UPPSALA UNIVERSITET

Department of Medical Cell Biology 2008/24 1:1

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2007

Avsändare/Fastställd av Institutionsstyrelsen 2008-04-03

Introduction

When living in times of electronic publishing one feels somewhat old-fashioned in writing an introduction to a printed version of our annual report. This type of information is already available on the home page of the department and then often in more sophisticated forms than the printed version allows for. Visiting that site, www.mcb.uu.se, you will find amongst other things 1; the so called "prefekt infos" delivered roughly every second months with information/remarks from the chairman on the activities at the department 2; all institutional board protocols 3; the annual budget 4; scientific reports from the research teams 5; links to the publication basis of the University – OPUS for the most updated information on all publications released from the department 6; announcements of seminars, dissertation defences 7; more detailed personnel information including members of different committees at the department 8; undergraduate teaching information etcetera. I would like to take this opportunity to acknowledge the splendid work of our web master, Martin Blixt, who constantly has been engaged in keeping it updated and of interest to all of us.

In one of these "prefektinfos" of last year I brought up the issue of the skewed demography of our employee. Twelve out of 33 persons (PhD students and postdocs excluded) with permanent positions were born 1945 or earlier. This in turn means that more than every third of our employees will retire within two years. How should we handle this situation? We have taken the advice of the faculty board ad notam to replace retired professors with "forskarassistenter". By such means we have now recruited three persons (Mia Phillipson, Fredrik Palm and Anca Dragomir), which means that we at present have got a total of six "forskarassistenter". We are glad to see that today the Swedish Research Council pays for three of them. But at the same time we have to keep in mind the steadily increasing tasks in undergraduate teaching, which is not that easy because almost all "lektorer" have become professors. In order to compensate for that we are at present recruiting two "universitetsadjunkter" for the daily teaching in anatomy and cell biology. Probably, there are more to come.

Another demographic change we are facing these days is that the number of PhD-students is decreasing. Last year seven PhD students defended their thesis but only four new students were recruited. This decrease seems to be on purpose since research education has become fairly complicated and also expensive – at our department less than half of the costs for one PhD student is paid for by the governmental funding. In general, the research teams seem to favour the idea of offering the new doctors a postdoc period after their thesis defence. By such means a higher efficiency can be achieved just by maintaining each individual for a longer time period in the lab.

One recurrent issue in these "prefektinfos" last year was KoF-07, the evaluation of research at Uppsala University. A lot can and has been said about this event but this is not the place to repeat the arguing. We are glad that Anders Tengholm was recognized as one of the "golden eggs" at the university and then awarded by the committee taking the final decisions.

Economy is also an interesting/depressing issue and especially so in times with decreasing support from the government. In an enterprise with personnel costs being more than half of the expenses it is of course detrimental when you have a less than 1% compensation for the salary increases, which have been at least 3% over the last years. When also faculty rules were changed in a negative way for us – why should a PhDstudent with a pharmacy diploma be twice as valuable for a pharmacy department as compared with one educated at our department?- we had a period when different savings had to be used. We ended up with a deficit of 3000 ksek, which

was not a surprise. This year we have to cut costs and with some retirements and also decreased costs for renting our laboratories – we are moving from four into three newly renovated corridors at Biomedicum – a balance should be reached. But still a prerequisite for that is that we can go on being successful in raising external grants for our research.

Finally, a few words on our undergraduate teaching. We have now welcomed four courses on the medicine programme with the new curriculum and we have gathered some experiences. No doubt, it is both challenging and rewarding to try new pedagogical methods. Some of us have clearly heard students asking for more traditional lectures and at least I myself have experienced that the lecture halls have been more filled up than previously and also the students more alert. As the chairman of the department, I have, however, seen the problem of finding people experienced enough for the group teaching. In the long run we cannot use our professors for these tasks. It is quite obvious that the increased work load in undergraduate teaching and its planning has, for a few persons, meant a decrease in scientific production.

I have already, in my "prefektinfos", congratulated all our prize-winners but I take this opportunity to do so once again. No one retired from the department during last year – the "release" will come this year. The administrative staff, Agneta, Marianne, Kärstin and Göran has done a magnificent job and I thank you all.

Uppsala March 30, 2008-03-30

Arne Andersson, Chairman

List of Contents

Organization

Chairman
Arne Andersson

Deputy chairmen
Erik Gylfe, (Director of graduate studies)
Örjan Källskog, (Director of undergraduate studies)

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(from July, 2005)
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Lisbeth Sagulin

Scientific Reports

Islet transplantation

Arne Andersson, Leif Jansson, Per-Ola Carlsson

The research of the group is mainly focused on the vasculature of the pancreatic islets and its relation to islet endocrine function during normal and diabetic conditions and after transplantation. The endothelial cells, which line all blood vessels, are important not only to distribute nutrients and oxygen to the islets, nut also to produce mediators which are involved in the regulation of hormone release, cell growth and the blood perfusion through the islets. Furthermore, endothelium-derived substances are likely to modulate the pathogenesis of both type 1 and type 2 diabetes. Much of our research within the last years have been devoted to the adaptation of transplanted islets of Langerhans (which contain the insulin-producing beta-cells) to the implantation organ, i.e. the so-called engraftment process, and how this may be affected by different conditions in the recipients. Such transplantations are performed also in humans, but the long-term results are disappointing, probably due to impaired engraftment. Our studies in this area include evaluations of the revascularization processes (with special emphasis on the circulatory physiology of the newly formed intra-graft blood vessels), reinnervation, growth and differentiation of the beta-cells and, finally, the ultimate specific function of the graft. Special attention is paid to the endothelial cells of the islets both before and after transplantation. In this context, we compare islets implanted into different organs of the recipients (under the renal capsule, into the spleen, muscle or liver) with corresponding endogenous islets within the pancreas. All these studies are made in animal models but some of the studies are also carried out on

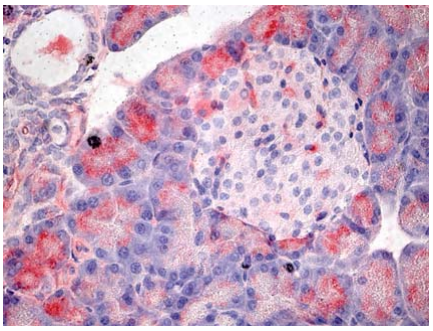

Figure: An islet of Langerhans stained with the lectin Bandeiraea simplicifolia that selectively stains blood vessels.

human islets transplanted into nude mice. The aim of the latter studies is to improve the outcome of human islet transplantations, by applying the knowledge gained from the experimental models. We also perform basic research, in collaboration with a group in Trondheim, Norway, on the possibilities to encapsulate isolated islets of Langerhans with different alginates, with the aim to prevent rejection of transplanted islets.

Another line of research on the islet vasculature is focussed on the regulation of pancreatic islet blood flow during normal conditions and in type 2 diabetes. We have found pronounced changes in the latter group, suggestive of an endothelial dysfunction, which seems to be related to the disturbed glucose and lipid homeostasis. Our working hypothesis is that disturbances in islet blood perfusion may modulate the development of type 2 diabetes, which is in line with the well known defects in endothelial cell function seen in diabetes. We have recently also initiated studies on the relation between white adipose tissue and the pancreatic islets, especially in experimental type 2 diabetes. So far we have found that there are marked disturbances also in white adipose tissue blood flow, which seem to mirror those in the islets, and we are at present investigating the possible connections between these findings.

Members of the group

Arne Andersson - Professor

Birgitta Bodin - Technician

Sara Bohman - Graduate Student

Carina Carlsson - Assistant Professor

Per-Ola Carlsson - Associate Professor

Leif Jansson - Professor

Ulrika Pettersson - Graduate Student

Joey Lau - Graduate Student

Johan Olerud - Graduate Student

Johanna Henriksnäs - Post Doc

Åsa Johansson - Graduate Student

Astrid Nordin - Laboratory Engineer

Richard Olsson - Post Doc

Monica Sandberg – Post Doc

Lisbet Sagulin -Technician

Eva Törnelius – Technician

Publications 2005-2007

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Agencies that support the work

The Swedish Research Council

The Swedish Diabetes Association

The Gunvor & Josef Ane’rs foundation

The Family Ernfors Foundation

Juvenile Diabetes Research Foundation

EFSD

Mechanisms of pancreatic β-cell dysfunction delineated by protein expression profiling

Peter Bergsten

Progression from health to disease is multi-factorial where environmental and genetic factors alter expression of many genes. Given the close relation between protein expression and cellular function, we are focusing on expression measurements at the protein level. In addition, when measuring at the protein level the biologically important post-translational modifications (PTMs) can be determined. In order to dissect complex disease processes, methods capable of separating, quantifying and identifying large number of proteins are required. In our laboratory several proteomic approaches including two-dimensional gel electrophoresis (2-DGE), matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), surface enhanced laser desorption/ionization (SELDI) TOF MS and, in collaborative arrangements, liquid chromatography Fourier transform ion cyclotron resonance (LC FT-ICR) MS are used for these purposes.

The obtained differences in expression of multiple identified proteins are bioinformatically analyzed. The analysis yields differentially expressed proteins, which are mapped onto signal transduction pathways and protein interaction databases. The proteomic measurements and subsequent analysis of the expression data sets give information about proteins, signaling pathways and highly interactive proteins specifically altered during disease progression.

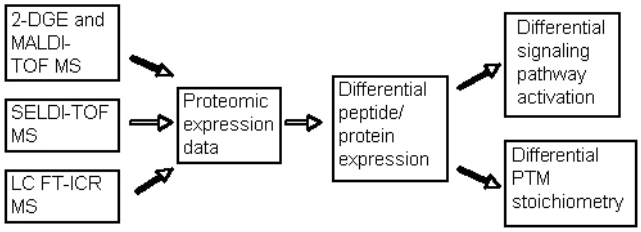

Figure 1. Identifying differentially expressed proteins

The proteomics- and bioinformatics-based information is used to generate hypotheses about mechanisms of disease development, which are tested in various animal and cellular models. Below are examples of some current projects, which were derived from the approach.

Members of the group

Peter Bergsten – professor

Johanna Westerlund – associate professor

Meri Hovsepyan – postdoctoral person

Hanna Nyblom – postdoctoral person

Ernest Sargsyan – postdoctoral person

E-ri Sol – graduate student

Tea Sundsten – graduate student

Kristofer Thörn – graduate student

Publications 2005-2007

1. Ortsäter H, Alberts P, Engblom LOM, Warpman U Abrahmsén L and Bergsten P. Regulation of 11b-hydroxysteroid dehydrogenase type 1 and glucose-stimulated insulin secretion in pancreatic islets of Langerhans. Diabetes/Metabolism Research and Reviews 21:359-366, 2005.
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Agencies that support the work

The Swedish Research Council

The Swedish Diabetes Association

The EFSD/MSD

The Swedish institute

Type 2 diabetes mellitus: Are elevated levels of apolipoprotein CIII causing pancreatic β-cell dysfunction?

Development of type 2 diabetes mellitus (T2DM) depends on both environmental and genetic factors. In an attempt to delineate genetic factors contributing to impaired pancreatic β-cell function, blood protein profiles were generated from individuals with or without family history of diabetes and with differences in β-cell function. Among the differentially displayed plasma proteins, apolipoprotein CIII was elevated in individuals with family history of diabetes and low b-cell function (13; see publications below). To investigate if the elevated levels of the apolipoprotein contributed to β-cell dysfunction β-cells were exposed to the apolipoprotein in vitro, which resulted in increased apoptosis. Different signaling pathways including MAPK are currently investigated for a role in this apolipoprotein CIII mediated enhanced b-cell apoptosis.

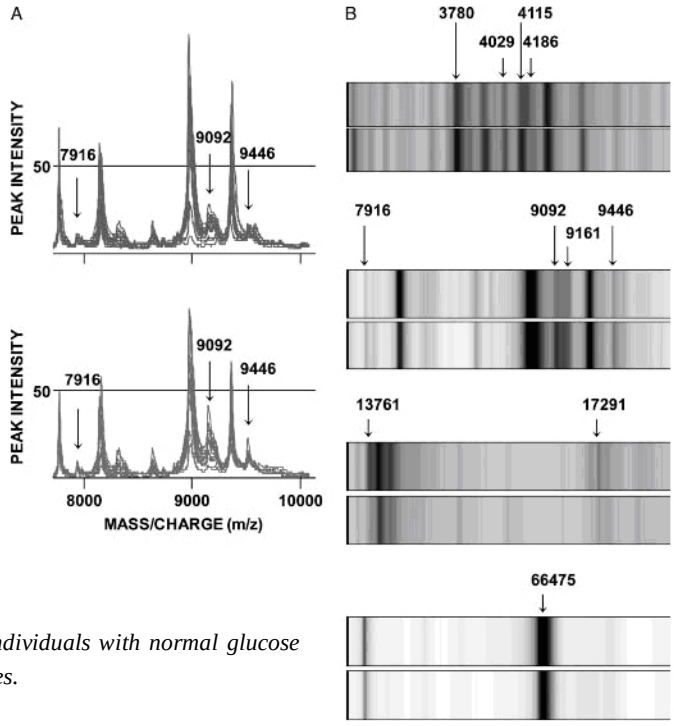

Figure 2. SELDI-TOF mass spectra from individuals with normal glucose tolerance and individuals with type 2 diabetes.

Type 2 diabetes mellitus: Is improved pancreatic b-cell function by "β-cell rest" mediated by lowered ER-stress?

T2DM is characterized by rising blood glucose and lipid levels, which impair pancreatic β-cell function. In individuals with T2DM improved glucose-stimulated insulin secretion has been observed after exposure to diazoxide. The compound hyperpolarizes the cell thereby inhibiting release of insulin. When isolated islets cultured in the absence or presence of elevated glucose or fatty acid levels were protein profiled, expression of chaperone proteins were altered (2,8). Such alterations are connected with endoplasmic reticulum (ER) stress. We hypothesized that diazoxide-induced improvement of b-cell involved lowering of ER stress. When isolated islets and b-cell lines were exposed to fatty acid palmitate, activation of the PERK signaling pathway of the unfolded protein response (UPR) was observed including enhanced expression of CHOP. When diazoxide was included in the culture medium, CHOP expression was reduced.

Type 2 diabetes mellitus: Why is deletion of the gene encoding fatty acid desaturation improving pancreatic β-cell function?

In T2DM fatty acid levels are elevated. Saturated but not unsaturated fatty acids are harmful for the β-cell. The conversion of saturated to unsaturated fatty acids is catalyzed by the enzyme stearoyl-CoA desaturase 1 (SCD1). Surprisingly, when this gene is disrupted, the individual handles fatty acid loads efficiently and becomes resistant to weight gain. To delineate mechanisms of this "protective effect", SCD1 was knocked down (KD) in b-cells. Protein profiles were obtained from such SCD1 KD cells and compared with profiles of control cells.

Among the identified differentially expressed proteins, proteins involved in protein synthesis were up-regulated in the SCD1 KD cells. Current experiments aim at determining to what extent over-expression of the specifically up-regulated genes in control cells serve a protective function against exposure to elevated levels of saturated fatty acids.

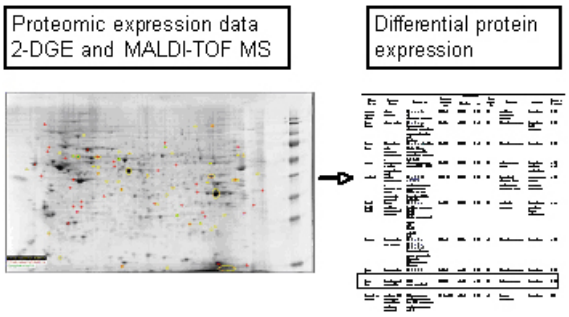

Figure 3. Identification of palmitate-regulated b-cell proteins.

Type 2 diabetes mellitus: Why are saturated but not unsaturated fatty acids detrimental to pancreatic β-cells?

Whereas saturated fatty acids have severe effects on pancreatic b-cell function including elevated apoptosis, unsaturated fatty acids have only mild effects. Novel mechanisms for this difference were delineated by protein profiling of β-cells cultured in the absence or presence of saturated fatty acid palmitate or unsaturated fatty acid oleate. Differentially expressed proteins were identified (10). We observed that palmitate but not oleate induced marked down-regulation of a protein with anti-apoptotic properties. We hypothesized that the preferential induction of apoptosis by the saturated fatty acid depended on the lowered levels of this protein. The hypothesis is currently addressed by over-expressing the protein in cells, which will subsequently be exposed to palmitate.

Type 2 diabetes mellitus: What proteins and signaling pathways are altered in islets from persons with type 2 diabetes mellitus?

Most hypotheses about pancreatic β-cell dysregulation stem from research conducted in β-cell lines or rodent islets. The relevance of these findings for human health and disease critically depends on verification in human islets. To obtain hypotheses about mechanisms of β-cell dysfunction in T2DM using islets obtained from individuals with the disease we made methodological amendments allowing protein profiling of as little as 100 islets. Human islets obtained from healthy and T2DM donors through collaboration with an islet transplant center were used to generate proteomic expression data sets (12). Differential activation of signaling pathways was identified e.g. in β-cell apoptosis, which will be the basis for work aiming at verifying these observations.

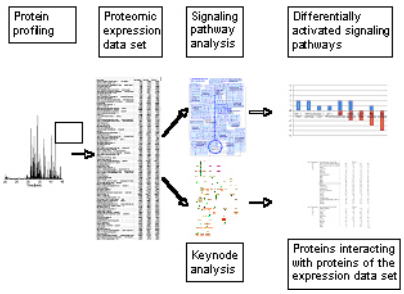

Figure 4. Identification of activated pathways and keynodes in islets obtained from individuals with T2DM.

Type 1 diabetes mellitus: Are proteins important for islet proliferation expressed in relation to hyperglycemia?

It was recently discovered that individuals with type 1 diabetes mellitus (T1DM) have insulin-positive cells. The cell number is very limited and need to be expanded significantly to meet insulin demands, however. The mechanisms for b-cell proliferation are poorly understood. In an attempt to delineate such mechanisms, islets from 3 month-old ob/ob-mice were used. At this age some animals were severely hyperglycemic and others only mildly hyperglycemic. We hypothesized that islets from animals with mild hyperglycemia were more proliferative compared to islets obtained from animals with accentuated hyperglycemia. Indeed, when stained for proliferation islets from the mildly hyperglycemic mice had more Ki67-positive cells than islets

from mice with accentuated hyperglycemia (11). To identify novel mechanisms for islet proliferation protein profiles of islets with marked proliferation were compared with islets with none or little proliferation. Several proteins involved in protein synthesis were down-regulated in islets from the severly hyperglycemic mice. Current experiments address to what extent the reduced levels of these proteins are important to islet proliferation.

Type 1 diabetes mellitus: What signaling pathways are differentially activated in engrafted and non-engrafted islets grafts?

In individuals with T1DM islet transplantation successfully normalizes blood glucose levels in a majority of cases. Five years after transplantation only 10% of the recipients have functional grafts, however. A major cause for graft failure is inadequate engraftment. Engraftment is the process when the graft is re-vascularized, re-innervated and cells are rearranged. To improve the percentage of surviving grafts islets transplanted under the kidney capsule were harvested 1, 4 and 24 weeks after transplantation. After 1 week engraftment has only started and there is extensive remodeling and necrosis/apoptosis within the graft. At 4 weeks engraftment is completed and islets have settled down. At time 16-24 weeks islet grafts that have successfully been engrafted. Harvested grafts were protein profiling and analysis revealed activation of pathways hitherto not connected with engraftment.

Physiology of pancreatic islet hormone secretion

Erik Gylfe, Anders Tengholm

Diabetes is widespread disease with rapidly increasing prevalence currently affecting >5 % of the world population. Diabetes is primarily due to insufficient or absent secretion of the blood glucose-lowering hormone insulin resulting in elevated blood glucose and glucose in the urine. Even if the acute symptoms of diabetes can be reversed by different therapies there are long-term complications like heart disease, stroke, kidney disease, eye complications with blindness, skin problems, nerve damage causing foot complications, gastrointestinal and sexual dysfunction.

Type 2 diabetes, which preferentially affects adult individuals, is the most common form and accounts for more than 90% of all diabetes. Type 2 diabetes is primarily characterized by insufficient insulin secretion from the pancreatic beta cells. Current therapy aims at maintaining or improving the secretory capacity of the beta cells and increasing the insulin sensitivity of the target organs. Elucidation of the mechanisms underlying insulin secretion and the malfunctions causing type 2 diabetes is expected to provide new strategies for restoring insulin secretion.

Type 1 diabetes mainly affects young individuals. It is a more severe disease than type 2 diabetes, since the beta cells are destroyed by an autoimmune attack. Apart from the lack of insulin, increased secretion of the blood glucose-elevating hormone glucagon contributes to rise of blood glucose in diabetes. Another dysfunction is that glucagon secretion is not appropriately stimulated when blood glucose falls to very low levels, as sometimes happens in insulin-treated type 1 diabetic patients. Clarification of the mechanisms underlying the failure of low glucose to stimulate glucagon release and the paradoxical hypersecretion of glucagon at high blood glucose may reduce acute illness and death after over-injection of insulin and help to prevent high blood glucose.

Members of the group

Helene Dansk -Research engineer

Oleg Dyachok – Postdoctoral fellow

Eva Grapengiesser - Associate professor

Erik Gylfe - Professor

Bo Hellman - Professor

Olof Idevall Hagren – Graduate student

Lisen Kullman - Assistant professor

Ing-Marie Mörsare - Technician

Jenny Sågetorp – Graduate student

Anders Tengholm - Associate professor

Geng Tian – Graduate student

Anne Wuttke – Graduate student

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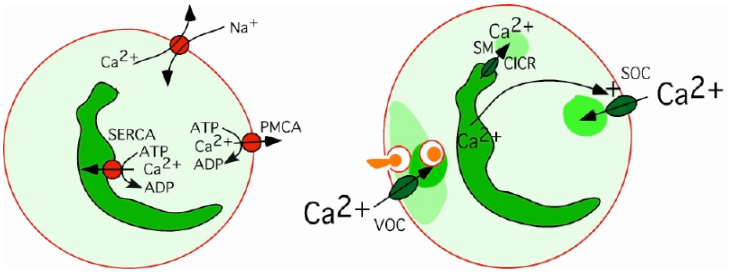
The Swedish Diabetes Association

Novo Nordic Foundation

Swedish Institute

Processes important for the role of Ca^{2+} as a universal cellular messenger

Ca^{2+} is a universal messenger that controls a variety of cell functions, including secretion. In most secretory cells rise of the cytoplasmic Ca^{2+} concentration stimulates secretion. However, the parathyroid cell is an exception to this rule, and we have shown that cytoplasmic Ca^{2+} is an inhibitory messenger for parathyroid hormone secretion.



Under basal conditions the cytoplasmic Ca^{2+} concentration is about 10 000-fold lower than the extracellular concentration. This low concentration is maintained by the activity of a Ca^{2+}-pumping ATPase (PMCA) and a Na^{+}/Ca^{2+} exchange mechanism in the plasma membrane. There is also a Ca^{2+}-pumping ATPase in the endoplasmic reticulum (SERCA). Activation of voltage-operated Ca^{2+} channels (VOC) results in influx of Ca^{2+} through the plasma membrane and a prominent rise of cytoplasmic Ca^{2+}. This is the major mechanism explaining the release of blood glucose-regulating hormones (orange). Intracellular messengers like inositol trisphosphate (IP3) and cyclic ADP ribose (cADPr) acting on specific receptors can also release Ca^{2+} from the endoplasmic reticulum. These receptors are also sensitive to Ca^{2+} itself causing Ca^{2+}-induced Ca^{2+} release (CICR). When the Ca^{2+} content of the endoplasmic reticulum decreases there is activation of store-operated Ca^{2+} influx in the

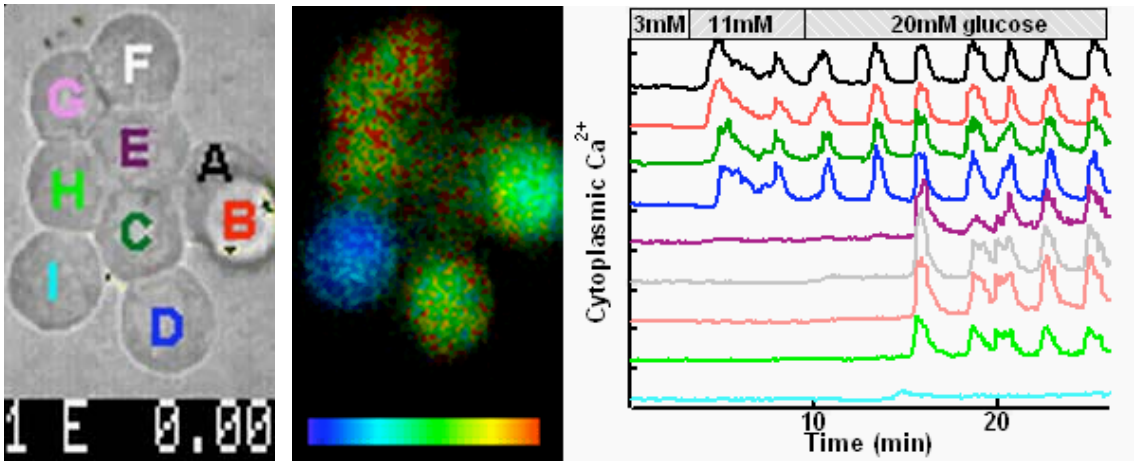
plasma membrane (SOC). We study all these aspects of Ca^{2+} signalling and their importance for hormone release and other physiological processes.

Selected publications

1. Sakwe AM, Rask L, Gylfe E. 2005. Protein kinase C modulates agonist-sensitive release of Ca2+ from internal stores in HEK293 cells overexpressing the calcium sensing receptor. J Biol Chem 280:4436-41.
2. Thore S, Dyachok O, Gylfe E, Tengholm A. 2005. Feedback activation of phospholipase C via intracellular mobilization and store-operated influx of Ca2+ in insulin-secreting beta-cells. J Cell Sci 118:4463-71.

Generation of pulsatile insulin secretion

The universal Ca^{2+} messenger is the main trigger of insulin secretion from pancreatic beta cells. Measuring the cytoplasmic Ca^{2+} concentration in individual cells we discovered that betacells have an endogenous rhythmic activity. Synchronization of the Ca^{2+} signals leads to pulsatile secretion of insulin, which is believed to be important for maintaining the sensitivity to the hormone in the target tissues. This project intends to clarify how the rhythmic signals are generated and currently focuses on defining the role of intracellular Ca^{2+} stores and store-operated entry of Ca^{2+} into the beta cells.



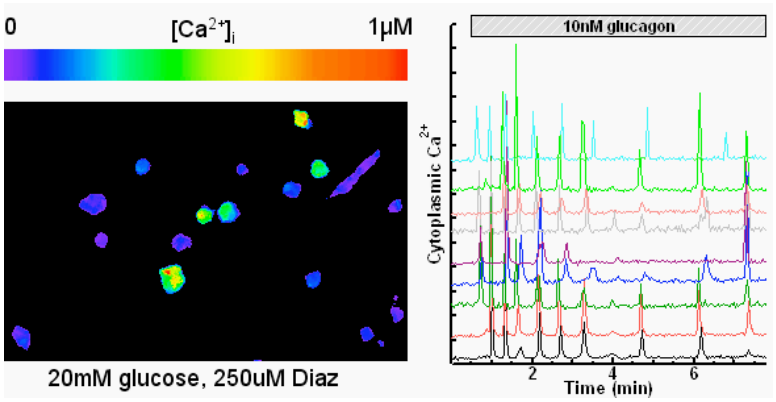
This experiment shows the effect of glucose on the cytoplasmic Ca^{2+} concentration in a cluster of 9 mouse b-cells (A-I). The cells are initially exposed to a non-stimulatory glucose concentration (3 mM). After elevation of glucose to 11 mM, pronounced slow Ca^{2+} oscillations occur in cells A-D due to periodic opening of voltage-dependent L-type Ca^{2+} channels. The oscillations propagate among the neighbouring cells by gap junctions and become synchronized. After further elevation of glucose to 20 mM another 4 cells (E-H) start oscillating and all active cells become synchronized. It is apparent that oscillations can start in different cells. These synchronized Ca^{2+} oscillations underlie pulsatile insulin release. The experiment supports the recruitment theory, implying that pulsatile insulin release increases in amplitude at higher glucose concentrations due to recruitment of an increasing number of beta cells from the resting to the active phase.

Selected publications

1. Hellman B, Gylfe E, Grapengiesser E, Dansk H, Salehi A. 2007. Insulinoscillationer – en kliniskt betydelsefull rytmik. Läkartidningen 104:2236-2239.
2. Larsson-Nyrén G, Grapengiesser E, Hellman B. 2007. Phospholipase A2 is important for glucose induction of rhythmic Ca2+ signals in pancreatic beta cells. Pancreas 35:173-179.

Synchronization of pulsatile insulin secretion among millions of pancreatic islets

Beta cells in close contact synchronize the oscillatory Ca^{2+} signals for insulin release by gap junctions. We have recently found that beta-cells communicate also in the absence of physical contact via diffusible factors. Similar molecules may participate in neural co-ordination of the oscillatory Ca^{2+} signaling underlying pulsatile insulin secretion from the pancreas.



This experiment shows physically separated pancreatic beta cells exposed to a stimulatory glucose concentration (20 mM) to promote Ca^{2+} sequestration in the endoplasmic reticulum (ER). However the cells are also exposed to the hyperpolarizing drug diazoxide, which prevents the potential-dependent slow Ca^{2+} oscillations typically observed in glucose-stimulated beta cells. After introduction of glucagon, to increase cAMP, pronounced Ca^{2+} transients occur in the cells due to inositol 3,4,5-trisphosphate-mediated mobilization of Ca^{2+} from the ER. Note that these transients rapidly propagate among the separated cell resulting in striking synchronization. ATP and NO/CO released from the beta cells are strong candidates as humoral factors causing this synchronization. Similar factors released from an intrapancreatic neuronal network may initiate regenerative Ca^{2+} signals in the different pancreatic islets resulting in co-ordination of the slow Ca^{2+} oscillations among all islets in the pancreas. Such coordination is required to explain pulsatile insulin release from the pancreas.

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1. Hellman B, Jansson L, Dansk H, Grapengiesser E. 2007. Effects of external ATP on Ca2+ signalling in endothelial cells isolated from mouse islets. Endocrine 2007 32:33-40.
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3. Larsson-Nyrén G, Grapengiesser E, Hellman B. 2007. Phospholipase A2 is important for glucose induction of rhythmic Ca2+ signals in pancreatic beta cells. Pancreas 35:173-179.
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5. Grapengiesser E, Salehi A, Qader SS, Hellman B. 2006. Glucose induces glucagon release pulses antisynchronous with insulin and sensitive to purinoceptor inhibition. Endocrinology 147:3472-7.

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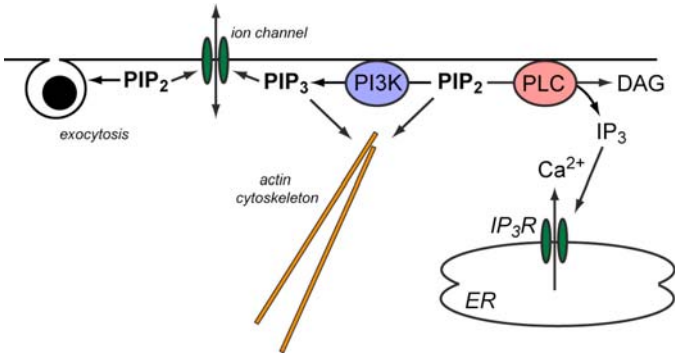
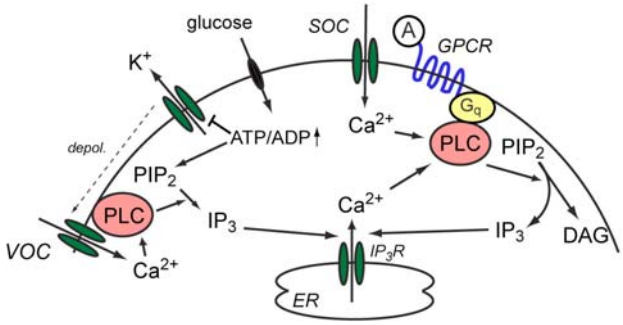
Signalling via plasma membrane phosphoinositides



Phosphatidylinositol 4,5-bisphosphate (PIP2) is a minor membrane component of eukaryotic cells constituting ~1% of the phospholipids in the inner leaflet of the plasma membrane. Nevertheless, the phospholipid plays important roles in the regulation of a variety of cell functions, including insulin secretion (Figure 1). For example, PIP2 serves as precursor for the messenger molecules inositol-1,4,5-trisphosphate (IP3) and diacylglycerol (DAG) generated upon activation of phospholipase C (PLC), as well as for phosphatidylinositol-3,4,5-trisphosphate (PIP3) generated by phosphoinositide-3-kinase (PI3-kinase). IP3 mobilizes Ca^{2+} from intracellular stores and DAG is important for activation of protein kinase C. Moreover, PIP2 and PIP3 regulate ion channel activity, proteins involved in the organization of the cytoskeleton and trafficking of vesicles in endo- and exocytosis. All these events influence the insulin secretory process.



To monitor changes in the concentrations of PIP2 and PIP3 in the plasma membrane we use evanescent wave microscopy and fluorescent biosensors based on GFP fused to isolated protein domains with high binding selectivity for the lipid of interest. Our studies have demonstrated that PIP2 undergoes rapid turnover and that its concentration is determined by cytoplasmic Ca^{2+} and the ATP/ADP ratio (Figure 2). Glucose stimulation of beta cells is associated with Ca^{2+}-dependent activation of PLC and oscillations of Ca^{2+} due to voltage-dependent influx is translated into oscillations of PLC activity. Also receptor-triggered PLC activity depends on Ca^{2+}, with strong positive feedback exerted by Ca^{2+} released from the ER and entering the cell through store-operated Ca^{2+} channels (Figure 2).

We have also demonstrated that glucose stimulation of beta cells results in pronounced oscillations of plasma membrane PIP3 concentration. This effect reflects co-activation of PI3-kinase by glucose and secreted insulin.

Work in progress focuses on the role of oscillatory phosphoinositide signals and on the regulation of phosphoinositide turnover by lipid kinases and phosphatases.

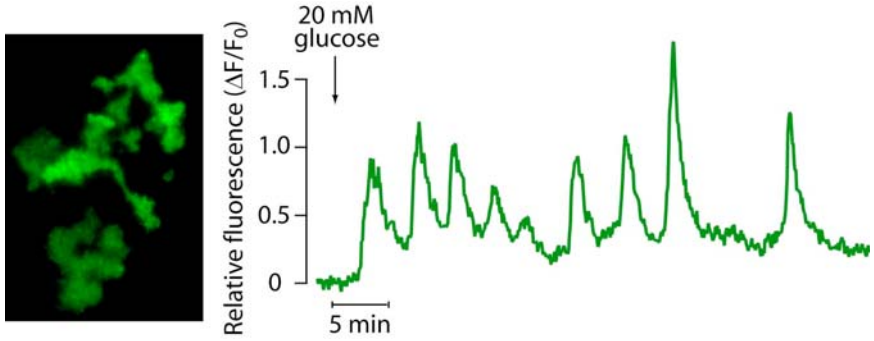


Figure 3 shows PIP3 oscillations induced by elevation of the glucose concentration from 3 mM to 20 mM in an individual insulin-secreting MIN6-cells expressing a biosensor based on a PIP3-binding protein domain conjugated to GFP.

Selected publications

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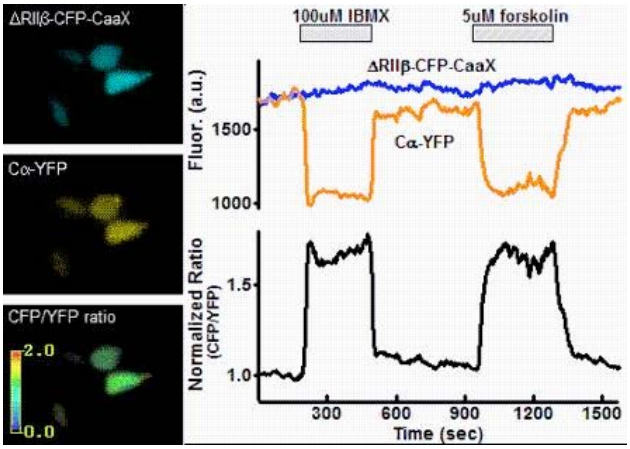
2. Idevall Hagren O, Tengholm A. 2006. Glucose and insulin synergistically activate PI3-kinase to trigger oscillations of phosphatidylinositol-3,4,5-trisphosphate in beta-cells. J Biol Chem 281:39121-7.
3. Thore S, Dyachok O, Gylfe E, Tengholm A. 2005. Feedback activation of phospholipase C via intracellular mobilization and store-operated entry of Ca2+ in insulin-secreting beta-cells. J Cell Sci 188:4463-71.

Spatio-temporal dynamics of cAMP signals

Cyclic AMP is a prototype second messenger that transduces signals from a variety of cell surface receptors to multiple intracellular targets. In pancreatic beta cells, cAMP strongly enhances insulin secretion by potentiating Ca^{2+}-dependent exocytosis. cAMP formation is catalyzed by adenylyl cyclases and the degradation mediated by phosphodiesterases. Protein kinase A (PKA) and cAMP-dependent guanine nucleotide exchange factors are the major cAMP effectors in beta cells. Little is known about the kinetics of cAMP signals. The lack of information stems from the difficulty to measure cAMP in individual living cells. We have recently developed a method that allows recording of cAMP concentration changes in the sub-plasma membrane space of individual cells. The technique is based on fluorescent protein-tagged PKA subunits, modified so that the catalytic subunit undergoes translocation to or from the plasma membrane upon changes in cAMP concentration. Fluorescence is selectively detected from a small volume adjacent to the membrane using evanescent wave microscopy. This approach allowed us to demonstrate that stimulation of beta cells with glucagon and glucagon-like peptide-1 (GLP-1) often triggered cAMP oscillations. We have also shown that different temporal patterns of cAMP signals could contribute to selective regulation of downstream events. Brief elevations of cAMP were sufficient to trigger Ca2+ spikes, but only prolonged cAMP elevation induced PKA translocation into the nucleus. The aim of ongoing work is to understand how the concentration of cAMP is regulated in beta cells by nutrients, hormones and neurotransmitters, and how the spatio-temporal pattern of the messenger is involved to control beta cell function.

This experiments demonstrates sequential increases of cAMP in individual insulin-secreting beta cells after inhibition of phosphodiesterases with IBMX and activation of adenylyl cyclases with forskolin. cAMP was monitored by measuring fluorescence in the submembrane space by evanescent-wave-microscopy. The blue fluorescence comes from cyan fluorescent protein fused to the regulatory subunit of PKA. Yellow fluorescent protein was fused with the catalytic subunit of PKA. Rise of cAMP triggers dissociation of the regulatory and catalytic subunits. Since the catalytic subunit was anchored to the plasma membrane the blue fluorescence remains membrane-associated whereas there is a loss of yellow florescence as the catalytic subunit diffuses into the cytoplasm. The black trace shows the plasma membrane-associated blue/yellow fluorescence ratio as a measure of cAMP.



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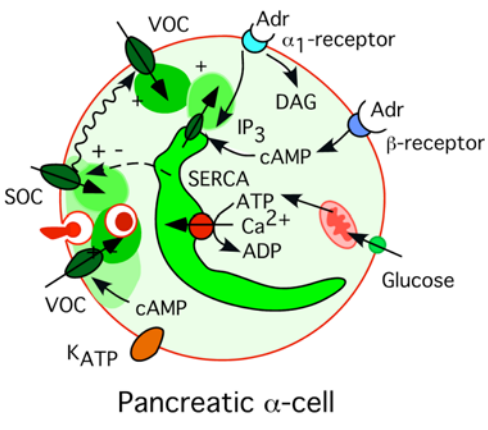
2. Dyachok O, Isakov Y, Sågetorp J, Tengholm A. 2006. Oscillations of cyclic AMP in hormone-stimulated insulin-secreting β-cells. Nature 439:349-52.
3. Dyachok O, Sågetorp J, Isakov Y, Tengholm A. 2006. cAMP oscillations restrict protein kinase A redistribution in insulin-secreting cells. Biochem Soc Trans 34:498-501.

Mechanisms controlling the release of glucagon, somatostatin and pancreatic polypeptide

In diabetes there is not only an impaired secretion of insulin but poor regulation of blood-glucose elevating glucagon contributes to the hyperglycemia underlying diabetes complications. Pancreatic polypeptide is another islet hormone of potential importance for blood glucose regulation by effects on gastric emptying. The fourth islet hormone somatostatin is a potent inhibitor of the release of the other hormones and probably has a paracrine function. Other paracrine event in the islets involve insulin-promoted inhibition of glucagon secretion and glucagon-potentiated insulin secretion. We were first to study Ca^{2+} signaling in all islet cell types and found that pulsatile release of the different hormones can be explained by Ca^{2+} oscillations. Recently we have proposed a new model for regulation of glucagon secretion.



Model for adrenaline stimulation and glucose inhibition of glucagon secretion. Adrenaline acts on alpha1- and beta-adrenoceptors activating Ca^{2+} release from the endplasmic reticulum (ER) and store-operated followed by voltage-dependent entry of Ca^{2+} leading to glucagon release. Glucose shuts off this stimulatory cascade by promoting Ca^{2+} sequestration in the ER.

The studies of glucagon secretion led to the unexpected discovery that glucose not only inhibits secretion but that high concentrations of the sugar have a paradoxical stimulatory effect. This phenomenon may explain why diabetic hyperglycaemia is often aggravated by inappropriate hyperglucagonemia. Glucose dependence of glucagon, insulin and somatostatin secretion from mouse islets. Note that glucagon secretion is maximally inhibited by 7 mM glucose, which stimulates somatostatin release but has little effect on insulin secretion. Glucose concentrations above 20 mM stimulated the release of all 3 hormones in parallel.

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Characterization of the Shb knockout mouse with particular reference to the function of hematopoietic cells, the vasculature, beta cells and oocyte maturation

Michael Welsh

We have previously characterized the Shb adapter protein. Shb is ubiquitously expressed and downstream of several tyrosine kinase receptors, such as VEGFR-2, the PDGF receptors, FGFR-1 and the T cell receptors. In vitro studies have suggested pleiotropic effects of Shb in survival, differentiation, cell migration and proliferation with particular reference to angiogenesis, T cell function and beta cell function. We have recently generated the Shb knockout mouse and note that it is viable when maintained on a mixed genetic background, although the knockout allele is not inherited by Mendelian genetics. A transmission ratio distortion was observed that could result from altered oocyte maturation. Our ongoing project aims at characterizing the Shb knockout mouse with respect to hematopoietic cells, endothelial cells, beta cells and oocytes.

Members of the group

Michael Welsh - Professor

Gabriela Calounova - Post-Doc

Björn Åkerblom - PhD-student

Nina Funa - PhD-student

Guangxiang Zhang – PhD-student

Ing-Britt Hallgren – Laboratory Engineer

Publications 2005-2007

1. Rolny, C., Lu, L., Ågren, N., Nilsson, I., Roe, C., Webb, G. C., Welsh, M. SHB promotes blood vessel formation in embryoid bodies by augmenting vascular endothelial frowth factor receptor-2 and platelet-derived growth factor beta-receptor signaling. Exp Cell Res, 308:381-93, 2005

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Agencies that support the work

Juvenile Diabetes Research Foundation International

The Swedish Research Council

The Swedish Cancer Foundation

The Swedish Diabetes Association

Complications in pregnancy

Ulf Eriksson

We are studying different types of pregnancy complications, such as preeclampsia, which affects both mother and child, and disturbed embryo-fetal development as a consequence of altered maternal metabolism (caused by diabetes, obesity, or ethanol intake). The short-term aim is to clarify and understand the mechanisms and patterns of damage; the long-term aim is to prevent the maternal and fetal damage. We work with animal models *in vivo*, and *in vitro* culture of embryos, tissues and cells.

Preeclampsia, which affects more than 5 % of all pregnant women, is characterized by hypertension in the mother and growth retardation in the offspring. In severe cases may the lives of both the mother and offspring be threatened. We have created and studied an animal model of preeclampsia and attempted to diminish the negative consequences of the disease by treatment with large doses of antioxidants.

Diabetes in the pregnant women is associated with an increased risk for congenital malformations. We have studied the mechanisms behind the disturbed development of the offspring in animal models, embryo culture, as well as by *in vitro* culture of embryonic tissues and cells. In earlier work, we reported the occurrence of oxidative stress in embryos exposed to a diabetic environment. We have been able to block the diabetes-induced damage to the embryo and fetus by several agents, such as arachidonic acid, inositol, N-acetylcysteine, BHT, vitamin E and C, and folic acid. We have also attempted to investigate the importance of genetic predisposition for the development of malformations, a project which is currently very active and yielding data dissecting the importance of the maternal and fetal genomes and epigenomes for the development of fetal dysmorphogenesis in diabetic pregnancy.

Obesity in the pregnant woman is associated with increased risk for congenital malformations, in particular the risk for neural tube defects and cardiac malformations been found to be increased. We are currently involved in creating an animal model for this type of pregnancy, as well as attempting to affect embryonic development *in vitro* by subjecting the embryos and embryonic cells to fatty acids and other lipid compounds.

Intake of ethanol during pregnancy can harm the offspring; the risk increases with increased consumption. We have studied this situation, and attempted to alter the maternal defense against free oxygen radicals *in vivo* and *in vitro*, in order to diminish the ethanol-induced damage. We

are currently studying possible biomarkers for maternal ethanol intake, by investigating embryonic tissues.

Members of the group

Ulf Eriksson, professor

Parri Wentzel, docent

Andreas Ejdesjö, graduate student

Sheller Zabihi, graduate student

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1. Placental dysfunction in Suramin-treated rats – a new model for pre-eclampsia.
   Nash P, Wentzel P, Lindeberg S, Naessén T, Jansson L, Olovsson M & Eriksson UJ
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2. Folic acid supplementation diminishes diabetes- and glucose-induced dysmorphogenesis in rat embryos in vivo and in vitro.
   Wentzel P, Gäreskog M & Eriksson UJ
   Diabetes 54: 546-553, 2005.

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   Wentzel P & Eriksson UJ
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   Wentzel P & Eriksson UJ
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14. Placental growth factor and soluble FMS-like tyrosine kinase-1 in early-onset and late-onset preeclampsia.
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Obstet Gynecol 109: 1368-1374, 2007.

15. Exposure of neural crest cells to elevated glucose leads to congenital heart defects, an effect that can be prevented by N-acetylcysteine.
Roest PAM, Van Iperen L, Vis S, Wisse LJ, Poelmann RE, Steegers-Theunissen RPM, Molin DGM, Eriksson UJ & Gittenberger-de Groot AC
Birth Defects Res A Clin Mol Teratol 79: 231-235, 2007.

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Eriksson UJ
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Reviews 2005-2007

1. Offspring of diabetic pregnancy.
Persson B, Eriksson UJ & Hanson U
In: Diabetology of Pregnancy, Djelmis, J (Ed), Karger, Basel, 2005, pp. 288-309.

2. Mechanisms of diabetic embryopathy.
Eriksson UJ
Diabetes & Pregnancy 5:10-16, 2005

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Eriksson UJ & Wentzel P
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4. Claes Hellerström: a friendly islet explerer.
Andersson A, Eriksson UJ, Jansson L, Sandler S, Welsh M & Welsh N
Diabetologia 50: 496-450, 2007.

5. Teratologi
Eriksson UJ & Wentzel P
In: Obstetrik, Hagberg H, Marsal K & Westgren M (eds.), Studentlitteratur, 2007

Agencies that support the work

The Swedish Labour Market Insurance Company

The Ernfors Family Foundation

The Swedish Diabetes Association

Novo Nordic Foundation

Pathogenesis of type 1 Diabete Mellitus

Stellan Sandler

The prevailing view is that an autoimmune reaction selectively destroys the insulin-producing β-cells in the pancreas in type 1 diabetes (T1DM). The aim of this project is to investigate cellular and molecular mechanisms involved in pancreatic b-cell damage and repair in this disease. We postulate that after certain types of damage β-cell function can be restored (Fig. 1). Furthermore, we believe that the β-cell is not a passive victim during a situation of potentially harmful exposure, but depending on gene expression and functional activity of the β-cell, the outcome can be affected. The aims of the present research projects are to investigate cellular and molecular mechanisms involved in pancreatic β-cell damage and repair in T1DM.

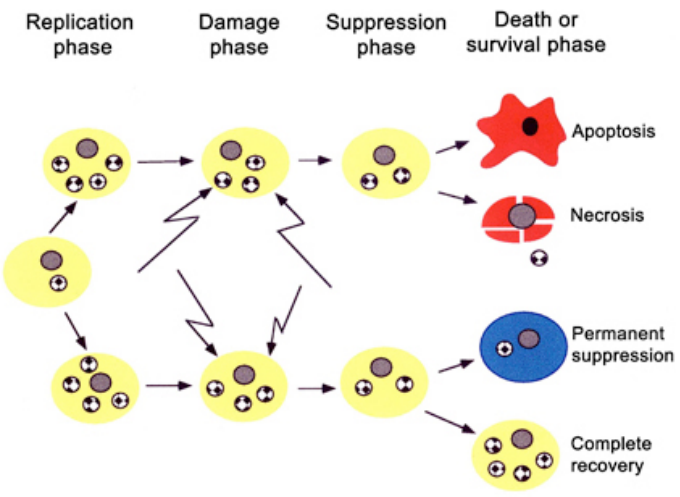

Fig. 1. Schematic view of the β-cell outcome following different immunologic or toxic assaults. In fetal and neonatal life, β-cell replication is increased, but later it becomes restricted. After birth β-cells acquire the full capacity to synthesise and release insulin (speckled symbols) upon appropriate stimuli. At one or several occasions in life, β-cells in some individuals are subject to damage (irregular arrows) which will lead to suppressed β-cell function and a reduction in insulin secretion. Depending on the genetic predisposition an autoimmune reaction will be launched which in certain individuals will cause extensive cell death leading to type 1 diabetes. In other individuals β-cells will survive, but their secretory function is impaired, which may have consequences for the glucose homeostasis. In some other individuals the β-cells may completely recover and the glucose tolerance will only be transiently disturbed. The latter outcome is most likely also dependent on genes regulating β-cell resistance to damage and β-cell repair.

It is anticipated that a deeper knowledge of these issues will lead to new strategies for intervention in the autoimmune β-cell destructive process in T1DM, as well as methods to enhance β-cell resistance against cytotoxic damage. We hope that by studying cytokine-induced cell signaling and the mechanisms leading to β-cell death, we will be able to elucidate which

factors that are crucial for β-cell survival and possibly identify candidate genes/proteins conferring β-cell susceptibility or resistance to destruction in T1DM.

Members of the group during 2007

Stellan Sandler - Professor

Martin Blixt - Graduate student

Andreas Börjesson - Graduate student

Ingbritt Hallgren - Laboratory technician

Jenny Larsson - Post-doc, part time

Ivana Cvetkovic - Post-doc, part time

Eva Ludvigsen - Post-doc, part time

Bo Niklasson - Adjunct professor

Tobias Rydgren - Post-doc

Lina Thorvaldsson - Post-doc

Recent publications

1. Ludvigsen E, Olsson R, Stridsberg M, Janson ET and Sandler S.Expression and distribution of somatostatin receptor suntypes in the pancreatic islets of mice and rats. J Histochem Cytochem 52: 391-400, 2004
2. Andersson AK, Thorvaldson L, Carlsson C and Sandler S.Cytokine-induced PGE2 formation is reduced from iNOS deficient murine islets.Mol Cell Endocrinol 220: 21-29, 2004
3. Andersson AK, Börjesson A, Sandgren J and Sandler S.Cytokines affect PDX-1expression, insulin and proinsulin conversion secretion in islets from iNOS deficient murine islets.Molec Cell Endocrinol 240: 50-57, 2005
4. Ludvigsen E, Stridsberg M, Janson ET and Sandler S. Expression of somatostatin receptor subtypes 1-5 in pancreatic islets of normoglycaemic and diabetic NOD mice. Eur J Endocrinol 153: 445-454, 2005
5. Thorvaldson L, Johansson SE, Höglund P and Sandler S.Impact of plastic adhesion in vitro on analysis of Th1 and Th2 cytokines and immune cell distribution from multiple low-dose streptozotocin diabetes. J Immunol Meth 307: 73-81, 2005
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7. Rydgren T, Bengtsson D and Sandler S. Complete protection against interleukin-1b-induced functional suppression and cytokine-mediated cytotoxicity in rat pancreatic islets in vitro using an interleukin-1 cytokine trap.Diabetes 55:1407-1412, 2006
8. Niklasson B, Samsioe A, Blixt M, Sandler S, Sjöholm Å, Lagerquist E, Lernmark Å and Klitz W. Prenatal viral exposure followed by adult stress produces glucose intolerance in a mouse model.Diabetologia 49: 2192-2199, 2006
9. Börjesson A, Andersson AK and Sandler S Survival of an islet allograft deficient in iNOS after implantation into diabetic NOD mice.Cell Transplant 15: 769-775, 2006

10. Ludvigsen E, Stridsberg M, Taylor JE, Culler MD, Öberg K, Janson ET and Sandler S. Regulation of insulin and glucagon secretion from rat pancreatic islets in vitro by somatostatin analogues.Regulatory Peptides 138: 1-9, 2007

11. Blixt M, Niklasson B, Sandler S. Characterization of beta-cell function of pancreatic islets isolated from bank voles developing glucose intolerance/diabetes: an animal model showing features of both type 1 and type 2 diabetes mellitus, and a possible role of the Ljungan virus. Gen Comp Endocrinol 154: 41-7, 2007

12. Rydgren T, Vaarala O, Sandler S. Simvastatin protects against multiple low-dose streptozotocin-induced type 1 diabetes in CD-1 mice and recurrence of disease in nonobese diabetic mice. J Pharmacol Exp Ther. 323: 180-5, 2007

Agencies that support the work/Funding

The Swedish Research Council

The Swedish Diabetes Association

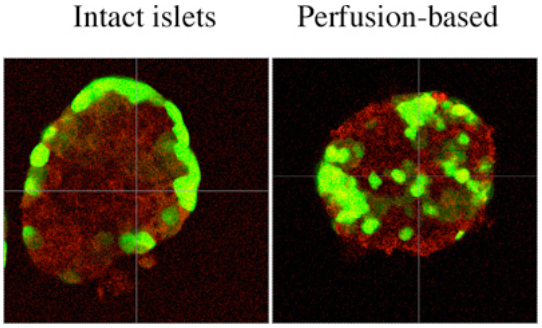
Novo Nordic Foundation

Current projects

- Evaluation of cytokine traps (hybride receptor molecules) in experimental T1DM.
- Novel KATP- channel openers (KCO) as rescue drugs during acute b-cell destruction and possible role of an ischemic preconditioning mechanism.
- T1DM development in mice transgenically overexpressing the SOCS-3 protein in b-cells.
- Cytokine gene expression during b-cell destruction in vivo by studying pancreatic islet grafts.
- Mechanism(s) of statin modulation in murine T1DM.
- Role of somatostatin receptor (sst) subtypes in diabetes models.
- Ljungan virus in development of diabetes in mice and bank voles.

Pancreatic β-cell research

Nils Welsh



Efficient transduction of islet cells

In this project we compare the efficency and safety of different adeno-, lenti- and AAV vectors for transduction of islet cells in vitro and in situ with the purpose to find the optimal gene delivery method for islet transduction purposes. The Figure below shows that GFP-expressing vectors reach only the outer cells of an intact islet when added in vitro (left panel), whereas using an in situ perfusion based protocol also centrally located cells are transduced.

Selected publications

1. Saldeen J, Curiel D, A. Andersson, Eizirik DL, Strandell E, Buschard K and Welsh N. Efficient gene transfer to human pancreatic islets in vitro using adenovirus/polylysine/DNA-conjugates or cationic liposomes. Diabetes, 45: 1197-1203 [1996].
2. Barbu AR, Akusjarvi G, Welsh N. Adenoviral-mediated transduction of human pancreatic islets: importance of adenoviral genome for cell viability and association with a deficient antiviral response. Endocrinology. 146:2406-14 (2005)
3. Hagerkvist R, Mokhtari D, Myers JW, Tengholm A, Welsh N. siRNA Produced by Recombinant Dicer Mediates Efficient Gene Silencing in Islet Cells. Ann N Y Acad Sci. 1040: 114-22 (2005)
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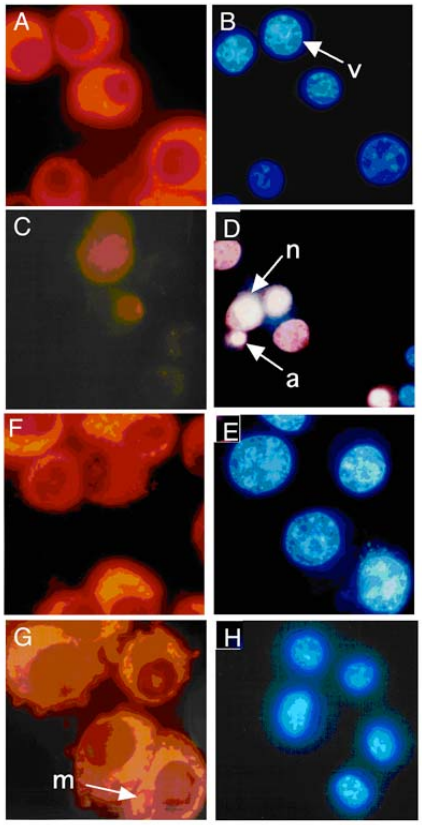
Genetic modification of beta-cells to prevent destruction by transplantation-induced stress or autoimmune reactions



In panels A-D insulin producing cells were transfected with a control vector and in panels E-H with a vector that promotes overexpression of the anti-apoptotic protein Bcl-2. In panels B, D, E and H cells were stained with bisbenzimide, which stains living cells blue, and propidium iodide, which stains dead cells pink. In panels A, C, F and G cells were stained with JC-1, which is a marker for a high mitochondrial membrane potential (m). Panels C, D, G and H are cells treated for 24 hours with a cytokine mixture. The figure shows that control cells die by apoptosis (a) and necrosis (n) in response to cytokines and that this is preceded by a loss of the mitochondrial membrane potential (m). In cells tranduced to overexpress Bcl-2, however, there is neither loss of mitochondrial membrane potential nor increased cell death. Other gene products that may influence beta-cell survival in diabetes and that are presently investigated are NF-kappaB, MIF-1, TGF-beta and FasL.

Selected publications

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2. Saldeen J, Sandler S, Bendtzen K and Welsh N. Liposome-mediated transfer of IL-1 receptor antagonist gene to dispersed islet cells does not prevent recurrence of disease in syngeneically transplanted NOD mice. Cytokine 12: (4) 405-408 (2000)

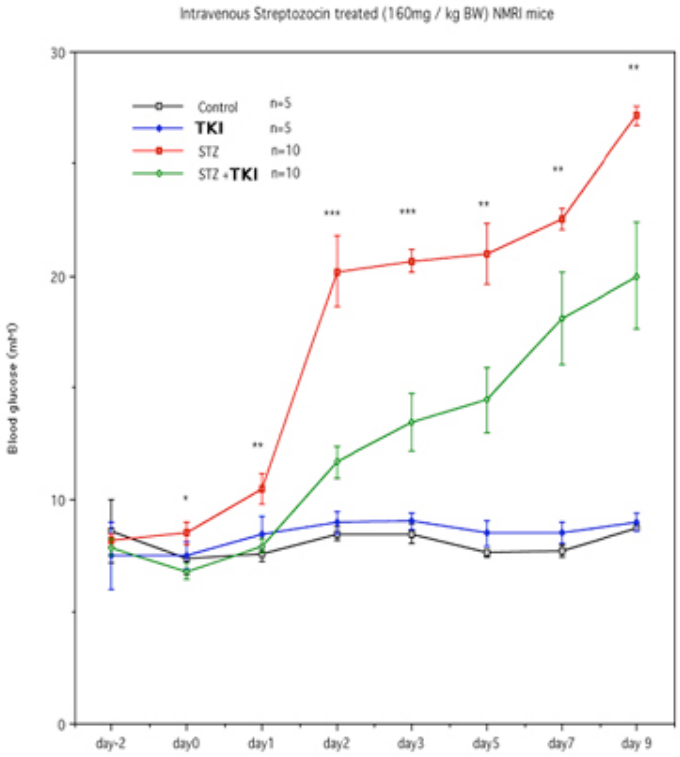
3. Barbu A, Welsh N, Saldeen J. Cytokine-induced cell death is preceded by disruption of the mitochondrial transmemebrane potential (DFm) in RINm5F cells:Prevention by Bcl-2. Mol Cell Endocrinol. 190, 75-82 (2002)

4. Welsh N, Makeeva N, Welsh M. Overexpression of the Shb SH2 domain-protein leads to altered signaling through the IRS-1 and IRS-2 proteins with consequences for phosphoinositide metabolism in insulin producing cells. Mol. Med. 8: 695-704 (2002).

5. Barbu A, Akusjärvi G, Welsh N. Adenoviral induced islet cell cytotoxicity is not counteracted by Bcl-2 overexpression. Mol. Med. 8(11): 733-741 (2002) Saldeen J, Curiel D, A. Andersson, Eizirik DL, Strandell E, Buschard K and Welsh N. Efficient gene transfer to human pancreatic islets in vitro using adenovirus/polylysine/DNA-conjugates or cationic liposomes. Diabetes, 45: 1197-1203 [1996].

Role of tyrosine kinases in beta-cell apoptosis

Tyrosine kinases seem to control beta-cell death and the tyrosine kinase inhibitor (TKI) Gleevec conteracts diabetes in both streptozotocin-injected mice (Figure right) and in NOD mice. It is the aim of this project to elucidate the mechanisms by which tyrosine kinases control beta-cell death.



Selected publications

1. Hagerkvist R, Makeeva N, Elliman S, Welsh N. Imatinib mesylate (Gleevec) protects against streptozotocin-induced diabetes and islet cell death in vitro. Cell Biol Int. 2006 Dec;30(12):1013-7

2. Hagerkvist R, Sandler S, Mokhtari D, Welsh N. Amelioration of diabetes by imatinib mesylate (Gleevec): role of beta-cell NF-kappaB activation and anti-apoptotic preconditioning. FASEB J. 2007 Feb;21(2):618-28

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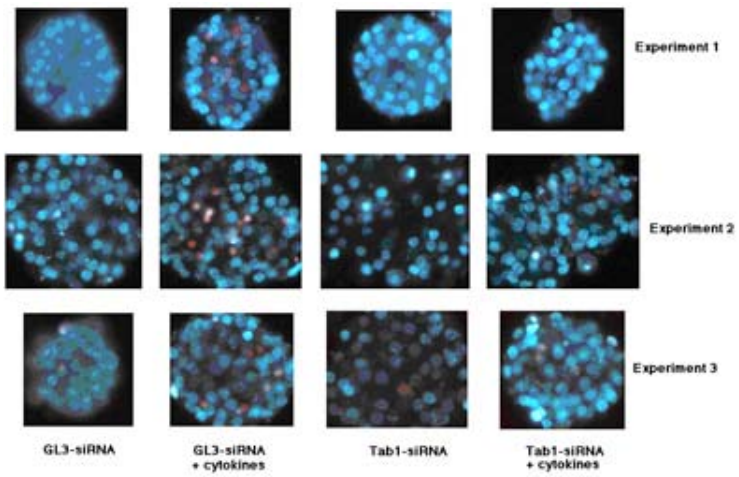
4. Robert Hägerkvist, Leif Jansson and Nils Welsh. Imatinib mesylate improves insulin sensitivity and glucose disposal rates in high-fat diet fed rats, Clinical Science, (Lond). 2008 Jan;114(1):65-71..

Role of p38 and JNK in beta-cell apoptosis

We have observed that both the two stress-activated MAP kinases p38 and JNK are activated in insulin producing cells in response to cytokines and nitric oxide. Furthermore, they seem to participate in beta-cell death as p38 down-regulation results in partial alleviation of the cytokine/nitric oxide-induced effect. Therefore, it is of great importance to better understand the mechanisms by which p38 and JNK are activated, and how these MAP kinases act in insulin producing cells. The figure below shows human islet cells treated with control (GL3) or Tab1-specific siRNA. Tab1 is a p38 activating protein and down-regulation of Tab1 results in partial protection against cytokines.



Selected publications

1. Welsh N. Interleukin-1b induced ceramide and diacylglycerol generation leads to activation of the Jun kinase and the transcription factor ATF2 in the insulin-producing cell line RINm5F. J. Biol. Chem, 271:8307-8312, [1996]
2. Saldeen J, Lee JC, Welsh N. Requirement of enhanced p38 mitogen-activated protein kinase activity in cytokine-induced apoptosis in rat islet cells in vitro. Biochemical Pharmacology, 61, 1561-1569 (2001)
3. Saldeen J, Welsh N. P38 MAPK inhibits JNK2 and mediates cytokine-activated iNOS induction and apoptosis independently of NF-kB translocation in insulin producing cells. European Cytokine Network 15: 47-52 (2004)
4. Welsh N, Cnop M, Kharroubi I, Bugliani M, Marchetti P, Eizirik DL. Is there a role for islet-produced interleukin-1 in the deleterious effects of high glucose to human pancreatic islets? Diabetes 54, 3238-3244 (2005).
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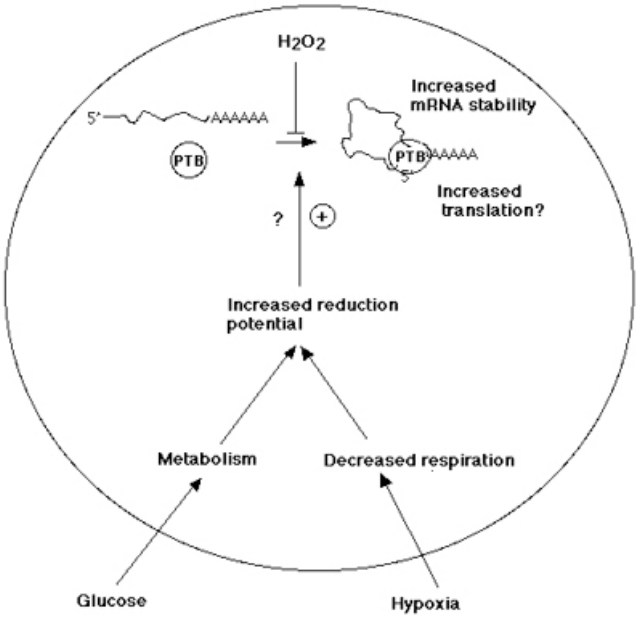
6. Makeeva N, Myers JW, Welsh N. Role of MKK3 and p38 MAPK in cytokine-induced death of insulin producing cells. Biochemical Journal 393, 129-139 (2006)
7. Makeeva N, Roomans G and Welsh N. Role of TAB1 in nitric oxide-induced p38 activation in insulin-producing cells. Int J Biol Sci. 2006 Nov 25;3(2):71-6.

8. Mokhtari D, Myers JW, Welsh N. The MAPK kinase kinase-1 is essential for stress-induced pancreatic islet cell death. Endocrinology. 2008 Feb 28; [Epub ahead of print]

Control of insulin mRNA stability by pyrimidine tract binding protein (PTB)

Most attempts to understand why insulin mRNA levels are decreased in diabetes have assumed a lowered transcription of the insulin gene. However, we have recently observed that insulin mRNA levels are mainly controlled by post-transcriptional mechanisms and that the 55 kDa pyrimidine tract binding protein (PTB) binds to the 3'-UTR of insulin mRNA. Hypoxia, glucose or mTOR stimulated this binding, and mutation of the core-binding site resulted in reporter mRNA destabilization. The over-all aim of this project is to understand how glucose regulates PTB activity in the control of insulin mRNA stability. This project might generate novel knowledge on the mechanisms behind decreased insulin production in certain types of diabetes.



Selected publications

1. Tillmar L, Carlsson C. Welsh N. Control of insulin mRNA stability in rat pancreatic islets: Regulatory role of a 3'-UTR pyrimidine-rich sequence. J. Biol. Chem. 277: 1099-1106 (2002)

2. Tillmar L, Welsh N. Hypoxia may increase insulin mRNA contents by stimulating the binding of polypyrimidine tract binding protein to the 3'-UTR of insulin mRNA. Mol. Med. 8:263-272 (2002)

3. Tillmar L, Welsh N. Glucose-stimulated binding of polypyrimidine-tract binding protein to the 3'UTR of insulin mRNA is inhibited by rapamycin. Mol. Cell. Biochem. 260: 85-90 (2004)

4. Tillmar L, Welsh N. Islet expression of the orphan endothelial cell tyrosine kinase receptor 1 is increased in response to hypoxia in vitro. Journal of the Pancreas. 5: 81-91 (2004)

5. Fred R and Welsh N. Increased expression of polypyrimidine tract binding protein in insulin-producing bTC-6 cells results in higher insulin mRNA levels. Biochem. Biophys. Res. Commun. 328: 38-42 (2005)

6. Fred RG, Tillmar L, Welsh N. The role of PTB in insulin mRNA stability control. Curr Diabetes Rev. 2006 Aug;2(3):363-6. Review.

Members of the group during 2007

Nils Welsh - Professor

Andreea Barbu – Post-doc

Dariush Mokthari – PhD-student

Rickard Fred – PhD student

Agencies that support the work

The Swedish Research Council

The Swedish Diabetes Association

Novo Nordic Foundation

The Knut & Alice Wallenberg Foundation

Renal function during hypertension and diabetes

Peter Hansell

The kidney is a main determinant of fluid/electrolyte balance and of mean arterial blood pressure. Hypertension is often caused by a renal inability to regulate fluid balance. The present research focuses on a matrix component (hyaluronan, HA) with extreme water binding properties in the regulation of fluid balance. In contrast to the renal cortex which is almost void of HA, the interstitium of the renal medulla contains high amounts of HA during normal physiological conditions which changes depending on the body hydration status. It is in the medulla where the main concentrating and diluting mechanisms occur. We have found that HA has an important dynamic role in normal renal water-handling (hydration/dehydration) and that the intrarenal distribution of HA is severely altered during diabetes and after ischemia-reperfusion injury which correlates to renal dysfunction and inflammation. The normal intrarenal distribution of HA is also severely altered if angiotensin II tonus is diminished neonatally (during nephrogenesis) in the rat which correlates to renal dysfunction and inflammation. We aim to: a) determine the physiological relevance of the glycosaminoglycan hyaluronan (HA) in the regulation of renal fluid/electrolyte balance; b) determine the pathophysiological relevance of HA in the renal dysfunction during diabetes (diabetic nephropathy) and after ischemia-reperfusion injury; c) determine if hyaluronidase-treatment and siRNA improves renal function during diabetic nephropathy and following renal ischemia-reperfusion; d) elucidate the time frame and mechanisms in the development of the intrarenal heterogenous distribution of HA which occur neonatally in the rat. Both in vivo and in vitro experiments are performed. Diabetes, ischemia, water loading and pharmacological treatment activate/deactivate the systems. In cooperation with the section of diagnostic radiology (assoc prof Per Liss) the mechanisms underlying diabetic nephropathy is studied and the increased sensitivity of the diabetic kidney to radiological contrast agents is elucidated. Cardiovascular disease is a dominant cause for invalidity and mortality. The results of the present projects may give rise to basic understanding of, and new treatment modalities in, fluid balance disorders and cardiovascular diseases.

Members of the group during 2007

Peter Hansell - Professor

Louise Rügheimer - Graduate Student

Malou Friederich – Graduate Student

Angelica Fasching - Laboratory Technician

Per Liss – Assoc Professor

Publications 2005-2007

1. Radiological contrast media and pancreatic islet blood flow in anaesthetized rats. Linder G, Carlsson PO, Källskog Ö, Hansell P, Jansson L & Källskog V. Acta Radiologica 2007; 48 (10): 1120-1124.
2. Hemodynamic effect of iopromide in pancreas-duodenum transplanted rats. Linder G, Carlsson PO, Källskog Ö, Hansell P, Jansson L & Källskog V. Acta Radiologica 2007; 48 (10): 1125-1130.
3. Hormonal Regulation of Renomedullary Hyaluronan. Rügheimer L, Johnsson C, Maric C & Hansell P. Acta Physiol (Oxford) 2007. In press.
4. Evaluation and comparison between visipaque (iodixanol) and hexabrix (ioxaglate) in coronary angiography. Persson PB, Liss P, Hansell P. J Am Coll Cardiol. 2007 Apr 17;49(15):1668-9; author reply 1669-70.
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6. Är de nya iso-osmolära röntgenkontrastmedlen mindre njurskadliga jämfört med de låg-osmolära? Liss P, Hansell P, Palm F, Lagerqvist B. Läkartidningen 2007, May;(20-21):1577.
7. Renal failure in 57,925 patients undergoing coronary procedures using iso-osmolar or low-osmolar contrast media. Liss P, Persson PB, Hansell P & Lagerqvist B. Kidney Int 2006 Nov;70(10):1811-7.
8. Prenatal exposure to interleukin-6 results in hypertension and alterations in the renin-angiotensin system. Samuelsson A-M, Alexanderson C, Mölne J, Haraldsson B, Hansell P, & Holmäng A. J Physiol 2006 Sep 15;575(Pt 3):855-67.
9. Lymphatic vessels in pancreatic islets implanted under the renal capsule of rats. Källskog Ö, Kampf C, Andersson A, Carlsson PO, Hansell P & Jansson L. Am J Transpl 2006 Apr;6(4):680-6.
10. Hyaluronan and renal ischemic damage. Hansell P, Rügheimer L., Johnsson C, Jacobson A, Heldin P, Hällgren R & Göransson V. In: Hyaluronan - Its Structure, Metabolism, Biological Activities and Therapeutic Applications, Editors: Endre A. Balazs and Vincent C. Hascall. Vol II, 2005, pp 747-750. Publishers: Winmar Enterprises. ISBN 0-9771359-0.
11. Nitric Oxide and Prostaglandins Influence the Renomedullary Hyaluronan Content. Rügheimer L, Johnsson C & Hansell P. In: Hyaluronan - Its Structure, Metabolism, Biological Activities and Therapeutic Applications, Editors: Endre A. Balazs and Vincent C. Hascall. Vol II, 2005, pp 773-776. Publishers: Winmar Enterprises. ISBN 0-9771359-0.

12. Influence of iothalamate on renal medullary perfusion and oxygenation in the rat. Liss P, Aukland K, Palm F, Carlsson PO & Hansell P. Acta Radiol. 2005 Dec;46(8):823-9.

13. CNS-induced natriuresis, neurohypophyseal peptides and renal dopamine and norepinephrine excretion in prehypertensive salt-sensitive Dahl rats. Sjöquist M, Lee SL & Hansell P. Exp Physiol 2005; 90(6):847-853.

14. Prolactin, a natriuretic hormone interacting with the renal dopamine system. Ibarra F, Crambert S, Eklöf A-C, Lundquist A, Hansell P & Holtbäck U. Kidney Int. 2005; 68(4): 1700-1707.

15. Pathophysiology of Contrast Medium Induced Nephropathy. Persson PB, Hansell P & Liss P. Kidney Int 2005; 68(1): 14-22.

16. The Effects of Carbon Dioxide versus Ioxaglate in the Rat Kidney. Palm F, Bergquist D, Carlsson PO, Hellberg O, Nyman R, Hansell P & Liss P. J Vasc Inter Radiol 2005 Feb;16(2):269-74.

17. The influence of intraarterial injection of CO2 and iodinated contrast media on renal function in patients undergoing renovascular intervention. A prospective randomized study. Liss P, Hellberg, O, Hägg A, Boström A, Löfberg Am, Olsson U, Örndahl P, Nilsson H, Hansell P, Eriksson LG, Bergquist D, Nyman R. J Vasc Inter Radiol 2005 Jan;16(1):57-65.

Agencies that support the work

The Swedish Research Council

Respiration Physiology

Marieann Högman

The primary aim of our research team is to study the endogenous production of nitric oxide (NO) as well as the effects of exogenous NO in the lung and airways. We have found that studying the NO molecule is quite intriguing, especially if the respiratory system is affected by disease, i.e. allergy, asthma, chronic obstructive pulmonary disease and adult respiratory distress syndrome. The goal of our research is to understand (a) the endogenous production of NO as a defense molecule and as an inflammatory marker of the airways and the nose cavity; and (b) the physiological effects of inhaled NO as a vasodilator, endogenous and exogenous in the lung. We have developed a method for diagnosis of airway diseases based on an algorithm using the exhaled NO at different breathing maneuvers. We have found an increase in airway NO in asthmatics together with an increase in diffusion rate from the airways of NO. This increase in diffusion rate was also found in persons with allergic rhinitis, without any symptoms from the lower airways. Interestingly persons with allergic rhinitis often develop asthma. In chronic obstructive pulmonary disease we found two distinct groups of patients. In the future we might be able to identify the difference between these patients like we today diagnose atopic and non-atopic asthma.

Inhaled NO has been studied in humans, rabbits, pigs and horses. We have a new delivery device that pulses NO in different parts of the inhalation gas. One fascinating finding is that pulse delivery will give a better match of ventilation and perfusion with greater increase in oxygenation

than the conventional method. We also study physiological effect during and after intensive care procedures.

Members of the group

Marieann Högman - Associate Professor

Birgitta Almgren – Post doc

Thomas Holmkvist - Graduate Student

Erkki Heinonen - Graduate Student

Andrei Malinovschi- Graduate Student

Publications 2005-2007

1. Kozlova I, Vanthanouvong V, Almgren B, Högman M, Roomans GM. Elemental composition of airway surface liquid in the pig determined by X-ray microanalysis. *Am J Respir Cell Mol Biol.* 2005; 32: 59-64.
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3. Kristjansson G, Högman M, Venge P, Hällgren R. Gut mucosal granulocyte activation precedes nitric oxide production. Studies in coeliac patients challenged with gluten and corn. *Gut* 2005; 54: 769-774.
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Renal Physiology

A. Erik Persson

For the fluid balance and blood pressure level in the body, the renal control of fluid excretion rate is essential. One factor of great importance in regulation of fluid excretion is the tubuloglomerular feedback (TGF) control mechanism. In the macula densa cells, located in the distal part of the nephron, the fluid flow rate is sensed. This information is used to activate the extraglomerular mesangial cells that modulate the response via influences from both hormones and fluid volume balance factors. Activation of the TGF mechanism finally leads to a contraction of the afferent arteriole. Renal renin release is controlled via the same mechanism.

Our group studies how hormones and other factors, e.g. nerves and NO, influence the overall function of the TGF mechanism and renin release using micropuncture techniques. We also employ isolated perfused tubule and arteriole techniques using fluorophores and digital imaging methods to determine calcium, chloride and NO in the macula densa cells and in the arteriolar smooth muscle cells. NO is also measured via microelectrodes. These techniques are used to investigate the sensing step in the TGF, the modulation step in the mesangial cells and the calcium release and contractile response of the arterioles. The juxtamedullary nephron preparation is used to visualise afferent arteriolar endothelial cells to measure calcium and NO. This method is employed to understand the important contribution of endothelial derived NO for renal function. We have also developed a model of hypertension with chronic treatment with 7-NI, a neuronal NO synthase inhibitor. The results of our studies aims at understanding how the TGF mechanism and renin release operates, the effect of renal NO and nerves on kidney function and the mechanism responsible for development of arterial hypertension.

Members of the group during 2007

A. Erik Persson - Professor

Mattias Carlström - Graduate Student

Johan Sällström - Graduate Student

Nils Wåhlin - Post Doc

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Agencies that support the work

The Swedish Research Council

Hjärt-Lungfonden

Gastro-intestinal protection mechanisms studied in vivo

Lena Holm

The gastrointestinal (GI) tract is being exposed to challenges, such as high gastric acidity and great amounts of bacteria and toxins in colon. We study the protective mucosal barrier (mucus and microcirculation) in an in vivo GI model, allowing direct access to the mucosa with intravital microscopy. Our focus is on barrier dysfunction and importance of bacteria in GI inflammation (gastritis and inflammatory bowel disease, IBD). Rats and mice, germ free or genetically modified are surgically prepared for intravital microscopy of the gastric or colonic mucosa. Mucus dynamics and pH at the epithelial cell surface are measured with microelectrodes. Blood flow, leukocyte-endothelial (L-E) and platelet-endothelial interactions are recorded. We have demonstrated that the adherent mucus gel in vivo can be divided in two layers, a firmly and a loosely adherent. We have shown that dietary nitrate, reduced to nitrite by bacteria in the oral cavity and further reduced to nitric oxide (NO) in the acidic stomach, is important in protecting the gastric mucosa against damaging agents and have indications that this is true even further down in the intestine. Earlier studies showed surprisingly low levels of L-E interactions in the superficial mucosal venules. This may be an important property given the very inflammatory nature of the gut contents. We now study mechanisms behind the activation of the mucosal endothelium resulting in L-E interactions and on-set of inflammation. The IBD models used are DSS and TNBS, resembling Ulcerative Colitis and Crohn's disease, respectively. Probiotics, prebiotics and different mechanisms proposed to have anti-inflammatory properties or in other ways protect the mucosa are or will be studied (NO, Prostaglandins, Protease Activated Receptors, adenosine receptors).

Members of the group during 2007

Lena Holm - professor

Annika Jägare - technician

Joel Petersson - graduate student

Mia Phillipson - assistant professor

Sara Rang - graduate student

Olof Schreiber - graduate student

Publications 2005-2007

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Agencies that support the work

The Swedish Research Council

Leukocyte-endothelial cell interactions

Mia Phillipson

The circulatory system not only transports oxygen and nutrients to tissues and washes away waste; it also transports thousands of millions white blood cells that circulate our body in search for alert signals. Traditionally leukocytes are associated with inflammatory conditions and clearing infections, but today we think they are important also for other physiological events such as cancer development and formation of new blood vessels.

The leukocyte recruitment cascade describes how leukocytes leave the circulation and enter tissue at the site of infection. We have recently identified a new step in this cascade in vivo,

called intravascular crawling. This step is crucial for the adherent neutrophils to get to optimal sites for emigration, the endothelial junctions. Our ongoing research aims at understanding how the intravascular crawling of leukocytes is regulated and the importance of the cross-talk between underlying endothelium and interacting leukocytes during angiogenesis as well as during inflammation.

Members of the group during 2007

Mia Phillipson - Assistant Professor

Gustaf Christoffersson - graduate student

Publications 2005-2007

(NB. M Phillipson was a post doctorate fellow at University of Calgary, Canada, 2005-2006)

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Agencies that support the work

Nanna Svartz fond

Harald och Greta Jeanssons stiftelse

Åke Wibergs stiftelse

Intravascular crawling to emigration sites

To understand the overall importance of the newly identified leukocyte-endothelial cell interaction, crawling, during pathophysiological events, we investigate the role of crawling during established inflammation (DSS induced colitis).We are also investigating how the direction of crawling is regulated, if it occurs along a gradient of chemokines expressed on the endothelium, what junctional molecules are responsible for guiding the neutrophil to emigration sites, and how the crawling leukocytes find the optimal sites for transmigration.

Dual functions of leukocytes – pancreatic islet graft angiogenesis and rejection

Transplantation of pancreatic islets is the only curative treatment of type 1 diabetes. Unfortunately, the long-term islet graft survival is poor, mainly due to low revascularization of the grafts. We recently developed a model where pancreatic islets are transplanted to the cremaster muscle. In this model we study the interplay between the endothelial cells lining the vasculature and the leukocytes in the blood in an attempt to understand and thereby try to improve the revascularization of the grafts.

Diabetic Nephropathy

Fredrik Palm

Diabetes mellitus is the most common cause for end-stage renal disease. The exact mechanisms mediating diabetes-induced kidney damage (diabetic nephropathy) are largely unknown despite intense research. The aim of this research program is to study effects of diabetes on renal metabolism and microcirculation in relation to functional changes. The ultimate goal is to find new treatment strategies to avoid the development of kidney dysfunction during diabetes.

We have recently observed a markedly decreased renal oxygenation in diabetic animals, and will now investigate mechanisms and importance of this for the development of diabetic nephropathy. Metabolic and functional alterations occurring in kidneys from diabetic animals (rats and genetically modified mice) are studied using in vivo techniques and molecular biology. Mitochondrial function and internal defence mechanisms are studied in diabetic animals and kidney tissue from diabetic patients. Renal blood flow and oxygen metabolism are studied using Magnetic Resonance Imaging (MRI) in animals as well as in diabetic patients.

By combining basic renal and diabetic research, we believe we can contribute to increase the understanding of the mechanisms involved in diabetic nephropathy which will facilitate development of novel therapies. Additionally, metabolic alterations always precede histological changes, which potentially can be used as a clinical diagnostic tool when identifying patients at increased risk to develop diabetic nephropathy. This would hopefully enable early treatment

modalities before the seemingly irreversible histological changes occur with manifest nephropathy.

Our results so far suggest:

A) Diabetic rats have reduced oxygen availability in the kidneys.
B) Reduced oxygen availability is mediated by increased oxidative stress and polyol pathway over-activity.
C) Increased oxygen utilization is mediated by increased mitochondrial uncoupling and NADPH oxidase activity.
D) Reduced NO levels, due to a reduction in plasma arginine as a result of increased hepatic metabolism, directly contributes to reduced oxygen availability in the diabetic kidney independently of hemodynamic alterations.

Members of the group during 2007

Fredrik Palm - Researcher, Ph.D.

Per Liss - Associate professor, MD, Ph.D.

Angelica Fasching - research engineer

Malou Friederich - Ph.D. student

Jenny Edlund - Ph.D. student

Publications 2005-2007

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Agencies that support the work

The Swedish Research Council

The Swedish Diabetes Association

National Institutes of Health/NIDDK

Fredrik och Ingrid Thurings stiftelse

Cystic Fibrosis

Godfried Roomans

Cystic Fibrosis (CF) is a congenital, hereditary disease with chronic obstructive lung disease and pancreatic insufficiency as main clinical symptoms. The disease is the most common lethal genetic disease in Sweden, and about 3% of the population carries the mutation for the defective gene. The disease is due to a defective chloride transport protein in the cell membrane of many epithelial cells, the CF transmembrane conductance regulator (CFTR). CFTR is a chloride channel that is activated by cyclic AMP. Clinically most important for CF patients is the defect in airway epithelium, where it results in viscous mucus. Similarly, clinical problems arise from defective chloride secretion in exocrine pancreas cells and intestinal epithelial cells. CFTR is also defective in sweat glands, and the resulting abnormally high Na and Cl content in sweat is used for diagnosis. In this project we study chloride secretion and the formation of airway surface liquid, in order to elucidate the connection between the genetic defect and the clinical symptoms. In addition, we use cultured epithelial cells to investigate pathways for chloride secretion, and we attempt to find compounds that may stimulate chloride secretion even in CF cells, in order to develop a pharmacological treatment of the disease. Currently, we are testing S-nitrosoglutathione, genistein and colchicines. In parallel, methods for gene therapy are being tested. We have developed minimally invasive methods to measure chloride secretion in nasal epithelial cells from CF patients, in order to be able to follow up the effects of pharmacological treatment or gene therapy in an objective way. Among the analytical methods used are electron probe X-ray microanalysis, a method for elemental analysis in the electron microscope, and localization of Ca and Cl ions by fluorescent probes.

Recent publications

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20. Nilsson H, Dragomir A, Ahlander A, Johannesson M, Roomans GM (2007) Effects of hyperosmotic stress on cultured airway epithelial cells. Cell Tissue Res **330**: 257-269.

Asthma and Allergy

Patients with asthma commonly display bronchial hyperreactivity and have extensive epithelial damage in their airways. We have recently investigated the inflammatory reaction in bronchial hyperactivity in patients with asthma or Sjögren's syndrome by electron microscopy and immunocytochemical techniques. Data from these studies indicate that there is a relationship between the inflammatory reaction and epithelial damage. An increased number of neutrophils and eosinophils in the airway wall is correlated with increased loss of columnar cells from the epithelium. We are therefore carrying out experimental studies to find out more about the mechanisms by which these granulocytes can damage the airway epithelium. We have found that products released by these cells, such as cytokines and polycations can cause generalized cell damage, but also specific changes in cell contacts such as tight junctions, desmosomes and cell attachment proteins. We are also investigating the effect of epithelial damage on fluid and ion homeostasis in the airway, and the effect that this can have on activation of mast cells and other cells that can play a role in the inflammatory process. Finally, we are extending the human studies to patients with allergic rhinitis, in collaboration with a group at the University of Riga.

Study group members

Godfried M. Roomans - Professor

Anders Ahlander - Research Engineer

Anca Dragomir – Post doc

Inna Kozlova – Graduate student

Harriet Nilsson – Graduate student

Zhanna Servetnyk – Graduate student

Leif Ljung - Senior Research Engineer

Marianne Ljungkvist - Research Engineer

Agencies that support the work

The Swedish Research Council

Hjärt-Lungfonden

Angiogenesis in childhood cancers

Rolf Christofferson

Angiogenesis, the formation of new blood vessels, is a tightly controlled and complex process involving several factors on the molecular and cellular levels with both stimulating and inhibiting steps, and is critical for tumor growth and metastasis. Inhibitors of angiogenesis are now being identified and today more than 17 angiogenic growth factors and their corresponding membrane receptors have been identified. Among the angiogenic growth factors, vascular endothelial growth factor A (VEGF-A) is probably the most important for the development, differentiation and maintenance of the vascular system. Specific inhibition of tumor induced angiogenesis should prevent growth of most types of solid tumors and thereby provide a novel approach for the treatment of cancer. Neuroblastoma is a childhood cancer that originates from immature neuroblasts in the peripheral nervous system. It is the second most common solid tumor in children. The tumors show considerable heterogeneity with respect to location, responsiveness to treatment and prognosis. The prognosis in infants and children with neuroblastoma is dependent on the age of the child and the clinical stage of the tumor at diagnosis. Since current therapy involves drugs with risk of serious side effects in the growing child, there is a clinical need for more effective and less toxic treatment strategies. In order to investigate the effects of angiogenic inhibitors in this disease, a new animal experimental model for human neuroblastoma was developed. This model is relevant, yields reproducible results and can be used for investigations of effects of angiogenic inhibitors, as well as of chemotherapy. When characterizing the model, we found that our experimental neuroblastomas express VEGF-A both at mRNA and protein levels, and that plasma VEGF-A levels correlates with tumor size. Several angiogenic inhibitors have been tested in the model: Avastin; a monoclonal VEGF antibody, SU5416; a novel antagonist for VEGFR-2, SU11657; a new orally available, synthetic small molecule multi-targeted tyrosine kinase inhibitor, CHS 828; a new cytotoxic drug, TNP-470; the synthetic analogue of fumagillin and furthermore, zoledronic acid; a bisphosphonate originally developed to reduce bone resorption showed to have more beneficial properties in patients with cancer apart from preventing bone resorption. The drugs gave a 31-94% reduction of the tumor growth rate. Thus, they could be valuable alone or in combination therapies in children with neuroblastoma. Most xenograft testing of anticancer agents, including those conducted in our laboratory between 1991 and 2003, has been done with tumors implanted s.c., an approach with a number of practical advantages (*e.g.*, the ease of measuring tumor volume). The biology of NB growing s.c. (a heterotopic site) may however differ from that of NB growing in an environment that mimics their site of origin. Such orthotopic ("in the right place") xenograft models are alternatives to s.c. model. Orthotopic xenografts models provide the most favourable condition for tumor progression and metastatic dissemination with more realistic tumor/stroma interaction, mimicking metastasic behavior and clinical responses to therapy. Thus, we have developed an orthotopic NB model in SCID (severe combined immunodeficiency) mice, which lack both T- and B-lymphocytes and therefore do not reject xenografted cells. MYCN-amplified human NB cells (IMR-32) were injected in the left adrenal medulla in these mice. All the mice developed an orthotopic NB in the adrenal gland. Currently, we are evaluating the effect of metronomic dosing with cytotoxic drugs on development of these tumors and the angiogenic index in the treated tumors compared to untreated controls. The results are promising. Our project will dissect the

biology and therapeutic intervention of metastasis in childhood cancers. New targets for restricting metastasis can be identified. New drugs, and new combinations of drugs and chemotherapy, will be evaluated with respect to their ability to inhibit angiogenesis, lymphangiogenesis, and metastatic spread and growth. We hope that our project improve survival and quality of life in children with cancer.

Recent publications

1. Svensson A, Azarbayjani F, Backman U, Matsumoto T and Christofferson R: Digoxin inhibits neuroblastoma tumor growth in mice: Anticancer research. *25 (1A)*: 207-212, 2005.

2. Segerström L, Fuchs D, Bäckman U, Holmquist K, Christofferson R, Azarbayjani F: The anti-VEGF antibody bevacizumab potently reduces the growth rate of high-risk neuroblastoma xenografts. Pediatr Res 60:576-81, 2006.

3. Azarbayjani F, Borg LA and Danielsson BR: Increased susceptibility to phenytoin teratogenicity: excessive generation of reactive oxygen species or impaired antioxidant defense? Basic & clinical pharmacology & toxicology. *99 (4)*: 305-311, 2006.

4. Svensson A, Backman U, Fuchs D, Christofferson R and Azarbayjani F: Angiogenesis can be reduced without significant reduction of tumor growth: Anticancer research. *27 (6B)*: 3883-3889, 2007.

5. Danielsson C, Azarbayjani F, Skold AC, Sjogren N and Danielsson BR: Polytherapy with hERG-blocking antiepileptic drugs: increased risk for embryonic cardiac arrhythmia and teratogenicity: Birth defects research. *79 (8)*: 595-603, 2007.

Study group members

Rolf Christofferson – Docent

Faranak Azerbayjani - Postdoctoral Fellow

Dieter Fuchs - Graduate Student

Barbro Einarsson - Research Engineer

Mats Hjortberg - PhD

Agencies supporting the work

Children’s Cancer Foundation of Sweden.

Dissertations

Lai, Enyin, Interaction between Adenosine and Angiotensin II in Renal Afferent Arterioles of Mice. – 2007

Nash, Peppi, Experimental and clinical studies of oxidative stress in preeclampsia. - 2007

Nordquist, Lina, Novel Approaches to Treatment and Prevention of Diabetic. – 2007

Nyblom, Hanna K, Glucotoxicity in Insulin-Producing Beta-Cells. – 2007

Rydgren, T, Experimental Studies Aiming to Prevent Type 1 Diabetes Mellitus. - 2007

Sandberg, Monica, Intracellular degradation of insulin in pancreatic islets. - 2007

Thorvaldson, Lina, Exploration of Conditions Affecting Cytokine Production in Experimental Type 1 Diabetes Mellitus. - 2007

Licentiate thesis

Börjesson, Andreas, Experimental studies of pancreatic islets deficient in inducible nitric oxide synthase. – 2007

Fuchs, Dieter, Angiogenesis inhibition: a novel strategy for treating neuroblastoma xenografts in mice. – 2007

Hultström, Michael, Interaction between contractile stimuli on tha isolated perfused afferent arteriole of the mouse kidney. - 2007

Mokhtari, Dariush, Role of MEKK-1 in beta-cell apoptosis. - 2007

Olerud, Johan, Interventions to improve the re-vascularization and function of transplanted pancreatic islets. – 2007

Servetnyk, Zhanna, Chloride transport in epithelial cells and its implications for pharmacological treatment of cystic fibrosis. – 2007

Sol, E-ri Maria, Mechanisms of palmitateinduced beta-cell glucolipotoxicity. – 2007

Sällström, Johan, The Effect of Nitric Oxide and Tubuloglomerular Feedback on Renin Release and Diabetes Induced Hyperfiltration. – 2007

Sågetorp, Jenny, Cyclic AMP oscillations in insulin-secreting cells. – 2007

Zabihi, Sheller, Yolk sac morphology and uterine blood flow are associated with fetal outcome in diabetes pregnancy. - 2007

Economy

(kSEK)

	2006	2007
Undergraduate Education Grant	24.803	24.755
Faculty Grant	22.066	20.902
External Grants	22.055	20.336
Others	3.725	3.237
Total	**72.737**	**69.641**

Undergraduate Teaching

IMCB participates in five different study programmes (utbildningsprogram): medicine, biomedicine, pharmacy, biomedical laboratory sciences and nursing sciences. In addition, it hosts a number of single subject courses (fristående kurser).

Medicine

The department contributes teaching in anatomy, cell biology and physiology in the form of lectures, seminars and laboratory experiments. Most of this teaching is given during terms 1-3 of the programme but extensive parts will also be given in the later integrated courses. The overall objective is to provide basic knowledge of the morphology and biological function of the human body and to create a basis for the following clinical studies. Some 80 students are enrolled every semester.

Biomedicine

This four-year programme aims to give students a thorough understanding of normal morphology and function of the human body. The programme gives the students training for future activity in research, information and education. The department takes part in the teaching of anatomy, embryology, cell biology and physiology. About 40 students are enrolled each year.

Pharmacy

The department is responsible for the teaching in physiology for the University Diploma of Pharmacy. Some 90 students are enrolled every semester.

Nursing sciences

The department is responsible for the teaching of anatomy, cell biology and physiology in the form of lectures and seminars. Some laboratory experiments are involved as well. Some 100 students are enrolled every second semester and 120 students are enrolled the other semester. Thus 220 students are enrolled every year.

Biomedical laboratory sciences

The aim of this programme is to produce technicians with appropriate training for a future task in diagnostic and research laboratories. The department is responsible for the teaching in anatomy, embryology, cellbiology and physiology. Some 35 students are enrolled each year.

Centres and Facilities

BMC Electron Microscopy Unit

Since the Biomedical center (BMC) was founded in 1968, a single organization has been responsible for the administration and service of the facilities electron microscopes. This organization, BMC - EM, is currently the responsibility of the Department of Medical Cell Biology, but other researchers take part in its activities. The equipment can be utilized by any microscopist in Uppsala. All equipment is connected to our computer central and to Internet.

The purpose of this site is to inform you about the various electron microscopes available at the BMC, and some practical details concerning the microscopic work. We hope that this information will make you aware of the excellent resources for electron microscopy at BMC and encourage you to exploit these resources in your own research. In addition, qualified and experienced staff is available to help you with any problems connected to specimen preparation and imaging. BMC - EM welcomes you at the electron microscopy center.

Godfried M. Roomans, PhD

Professor of Medical Ultrastructure Research

For technical information and booking, please contact Leif Ljung, Research Engineer, 018-4714150 Leif.Ljung@mcb.uu.se

Awards and Appointments 2007

Joey Lau	**Young Investigators Award, Scandinavian Society for the Study of Diabetes**
Dieter Fuchs	**Best oral presentation – Nordic Society of Pediatric Hematology and Oncology**
Ina Kozlova et al.	**Best publication in Upsala Journal of Medical Sciences**
Louise Rügheimer	**Pedagogiskt pris vid Uppsala Universitet**

ADDRESS LIST

Department of Medical Cell Biology

www.mcb.uu.se

Address: Uppsala University, Biomedical Center, Box 571, 751 23 Uppsala, Sweden

Office: Fax +46 18 471 4059, Phone +46 18 471 4328, +46 18 471 4431

AHLANDER ANDERS	Anders.Ahlander@mcb.uu.se
ANDERSSON ARNE	Arne.Andersson@mcb.uu.se
AZARBAYJANI FARANAK	Faranak.Azarbayjani@mcb.uu.se
BARBU ANDREEA	Andreea.Barbu@mcb.uu.se
BERGSTEN PETER	Peter.Bergsten@mcb.uu.se
BLIXT MARTIN	Martin.Blixt@mcb.uu.se
BODIN BIRGITTA	Birgitta.Bodin@mcb.uu.se
BOHMAN SARA	Sara.Bohman@mcb.uu.se
BORG HÅKAN	Hakan.Borg@mcb.uu.se
BÖRJESSON ANDREAS	Andreas.Borjesson@mcb.uu.se
CALOUNOVÁ GABRIELA	Gabriela.Calounova@mcb.uu.se
CARLSSON CARINA	Carina.Carlsson@mcb.uu.se
CARLSTRÖM MATTIAS	Mattias.Carlstrom@mcb.uu.se
CHRISTOFFERSSON GUSTAF	Gustaf.Christoffersson@mcb.uu.se
DANSK HELENE	Helene.Dansk@mcb.uu.se
DRAGOMIR ANCA	Anca.Dragomir@mcb.uu.se
DYACHOK OLEG	Oleg.Dyachok@mcb.uu.se
EDLUND JENNY	Jenny.Edlund@mcb.uu.se
EINARSSON BARBRO	Barbro.Einarsson@mcb.uu.se
EJDERSJÖ ANDREAS	Andreas.Ejdersjo@mcb.uu.se
ERIKSSON ULF	Ulf.Eriksson@mcb.uu.se
FASCHING ANGELICA	Angelica.Fasching@mcb.uu.se
FLINK KÄRSTIN	Karstin.Flink@mcb.uu.se
FRED RIKARD	Rikard.Fred@mcb.uu.se
FUCHS DIETER	Dieter.Fuchs@mcb.uu.se
FUNA NINA	Nina.Funa@mcb.uu.se
GRAPENGIESSER EVA	Eva.Grapengiesser@mcb.uu.se
GYLFE ERIK	Erik.Gylfe@mcb.uu.se
HALLGREN ING-BRITT	Ing-Britt.Hallgren@mcb.uu.se
HANSELL PETER	Peter.Hansell@mcb.uu.se
HELLMAN BO	Bo.Hellman@mcb.uu.se
HENRIKSNÄS JOHANNA	Johanna.Henriksnas@mcb.uu.se
HOLM LENA	Lena.Holm@mcb.uu.se
HOVSEPYAN MERI	Meri.Hovsepyan@mcb.uu.se
IDEVALL OLOF	Olof.Idevall@mcb.uu.se
ISAKSSON BRITTA	Britta.Isaksson@mcb.uu.se
JANSSON LEIF	Leif.Jansson@mcb.uu.se

JOHANSSON ÅSA	Asa.Johansson@mcb.uu.se
JÄGARE ANNIKA	Annika.Jagare@mcb.uu.se
KOZLOVA INNA	Inna.Kozlova@mcb.uu.se
KULLMAN LISEN	Lisen.Kullman@mcb.uu.se
KÄLLSKOG ÖRJAN	Orjan.Kallskog@mcb.uu.se
LAU JOEY	Joey.Lau@mcb.uu.se
LJUNG LEIF	Leif.Ljung@mcb.uu.se
LJUNGKVIST MARIANNE	Marianne.Ljungkvist@mcb.uu.se
MALINOVSCHI ANDREI	Andrei.Malinovschi@mcb.uu.se
MOKHTARI DARIUS	Dariush.Mokhtari@mcb.uu.se
MÖRSARE ING-MARIE	Ing-Marie.Morsare@mcb.uu.se
NILSSON GUNNO	Gunno.Nilsson@mcb.uu.se
NILSSON HARRIET	Harriet.Nilsson@mcb.uu.se
NORDIN ASTRID	Astrid.Nordin@mcb.uu.se
NYBLOM HANNA	Hanna.Nyblom@mcb.uu.se
OLERUD JOHAN	Johan.Olerud@mcb.uu.se
PALM FREDRIK	Fredrik.Palm@mcb.uu.se
PERSSON ERIK	Erik.Persson@mcb.uu.se
PETERSSON JOEL	Joel.Petersson@mcb.uu.se
PETTERSSON ULRIKA	Ulrika.Pettersson@mcb.uu.se
PHILLIPSON MIA	Mia.Phillipson@mcb.uu.se
RISBERG ANITHA	Anitha.Risberg@mcb.uu.se
ROOMANS GODFRID	Godfried.Roomans@mcb.uu.se
RYDGREN TOBIAS	Tobias.Rydgren@mcb.uu.se
RÜGHEIMER LOUISE	Louise.Rugheimer@mcb.uu.se
SAGULIN LISBETH	Lisbeth.Sagulin@mcb.uu.se
SANDBERG MONICA	Monica.Sandberg@mcb.uu.se
SANDLER BÄFWE AGNETA	Agneta.Bafwe@mcb.uu.se
SANDLER STELLAN	Stellan.Sandler@mcb.uu.se
SARGSYAN ERNEST	Ernest.Sargsyan@mcb.uu.se
SCHREIBER OLOF	Olof.Schreiber@mcb.uu.se
SERVETNYK ZHANNA	Janna.Servetnyk@mcb.uu.se
SJÖQUIST MATS	Mats.Sjoquist@mcb.uu.se
SOL E-RI	E-ri.Sol@mcb.uu.se
STÅHL GÖRAN	Goran.Stahl@mcb.uu.se
SUNDSTEN TEA	Tea.Sundsten@mcb.uu.se
SÅGETORP JENNY	Jenny.Sagetorp@mcb.uu.se
SÄLLSTRÖM JOHAN	Johan.Sallstrom@mcb.uu.se
TENGHOLM ANDERS	Anders.Tengholm@mcb.uu.se
THORVALDSON LINA	Lina.Thorvaldson@mcb.uu.se
THÖRN KRISTOFER	Kristofer.Thorn@mcb.uu.se
TIAN GENG	Geng.Tian@mcb.uu.se
TÖRNELIUS EVA	Eva.Tornelius@mcb.uu.se
WELSH MICHAEL	Michael.Welsh@mcb.uu.se
WELSH NILS	Nils.Welsh@mcb.uu.se
WENTZEL PARRI	Parri.Wentzel@mcb.uu.se

WUTTKE ANNE	Anne.Wuttke@mcb.uu.se
ZABIHI SHELLER	Sheller.Zabihi@mcb.uu.se
ÅKERBLOM BJÖRN	Bjorn.Akerblom@mcb.uu.se