ANNUAL REPORT 2009

Department of Pharmaceutical Biosciences
The localization of several neuropeptides and small proteins in a single tissue section can be determined with MALDI imaging mass spectrometry.
Introduction

The large diversity of research activities at the department can be observed in this report. The experimental research covers both basic studies on for example mechanism of oxygenation and bioactivation of fatty acids as well as studies on the transfer of drugs across the blood-brain-barrier. In addition, there is a growing focus on pharmaceutical bioinformatics and proteomics as well as modelling and simulations used in drug development. All these research activities require funding from research councils, pharmaceutical industries and the government. For example, in 2009 the Swedish council for working life and social research allocated funding lasting several years to a program on characterization of novel mechanisms of alcohol binge drinking-induced cognitive impairment at the department. This funding makes it possible for professors Georgy Bakalkin and Fred Nyberg to study the relation between alcohol-related impaired cognition and dysregulations in neurotransmission that is controlled by the endogenous opioid systems. All research groups at the department also maintain ongoing international collaboration within their research areas. For example, professor Jarl Wikberg and coworkers have discovered a new class of natural regulators of sexual behavior, the libiguins, in collaboration with Institut Malgache de Recherches Appliquées, Madagascar. The libiguins are now being developed into pharmaceuticals for treatment of sexual dysfunction under support from Uppsala Bio for which Jarl Wikberg during 2009 was given the Bio-X award.

The staff is a key resource for the development of the department and all scientific and technical staff members are highly motivated to contribute with their special skills. During 2009 the following new academic staff members joined the department: Erika Roman who was appointed as a postdoctoral research fellow and Jonna Olsson who was appointed as a junior lecturer. Georgy Bakalkin was promoted to professor in molecular drug dependence research. We look forward to working with them.

Graduate education

The department has a high priority for research training. The aim is to train the PhD students so that they will be able to make significant contributions in the academia and pharmaceutical industry in the future. In order to improve oral presentation skills and promote scientific interactions within our department we have started a new weekly seminar series for PhD students and young scientists. The seminar series has attracted interest and proved to be successful. During the summer eleven of our PhD students participated in the 9th ULLA summer school in Copenhagen. The Faculty of Pharmaceutical Sciences at the University of Copenhagen had organised an inspiring programme consisting of courses, poster sessions and a symposium day for 150 PhD students who came from the ULLA partners in Uppsala, Copenhagen, London, Leiden/Amsterdam, Paris Sud, Parma and Leuven. The programme covered future pharmaceutical issues and crossing borders between disciplines, between countries, between academia and industry.

There is also a new European graduate education in Safety sciences. The SafeSciMet initiative is a large EU-funded programme where European universities and pharmaceutical industry make joint efforts to train especially employees at the industry, but also at regulatory agencies and universities in the safety sciences of medicines development. Professor Lennart Dencker and coworkers will organize and be responsible for the Student Office for this education and training network programme.
Undergraduate education

How do we prepare our students for the future in the 21st century? In order to address this question the faculty education committee has decided to start a renewal of the Master of Science of Pharmacy programme. The pharmacy education should be research-based and it is our aim to ensure that there is a synergy between teaching and research. The challenge for teachers in pharmaceutical biosciences is to include both basic knowledge in various life science subjects as well as advanced knowledge about novel biological pharmaceuticals, as well as personalized medications. An increased focus on drug safety is also expected. We have to introduce novel teaching areas and to reform traditional teaching areas. Without the breadth of research at the department we would not be prepared to meet these teaching challenges.

Uppsala University confers annual pedagogical prizes for outstanding accomplishments in undergraduate education. Ann-Marie Falk, Emma Lundkvist and Maria Swartling, directors of study in pharmacotherapy, received the teaching award in 2009. They have collaborated with the teachers to develop novel forms of grading criteria for examinations in pharmacotherapy. The prize was delivered at the public inauguration ceremony of professors in the university grand auditorium.

After graduation, our pharmacy students can find a wide variety of positions in pharmacies, drug industries, and governmental agencies. Notably this year has been the de-regulation of the Swedish pharmacies. The de-regulation aims to increase the number of pharmacies per capita and to increase the availability of pharmaceutical drugs. Previously all pharmaceutical drugs were sold only in publicly owned pharmacies. Both publicly owned and private pharmacies will be allowed from 2010 onward and some OTC drugs will also be available in grocery stores and petrol stations. The number of pharmacy positions at the pharmacies is expected to rapidly increase due to the de-regulation. In the years to come we will know if the de-regulation and the entry of novel retail pharmacy chains will also be successful in increasing the quality of pharmacy services. Besides providing professional pharmacy education, the department also offers graduate pharmacy students specialization in clinical pharmacy. During the next few years we expect the number of clinical pharmacists positions at hospitals will increase.

Other activities

The university encourages alumni to keep in touch with the faculty members by way of dedicated web pages and a database containing contact information. During the spring we had the pleasure of hosting two jubilees for Masters of Science in Pharmacy classes who graduated in 1974 and 1979. In December we spent one department afternoon at the Department of Psychology listening to a number of interesting lectures about studies in the baby lab, studies on brain function and emotion, as well as anorexia. In the evening we had an informal department dinner in the university building.

Organization and financial review

In the beginning of 2009 the organizational structure of the department was changed due to the introduction of a new accounting system at the university. The department is now divided on the basis of core activities i.e. research and education and the collected support activities i.e. management, economy/staff administration, education administration and infrastructure. The support activities are funded by a per cental overhead on all salaries and operating costs. The new organizational structure has been implemented during the year and it will be revised gradually during the next few years. A short summary of the income and expenditure 2009 is given below.
**Income 2009 (kSEK)**
Research and graduate education (government) 28 300  
Research grants 22 256  
Research contracts 4 353  
Education – basic and advanced level (government) 35 703  
Education contracts 227

**Expenditure 2009 (kSEK)**
Staff costs 48 984  
Operating expenses etc 15 100  
Premises 12 920  
University/faculty support activities 8 889  
Library 2 156  
Depreciation 3 062

**Future development**

The years ahead promise many changes in terms of research and education. A number of our senior professors are expected to retire between 2012-2016. Their commitment to research work have yielded important contributions and have helped build the very foundation of the department. The search for and incorporation of highly innovative researchers in the field of pharmaceutical biosciences to complement the existing research areas will be of vital importance for the development and competitiveness of the department. Keywords to take into the future are biological drugs and personalized drug treatment as well as national and international collaboration.

Uppsala March 3, 2009

Eva Brittebo
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Organization

Chairman
Eva Brittebo

Deputy chairman
Mats Karlsson

Department board
Eva Brittebo, chairman
Marianne Danersund, secretary
Mathias Hallberg, teacher representative
Margareta Hammarlund-Udenaes, teacher representative
Mats Karlsson, teacher representative
Anne-Lie Svensson, teacher representative
Björn Hellman, teacher representative
Lennart Dencker, teacher representative, deputy
Maria Norlin, teacher representative, deputy
Raii Engdahl, technical/administrative representative
Agneta Hortlund, technical/administrative representative
Marina Rönggren, technical/administrative representative, deputy
Mats Nilsson, graduate student representative
Angelica Quartino, graduate student representative
Sadie Oreland, graduate student representative, deputy
Marie Heidenvall, student representative
Ida Hemmingsson, student representative, deputy

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

Professor emeritus
Lennart Paalzow

Adjunct professors
Per Andrén
Bengt RG Danielsson
Anders Grahnén
Niclas Jonsson
Nils Gunnar Lindquist

Assistant professors
Malin Andersson
Érika Roman

Senior and junior lecturers
Matts Balgård
Jörgen Bengtsson
Lena Bergström
Ann-Marie Falk
Lena Friberg
Agneta Freij
Ulrika Gillespie
Ronnie Hansson
Björn Hellman
Annika Hipeli
Andrew Hooker
Lena Klarén
Emma Lundkvist
Jonna Olsson
Ulrika Simonsson
Anne-Lie Svensson
Maria Swartling
Directors of undergraduate studies
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Sven Björkman
Ann-Marie Falk
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Björn Hellman
Ingrid Nylander
Anne-Lie Svensson
Maria Swartling
Kjell Wikvall

Working group on graduate studies
Margareta Hammarlund-Udenaes, chairman
Maria Norlin
Angelica Quartino
Tomas Nilsson
Anna Carlsson
Erica Johansson
Marianne Danersund

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Raili Engdahl
Britt Jansson
Birgit Jansson
Britt-Marie Johansson
Lena Norgren
Jessica Strömgren

Safety officers
Raili Engdahl
Ronnie Hansson
Britt-Marie Johansson
Lena Norgren
Henrik Wadensten
Sviatlana Yahorava

Working group on gender equality and other policy issues
Loudin Daoura, chairman
Annika Häger
Erica Johansson
Uwe Rossbach
Claes Pettersson
Emma Lundqvist
Marie Heidenvall
Ulrika Simonsson
Hanna Wärner

Gender equality representative
Anne-Lie Svensson

Technical and administrative staff
Agneta Bergström
Ulrica Bergström
Marianne Danersund
Agneta Hortlund
Annika Häger
Magnus Jansson
Erica Johansson
Marina Rönggren
Karin Tjäder
Kjell Åkerlund

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    eg fred.nyberg@farmbio.uu.se
Awards and Appointments

- Per Andrén: Guest Professor Honor at Delft University, Dept. of Biotechnology, Delft, the Netherlands
  Strategic Research Partner Acknowledgment Award in Imaging Mass Spectrometry by AstraZeneca, Sweden
- Anna Nilsson: European Proteomics Association (EuPA) Young Investigator Prize 2009, Stockholm.
- Uppsala University confers annual pedagogical prizes for outstanding accomplishments in undergraduate education. The 2009 prize in Medicine and Pharmacy was awarded Ann-Marie Falk, Emma Lundkvist and Maria Swartling, teachers in Pharmacotherapy and Clinical Pharmacy, for their development of grading criteria for examinations in pharmacotherapy as an alternative to marks/scores. They have according to the nomination text "in an independent way, with very high ambitions and high quality, approached the work to develop control instruments in the grading of exams in the form of grading criteria."
- Fred Nyberg was appointed as guest professor at the Hoshi University, Tokyo
**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, Pharmaceutical bioinformatics, Pharmacology, Pharmacokinetics, Pharmacotherapy, Physiology, Toxicology. A mix of traditional teaching and problem-based learning with lectures, laboratory sessions, seminars, workshops and computer sessions characterizes the teaching. In addition, the teachers are involved in interdisciplinary training in laboratory practice, communication skills and professional development. 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 2009, the faculty of Pharmacy put two new master programs into practice, the Master programme in drug development and the Master programme in drug management and safety. These programmes comprise courses and undergraduate projects at the advanced level. The Department of Pharmaceutical Biosciences contributes to teaching at both programmes. The department was also responsible for the first course Drug management and safety. In addition to these programmes at the advanced level, the division of Pharmacotherapy is responsible for the one-year programme in Clinical Pharmacy.

Uppsala February 28, 2010

Ingrid Nylander
Course List

List of courses on basic and advanced levels

Advanced Pharmacotherapy B, 7.5 c
Advanced Pharmacotherapy D, 7.5 c
Adverse Drug Reactions and Pharmacovigilance, 7.5 c
Analytical Toxicology, 30 c
Applied Pharmacotherapy, Pharmacokinetics and Therapeutics D, 15 c
Biochemistry of Gene Regulation B, 7.5 c
Biochemistry of Gene Regulation C, 7.5 c
Clinical Attachment and Service Development D, 18 c
Clinical Drug Trials with Applied Biostatistics C, 7.5 c
Clinical Pharmacokinetics and Pharmacodynamics C, 7.5 c
Clinical Pharmacy C, 7.5 c
Clinical Pharmacy D, 7.5 c
Degree Project in Drug usage D, 15 c
Degree Project in Drug usage D, 30 c
Degree Project in Pharmaceutical Biochemistry C, 15 c
Degree Project in Pharmaceutical Biochemistry D, 30 c
Degree project in Pharmaceutical Bioinformatics, 30 c
Degree Project in Pharmaceutical Bioscience D, 20 c
Degree project in Pharmaceutical Pharmacology D, 30 c
Degree project in Pharmacokinetics C, 15 c
Degree project in Pharmacokinetics C, 30 c
Degree project in Pharmacokinetics D, 30 c
Degree Project in Pharmacotherapy C, 15 c
Degree Project in Pharmacotherapy C, 30 c
Degree Project in Pharmacotherapy D, 30 c
Degree Project in Toxicology, 15 c
Degree Project in Toxicology, 30 c
Degree Project, Pharmacology C, 15 c
Drug Development and Drug Usage, 7.5 c
Drug management, 7.5 c
Drugs and Dependence C, 7.5 c
Drugs and Dependence, Advanced Course D, 7.5 c
Drugs and the Elderly B, 7.5 c
Drugs and the Elderly D, 7.5 c
Drugs: Use, Abuse and Dependence A, 10.5 c
Embryotoxicology, Advanced Course, 7.5 c
Embryotoxicology, Intermediate Course, 7.5 c
Evidence Based Clinical Pharmaceutical Methods D, 12 c
Evidence-based treatment with cognitive approach A, 15 c
Models for Biological Systems C, 7.5 c
Molecular Mechanisms for Enzymatic Activation B, 7.5 c
Molecular Mechanisms for Enzymatic Activation C, 7.5 c
Molecular Pharmacology B, 7.5 c
Neuropharmacology C, 7.5 c
Neuropharmacology D, 7.5 c
Pharmaceutical Biochemistry B, 9 c
Pharmaceutical Biochemistry and Cell Biology A, 7.5 c

16
Pharmaceutical Bioinformatics C, 7.5 c
Pharmaceutical Bioinformatics D, 7.5 c
Pharmacokinetics B, 7.5 c
Pharmacokinetics B, 3 c
Pharmacokinetics C, 7.5 c
Pharmacokinetics and Statistics B, 9 c
Pharmacology A, 7.5 c
Pharmacology A, 15 c
Pharmacology B, 16.5 c
Pharmacology B, 15 c
Pharmacotherapy B, 7.5 c
Pharmacotherapy in Self-Treatment B, 9 c
Toxicology B, 7.5 c
Toxicology, Advanced Course D, 30 c
Toxicology, Drug Metabolism and Safety Assessment A, 4.5 c
Toxicology, Drug Metabolism and Safety Assessment B, 7.5 c
Toxicology, Intermediate Course, 15 c
Veterinary Pharmacology, 7.5 c
Scientific Reports
Biochemical Pharmacology

Ernst H. Oliw

Hydrolysis of allene oxides to α-ketols via 9R- and 9S-hydroperoxylinoleic acids and separation by chiral phase HPLC. The allene oxide synthase and 9R-dioxygenase of Aspergillus terreus were original discoveries in 2009.

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 use 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, PGH₂, can be transformed by cytochromes P450 to thromboxanes, prostacyclins and to 19-hydroxy-PGH₂, the precursor of 19-hydroxy-PGE₂. 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 syntheses are described in our recent papers in *The Journal of Biological Chemistry* (Refs. 13 and 24). Site-directed mutagenesis of 7,8-LDS revealed structural and mechanistic similarities to cyclooxygenases (Refs. 15 and 17). A novel observation is the allene oxide synthase of *A. terreus* (Ref. 25).

**Members of the group during 2009**

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

**Publications 2007-2009**


**Dissertations 2009**

1. Tomas Nilsson
   *Mass Spectrometric Analysis of Oxylipins: Application to Cytochrome P450-Dependent Metabolism*  
   Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy 114  
   [http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-109715](http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-109715)

2. Ulrike Garscha  
   *Catalytic and Structural Properties of Heme-containing Fatty Acid Dioxygenases: Similarities of Fungal Dioxygenases and Cyclooxygenases*  
   Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy 109  
   [http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-108770](http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-108770)

**Agencies that support the work/Funding 2009**

The Swedish Research Council Medicine

**Other commitments/assignments of staff members**

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 isomerase 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.

Characterization of oleic acid 7,10-diol synthase of Pseudomonas aeruginosa

Eriel Martinez, Ernst Oliw
P. aeruginosa is a Gram negative pathogen, which transforms oleic acid to a 7,10-dihydroxy metabolite by unknown mechanisms. Our aim is to characterize the reaction mechanism.
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 2009
Fred Nyberg, Professor
Mathias Hallberg, PhD Associate Professor
Qin Zhou, PhD, Researcher
Milad Botros, PhD, Researcher
Publications 2007-2009


Reviews 2007-2009

Dissertations 2007-2009
1. Kristina Magnusson
The impact of nandrolone decanoate on peptidergic mechanisms related to cognition, aggression, reward and dependence
Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy 103
http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-107483

Funding 2009
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/assignments of staff members
Fred Nyberg: Director of Research Issues at the Swedish National Drug Policy Coordinator 2002-2007, Member of the Governmental Advisory Board for
Addictive drugs (ANT-Advisory Board). Member of the Uppsala University Center for Studies of the Religion in the Society since 2006 and the National Center for Mens Violence against women since 2006. Member of the Board for the Medical Committee of the Swedish Criminal Care. Member of the Executive committee for the International Narcotics Research Conference (INRC) from 2006 to the present.

Member of Editorial Board of Scientific journals (Fred Nyberg): Peptides, Open J Endocrinology (Editor in Chief), Pharmacology-on-line, J Musc Skel. Pain.

Mathias Hallberg: Curr Protein Pept Sci.

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 (100 milj, SEK 2006-2015); PI at the Linne project Impact of Religion: Challenges for Society, Law and Democracy (50 milj. SEK 2008-2017); PI at the FAS supported project on alcohol effects on cognitive functions (6 milj. 3 years).

Swedish Research Council/Medicin for peptidergic mechanism in the development of drug dependence (2009 additional grant 2.3 milj SEK for 2009)
Molecular Neuropsychopharmacology

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

Members of the group during 2009
Tatiana Yakovleva, PhD, Researcher
Alexander Kuzmin, PhD, Researcher
Igor Bazov, PhD, Scientist
Hiroyuki Watanabe, PhD, Postdoctoral scientist
Richard Henriksson, PhD student at the Karolinska Institutet – NIH collaborative program
Malik Mumtaz Hussain Taqi, PhD student
Muhammad Zubair Hussain, PhD student

Publications 2007-2009


Reviews 2007-2009


Agencies that support the work/Funding 2009-2010

The Swedish Science Council / Senior Scientist / Forskare Position
The Swedish Science Council (#12190)
The Swedish Council for Working Life and Social Research (FAS)
The Swedish Institute (grant for collaboration with Ukraine)
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.
Embryotoxicology

Lennart Dencker

Embryogenesis  The embryo is not protected from pharmaceuticals and environmental pollutants. Intended and unintended pharmacological effects of drugs are often exerted in the conceptus as well. They can be reversible, but have occasionally detrimental morphological and functional downstream effects. We aim to improve the mechanistic understanding of teratogenic processes and develop improved in vitro methodologies in developmental toxicology.

One project is to develop improved image analysis software for the characterization and scoring of rodent embryos undergoing organogenesis in whole embryo culture (WEC). This is performed in collaboration with Professor Ewert Bengtsson at the Centre of Image Analysis, Uppsala University. By combining image analysis with multivariate analysis to assess adverse effects of embryonic development in vitro, we believe that the objectivity and the sensitivity of the method will increase. One aspect of the image analysis software project is to develop better ways of analyzing the developmental toxicity effect of drugs on heart rate in WEC conditions. In addition, a more specific developmental heart rate associated project concentrated on the properties of potassium (IKr)-channels reached its conclusion during 2009. This latter research was lead by adjunct professor Bengt Danielsson.

A second approach concerns the proteomic characterization of the WEC culture medium (rat serum based). Increased knowledge of the culture medium properties will be the first step for the establishment of a defined culture medium, which will both reduce variability in the WEC method and the number of required animals. This is done in collaboration with Professor Peter Bergsten at the department of
To improve scoring and data handling, we are also developing and anatomical ontology and controlled vocabulary that incorporates the different possible malformation phenotypes observed during organogenesis (in vivo and in vitro). These approaches will, when appropriate, be combined with our molecular biology data (functional genomics) and enable an improved monitoring of the “symphony” and its different tunes concerted by the collected expression of genes and proteins governing embryonic development and brain maturation.

Cell studies In addition, we use the 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. Using the antiepileptic and teratogenic drug valproic acid, an Histone deacetylase inhibitor (HDACi), and some less teratogenic analogues of valproic acid, we try to visualize which categories of genes may be representative for the teratogenic action (such as neural tube defects). Presently, a battery of teratogenic and non-teratogenic compounds are tested with respect to their gene (de)regulation in murine embryonic stem cells. A third cell type, mesenchymal stem cell-like pericytes isolated from human full term placentas are also being evaluated for cellular and molecular VPA effects. The later project was performed in the context of a EU FP6 project (ReproTect) and in collaboration with Dr Christian Sundberg at IMBIM, Uppsala University and ended during 2009. We are at the same time exploring the global epigenomic effects of VPA:s HDACi capacity in collaboration with Professor Claes Wadelius at Rudbeck laboratory. Here, we have used chromatin immunoprecipitation (ChIP) on chip (ChIP-chip) to study histone modification changes in the model system (human hepatoma cell lines) used by Wadelius group as a precursor for later studies in a more embryonic context. These studies have shown us that VPA as an HDAC inhibitor has unsuspected complex genome wide effects outside the prediction of the literature so far by removing large regions of histone acetylation instead of promoting it. This project was ended during 2009.

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 behaviors. 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
Dept. of Pharm. Biosciences

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. Although not conclusive, the data indicates that behavioral alterations induced by neonatal exposure to PBDE occur at non-cytotoxic levels. In addition, we are evaluating the effects of PBDE-99 and DE-71 (a commercial mixture of PBDE congeners) exposure on neuritogenesis. 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.

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

Members of the group during 2009
Lennart Dencker, Professor
Bengt R Danielsson, Adjunct Professor
Michael Stigson, Researcher
Henrik Alm, PhD student
Birger Scholz, Researcher
Måns Jergil, PhD student
Mats Nilsson, PhD Student

Publications 2007-2009

Reviews 2007-2009

Dissertations 2009

1. Måns Jergil
   Pluripotent Stem Cells of Embryonic Origin: Applications in Developmental Toxicology.
   Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy 116
   http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-109946

2. Henrik Alm
   Proteomic Characterization of Induced Developmental Neurotoxicity.
   Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy 99
   http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-99652

Supporting the work/Funding 2009

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

Other commitments/assignments of staff members

Vice Chairman, Domain of Medicine and Pharmacy at Uppsala University
ExCo member of an EU-project within IMI JU, planning a pan-European training programme in safety of medicines (see SafeSciMET.eu).
ExCo member of MRA, (http://www.medicinesacademy.org/index.php/Home/8/0/), a newly established industrial oriented medicines research education cooperation between Lund University, Technical University of Denmark, University of Copenhagen and Uppsala University.
ExCo member of EUFEBS (http://www.eufeps.org/), an organization representing and serving the pharmaceutical sciences community/ies and innovative drug research in Europe.
Bioactivation and Toxicity

Eva Brittebo

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

Members of the group during 2009

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

Publications 2007-2009


4. Andersson H, Piras E, Demma J, Hellman B, Brittebo E. Low levels of the air pollutant 1-nitropyrene induce DNA damage, increased levels of reactive oxygen species and endoplasmic reticulum stress in human endothelial cells. Toxicology. 2009;262:57-64.


Reviews 2007-2009


Funding 2009

The Research Council FORMAS
EU FP6 - ReProTect
**Other commitments/Assignments of staff members**

Head of the Department  
Member of the Pharmaceutical Faculty committee, Uppsala University  
Member of Open Access reference group, Uppsala University  
Member of the panel at the Norwegian Research Council ranking grant applications in Environment, genetics and health

**Projects**

**Bioactivation and toxicity of pollutants and drugs in vascular tissues (Helén Andersson)**

Both epidemiological and experimental studies suggest that exposure to high levels of air pollution is a risk factor associated with cardiovascular disease. Traffic emission is a major source of exposure to persistent air pollutants such as polycyclic aromatic hydrocarbons (PAH) and nitrated polycyclic aromatic hydrocarbons (nitro-PAHs). We have previously reported that PAHs are bioactivated and induce DNA damage in cultured human umbilical vein endothelial cells (HUVEC) following pretreatment with cytochrome P450 (CYP) 1A1-inducers. In a recent study we have also examined the metabolic pathways and effects of one of the most abundant nitro-PAHs in diesel exhausts, 1-nitropyrene (1-NP) in HUVEC. The results revealed that low levels of 1-NP induced DNA damage, increased the levels of reactive oxygen species and increased the protein expression of the endoplasmic reticulum stress chaperone GRP78. The results also suggested that the effects were mediated by metabolites mainly formed at nitroreduction. These findings suggest that the human blood vessel endothelium is a sensitive target tissue for 1-NP.

Data on the expression of major endometrial CYP forms is useful in the development of novel tests for detecting chemicals affecting the embryo implantation process. A local variation in drug metabolism due to cell specific expression of CYP enzymes might be one important reason for variability in tissue response and toxicity. The cell specific expression of drug metabolizing CYP enzymes in the highly vascularized human endometrium and in primary human endometrial endothelial cells is presently examined. In addition, the bioactivation and early onset stress protein induction in human endometrial explants following a short-term incubation of tamoxifen which is an agent for the prevention and treatment of breast cancer, is investigated.

**Effects of neurotoxicants in pigmented tissues and brain (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 in humans. 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 $^3$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 $^3$H-BMAA to Sepia melanin and to synthetic melanin revealed a stronger interaction of $^3$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.

Our recent studies demonstrated a poor transfer of BMAA across the blood-brain barrier (BBB) with no specific localization in discrete brain regions in adult mice. In neonatal mice, however, there was an efficient transport across the BBB and a selective uptake of BMAA in discrete brain regions such as the hippocampus and striatum. Moreover, a high placental transfer of BMAA occurred at gestation day 14, resulting in a pronounced enrichment of BMAA in the fetal brain. We have also reported that BMAA-treatment of neonatal rats induced acute alterations, such as impaired locomotor ability and hyperactivity. In addition, there were persistent cognitive deficits suggesting impaired learning abilities in adult animals following neonatal exposure. These data imply that the developing brain is particularly sensitive to BMAA exposure. The corresponding period in humans, starts during the last trimester of pregnancy and continues until at least two years after birth.

**Olfactory transfer of therapeutic agents (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 therapeutic agents into the brain is a novel principle for drug delivery. We have previously demonstrated a transfer of morphine and dopamine via the olfactory pathways to the brain following intranasal administration in rodents. The olfactory transfer of other therapeutic agents is presently under study.
Genetic Toxicology

Björn Hellman

When testing the potential DNA-damaging effects by pharmaceutical drugs and other chemicals, the test systems are generally based on experimental animals, bacteria or various kinds of transformed cells. For the safety evaluation it would be of advantage if healthy cells from humans could be used instead, since they have a normal and stable set of chromosomes. In case of using primary cultures of human lymphocytes, it is hardly possible to use a blood sample on more than one single testing occasion. This will often give varying results from different testing occasions, since new blood samples (in general from different donors) have to be taken all the time. Our extended-term cultures of lymphocytes allow one single blood sample to be used for up to 50 different experiments. We measure the DNA-damage with the so called Comet Assay, which is a relatively quick, simple and cheap method for evaluating DNA-strand breaks in individual cells. The major objective of our in vitro studies using the comet assay in various cell lines is to improve the risk assessment regarding exposures to genotoxic agents. One example of this is our recent study (Durling et al., 2009) 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., 2009), a naphtoquinone 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 provide further support for the idea that plumbagin may act as an antioxidative agent at low non-DNA damaging concentrations.

Members of the group during 2009
Björn Hellman, Associate Professor; Ph.D.
Jemmal Demma; Ph.D. student
Lena Norgren; Laboratory assistant

Publications 2007-2009
8. H. Andersson, E. Piras, J. Demma, B. Hellman & E. Brittebo. Low levels of the air pollutant 1-nitropyrene induce DNA damage, increased levels of reactive oxygen species and endoplasmatic reticulum stress in human endothelial cells. *Toxicology*, 262(2009)57-64.

Reviews 2007-2009

Agencies that support the work/Funding 2009
SIDA/SAREC
Other commitments/assignments of staff members
Member of the local committee for scholarships at the Faculty of Pharmacy, Uppsala University
Member of the committee for undergraduate courses (GRUFF) at the Faculty of Pharmacy, Uppsala University
Deputy member of the ethical committee for animal experiments in Uppsala
Neurotoxicology

Malin Andersson

We use 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. In particular we focus on Parkinson’s disease which is characterized by degeneration of dopaminergic neurons accompanied by a dramatic loss of DA in the striatum, particularly in the putamen. About 1% of the population over 65 years suffers from PD and the cardinal symptoms that include akinesia, bradykinesia, muscle rigidity, and tremor. DA replacement therapy with L-DOPA is currently the most effective pharmacotherapy for patients with PD, however with PD disease progression and long-term L-DOPA treatment complications occur in many patients. The main complications are troublesome motor complications such as “wearing off” fluctuations and L-DOPA-induced dyskinesia (LID). Despite large efforts in the field of LID research, the difficulties we face today remain as how to dissociate the molecular changes that arise by the motor execution of LID from causative changes that induce or predispose to dyskinesias. Our group studies the brain structures of the basal ganglia for molecular correlates of the incidence and severity of LID in an experimental model of Parkinson’s disease.

Members of the group during 2009

Malin Andersson, Assistant professor
Anna Karlsson, PhD student
Master students: Theodora Kallak (UGSBR); Rickard Kaugesaar (Master of Science in Pharmacy); Nooshin Talebi Zadeh (Pharm Biosciences); Helena Cordeiro (ERASMUS)
Publications 2007-2009

Reviews 2007-2009

Agencies that support the work/Funding 2009
Swedish Research Council, Disciplinary Domain of Medicine and Pharmacy, The Royal Swedish Academy of Sciences, Astrid och Gustaf Kaleens fond, Åke Wibergs Stiftelse, Parkinsonfonden

Other commitments/assignments of staff members
Director of studies for the Biomedicine Program (180 hp)
Chairman of the National Network of Junior Scientists.

Projects
In collaboration with Professor Jonas Bergquist (Dept. Analytical Chemistry) we study L-DOPA-induced dyskinesias in experimental Parkinson’s disease (Anna Karlsson and Jörg Hanreider). Other collaborations include; glia cell identification in culture and in tissue sections (Jörg Hanreider, Grzegorz Wicher, Åsa Fex-Svenningson), PDBE-induced neurotoxicity (Henrik Alm and Prof. Lennart Dencker), BMAA-induced neurotoxicity (Oskar Karlsson and Prof. Eva Brittebo), neuropeptides in drug dependence (Uwe Rossbach and Prof. Fred Nyberg), and maternal separation (Prof. Ingrid Nylander).
Medical Mass Spectrometry

Per Andrén

Imaging Mass Spectrometry and Peptidomics in Neurodegenerative Disorders and Drug Discovery. Our research group focus on new approaches in mass spectrometry (MS), i.e. matrix-assisted laser desorption ionization (MALDI) 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 aims is to define neuropeptides and proteins that are differentially expressed in the basal ganglia complex of animals with striatal dopamine depletions, and to determine which of these proteins are regulated by loss of dopamine signaling, as well as to investigate protein and peptide expression patterns in subjects with and without LID symptoms.

Understanding the relationship between pharmacokinetics and pharmacodynamics is crucial in the development of effective drug therapies. Current technologies only provide information on the total amount of drug in the whole tissue with no possibility to address micro-environmental localization of the compound or metabolic derivatives. The application of imaging mass spectrometry in drug discovery 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 for the imaging mass spectrometry applications.
Members of the group
Anna Nilsson, post-doc
Henrik Wadensten, researcher
Patrik Källback
Johan Gustavsson, visiting PhD student, University of Adelaide, Australia
Allessandro Fioni, visiting PhD student, University of Parma, Italy
Sophie Wavrin, visiting undergraduate student, Université Paris – Sud 11, Faculté de Pharmacie de Châtenay-Malabry

Publications 2007-2009


Reviews 2007-2009

Agencies that support the work/Funding 2009
The Swedish Research Council (VR), Natural Science
The Swedish Research Council (VR), Medicine
The Swedish Research Council (VR), Research infrastructures
The Swedish Research Council (VR), Medicine: Senior Research Positions in Functional Protein Chemistry
The National Institute of Health (NIH)/the National Institute on Drug Abuse (NIDA)
AstraZeneca, Lund.
Denator AB, Gothenburg, Sweden.
The Knut & Alice Wallenberg (KAW) Foundation.

Other commitments/assignments of staff members
Member of the Board, the Swedish Academy of Pharmaceutical Sciences, Section for Drug Analysis
Member of the Board, the Swedish Proteomics Society (Founding member)
Member of the Board, the Swedish Mass Spectrometry Society
Editorial Board, Journal of Proteomics
Member of the Board, European Imaging Mass Spectrometry Infrastructure
Member of the Board, Organizing Committee, the 3rd European Proteomics Association (EuPA) Congress, Stockholm, June 14th – 17th.
Organizer of MALDI Imaging Workshop, Uppsala, June 13th – 14th.
Projects

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

Per Andrén, Anna Nilsson, Patrik Källback, Maria Fälth, Henrik Wadensten, MMS, Per Svenningsson, Karolinska Institutet, Alan Crossman, University of Manchester, UK, Erwan Bezard, Univ. of Bordeaux 2, France.
The main objective of the present research is to study neurochemical processes in Parkinson disease and specifically L-Dopa-induced dyskinesias (LID). No treatment exists yet for the management of LID, a debilitating complication of L-dopa therapy for PD. The aim is to define neuropeptides and proteins that are differentially expressed in the basal ganglia complex of animals with striatal dopamine depletions, with and without LID.

Integration of resources and studies to elucidate neuropeptide signaling.

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

Identification and functional characterization of protein-protein interactions in cerebrospinal fluid and brain tissue from Parkinson’s disease (PD) patients and experimental PD models

Per Andrén, Anna Nilsson, MMS, Per Svenningsson, Karolinska Institutet, Peter Verhaert, University of Delft, the Netherlands
Using surface plasmon resonance technique (Biacore 3000) coupled to mass spectrometry technology, new protein partners of α-synuclein and parkin have been captured and identified in cerebrospinal fluid or post-mortem human tissue from PD patients. In addition to using native α-synuclein and parkin, mutated forms of these proteins seen in familiar forms of PD will be immobilized on the sensor chip and used as baits.

Fine mapping the spatial distribution and concentration of unlabeled drugs within tissue micro-compartmentss using imaging mass spectrometry

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 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, Uppsala Applied Science Lab, GE Healthcare, Lars Pählman, Ulrik Wallin, Department of Surgery*

This collaborative project aims at developing new methods for the clinical diagnosis of colon 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 68Ga-labeled tracers for the early diagnosis of colon 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, Patrik Källback, Anna Nilsson, Henrik Wadensten, 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.
Neuropharmacology - neurobiology, dependence and behaviour

The research within the group is focused on neurobiology and neuropharmacology according to the following areas:

• Neuropharmacology (L Bergström)
• Neurodegeneration (AL Svensson)
• Drug dependence mechanisms (I Nylander)
• Behavioral science (E Roman)

Projects

• Neurobiology and neuropharmacology of relevance for reward and reinforcement
• The role of neurosteroids on neurogenesis, neuroprotection and on interactive processes, which are ongoing in neurodegenerative disorders and drug dependence
• The influence of early environmental factors on the vulnerability to develop drug dependence
• Ethoexperimental studies of behavioural profiles and aspects of exploration and motivated behaviours using a multivariate approach
Drug Dependence

Ingrid Nylander

The projects within the research group focus on the neurobiological substrates for individual differences in vulnerability for alcohol dependence. Of particular interest is the impact of early environmental factors, such as rearing environment and the consequences of adolescent drug intake. 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 or prolonged periods during the first postnatal weeks. Short periods of MS are similar to natural conditions where the mother regularly leaves the litter for shorter periods of time. Previous results within the group provide evidence for such rearing conditions being protective. Adult rats subjected to short MS the first weeks of life have a low alcohol intake. In addition, genetically predisposed rats subjected to short periods of MS 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. This rearing condition is associated with an increased risk for excessive alcohol intake and altered risk-taking behavior. Rats subjected to prolonged MS have higher alcohol consumption, preference for high alcohol concentrations and in alcohol-preferring rats, this rearing environment 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 either by rearing factors or drug intake early in life, cause long-term changes in behavior and, in turn, affects alcohol consumption later in life.

Members of the group during 2009
Ingrid Nylander, Professor
Chris Pickering, post doc
Sadia Oreland, PhD Student
Loudin Daoura, PhD Student
Sara Palm, PhD student
Marita Berg, Technician
**Dissertations 2009**

1. Sadia Oreland
   Maternal separation in the rat. The short- and long-term effects of early-life experience on neuropeptides, monoamines and voluntary ethanol consumption, 2009
   Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy 106
   http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-108678

**Publications 2007-2009**


**Agencies that support the work/Funding 2009**

The Swedish Research Council (K2005-04X-12588-08A)

**Other commitments/assignments of staff members**

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, Sara Palm, 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 enhanced response to alcohol. The consequences of early environment on adolescence and adult 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 reported 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.

**Behavioral studies**

*Loudin Daoura, Sara Palm, 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. In addition, the phenotype of five different Wistar rats is examined, including behavioral profiling and assessment of voluntary alcohol consumption.
Neuropharmacology

Lena Bergström

Neurotransmission in the central nervous system involves a large amount of different neurotransmitters and neuromodulators, some of which have been closely associated with different psychiatric diseases like psychosis, depression, anxiety and drug dependence. In a recently completed study Carolina Birgner (who finished her thesis in September 2008) and I studied changes in the dopamine and serotonin systems in the brain from rats long-term treated with the androgenic anabolic steroid (AAS), nandrolone decanoate. The results from those studies demonstrated changes in both the dopamine as well as the serotonin system (see list of publications).

The serotonin system in the brain has had a renaissance during the last decade and more and more reports point at a close connection between a changed serotonin activity and psychiatric illness. In addition to changes in serotonin activities in the brain there are also several reports demonstrating different genotypes including serotonin reuptake transporter (5-HTT), synthesising enzyme (TPH) and degrading enzyme (MAO). There are however, less studies focusing on whether those genotypes in fact reflect different phenotypes. I am therefore involved in some projects elucidating if different genotypes of 5-HTT and TPH-2 in patients suffering from social phobia leads to increased or decreased uptake or synthesis of 5-HT, respectively.

Members of the group during 2009
Lena Bergström, Associate Professor

Publications 2007-2009

Agencies that support the work/Funding 2009
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-β-induced toxicity and underlying mechanism(s). Moreover, the effects of other compounds like growth hormones on amyloid-β-induced toxicity as well as aggregation of amyloid-β are of interest.

Another part of my research is focused on the cholinergic involvement in drug addiction, with emphasis on neuronal nicotinic receptors and their interaction with the dopaminergic reward system in the ventral tegmental area, nucleus accumbens and prefrontal cortex.

Members of the group during 2009
Anne-Lie Svensson, Lecturer
Marie Eketjäll, PhD Student
Karina Dudko, Laboratory assistant

Publications 2007-2009

Agencies that support the work/Funding 2009
Gun och Bertil Stohnes stiftelse
Åhlén-stiftelsen

Other commitments/assignments of staff members
Member of the Faculty Board
Member of the Gender Equality Committee at the Disciplinary Domain of Medicine and Pharmacy, Uppsala University.

Projects

Neurosteroids and Alzheimer’s disease: Mechanistic studies of neuroprotection and amyloid-β-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-β peptide, which is known to play a central role in the pathogenesis of AD, through the ability of amyloid-β monomers to aggregate and form protofibrils. Amyloid-β has been implicated in cell death during the course of AD and exerts toxic effects on neurons both in vivo and in vitro. An important goal of the therapeutic strategies of AD is to identify compounds able to prevent Aβ formation, aggregation and thereby prevent protofibril formation.

The significance of neurosteroidogenesis in regulating neurodegenerative mechanisms is unknown. Accumulation of amyloid-β, induced by toxic events in cells might be able to reduce the synthesis of neuroprotective 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-β-induced toxicity, as well as the underlying molecular mechanism(s), with focus on neurogenesis and apoptosis.
The concept of ethoexperimental studies of behaviour promotes the advantage of integrating ethology and experimental psychology. With this approach, the aim is to use test conditions and procedures that are based on the circumstances and challenges the animal meets under natural conditions. The behaviour laboratory moved to new facilities during 2009 and is under continuous development with regard to tests and techniques. 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 2009

Erika Roman, Assistant Professor
Bengt J Meyerson, Professor Emeritus
Marita Berg, Technician
Publications 2007-2009


Agencies that support the work/Funding 2009

The Swedish Society for Medical Research, post-doc stipend
The Alcohol Research Council of the Swedish Alcohol Retailing Monopoly
Magnus Bergvall Foundation
Fredrik and Ingrid Thuring Foundation
Facias Foundation
Other commitments/assignments of staff members
International Associate, Department of Psychology, Indiana University Purdue University at Indianapolis (IUPUI), Indianapolis, IN, USA.
Member of the board, The Society for Swedish Alcohol and Drug Research (SAD).
Member of the Uppsala Animal Ethical Committee.
Member of the board of Uppsala University Laboratory Animal Resources (SUUF).

Projects

Behavioural profiling of selectively bred alcohol-preferring and alcohol-avoiding rodent lines
Erika Roman, Robert Stewart, 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. This experiment involves 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.

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.

Development and validation of the MCSF test
Erika Roman, Bengt J Meyerson
The MCSF test is under continuous development for studies of exploration and motivated behaviours. Ongoing work aims at developing an automatic tracking and scoring system as well as expanding the use of the MCSF for studies of learning and memory.

Maternal and neonatal behaviour
Loudin Daoura, Ingrid Nylander, Erika Roman
The project comprises establishment and development of animal experimental models to assess maternal behaviour, neonatal behaviour and behavioural consequences of different early rearing environmental conditions.
Acute and long-term effects of environmental neurotoxins on behaviour

Oskar Karlsson, Eva Brittebo, Erika Roman

The project uses various behavioural tests for assessment of acute and long-term effects of environmental neurotoxins on behaviour. Within the frame of this project the MCSF test has been evaluated for studies of learning and memory mechanisms and tests including radial arm maze, water maze, and novel object recognition have been established.
Pharmacokinetics/Pharmacodynamics

Margareta Hammarlund-Udenaes and Sven Björkman

The blood-brain barrier is essential for regulation of drug transport to and from the brain.

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 2009
Margareta Hammarlund-Udenaes, Professor
Sven Björkman, Professor
Jörgen Bengtsson, PhD Student (PhD Nov 2009)
Annika Borgs, PhD Student
Markus Fridén, PhD Student
Ulrika Gillespie, PhD Student
Britt Jansson, Lab Engineer
Jessica Strömgren, Lab Assistant
Muhammad Waqas Sadiq, PhD Student

Publications 2007-2009


Reviews 2007-2009


Dissertations 2009


Agencies that support the work/Funding 2009

AstraZeneca

LIF

Other commitments/assignments of staff members


Projects

Blood-brain barrier transport of drugs – mechanisms and methods
Margareta Hammarlund-Udenaes, Sven Björkman, Jörgen Bengtsson, Annika Borgs, Markus Fridén, Stina Syvänen (the Netherlands), 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 focus on unbound drug concentrations and is important for the drug industry that has problems in finding good drug candidates for brain diseases, partly due to a lack of understanding of which parameters to optimize for.

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. Publications during 2009 has centered around in vitro methods for estimating blood-brain barrier transport and brain distribution parameters in early discover. CSF was shown to be a reasonably good marker for unbound drug concentrations in the brain.

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

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

Clinical pharmacokinetics of coagulation factors VIII and IX

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

The aim of the project is to optimize the prophylactic treatment of haemophilia with coagulation factors VIII and IX by the use of individually tailored dosing. Optimizing the dosing by means of clinical pharmacokinetic (PK) principles can potentially yield important benefits both from a purely medical as well as from an economical point of view. The project started in 1989 and has resulted in widespread international acceptance of “pharmacokinetic dosing” in this particular field of disease management. The activity during 2009 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.
• Evaluating the PK and clinical information obtained during extensive licensing studies on a novel factor VIII preparation, Advate (Baxter Inc.).
• Using the Advate data to illustrate the influence of differences in PK and dosing schedules on achieved factor VIII levels during prophylactic treatment.
• Designing and applying limited blood sampling schedules for the dose tailoring of factor VIII and factor IX in clinical practice.
• Disseminating knowledge of PK dose tailoring to physicians, at meetings or courses in Stockholm, Malmö, Helsinki, Oslo, Budapest, London and San Antonio (TX).
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, and a set of hierarchical variability components, accounting for variability between subjects, within subjects over time and remaining between observation variability. Research at the pharmacometrics group can be divided into four main areas. First, development and evaluation of methods for efficient and robust model building. This involves development of estimation algorithms, methods for model diagnosis and sequential procedures for model building. The result of the research, when applicable, is made available as free software. 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 2009
Mats O Karlsson, Professor
Lena Friberg, Researcher, Associate Professor
Andrew Hooker, Senior Lecturer
Ulrika Simonsson, Senior Lecturer, Associate Professor
Anders Grahnén, Adjunct Professor
Niclas Jonsson, Adjunct Professor
Siv Jönsson, Researcher
Britt Jansson, Lab Engineer
Kajsa Harling, System Developer
Radojka Savic, Researcher
Rocio Lledo, Researcher
Stefanie Hennig, Researcher
Emilie Henin, Post-doctoral fellow
Robert Kalicki, Post-doctoral Fellow  
Guangli Ma, Post-doctoral Fellow  
Joseph Standing, Post-doctoral Fellow  
Paul Westwood, Post-doctoral Fellow  
Martin Bergstrand, PhD Student  
Kristin Karlsson, PhD Student  
Angelica Quartino, PhD Student  
Emma Hansson, PhD Student  
Åsa Johansson, 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  
Sebastian Ueckert, PhD Student  
Jan-Stefan van der Walt, PhD Student  
Brigitte Lacroix, PhD Student  
Johan Wallin, PhD Student  
Elisabet Nielsen, PhD Student  
Marcus Björnsson, PhD Student  
Matts Kågedal, PhD Student  
Anna Löennebo, PhD Student  
Patanjali Ravva, PhD Student  
Al Maloney, PhD Student  
Steve Ernest, PhD Student  
Roberto Bizzotto, Visiting Scientist  
Xiujin Shi, Visiting Scientist  
Aurora d’Apuzzo, ERASMUS student  
Alexandre Sostelly, MSc student  
Yang Sun, MSc student  

Publications 2007-2009


**Dissertations 2009**

1. Silber Hanna  
*Integrated Modeling of Glucose and Insulin Regulation Following Provocation Experiments*  
Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy 92  
http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-99195

2. Wallin Johan  
*Dose adaptation based on pharmacometric models*  
Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy 93  
http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-100569

**Agencies that support the work/Funding 2009**

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

**Other commitments/assignments of staff members**

Lena Friberg, Organizing committee, PAGE conference, St Petersburg  
Andrew Hooker, Organizing committee, PODE conference, St Petersburg  
Mats Karlsson, Vice-chair COST Action B25  
Mats Karlsson, Deputy Head of Department  
Mats Karlsson, Scientific committee, PAGE conference, St Petersburg  
Mats Karlsson, Scientific committee, AAPS conference, Washington DC  
Mats Karlsson, Editor Journal of Pharmacokinetics and Pharmacodynamics  
Projects

Clinical modelling of pharmacokinetics in HIV, TB and malaria therapy

Mats Karlsson, Ulrika Simonsson, Stefanie Hennig, Jan-Stefan van der Walt, Joe Standing

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 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 know 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**

*Mats Karlsson, Hanna Silber, Rocio Lledo, Robert Kalicki*

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 different trial types 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 glucosylated hemoglobin (HbA1c). As hemoglobin in red blood cells has a life-span in the body of a few months and the glucosylation 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 charaterised 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 transplantation**

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

Cyclosporine 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, Rocio Lledo Martin Bergstrand, Emilie Henin, 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.

Oncology

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

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, s-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 cefturoxime 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, Sebastian Ueckert*

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 designed 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 off 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, Elodie Plan, Rada Savic, Joakim Nyberg*

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 automization 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.
Proteochemometrics

Jarl Wikberg

Research during 2009 was concentrated on 1) a natural products project and 2) the continued development of the Bioclipse platform.

The most prominent discovery during 2009 was the discovery of the libiguins, a new class of natural compounds that profoundly regulate sexual behavior. The libiguins are low molecular weight terpenoid compounds with extraordinary high potency and long duration of effect. The libiguins are now being developed into pharmaceuticals for treatment of sexual dysfunction.

During the year Bioclipse 2.2 was released was released (available at 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.

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 2009

Jarl Wikberg, Professor
Sviatlana Yahorava, PhD, Visiting Post Doc
Egon Willighagen, PhD, Visiting Post Doc
Maris Lapins, PhD, Researcher
Ola Spjuth, PhD Student
Jonathan Alvarsson, PhD student
Stephy Prakash, PhD student
Martin Eklund, PhD student
Eskil Andersen, Programmer
Carl Måsak, Programmer
Muhammad Junaid, PhD student

Publications 2007-2009


Reviews 2007-2009


Dissertations 2009

1. Ola Spjuth

Bioclipse : Integration of Data and Software in the Life Sciences.
Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy 111.
http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-109305
2. Martin Eklund
   *eScience Approaches to Model Selection and Assessment: Applications in Bioinformatics*
   Summaries of Uppsala Dissertations from the Faculty of Pharmacy 112. http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-109437

*Agencies that support the work/Funding 2009*
The Swedish Research Council
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.

Members of the group during 2009
Kjell Wikvall, MD, PhD, Professor
Maria Norlin, PhD, Associate Professor
Johan Lundqvist, PhD Student
Hanna Pettersson, PhD Student

Publications 2007-2009
Reviews 2007-2009


Dissertation 2009

1. Hanna Pettersson

   *Steroid-Metabolizing cytochrome P450 (CYP) Enzymes in the Maintenance of Cholesterol and Sex Hormone Levels*

   Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy 96

   http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-100787

Agencies that support the work/Funding 2009

The Swedish Research Council-Medicine

Projects

**Bioactivation and metabolism of vitamin D and cholesterol**

*Kjell Wikvall*

This research is focused on enzymes and genes of importance for vitamin D bioactivation and cholesterol homeostasis. Effects on these processes by endogenous and pharmacological compounds are studied. Vitamin D is needed for regulation of calcium levels in the body and vitamin D deficiency leads to skeletal diseases such as rickets in children and osteomalacia/osteoporosis in adults. The biologically active form, 1α,25-dihydroxyvitamin D3, 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.

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

*Maria Norlin*

This research concerns steroids involved in hormonal signalling in connection with sex hormone biosynthesis, neurosteroid function and cellular 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 5α-androstane-3β,17β-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. 5α-androstane-3β,17β-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.
Stress Adaption

Matti Lang

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

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

Stress responding genes used as models include;

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

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

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

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

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

Members of the group during 2009

Matti Lang, Professor

Publications 2007-2009


**Agencies that support the work/Funding 2009**

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

**Other commitments/assignments of staff members**

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.
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