UPPSALA
UNIVERSITET

Annual Report 2013

Department of Pharmaceutical Biosciences

Fastställd av styrelsen 2015-03-17

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Introduction

At little bit late, this annual report highlights the research activities in the department of pharmaceutical biosciences during 2013. The research areas cover many different areas, including research on basic mechanisms of drug action, drug dependency and adverse health effects, as well as studies on drug metabolism, pharmacokinetics and pharmacodynamics. In addition, there is significant focus on pharmaceutical bioinformatics and proteomics, as well as pharmacometric modeling and simulations used in drug development. Several research groups are developing tools directly applicable to the pharmaceutical industry, while others are studying issues such as drug dependency and environmental contaminants with important socioeconomically implications. This report confirms that the research groups continue to have national and international collaborations within their research areas. Another important activity is the teaching we promote, and most of our junior lecturers, senior lecturers, and professors and also some of our researchers are heavily engaged in our education on various programs (focusing on pharmacy students). Most of our education is research-based and it is our aim to ensure that there is a synergy between teaching and research. Besides providing professional pharmacy education, the department also offers graduate pharmacy students specialization in clinical pharmacy.

Funding

All research activities require funding from national and international research councils, pharmaceutical companies, and the government. For example, in 2013 major grants were obtained from various national and international research councils such as the Swedish Research Council, the Swedish Foundation for Strategic Research, the Swedish Institute, FORMAS, the European Foundation for Alcohol Research, EU, the Swedish Cancer Institute, VINNOVA, National Institute of Health and the Swedish Society for Working Life and Social Research. Many projects also have financial support from pharmaceutical companies such as AstraZeneca, Janssen Pharmaceuticals, Novartis, Pfizer and Roche.

Organisation and financial review

The department is organised on the basis of our main core activities, i.e., research, teaching and the collected support activities (i.e., management, finance/staff administration, education administration and infrastructure). All support activities are funded by a percentile overhead on salaries and operating costs. The government provides the basic funding for teaching and research. The peer-reviewed scientific publications, PhD examinations and the external grants provide the foundation for any decisions concerning the allocation of university funding of research. The revenues from research councils, EU, foundations and pharmaceutical companies are very important to increase the research activities at the department. Below follows a short summary of incomes and expenditures 2013.

Major incomes 2013 (kSEK)	
Research and graduate education- government	33 919
Research grants – research councils	38 385
Research – commissioned	7 318
Education – basic and advanced level –government	37 135

Major expenditures 2013 (kSEK)	
Staff costs	67 865
Operating expenses etc.	12 372
Premises	13 549
University/faculty support activities	12 197
Library	2 304
Depreciation	2 381
Travels	3 188

Core facilities, UFOLD and SafeSciMet

Apart from being involved in regular research and teaching activities, our department also host two major core facilities, The National Centre for Mass Spectrometry Imaging (NCMSI) and Uppsala University Behavioural Facility (UUBF). The department is also engaged in "the third mission", mainly by hosting and leading the activities of U-FOLD. In addition, the department of Pharmaceutical Biosciences is also one of the participants in the EU-supported education project SafeSciMet, involving both the Academy and Pharmaceutical Industry in several European countries.

The NCMSI resource was founded at the Uppsala University in 2010 with funds provided by the Swedish Research Council-Research Infrastructure in 2010 and Uppsala University-Infrastructure in 2013. The mission of the Centre is to accelerate the adoption of MALDI (matrix-assisted laser desorption ionization)-MSI technology by the biological and medical research community by providing access to advanced technologies and promoting interactions between scientists who are experts in these technologies and researchers conducting focused biological investigations. Such collaborations provide new insights into the targeted biological problems and a better understanding of health and disease at the molecular level. Another important activity of the NCMSI is training, informing scientists about the capabilities and limitations of the technology, good practices, and established methods and protocols. NCMSI uses state-of-the art mass spectrometry technologies and cutting edge research to help projects in e.g., Drug Discovery and Development, Neuroscience, Oncology and Pathology applications. NCMSI is located at Dr. Per Andrén´s Biomolecular Imaging and Proteomics research group at the Dept. of Pharmaceutical Biosciences.

UUBF was established in 2011 through strategic funding from the Faculty of Medicine and Pharmacy. UUBF functions as a non-profit core facility that administers and organizes experimental behavioral testing of mice, rats and fish. UUBF have a large variety of behavioral tests and can assist with everything from providing the experimental apparatus to performing the complete experiment. The aim of UUBF is to

optimize access to well validated tests for research groups at Uppsala University as well as for external researchers, and promote the further development of high quality behavioral tests. UUBF provides equipment and professional assistance for behavioral experiments as well as data analysis and interpretation. At the core facility, several tests for motor behavior, learning and memory tests, sensorimotor tests and cognition can be run. UUBF also provide the use of specific software programs for behavioral tracking and recording, and advanced statistical analyses. The primary contact persons at our department are: Associate Professor Erika Roman and Dr Sara Ekmark-Lewen.

E-mail: uubf@farmbio.uu.se. Homepage: http://www.farmbio.uu.se/Corefacility/uubf/

U-FOLD is a forum for research on addiction to medical products and illegal drugs. This network is the link between research, prevention and treatment activities in Uppsala regarding different types of addiction. The network brings broad experience and different perspectives and the major aim of the activities within U-FOLD is to contribute to greater understanding and a common approach regarding the underlying chain of events leading to addiction. Within the network we can create new methods to prevent drug addiction, including preventive measures, care and legal actions. U-FOLD gather today twenty participants from academia, government, and nonprofit organizations. U-FOLD has lowered the threshold for new appointments and flow of ideas with clear practical effects. During 2013, the first education in cannabis prevention was initiated, and Uppsala University and Regionförbundet initiated the project Children in abusive environments. More information (still only in Swedish) can be found on http://www.ufold.uu.se

SafeSciMET constitutes a new and unique pan-European education and training network, developing and establishing a comprehensive Modular Education and Training Programme in Safety Sciences for Medicines. This programme addresses the needs in small and large pharmaceutical companies, regulatory authorities, academic institutes and health care. SafeSciMET courses are especially designed to support scientists and professionals in drug/medicines research and development. The Student Office manages the SafeSciMET website, course application and registration and it also contributes to information and communication of the SafeSciMET programme. Professor Lennart Dencker is responsible for the Student Office.

Future development

The years ahead promise many changes in terms of research and education and one can only hope that we will be successful in our attempts to get our different types of activities financially supported so that we can keep the high level of research and teaching also comming years. What we do know is that quite many professors, teachers and members of our technical and administrative staff will retire 2014/2015/2016 – and even if it is a real challenge to find equally good replacements, it will also provide many possibilities for renewal.

In memoriam

During 2013, two of our active collegues at the Department sadly passed away: Jonna Lübcke, a teacher in Clinical Pharmacy, and Sven Björkman, a Professor of Pharmacokinetics.

Jonna Lübke sadly passed away in April of 2013 at the age of 34. During her time at the Department she was a driving force in shaping the new Master Program in Clinical Pharmacy into what it is today. She divided her time between the University and the hospital, where she worked as a clinical pharmacist, being the Faculty of Pharmacy's first "teacher-practitioner". Her experience from the hospital setting was essential for the development of the program. She was much appreciated by the students and had a genuine interest in, and commitment to, delivering high quality education. She was an invaluable part of the team of teachers working close to her, and is much missed by the collegues and friends at the Department.

Sven Björkman sadly passed away in July of 2013 at the age of 62. His expertise was within the areas of physiologically based pharmacokinetic modeling and dosing of Factor VIII and IX in hemofilia. Starting with a PhD in Organic Chemistry and working for 25 years as a Research Scientist at the Hospital Pharmacy of Malmö General Hospital, before joining the Department, he had a broad knowledge on all aspects of the use of drugs. His wisdom was much sought after not only from his fellow scientists and the PhD students he supervised, but also from hospital staff and international colleagues within his expertise areas. He is much missed by his colleagues at the Department.

Uppsala March 4, 2015

Björn Hellman

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Organisation 2013

Chairman

Eva Brittebo (until 20130831)
Björn Hellman (from 20130701)

Deputy chairman

Mats Karlsson

Department board

Eva Brittebo, *chairman* (until 20130631)
Björn Hellman, *chairman* (teacher representative until 20130631)
Mikaela Andersson, *secretary*
Mats Karlsson*, teacher representative*
Margareta Hammarlund-Udenaes*, teacher representative*
Maria Kjellsson*, teacher representative*
Siv Jönsson, *teacher representative*
Jörgen Bengtsson, *teacher representative*
Mathias Hallberg, *teacher representative* (deputy until 20130631)
Lena Bergström, *teacher representative, deputy* (from 20130701)
Karin Tjäder*, technical/administrative representative*
Magnus Jansson *technical/administrative representative*
Agneta Hortlund, *technical/administrative representative*, deputy
Patrik Källback, *graduate student representative*
Ida Netterberg*, graduate student representative, deputy*
Erika Brolin, *graduate student representative*
Vincent Ferrigan, *student representative*
Sebastian Axelsson, *student representative, deputy*
Marina Rönngren*, adjungerad personnel coordinator*

Professors

Georgy Bakalkin
Sven Björkman (deceased 20130731).
Eva Brittebo
Lennart Dencker (until 20130531)
Margareta Hammarlund-Udenaes
Björn Hellman
Mats Karlsson
Matti Lang
Ingrid Nylander
Ernst Oliw
Jarl Wikberg
Kjell Wikvall

Professor emeriti

Lennart Paalzow
Lennart Dencker (from 20130601)

Senior Professor

Fred Nyberg

Adjunct and Guest Professors

Staffan Eksborg (*adjunct*)
Niclas Jonsson (*adjunct*)
Jan Kehr (*adjunct*)
William Webster (*guest*)

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

Postdocs, Researchers and PhD students
Listed in the Scientific report

Junior lecturers
Ann-Marie Falk
Lena Klarén
Anna-Karin Lidehäll*
Emma Lundkvist
Jonna Lübcke (deceased 20130422)
Maria Swartling
Maria Ellgren*
Åsa Johansson*
Srebrenka Dobric*
Oskar Karlsson*
Loudin Daoura*

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

Laboratory staff
Jessica Dunhall
Raili Engdahl
Britt Jansson
Lena Norgren

Technical and administrative staff
Ulrica Bergström
Marianne Danersund (until 20130631)
Agneta Hortlund
Johanna Svensson
Magnus Jansson
Marina Rönngren
Karin Tjäder
Karin Elg*
Kjell Åkerlund
Myron Zaluha
Birgitta Hellsing*
Mikaela Andersson
Malin Gadeborg*
Elisabeth Jonsson
Arvid Berg
Klas Jönsson*
Kajsa Harling
Valentin Georgiev*
Polina Georgiev*
Hans Lindén*
Johanna Elnersson*
Anna Iatsyshyna*
Wei Sun*
Rikard Nordgren
Samuel Lampa*

Safety officers
Marianne Danersund (until 20130631)
Marina Rönngren
Raili Engdahl
Ronnie Hansson
Lena Norgren
Henrik Wadensten
Sviatlana Yahorava
Kjell Åkerlund

The work environment group
Eva Brittebo, *chairman* (until 20130631)
Björn Hellman, *chairman* (from 20130701)
Raili Engdahl
Ernst Oliw
Marina Rönngren
Patrik Källback

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

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

**Temporary position*

Scientific Reports 2013

Biochemical Pharmacology

Ernst H. Oliw

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

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. Common drugs such as aspirin, acetaminophen (paracetamol, APAP) and ibuprofen inhibit biosynthesis of prostaglandins and reduce symptoms of disease, but may also cause side effects related to their actions. Other drugs are based leukotriene receptor antagonists (e.g., montelukast), which are used for treatment of bronchial asthma.

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

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

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

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

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

Members of the group during 2013

Ernst H. Oliw, MD, PhD, Professor
Inga Hoffmann, PhD
Saeid Karkehabadi, PhD
Mirela Jonsson, PhD
Anneli Wennman, PhD student

Publications 2011 - 2013

1. Hoffmann, I., Jernerén, F., Garscha, U., and Oliw, E. H. (2011) Expression of 5,8-LDS of Aspergillus fumigatus and its dioxygenase domain. A comparison with 7,8-LDS, 10-dioxygenase, and cyclooxygenase. *Arch. Biochem. Biophys.* **506**, 216-222
2. Oliw, E. H., Jernerén, F., Hoffmann, I., Sahlin, M., and Garscha, U. (2011) Manganese lipoxygenase oxidizes bis-allylic hydroperoxides and octadecenoic acids by different mechanisms. *Biochim. Biophys. Acta* **1811**, 138-147
3. Oliw, E. H., Wennman, A., Hoffmann, I., Garscha, U., Hamberg, M., and Jernerén, F. (2011) Stereoselective oxidation of regioisomeric octadecenoic acids by fatty acid dioxygenases. *J. Lipid. Res.* **52**, 1995-2004
4. Palmieri-Thiers, C., Alberti, J. C., Canaan, S., Brunini, V., Gambotti, C., Tomi, F., Oliw, E. H., Berti, L., and Maury, J. (2011) Identification of putative residues involved in the accessibility of the substrate-binding site of lipoxygenase by site-directed mutagenesis studies. *Archives of biochemistry and biophysics* **509**, 82-89
5. Hoffmann, I., Hamberg, M., Lindh, R., and Oliw, E. H. (2012) Novel insights into cyclooxygenases, linoleate diol synthases, and lipoxygenases from deuterium kinetic isotope effects and oxidation of substrate analogs. *Biochim. Biophys. Acta* **1821**, 1508-1517
6. Jernerén, F., Eng, F., Hamberg, M., and Oliw, E. H. (2012) Linolenate 9R-dioxygenase and allene oxide synthase activities of Lasiodiplodia theobromae. *Lipids* **47**, 65-73
7. Jerneren, F., and Oliw, E. H. (2012) The fatty acid 8,11-diol synthase of Aspergillus fumigatus is inhibited by imidazole derivatives and unrelated to PpoB. *Lipids* **47**, 707-717
8. Wennman, A., Jernerén, F., Hamberg, M., and Oliw, E. H. (2012) Catalytic convergence of manganese and iron lipoxygenases by replacement of a single amino acid. ***J. Biol. Chem***. **287**, 31757-31765
9. Hoffmann, I., Jerneren, F., and Oliw, E. H. (2013) Expression of fusion proteins of Aspergillus terreus reveals a novel allene oxide synthase. ***J. Biol. Chem.*** **288**, 11459-11469
10. Hoffmann, I., and Oliw, E. H. (2013) 7,8- and 5,8-Linoleate diol synthases support the heterolytic scission of oxygen-oxygen bonds by different amide residues. *Arch. Biochem. Biophys.* **539**, 87-91
11. Hoffmann, I., and Oliw, E. H. (2013) Discovery of a linoleate 9S-dioxygenase and an allene oxide synthase in a fusion protein of Fusarium oxysporum. *J. Lipid Res.* **54**, 3471-3480
12. Wennman, A., and Oliw, E. H. (2013) Secretion of two novel enzymes, manganese 9S-lipoxygenase and epoxy alcohol synthase, by the rice pathogen Magnaporthe salvinii. *J. Lipid Res.* **54**, 762-775

Dissertations 2013

1. Inga Hoffmann. *Discovery of Novel Fatty Acid Dioxygenases and Cytochromes P450. Mechanism of Oxylipin Biosynthesis in Pathogenic Fungi.* Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy, 176, 2013. ISSN 1651-6192.

Agencies that support the work/Funding 2013

The Swedish Research Council Medicine

Projects

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

Fungi are severe pathogens of man and can be devastating for important crops. Aspergillus causes farmer´s lung disease and invasive aspergillosis of immunocompromized patients. Rice blast disease is caused by *Magnaporthe oryzae*, and destroys ~25% of rice crops worldwide. Aspergillus and *M. oryzae* contain cyclooxygenase-related enzymes, diol synthases and dioxygenases, which transform linoleic acid into hydroperoxides and dihydroxy fatty acids. Our aim is to characterize the enzymes by enzyme expression, gene targeting and by studies on their biological importance.

(i) Characterization of 9R- and 9S-dioxygenases linked to allene oxide synthases of the Aspergillus and Fusarium complexes*:* The quest for genes with homology to 7,8-LDS led us to investigate *A. terreus*, where we found a novel *9R*-dioxygenase linked to an allene oxide synthase (JBC, 2013). This enzyme is also present in *A. niger*, and homologues with 9S-dioxygenase-allene oxide synthase activities are found in the Fusarium complex. We are now investigating the reaction mechanisms.
(ii) Charaterization of a novel subfamily with epoxy alcohol synthase activity: Our most recent discovery is the 10R-dioxygenase-epoxy alcohol synthase of M. oryzae, and we are now investigating the catalytic mechanism.
(iii) *EPR analysis of the protein radicals of CYP-DOX fusion enzymes.*

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

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

(III) Novel transformations of eicosanoids (Ernst Oliw, Johan Bylund).

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

Biological Research on Drug Dependence

Fred Nyberg & Mathias Hallberg

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

The overall goal of research is to increase the understanding of brain mechanisms of relevance for the aetiology of drug addiction and to develop strategies for relevant treatment. Studies of drug effects on brain circuits involved in reward and dependence are combined with attempts to develop peptidomimetics reducing the expression of opioid withdrawal, an important aspect in the treatment of opioid addiction. Studies are also aimed to find strategies to repair drug-induced brain damages, i.e. using peptidomimetics or growth factors counteracting apoptosis and stimulating neurogenesis. As animal models do not reflect all complexity of addiction current research also involves epigenetic adaptations and regulation of plastic neuropeptide genes in human brain.

Members of the group during 2013

Fred Nyberg, Professor
Mathias Hallberg, PhD Associate Professor
Jenny Johansson, PhD
Alfhild Grönbladh, PhD
Anna Carlsson PhD student
Erika Brolin, PhD student
Anna Lesniack, Postdoc
Johanna Elnersson, Project assistant
Myron Zaluha, Project leader

Publications 2011-2013

1. Andersson H, Demaegdt H, Johnsson A, Vauquelin G, Lindeberg G, Hallberg M, Erdelyi M, Karlen A, Hallberg A. Potent macrocyclic inhibitors of insulin-regulated aminopeptidase (IRAP) by olefin ring-closing metathesis. J Med Chem. 2011 Jun 9;54(11):3779-92.
2. Elfverson M, Johansson T, Zhou Q, Le Grevès P, Nyberg F. Chronic administration of the anabolic androgenic steroid nandrolone alters neurosteroid action at the sigma-1 receptor but not at the sigma-2 or NMDA receptors. Neuropharmacology. 2011 Dec;61(7):1172-81. Epub 2011 Jan 18.
3. Ghasemzadeh N, Rossbach UL, Johansson BM, Nyberg F. Application of artificial gel antibodies for investigating molecular polymorphisms of human pituitary growth hormone. Amino Acids. 2011 Apr;40(4):1249-55. Epub 2011 Feb 11.
4. Taqi MM, Bazov I, Watanabe H, Nyberg F, Yakovleva T, Bakalkin G. Prodynorphin promoter SNP associated with alcohol dependence forms noncanonical AP-1 binding site that may influence gene expression in human brain. Brain Res. 2011 Apr 18;1385:18-25.
5. Taqi MM, Bazov I, Watanabe H, Sheedy D, Harper C, Alkass K, Druid H, Wentzel P, Nyberg F, Yakovleva T, Bakalkin G. Prodynorphin CpG-SNPs associated with alcohol dependence: elevated methylation in the brain of human alcoholics. Addict Biol. 2011 Jul;16(3):499-509. doi: 10.1111/j.1369-1600.2011.00323.x. Epub 2011 Apr 26.
6. Kehr J, Ichinose F, Yoshitake S, Goiny M, Sievertsson T, Nyberg F, Yoshitake T.Mephedrone, compared with MDMA (ecstasy) and amphetamine, rapidly increases both dopamine and 5-HT levels in nucleus accumbens of awake rats. Br J Pharmacol. 2011 Dec;164(8):1949-58. doi: 10.1111/j.1476-5381.2011.01499.x.
7. Zhou Q, Carlsson A, Hallberg M, Nyberg F. Substance P N-terminal fragment SP(1-7) attenuates chronic morphine tolerance and affects dynorphin B and nociceptin in rats. Peptides. 2011 Aug;32(8):1661-5. Epub 2011 Jul 7

8. Bazov I, Kononenko O, Watanabe H, Kuntić V, Sarkisyan D, Taqi MM, Hussain MZ, Nyberg F, Yakovleva T, Bakalkin G. The endogenous opioid system in human alcoholics: molecular adaptations in brain areas involved in cognitive control of addiction. Addict Biol. 2011 Sep 28. doi: 10.1111/j.1369-1600.2011.00366.x. [Epub ahead of print]
9. Ohsawa M, Carlsson A, Asato M, Koizumi T, Nakanishi Y, Fransson R, Sandström A, Hallberg M, Nyberg F, Kamei J. The dipeptide Phe-Phe amide attenuates signs of hyperalgesia, allodynia and nociception in diabetic mice using a mechanism involving the sigma receptor system. Mol Pain. 2011;7:85
10. Ohsawa M, Carlsson A, Asato M, Koizumi T, Nakanishi Y, Fransson R, Sandström A, Hallberg M, Nyberg F, Kamei J.The effect of substance P1-7 amide on nociceptive threshold in diabetic mice. Peptides. 2011 32(1):93-8.
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12. Enhamre E, Carlsson A, Grönbladh A, Watanabe H, Hallberg M, Nyberg F. (2012) The expression of growth hormone receptor gene transcript in the prefrontal cortex is affected in male mice with diabetes-induced learning impairments. Neurosci Lett. 523:82-6.
13. Grönbladh A, Johansson J, Nyberg F, Hallberg M (2013) Recombinant human growth hormone affects the density and functionality of GABA(B) receptors in the male rat brain. Neuroendocrinology. 97:203-211.
14. Heddini U, Bohm-Starke N, Grönbladh A, Nyberg F, Nilsson KW, Johannesson U. GCH1-polymorphism and pain sensitivity among women with provoked vestibulodynia. Mol Pain. 2012 Sep 12;8:68.
15. Isaksson J, Nilsson KW, Nyberg F, Hogmark A, Lindblad F. J Psychiatr Res. Cortisol levels in children with attention-deficit/hyperactivity disorder. 2012 Nov;46(11):1398-405.
16. Murugaiah AM, Wu X, Wallinder C, Mahalingam AK, Wan Y, Sköld C, Botros M, Guimond MO, Joshi A, Nyberg F, Gallo-Payet N, Hallberg A, Alterman M. From the first selective non-peptide AT(2) receptor agonist to structurally related antagonists. J Med Chem. 2012 Mar 8;55(5):2265-78.
17. Pettersson FD, Grönbladh A, Nyberg F, Sundström-Poromaa I, Åkerud H. The A118G single-nucleotide polymorphism of human μ-opioid receptor gene and use of labor analgesia. Reprod Sci. 2012 Sep;19(9):962-7.
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1. Jenny Johansson. *The Impact of Growth Hormone and Gamma-Hydroxybutyrate (GHB) on Systems Related to Cognition.* 2013. Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy, 168. ISSN 1651-6192.
2. Alfhild Grönbladh. *Growth Hormone and Anabolic Androgenic Steroids: Effects on Neurochemistry and Cognition*, 2013. Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy, 175. ISSN 1651-6192; 175

Agencies that support the work/Funding 2013

Swedish Research Council Medicine
Swedish Council for Working Life and Social Research
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Berzelii Centre for Biotechnological Research
Swedish Institute, Visby Program
Disciplinary Domain of Medicine and Pharmacy
The Alcohol Research Council of the Swedish Alcohol Retailing Monopoly
The Research Council of Swedish Criminal Care

Other commitments/assignments of group members 2013

Fred Nyberg: Member of the Governmental Advisory Board for Addictive drugs (ANT-Advisory Board). Member of the Uppsala University Center for Studies of the Religion in the Society since 2006 and the National Center for Mens Violence against women since 2006. Member of the Board for the Medical Committee of the Swedish Criminal Care. Member of the Executive committee for the International Narcotics Research Conference (INRC) from 2006 to the present.
Member of the Board of the Alcohol Research Council of the Swedish Alcohol Retailing Monopoly
Member of the Board of The Research Council of the Swedish Criminal Care
Member of Editorial Board of Scientific journals (Fred Nyberg): Peptides, Open J Endocrinology (Editor in Chief), Pharmacology-on-line, J Musc Skel. Pain. PI at the Uppsala Berzelii Technology Center for Neurodiagnostics (100 milj, SEK 2006-2015); PI at the Linne project Impact of Religion: Challenges for Society, Law and Democracy (50 milj. SEK 2008-2017); PI at the FAS supported project on alcohol effects on cognitive functions (12 milj. 6 years). Swedish Research Council/Medicin for peptidergic mechanism in the development of drug dependence: 1.95 mil, SEK 2009-2011 (2009 additional grant 2.3 milj SEK for 2009) and 2.4 milj SEK for 2012-2014.
Mathias Hallberg: Referee: Curr Protein Pept Sci. and The Open Biochemistry Journal

Projects

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

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

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

(IV) Studies on 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*. The AT4 studies a in particular focused on the memory promoting effects.

Drug Safety and Toxicology

Lennart Dencker & Eva Brittebo

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

The research group Drug Safety and Toxicology are revealing mechanisms of adverse effects of environmental contaminants as well as pharmaceuticals and traditionally used medicinal plants, by the use of various in vitro and in vivo models. The overall aim is to understand the mechanisms of toxicity, focusing on the following four research areas:

- Developmental toxicology (Lennart Dencker)
- Bioactivation and toxicity (Eva Brittebo)
- Genetic toxicology (Björn Hellman)
- Neurotoxicology (Malin Andersson)

Developmental toxicology

Lennart Dencker

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

Members of the group during 2013

Lennart Dencker, Professor
Michael Stigson, PhD, Researcher
Henrik Alm, PhD Researcher
Birger Scholz, PhD, Researcher
Mats Nilsson, PhD student
Raili Engdahl, Technician
Lena Norgren, Technician

Publications 2011-2013

1. Scholz B, Sköld K, Kultima K, Fernandez C, Waldemarson S, Savitski MM, Söderquist M, Borén M, Stella R, Andrén P, Zubarev R, James P. Impact of temperature dependent sampling procedures in proteomics and peptidomics - a characterization of the liver and pancreas post

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Agencies that support the work/Funding 2013

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

Other commitments/assignments of group members 2013

Lennart Dencker: ExCo member of an EU-project within IMI JU, planning a pan-European training programme in safety of medicines (see SafeSciMET.eu). ExCo member of MRA, (http://www.medicinesacademy.org/index.php/Home/8/0/), a newly established industrial oriented medicines research education cooperation between Lund University, Technical University of Denmark, University of Copenhagen and Uppsala University. President of EUFEBS (http://www.eufeps.org/), an organization representing and serving the pharmaceutical sciences community/ies and innovative drug research in Europe. Member of Toxikologiska Rådet, Kemikalieinspektionen. Member of Läkemedelsnämnden, Läkemedelsverket (Medical Products Agency).

Projects

(I) In vitro system development for prenatal cardiotoxicity

The embryo is not protected from pharmaceuticals and environmental pollutants. Intended and unintended pharmacological effects of drugs are often exerted in the conceptus as well. They can be reversible, but have occasionally detrimental morphological and functional downstream effects. We aim to improve the mechanistic understanding of teratogenic processes and develop improved in vitro methodologies in developmental toxicology. One project is to develop improved image analysis software for the characterization and scoring of rodent embryos undergoing organogenesis in whole embryo culture (WEC). By combining image analysis with multivariate analysis to assess adverse effects of embryonic development *in vitro*, we believe that the objectivity and the sensitivity of the method will increase. One aspect of the image analysis software project is to develop better ways of analysing the developmental toxicity effect of drugs on heart rate in WEC conditions. For the purpose of standardization and to improve scoring and data handling in the assessment of WEC, we are also developing a controlled vocabulary in the form of a OBO and OWL compatible upper ontology (Phenosemantic ontology, PSO) together with a phenotypic domain ontology (Rodent Organogenesis and Toxicology Ontology, ROTO) that encompasses observable physiological and morphological endpoints during organogenesis (applicable both *in vivo* and *in vitro*).

(II) Developmental neurotoxicity during the postnatal synaptogenic sensitivity period

Brain development includes key neurodevelopmental prenatal and postnatal stages where environmental stimuli (such as neurotoxicants) can be particular efficient in inducing long lasting changes in neurodevelopmental trajectories. During the first two to three postnatal weeks of rodent life, there is a synaptogenic sensitivity period, corresponding to the first ~2-3 years of human life.

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

(III) Developmental contingencies and DNT in vitro

The issue of in-vitro extrapolation to in-vivo conditions is of great importance in predictive toxicology considering how resource-intensive animal studies are. Unfortunately, the majority of todays in silico and in vitro assays suffer from weak predictive power for more complex toxicological endpoints. The mechanistic information from the postnatal sensitivity project is to be applied to the development of a range of more representative and specific DNT in vitro tests. To this end, this project investigates how the developmental contingency of the original conditions (i.e. environmental experiences of the donor, age of cell isolation, brain region, sex, and species) influences regular and DNT properties of neural stem/progenitor cells (NSPCs) derived from prenatal and postnatal mice and rats. Neurite outgrowth and synaptogenesis is a fundamental event in postnatal brain development. We have previously shown, both in mouse in vivo and in primary CNS cell cultures in vitro, that PBDEs disrupt the normal expression of proteins necessary for neuritogenesis and synapse formation. These findings, together with the ability of certain PBDEs to accumulate in CNS cells, particularly in astrocytes, and to elicit stress in these cells has prompted us to investigate the effects of PBDEs on astrocyte-neuron interactions leading to altered neuritogenesis and synaptogenesis. NSPC cultures are neural cell mixes that – depending on their developmental contingency- are composed of different proportions of neurons, astrocytes and oligodendrocytes. The analysis involves both functional genomic characterizations and the use of high content imaging (HCA) systems and is conducted with Kim Kultima at the department of Medical sciences, Uppsala University. Besides rodent NSph, the project will also use primary human NSph derived from human glioblastoma biopsies.

(IV) Stem cells for embryotoxicity testing

In addition, we use the information from embryos (cultured in vitro, or exposed in vivo), and apply it on mouse and human embryonic stem (ES) cells, to develop mechanism-based in vitro cell test systems to reveal the teratogenic potential of substances. Using the antiepileptic and teratogenic drug valproic acid (VPA), an histone deacetylase inhibitors (HDACi), together with some analogs of valproic acid, we try to visualize which categories of genes may be representative for the teratogenic action (such as neural tube defects) of VPA, and in addition find coherent responses, on the level of gene regulation, to these compounds in the two species. Presently, larger batteries of teratogenic and non-teratogenic compounds are tested with respect to their gene (de)regulation in murine embryonic stem cells. For the purpose to screen in vitro for teratogenic action on specific developmental processes, we use differentiation of murine ES cells along a verity of lineages under the influence of teratogenic compounds. To further extend the usefulness, and facilitate the implementation of murine ES cells in HTS of developmental toxicity assays, we have adopted these cells to culture conditions free of animal products, such as serum and feeder cells.

(V) Mechanistic studies of CRABP1

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

(VI) Resilience of memory traces

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

Bioactivation and toxicity

Eva Brittebo

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

Members of the group during 2013

Eva Brittebo, Professor
Helén Andersson, PhD
Oskar Karlsson, PhD
Lisa Ersson, PhD Student
Elena Piras, PhD student

Publications 2011-2013

1. Andersson M, Karlsson O, Bergström U, Brittebo EB, Brandt I. Maternal transfer of the cyanobacterial neurotoxin β-N-methylamino-L-alanine (BMAA) via milk to suckling offspring. PLoS One. 2013;8:e78133.
2. Roman E, Karlsson O. Increased anxiety-like behavior but no cognitive impairments in adult rats exposed to constant light conditions during perinatal development. Ups J Med Sci. 2013;118:222-7.
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10. Karlsson O, Roman E, Berg AL, Brittebo EB. Early hippocampal cell death, and late learning and memory deficits in rats exposed to the environmental toxin BMAA (beta-N-methylamino-L-alanine) during the neonatal period. Behav Brain Res. 2011; 219: 310-20.
11. Andersson H, Garscha U, Brittebo E. Effects of PCB126 and 17-beta-oestradiol on endothelium-derived vasoactive factors in human endothelial cells. Toxicology. 2011; 285: 46-56.

Agencies that support the work/Funding 2013

The Swedish Research Council Formas.

Other commitments/assignments of group members 2013

Eva Brittebo: Associate Head of the Department; Deputy member of the Education committee Faculty Pharmacy; Member of the working group “Goals and strategies for Uppsala University”

Projects

(I) Bioactivation and toxicity of pollutants and drugs in vascular tissues (Helén Andersson and Eva Brittebo)

The persistent industrial chemicals PCBs were banned in the 1970s but they are still present in the environment. Epidemiological and experimental studies suggest an association between elevated serum levels of co-planar PCBs and hypertension. Our data demonstrate that PCB126 increased the expression of the endothelial vasoconstriction factors COX-2 and reactive oxygen species and stimulated the formation of the COX-2 derived vasoconstrictor prostaglandin PGF_2 in blood vessel endothelial cells (HUVEC). This indicates that PCB126 can modulate the expression and production of vasoconstriction factors in the human endothelium in a way that is characteristic for endothelial dysfunction related to human hypertension. Bisphenol A (BPA) is widely used in the manufacturing of consumer products such as plastic food containers and food cans. Epidemiological studies report an association between elevated exposure to BPA and cardiovascular disease and diabetes. Although alterations in the vascular endothelium are implicated in pathological conditions associated with BPA, little is known about the effects of BPA in the human endothelium. Our data demonstrate that BPA increased the mRNA expression of the proangiogenic genes VEGFR-2, VEGF-A, eNOS, and Cx43 and increased the production of nitric oxide in HUVEC. Furthermore, BPA increased the expression of phosphorylated eNOS and endothelial tube formation in HUVEC. These studies demonstrate that environmentally relevant levels of BPA have direct proangiogenic effects on human primary endothelial cells in vitro suggesting that the human endothelium may be an important target for BPA.

The selective estrogen receptor modulator tamoxifen is the most widely used agent for treatment and prevention of oestrogen receptor positive breast cancer. However, the beneficial effects are compromised by an increased risk for endometrial polyps, hyperplasia, and cancer in the endometrium. The adverse effects of tamoxifen include vaginal endometrial bleeding, endometrial hyperplasia, and cancer, conditions associated with angiogenesis. We have examined the effects of tamoxifen on cell migration and angiogenesis-related gene expression in human endometrial endothelial cells (HEECs). The data suggest that tamoxifen changes the regulation of angiogenesis in the endometrium, most likely by reducing angiogenic activity and that endometrial stromal cells regulate some of tamoxifen's effects in HEECs. These studies were performed in collaboration with Professor Matts Olovsson at the Department of Women's and Children's Health, Uppsala University.

(II) Neurodegeneration following neonatal exposure to a cyanobacterial toxin (Oskar Karlsson, Lisa Ersson and Eva Brittebo)

BMAA (beta-N-methylamino-L-alanine) is a neurotoxic amino acid that is produced by cyanobacteria present in terrestrial and aquatic environments. This neurotoxin has been suggested to contribute to a particular neurodegenerative disease in humans. Our studies demonstrated a poor transfer of radiolabelled BMAA across the blood-brain barrier (BBB) with no specific localisation in discrete brain regions in adult mice. In neonatal mice, however, there is an efficient transport across the BBB and a selective uptake of radioactivity in the hippocampus and striatum. Neonatal exposure to BMAA also induces cognitive impairments such as reduced spatial learning and memory abilities in adulthood without any effects on recognition memory. In addition, neonatal rat pups treated with a high dose BMAA show early neuronal cell death in the hippocampus, retrosplenial and cingulate cortices. These brain areas are all important for cognitive function. Histopathological analysis identified major changes i.e., neuronal degeneration, cell loss, calcium deposits and astrogliosis in the hippocampus of adult animals following neonatal exposure. Lower doses of BMAA cause distinct impairments in learning and memory function without any acute morphological changes in the brain. However, MALDI imaging studies revealed that BMAA decreased the expression of proteins involved in energy metabolism and intracellular signalling in the adult hippocampus at a dose (150 mg/kg) that gave no histopathological lesions. Developmental exposure to a higher dose (460 mg/kg) also induces changes in the expression of S100β, histones, calcium and calmodulin-binding proteins as well as guanine nucleotide-binding proteins. In addition, mass spectrometric quantification of the relative levels of endogenous neuropeptides in the neonatal striatum revealed

that 25 peptides from 13 neuropeptide precursors were significantly changed. The exposure to noncytotoxic doses of BMAA induced a dose-dependent increase of neurosecretory protein VGF-derived peptides, and changes in the relative levels of cholecystokinin, chromogranin, secretogranin, MCH, somatostatin and cortistatin-derived peptides were observed at the highest dose. Because several of these peptides play a critical role in the development and survival of neurons, the observed neuropeptide changes might be possible mediators of BMAA-induced behavioral changes.

In another study we characterized changes of major intermediary metabolites in serum following neonatal exposure to BMAA using a non-targeted metabolomic approach. Multivariate data analysis of binned NMR data indicated metabolic pattern differences between the treatment groups. In particular d-glucose, lactate, 3-hydroxybutyrate, creatine and acetate, were changed in serum of BMAA-treated neonatal rats. These metabolites are associated with changes in energy metabolism and amino acid metabolism. The neonatal rat model used in this study is so far the only animal model that displays significant biochemical and behavioral effects after a low short-term dose of BMAA. Finally, we have observed that secretion into milk is an important elimination pathway of BMAA in lactating mothers. Following secretion of radiolabelled L-BMAA into milk, the levels of radioactivity in the brains of the suckling neonatal mice significantly exceeded the levels in the maternal brains. In vitro studies using the mouse mammary epithelial HC11 cell line confirmed a more efficient influx and efflux of L-BMAA than of D-BMAA in cells, suggesting enantiomer-selective transport. Competition experiments with other amino acids and a low sodium dependency of the influx suggest that the amino acid transporters LAT1 and LAT2 may contribute to the transport of L-BMAA into milk.

Overall, our studies imply that the developing brain is particularly sensitive to BMAA. The risk posed by BMAA as a potential human neurotoxin merits further consideration, particularly if the proposed biomagnification in the food chain is confirmed. These studies were performed in collaboration with the Department of Medicinal Chemistry and Department of Environmental Toxicology, Uppsala University, and with the associate professor Anna-Lena Berg and coworkers at AstraZenca, Södertälje.

(III) Nasal transfer of therapeutic agents (Elena Piras and Eva Brittebo)

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

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

Genetic Toxicology

Björn Hellman

When testing the potential DNA-damaging effects by pharmaceutical drugs and other chemicals, the test systems are generally based on experimental animals, bacteria or various kinds of transformed cells. For the safety evaluation it would be of advantage if healthy cells from humans could be used instead, since they have a normal and stable set of chromosomes. In case of using primary cultures of human lymphocytes, it is hardly possible to use a blood sample on more than one single testing occasion. This will often give varying results from different testing occasions, since new blood samples (in general from different donors) have to be taken all the time. Our extended-term cultures of lymphocytes allow one single blood sample to be used for up to 50 different experiments. We measure the DNA-damage with the so called Comet Assay, which is a relatively quick, simple and cheap method for evaluating DNA-strand breaks in individual cells. The major objective of our *in vitro* studies using the comet assay in various cell lines is to improve the risk assessment regarding exposures to genotoxic agents. In our most recent project evaluating the potential genotoxicity of water samples intended to be used as drinking water, we have introduced HepG2-cells as an additional cell line to be used in our tests. The latter cells are liver cells of human origin, commonly used when screening for toxicological profiles of xenobiotics in vitro. The main objective of the drinking water project (which we only are a small part of) is to develop methods for assessment of hazardous chemicals in drinking water by integrating chemical analysis and in vitro toxicity testing. Read more about the "SafeDrink project" on http://www.slu.se/en/departments/aquatic-sciences-assessment/research/safedrink/

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

In collaboration with colleagues from the Ångström Laboratory at Uppsala University, and FOI, Division of CBRN Defence and Security in Umeå, we have also evaluated cellular and nuclear uptake and DNA damage in human lung cancer cells exposed to reactive nanoparticles of titanium dioxide using Raman microspectroscopy and the Comet Assay. The results clearly indicated a nuclear uptake of the nanoparticles, and that a direct interaction between reactive TiO_2 nanoparticles and DNA cannot be ruled out as one of the mechanisms leading to an increased level of DNA damage in cells that had been exposed to a rather low concentration of particles.

An important issue in the safety evaluation of drugs is if there are threshold doses or not for agents found to be genotoxic *in vitro* or *in vivo*. In the most recent project we are therefore also evaluating the effect of oxidative stress on the nucleotide pool and the integrity of DNA using various compounds previously shown to induce gene mutations, but only at rather high concentrations

Members of the group during 2013

Björn Hellman, Professor
Rikard Åsgård, Ph.D. student
Jasem Al Shemaili, Ph.D. student
Lena Norgren, Technician

Publications 2011-2013.

1. Yeshak MY, Göransson U, Burman R, Hellman B (2012). Genotoxicity and cellular uptake of cyclotides: Evidence for multiple mode of action. *Mutat. Res*. 747: 176-181.
2. Demma J, Elseedi H, Engidawork E, Leta Aboye T, Göransson U, Hellman B (2013) An in vitro study on the DNA damaging effects of phytochemicals partially isolated from an extract of Glinus lotoides. *Phytother. Res*., 27: 507-514.
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Other commitments/assignments of group members 2013

Björn Hellman: Head of department (from July 2013); Member of the local committee for scholarships at the Faculty of Pharmacy; Member of the committee for undergraduate courses (GRUFF) at the Faculty of Pharmacy (until June 2013); Deputy member of the ethical committee for animal experiments in Uppsala (until June 2013); Deputy member of "Docenturkommittén" at the Disciplinary Domain of Medicine and Pharmacy (until June 2013); Study director in toxicology.

Neurotoxicology

Malin Andersson

In the group, MALDI –TOF imaging mass spectrometry (MALDI IMS) is used for the topographical elucidation of proteins, neuropeptides and neurotransmittors and their changing concentrations in brain during physiological and pathophysiological events. In particular we focus on Parkinson's disease, which is characterized by degeneration of dopaminergic neurons and accompanied by a dramatic loss of DA in the striatum, particularly in the putamen. About 1% of the population over 65 years suffers from PD and the cardinal symptoms that include akinesia, bradykinesia, muscle rigidity, and tremor. DA replacement therapy with L-DOPA is currently the most effective pharmacotherapy for patients with PD, however with PD disease progression and long-term L-DOPA treatment complications occur in many patients. The main complications are troublesome motor complications such as "wearing off" fluctuations and L-DOPA-induced dyskinesia (LID). Despite large efforts in the field of LID research, the difficulties we face today remain as how to dissociate the molecular changes that arise by the motor execution of LID from causative changes that induce or predispose to dyskinesias. The 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 2013

Malin Andersson, Associate Professor

Publications 2011-2013.

1. Hanrieder J, Wicher G, Bergquist J, Andersson M, Fex-Svenningsen A. (2011). MALDI mass spectrometry based molecular phenotyping of CNS glial cells for prediction in mammalian brain tissue. Anal Bioanal Chem. 2011 Jul;401(1):135-47.
2. Ljungdahl A, Hanrieder J, Fälth M, Bergquist J, Andersson M. (2011). Imaging Mass Spectrometry Reveals Elevated Nigral Levels of Dynorphin Neuropeptides in L-DOPA-Induced Dyskinesia in Rat Model of Parkinson's Disease. PLoS One. 2011;6(9):e25653.

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5. Hanrieder J, Ljungdahl A, Andersson M. (2012). MALDI Imaging Mass Spectrometry of Neuropeptides in Parkinson's Disease. JOVE, J Vis Exp. 2012 Feb 14;(60). pii: 3445
6. Hanrieder J, Ekegren T, Andersson M, Bergquist J (2013) MALDI Imaging of Post Mortem Human Spinal Cord in Amyotrophic Lateral Sclerosis. J. Neurochem. J Neurochem. 2012 Sep 19. PMID: 22994484.
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Agencies that support the work/Funding 2013

The Swedish Research Council

Projects

(I) Imaging Mass Spectrometry Study of Basal Ganglia Levels of Neuropeptides in L-DOPA-induced Dyskinesia in experimental Parkinson's Disease (Anna Ljungdahl, Madelene Svedin, Kristen Burnum (PNLL), Jonas Bergquist, and Malin Andersson)

We study neuropeptides and proteins involved in the development of L-DOPA-induced dyskinesias in experimental Parkinson's disease. Here we use MALDI-TOF imaging mass spectrometry (IMS) for unbiased assessment and topographical elucidation of neuropeptides and proteins in the basal ganglia of high and low dyskinetic animals. MALDI-IMS has the unique advantage of high sensitivity, high molecular specificity, and the detection of hundreds of molecular species in a single tissue section. Indeed, several dynorphins and enkephalins have been detected in these studies, including dynorphin B, dynorphin A(1-8), alpha-neoendorphin, MetEnkRF, MetEnkRGL, PEnk (198-209, 219-229).

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

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

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

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

(IV) Assessment of neuropeptides and proteins rat spinal cord (Ping Sui, Jonas Bergquist, Georgy Bakalkin and Malin Andersson)

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

Medical Mass Spectrometry

Per Andrén

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

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

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

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

The objective of our research is to utilize MSI and peptidomics approaches to study neurochemical processes in Parkinson's disease (PD) and specifically L-Dopa-induced dyskinesias (LID) (VR-M grant 2011-3170). The aim is to define neuropeptides and proteins that are differentially expressed in the basal ganglia complex of animals with striatal dopamine depletions, and to determine which of these neuropeptides and proteins are regulated by loss of dopamine signaling, as well as to investigate protein and peptide expression patterns in subjects with and without LID symptoms.

Understanding the relationship between pharmacokinetics and pharmacodynamics is crucial in the development of effective drug therapies. Current technologies only provide information on the total amount of drug in the whole tissue with no possibility to address micro-environmental localization of the compound or metabolic derivatives. The application of MALDI MSI in drug discovery studies (VR-NT grant 2010-5421) provide a new tool to accurately measure local tissue concentrations of drug/drug metabolite in context with efficacy achieved with the targeted biology or in the context of safety.

Our laboratory is equipped with the latest separation and MS technologies, i.e., two MALDI MS imaging instruments, Ultraflextreme and UltraFlex II (Bruker Daltonics) and two electrospray ionization mass spectrometers, LTQ (Thermo) and a high-resolution Q-Tof mass spectrometer (Maxis Impact, Bruker Daltonics).

Members of the group during 2013

Per Andrén, Associate Prof.
Anna Nilsson, Researcher
Henrik Wadensten, Researcher
Henrik Lodén, Reseacher
Mohammadreza Shariatgorji, Researcher
Cecilia Eriksson, Post-doc
Richard Goodwin, Post-doc
Sara Ståhl, Post-doc
Patrik Källback, PhD student

Publications 2011-2013.

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14. Shariatgorji M, Nilsson A, Goodwin RJ, Svenningsson P, Schintu N, Banka Z, Kladni L, Hasko T, Szabo A, Andren PE (2012) Deuterated matrix-assisted laser desorption ionization matrix uncovers masked mass spectrometry imaging signals of small molecules. Anal Chem. 84:7152-7157. doi: 10.1021/ac301498m.
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16. Nilsson A, Forngren B, Bjurström S, Goodwin RJ, Basmaci E, Gustafsson I, Annas A, Hellgren D, Svanhagen A, Andren PE, Lindberg J (2012) In situ mass spectrometry imaging and ex vivo characterization of renal crystalline deposits induced in multiple preclinical drug toxicology studies. PLoS One. 2012;7(10):e47353. doi: 10.1371/journal.pone.0047353.
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Reviews 2011-2013

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

Agencies that support the work/Funding 2013

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

Other commitments/assignments of group members 2013

Per Andrén: Swedish Academy of Pharmaceutical Sciences, Section for Drug Analysis (member of the board); Swedish Proteomics Society (member of the board); Journal of Proteomics (editorial board); Peptidomics (editorial board); European Proteomics Association (EuPA, chairman for mass spectrometry imaging initiative cooperation in science and technology); Mass Spectrometry Imaging: New Tools for Healthcare Research Infrastructure (member of the management committee).

Projects

(I) Neurochemical characterization of basal ganglia neuropeptides and proteins in levodopa-induced dyskinesia in experimental Parkinson's disease using Imaging Mass Spectrometry and Peptidomics (Collaboration with Per Svenningsson, Karolinska Institutet, Alan Crossman, University of Manchester, UK, Erwan Bezard, Univ. of Bordeaux 2, France)

The main objective of the present research is to study neurochemical processes in Parkinson disease and specifically L-Dopa-induced dyskinesias (LID). No treatment exists yet for the management of LID, a debilitating complication of L-dopa therapy for PD. The aim is to define neuropeptides and proteins that are differentially expressed in the basal ganglia complex of animals with striatal dopamine depletions, with and without LID. Project supported by VR-MH grant 2011-3170.

(II) Imaging Mass Spectrometry: Direct analysis of peptides, proteins and drugs in tissue sections (Collaboration with Per Svenningsson, Karolinska Institutet, Liam McDonnell, Leiden University, the Nedtherlands)

The aim of this project is to create an approach to solve complex clinical problems by developing novel clinical tools utilizing mass spectrometry imaging directly on biological tissue sections. The mass spectrometry imaging technology is used to identify individual peptides and proteins and their 2-dimensional expression patterns in tissue sections as well as drugs and their metabolites in different animal disease models, but also in human tissues. We describe the development of new biomarkers and technologies in personalized medicine for marker-assisted diagnosis, prognosis and targeted therapies derived from an individual´s molecular profile. Conceptually, the project integrates in vivo disease models, biomarker discovery, personalized drugs which translate to clinic. Project supported by VR-NT grant 2010-5421.

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

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

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

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

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

In respiratory inhalation drug discovery projects, one key objective is to optimize retention of compound in the lung and consequently achieve duration of effect. Current technologies only provide information on the total amount of compound in the whole lung with no possibility to address micro-environmental localization of the compound or metabolic derivatives. The application of MALDI 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.

(VI) Characterization of drug-induced kidney toxicity using MS-Imaging (Collaboration with AstraZeneca)

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

(VII) Characterization of PET-ligands using MS-Imaging (Collaboration with AstraZeneca, Mats Larhed, UU)

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

(VIII) Novel inactivation technology stabilizes the in vivo levels of proteins, peptides, phosphorylations, lipids in tissue samples (Collaboration with Per Svenningsson, Karolinska Institutet, Denator AB, Uppsala and Göteborg, Sweden)

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

Molecular Neuropsychopharmacology

Georgy Bakalkin

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

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

Members of the group during 2013

Georgy Bakalkin, PhD, Professor
Tatiana Yakovleva, PhD, Senior scientist
Hiroyuki Watanabe, PhD, Research scientist
Daniil Sarkisyan, PhD, Research scientist
Qin Zhou, PhD, Research scientist
Richard Henriksson, PhD, Postdoctoral scientist
Wei Sun, Ph.D., Postdoctoral scientist
Anna Iatsyshyna, PhD, Postdoctoral scientist
Helena Kadyrova, PhD, Postdoctoral scientist
Igor Bazov, PhD student
Muhammad Zubair Hussain, PhD student
Olga Kononenko, PhD student
Vlad Tashbulatov, Research assistant

Publications 2011-2013

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Dissertations 2013

Muhammad Zubair Hussain. *Molecular Adaptations in the Endogenous Opioid System in Human and Rodent Brain.* 2013. Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy, 174. ISSN 1651-6192

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The Swedish Council for Working Life and Social Research
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The Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning

Other commitments/assignments of group members 2013

Georgy Bakalkin: Editor in Addiction Biology journal

Projects

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

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

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

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

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

Much of our knowledge about functions of genes and proteins in human brain has come from analysis of rare human and mouse mutations. Such mutations represent entry points into novel signaling pathways and molecular mechanisms. In collaboration with Dr. Dineke Verbeek, Dept. Genetics, Univ. Med. Center Groningen, The Netherlands, we have identified missense mutations in the human prodynorphin gene (*PDYN*) coding for a precursor to opioid peptides dynorphins (Bakalkin et al., 2010). These mutations cause profound neurodegeneration in the cerebrum and cerebellum underlying the development of spinocerebellar ataxia type 23, a dominantly inherited neurodegenerative disorder. Remarkably, seven out of eight mutations are located in dynorphins which also have non-opioid neurodegenerative activities. This is the first finding on neuropeptide mutations underlying human neuropathology. The best-established function of the dynorphins in the brain is regulation of the anti-reward system by inducing dysphoria that counterbalances positive hedonic effects of endorphins and enkephalins, and addictive substances. Analysis of PDYN mutations could uncover novel molecular and cellular mechanisms of anti-reward - reward regulation that are generally unknown. We focus on two mechanisms. First, the mutations may impair correct folding of PDYN molecules in the endoplasmic reticulum, resulting in PDYN aggregation, cellular stress and the unfolded protein response. Long-lasting activation of the

unfolded protein response by mutant PDYNs, or by wild-type - PDYN excessively produced under pathological conditions such as substance addiction, may lead to neuronal dysfunction, atrophy and neurodegeneration. Second, *PDYN* mutations may enhance non-opioid pathogenic activities of dynorphins. Our previous analysis suggests that dynorphins may mediate communications between neurons through non-receptor excitatory mechanism. This mechanism may be engaged in pathogenic effects of upregulated wild-type - dynorphins and mutant peptides. Our general goal is to understand pathogenic mechanisms of PDYN mutations and, in the following studies to evaluate whether wild-type - PDYN and dynorphins engage these mechanisms to regulate reward circuits under normal conditions and in substance addiction when these neuropeptides are excessively produced. We explore pathogenic mechanisms underlying actions of wild-type- and mutant-PDYN in cellular and in vitro biochemical/ biophysical studies, and are planning to use in vivo transgenic mice expressing human wild-type- or pathogenic mutant-PDYN that have been produced by Dr. Verbeek. Generalized pathological changes including cerebral cortical and subcortical atrophy, and agenesis of corpus callosum in patients caring PDYN mutations emphasize the fundamental role of neuropeptides in brain functions, and propose that the essential molecular mechanisms are affected; the phenomenon that requires detailed investigation. Dissection of these mechanisms is critically important for understanding of reward – anti-reward, substance addiction, depression and chronic pain, all neuropathological conditions in which dynorphins play a critical role, as well as for the neuropeptide and neurodegeneration fields in general because of the novelty of mechanisms identified.

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

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

Neuropharmacology, Addiction & Behaviour

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

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

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

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

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

Members of the group during 2013

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

Jenny Gustavsson, Research assistant
Stina Lundberg, Research assistant
Hanna Eriksson Röhnisch, Research assistant

Publications 2011-2013

1. Palm S, Roman E*, Nylander I*. Differences in voluntary ethanol consumption in Wistar rats from five different suppliers. Alcohol (2011) 45:607-614, *shared senior authorship.
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4. Palm S, Hävermark Å, Meyerson BJ, Nylander I*, Roman E*. When is a Wistar a Wistar? Behavioral profiling of outbred Wistar rats from five different suppliers using the MCSF test. Appl Animal Behav Sci (2011) 135:128-137 *shared senior authorship.
5. Daoura L, Nylander I. The response to naltrexone in ethanol-drinking rats depends on early environmental experiences. Pharmacol Biochem Behav (2011) 99:626-633
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Dissertations 2013

Loudin Daoura *Early environment and adolescent ethanol consumption; Effects on endogenous opioids and behaviour in rats*, 2013. Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy, 171. ISSN 1651-6192.

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ERAB - The European Foundation for Alcohol Research (PI Nylander, co-applicants Roman and Svensson)
The Swedish Research Council (Nylander)
The Alcohol Research Council of the Swedish Alcohol Retailing Monopoly (two separate projects; Nylander and Roman)
Facias Foundation (three separate projects; Momeni and Roman)
Gun och Bertil Stohnes stiftelse (Svensson)
Stiftelsen för Gamla Tjänarinnor (Svensson)
Åke Wibergs stiftelse (Roman)

Other commitments/assignments of group members 2013

Lena Bergström: Member of the Academic Senate, Uppsala University

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

Erika Roman: International Adjunct Associate, Department of Psychology, Indiana University, USA; Review Editor: Frontiers in Addictive Disorders and Behavioural Dyscontrol; External mentor Salvatore Magara, KI; Member of the board: The Society for Swedish Alcohol and Drug Research; Member of the board of Uppsala University Laboratory Animal Resources; Member of the Animal Welfare Body, Uppsala University; Member of the Postgraduate Programs Committee (KUF), Uppsala University; Member of the Uppsala Animal Ethical Committee; Approved Supervisor by the Swedish Board of Agriculture; Representative in the National Committee, the Swedish Board of Agriculture; Member of the expert panel, Swedish Centre for Animal Welfare; One of three coordinators of Uppsala University Behavioural Facility (UUBF), Uppsala University

Anne-Lie Svensson: Member of the committee for undergraduate education (GRUFF) at the Faculty of Pharmacy, Uppsala University; Member of the gender equality committee at the Disciplinary Domain of Medicine and Pharmacy, Uppsala University

Projects

(I) The impact of early environmental experiences on endogenous opioids, alcohol consumption and alcohol-induced effects (Loudin Daoura, Sara Palm, Linnea Granholm, Lova Perup-Segerström, Maria Ellgren, ***Ingrid Nylander****)*

We have previously shown that adverse experiences early in life cause long-term neurobiological and behavioural alterations. These changes may contribute to the increased vulnerability for drug addiction that is seen in the clinic, but also provide protection in a predisposed individual. Currently, we study the mechanisms underlying long-term consequences of early life stress. A rodent maternal separation (MS) model is used to simulate different environmental settings. Rearing conditions are used that are associated with resilience (after short MS) or vulnerability (after longer MS) in terms of adult risk consumption. We have shown that central neuropeptides and monoamines may contribute to these differences. Rats reared in a stressful environment have signs of a dysfunctional opioid system with low basal enkephalin levels and enhanced response to alcohol in a voluntary alcohol consumption paradigm. They also respond differently to treatment with naltrexone; animals reared in a risk environment reduce their alcohol intake whereas other individuals do not respond. These results highlight the importance of the early environment in drug consumption, drug-induced affects and treatment outcome. The consequences of adolescent voluntary alcohol consumption and of alcohol exposure through gavage on the brain and behaviour are currently examined in animals subjected to early life stress or no stress. We assess the effects of adolescent drug exposure on opioid networks, expression and methylation of genes involved in stress and reward processes and on alcohol consumption, motivation and relapse in adulthood.

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

Fast Analytical Sensing Technology (FAST) is currently used for *in vivo* analysis of dopamine in the brain. FAST is a chronoamperometric technique that enables *in vivo* electrochemical detection of transmitters in anaesthetized or awake animals. Microelectrodes are used to measure electrochemically active substances like dopamine. FAST offers unique advantages as compared to *in vivo* microdialysis: high temporal resolution and close spatial orientation enables a second-by-second analysis instead of several minutes; high sensitivity allows measurement of resting levels; high specificity by the use of different electrodes; the brain damage is reduced to a minimum. The technique is currently tested in awake animals. FAST has used in anaesthetized animals to analyse effects of exposure to early life stress including adolescent alcohol exposure. It is also used to correlate *in vivo* dopamine dynamics with behaviour.

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

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

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

Post-mortem metabolism is a major concern in the analysis of opioid peptides in biological samples and it is vital to find reliable, reproducible and easy to use procedures to avoid peptide metabolism in the handling of fresh tissues. A new project utilizes the Stabilizor T1 instrument (Denator AB, Gothenburg, Sweden) for heat stabilization of tissue samples to establish a procedure for rapid and efficient enzyme inactivation. The aim is to optimize the measurements of opioid peptides in the brain and achieve detection of peptide levels more similar to the *in vivo* concentration. To this end,

various *post-mortem* handling procedures are used to systematically evaluate the effect of heat stabilization on tissue levels of several opioid peptides in the brain.

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

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

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

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

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

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

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

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

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

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

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

Neurosteroids are endogenous modulators of neuronal functions responsible for many biological and pathophysiological effects. Plausible links between neurosteroids and neurodegenerative disorders, like Alzheimer´s disease (AD), are suggested. Accumulation of amyloid-b (the principal component of amyloid plaques) 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 study neuroprotective properties of neurosteroids and their metabolites, against amyloid-b-induced toxicity, as well as the underlying molecular mechanism(s), with focus on neurogenesis and apoptosis. Since neurosteroids most likely affects neuronal and glial cells differently, their effects on amyloid-b-induced toxicity are also investigated in numerous cell types.

Pharmaceutical Bioinformatics

Jarl Wikberg

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

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

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

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

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

The libiguin project also continued. A series of libiguins A-H were produced semi-synthetically and evaluated for their highly potent and powerful ability to stimulate of sexual behaviour. An [^{11}C]-labelled PET probe of the libiguins was developed and characterized biologically. Studies were continued to understand the molecular basis of the powerful actions of the libiguins. Further natural products were isolated from different *meliace* and determined to their structure. These studies included the discovery of a novel protolimonoid, as well as other novel natural compounds, as well as novel semi-synthetic compounds, with highly interesting novel pharmacological properties.

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Polina Georgieva, PhD
Klas Jönsson, Programmer
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The Swedish Research Council
AstraZeneca
Swedish Institute

Other commitments/assignments of group members 2013

Ola Spjuth: Deputy Director, UPPMAX

Projects

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

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

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

(IV) The Bioclipse Workbench (Ola Spjuth et al.)
Development of the Bioclipse workbench for e-Science. Main focus is on drug discovery, safety assessment, and predictive modelling, but other developments include plugins for Next-Generation Sequencing in collaboration with SciLifeLab and UPPMAX.

(V) Predictive toxicology (Ola Spjuth et al.)
Studies on predictive modelling in toxicology, mainly drug safety. Collaboration with the EU FP7 project OpenTox, AstraZeneca R&D, Karolinska Institutet, and the National Food Agency.

(VI) Prediction of metabolic sites (Ola Spjuth et al.)
Studies on predictions of site-of-metabolism, manifested in the MetaPrint2D method. Collaboration with AstraZeneca R&D and University of Copenhagen.

Pharmacometrics

Mats Karlsson

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

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

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Anders Burild, Visiting PhD student
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to treatment-naive HIV-1 infected patients. J Pharmacokinet Pharmacodyn 38, 727-742, doi:10.1007/s10928-011-9217-1 (2011).
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Dissertations 2013

1. Björnsson, M. *Pharmacometric Models in Anesthesia and Analgesia*, 2013. Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy, 173. ISSN 1651-6192.
2. Ernest II, C. S. *Benefits of Non-Linear Mixed Effect Modeling and Optimal Design: Pre-Clinical and Clinical Study Applications*, 2013. Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy, 181. ISSN 1651-6192.
3. Syed Mohamed, A. F. *Pharmacokinetic and Pharmacodynamic Modeling of Antibiotics and Bacterial Drug Resistance*, 2013. Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy, 170. ISSN 1651.

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Other commitments/assignments of group members 2013

Lena Friberg: Organizing committee, chair scientific program, PAGE conference, Glasgow 2013 & Alicante 2014; Deputy Editor-in-chief, CPT: Pharmacometrics & Systems Pharmacology

Andrew Hooker: Organizing committee, PODE conference

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

Mats Karlsson: Deputy Head of Department; Department Board Member; Scientific committee, PAGE conference, Alicante 2014; Organizing committee, WCoP conference, Brisbane 201; Scientific committee, ASCPT, Pharmacometric Section Committee member; Editor Journal of Pharmacokinetics and Pharmacodynamics; Editorial Board on Clin Pharmacol Ther, Eur J Pharm Sci, Basic Clin Pharmacol Toxicol, Adv Pharmacol Sci, CPT: Pharmacometrics & Systems Pharmacology

Maria Kjellsson: Department Board Member

Elisabet Nielsen: Board Member: Swedish Academy of Pharmaceutical Sciences, Section for Hospital Pharmacy; Executive Committee member, EPASG, ESCMID PK/PD Study Group

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

Projects

Methodological research

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

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

The second way of evaluating and optimizing trial designs is through the use of optimal experimental design methodologies. These methods often rely on calculations of an Information Matrix (e.g. the Fisher Information Matrix), which characterizes the information content of any possible design. Each calculation is much quicker than clinical trial simulation, thus one can investigate the landscape of possible designs (within constraints) potentially available for an experiment, and even optimize a design based on this information. We have developed methods and software (PopED) that utilize these methods with both local and global design criteria (e.g. API-optimal designs, which take into account that the underlying system (model) is not known before the study takes place). Additionally, while optimal design has previously focused on optimization of

sampling times in an experiment, we have extended the methodology to apply to other aspects of trial designs, such as the dose administered and the length of run-in, treatment and wash-out phases of an experiment. Further, we have extended optimal design methodology to optimize a study for power, as opposed to the traditional optimization based on model parameter estimation uncertainty.

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

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

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

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

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

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

(IV) Software development (Andrew Hooker, Mats Karlsson, Joakim Nyberg)

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

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

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

In this project we aim to describe disease progression and treatment exposure-response, and to develop new models for simulations of future studies. The time course of sleep stages and its relation

to placebo and drug effects has been analysed using Markov models in patients with insomnia. Pain scores rated on a Likert scale by neuropathic patients have been modelled by including features for under-dispersion and serial correlation. Daily numbers of seizures have been used in the investigation of over-dispersion and Markov patterns in count data. Simultaneous characterization of drug effect on severity and time to acid reflux events has been made possible and resulted in good simulation properties and high power.

In parallel we also investigate the performance of available estimation methods with discrete models. We have pointed out the fact that the Laplacian estimation method in NONMEM and NLMIXED results in biased parameter in situations with non-even distributions of the response categories. In another study the Laplace method produced accurate parameter estimation for Poisson models, with or without Markov elements and mixture distribution, whereas we identified a small bias in the random effect of zero-inflated Poisson, generalized Poisson and negative binomial models. The performance of the SAEM and importance sampling have been shown to be generally higher than Laplace in repeated time-to-events models where the frequency of individuals with events was low, while at high frequencies all methods were equal in performance. We have also conducted a study investigating all methods available in NONMEM version 7 for all types of discrete models, where we highlighted the overall fast and robust results obtained with Laplace.

Currently we are interested in handling the within-individual variability for discrete models. More specifically we adapted methods to handle inter-occasion variability in count models. Since count data are often collected on a regular basis (e.g. daily) in clinical trials, the time and length of potential occasions is not predefined but can be estimated through dynamic implementations. Alternatively the noise present in these types of records can be characterised using stochastic differential equations.

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

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

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

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

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

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

Applied research/Disease areas

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

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

Today, dosing regimens are typically selected based on PK/PD indices that discard information on dynamic changes in the drug-bacteria interaction. Mechanism-based models describing time-kill curves from *in vitro* experiments form the basis for the modelling. The developed model structure has been shown to be applicable across drugs and bacteria strains, for both static and dynamic concentration experiments, and for different sizes of start inocula. The model has been extended to describe different types of resistance; the adaptive resistance development of gentamicin and colistin, pre-existing mutants resistant to ciprofloxacin in starting inocula and resistance mutants from clinical isolates of meropenem. For ciprofloxacin and *E.coli*, the model has been successfully applied for wild-type and 9 well-characterized mutants, in addition to 3 clinical isolates. The model can also be used to predict competition experiments of wild-type and mutants. There is limited knowledge on combination treatments of antibiotics and predictions from PKPD-models based on *in vitro* data are performed to guide in the selection process of potential drug combinations to test clinically. Optimal experimental design techniques are applied to find experimental protocols that increase the efficiency of both static and dynamic time-kill curve experiments and in clinical studies of colistin.

Colistin has regained interest in recent years as a promising drug to overcome antibiotic drug resistance. With an in-house developed LC-MS-MS method we can quantify colistin and its prodrug CMS in both clinical plasma samples and in samples from *in vitro* experiments. Developed pharmacokinetic models for CMS and colistin in different subpopulations show that the drug is typically underdosed and a loading dose of 6-9 MU has been shown to be applicable and of value. The non-linear protein binding of colistin has been quantified as well as unspecific binding to lab material. Whole-body Physiology-based Pharmacokinetic (WBPBK) models for CMS, colistin and ciprofloxacin have been developed based on data from various sources, including patients, healthy volunteers and several animal species. Such a model can be used to understand the time-courses of the antibiotics, and thereby the bacterial killing, in different tissues.

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

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

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

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

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

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

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

been used for translational simulations of expected treatment outcome in vulnerable populations such as children and pregnant and to optimize the treatment regimen.

(III) Diabetes (Mats Karlsson, Maria Kjellsson)

Diabetes is a chronic disease, affecting more than 220 million people worldwide and the "diabetic epidemic" is projected to affect 366 million people in 2030. The disease occurs when the body does not produce enough insulin or cannot effectively use the insulin produced, resulting in increased blood glucose levels which in the extension leads to a multitude of conditions; e.g. cardio-vascular diseases (CVD). The aim with all treatment against diabetes is to bring the glucose level in blood down to the healthy levels. The success of a treatment is assess both on short and long term measurements; the most common biomarkers being fasting plasma glucose concentration (FPG) and the fraction glycosylated haemoglobin (HbA1c) for short and long term assessment respectively.

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

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

The overall endpoint of most treatments against type 2 diabetes is to lower the risk of long-term complications, such as CVD, retinopathy and chronic kidney disease. Long term studies commonly involve assessments of the risk of CVD in relation to elevated levels of HbA1c or FPG. We are developing parametric risk models, using registry data, quantifying the relationship between CVD and time-varying covariates such as HbA1c and other predictors of CVD, i.e. blood pressure, blood lipids, etc.

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

(IV) Oncology (Lena Friberg, Mats Karlsson)

Within the oncology area, we are working on PK and PKPD models describing the time-courses of biomarkers drug-induced toxicity, tumour size measurements (SLD, sum of longest diameter), tumor activity measurements (SUV, standard uptake value) and overall survival. The models are aimed to be mechanism-based and be applicable across different drugs, and thereby valuable in development of new and existing drugs, including individualization of the therapy. By integrating information of different variables in to a modelling framework the variables' relations and predictive value can be tested, and a better overview of both desired and adverse effects from a changed dosing regimen can be obtained. The models can also be used to explore different concepts of study design in oncology.

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

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

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

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

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

Pharmacokinetic-pharmacodynamic models in the therapeutic area of pain relief are investigated. The aim is to characterize the exposure-response relation of individual drugs as well as develop

models for simulation of study design of future studies and drugs. (Repeated) Time to event modelling of drop out in depression and pre-clinical addiction studies have been described.

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

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

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

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

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

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

Steroid P450

Maria Norlin & Kjell Wikvall

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

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

Primary missions and goals are to find new drug targets, understand the mechanisms for effects of steroids and steroid-related enzymes, compare effects of endogenous molecules and drugs, examine effects of drugs and drug candidates on steroid-related processes and study molecular mechanisms behind adverse drug effects.

Our research activities, focusing on basic research, provide important information that increases the understanding of mechanisms for steroid action. This should improve drug management (e.g. counteract/avoid adverse side-effects) and can be used for development of novel, better drugs.

Members of the group during 2013

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

Publications 2011-2013

1. Norlin M, Pettersson H, Tang W, and Wikvall K. Androgen receptor-mediated regulation of the anti-atherogenic enzyme CYP27A1 involves the JNK/c-jun pathway. *Arch Biochem Biophys. 506, 236-241 (2011)*
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Reviews 2011-2013

1. Norlin M and Wikvall K. Tissue-specific regulation of sex hormone biosynthesis and metabolism: novel aspects on hormonal signalling and maintenance of cellular steroid levels in "Sex Hormones", Ed. R.K. Dubey, ISBN 978-953-307-856-4. InTech, Rijeka, Croatia, 2012.
2. Nebert DW, Wikvall K and Miller WL. Human cytochromes P450 in health and disease. Philos Trans R Soc Lond B Biol Sci. 368, 1-21 (2013) doi: 10.1098/rstb.2012.0430

Other commitments/assignments of staff members 2013

Kjell Wikvall: Chair of the Scholarships committee for the Faculty of Pharmacy;
Member of the Scholarships committee for Uppsala University; Study director in Pharmaceutical Biochemistry

Maria Norlin: Senior member of the Departmental Committee for PhD education (FUG).

Projects

(I) Functions and regulation of neurosteroids and steroid-metabolizing enzymes in CNS cells (Ida Emanuelsson, Mokhtar Almokhtar and Maria Norlin)

Steroids produced locally in the nervous system, such as DHEA (dehydroepiandrosterone), pregnenolone or 27-hydroxycholesterol, have been termed neurosteroids and are considered of particular importance for brain development and function. The mechanisms behind the effects of neurosteroids and the regulation of neurosteroid levels remain unclear. Vitamin D has recently been suggested as a neurosteroid and is believed to play a role in psychiatric as well as neurodegenerative disease, e g Parkinson's disease. The biologically active form of vitamin D, 1a,25-dihydroxyvitamin D_3, is formed through metabolic activation. We study enzymes and genes in activation and metabolism of vitamin D and the roles of the formed metabolites. The studies include cellular effects of vitamin D and vitamin D analogues. Our studies using cultured cells and animal studies, are directed to understand the processes of enzymatic actions and gene regulation that affect cellular steroid levels and steroid hormone signaling in the brain. Regulators of interest include endogenous compounds as well as drugs e g SERM (selective estrogen receptor modulators).

(II) Mechanisms behind adverse drug effects on bone health: roles of gene regulation in vitamin D homeostasis (Christine Wegler, Kjell Wikvall and Maria Norlin)

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

Translational Pharmacokinetics/Pharmacodynamics

Margareta Hammarlund-Udenaes

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

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

Members of the group during 2013

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

Publications 2011-2013

1. Björkman S: Evaluation of the TCIWorks Bayesian computer program for estimation of individual pharmacokinetics of FVIII. *Haemophilia* **17:** e239-e240 (2011).
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9. Gillespie U, Morlin C, Hammarlund-Udenaes M, Hedstrom M. Perceived value of ward-based pharmacists from the perspective of physicians and nurses. *Int J Clin Pharmacy* **34**:127-135 (2012).
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13. Stevens J, Ploeger BA, Hammarlund-Udenaes M, Osswald G, van der Graaf PH, Danhof M, de Lange EC. Mechanism-based PK-PD model for the prolactin biological system response following an acute dopamine inhibition challenge: quantitative extrapolation to humans. *J Pharmacokin Pharmacodyn* **39**:463-477 (2012).
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16. Björkman S. Population pharmacokinetics of recombinant factor IX: implications for dose tailoring. *Haemophilia* **19**:753-7 (2013)
17. Björkman S. Pharmacokinetics of plasma-derived and recombinant factor IX - implications for prophylaxis and on-demand therapy. *Haemophilia* **19**:808-13 (2013).
18. Björkman S. Comparative pharmacokinetics of factor VIII and recombinant factor IX: for which coagulation factors should half-life change with age? *Haemophilia* **19**:882-6 (2013).
19. Gillespie U, Alassaad A, Hammarlund-Udenaes M, Mörlin C, Henrohn D, Bertilsson M, Melhus H. Effects of pharmacists' interventions on appropriateness of prescribing and evaluation of the instruments' (MAI, STOPP and STARTs') ability to predict hospitalization-analyses from a randomized controlled trial. *PLoS One* **8**:e62401 (2013).
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4. Hammarlund-Udenaes M, Brouwer K, Nakashima E, Terasaki T. Perspectives on a pharmacokinetics legend: C versus T (contributions over time). *J Pharm Sci*. **102**:2889-94 (2013).

Discussion articles and blogs 2013

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

Agencies that support the work/Funding 2013

Janssen Pharmaceuticals
Swedish Research Council
AstraZeneca

Other commitments/assignments of staff members 2013

Margareta Hammarlund-Udenaes: Associate Editor of the Journal Pharmaceutical Research, Editorial Advisory Board member of the Journal of Pharmaceutical Sciences and of Fluids and Barriers of the CNS.

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

Projects

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

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

Neuropharmacokinetic parameters such as unbound brain-to-plasma concentration ratio (Kp,uu,brain), unbound volume of distribution in brain (Vu,brain), and permeability clearance into the brain (CLin), are descriptors of BBB function and intracerebral distribution established by the group. Kp,uu,cell is another unique parameter characterizing the extent of cellular barrier transport. These parameters are identified by means of systematic PKPD analyses.

For quantitative evaluation of BBB transport we are using cerebral microdialysis, recognized as the "gold standard" for measurement of unbound drug concentration in the brain. An alternative strategy is based on combination of high-throughput in vitro techniques, such as brain homogenate equilibrium dialysis and brain slices.

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

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

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

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

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

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

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

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

diseases and other pathological conditions associated with BBB impairment.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

(IX) Clinical pharmacokinetics of coagulation factors VIII and IX (Sven Björkman in collaboration with Erik Berntorp, Jan Astermark, Karin Lindvall, Malmö, Peter Collins, Cardiff, Kathelijn Fischer, Utrecht, and Victor Blanchette, Toronto). This project has during 2013 been taken over by Siv Jönsson and Elisabet Nielsen at the Department of Pharmaceutical Biosciences

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

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

Undergraduate Teaching 2013

During 2013 the teachers have devoted extra time to the evaluation of the Master of Science in Pharmacy program (Högskoleverkets utvärdering av apotekarprogram). The background documentation was collected from all teachers and summarized by the two program coordinators, Emma Lundkvist and Ronnie Hansson, and the vice dean with responsibility for undergraduate teaching. In addition, the teachers were involved in the revision of the two pharmacy programmes. The work is led by a group consisting of members from the three departments and from our department Ingrid Nylander and Lena Klarén (Maria Swartling) participate. Finally, two teachers devoted time to improvement of the bachelor project in the Bachelor of Science in Pharmacy programme. The evaluation of this programme pointed out weaknesses in the outcome of the project and the faculty gave an assignment to Anne-Lie Svensson and Jörgen Bengtsson to work with quality improvement measures with the aim to increase the quality of the bachelor project.

The major part of the undergraduate teaching is within the two Pharmacy programmes. During 2013 the extent of undergraduate teaching was 429 *hst* (full-time equivalents) and that represents 42% of the total number of hst within the Faculty of Pharmacy. In addition, the department is involved in teaching at the Master of Science in Chemical Engineering with specialization in drugs and the Biomedical programme. Students attending internet-based courses during 2013 comprised 81 hst.

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

Teaching at the basic level

The main teaching is within the Bachelor of Science in Pharmacy programme that comprises three years studies (180 hp). Completed studies at the programme provide the necessary theoretical and practical competence that is required to apply for a pharmacist license as *receptarie*. The teachers also instruct in undergraduate projects (15 hp) at the basic level. These projects are individual and they are examined by an oral presentation and a written report. A new course, Drug abuse and addiction, started and attracted many participants. The course is open for students from all disciplines at the Uppsala University and the aim is to increase the basic knowledge about drugs of abuse and of addiction. During 2013, the teachers within the department supervised 72% (31 students) out of the total number of undergraduate projects within the Bachelor of Science in Pharmacy programme.

Teaching at the advanced level

The department gives courses at the Master of Science in Pharmacy programme that comprises five years studies (300 hp). Completed studies at the programmes provide the necessary theoretical and practical competence that is required to apply for a pharmacist

license as *apotekare*. The teachers instruct in undergraduate projects (30 hp) at the advanced level. These projects are individual and are examined by an oral presentation and a written report. The projects are laboratory-based and involve the student in ongoing research projects. During 2013, the teachers supervised 60% (70 students) of the total number of undergraduate projects within the Master of Science in Pharmacy programme.

The teachers lead and teach a number of elective courses and single subject courses at the advanced level. These courses mirror research profiles within the department, such as Bioinformatics, Clinical pharmacy, Drug metabolism and safety, Drug addiction and Pharmacokinetics. The courses attract a large number of students, not only pharmacy students but also other students showing the proper prerequisites. During 2013, 69% (286 out of 416 students) of the students within the Master of Science in Pharmacy programme and 58% (50 out of 86 students) of the students within the Bachelor of Science in Pharmacy programme participated in courses given by the department.

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

Uppsala May 2014

Ingrid Nylander

Course List 2013

List of courses on basic and advanced levels (first and second cycles)

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

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

Research Education 2013

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

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

Many PhD students also perform important tasks in undergraduate teaching, comprising around 20 % of their time, making the time for a PhD exam be around 5 years in total. The teaching assignments give the students a broader knowledge base than their own thesis area. It also gives them leadership and communication skills that are of importance in further professional life. PhD students may also tutor master students in experimental research work. During 2013 there were 57 PhD students registered, and 11 of these defended their theses during the year.

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

Uppsala April 2013

Margareta Hammarlund-Udenaes

Awards and Appointments 2013

1. Mathias Hallberg was awarded Uppsala Universities Pedagogical Prize (category Medicine and Pharmacy)
2. Mathias Hallberg was awarded The Special Award for “Studentbemötande” from the Pharmacy Student Union
3. Richard Goodwin. AstraZeneca Innovative Medicines Global Science Awards - Post-Doc of the Year. Development of innovative of mass spectrometry imaging techniques for whole body and intact body tissue sections.