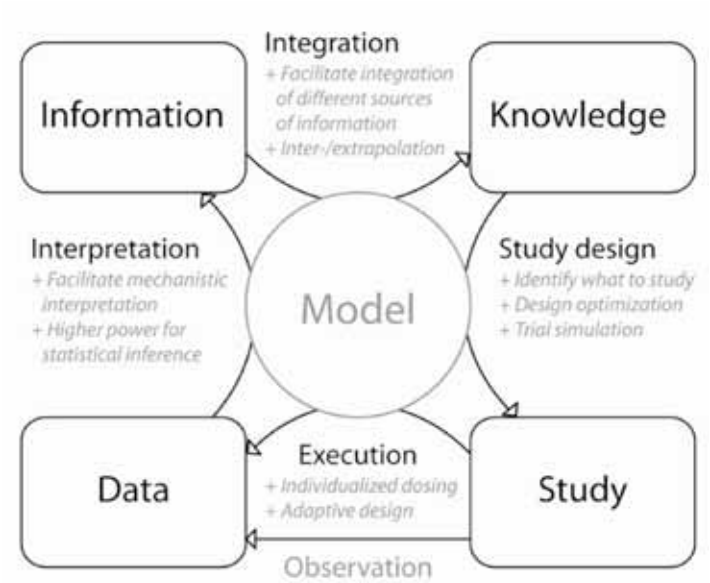
### *Clinical pharmacokinetics of coagulation factors VIII and IX*

*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 2011 included:

- Testing the feasibility to dose-tailor haemophilia prophylaxis by daily self-injection of factor concentrate.
- Evaluating the PK and clinical information obtained during extensive licensing studies on a novel factor VIII preparation, Advate (Baxter Inc.).
- Examination of the disposition and dosing requirements of factor VIII (Advate) as a function of age of the patient by means of population PK modelling.
- 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 plasma-derived factor IX.
- Disseminating knowledge of PK dose tailoring to physicians, at national and international meetings and courses and through writing of reviews and commentaries.

# Pharmacometrics

*Mats Karlsson*




*Pharmacometric model based scientific learning. Learning in science is a circular process: A study/experiment is initialized to address one or several missing pieces of information. The design of the study is based on previous knowledge and the observations made according to that design constitute the study data. Interpretation of study data by statistical summarization and comparison generate new information out of the raw data. By integrating different sources of information from the study with previous information new knowledge is generated. Based on the extended knowledge new studies can be designed to address other pieces of missing information. Advantages with a model based approach to scientific learning are pointed out with "+" in the figure. From: Martin Bergstrand. Application of Mixed-Effect Modeling to Improve Mechanistic Understanding and Predictability of Oral Absorption Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy, http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-149314*

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.

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### *Dissertations 2011*

1. Paul Baverel
   *Development and Evaluation of Nonparametric Mixed Effects Models*
   Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy, ISSN 1651-6192; 136
   http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-144583
2. Elisabet I. Nielsen
   *Pharmacometric Models for Antibacterial Agents to Improve Dosing Strategies*
   Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy, ISSN 1651-6192; 138
   http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-144909
3. Martin Bergstrand
   *Application of Mixed-Effect Modeling to Improve Mechanistic Understanding and Predictability of Oral Absorption*
   Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy, ISSN 1651-6192; 142
   http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-149314

4. Angelica L. Quartino
   *Pharmacometric Models for Improved Prediction of Myelosuppression and Treatment Response in Oncology*
   Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy, ISSN 1651-6192; 144
   http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-150431
5. Elodie L. Plan
   *Pharmacometric Methods and Novel Models for Discrete Data*
   Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy, ISSN 1651-6192; 145
   http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-150929
6. Joakim Nyberg.
   *Practical Optimal Experimental Design in Drug Development and Drug Treatment using Nonlinear Mixed Effects Models*
   Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy, ISSN 1651-6192; 149
   http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-160481

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### *Other commitments/assignments of staff members*

Lena Friberg, Organizing committee, chair scientific program, PAGE conference, Athens
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
Mats Karlsson, Deputy Head of Department
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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
Ulrika Simonsson, Board Member, Swedish Academy of Pharmaceutical Sciences, Section for Pharmacokinetics and Drug Metabolism
Ulrika Simonsson, Member of Department Policy Group

## Projects

### *Clinical modelling of pharmacokinetics in HIV, TB and malaria therapy*

*Mats Karlsson, Maria Kjellsson, 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 subtherapeutic 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.

### *Development of integrated models for Type II 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 as well as inclusion of exogenous insulin for insulin treated patients.

Medium 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 reaction depending on the plasma glucose concentration; the higher the more glucose available in plasma,, the higher the fraction. As the life-span of red blood cells ranges from 2 to 4 months, the HbA1c supplies a measurement of the glycemic 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 assessing the risk of CVD in relation to elevated levels of HbA1c or FPG. We are developing repeated time-to-event models, using registry data, quantifying this 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 able to quantify changes in the system following interventions (drug administration, diet changes, etc) and associate these 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.

### *Dose individualisation in transplantation*

*Siv Jönsson, Kristin Karlsson, 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. At present we are outlining the relationship between plasma drug concentration and biomarkers/clinical endpoints in order to allow better decision criteria for dose adjustments.

### *Multiple sclerosis*

*Mats Karlsson, Rada Savic*

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. Because the clinical manifestations and pathological consequences of disease as well as treatment response to existing therapies vary dramatically from individual to individual, these differences pose unique challenges for therapeutic interventions in terms of delineating specific targets related to disease mechanisms and developing safe and effective interventions for clinical application. Efforts to implement biomarker strategies for the divergent MS patient populations underscore the need for improved understanding of precise factors involved in disease onset and progression. Substantial work has been done trying to identify relevant Magnetic Resonance Imaging (MRI) readouts that can predict relapses and disease progression. Prior to this project, there has not been work that links the dynamics of the clinical course of the disease with the time course of MRI data or lymphocyte counts that are responsible for evolution of relapses. This was probably due to numerous challenges associated with the types of data available and the limitations of traditional statistical methods used to analyse these data.

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.

Anticipated challenges of this work include application and further development of state-of-the-art methodologies in the area of nonlinear mixed effects techniques that are required for successful development of the model. They are: (i) methodology for simultaneously analysing of continuous, categorical/count and time to event data in the non-linear mixed effects framework; (ii) application of the mixed Hidden Markov Model for describing exacerbations or flares in relapsing MS patients, and (iii) application of powerful new algorithm for maximum likelihood parameter estimation in the complex system - stochastic approximation of expectation-maximization (SAEM) algorithm coupled with Markov Chain Monte Carlo (MCMC) and Simulated Annealing.

### *Pharmacodynamic modelling of discrete outcomes*

*Mats Karlsson, Elodie Plan, Andrew Hooker*

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 underdispersion and serial correlation. Daily numbers of seizures have been used in the investigation of overdispersion 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*

*Mats Karlsson, Martin Bergstrand*

Clinical pharmacokinetic experiments typically measures drug concentrations in plasma only. As a consequence, pharmacokinetic models typically used in drug development aim to describe, with the minimum model complexity, these observations of drug concentration in plasma. 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.

The impact of induction properties for a drug candidate in drug development or for a drug already in clinical use can influence the use of the drug itself and have major impact on the metabolism and transport of other drugs used in combination with the inducing drug. Clinical interactions studies with type substrates for specific enzymes e.g. midazolam (CYP3A4), caffeine (CYP1A2) and transport protein such as digoxin (Pgp) have been used as the basis for developing mechanism based induction models. The developed models have included the kinetics of induced enzyme(s) and transporters and the pharmacodynamics of the inductions. This approach has been successful in predicting pharmacokinetics in the presence of auto induction and the risk of potential drug-drug interactions. Prediction of the time course and extent of induction is complex. It depends on the half-life of the induced enzyme(s) and transporters, the pharmacokinetics and dosing regimen of the inducing agent, and the relationship between the plasma concentration of the inducer and extent of the induction. If it is to be possible to predict the activity of enzymes and transporters at any point in time during and after the induction, all of these aspects have to be understood. By developing mechanistic models, the key elements of these systems can be isolated, and their contribution to the induction

process can be determined. This is done by using methods to assess the activity of the enzymes and transporters *in vivo* by using different probes that have specific reactions for certain enzyme such as midazolam (CYP3A4), caffeine (CYP1A2) or that are transported by a specific transport protein such as digoxin (Pgp). *In vitro* data from liver microsomes can also successfully be incorporated into the models to explain and predict the kinetics of the drug and the risk of potential drug-drug interactions.

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, eg. 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* dissolutions data.

### *Oncology*

*Lena Friberg, Mats Karlsson*

Within the oncology area, we are working on PK and PD models describing the time-courses of biomarkers drug-induced toxicity, tumour response 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. We have several projects around extensions and applications of a semi-physiological model that can describe neutropenia for numerous anticancer drugs. Based on data from a clinical study that has been performed, a model, that integrates the G-CSF levels and neutrophil counts, has been developed to provide a better description of the feedback mechanism of G_CSF on neutrophils. IL-6 and AAG are also being evaluated for their predictive value in chemotherapy-induced neutropenia. Other extensions of the model have been explored, for example the application to platelet counts and dose recommendations for docetaxel-treated patients with reduced liver function.

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) to make early predictions of tumour response, toxicity and overall survival have been investigated. sVEGFR-3 was the best variable in predicting survival (better than tumour response) and the time-courses were shown to be better predictors than fixed time-points. However, absolute neutrophil counts, which are routinely measured, provide nearly as (indirect) good prediction of survival as sVEGFR-3. The models provide a framework fo simulation that will be useful for understanding which biomarkers to measure and which patients benefit from a continuation of the therapy The pharmacokinetics of conjugated antibodies (mixture of 8 different species) and the influence of transporters are also investigated for taxanes.

### *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. Semi-mechanistic models describing time-kill curves from *in vitro* experiments form the basis for the modelling, and the basic structure has been shown to be applicable across a number of drugs with different mechanisms of action as well as well fit data from both static and dynamic concentration experiments. 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, a model has been developed that can characterize the time-course of bacteria growth and kill for

wild-type and 6 well-characterized mutants. The effects of filamentation at early hours of exposure are also considered. Other complexities are being investigated such as competition of wild-type and mutants and effects of high inocula. 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 samples from *in vitro* experiments to determine the actual concentrations. Developed pharmacokinetic models for CMS and colistin in different subpopulations show that the drug is typically underdosed and a loading dose 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. Voriconazole dosing recommendations in children and adolescence have also been developed.

### *Clinical trial design*

*Andrew Hooker, Kristin Karlsson, Mats Karlsson*

There are two principle ways by which models can be used to help optimize trial designs. 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, thus 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 optimizing trial designs is through a more formal investigation of the landscape of possible designs (within constraints) potentially available for an experiment (as opposed to simply choosing a few designs to investigate through simulation, as described above). This method, known as optimal experimental design, often relies on calculations of an Information Matrix (e.g. the Fisher Information Matrix), which characterizes the information content of any possible design. A number of different criteria, based on these Information Matrices, can be used to optimize designs. We have developed methods and software (PopED) that use 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.

### *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 between occupancy, prolactin response, and extrapyramidal side effects are being investigated as well as the relationship to the disease state variable PANSS.

The possibility to apply the prolactin animal data to even further increase the *in vitro*-patient prediction is being investigated.

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. A longitudinal transition model describing the probability of ACR20, ACR50 and ACR70 responses have been developed.

The time course of sleep stages has been characterized and its relation to placebo and drug effects using Markov models in patients with insomnia. Good simulation properties of the model were demonstrated and simulations have been performed to investigate the efficacy of different dose levels.

The efficacy of anti-epileptic drugs are measured as number of seizures per day, i.e. count data. A Poisson model for the count data has been developed and the significance of including overdispersion and Markovian elements has been shown. 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 the disease area of, among others, insomnia, epilepsy, and gastro-oesophageal reflux have also been developed and are described in the section "Pharmacodynamic modelling of discrete outcomes".

### *Model building methodologies and estimation methods*

*Andrew Hooker, Mats Karlsson, Rada Savic*

This project aims at developing methods for pharmacometric model development and evaluation. 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 of using pharmacometric models to analyse data from clinical trials, for example the ability to handle sparse data and to integrate different types of observations into one model. 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 hundreds. One integral part of these research activities is the implementation and automation of important modelling tasks through the use of new algorithms developed in our research group. A second part of these research activities involves 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.

A second main problem for these complex models and complex 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.

We have an active research going on in the area of model evaluation and assessment of model fit. Significant amount of research was done and is on-going

to evaluate performance and usefulness of available graphical and numerical diagnostics as well as development of new improved diagnostic tools. Range of new methods and tools has been developed and evaluated to improve on model building of stochastic components of the model, with special emphasis on non-parametric estimation methods.

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.

One integral part of these 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).

# Pharmaceutical Bioinformatics

*Jarl Wikberg*

During the year the Bioclipse workbench (www.bioclipse.net) was further developed, with a focus on sustainability and core cheminformatics functionality. The new developments were mainly centered round the novel Bioclipse Decision Support, which is a platform and workbench for building and using predictive models. This was demonstrated on several toxicological endpoints including mutagenicity, AHR, and carcinogenicity as well as ADME. During late 2011, AstraZeneca acknowledged that: "Bioclipse Decision Support is now being used for ADME-T predictions within AstraZeneca R&D".

Bioclipse was also established as a workbench for the EU FP7 project OpenTox, and was used in several workshops and tutorials. The acceptance implies that European predictive toxicology models are made available from within Bioclipse. Our group also participated in the establishment of a toxicology ontology roadmap, in collaboration with the European Bioinformatics Institute, OpenTox, and 15 other institutions and major pharmaceutical companies.

A project for prediction of secondary pharmacology was initiated, funded by AstraZeneca R&D, Mölndal. Other projects involved standardization and interoperability in drug discovery informatics, including semantic web and linked data. Several studies involved the use of national high-performance computing and storage resources at UPPMAX. A pilot study was conducted to demonstrate the benefits of cloud computing in predictive toxicology by providing on-demand computational resources for large modeling problems. These findings can enable scientists without a supercomputer center to build predictive models on large collections (>100,000 molecules) within feasible time and in a cost-efficient way.

In collaboration AstraZeneca R&D, Mölndal, we also worked on statistical methods development for toxicity prediction. The project has focused on two main research tracks: (1) to reduce the dimensionality of the problem in order to achieve more accurate results faster, and (2) to adhere a prediction with prediction intervals representing its confidence. Both of these tracks represent important advances for the practical use of toxicity predictions.

The wet-laboratory project on libuguins was also continued. Methods for productions of libiguins were improved and studies on mechanism of actions for the prominent effects of the libiguins on sexual behavior were conducted with highly interesting results achieved.

Several studies relating to dengue and Japanese encephalitis virus proteases were conducted in relation to the possibility to develop antivirals for these by use of the proteochemometric technology.

*Members of the group during 2011*

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The Swedish Research Council
Uppsala Bio
AstraZeneca
Swedish Institute

*Other commitments/assignments of staff members*

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.

*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.

*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

*Kjell Wikvall and Maria Norlin*

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 2011*

Kjell Wikvall, MD, PhD, Professor
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Johan Lundqvist, PhD Student

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### *Agencies that support the work/Funding 2011*

The Swedish Research Council-Medicine

## Projects

### *Bioactivation and metabolism of vitamin D and cholesterol, including vitamin D-mediated effects on cellular function*

*Kjell Wikvall*

This research is focused on enzymes and genes of importance for vitamin D bioactivation and cholesterol homeostasis. Effects on these processes by endogenous and pharmacological compounds are studied. Vitamin D is needed for regulation of calcium levels in the body and vitamin D deficiency leads to skeletal diseases such as rickets in children and osteomalacia/osteoporosis in adults. The biologically active form, 1α,25-dihydroxyvitamin $D_3$, is formed through metabolic activation. The activated form of vitamin D blocks cell division and increases cell differentiation. Vitamin D analogues are used in the treatment of psoriasis and are of potential interest in cancer treatment. In addition, epidemiological data during recent years have indicated that vitamin D may have many more targets than previously known. For these reasons, it is important to obtain more knowledge about the enzymes that activate and metabolize vitamin D and the roles of the formed metabolites. Part of this project is focused on cellular effects of vitamin D and vitamin D-like drugs in order to explore previously unknown functions of these compounds.

Excess cholesterol and disturbances in cholesterol balance may lead to health problems such as heart disease, gall stones and neurological disease. Cholesterol is eliminated from the body by being converted into bile acids. Bile acids and a type of cholesterol derivatives called oxysterols have been shown to be ligands to nuclear receptors which regulate genes in lipid homeostasis and drug metabolism. It is therefore important to obtain more information about the enzymes that form and metabolize bile acids and oxysterols, such as their properties and how they are regulated. During the last couple of years these processes have gained increasing interest in connection with development of new drugs to treat abnormal cholesterol levels.

In one of our projects we have studied a new group of side-chain modified 15-oxosterols, synthetic inhibitors of cholesterol biosynthesis, which are potential drugs for treatment of high cholesterol levels. Other studies involve influences of anti-epileptic and anti-viral drugs on the processes described above, which result in adverse side-effects in some patients.

### *Functions of steroids and steroid-metabolizing enzymes in endocrine signalling*

*Maria Norlin*

This research concerns steroids involved in hormonal signalling in connection with sex hormone biosynthesis, neurosteroid function and cellular viability and growth. The studies are focused on physiological and pharmacological control of steroid levels, effects of metabolic events and regulation of gene expression. In particular, mechanisms involving estrogenic and androgenic signalling are studied. The project concerns endogenous steroids, steroid drugs and drugs affecting steroid hormone receptors such as SERMs (selective estrogen receptor modulators).

One of the steroids of interest in this area is dehydroepiandrosterone (DHEA). This steroid is well-known as a precursor for androgens and estrogens but also plays roles in brain function and in connection with cell growth and viability, e g in neurodegenerative processes. Tissue-specific metabolism of this steroid leads to a number of metabolites with differential effects on cell functions.

Steroids of importance for functions of the prostate and other endocrine tissues also are studied in this project. Transformation into malignancy may elicit changes in both sex hormone concentrations and effects of sex hormones on growth. Sex hormones are essential for function and growth of the prostate, and treatment to control hormonal action is of great interest for prostate cancer therapy.

Several enzymatic reactions of interest, involving the steroids mentioned above, are catalyzed by CYP7B1, a multifunctional enzyme which impacts the levels of a

number of steroids in many different tissues. For instance, CYP7B1 is responsible for enzymatic conversions that affect prostate hormone levels and the concentration of neuroactive steroids in the brain. This enzyme may be a future target for therapy aimed at regulating the levels of steroids of importance for abnormal cell growth, immune function or in neurodegenerative processes. Some of the current studies involve regulation of the levels of DHEA and other neurosteroids in neurons and glial cells and mechanisms for estrogen receptor-mediated control of the CYP7B1 gene by endogenous steroids and pharmaceutical compounds.

# Stress Adaption

*Matti Lang*

Cells adapt to stress by modulating the expression of genes which are part of their defense and adaptation machinery.

We are investigating the molecular mechanisms of how stress, in the form of toxic chemicals, oxidative stress and experimental psychological stress modulate gene expression.

Stress responding genes used as models include;

***Cyp2a5 /Cyp2a6****;* The encoded enzymes are involved in detoxification of xenobiotics. The genes were chosen because they are inducible by a variety of toxic chemicals and also by viral and bacterial infection and by oxidative stress. In addition, conditions which disturb transcriptional activity lead to sustained high expression of these genes via mRNA stabilization.

***iNos;*** the enzyme: inducible nitric oxide syntase protects organisms against microorganisms and is up regulated by oxidative stress under infestation.

***P53***; the protein is a transcription factor playing a central role in control of cell growth. The gene is upregulated for example by DNA damage caused by xenobiotics.

***Cyp2B1, CYP2E1, CYP2D1*** ; genes encoding for drug metabolizing enzymes are used as models to see how psychological disorders such as psychosis and depression influence their level of expression and the level xenobiotic metabolism, and thereby affect patients sensitivity to drugs.

A central part of our research strategy and goals is to seek and identify stress response elements (stress sensors) on the mRNA and DNA of these model genes, responsible for their regulation under stress. And to identify transacting factors interacting with these elements. Our working hypothesis is that genes responding similarly to stress should have similar stress sensors and transacting, stress activated, factors.

*Members of the group during 2011*

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