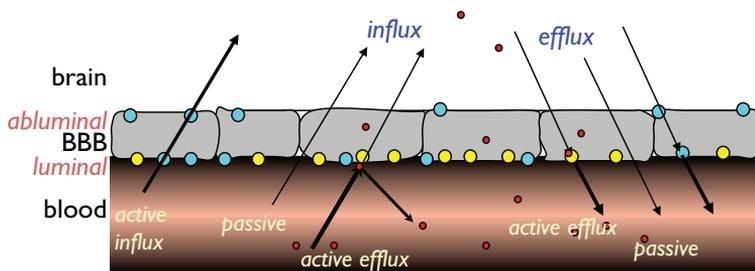




# ANNUAL REPORT 2008

Department of Pharmaceutical  
Biosciences







UPPSALA  
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Farmbio 2009/35

# ANNUAL REPORT 2008

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Department of Pharmaceutical  
Biosciences

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*"The blood-brain barrier regulates drug  
transport to and from the brain through  
passive transport, active uptake and/or  
active efflux."*

## Introduction

The discovery and development of novel drugs is a complex process that involves many aspects of pharmaceutical biosciences. Recently an overall evaluation of research at Uppsala University was carried out by a large number of international reviewers. The objective of the review was to identify emerging research areas and promising initiatives for future research. Based on the evaluation report, proteochemometrics, pharmacometrics, neuroscience and drug dependence were considered as areas of strength at our department. These research areas also play an important role in the development of novel drugs. Proteochemometrics is an emerging methodology for drug target interaction analysis. Research activities in neuroscience and drug dependence are of importance for the comprehension of drug addiction mechanisms. Pharmacometrics is of high relevance to the pharmaceutical industry and the demand for knowledge within this area has risen. The special support from the university to the strategic research areas was used to fund a number of novel positions. We are now pleased to welcome some young researchers to conduct their projects within these strategic research areas.

The researchers, teachers, laboratory, technical and administrative staff and PhD students are the key asset in the department. Some department members have moved on and others have joined. In the beginning of 2008 Georgy Bakalkin's research group from Karolinska Institute joined the department to start collaboration with Fred Nyberg's research group on drug dependence studies. The Bakalkin group also includes Tatjana Iakovleva, Alexander Kuzmin, a number of PhD students and postdocs. In addition, the following senior academic staff members joined the department: Per Andrén was appointed Adjunct Professor in Medical Mass Spectrometry and Henrik Wadensten was appointed as a Researcher in the same group. Lena Friberg was appointed Researcher in Pharmacometrics and Erika Roman was appointed Researcher in Neurobiological Behavioural Science. Egon Willighagen started to work with software development within the Bioclipse project and in the end of 2008 Anne-Lie Svensson was appointed Lecturer in Pharmacology. We look forward to working with all of them.

The department has been involved in the organization of some important events during this year. In April 2008 we celebrated the 40 year Jubilee of the Faculty of Pharmacy at Uppsala University. The Pharmaceutical Institute in Stockholm joined the university in Uppsala in 1968. The incorporation of the faculty into Uppsala University that has a tradition of research and education stretching back over 500 years has been successful. The Jubilee Meeting was held in the Grand Auditorium at the University Main Building and was attended by 500 participants. The distinguished speakers were from academia, pharmaceutical organizations and industries and they had the opportunity to reflect on history, scientific progress, and future directions of the faculty. A comprehensive jubilee publication covering the achievements of the faculty during the last 40 years and described by several authors was distributed to all participants. The book was edited by the Dean Fred Nyberg and the pharmacy student Jenny Carlsson. The celebration continued in the evening with a Jubilee Banquette in the former Hall of State at Uppsala Castle. The dinner with live entertainment was enjoyed by many guests including Nobel laureates, honorary doctors and guests, faculty staff, alumni, students, and friends.

In December 2008 over 110 participants from academic communities gathered in the lecture halls of BMC to honour professor emeritus Bengt Meyersson. The meeting "Research frontiers in behavioural neuroscience" was organized and chaired by Erika Roman and Dan Larhammar. Besides listening to lectures from distinguished speakers in the field of behavioural neuroscience the participants attended a symposium dinner. In connection to this symposium, a PhD course in "Adaptive mechanisms in brain disorders" was organized by Georgy Bakalkin and Lena Bergström. The course had many participants from various departments and universities.

The department has a solid basis in life science disciplines. Without research projects in pharmaceutical bioscience we would not be prepared to meet the needs of the students in pharmacy, biomedicine, medicine and chemical engineering. The department has a high priority for research training and the members of the staff are dedicated to foster students and PhD students in various aspects of the drug discovery and development processes. The main part of our undergraduate educational activities is focused on the professional pharmacy programs (three or five years). The courses are continuously evaluated by the students and updated to provide the participants with the tools and concepts needed to work at pharmacies, drug companies, or medicine agencies. In addition, some master programs are presently developed to cover Drug management (two years) and Clinical pharmacy (one year). Members of the staff are also involved in the development of a master program in Forensic Science.

The research groups maintain ongoing international collaboration within their research areas. For example, the pharmacometrics research group has an extensive collaboration on HIV and tuberculosis research with the Department of Clinical Pharmacology, University of Cape Town, in South Africa. The pharmacometrics research group conducts yearly training in South Africa as well as training in Sweden. The collaboration also involves co-supervision of PhD students. The department also has an ongoing collaboration with ULLA – the European consortium for training and research in the field of pharmaceutical science. The ULLA partners include the School of Pharmacy, London, the Leiden/Amsterdam Center for Drug Research, the Faculty of Pharmaceutical Sciences, Copenhagen, the Faculty of Pharmacy, Paris, the Faculty of Pharmacy, Parma, and the Faculty of Pharmaceutical Sciences, Leuven.

The department has a broad research environment and it has been my pleasure to cooperate with all members of the staff. The achievements over the past year form a strong foundation for future research as well as education and training.

Uppsala March 15, 2009

Eva Brittebo

# List of Contents

|   |    |
|---|----|
| Introduction .....  | 5  |
| Organization .....  | 9  |
| Address List .....  | 11 |
| Undergraduate Teaching.....                               | 13 |
| Awards and Appointments.....                              | 14 |
| Scientific Reports .....                                  | 15 |
| <b>Division of Biological Research on Drug Dependence</b> |    |
| Biological Research on Drug Dependence .....              | 15 |
| Molecular Neuropsychopharmacology .....                   | 21 |
| Medical Mass Spectrometry (MMS) .....                     | 23 |
| <b>Division of Pharmaceutical Biochemistry</b>            |    |
| Stress Adaption.....                                      | 28 |
| Steroid P450 .....  | 31 |
| <b>Division of Pharmacology</b>                           |    |
| Proteochemometrics.....                                   | 34 |
| Prostaglandin .....                                       | 37 |
| Drug Dependence.....                                      | 40 |
| Neuropharmacology .....                                   | 43 |
| Neurodegeneration .....                                   | 45 |
| Behaviour.....  | 47 |
| <b>Division of Pharmacokinetics and Drug Therapy</b>      |    |
| Pharmacokinetics/Pharmacodynamics .....                   | 50 |
| Pharmacometrics .....                                     | 57 |
| <b>Division of Toxicology</b>                             |    |
| Embryotoxicology.....                                     | 70 |
| Bioactivation and Toxicity .....                          | 75 |
| Genetic Toxicology.....                                   | 78 |
| MALDI imaging mass spectrometry .....                     | 81 |



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## Undergraduate Teaching

The Department of Pharmaceutical Biosciences is responsible for education in bioscience at two main programmes, the Bachelor of Science in Pharmacy programme (three years, 180 hp) and the Master of Science in Pharmacy programme (five years, 300 hp). Completed studies at the Pharmacy programmes provide the necessary theoretical and practical competence that is required to apply for a licence as a Pharmacist, either *receptarie* (after the Bachelor programme) or *apotekare* (after the Master programme). In addition, teachers within the department are involved in teaching at the Master of Science in Chemical Engineering, with specialization in drugs, and the Biomedical programme.

The current Swedish educational system was put into practice in July 2007. In this system, undergraduate studies are divided into two levels, basic (first 3 years of studies) and advanced level (additional 1-2 years). The grading system within the Faculty of Pharmacy was also revised in 2007. Examinations are from July and onwards graded with three grades, U (not passed), G (passed) and VG (passed with distinction). At present, and for the next coming years, teaching is in progress both according to the previous and the new educational system.

The main subject taught at the department is Pharmaceutical Biosciences that comprises a large number of courses: Drug development and Drug usage, Drug metabolism and safety, Gene technology, Immunology, Infection Biology, Microbiology, Molecular Biology, Pharmaceutical Biochemistry, Pharmacology, Pharmacokinetics, Pharmacotherapy, Physiology, Toxicology. A mix of traditional teaching and problem-based learning with lectures, laboratory sessions, seminars, group work and computer sessions characterizes the teaching. In addition, the teachers are involved in interdisciplinary training in laboratory practice, communication skills and professional development. Besides the mandatory courses at the programmes, the department gives many elective courses on the advanced level. The courses attract a large number of students, not only Pharmacy students, but also other students showing the proper prerequisites in biosciences. These courses mirror research profiles within the department, such as bioinformatics, clinical pharmacy, drug metabolism and safety, drug dependence, and pharmacokinetics. The teachers also instruct in undergraduate projects. These projects comprise 15 hp or 30 hp and are examined by an oral presentation as well as a written report. Most of the projects within biosciences involve laboratory-based projects in which the student is involved in ongoing research projects.

During 2008, the teachers have been involved in development of two new master programs, Master programme in drug development and Master programme in drug management and safety. These programs have been available for students during 2008 as one-year programmes and they are now extended to full two-year master programmes. These programmes comprise courses and undergraduate projects at the advanced level and the first students start in 2009. In addition to these programmes at the advanced level, the division of Pharmacotherapy is responsible for the one-year programme in Clinical Pharmacy.

Uppsala March 8, 2009

Ingrid Nylander

## Awards and Appointments

1. Fred Nyberg "Top Reviewer in 2007" in Behavioural Brain Research, Elsevier
2. Mathias Hallberg Oscarspriset 2008, Uppsala University
3. Mats Karlsson "Honorary Fellowship Award" American College of Clinical Pharmacology
4. Martin Bergstrand "*Outstanding modelling and simulation abstract award*" American Conference on Pharmacometrics

# Scientific Reports

## Division of Biological Research on Drug Dependence

### Biological Research on Drug Dependence

*Fred Nyberg and Mathias Hallberg*

(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, autoradiography, 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*.

### **Members of the group during 2008**

Fred Nyberg, Professor  
Mathias Hallberg, PhD Associate Professor  
Qin Zhou, PhD Researcher  
Tobias Johansson, PhD, Researcher  
Milad Botros, PhD, Researcher  
Kristina Magnusson, PhD Student  
Uwe Rossbach, PhD Student  
Martin Elfverson, PhD Student  
Dan Henrohn, PhD Student  
Jenny Johansson, PhD Student  
Alfhild Grönbladh, PhD, Student  
Britt-Marie Johansson, Technician  
Nasim Ghasemzadeh, PhD Student

### **Publications 2006-2008**

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3. Lai Z and Nyberg F.  
Purification of growth hormone receptors from human brain tissues; in Nyberg F (ed.): The somatotrophic axis in brain function. *Elsevier Academic Press, San Diego, 2006, pp 99-107.*
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Neuropeptides in hyperthermia. *Progr Brain Res* 162, 2007; 162:277-293.

### Dissertations 2008

1. Tobias Johansson, PhD  
*Neurosteroids Induce Allosteric Effects on the NMDA Receptor: Nanomolar Concentrations of Neurosteroids Exert Non-Genomic Effects on the NMDA Receptor Complex*  
Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy 69  
<http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-8503>

2. Milad Botros, PhD  
*Characterization of Substance P (SP) Aminoterminal SP (1-7) Binding in Brain Regions and Spinal Cord of the Male Rat: Studies on the Interaction with Opioid Related Pathways*  
Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy 85  
<http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-9401>
3. Martin Elfverson  
*Neurosteroids in Nanomolar Concentrations Modulate the NMDA Receptor - Functional Effects of the Allosterically Altered Ifenprodil Binding to the NR2B Subunit*  
Licentiate Thesis

### **Funding 2008**

Swedish Research Council Medicin  
Precision Science System  
Swedish Foundation for Strategic Research  
Berzelii Centre for Biotechnological Research  
Disciplinary Domain of Medicine and Pharmacy  
Swedish National Drug Policy Coordinator  
The Alcohol Research Council of the Swedish Alcohol Retailing Monopoly  
The Research Council of Swedish Criminal Care

### **Other commitments**

Fred Nyberg: Dean of the Faculty of Pharmacy, Chairman of the Faculty of Pharmacy Committee, Member of the Faculty Board, Chairman of a number of subcommittees within the University, 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: Peptides, Open J Endocrinology (Editor in Chief), Pharmacology-on-line, J Musc Skel. Pain.

Mathias Hallberg: Curr Protein Pept Sci., 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.

### **Projects**

*Fred Nyberg*

PI at the Uppsala Berzelii Technology Center for Neurodiagnostics

PI at the Linne project Impact of Religion: Challenges for Society, Law and Democracy

## Molecular Neuropsychopharmacology

### Georgy Bakalin

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 2008

Tatiana Yakovleva, PhD, Senior scientist.

Alexander Kuzmin, PhD, Senior scientist.

Igor Bazov, PhD, Scientist.

Hiroyuki Watanabe, PhD, Postdoctoral scientist.

Richard Henriksson, PhD student

Malik Mumtaz Hussain Taqi, PhD student

### Publications 2006-2008

1. Nazira E.H., Kelps K.A., Zou S.P., Zhao T.Y., Bruce-Keller A.J., Yakovleva T., Bakalkin G., Knapp P.E. and Hauser KF. Convergent [Ca<sup>2+</sup>]<sub>i</sub> PI3K, and NF-κB Signals Underlie Morphine and HIV-1 Tat Synergistic Increases in MCP-1 and IL-6 Release by Astrocytes. *PLoS ONE* 2008, 3(12):e4093.
2. Henriksson R., Kuzmin A., Ökvist A., Haper C., Sheedy D., Garrick T., Yakovleva T. and Bakalkin G. Elevated Synaptophysin I in the Prefrontal Cortex of Human Chronic Alcoholics. *Synapse* 2008, 62: 829-833.
3. Hugonin L, Vukojevic V. Bakalkin G. and Graslund A. Calcium influx into phospholipid vesicles caused by dynorphin neuropeptides. *Biochem. Biophys. Acta* 2008, 1778: 1267-1273.

### Funding 2008

The Swedish Research Council

AFA Insurance

NIH/NIDA – Karolinska Inst. neuroscience program

Torsten och Ragnar Söderbergs stiftelser

### Projects

#### ***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.

***Mechanisms of neurodegeneration and cognitive impairments induced by alcohol: development of neuroprotective pharmacotherapy.***

After many years of heavy drinking, alcohol produces pathological alterations in the brain that culminate in social deterioration and disorders in memory and learning. An important issue is how alcohol damages neural systems and whether this damage underlies the psychological and cognitive disturbances associated with alcoholism. Our hypothesis is that chronic alcohol consumption affects specialized molecular systems that control neuronal death and viability in the brain, which leads to the destruction of selected populations of neurons and eventually results in cognitive impairments. We aim to identify these molecular systems among endogenous regulators of neuronal death and viability, and to map specialized functional circuits in which these systems are affected in the human brain. We are testing whether chemical inhibitors of these molecular systems attenuate alcohol-induced impairments of cognitive functions in animal models of alcohol neurotoxicity. Relevant molecular systems include transcriptional factors NF-kappaB and the tumor suppressor p53 protein, and endogenous opioid peptides dynorphins, which have neurotoxic activity mediated through glutamate receptors. Innovative epigenetic approach is applied to characterize mechanisms underlying alterations in patterns of gene transcription induced by alcohol in human brain.

## Medical Mass Spectrometry (MMS)

*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) imaging MS of biological tissue sections, and peptidomics, the comprehensive study of endogenous peptides.

**Imaging mass spectrometry (IMS)** 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). 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 investigate protein and peptide expression patterns in subjects with and without LID symptoms. Furthermore, 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 IMS 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.

Our laboratory is equipped with the latest separation and MS technologies (4 capillary nanoLC instruments, two Q-ToF mass spectrometers, one LTQ mass spectrometer. In addition, K&A Wallenberg Foundation has recently funded two MALDI mass spectrometers (AutoFlex III MALDI TOF MS and UltraFlex II MALDI TOF-TOF MSMS, Bruker), specifically the for imaging mass spectrometry approach.

### **Members of the group 2008**

Anna Nilsson, PhD student

Maria Fälth, PhD student

Henrik Wadensten, researcher

Johan Gustavsson, visiting graduate student

Allessandro Fioni, visiting graduate student

### **Publications 2006-2008**

1. Zhang X, Andren PE, Greengard P, Svenningsson P (2008) Evidence for a role of the 5-HT1B receptor and its adaptor protein, p11, in L-DOPA treatment of an animal model of Parkinsonism. *Proc Natl Acad Sci USA*, 105: 2163-2168. Epub 2008 Feb 6.
2. Scholz B, Svensson M, Alm H, Sköld K, Fälth M, Kultima K, Guigoni C, Doudnikoff E, Li Q, Crossman AR, Bezdard E, Andrén PE (2008) Striatal Proteomic Analysis Suggests that First L-Dopa Dose Equates to Chronic Exposure. *PLoS ONE*. 3: e1589
3. Sundström I, Andren PE, Westerlund D (2008) Method development for identification of ketobemidone metabolites in microdialysate samples by coupled-column capillary liquid chromatography–tandem mass spectrometry. *J Chromatogr A*, 1189:503-513. Epub 2008 Feb 16.
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5. Fälth M, Svensson M, Nilsson A, Sköld K, Fenyö D, Andren PE (2008) Validation of endogenous peptide identifications using a database of tandem mass spectra. *J Proteome Res*, 7: 3049-3053. Epub 2008 Jun 13.
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7. Fälth M, Savitski MM, Nielsen ML, Kjeldsen F, Andren PE, Zubarev RA (2008) SwedECD, a New Database of ECD Spectra. *Analytical Chemistry*, 80:8089-8094. Epub 2008 Oct 7.
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10. Nilsson A, Fälth M, Zhang X, Kultima K, Sköld K, Svenningsson P, Andren PE (2008) Striatal alterations of secretogranin-1, somatostatin, prodynorphin and cholecystokinin peptides in an experimental mouse model of Parkinson's disease. *Mol Cell Proteomics*, Accepted for publication, Dec
11. Svensson M, Borén M, Sköld K, Fälth M, Sjögren B, Andersson M, Svenningsson P, Andrén PE (2008) Heat stabilization of the tissue proteome: a new technology for improved proteomics. *J Proteome Res.*, Accepted for publication, Dec

### Reviews 2006-2008

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(2008) MALDI Imaging and Profiling Mass Spectrometry in Neuroproteomics. In: *Frontiers in Neuroscience: Neuroproteomics*. Eds. Simon S, Nicoletis M. Frontiers Research Foundation, Lausanne, Switzerland, In press

### Dissertations 2008

1. Anna Nilsson  
*Molecular Profiling and Imaging of Peptides, Proteins and Drugs in Biological Tissue using Mass Spectrometry*.  
Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy 84  
<http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-9337>
2. Maria Fälth Savitski  
*Improved Neuropeptide Identification: Bioinformatics and Mass Spectrometry*.  
Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy 86  
<http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-9400>

### Funding 2008

The Swedish Research Council (VR)  
Denator AB,  
GE Healthcare

### Other commitments

Member of the Board, the Swedish Academy of Pharmaceutical Sciences, Section for Drug Analysis  
Member of the Board, the Swedish Proteomics Society  
Member of the Board, the Swedish Mass Spectrometry Society  
R&D Science Committee, GE Healthcare Life Science, Uppsala  
Processes & Applications Management Group, GE Healthcare Life Science, Uppsala  
Editorial Board, Journal of Proteomics

### Projects

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

*Per Andréén, Anna Nilsson, Maria Fälth, Henrik Wadensten, MMS, Per Svenningsson, Karolinska Institutet, Alan Crossman, University of Manchester, UK, Erwan Bezdard, 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.

### **Neuropeptidomics strategies for specific and sensitive identification of novel endogenous peptides.**

*Per Andrén, Maria Fälth, Anna Nilsson, Henrik Wadensten, MMS, David Fenyö, Rockefeller University, New York, USA*

A new database, SwePep, specifically designed for endogenous peptides, has been constructed to significantly speed up the identification process from complex tissue samples utilizing mass spectrometry. In the identification process the experimental peptide masses are compared with the peptide masses stored in the database both with and without possible post-translational modifications. A new approach using targeted sequence collections has been developed for identifying endogenous peptides. This approach enables a fast, specific, and sensitive identification of endogenous peptides. Three different sequence collections were constituted in this study to mimic the peptidomic samples. A number of potential bioactive neuropeptides have been identified.

### **Identification and functional characterization of protein-protein interactions in cerebrospinal fluid and brain tissue from Parkinson's disease patients**

*Per Andrén, Anna Nilsson, MMS, Per Svenningsson, Elisabeth Öhman, Alexandra Madeira, Benita Sjögren, Karolinska Institutet*

Using surface plasmon resonance technique (Biacore 3000) coupled to mass spectrometry (BIA/MS) technology, new protein partners of  $\alpha$ -synuclein and parkin have been captured and identified in cerebrospinal fluid or post-mortem human tissue from PD patients. In addition to using native  $\alpha$ -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.

### **The definition of drug distribution within tissue using imaging mass spectrometry and histology**

*Per Andrén, Anna Nilsson, MMS, AstraZeneca, Lund*

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 microenvironmental 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.

### **Novel Molecular imaging approaches for the development of new diagnostic tools for colon and prostate cancer and Alzheimer's disease using Imaging Mass Spectrometry.**

*Per Andrén, Anna Nilsson, Henrik Wadensten, MMS, Bengt Långström, Department of Biochemistry & Organic Chemistry, and Uppsala Applied Science Lab, GE Healthcare, Håkan Hall, Oleksiy Itsenko, Azita Monazzam, Obaidur Rahman, Irina Velikyan, Pasha Razifar, Uppsala Applied Science Lab, GE Healthcare, Lars Pählman, Ulrik Wallin, Department of Surgery, Bengt Glimelius, Department of Oncology, Christer Busch, Department of Pathology, Maréne Landström, Department of Pathology*

This collaborative project aims at developing new methods for the clinical diagnosis

of colon and prostate cancer and Alzheimer's disease using molecular imaging leading aiming at finding new therapeutic avenues. Advanced biochemical, imaging mass spectrometry, cell culturing and imaging methods will be used in the search of new  $^{68}\text{Ga}$ -labeled tracers for the early diagnosis of colon and prostate cancer, and Alzheimer's disease with positron emission tomography.

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

*Per Andrén, Maria Fälth, MMS, 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.

***Anatomical and neurochemical characterization of neuropeptides and proteins in striatum and n. accumbens in morphine withdrawal using Peptidomics and Imaging Mass Spectrometry.***

*Fred Nyberg, Uwe Rossbach, Biological Research on Drug Dependence, Per Andrén, Anna Nilsson, Maria Fälth, MMS*

Repeated administration of morphine may lead to neuroadaptive changes in the brain that are thought to underlie molecular mechanisms of the development of morphine tolerance and physical dependence. Here, we employ peptidomics and MALDI imaging approaches to detect peptide and protein expression changes of the brain in rats that had developed morphine tolerance.

## Division of Pharmaceutical Biochemistry

### 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 synthase 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.

#### Current status;

We have identified hnRNPA1 (heterogenous nuclear ribo nucleoprotein A1) as a key regulator of the *Cyp2a5 and Cyp2a6*. The protein is activated by different toxic insults and can regulate the *Cyp2a5* expression both at transcriptional and posttranscriptional levels: by interacting with the promoter alternatively with the 3'-UTR of the corresponding mRNA. As the hnRNPA1 is multifunctional, it impossible that it controls the gene expression at different levels on the gene expression pathway ( for refs., see below).

Two other members of the hnRNP family; the hnRNPI and hnRNPL, were found to interact with the iNOS mRNA in an infection dependent manner, and with a possible regulatory function (see ref, below). We are currently working on the detailed mechanisms of gene regulation by these proteins.

We have identified hnRNPC as an important regulator of P53 via a regulatory element in the first exon of the encoding mRNA. HnRNPC is activated by DNA

damage and disturbed transcription thereby linking the toxic reaction to up regulation of P53.

A strong down regulation of CYP2E1 and CYP2B1 has been shown in relation to psychosis, and evidence has been obtained for the involvement of the dopaminergic signaling pathway in this process.

### **Members of the group during 2008**

Matti Lang, Professor

Kyle Christian, PhD student

Silvia Visoni, Ph.D student

### **Publications 2008**

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8. Konstandi, M., Lang, M.A., Kostakis, D., Johnson, E.O. and Marselos, M. (2007)  
Predominant role of peripheral catecholamines in the stress induced modulation of CYP1A2 inducibility by benzo(a)pyrene. *Basic and clinical Pharmacology and toxicology. In press*
9. Konstandi, M. Harkitis, P., Kostakis, D., Marselos, M., Johnson, E.O. and Lang, M.A. (2007)  
D2-receptor linked signaling pathways regulate the expression of hepatic CYP2E1, *Life Sciences, In press*

10. Christian, K., Lang, M, A., Raffalli-Mathieu, F. (2008)  
Interaction of hnRNPC1/C2 with a novel cis-regulatory element within P53 mRNA as a response to cytostatic drug treatment. *Mol. Pharmacol.* 73, 1-10.
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### **Dissertations 2008**

1. Kyle Christian  
*The role of hnRNP A1 and hnRNP C1/C2 in the regulation of the stress responsive genes Cyp2a5/2A6 and p53.*  
Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy 74  
<http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-8722>

### **Funding 2008**

University of Queensland Australia and Natl. Centre for Env. Toxicology in Brisbane Australia

### **Other commitments**

Honorary professor, Univ Queensland

### **Projects**

Bilirubin, substrate and regulator of CYP2A5, Biological significance in cells exposed to oxidative stress.

Regulation of P53 by hnRNPC1, a mechanistic link between disturbed transcription, arrested cell growth and apoptosis.

Impact of mental disorders on the expression of CYP2E1, mechanistic evidence on mental status influencing the metabolism of xenobiotics.

## Steroid P450

*Kjell Wikvall and Maria Norlin*

Our research is focused on the properties and regulation of cytochrome P450-mediated enzymatic processes involving steroids.

1. Bioactivation and metabolism of vitamin D and cholesterol (Principal investigator: Kjell Wikvall)
2. Functions of steroids and steroid-metabolizing enzymes in endocrine signalling (Principal investigator: Maria Norlin)

The Steroid P450 group is part of UBAP (Uppsala Bioactivation Program), a scientific program for bioactivation (metabolic activation) at the Faculty of Pharmacy.

### 1) Bioactivation and metabolism of vitamin D and cholesterol

(Principal investigator: 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,  $1\alpha,25$ -dihydroxyvitamin D<sub>3</sub>, 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. 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.

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 recently 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.

An ongoing project concerns 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.

### 2) Functions of steroids and steroid-metabolizing enzymes in endocrine signalling (Principal investigator: Maria Norlin)

This research concerns steroids involved in hormonal signalling in connection with sex hormone biosynthesis, neurosteroid function and cellular proliferation. The studies are focused on physiological and pharmacological control of steroid levels, effects of metabolic events and mechanisms for regulation of gene expression. Some of the steroids of interest in this area are dehydroepiandrosterone (DHEA) and  $5\alpha$ -androstane- $3\beta,17\beta$ -diol.

DHEA is well-known as a precursor for androgens and estrogens but also plays roles in brain function and in connection with cell growth and functions of the immune system. This steroid has been proposed as a potential drug for treatment of several diseases, for instance systemic lupus erythematosus, an autoimmune disease.  $5\alpha$ -androstane- $3\beta,17\beta$ -diol, an estrogenic hormone, is believed to play a role for hormone-dependent proliferation, particularly in the prostate.

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.

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.

### **Members of the group during 2008**

Kjell Wikvall, MD, PhD, Professor  
Maria Norlin, PhD, Associate Professor  
Maria Ellfolk, PhD Student  
Johan Lundqvist, PhD Student  
Hanna Pettersson, PhD Student

### **Publications 2006-2008**

1. Tang, W., Eggertsen, G., Chiang, J.Y.L., and Norlin, M.  
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3. Tang, W. and Norlin, M.  
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3. Norlin, M.  
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### Dissertation 2008

1. Maria Ellfolk  
*Regulation of vitamin D 25-hydroxylases: Effects of vitamin D metabolites and pharmaceutical compounds on the bioactivation of vitamin D*  
Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy 87  
<http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-9412>

### Funding 2008

The Swedish Research Council-Medicine

### Other commitments

Kjell Wikvall: Director of studies, Chair of the Scholarship committee (Faculty of Pharmacy)

Maria Norlin: Deputy member of the Department board, Member of the Post-graduate study committee (FUG), Deputy member of the Board for Uppsala Graduate School in Biomedical Research (UGSBR)

## Division of Pharmacology

### Proteochemometrics

*Jarl Wikberg*

Research during 2008 was concentrated on 1) the continued development of the **Bioclipse** platform and 2) the further development of proteochemometrics.

During the year Bioclipse 2.0 beta3 was released (available at [www.bioclipse.net](http://www.bioclipse.net)). The new platform provides stability and eliminates technical shortcomings of the v. 1 series of Bioclipse while the same functionalities are available and extended.

During the year many proteochemometrics studies directed to validation of the technology in drug design, drug monitoring and protein engineering were completed. A prediction server based on Bioclipse software for prediction of drug resistance based on HIV reverse transcriptase sequence was set up and is available at [www.hivdrc.org](http://www.hivdrc.org)

In ongoing studies assay systems for a multitude of resistance mutated HIV proteases was also set up and a large number of organic compounds were synthesized based under experimental design and proteochemometric design principles with the aim to develop new HIV protease inhibitors with a broad spectrum over a large array of resistant HIV forms.

Development of Bioclipse and validation of proteochemometrics continues.

### *Members of the group during 2008*

Jarl Wikberg, Professor  
Felikss Mutulis, PhD, Visiting Post Doc  
Sviatlana Yahorava, PhD, Visiting Post Doc  
Maris Lapins, PhD, Researcher  
Anton Sholuk, PhD, Visiting Post Doc  
Egon Willighagen, PhD, Post Doc  
Aleksejs Kontijevskis, PhD Student  
Ola Spjuth, PhD Student  
Jonathan Alvarsson, PhD Student  
Stephy Prakash, PhD Student  
Martin Eklund, PhD Student  
Eskil Andersen, Programmer  
Carl Mäsak, Programmer  
Bjarni Juliusson, Programmer  
Ramona Petrovska, Technician

### *Publications 2006-2008*

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4. Eklund M, Spjuth O, Wikberg JE. The C1C2: A framework for simultaneous model selection and assessment. *BMC Bioinformatics*. 2008 Sep 2;9(1):360.
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21. Kopanchuk S, Veiksina S, Mutulis F, Mutule I, Yahorava S, Mandrika I, Petrovska R, Rinken A, Wikberg JE.  
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24. Muceniece R, Zvejniece L, Liepinsh E, Kirjanova O, Baumane L, Petrovska R, Mutulis F, Mutule I, Kalvinsh I, Wikberg JE, Dambrova M  
The MC(3) receptor binding affinity of melanocortins correlates with the nitric oxide production inhibition in mice brain inflammation model. *Peptides*. 2006 Jan 13.
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Rough set-based proteochemometrics modeling of G-protein-coupled receptor-ligand interactions. *Proteins*. 2006, 63, 24-34.

### **Funding 2008**

The Swedish Research Council  
The Swedish International Cooperation Agency  
The Swedish Research Links  
The Swedish Institute

## Prostaglandin

Ernst H. Oliw

Arachidonic acid and a few other polyunsaturated fatty acids are bio activated 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 during reproduction and in many other organs. Common drugs such as aspirin, acetaminophen (paracetamol) and ibuprofen inhibit biosynthesis of prostaglandins and reduce symptoms of disease, but may also cause side effects related to their actions. Other drugs are based leukotriene receptor antagonists (e.g., montelukast), which are used for treatment of bronchial asthma. Bioactivation of polyunsaturated fatty acids also occur in plants and fungi where oxygenation of linoleic and linolenic acids is important for the plant-pathogen interaction and for fungal reproduction and pathogenicity. The goal of our research is to investigate the mechanism of oxygenation and bioactivation of fatty acids and to determine their biological function.

We investigate mainly three groups of enzymes: (i) lipoxygenases, (ii) cytochromes P450 and (iii) heme-containing dioxygenases. These enzymes occur in man but also in important fungal pathogens, e.g., *Aspergillus fumigatus* causing farmer's lung disease and *Magnaporthe grisea*, 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 and pathophysiological functions and to develop new drugs.

In humans, the prostaglandin endoperoxide,  $\text{PGH}_2$ , can be transformed by cytochromes P450 to thromboxanes, prostacyclin and to 19-hydroxy- $\text{PGH}_2$ , the precursor of 19-hydroxy- $\text{PGE}_2$ . 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. CYP4F8 and CYP4F22 are also expressed in skin and we investigate their oxygenation of fatty acids.

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 grisea*, and *Aspergillus fumigatus*. These fungi also contain oxygenate cyclooxygenase-related enzymes, which oxidized linoleic acid by to a series of vicinal diols (5,8-dihydroxy-, 7,8-dihydroxy-, and 8,11-dihydroxyoctadecadienoic acids) via formation of hydroperoxides (8-hydroperoxy- and 10-hydroperoxylinoleic acid), which likely function as sporulation hormones. The reaction mechanism and identification of these diol synthases are described in our paper in *The Journal of Biological Chemistry* (Ref. 13). Site-directed mutagenesis of 7,8-LDS revealed structural and mechanistic similarities to cyclooxygenases (Refs. 15 and 17).

### Members of the group during 2008

Ernst H. Oliw, MD PhD, Professor  
 Erica Johansson, administrative assistant  
 Ulrike Garscha, PhD student  
 Tomas Nilsson, PhD student  
 Fredrik Jernerén, PhD student

### **Publications 2006-2008**

1. Karlgren M, Gomez A, Stark K, Svard J, Rodriguez-Antona C, Oliw E, Bernal ML, Y Cajal SR, Johansson I, Ingelman-Sundberg M (2006). Tumor-specific expression of the novel cytochrome P450 enzyme, CYP2W1. *Biochem. Biophys. Res. Commun.* 341, 451-8.
2. Stark, K. Törmä, H. and Oliw, E.H. (2006) Co-localization of COX-2, CYP4F8, and mPGES-1 in Epidermis with prominent expression of CYP4F8 mRNA in psoriatic lesions, *Prostaglandins Other Lipid Mediat.* 79, 114-125.
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6. Cristea, M. and Oliw, E.H. (2007) On singular, dual and multiple positional specificity of manganese-lipoxygenase and its Gly316Ala mutant. *J. Lipid Res.*, 48, 890-903.
7. Garscha, U. and Oliw, E.H. (2007) Steric analysis of 8-hydroxy- and 10-hydroxyoctadecadienoic acids formed from 8R-hydroperoxyoctadecadienoic acid by hydroperoxide isomerase. *Anal. Biochem.* 369, 238-246.
8. Garscha, U., Jernerén, F., Chung, D., Keller, N.P., Hamberg, M., and Oliw, E.H. (2007) Identification of dioxygenases required for *Aspergillus* development: Studies of products, stereochemistry, and the reaction mechanism. *J. Biol. Chem.* 282, 34707-34718.
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11. Garscha, U., Nilsson, T., and Oliw, E.H. (2008) Enantiomeric separation and analysis of unsaturated hydroperoxy fatty acids by chiral column chromatography-mass spectrometry, *J. Chromatogr. B*, 872, 90-98
12. Garscha, U. and Oliw, E. H. (2008) Critical amino acids for the 8(R)-dioxygenase activity of linoleate diol synthase. A comparison with cyclooxygenases. *FEBS Lett.* 582, 3547-3551.

### **Dissertations 2008**

13. Tomas Nilsson  
*Mass Spectrometrical Analysis of Hydroperoxides, Epoxyalcohols, and other Oxygenated Fatty Acids in the Investigation of Catalysis by CYP4 Isozymes*  
Licentiate Thesis

**Funding 2008**

The Swedish Research Council Medicine  
The Research Council Formas

**Other commitments**

Chairman, The Linnaeus Library Board, Uppsala University

**Projects*****Novel transformations of polyunsaturated fatty acids and eicosanoids.***

*Ernst Oliw, Johan Bylund and 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.

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

*Ernst Oliw, Ulrike Garscha, Fredrik Jernerén, Inga Hoffmann*

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.

## Drug Dependence

*Ingrid Nylander*

The projects within the research group focus on the neurobiological substrates for individual differences in vulnerability for alcohol dependence, with special emphasis on the impact of early environmental factors. Alcohol dependence is a complex trait and the phenotype related to vulnerability for dependence is based on the interaction of multiple genes and environmental factors. Adverse experiences during the critical childhood and adolescence periods can cause long-term neurobiological and behavioral alterations and increased vulnerability for psychopathology, including drug dependence. The environment may also provide protection, for instance in a predisposed individual. The mechanisms underlying the environmental influence are not fully understood. The projects within the research group aim to elucidate mechanisms underlying protective and risk factors for excessive alcohol consumption. For that purpose animal experimental models are used in combination with extensive evaluation of neurobiological and behavioral consequences of different early environmental conditions.

A rodent maternal separation (MS) model is used to simulate different environmental settings. Rat pups are separated from the caregiver short (15 min, MS15) or prolonged (360 min, MS360) periods during the first postnatal weeks. MS15 is more similar to natural conditions where the mother regularly leaves the litter for shorter periods of time. Previous results within the group provide evidence for MS15 being protective. Adult rats subjected to MS15 during the first weeks of life have a low alcohol intake. In addition, genetically predisposed rats subjected to MS15 exhibit a slower acquisition of alcohol intake. The prolonged separations interfere with early social interactions and are used to simulate an emotional stressful environment for the rat pups and/or the mother. MS360 is associated with an increased risk for excessive alcohol intake and altered risk-taking behavior. Rats subjected to MS360 have higher alcohol consumption, they prefer higher alcohol concentrations and in alcohol-preferring rats, MS360 rearing adds to the risk as evidenced by an even higher adult alcohol intake. Some rats do not respond to the emotional stressful early environment and the reason for these differences in responsiveness are to date not known. In projects within the group, possible brain target systems mediating the early environmental influence are studied. Focus is on neuropeptides, such as opioids, oxytocin and vasopressin, and monoamines that are important for early social behavior and normal neuronal development. It is hypothesized that disruption of early developmental processes in these transmitter networks cause long-term changes in behavior and, in turn, alcohol consumption later in life.

### *Members of the group during 2008*

Ingrid Nylander, Professor

Chris Pickering, PhD

Stefan Schlussmann, PhD

Sadia Oreland, PhD Student

Loudin Daoura, PhD Student

Marita Berg, Technician

### **Publications 2006-2008**

1. Roman E, Ploj K, Gustafsson L, Meyerson BJ, Nylander I.  
Variations in opioid peptide levels during the estrous cycle in Sprague-Dawley rats. *Neuropeptides* 40 (2006) 195-206.
2. Roman E, Gustafsson L, Berg M, Nylander I.  
Behavioral profiles and stress-induced corticosteroid secretion in male Wistar rats subjected to short and prolonged periods of maternal separation. *Hormones and Behavior* 50 (2006) 736-747.
3. Gustafsson L, Nylander I.  
Time-dependent alterations in ethanol intake in male Wistar rats exposed to short and prolonged daily maternal separation in a four-bottle free choice paradigm. *Alc Clin Exp Res* 30 (2006) 2208-2016.
4. Pickering C, Gustafsson L, Cebere A, Nylander I, Liljequist S.  
Repeated maternal separation of male Wistar rats alters glutamate receptor expression in the hippocampus but not the prefrontal cortex. *Brain Res* 1099 (2006) 101-108.
5. Drakenberg K, Nikoshkov N, Horváth MC, Gharibyan A, Fagergren P, Gharibyan A, Saarelainen K, Rahman S, Nylander I, Bakalkin G, Rajs J, Keller E, L Hurd YL.  
Mu opioid receptor A118G polymorphism in association with striatal opioid neuropeptide gene expression in heroin abusers. *PNAS* 103:20 (2006) 7883-7888.
6. Gustafsson L, Zhou Q, Nylander I.  
Ethanol-induced effects on opioid peptides in adult male Wistar rats are dependent on early environmental factors. *Neuroscience* 146 (2007) 1137-1149.
7. Roman E, Meyerson BJ, Hyytä P, Nylander I.  
The multivariate concentric square field test reveals different behavioral profiles in male AA and ANA rats with regard to risk taking and environmental reactivity. *Behav Brain Res* 183 (2007) 195-205.
8. Gustafsson L, Orelund S, Hoffmann P, Nylander I.  
The impact of postnatal environment on opioid peptides in young and adult male Wistar rats. *Neuropeptides* 42 (2008) 177-191.

### **Funding 2008**

The Swedish Research Council

The Alcohol Research Council of the Swedish Alcohol Retailing Monopoly

AFA Insurance

### **Other commitments**

Vice dean with responsibility for undergraduate programs at the Faculty of Pharmacy

Member of the Faculty Board

Chairman of the quality assurance group at the Disciplinary Domain of Medicine and Pharmacy

## **Projects**

### ***Consequences of early environmental factors on alcohol consumption and neuropeptides***

*Loudin Daoura, Stefan Schlussmann, Ingrid Nylander*

Endogenous opioid peptides and oxytocin are sensitive to early environmental factors as evidenced by specific short- and long-term MS-induced effects. Rats reared in a stressful environment have signs of a dysfunctional opioid system. They have characteristics relating to high alcohol preference, lower basal opioid levels and an enhanced response to alcohol. The consequences of early environment on adolescence voluntary alcohol consumption and alcohol-induced effects on neuropeptides are currently examined.

### ***Consequences of early environmental factors on alcohol consumption and central monoamines***

*Sadia Oreland, Ingrid Nylander*

Recent results provide evidence for pronounced effect of the early rearing environment on gene expression of 5-HT receptors. In particular, specific alterations are shown in rats reared in the protective environment, MS15, as compared to other rats and these results may give further insight in protective mechanisms. In ongoing experiments, a comprehensive evaluation of alcohol-induced effects on dopamine, noradrenalin and 5-HT systems is performed in animals reared in different environmental settings.

### ***Consequences of early environmental factors on neuronal development***

*Chris Pickering, Sadia Oreland, Ingrid Nylander*

Previous studies have found an affect of early life stress on development of the nervous system. We have observed decreases in expression of hippocampal NMDA and AMPA receptors, suggesting dramatic changes in glutamate, the excitatory neurotransmitter in the brain. We are currently investigating developmental effects both during and immediately following maternal separation. By measuring changes in neuron or glia number and several markers of synapse formation, we can examine effects induced by early environmental factors and how these contribute to the differences in alcohol consumption that we observe in older animals.

### ***Maternal and neonatal behavior***

*Loudin Daoura, Ingrid Nylander, Erika Roman*

The projects comprise establishment and development of animal experimental models to assess maternal behavior, neonatal behavior and behavioral consequences of different early rearing environmental conditions. Current experiments analyze behavioral effects of maternal separation.

## Neuropharmacology

Lena Bergström

Reports from police, Customs, and medical services all point to the fact that abuse of anabolic androgenic steroids (AAS) in the society is increasing. The abuse leads to personality changes often of violent nature. There are investigations showing that misuse of AAS often is associated with an increased abuse of other addictive drugs including alcohol, amphetamine and opiates. There is a very limited knowledge how AAS affects the brain and the aim of this project is to study the effects of AAS on the reward system in the brain and in regions associated with aggressive behaviour. The reward system i.e. neuronal circuits which are activated following a positive stimuli (including drugs of abuse) are principally very similar in all mammals. The studies are therefore performed on laboratory rats long-term treated with AAS in different doses. We have started studies in order to investigate whether long-term treatment with AAS will change the activity in the reward system and done some interesting observations. In one study dopamine and its metabolites, HVA and DOPAC, were measured in the extracellular fluid in the nucleus accumbens using a microdialysis technique. We found reduced levels of the dopamine metabolites which we interpreted as a reduced dopaminergic activity, and the observation might explain why abusers of AAS also consume other illegal drugs in a higher extent. The results are followed up with measurements of mRNA for monoaminergic enzymes, transporters and receptors in order to conclude the mechanisms behind the decreased dopaminergic activity. Like humans, rodents show an increased aggressive behaviour when injected with AAS and there is known that certain brain regions in the brain (amygdala, hypothalamus, PAG) are activated during an aggressive attack. Aggressive behaviour increases when the level of the neurotransmitter serotonin is decreased or when serotonin receptors are lacking. On the contrary drugs that increase the serotonin activity will decrease aggression. GABA and glutamate are important transmitters in the these neuronal circuits having inhibitory and excitatory functions, respectively. We are measuring markers for these transmitter systems using quantitative real time PCR in purpose to find specific changes.

### *Members of the group during 2008*

Lena Bergström, Associate Professor  
Carolina Birgner, PhD student

### *Publications 2006-2008*

1. Birgner C., Kindlundh-Högberg A., Ploj K., Lindblom J., Nyberg F., and Bergström L.  
Effects on rat brain dopamine and DOPAC levels after sub-chronic nandrolone administration followed by an amphetamine challenge., *Pharmacologyonline*, 2007
2. Birgner C., Kindlundh-Högberg A., Nyberg F., and Bergström L.  
Altered extracellular levels of DOPAC and HVA in the rat nucleus accumbens shell in response to sub-chronic nandrolone administration and a subsequent amphetamine challenge. *Neuroscience Lett.*, 412:168-172 (2007)
3. Birgner C., Kindlundh-Högberg AM., Orelund L., Alsiö J., Lindblom J., Schiöth HB., and Bergström L., Reduced activity of monoamine oxidase in the rat brain following repeated nandrolone decanoate administration. *Brain Res.* 1219: 103-110 (2008)

4. Birgner C., Kindlund-Högberg AM., Alsiö J., Lindblom J., Schiöth HB, and Bergström L., The anabolic androgenic steroid nandrolone decanoate affects mRNA expression of dopaminergic but not serotonergic receptors. *Brain Res. 1240: 221-228 (2008)*

### **Dissertations 2008**

1. Carolina Birgner  
*Anabolic androgenic steroids and central monoaminergic systems:  
Supratherapeutic doses of nandrolone decanoate affect dopamine and serotonin.*  
Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty  
of Pharmacy 77  
<http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-9208>

### **Funding 2008**

Magnus Bergwalls Stiftelse  
Åhlén-stiftelsen  
Lars Hiertas Minnesfond

## Neurodegeneration

*Anne-Lie Svensson*

My scientific interest is in the fields of neurodegeneration and neurogenesis. Neurodegeneration is a core problem in Alzheimer's disease (AD), resulting in learning and memory impairments, as a result of apoptotic and necrotic events.

One part of the research is focused on the role of neurosteroids on neurogenesis and of the interactive processes which are ongoing in neurodegenerative disorders like Alzheimer's disease, with emphasis on neuroprotective properties of neurosteroids against amyloid- $\beta$ -induced toxicity and underlying mechanism(s). Moreover, the effects of other compounds like growth hormones on amyloid- $\beta$ -induced toxicity as well as aggregation of amyloid- $\beta$  are of interest.

Another part of my research is focused on investigation of the effect of opioid treatment on neurogenesis, as treatment with opioids may inhibit cell growth and trigger apoptosis, ending up with cognitive impairment. Neuroprotective properties of growth hormones as well as neurosteroids against opioid-induced toxicity are of interest.

### *Members of the group during 2008*

Anne-Lie Svensson, Lecturer

Marie Eketjäll, PhD Student

Elise Persson, Research assistant

Shima Momeni, Laboratory assistant

### *Publications 2006-2008*

1. Reversal of opiate-induced apoptosis by human recombinant growth hormone in murine foetus primary hippocampal neuronal cell cultures, A-L Svensson, N Bucht, M Hallberg and F Nyberg, PNAS, 105 (2008) 7304-7308.

### *Funding 2008*

Gun och Bertil Stohnes stiftelse

### *Other commitments*

Member of the Faculty Board

Member of the Gender Equality Committee at the Disciplinary Domain of Medicine and Pharmacy, Uppsala University.

### *Projects*

#### *Neurosteroids and Alzheimers's disease: Mechanistic studies of neuroprotection and amyloid- $\beta$ -modulation*

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- $\beta$  peptide, which is known to play a central role in the pathogenesis of AD, through the ability of amyloid- $\beta$  monomers to aggregate and form protofibrils. Amyloid- $\beta$  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 A $\beta$  formation, aggregation and thereby prevent protofibril formation.

The significance of neurosteroidogenesis in regulating neurodegenerative mechanisms is unknown. Accumulation of amyloid- $\beta$ , induced by toxic events in cells might be able to reduce the synthesis of neuroprotective neurosteroids, 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- $\beta$ -induced toxicity, as well as the underlying molecular mechanism(s), with focus on neurogenesis and apoptosis.

### ***Effects of growth hormone and opioids on neuronal cell survival***

During long-term opioid intake by patients with chronic pain, brain disturbances might occur, such as cognitive dysfunctions. The altered brain function in those patients can arise due to fact that long-term opioid treatment might negatively influence neurogenesis and trigger apoptotic mechanisms in hippocampus, which is the brain region associated with learning, memory and cognition. Opioids have also been shown to disturb growth hormone (GH) function.

GH is involved in many functions related to the CNS and may improve memory and cognitive capabilities in rats and humans. GH-deficient patients show lack of concentration, memory and cognitive disabilities. GH replacement therapy in these patients seems to improve their memory and cognitive efficiency. Recent *in vitro* studies have demonstrated that GH is capable of preventing or even repair morphine-induced damage of hippocampal cells.

The aim of this project is to determine the effects and underlying mechanisms of GH and opioid administration on neuronal cell survival.

## Behaviour

*Erika Roman*

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. A laboratory for behavioural tests in rodents has been established and is under continuous development. 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 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.

Besides the listed projects, advice is given and collaborations are established in projects related to behavioural neuroscience.

### **Members of the group during 2008**

Erika Roman, Ph.D.

Bengt J Meyerson, Professor Emeritus

Marita Berg, Technician

Hannah Karlsson, Research Assistant

### **Publications 2006-2008**

1. Roman E, Ploj K, Gustafsson L, Meyerson BJ, Nylander I.  
Variations in opioid peptide levels during the estrous cycle in Sprague-Dawley rats. *Neuropeptides* (2006), 40, 195-206.
2. Roman E, Gustafsson L, Berg M, Nylander I.  
Behavioral profiles and stress-induced corticosteroid secretion in male Wistar rats subjected to short and prolonged periods of maternal separation. *Hormones and Behavior* (2006), 50, 736-747.
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The multivariate concentric square field test reveals different behavioural profiles in male AA and ANA rats with regard to risk taking and environmental reactivity. *Behavioural Brain Research* (2007), 183, 195-205.
5. Sundberg B, Wååg E, Jacobsson JA, Stephansson O, Rumaks J, Svirskis S, Alsjö J, Roman E, Ebendal T, Klusa V, Fredriksson R.  
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6. Haitina T, Olsson F, Stephansson O, Alsiö J, Roman E, Ebendal T, Schiöth HB, Fredriksson R.  
Expression profile of the entire family of Adhesion G protein-coupled receptors in mouse and rat. *BMC Neuroscience* (2008), 9, 43.
7. Alsiö J, Roman E, Olszewski PK, Jonsson P, Fredriksson R, Levine AS, Meyerson BJ, Hulting AL, Lindblom J, Schiöth HB.  
Inverse association of high-fat diet preference and anxiety-like behavior involving urocortin 2. *Genes, Brain and Behavior* (2008), *accepted for publication*.

### **Funding 2008**

The Swedish Brain Foundation, post-doc stipend  
The Swedish Society for Medical Research, post-doc stipend  
Disciplinary Domain of Medicine and Pharmacy  
The Alcohol Research Council of the Swedish Alcohol Retailing Monopoly  
The Magnus Bergvall Foundation  
The Facias Foundation

### **Other commitments**

Organizer of the symposium "Research Frontiers in Behavioral Neuroscience. A tribute to Bengt J Meyerson", Uppsala, Dec 5-6.

### **Projects**

#### ***Development and validation of the MCSF test***

*Erika Roman, Bengt J Meyerson*

The MCSF test has been developed for studies of exploration and motivated behaviours. Ongoing work aims at developing an automatic tracking and scoring system. The MCSF arena gained increasing international attention and was sold to two independent laboratories in the USA, which generated new collaborations.

#### ***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.

#### ***Behavioural profiling of selectively bred alcohol-preferring and alcohol-avoiding rodent lines***

*Erika Roman, Giancarlo Colombo, Petri Hyytiä, Lawrence Lumeng*

Alcoholism is a complex disorder determined by the combination of genetic and environmental factors that also influence personality trait characteristics. Several different lines of rodents have been selectively bred for high and low oral alcohol

preference and intake and have proven to be valuable animal models. Previous work has demonstrated different behavioural strategies in the alcohol-preferring AA and sP rats. This work has been extended to also include selectively bred rodent lines from breeding programs in the USA. The fall was spent at Indiana University Purdue University at Indianapolis (IUPUI), Indianapolis, USA. This experiment involved behavioural characterization of five different pairs of selectively bred rat lines and two different pairs of selectively bred mouse lines in the MCSF test. This extensive behavioural characterization enables a deeper understanding of behavioural traits of importance for understanding of alcoholism.

### ***Maternal and neonatal behaviour***

*Loudin Daoura, Ingrid Nylander, Erika Roman*

The projects comprise establishment and development of animal experimental models to assess maternal behavioural, neonatal behaviour and behavioural consequences of different early rearing environmental conditions. Current experiments analyze behavioural effects of maternal separation.

## Division of Pharmacokinetics and Drug Therapy

### 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. 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 2008*

Margareta Hammarlund-Udenaes, Professor

Sven Björkman, Professor

Malin Alenius, PhD Student

Jörgen Bengtsson, PhD Student

Markus Fridén, PhD Student

Ulrika Gillespie, PhD Student

Muhammad Waqas Sadiq, PhD Student

Stina Syvänen, PhD Student

Britt Jansson, Laboratory Engineer

Jessica Strömberg, Laboratory Assistant

#### *Publications 2006-2008*

1. Bergstrom, M., R. Yates, A. Wall, M. Kagedal, S. Syvanen, and B. Langstrom  
Blood-brain barrier penetration of zolmitriptan--modelling of positron emission tomography data. *J Pharmacokinet Pharmacodyn*, 2006. 33(1): p. 75-91.
2. Bjorkman, S.  
Prediction of cytochrome p450-mediated hepatic drug clearance in neonates, infants and children : how accurate are available scaling methods? *Clin Pharmacokinet*, 2006. 45(1): p. 1-11.
3. Bostrom, E., U.S. Simonsson, and M. Hammarlund-Udenaes  
In vivo blood-brain barrier transport of oxycodone in the rat: indications for active influx and implications for pharmacokinetics/pharmacodynamics. *Drug Metab Dispos*, 2006. 34(9): p. 1624-31.
4. Dyhre, H., L. Soderberg, S. Bjorkman, and C. Carlsson  
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5. Eriksson, O., T. Backstrom, M. Stridsberg, M. Hammarlund-Udenaes, and T. Naessen  
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6. Gupta, A., P. Chatelain, R. Massingham, E.N. Jonsson, and M. Hammarlund-Udenaes  
Brain distribution of cetirizine enantiomers: comparison of three different tissue-to-plasma partition coefficients:  $K(p)$ ,  $K(p,u)$ , and  $K(p,uu)$ . *Drug Metab Dispos*, 2006. 34(2): p. 318-23.
7. Gupta, A., M. Hammarlund-Udenaes, P. Chatelain, R. Massingham, and E.N. Jonsson  
Stereoselective pharmacokinetics of cetirizine in the guinea pig: role of protein binding. *Biopharm Drug Dispos*, 2006. 27(6): p. 291-7.
8. Lundquist, P., G. Blomquist, P. Hartvig, G.E. Hagberg, R. Torstenson, M. Hammarlund-Udenaes, and B. Langstrom  
Validation studies on the 5-hydroxy-L-[beta-11C]-tryptophan/PET method for probing the decarboxylase step in serotonin synthesis. *Synapse*, 2006. 59(8): p. 521-31.
9. Magnusson, M., I.C. Bergstrand, S. Bjorkman, A. Heijl, B. Roth, and P. Hoglund  
A placebo-controlled study of retinal blood flow changes by pentoxifylline and metabolites in humans. *Br J Clin Pharmacol*, 2006. 61(2): p. 138-47.
10. Norrgren, K., M. Sjolín, S. Bjorkman, J. Areberg, A. Johnsson, L. Johansson, and S. Mattsson  
Comparative renal, hepatic, and bone marrow toxicity of Cisplatin and radioactive Cisplatin (191Pt) in Wistar rats. *Cancer Biother Radiopharm*, 2006. 21(5): p. 528-34.
11. Soderberg, L., H. Dyhre, B. Roth, and S. Bjorkman  
Ultralong peripheral nerve block by lidocaine:prilocaine 1:1 mixture in a lipid depot formulation: comparison of in vitro, in vivo, and effect kinetics. *Anesthesiology*, 2006. 104(1): p. 110-21.
12. Soderberg, L., H. Dyhre, B. Roth, and S. Bjorkman  
The "inverted cup" -- a novel in vitro release technique for drugs in lipid formulations. *J Control Release*, 2006. 113(1): p. 80-8.
13. Syvanen, S., G. Blomquist, L. Appel, M. Hammarlund-Udenaes, B. Langstrom, and M. Bergstrom  
Predicting brain concentrations of drug using positron emission tomography and venous input: modeling of arterial-venous concentration differences. *Eur J Clin Pharmacol*, 2006. 62(10): p. 839-48.
14. Syvanen, S., G. Blomquist, M. Sprycha, A.U. Hoglund, M. Roman, O. Eriksson, M. Hammarlund-Udenaes, B. Langstrom, and M. Bergstrom  
Duration and degree of cyclosporin induced P-glycoprotein inhibition in the rat blood-brain barrier can be studied with PET. *Neuroimage*, 2006. 32(3): p. 1134-41.
15. Syvanen, S., R. Xie, S. Sahin, and M. Hammarlund-Udenaes  
Pharmacokinetic consequences of active drug efflux at the blood-brain barrier. *Pharm Res*, 2006. 23(4): p. 705-17.
16. Westin, U.E., E. Bostrom, J. Grasjo, M. Hammarlund-Udenaes, and E. Bjork  
Direct nose-to-brain transfer of morphine after nasal administration to rats. *Pharm Res*, 2006. 23(3): p. 565-72.
17. Alenius, M., M. Wadelius, M.L. Dahl, P. Hartvig, L. Lindstrom, and M. Hammarlund-Udenaes  
Gene polymorphism influencing treatment response in psychotic patients in a naturalistic setting. *J Psychiatr Res*, 2007.

18. Bengtsson, J., E. Bostrom, and M. Hammarlund-Udenaes  
The use of a deuterated calibrator for in vivo recovery estimations in microdialysis studies. *J Pharm Sci*, 2007.
19. Bjorkman, S., A. Folkesson, and E. Berntorp  
In vivo recovery of factor VIII and factor IX: intra- and interindividual variance in a clinical setting. *Haemophilia*, 2007. 13(1): p. 2-8.
20. Chaurasia, C.S., M. Muller, E.D. Bashaw, E. Benfeldt, J. Bolinder, R. Bullock, P.M. Bungay, E.C. DeLange, H. Derendorf, W.F. Elmquist, M. Hammarlund-Udenaes, C. Joukhadar, D.L. Kellogg, Jr., C.E. Lunte, C.H. Nordstrom, H. Rollema, R.J. Sawchuk, B.W. Cheung, V.P. Shah, L. Stahle, U. Ungerstedt, D.F. Welty, and H. Yeo  
AAPS-FDA Workshop White Paper: microdialysis principles, application, and regulatory perspectives. *J Clin Pharmacol*, 2007. 47(5): p. 589-603.
21. Chaurasia, C.S., M. Muller, E.D. Bashaw, E. Benfeldt, J. Bolinder, R. Bullock, P.M. Bungay, E.C. DeLange, H. Derendorf, W.F. Elmquist, M. Hammarlund-Udenaes, C. Joukhadar, D.L. Kellogg, Jr., C.E. Lunte, C.H. Nordstrom, H. Rollema, R.J. Sawchuk, B.W. Cheung, V.P. Shah, L. Stahle, U. Ungerstedt, D.F. Welty, and H. Yeo  
AAPS-FDA workshop white paper: microdialysis principles, application and regulatory perspectives. *Pharm Res*, 2007. 24(5): p. 1014-25.
22. Friden, M., A. Gupta, M. Antonsson, U. Bredberg, and M. Hammarlund-Udenaes  
In vitro methods for estimating unbound drug concentrations in the brain interstitial and intracellular fluids. *Drug Metab Dispos*, 2007. 35(9): p. 1711-9.
23. Gupta, A., M. Gillard, B. Christophe, P. Chatelain, R. Massingham, and M. Hammarlund-Udenaes  
Peripheral and central H1 histamine receptor occupancy by levocetirizine, a non-sedating antihistamine; a time course study in the guinea pig. *Br J Pharmacol*, 2007. 151(7): p. 1129-36.
24. Hammarlund-Udenaes, M.  
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25. Hammarlund-Udenaes, M., M. Friden, S. Syvanen, and A. Gupta  
On The Rate and Extent of Drug Delivery to the Brain. *Pharm Res*, 2007.
26. Lundquist, P., P. Hartvig, G. Blomquist, M. Hammarlund-Udenaes, and B. Langstrom  
5-Hydroxy-L-[beta-11C]tryptophan versus alpha-[11C]methyl-L-tryptophan for positron emission tomography imaging of serotonin synthesis capacity in the rhesus monkey brain. *J Cereb Blood Flow Metab*, 2007. 27(4): p. 821-30.
27. Lundquist, P., M. Roman, S. Syvanen, P. Hartvig, G. Blomquist, M. Hammarlund-Udenaes, and B. Langstrom  
Effect on [11C]DASB binding after tranlycypromine-induced increase in serotonin concentration: positron emission tomography studies in monkeys and rats. *Synapse*, 2007. 61(6): p. 440-9.
28. Rudin, A., J.F. Lundberg, M. Hammarlund-Udenaes, P. Flisberg, and M.U. Werner  
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29. Syvanen, S., J. Eriksson, T. Genchel, O. Lindhe, G. Antoni, and B. Langstrom  
Synthesis of two potential NK1-receptor ligands using [1-11C]ethyl iodide and [1-11C]propyl iodide and initial PET-imaging. *BMC Med Imaging*, 2007. 7: p. 6.
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## Reviews 2006-2008

1. Björkman, S.  
Prediction of cytochrome p450-mediated hepatic drug clearance in neonates, infants and children : how accurate are available scaling methods? *Clin Pharmacokinet*, 2006. 45(1): p. 1-11.
2. Hammarlund-Udenaes, M., Friden, M., Syvanen, S. and Gupta, A. (2008)  
On the rate and extent of drug delivery to the brain. *Pharmaceutical research*, 25, 1737-1750.

## Dissertations 2008

1. Stina Syvänen  
*Blood-Brain Barrier Transport: Investigation of Active Efflux using Positron Emission Tomography and Modelling Studies.*  
Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy 70  
<http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-8562>

## Funding 2008

AstraZeneca  
Apoteket AB  
Swedish Academy of Pharmaceutical Sciences  
LIF

## Other commitments

Margareta Hammarlund-Udenaes: Field Editor of *Pharmaceutical Research*, Member of the LIF/SFF group “Aktionsgrupp kring äldres läkemedel”, Member of the Research Education Board of the area Medicine and Pharmacy, Representative from the Faculty of Pharmacy in the Academic Senate of Uppsala University, Chair of the Research Education Group at the Department of Pharmaceutical Biosciences, Representative from the Faculty of Pharmacy in the group organizing the Development in Teaching conference of Uppsala University 2009.

Sven Björkman: Responsible for updating the drug-drug interactions database in Fass.se. Co-supervisor of Lars Söderberg, Lund University. Ph.D. dissertation: In vitro – in vivo correlation of injectable lipid formulations (17 October 2008).

## Projects

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

*Margareta Hammarlund-Udenaes, Jörgen Bengtsson, Markus Fridén, Stina Syvänen, Ulf Bredberg (AstraZeneca), Mats Bergström (GSK), Bengt Långström (Uppsala Imanet), Yoshiharu Deguchi (Japan), Tetsuya Terasaki (Japan)*

Our research is focused on understanding how the blood-brain barrier (BBB) functions regarding drug transport in health and disease, and to optimize methods to measure brain penetration of drugs. The research 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.

One of our purposes is to find the key parameters that describe BBB transport of drugs. This has been accomplished and is summarized in a publication in *Pharmaceutical Research*, “On the rate and extent of drug transport to the brain”,

published in *Pharm Res* in 2008. During 2008 the research was presented at several international conferences. Positron Emission Tomography (PET) is also used as a way of understanding BBB transport of drugs. Stina Syvänen defended her thesis within the PET - BBB in 2008. We earlier found that the opioid drug oxycodone is actively taken up at the BBB, resulting in 3 times higher unbound concentrations in the brain than in blood. The transporter responsible for this uptake is investigated in research collaboration with researchers in Japan, with a first publication in 2008. This research is ongoing by studying other drugs with the same properties. When identified and connected to the properties of the drug substances, it could be used to optimize active uptake of drugs into the brain.

### **Clinical Pharmacy Research**

*Margareta Hammarlund-Udenaes, Malin Alenius, Ulrika Gillespie, Håkan Melhus (Clinical Pharmacology, Uppsala), Claes Mörlin (Medicine, Uppsala), Åsa Kettis-Lindblad (Social Pharmacy, Uppsala), Per Hartvig (Univ of Copenhagen) and Leif Lindström (Uppsala)*

This research is divided into two parts, the first being a development of a method to describe drug effects in a naturalistic setting of psychosis patients. This is an area where clinical drug development today uses selected subpopulations to measure new drug effects. The research is also aiming at finding correlations between genetic factors, and other treatment factors between responders and non-responders, and between those without and with side effects, in order to optimize drug treatment to this patient group.

In the other part, 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.

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

*Sven Björkman, with professor Erik Berntorp, M.D. and research associate Karin Lindvall, R.N., Malmö.*

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. Prophylactic treatment of severe haemophilia with coagulation factor concentrates is effective but very expensive, with a cost approaching or even exceeding SEK 1 million per patient per year. Optimizing the dosing of factor VIII or factor IX 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 acceptance of "pharmacokinetic dosing" in this particular field of disease management. The activity during 2008 comprised:

- Examination of the disposition and dosing requirements of factor VIII as a function of age of the patient by means of population PK modelling.
- Testing the feasibility to achieve dose-tailored haemophilia prophylaxis by daily self-injection of factor concentrate instead of by the conventional every two days or three times per week treatment schedules.

- Evaluating the PK and clinical information obtained during extensive licensing studies on a novel factor VIII preparation, Advate (Baxter Inc.).
- Designing limited blood sampling schedules for the dose tailoring of factor VIII and factor IX in clinical practice.

## Pharmacometrics

*Mats Karlsson*

Pharmacometric research focuses on nonlinear mixed effects ("population") models. Such models describe data, generally is the response-time profiles observed in a clinical trial, by a basic model, accounting for the general structure of the underlying system, 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. Secondly, 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 a system biomarker or a set of such biomarkers during normal, diseased 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. Last, we also do analyses of dose-concentration-response data from trials to understand therapies with existing drugs with the aim of allowing improved therapy.

### *Members of the group during 2008*

Mats O Karlsson, Professor  
 Lena Friberg, Researcher, Associate Professor  
 Andrew Hooker, Senior Lecturer  
 Rikard Sandström, Senior Lecturer  
 Ulrika Simonsson, Senior Lecturer  
 Anders Grahnén, Adjunct Professor  
 Niclas Jonsson, Adjunct Professor  
 Dominik Uehlinger, Visiting Professor  
 Siv Jönsson, Researcher  
 Britt Jansson, Lab Engineer  
 Pontus Pihlgren, System Administrator  
 Kajsa Harling, System Administrator  
 Robert Kalicki, Post-doctoral Fellow  
 Rocio Lledo, Post-doctoral Fellow  
 Stefanie Hennig, Post-doctoral Fellow  
 Guangli Ma, Post-doctoral Fellow  
 Joseph Standing, Post-doctoral Fellow  
 Lotfi Slimani, Post-doctoral Fellow  
 Martin Bergstrand, PhD Student  
 Doaa Elsherbini, PhD Student  
 Kristin Karlsson, PhD Student  
 Maria Kjellsson, PhD Student  
 Angelica Quartino, PhD Student  
 Radojka Savic, PhD Student  
 Emma Hansson, PhD Student  
 Joakim Nyberg, PhD Student  
 Hanna Silber, PhD Student  
 Klas Petersson, PhD Student

Paul Baverel, PhD Student  
Elodie Plan, PhD Student  
Ami Mohammed, PhD Student  
Brigitte Lacroix, PhD Student  
Johan Wallin, PhD Student  
Elisabet Nielsen, PhD Student  
Bengt Hamrén, PhD Student  
Marcus Björnsson, PhD Student  
Mattis Kågedal, PhD Student  
Anna Lönnebo, PhD Student  
Petra Jauslin, PhD Student  
Al Maloney, PhD Student  
Steve Ernest, PhD Student

### **Publications 2006-2008**

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### Dissertations 2008

1. Elsherbiny, Doaa  
*Pharmacokinetic drug-drug interactions in the management of malaria, HIV and tuberculosis*  
Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy 68  
<http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-8426>
2. Jauslin-Stetina, Petra  
*Mechanism-Based Modeling of the Glucose-Insulin Regulation during Clinical Provocation Experiments*  
Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy 73  
<http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-8719>
3. Hamrén, Bengt  
*Safety and Efficacy Modelling in Anti-Diabetic Drug Development*  
Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy 71  
<http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-8648>
4. Savic, Radojka  
*Improved pharmacometric model building techniques*  
Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy 80  
<http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-9272>

5. Kjellsson, Maria C.  
*Methodological Studies on Models and Methods for Mixed-Effects Categorical Data Analysis*  
Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy 83  
<http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-9333>

### **Funding 2008**

AstraZeneca, Swedish Cancer Society, Novartis, Pfizer, Roche, UCB Pharma, Johnson & Johnson, The Swedish Research Council/Sida, Knut & Alice Wallenberg Foundation, WHO

### **Other commitments**

Mats Karlsson: Vice-chair COST Action B25, Chairman for the Docent Committee at the Faculties of Medicine and Pharmacy, Deputy Head of Department, Scientific committee, PAGE conference, Marseille, Scientific committee, ACOP conference, Tucson, AZ, Editor Journal of Pharmacokinetics and Pharmacodynamics, Editorial Board on Clin Pharmacol Ther, Eur J Pharm Sci, Basic Clin Pharmacol Toxicol, Eur J Clin Pharmacol.

Lena Friberg: Organizing committee, PAGE conference, Marseille

Andrew Hooker: Organizing committee, PODE conference, Paris

### **Projects**

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

*Doaa Elsherbiny, Mats Karlsson, Ulrika Simonsson*

Plasmodium falciparum, the human immunodeficiency virus (HIV), and Mycobacterium tuberculosis are three devastating pathogens in tropical areas. Due to the geographical overlap of malaria, HIV and TB prevalence, the diseases are likely to co-exist in a great number of individuals. For these individuals, there is an obvious need for concomitant use of antimalarial, antiretroviral and antitubercular drugs. Drug-drug interactions may result from concurrent administration of drugs leading to diminished therapeutic efficacy of or increased toxicity from one or more of the administered drugs. Drug-drug interactions are an important concern in the management of patients with HIV because of the large number of antiretroviral drugs and other drugs that are required by these patients for the management of co-morbidities and opportunistic infections. Combination therapy has also been introduced in the management of malaria and TB, to overcome drug resistance. 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. 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 glucose-insulin models for Type II diabetes***

*Bengt Hamrén, Petra Jauslin, Mats Karlsson, Jakob Ribbing, Hanna Silber, Ulrika Simonsson*

To characterize the functionality of the glucose insulin system in Type II diabetic mellitus (T2DM) patients and healthy volunteers a multitude of clinical trial types are used. Experimental provocation studies include: clamping of glucose or insulin by variable rate infusions, intravenous bolus administration of glucose or insulin, administration of oral glucose solution or administration of test meals with well-characterised nutrient content. The studies vary in length from a few hours to about a day. In each subject the time-profile of glucose and insulin is measured. We have developed an integrated mathematical model that based on simultaneous analysis of both glucose and insulin time-profiles in all subjects can quantitatively describe the result of such experiments. This model, which includes production, disposition and control (homeostatic) mechanisms have shown to be able to realistically simulate the outcome of trial studies at the raw data level.

Medium-term clinical trials in T2DM patients, varying in length from a few weeks to about a year usually focus on measured longitudinal changes in fasting plasma glucose, fasting insulin and the fraction glycosylated hemoglobin (HbA1c). As hemoglobin in red blood cells has a life-span in the body of a few months and the glycosylation of hemoglobin is a reaction directly dependent on the concentration of glucose HbA1c is a suitable marker of long-term glycemic control. It is elevated in T2DM patients. Based on data from large-scale clinical trials in T2DM patients and non-diabetic subjects with hypertriglyceridaemia and abdominal obesity we have developed a mathematical model that quantifies the link between plasma glucose concentration and HbA1c. This model is based on mechanistic aspects of the production and elimination of red blood cells and hemoglobin as well as relationships between fasting glucose and daily average glucose. In a complementary model the relationship between insulin sensitivity, glucose production and disposition and changes in beta-cell mass has been characterised and quantified in the same populations. Both models can realistically simulate the outcome of clinical trials with respect to glucose, insulin and HbA1c.

The models have been developed for the purpose of being able to quantitate changes in the system following interventions (drug administration, diet changes) 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 paediatric transplantation***

*Lena Friberg, Stefanie Hennig, Siv Jönsson, Mats Karlsson, Johan Wallin*

Cyclosporin and tacrolimus are two commonly used drugs in pediatric transplantation. 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, we are analyzing the data with the following aims: (i) to optimize a pre-transplantation test procedure with respect to convenience and information content, (ii) to characterize determinants of variability in pharmacokinetics for this population over time after transplantation, and (iii) to outline the relationship between plasma drug concentration and biomarkers/clinical endpoints in order to allow better decision criteria for dose adjustments.

The pharmacokinetics of tacrolimus in bone marrow transplant and liver transplant pediatric patients has been characterized from data collected up to 1 year after transplantation. The half-life of tacrolimus has been shown to be relatively long (approx 50 hours) making dose adaptations difficult without considering the full dosing history. An Excel macro for dose individualization following i.v. administration in bone marrow transplant children has been developed to facilitate individualized therapy to avoid rejection and toxicity.

### ***Mechanism-based pharmacokinetic models***

*Mats Karlsson, Grant Langdon, Rocio Lledo, Mats Magnusson, Rikard Sandström, Lotfi Slimani, Joseph Standing*

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 to predict concentration-time profiles in tissues and organ. 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 not used for analyzing clinical data. 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. 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.

### **Oncology**

*Lena Friberg, Emma Hansson, Mats Karlsson, Angelica Quartino, Johan Wallin*

Within the oncology area, we are working on projects related to extensions and applications of an earlier developed semi-physiological model describing the time-course of myelosuppression that has been successfully applied for numerous anticancer drugs. We have now shown that the model can also be used to characterize the time-course of absolute neutrophil counts when the actual neutrophil counts (i.e. continuous data) is not available but the degree of toxicity has been summarized as grade of neutropenia (i.e. categorical data).

The variability in myelosuppression within patients from course to course has been shown to be lower than the variability between individuals, indicating that dose-individualization based on observed neutrophil counts may be valuable. An Excel macro has been developed for this purpose where observed neutrophil counts are used to calculate individual parameter values and a suitable dose to reach a by the clinician determined nadir in the following treatment course. In a simulation study we have shown that there is limited additional information to collect pharmacokinetic samples when neutrophil counts are available for forecasting the neutrophil counts in the next cycle. Model-based dose-adaptation that allows for both dose escalation and dose reductions appear to increase the number of patients within the target degree for neutropenia compared with standard 25% dose decrements. In patients who develop febrile neutropenia, we have seen that they have a faster decline and a shorter maturation time of neutrophils than in other patients who develop Grade 4 neutropenia.

In collaboration with the Department of Oncology, a clinical study where endogenous G-CSF concentrations following chemotherapy are determined has been ongoing and recruitment is expected to finish in 2009.

We have started to characterize how angiogenetic biomarkers (VEGF<sub>s</sub>-VEGFR-2, s-VEGFR-3 and s-KIT) are changing over time in gastrointestinal stromal cancer following therapy with sunitinib. The correlations between the biomarkers and with tumour response and survival will be investigated to determine which of the biomarker(s) that are suitable for early prediction of drug effects.

### **Antibiotics**

*Lena Friberg, Mats Karlsson, Ami Syed Mohamed, Elisabet Nielsen*

We aim to improve on the understanding of pharmacokinetic-pharmacodynamic relationships for antibiotics of value for improving dosing recommendations and minimizing resistance development. A semi-mechanistic model including one

population of growing, susceptible bacteria and one population of resting bacteria, originally developed for experiments with static drug concentrations, has been applied to in vitro experiments where the drug concentrations diminish with half-lives observed in patients. For three antibiotics the model gave a good fit to the data while for cefuroxime and vancomycin the bacteria kill appear to slightly dependent on the experimental system.

Colistin is a drug that appears promising to overcome antibiotic drug resistance because of its synergistic effects. A novel LC-MS-MS method to quantify colistin and its prodrug CMS in plasma and broth have been developed and validated. The method has been used to determine colistin and CMS after 1-3 doses in the therapy of 18 patients. A pharmacokinetic model has been built and we have shown that it takes several days of treatment before the patients reach the MIC. For an additional 5 patients on haemodialysis, the clinically used dose also appear to generate subtherapeutic colistin concentrations.

### ***Clinical trial design***

*Andrew Hooker, Stefanie Hennig, Kristin Karlsson, Mats Karlsson, Rocio Lledo, Joakim Nyberg, Hanna Silber*

There are two principled ways by which models can be used to help optimizing trial designs for information regarding parameter estimates. The first is by simulation from the model and a proposed design followed by parameter estimation from the resulting data set. The simulation, repeated many times with different random seeds, thus provides measures of precision and bias of parameter estimates. With this methodology we have investigated differences in different randomization schemes for dose-finding trial. It was found that dose-randomized trials are more powerful to characterize the underlying relation. 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 formal estimation of design parameters. A number of different criteria can be used to optimize designs. We have developed methods and software (PopED) to do so for ED-optimal designs, which take into account that the underlying system (model) is not known before the study takes place. While optimal design previously has focused on optimization of sampling times, we have extended this to apply also to other aspects of trial designs, such as the dose administered and the length of run-in, treatment and wash-out phases.

### ***Pharmacodynamic modelling in other disease areas***

*Lena Friberg, Robert Kalicki, Mats Karlsson, Maria Kjellsson, Brigitte Lacroix, Guangli Ma, Klas Petersson, Elodie Plan, Marcus Björnsson, 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 we have earlier developed to describe the effect of risperidone and paliperidone on the prolactin levels have been compared to an earlier developed pool model with the remoxipride dataset used to develop the pool model. Both models describe the data well and further model refinement has been shown to improve the model fit. The agonist-antagonist model has also been applied for drugs in clinical development and the relationship between the efficacy parameter and k<sub>off</sub> values determined in vitro has been investigated.

A longitudinal transition model describing the probability of ACR20 response and dropout in rheumatoid arthritis has been applied and optimized for ACR50 response following certolizumab pegol treatment. Modeling of CRP in rheumatoid

arthritis with and without certolizumab pegol treatment has been initiated.

The pharmacokinetics of exogenous IGF-1/IGF-BP3 in preterm neonates has been characterized and results have been used in the design of a Phase II trial.

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.

### ***Model building methodologies and estimation methods***

*Paul Baverel, 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 analyze 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 automatization of important modeling 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.

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. Examples of software developed by the group are PsN and Xpose.

## Division of Toxicology

### Embryotoxicology

Lennart Dencker

**Embryogenesis** The embryo is unprotected from pharmaceuticals and environmental pollutants. The intended pharmacological effects of drugs are often exerted in the conceptus as well. They can be reversible, but have occasionally detrimental morphological downstream effects. We try to better understand both the morphological aspects of the teratogenic process in rodents by improving the methodology of whole embryo culture and the molecular background of malformations by monitoring the “symphony” and its different tunes concerted by the collected expression of genes and proteins governing embryonic development and brain maturation. 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. In collaboration with Professor Ewert Bengtsson at the Centre of Image Analysis, Uppsala University, we work on developing image analysis tools for whole embryo culture.

In addition we use information from embryos (cultured *in vitro*, or exposed *in vivo*), and apply it on murine and human embryonic stem cells, to develop mechanism-based *in vitro* cell test systems to reveal the teratogenic potential of substances. Conventional murine embryonic stem cells may not be optimal as test cells, why we try other embryonic stem cells derived from somewhat more advanced embryonic layer than the conventional ones derived from the inner cell mass. Using the antiepileptic and teratogenic drug valproic acid, an HDAC inhibitor, and some less teratogenic analogues of valproic acid, we try to visualize e.g. by gene ontology studies which categories of genes may be responsible for the teratogenic action (such as neural tube defects). They seem not necessarily to be HDAC-related. Such studies in the embryo and in embryonic stem cells in parallel, combined with the extensive literature on the role of individual genes and pathways in morphogenetic processes, give us new biological information which can be applied in creating tools for screening purposes in drug development and classification of environmental chemicals.

We are at the same time exploring the global epigenomic effects of VPA:s HDAC inhibitor capacity in collaboration with Professor Clas 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. The properties of VPA on stem cells besides embryonic stem cells have also been investigated through collaboration on human placenta derived mesenchymal stem cell-like pericytes with Christian Sundbergs group at IMBIM, BMC.

In addition, physiological systems such as hemodynamics during development is approached, in this case the widespread property among pharmaceuticals to

affect potassium (IKr)-channels thereby affecting rhythm also in the embryonic heart, leading to blood pressure and circulatory fluctuations, and thus oxygen supply, being deleterious to the morphological development of vessels and other embryonic tissues/organs. This latter research is lead by adjunct professor Bengt Danielsson.

**Neurogenesis** Several chemicals exert estrogenicity, being a potential problem esp. during development (reproductive organs and sex-specific behaviour). There is relatively little known about the mechanisms behind their sex specific brain development in general, especially regarding the impact of the sex chromosomes, on future sex specific behaviours. We have been using Chicken and Japanese quail as models, to study basic sex differences (with and without estrogen exposure) in gene expression in the developing brain. We were the first to report that there is strong gonadal hormone independent sex chromosome based component in avian sex specific brain development. Although there were no clear-cut effects of estrogens in gene expression, there were some estrogen induced differences in the developing neuropeptidome, implicating posttranscriptional regulation. There was an overall up-regulation of peptides (about 60 identified) in diencephalons with embryonic age. One of the most interesting candidates for estrogenic effects on the developing diencephalon was the GnIH-RP2 peptide which is speculated to be involved in the establishment of the HPG axis during development. Many chemicals (incl. drugs) given to newborn mice disrupts normal brain (growth spurt) development, resulting in disturbed spontaneous behavior in adulthood. Polybrominated diphenyl ethers (PBDEs) are environmental contaminants found in human and animal tissues worldwide. We have investigated their short-term effects on protein expression in hippocampus, striatum and cortex by using two-dimensional difference gel electrophoresis (2D-DIGE). We determined the identity of 111 differentially expressed proteins in cortex, 39 (35%) of which are known to be cytoskeleton-related. As in striatum, we found elevated levels of the neuron growth-associated protein Gap43 in the cortex. A more recent in vitro approach has generated some new insights to developmental PBDE neurotoxicity. Based on studies using fetal rat cortical cells, we have further strengthened our hypothesis regarding direct effects of PBDEs on cytoskeletal organization. In addition, we have evaluated the effects of PBDE exposure on neurite expression. Although not conclusive, the data show trends such that low concentrations of PBDE-99 may increase the neurite sprouting of cultured cerebral cortex cells. Although much work is required to make a complete picture of these early changes, it is a beginning of a mechanistic approach to a potentially important general health problem caused by environmental chemicals as well as drugs.

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, where 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.

### **Members of the group during 2008**

Lennart Dencker, Professor  
Bengt R Danielsson, Adjunct Professor  
Michael Stigson, Researcher  
Kim Kultima, Researcher  
Henrik Alm, PhD Student  
Måns Jergil, PhD Student  
Mats Nilsson, PhD Student  
Birger Scholz, PhD Student  
Raili Engdahl, Laboratory Assistent

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1. Birger Scholz  
*Genomic and Peptidomic Characterization of the Developing Avian Brain*  
Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine 317  
<http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-8507>

### Funding 2008

Swedish Animal Welfare Agency  
Formas The Swedish Research Council for Environment  
EU  
AstraZeneca AB  
The Swedish Association of the Pharmaceutical Industry  
The Swedish Medical Product Agency

***Other commitments***

Deputy Vice rector, Domain of Medicine and Pharmacy, Uppsala University  
Chairman of a number of subcommittees within the University  
Uppsala University Library, Member of Board  
Medical Products Agency, Member of Scientific Board  
Chemical Inspectorate, Member of Scientific Board  
National Food Administration, Member of Scientific Board  
Member of Editorial Board, Toxicology Letters  
Member of the Faculty of Pharmacy Committee

## Bioactivation and Toxicity

*Eva Brittebo*

Our current studies are directed towards characterization of toxicant-induced changes leading to cell damage in the nasal mucosa, vascular tissues and pigmented neurons. In addition, the delivery of drugs and chemicals to the brain via the nasal olfactory pathways is also being examined.

### *Members of the group during 2008*

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

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1. Karlsson O, Berg C, Brittebo EB, Lindquist NG.  
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In vitro tests for detecting chemicals affecting the embryo implantation process. The report and recommendations of ECVAM workshop 62 -- a strategic workshop of the EU ReProTect project. *Altern Lab Anim.* 2007;35:421-39.
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### *Funding 2008*

The Research Council FORMAS  
 EU FP6

### **Other commitments**

Head of the Department

Member of the Pharmaceutical Faculty of Pharmacy Committee, Uppsala University

Member of Open Access reference group, Uppsala University

### **Projects**

#### ***Bioactivation and toxicity of xenobiotics***

*Helén Andersson*

Epidemiological and experimental animal studies suggest that increased air pollution contributes to cardiovascular diseases. Humans are exposed to polyaromatic hydrocarbons (PAH) and nitro-PAHs by inhalation of diesel exhausts. We have previously reported that PAHs are bioactivated and induce DNA damage in cultured human endothelial cells (HUVEC) following pretreatment with CYP1A1-inducers. We are currently studying the bioactivation and effects of nitro-PAHs in HUVEC. In addition, the cellular expression of drug metabolizing cytochrome P450 enzymes (CYP) in the highly vascularized human endometrium is examined. Data on the cellular expression of major endometrial CYP forms will be useful when human in vitro test are to be developed for detecting chemicals affecting the embryo implantation process.

The high expression of drug-metabolizing CYP enzymes in specific cells in the upper respiratory tract makes this tissue sensitive to chemicals that are bioactivated. We have identified a number of nasal olfactory toxicants, e.g. dichlobenil, chlorthiamid, 2,6-dichlorophenyl methylsulfone and methimazole. All these compounds are bioactivated by nasal CYP to reactive metabolites that become irreversibly bound to the airway epithelium in vivo. In vitro studies revealed an extensive CYP2A5-mediated bioactivation of dichlobenil and 2,6-dichlorophenyl methylsulfone in olfactory microsomes whereas no activation occurred in liver microsomes. We observed that upregulation of the ER stress protein GRP78 and activation of the ER resident caspase 12, are early and cell specific events following exposure to nasal olfactory toxicants activated by CYP2A5. The cellular upregulation of GRP78 and activation of caspase 12 as well as the cellular formation of protein adducts in nasal glands colocalize with the early lesions induced by nasal toxicants.

#### ***Effects of neurotoxicants in pigmented tissues***

*Oskar Karlsson and Nils Gunnar Lindquist*

We are currently examining the uptake and effects of an algal neurotoxin BMAA (beta-N-methylamino-L-alanine) in the brain of rodents. BMAA is a non-protein amino acid that is produced by cyanobacteria. This neurotoxin has been suggested to contribute to neurodegenerative disease.

The neuromelanin-containing neurons in substantia nigra are degenerated and many patients also have an uncommon pigmentary retinopathy. Using autoradiography, we have demonstrated a distinct retention of <sup>3</sup>H-BMAA in melanin-containing tissues such as the eye in pigmented mice and neuromelanin-containing neurons in frog brain. Analysis of the interaction of <sup>3</sup>H-BMAA to Sepia melanin and to synthetic melanin revealed a stronger interaction of <sup>3</sup>H-BMAA with melanin during synthesis than with preformed melanin. The studies suggest that long-term exposure to BMAA may lead to bioaccumulation in melanin- and

neuromelanin-containing cells causing high intracellular levels, and potentially changed melanin characteristics via incorporation of BMAA into the melanin polymer.

The  $\beta$ -carbolines norharman and harman are formed at cooking of food. These compounds structurally resemble the Parkinson-inducing toxicant MPTP, known for its ability to damage neuromelanin-containing neurons of the substantia nigra. The  $\beta$ -carbolines showed an affinity to melanin and were retained for at least a month in neuromelanin-containing neurons. Norharman was found to induce neurodegeneration, glial cell activation and motor impairment in mice. Furthermore, these compounds induced ER stress and cell death in cultured PC12 cells. An *in vitro* model of dopamine melanin-loaded cultured PC12 cells was developed in order to study the effects of melanin on norharman-induced toxicity. In this model, melanin was found to attenuate the toxicity induced by low concentration of norharman. Following a high concentration of norharman, melanin still attenuated necrosis but also gave rise to a high level of cellular stress and apoptosis.

### ***Olfactory transfer of drugs***

*Elena Piras*

The olfactory pathway is a potential route of delivery of therapeutic agents that do not easily pass the blood-brain barrier. The olfactory neurons have direct contact with the external environment via dendrites in the nasal mucus and with the brain via axons that reach the olfactory bulb without synaptic connections. The olfactory transfer of CNS-active drugs into the brain is a novel principle for drug delivery. We have demonstrated a transfer of morphine and dopamine via the olfactory pathways to the brain following intranasal administration in rodents.

## Genetic Toxicology

Björn Hellman

When testing the potential DNA-damaging effects by medical 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. One example of this is our recent study (Durling et al., in press) on the DNA damaging effect of 5-hydroxy-methylfurfural (HMF), a heat-induced food toxicant present in a vast number of food items. Since HMF has been suggested to be genotoxic after being bioactivated by the sulfotransferase SULT1A1, the comet assay was used to evaluate the DNA damaging effect of HMF in cell lines with different activities of SULT1A1. We found that HMF induced significant DNA damage in all cell lines independent of the activity of SULT1A1 in the cells, and that the HMF-induced DNA damage was observed only at rather high concentrations, which in most cases were associated with a concomitant decrease in cell viability.

From point of view of risk assessment, it is important to differ between genotoxic carcinogens and other substances that increase the risk of cancer by other mechanisms. In the case of drug-induced oxidative DNA-changes, for instance, one can distinguish two different main groups of substances: those who cause various types of reactive oxygen radicals in the cells directly and those who cause oxidative stress indirectly, as a consequence of general cytotoxicity. The research of recent years has also shown that the DNA repair has a great impact on whether the DNA-damage is manifested as a mutation or not, and there is reason to believe that there is a great variation in individual sensitivity to genotoxic agents, due to individual differences in DNA repair, metabolic bioactivation/detoxification pattern and/or other defense mechanisms in the cells. All those aspects are studied in this project.

In a recent project, supported by SIDA/SAREC, we are currently also evaluating the genotoxic and antigenotoxic 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., in press), a naphthoquinone present in the roots of *Plumbago zeylanica*, a traditionally used medicinal plant which 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.

### **Members of the group during 2008**

Björn Hellman, Associate Professor

Jemmal Demma, Ph.D. student

Lena Norgren, Laboratory assistant

### **Publications 2006-2008**

1. M. A. Andersson, K. V. Peterson-Grawé, O. M. Karlsson, L. A. G. Abramsson-Zetterberg & B. E. Hellman.  
Evaluation of the potential genotoxicity of chromium picolinate in mammalian cells in vivo and in vitro. *Food Chem. Toxicol.*, 45(2007)1097-1106.
2. M.A. Andersson & B.E. Hellman.  
Evaluation of catechol-induced DNA damage in human lymphocytes: A comparison between freshly isolated lymphocytes and T-lymphocytes from extended-term cultures. *Toxicology In Vitro*, 21(2007)716-722.
3. K. Johansson, S. Olsson, B. Hellman & R.H.B. Meyboom.  
An analysis of Vigimed, a global E-mail system for the exchange of pharmacovigilance information. *Drug Safety*, 30(2007)883-889.
4. M. Andersson, P. Stenqvist & B Hellman.  
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5. L. J. K. Durling, L. Busk & B. Hellman.  
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7. J. Demma, K. Hallberg & B.Hellman.  
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### **Reviews 2006-2008**

1. B. Hellman. General toxicology. In: Pharmaceutical Toxicology: Safety Science of drugs. Eds: G. J. Mulder and L. Dencker. Pharmaceutical Press, London, 2006, pp 1-39.
2. B. Hellman. Genotoxicity. In: Pharmaceutical Toxicology: Safety Science of drugs. Eds: G. J. Mulder and L. Dencker. Pharmaceutical Press, London, 2006, pp 105-121.
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***Funding 2008***

SIDA/SAREC

***Other commitments***

Member of the board of the Department of Pharmaceutical Biosciences, Uppsala University.

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  
Study director.

## MALDI imaging mass spectrometry

*Malin Andersson*

My projects utilizes MALDI imaging mass spectrometry for the topographical elucidation of proteins, neuropeptides and neurotransmitters and their changing concentrations in the brain during physiological and pathophysiological events. The structural heterogeneity and complexity of many tissues, such as brain, requires an approach that provides high anatomical resolution coupled with quantitative analysis of proteins in the context of unbiased assessment.

Ultimately, visualizing molecular expression in 2 and 3-dimensional space using MALDI IMS will provide insights into the neural substrates of neurodegenerative disease pathogenesis and help pave the road toward improved and tailored treatments.

### *Members of the group during 2008*

Malin Andersson, Assistant Professor

Anna Karlsson, PhD student

### *Publications 2006-2008*

1. Groseclose, R. M., Andersson, M., Hardesty, W. M., and Caprioli, R. M. (2007). Identification of Proteins Directly from Tissue: In Situ Tryptic Digestions Coupled with Imaging Mass Spectrometry. *Journal of Mass Spectrometry* 42 (2):254-262.
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4. Andersson, M., and Caprioli, R. M. (2007) Microdialysis Coupled with Liquid Chromatography / Mass Spectrometry; Handbook of Microdialysis: Methods, Applications and Perspectives, *Elsevier Science*. 16: 251-266
5. Andersson, M., Groseclose, R. M., Deutch, A. Y., and Caprioli, R. M. (2008). Imaging mass spectrometry of proteins and peptides: 3D volume reconstructions. *Nature Methods* 5 (1):101-8.
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### *Funding 2008*

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