ABSTRACTS POSTER PRESENTATIONS

P1 KV7 channel impairment occurs before the onset of hypertension in rats on a high fat and fructose diet.

Saereh S. Khanamiri[1], Ewa Soltysinska[1], Thomas A. Jepps[1], Bo H. Bentzen[1], Iain A. Greenwood[2], Søren-Peter Olesen[1], Nicole Schmitt[1]
[1]*Danish National Research Foundation Centre for Cardiac Arrhythmia and Dept. of Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark*
[2]*Pharmacology & Cell Physiology Research Group, Division of Biomedical Sciences, St. George's University of London, London, UK*

Rationale: Western style high fat/high fructose diets are known to lead to cardiovascular complication but the underlying mechanisms are unclear. Impaired activity of voltage-gated K_V7 channels critically determine sensitivity to the key regulators of coronary tone[9]. Therefore we hypothesized that K_V7 function in different arterial beds could be detected before gross cardiovascular changes develop.
Aims: The aim of this study was to clarify if the *KCNQ*-encoded K_V7 channels are compromised in rats on a high fat/fructose diet prior to development of hypertension.
Methods and results: Sprauge Dawley rats on a high fat and fructose diet (FFFR) for 6 weeks exhibited raised blood glucose compared with rats fed on normal chow but similar blood pressure and heart rates. Isometric tension recording was performed on segments of left anterior descending coronary arteries, mesenteric arteries, and thoracic aorta segments from Sprague Dawley rats fed either normal chow (CTRL) or on a high fat and fructose diet (FFFR) for 6 weeks. In arteries from FFFRs the relaxant response to two structurally different K_v7 activators S-1 or ZnPy was attenuated compared to controls. Similarly experiments on whole hearts from FFFRs revealed significant increase in basal coronary flow, but no change in the reactive hyperemic response as compared to the control. Incubating left coronary descending arteries from control rats in a high glucose Krebs' Solution for 6 hours led to an impaired response to the K_v7 activators. The functional impairment of K_V7 channels was not caused by differences in the expression levels of *KCNQ1-5*.
Conclusion: These data show that diet-induced impairment in K_v7 activity precedes cardiovascular changes.
Key Words: K_v7 channels, FFFR; high-fructose and fat fed rat, LAD; left anterior descending arteries, Western style diet, Pre-diabetes,

P2 Hemodynamic effects of intracardially infused clusterin in the anesthetized rat

Aadne Daehlin[1], E. Helgeland[1], A.K. Jonassen[1,2]
[1]*Department of Biomedicine, University of Bergen (UiB), Bergen, Norway.*
[2]*Faculty of Health Care and Nursing, Gjøvik University College, Gjøvik, Norway.*

The protein clusterin (apolipoprotein J) has been identified in most human tissues, and has been associated with apoptosis in various settings. Previous ischemia-reperfusion experiments on the *ex vivo* perfused rat heart have indicated that this protein could be a humoral mediator for cardioprotection from ischemia-reperfusion injury (IRI). As an introduction to investigations into the cardioprotectivity of clusterin *in vivo*, experiments were performed to investigate whether clusterin has any unwanted effects on hemodynamics. The animals were anesthetized using a mixture of fentanyl, fluanisone and midazolam (FFM), laid on water-temperated pads, ventilated, and blood gases, rectal temperature and hemodynamics were monitored. Following transsternal thoracotomy, clusterin was administered intracardially via a transmural plastic tube to the left ventricle. The dose was calculated from distribution of cardiac output and the smallest effective dose *ex vivo*. The hemodynamics was observed during 20 minutes pre---ischemia, 30 minutes of ischemia and 120 minutes

after reperfusion. Heart rate, systolic and diastolic pressure and the slope of pressure (dP/dt) were monitored. All parameters were measured inside the left ventricle as well as in the right carotid artery. Clusterin administration was initiated at the time of reperfusion and given continuously for 15 minutes. A total of 0.5 µg clusterin was administered per animal. The control group was given vehicle in the same manner. Clusterin did not negatively impact the hemodynamic parameters measured, nor did we observe any significant differences in hemodynamics as compared to the control group. Further studies to evaluate hemodynamic responses to higher doses of clusterin are called-for.

P3 Initial investigations into the effect of clusterin on cardiac ischemia--- reperfusion injury *in vivo*

Aadne Daehlin[1], E. Helgeland[1], A.K. Jonassen[1,2]
[1]*Department of Biomedicine, University of Bergen (UiB), Bergen, Norway.*
[2]*Faculty of Health Care and Nursing, Gjøvik University College, Gjøvik, Norway.*

Much research has been conducted on the topic of finding a factor that might convey protection from ischemia---reperfusion injury (IRI). So far no such factor has been identified, at least not to the extent that it has incorporated as part of treatment of ischemic disease. An investigation performed on isolated perfused rat hearts *ex vivo*, has produced promising results in regards to the ability of the endogenous protein clusterin in reducing IRI. Clusterin is also identified as one of many factors that human tissue produces as a response to ischemia. It is further established that humoral factor(s) with the ability to salvage tissue from IRI do actually exist. Using an *in vivo* protocol, hearts of anesthetized rats were exposed to 30-minute ischemic periods, and then reperfused while having a clusterin-containing isotonic solution administered intracardially for the first 15 minutes. The dose of clusterin (a total of 0.5 µg per animal) was calculated based on the smallest effective dose *ex vivo* and the distribution of cardiac output. After a total of 120 minutes of reperfusion, the hearts were extracted and stained using Evans blue and triphenyl tetrazolium chloride (TTC). Infarct size of the ischemic area was quantified using Photoshop software. The control group went through the same procedure, but received a vehicle instead of clusterin. With the concentration of clusterin used, no significant change in infarct size was observed between the groups. Further investigations using higher concentrations of clusterin are needed in order to conclude.

P4 Action of somatostatin and analogues on human lymphatic vessels

Frederik T. Skov[1], Vibeke E. Hjortdal[2], 1Donna B. Bødtkjer[1]
[1]*Department of Biomedicine, Aarhus University, Denmark*
[2]*Department of Cardiothoracic and Vascular Surgery, Aarhus University Hospital Skejby, Denmark*

Lymph is actively transported from lymphatic capillaries to the venous circulation by intrinsic contractile activity in the collecting lymphatic vessels. The largest collecting lymphatic is the thoracic duct (TD), which terminates into the veins of the neck. Accumulation of lymph in the thorax – chylothorax – caused by damage to the TD occurs in congenital heart diseases, e.g. univentricular circulation, as well as in connection with thoracic operations or trauma. With protracted resolution of chylothorax a pharmacological treatment to accelerate healing is desired. Since the first reports of somatostatin (SST) infusion to treat chylothorax, many clinicians worldwide have treated patients with SST or its stable synthetic analogue octreotide. The therapeutic rationale is that SST or octreotide reduce lymph production: we hypothesize that an additional direct action of SST on lymphatic contractility also occurs. To investigate SST reactivity we use isolated lymphatic vessels – thoracic duct (from oesophagus cancer) and intestinal lymphatics (from gastric bypass) mounted in wire myographs for isometric force measurement. Spontaneous and agonist-induced contractile activity is studied in vessels normalized to a transmural pressure of 21 mmHg. After equilibration vessels are challenged by acute and prolonged incubations with somatostatin (SST-14 and -28) and octreotide. Results to date suggest that octreotide and SST-14 enhance both force and rate of spontaneous lymphatic contractility in concentrations corresponding to the

attainable plasma level in humans undergoing treatment; 1– 10nM. We tentatively conclude that human lymphatic vessels respond to acute SST exposure by increased activity, which would be contrary to reducing lymph flow *in vivo*.
We gratefully acknowledge Einar Pahle and the operation team from Viborg Hospital and Hans Pilegaard and operation team from Aarhus University Hospital Skejby.

P5 Involvement of hydrogen sulfide in perivascular and hypoxia-induced inhibition of endothelin contraction in porcine retinal arterioles

Anna K. Winther, T. Dalsgaard, E.R. Hedegaard, U. Simonsen
Department of Biomedicine, Aarhus University, Aarhus, Denmark.

Perivascular retina has been shown to regulate retinal vascular tone. In the present study, we evaluated an *ex vivo* retina preparation, and investigated whether hydrogen sulfide (H_2S) mediates an inhibitory effect of retina and/or hypoxia on arteriolar tone. In retina, immunolabeling showed an increase of glial fibrillary acidic protein, but not vimentin over time in Müller cells, and the presence of necrotic cells after 2hrs and apoptotic cells after 8hrs. Isometric tension recordings showed endothelin-1(ET-1) to induce concentration-dependent contractions, which were reduced in the presence of retina. In arterioles with retina no change was observed in ET-1 contractions after 5 hrs compared to 8hrs. Hypoxia (1% O_2) reduced ET-1 contraction in arterioles with and without retina. The H_2S donor, GYY4137 and the salt, sodium hydrogen sulfide, induced concentra-tion-dependent relaxations in ET-1 contracted retinal arterioles. Inhibition of the H_2S producing enzymes, cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE), with carboxymethoxyl-amine (AOA) and L-propargylglycine (PPG) enhanced ET-1 contractions. This effect was more pro-nounced in hypoxic conditions. However, even in the presence of AOA and PPG ET-1 induced less contraction in the presence of perivascular retina compared to isolated vessels. These findings suggest that both the presence of perivascular retina and hypoxia reduce arteriolar vasocon-striction and that both H_2S and another factor mediate this effect. Finally, H_2S donors, as well as endogenous H_2S, can reduce retinal arteriolar tone, suggesting a potential therapeutic role for enhanced H_2S bioavailability in the treatment of retinal disease.

P6 The Role of Adenosine Receptors in Hypoxic Vasodilatation and Cardioprotection

Vibeke Bjerg Pedersen, Ulf Simonsen, Elise Røge Hedegaard
Aarhus University, Department of Biomedicin

During ischemia in the heart the arteries respond with hypoxic vasodilatation. This increase blood flow and thereby the oxygen supply. This mechanism can reduce the complications caused from long duration of ischemia. Adenosine is a metabolite suggested to play a key role in hypoxic vasodilation. When adenosine works through the A_{2A} receptor it produces relaxation. The aim of this study is to clarify how adenosine acts under hypoxic vasodilatation.
Small porcine coronary arteries are mounted for isometric tension recordings in a microvascular myograph. This set-up allows us to determine the response in contractility to pharmacologic treatment and hypoxia (1% O_2). We use adenosine or the A_{2A} selective agonist CGS21680 to do a concentration-response curve. Smooth muscle cells from the small porcine coronary arteries are cultured for immunofluorescenct staining with an A_{2A} selective antibody to examine the localization of the receptor. We plan to compare the expression of A_{2A} receptors on hypoxic and normoxic cells.
Hypoxia increase the sensitivity to adenosine and CGS21680. Hypoxia shifted the adenosine and CGS21680 response curve to the left but did not change maximum relaxation. When using the selective A_{2A} blocker ZM241386 we were able to block some of the CGS21680 induced vasodilatation. So far we have been able to visualize the A_{2A} receptors inside the smooth muscle cell.

Our results suggest that in hypoxia adenosine A_{2A} receptors are involved in the increased sensitivity towards adenosine and this may be caused either by interactions with other adenosine receptors or by different adenosine A_{2A} receptor populations.

P7 Placental vessels from type---1 diabetics and the surrounding tissue

Daniel Kristensen[1,2],Torbjørn Brøgger[1,2] ,Donna Briggs Bødtkjer[1,2], Karl---Erik Andersson[1,2], Per Glud Ovesen[1], Axel Forman[1] and Christian Aalkjær[2]
[1]Department of Gynaecology and Obstetrics, Aarhus University Hospital Skejby, Aarhus, Denmark [2]Department of Biomedicine, Aarhus University, Aarhus, Denmark E---

Background: During pregnancy the placenta serves as the site of communication between the ma--- ternal and the foetal cardiovascular systems. In pregnancies complicated by type-1 diabetes mellitus (T1DM) conditions such as pre-eclampsia occur more frequently. A profound effect of the perivascular tissue (PVT) of the stem villous arteries (SVA) tone has been observed in previous studies. The effect could partly be related this to the local release of nitric oxide (NO) from the PVT to the smooth muscle cells of the stem villous arteries (SVA). We hypothesize that the SVAs from pregnant women with T1DM have altered NO response from the PVT compared to uncomplicated pregnancies.
Methods: Fresh delivered placentas were obtained from cases (T1DM pregnancies) and controls (uncomplicated pregnancies) and the SVA are dissected free under a stereomicroscope. The SVA segment was then divided and prepared into two paired vessel preparations (±PVT) before mounting in a wire myograph for recording of isometric force development. Cumulative concentration-response curves (10^{-8} – 10^{-4} M) to prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) were made in and without presence of 100 µM of N^{ω}-nitro-L-arginine (L-NNA). The maximal response to $PGF_{2\alpha}$ was used to assess the vasodilatory effect of a single dose (50 µM) of sodium nitroprusside (SNP).
Results: Our preliminary data (4 case SVA and 4 control SVA) demonstrate that SVAs ± PVT obtained from T1DM pregnancies achieve lower maximal contraction to $PGF_{2\alpha}$. Stimulation with 50 µM SNP on the SVA + PVT preconstricted with 100 µM $PGF_{2\alpha}$ resulted in a prolonged and inferior dilatory response in the SVAs obtained from T1DM.
Conclusion: The vascular response to $PGF_{2\alpha}$ and SNP is seemingly impaired in placental stem villi arteries obtained from pregnancies complicated by T1DM. Data collection is on going.

P8 In vitro study of the vasodilatory effect of pomegranate flower---polyphenols on the aorta ring of Sprague-Dawley rats

Wei Yuan-Yuan[1], T. Abudureyimu[1], S. Hongjie[1], W. Yanjun[1], D. Minghui[1]， A. Ajieaikebaer[2]
[1]Department of Physiology, School of Basic Medical Science, Xinjiang Medical University, Urumqi, China [2]Xinjiang Technical Institute of Physics and Chemistry, Chinese Academy of Science, Urumqi, China

Background: Diets rich in polyphenols has shown to be cardiovascular protective. In this study, we have investigated the direct effect of the pomegranate flower polyphenols (PFP) on the isolated rat aorta and examined its possible mechanisms involved in the action of vasodilation.
Methods: Rings of aorta from male Sprague-Dawley rats were mounted for isometric force recording with or without endothelium present. Phenylephrine (PE) was used for the vasoactivation and the PFP-induced relaxation rate was normalized to 1uM forskolin-induced relaxation.
Results: PFP (100-900mg/L) induced dose-dependent and endothelium-independent relaxation in the pre-activated aortic rings. The maximum relaxation in the endothelium removed aortic rings reached to 88.78% while it was approximately 64.37% in the endothelium intact preparations. The PFP–induced relaxation was not

affected by the presence of NO inhibitor (L-NAME, 10mM), but partly abolished with the potassium channel inhibitors: glibenclamide (10uM), tetraethylammonium (TEA, 3 mM) and $BaCl_2$ (100uM) respectively. In the calcium ion free solution, the PFP significantly increased the PE---induced contraction, and inhibited the high KCl–induced force development in the endothelium removed aortic rings.
Conclusion: Our preliminary results suggested that, PFP induces vasorelaxation independent of endothelium possibly via effecting the activity of calcium activated potassium channels, and/or inhibition of receptor-operated and voltage-activated calcium channels in the smooth muscle cell membrane.
Source of financial support: The National Natural Science Foundation of China (NNSF, project number: 81360669)

P9 Pro-contractile action of the ouabain-sensitive Na,K-ATPase in the vascular wall is associated with *Src*-kinase activation.

Elena V. Bouzinova[1], L. Hangaard[1], Z. Xie[2], C.Aalkjær[1], V. V. Matchkov[1]
[1]*Dept. of Biomedicine, Health, Aarhus University, Aarhus, Denmark*
[2]*Marshall Institute for Interdisciplinary Research, Marshall University, Huntington, West Virginia, USA*
Na,K-ATPase is essential for maintaining the transmembrane ion gradient and has been recently suggested to initiate various intracellular signaling. These signals possibly act through a modification of the local ion concentrations or via *Src*-kinase activation. It has previously been shown that inhibition of the α-2 isoform of Na,K-ATPase by ouabain elevates blood pressure. Consequently, ouabain was shown to potentiate arterial contraction *in vitro*. In contrast, we have demonstrated that siRNA-induced down-regulation of the α-2 isoform Na,K-ATPase expression reduced arterial sensitivity to agonist stimulation and prevented the effect of ouabain.
Here we demonstrate results of our research on the mechanisms involved in the modulation of vascular wall contractility by ouabain-sensitive Na,K-ATPase. The experiments were performed using rat mesenteric arteries in isometric myograph conditions.
Using a *Src*-family selective tyrosine kinase inhibitor, PP2, and pNaKtide - a membrane-permeable small peptide which antagonizes ouabain-induced activation of *Src*-kinase, we demonstrate that the pro-contractile action of ouabain is associated with activation of *Src*. This is supported by Western blot analyses showing activation of *Src* by ouabain and its inhibition by pNaKtide. *Src* was also activated by agonist (noradrenaline) stimulation. *Src*-dependent potentiation of vasoconstriction was associated with sensitization of contractile machinery to $[Ca^{2+}]i$, as evident from MYPT (myosin phosphatase targeting protein) phosphorylation assay. Down-regulation of the α-2 isoform Na,K-ATPase prevented the inhibitory effect of *Src* inhibitors on arterial contraction.
Thus, the pro-contractile action of ouabain-sensitive Na,K-ATPase inhibition is associated with *Src*-kinase inhibition suggesting the role of this signaling pathway in regulation of vascular tone and peripheral resistance.

P10 Validation of Near Infrared Fluorescence (NIRF) Imaging of lymphatic vessels in humans

Jacob Grønlund, N. Telinius, S Skov, M Ølgaard, V Hjortdal
Department of Cardiothoracic and Vascular Surgery, Aarhus University Hospital, Denmark

NIRF imaging is new imaging technique to visualise lymphatic vessels in humans. In contrast to lymphoscintigraphy, the gold standard, NIRF imaging has a higher spatial resolution and a temporal resolution allowing real time visualisation of lymph flow. The current study investigated the intra and inter individual variability of the technique and how local hyperthermia as well as exercise affects lymph transport. In our study ten healthy volunteers were studied twice with two weeks between. NIRF imaging was conducted using intradermal indocyanine green injections and a custombuilt camera setup. All data was blinded prior to analysis and presented as mean±SD. Mean contraction frequency and lymph propulsion velocity were 0.59±0.13min^{-1}

and 1.51±0.24cm/s with no significant difference ($p<0.05$) during each 3.5h examination or between the two visits. The maximal pressure the vessels could overcome on test day 1 and 2 was 56±9mmHg and 57±9mmHg ($p=0.4961$). Local hyperthermia, induced via immersion of the foot in 40°C water, increased contraction frequency from 0.62±0.4min to 1.46±0.5min^{-1} ($p<0.05$). Immediately after exercise (1.4km on cycle ergomenter) an increase in lymph propulsion velocity from 1.5±0.49 to 2.2±0.63cm/s was observed ($p<0.05$) whereas contraction frequency was unaltered. A decrease in contraction frequency from 0.68±0.25 to 0.35±0.19min^{-1} was observed 10 minutes after exercise without a change in velocity. NIRF imaging results show repeatability can be conducted over at least 3.5 hours without a change in lymphatic activity. It has furthermore the sensitivity to detect changes in lymphatic activity by local hyperthermia.

P11 The cardiovascular pharmacology of the venom of the Papuan black snake *Pseudechis papuanus*

Makhala M. Khammy[1], C.E. Wright[1,2]
[1]*Cardiovascular Therapeutics Unit, Department of Pharmacology and Therapeutics, University of Melbourne, Victoria 3010, Australia*
[2]*Australian Venom Research Unit, Department of Pharmacology and Therapeutics, University of Melbourne, Victoria 3010, Australia*

This study assessed the cardiovascular pharmacology of the venom of the Papuan black snake, *Pseudechis papuanus*. In rat isolated small mesenteric arteries (250-300 µm i.d.) precontracted with U46619 to 80-90% of their maximum contraction, venom caused potent relaxation with % decreases in tone of 25±6, 66±16, 85±5 and 84±7 with 0.03, 0.1, 0.3 and 1 µg/ml, respectively (single concentration per vessel, *n*=4 each). Venom also inhibited arterial contractile responses to field stimulation in a concentration- and time-dependent manner. In rat isolated right and left atria, venom (0.01-10 µg/ml) caused concentration-dependent tachycardia and increases in contractile force, respectively; with 10 µg/ml, changes were +48±14 b/min and +85% increase in contractility. In anaesthetised rats, venom (100 µg/kg i.v.) caused an immediate fall in blood pressure (-30±1 mmHg; *n*=5) and increased hindquarter vascular conductance (HVC; +30%), indicative of vasodilatation. By 50 min post-dose, compared with pre-venom baseline values, blood pressure was elevated (+29±5 mmHg), consistent with a (by then) decreased HVC (-37%) and raised heart rate (+48±12 b/min). The mechanism(s) of venom-induced positive chronotropy, positive inotropy, vasodilatation and late vasoconstriction are unknown. In the heart, effects are independent of β-adrenoceptor activation; the β-adrenoceptor antagonist propranolol (1 µM) did not affect responses to venom. In resistance arteries, venom-induced relaxation was markedly tachyphylactic and significantly attenuated in the presence of the calcitonin gene-related peptide (CGRP) receptor antagonist, CGRP 8-37 (3 µM), or indomethacin (3 µM), suggesting a role for the release of endogenous dilator agents such as CGRP, prostaglandin I_2 and E_2.

P12 Pharmacological analysis of endothelial influences in basilar artery of C57BL/6 mice

Oxana O. Kiryukhina, O.S. Tarasova
Faculty of Biology, M.V. Lomonosov Moscow State University and Laboratory of Exercise Physiology, SRC RF Institute for Biomedical Problems RAS, Moscow, Russian Federation

The basilar artery supplies blood to vital nervous centers of the medulla. C57BL/6 mice are widely used for studying brain circulatory disorders and also as genetic background for various transgenic models. We aimed at exploring the mechanisms of endothelial influences in basilar artery of C57BL/6 mice, which have not been characterized yet. Experiments were performed on 2–3 months old male C57BL/6 mice. The segments of basilar artery were mounted in wire myograph (DMT). Endothelial pathways were dissected using NO-synthase inhibitor L-NNA, cyclooxygenase inhibitor indomethacin and the combination of IK_{Ca}/SK_{Ca} channels blockers

(TRAM-34 + UCL-1684). The preparations did not respond to phenylephrine and 5-hydroxytryptamine, but developed strong contractions to KCl and U46619, thromboxane A2 receptor agonist. L-NNA prominently increased maximal force (by 40%) and the sensitivity to U46619 (5.3-fold decrease of EC_{50}); conversely, indomethacin slightly reduced the sensitivity to U46619, while IK_{Ca}/SK_{Ca} blockade had no effect. The relaxation response was studied using acetylcholine after U46619-induced precontraction; it was not observed after blockade of all three pathways. During isolated blockade, the response to acetylcholine was mostly affected by IK_{Ca}/SK_{Ca} blockers and, to the less extent, by L-NNA, while indomethacin was without effect. Maximal responses to acetylcholine (observed at concentration of 5*10-6 M) in control, after L-NNA and after IK_{Ca}/SK_{Ca} blockade were 64%, 56% and 30% respectively. Our data show the dominant role of NO in endothelial anticontractile effect and dominant role of EDHF in relaxation during pharmacological activation of the endothelium.
Supported by the Russian Foundation for Basic Research (grant N13-04-02087).

P13 Perivascular tissue affects regulation of coronary artery tone

Lisbeth Bonde, C. Aalkjaer, E. Boedtkjer
Department of Biomedicine, Aarhus University, Aarhus, Denmark

Background: Diabetic cardiomyopathy is a well-described complication of diabetes, characterized by malfunctioning myocardium. However, it is unclear whether this cardiac complication is caused by direct effects of diabetes on the heart or is secondary to changes in coronary artery perfusion. This study investigates the effects of perivascular tissue (PVT) on regulation of coronary artery tone in a model of diabetic cardiomyopathy.
Method: Zucker diabetic fatty (ZDF) rats (homozygote fat (fa/fa)) and their respective controls (heterozygous lean (fa/+)) were purchased at age 6 weeks and kept for 11 weeks before sacrifice. One segment of the septal coronary artery was dissected with and without PVT from each heart and mounted for isometric force recordings. All values presented below are means ±SEM.
Results: For lean ZDF rats, the maximum contractile response to serotonin decreased significantly from 3.33 ± 0.1 N/m in coronary arteries without PVT to 2.05 ± 0.2 N/m in coronary arteries with PVT ($p<0.0001$). In fat ZDF rats, this effect of PVT was diminished ($p<0.0001$): PVT lowered the maximum contractile response to serotonin from 3.08 ± 0.1 N/m to 2.56 ± 0.1 N/m ($p<0.0001$).
Conclusion: PVT inhibits serotonin-induced contractile responses of coronary arteries, and this effect is lower in coronary arteries from fat ZDF rats compared to their lean controls. This finding indicates that normal myocardium contributes to regulation of coronary artery tone and that this regulation is compromised in diseased hearts. An attenuated interplay between PVT and coronary arteries may play a role in the pathogenesis of diabetic cardiomyopathy.

P14 Effects of an opener of small and intermediate calcium-activated K channels NS309 on cardiac rhythm and erectile function in rats

Simon Comerma-Steffensen, T. Dalsgaarad, E.R. Hedegaard and U. Simonsen
Department of Biomedicine, Pulmonary and Cardiovascular Pharmacology, Aarhus University, Denmark.

Erectile dysfunction (ED) is considered an early clinical manifestation of vascular disease, due to its high prevalence in patients with cardiovascular risk factors. Pharmacological modulation of endothelial potassium channels had been shown to improve endothelium-dependent vasodilatation (Simonsen et al., 2009). However, only few studies have investigated the effect on cardiovascular hemodynamics of potassium channel modulators. Therefore, we examined the effect of NS309 an opener of KCa2.3 (SK) and KCa3.1 (IK) channels, on cardiac rhythm and blood pressure as well as erectile function. Mean arterial pressure (MAP), intracavernosal

pressure (ICP) and electrocardiography were measured in anesthesized rats, corpus cavernosum strips were mounted for isometric tension recording and processed for immunoblotting. Erectile function measured as (ICP/MAP) was increased during administration of NS309, while increases in ICP by stimulation of the cavernous nerve were unchanged. Heart sinus node and atrial ventricle conduction changed with an increment in depolarization and conduction, ventricle depolarization increase in association with the heart rate (QTc). Corpus cavernosum relaxation improved upon NS309 administration, and involved activation of SK and large conductance potassium channels (BK). Immunoblotting revealed the presence of SK channels in the corpus cavernosum. Our findings suggest that NS309 improves erectile function during infusion and may restore erectile function in disease. However, the effect on cardiac rhythm needs to be taken into account in the development of drugs of this class with longer duration of action.

P15 Lamin A/C disruption in white blood cells augments leukocyte extravasation and atherosclerosis development in *Ldlr*-KO mice

Cristina Rius, Alberto del Monte, Jose Maria Gonzalez-Granado, Vicente Andrés
Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain

A-type lamins (A/C) are proteins localized in the inner nuclear membrane of differentiated cells. Nuclear Lamin A/C have structural and functional roles in many cell types. We have shown that lamin A/C are essential for optimal activation of naïve T-cells and have detected lamins A/C expression in other types of human and mouse circulating leukocytes. We have investigated the role of lamins A/C in immune cells and if their expression affects atherosclerosis development. With this aim, lethally-irradiated low-density-lipoprotein receptor (*Ldlr)*-KO mice were reconstituted with wild-type or lamin A/C-KO bone marrow (BM). Our results show that circulating leukocytes, lipid profile, and mouse body weight were similar in both groups. However, atherosclerotic plaque formation was increased in fat-fed *Ldlr*-KO mice reconstituted with lamin A/C-KO BM compared with controls receiving wild-type BM. The absence of lamin A/C did not affect rolling or adhesion properties of neutrophils and monocytes, but increased leukocyte extravasation through the vessel wall (although their migration velocity within the tissue was lower). In addition, lamin A/C-deficiency in transplanted leukocytes resulted in a reduced content of macrophages in atherosclerotic plaques. We also find that lamin A/C expression is not required for in vitro macrophage polarization to M1 (pro-inflammatory) or M2 (anti-inflammatory); however, once differentiated, M2 express higher levels of lamins A/C than M1, suggesting that lamin A/C disruption might affect M2 function or viability in atherosclerotic lesions. We are currently examining the mechanisms underlying these new regulatory functions of lamin A/C in atherosclerosis, with particular emphasis in monocyte/macrophage migration and functionality.

P16 Late onset vascular dysfunction in the R6/1 model of Huntington's disease

Reheman Awahan[1], E. Mari[1], S. Yulia[1], A. Kristina[1], M. Matthias[2], E. Jonas[1], B. Patrik[3], J. Yili[4], S. Karl[1] *[1]Section for Cell and Tissue Biology, Department of Experimental Medical Science, Lund University BMC D12, Sweden*
[2]Department of Clinical Sciences Lund, Division of Infection Medicine, Lund University, Sweden,
[3]Neuronal Survival Unit, Department of Experimental Medical Science, Wallenberg Neuroscience Center, Lund University BMC B11, Sweden
[4]Neural Plasticity and Repair Unit, Department of Experimental Medical Science, Wallenberg Neuroscience Center, Lund University BMC A10, Sweden

Objective: Huntington's disease (HD) is a neurodegenerative disorder that also gives rise to widespread changes in peripheral organs and tissues. We tested the hypothesis that vascular dysfunction may occur in HD by studying R6/1 mice which express *exon 1* of mutant huntingtin.

Methods and Results: We assessed arterial function in R6/1 and wild type (WT) mice using myography. Arterial contractility was largely unaltered in R6/1 arteries at 15 and 32 weeks of age. By 40 weeks contractility was impaired in several arteries irrespective of vasoconstrictor. Endothelium---dependent relaxation was not affected, and we observed no changes in arterial geometry or expression of contractile proteins. The frequency of calcium oscillations in arterial smooth muscle cells was higher in R6/1 mice after agonist stimulation, whereas myosin phosphorylation was unaltered. Impairment of the force by the mitochondrial inhibitors cyanide and rotenone was less pronounced in R6/1 than in WT arteries and mitochondria were enlarged, in keeping with an effect related to altered mitochondrial function.
Conclusions: Our results reveal that arteries in the R6/1 model of Huntington's disease exhibit an age---dependent impairment of contractility and that they depend less on mitochondrial function when they contract.

P17 TMEM16A is upregulated, but does not translate into increased contractility of mesenteric arteries in streptozotocin model of type 1 diabetes

Dmitry Kamaev, A. Bisgaard Jensen, L. Bonde, C. Aalkjær, D. M. Boedtkjer, V. V. Matchkov
Department of Biomedicine, Aarhus University, Aarhus Denmark

Background: Vascular pathology is among the list of chronic complications associated with diabetes mellitus although the mechanism behind it is debated. Our study attempts to investigate the contribution of calcium-activated chloride channel protein, TMEM16A, to functional changes in mesenteric arteries from diabetic rats.
Methods: Model of type 1 diabetes was made by a single injection of streptozotocin; age-matched male control Wistar rats were injected with citratebuffer. Concentration response curves (CRC) to noradrenaline (NA) of small mesenteric arteries from STZ-treated and control rats were compared under normal and chloride-free conditions in wire myograph.
Results: We have observed an increased expression of TMEM16A in mesenteric arteries of STZ rats. TMEM16A mRNA and protein concentrations in STZ rats were increased to 182±10 % (n=4, P<0.01) and 195±23 % (n=9, P<0.01), respectively, compared to the control. There was no significant difference between NA CRCs for mesenteric arteries from STZ and control rats under normal conditions (n=9). A substitution of extracellular chloride significantly reduced the sensitivity to NA of mesenteric arteries from both groups; pD_2 for control and STZ groups shifted from 5.94±0.06 to 5.63±0.07 (P<0.01) and from 5.95±0.08 to 5.58±0.04 (P<0.001), respectively. No difference between CRCs for control and STZ groups in Cl^--free solution was seen, also there was tendency for elevated maximal contraction in STZ rats (3.1±0.2 vs. 3.7±0.2, P=0.07, n=9). Blockade of endothelium-derived relaxation factors (NO, prostacyclin and endothelium-derived hyperpolarization) resulted in suppression of contraction in Cl--free solution. No difference between CRCs for arteries from control and STZ rats was seen under these conditions.
Conclusions: We have found that an increased expression of TMEM16A in mesenteric arteries of diabetic rats does not directly translate into increased contractility of those arteries.

P18 Deficiency of thyroid hormones in early development provokes alterations in nervous control of cardiovascular system

Ekaterina K. Selivanova, A.A. Martyanov, A.A. Shvetsova, S.I. Sofronova, E.V. Lukoshkova, O.S. Tarasova
Faculty of Biology, M.V. Lomonosov Moscow State University and Laboratory of Exercise Physiology, SRC RF Institute for Biomedical Problems RAS, Moscow, Russian Federation

The deficiency of thyroid hormones (TH) during critical periods of ontogeny impairs neural development, but long-term effects of early hypothyroidism on cardiovascular system are poorly understood. The aim of our

study was to evaluate the influence of early hypothyroidism on cardiovascular system control in adult rats. We administered propylthiouracil (PTU) in dams from the 1st gestation day to 14th day after delivery to maintain early hypothyroidism in their offspring. PTU treatment depressed the thyroid function in 2-week male progeny: blood levels of total T_4 were 76.6±7.7 and 15.6±1.0 nM, and the levels of free T_3 were 3.3±0.4 and 0.1±0.1 pM in control and PTU pups, respectively. PTU offspring aged 10 weeks did not show any differences in blood TH and body weight from control offspring and demonstrated only moderate behavioral changes in elevated plus-maze and extrapolation escape task tests: decreased anxiety level and increased locomotor activity in spite of the unchanged escape time. However, catheter-instrumented awake PTU offspring compared to control showed smaller response to sympathomimetic drug tyramine along with unchanged response to α_1-adrenoceptor agonist methoxamine, which indicates abnormal sympathetic regulation in the cardiovascular tone. Besides that, they showed impaired vagal heart rate control, as was seen from the shift of sympathovagal balance towards sympathetic component and smaller tachycardic response to M-cholinoceptor antagonist. In conclusion, the early hypothyroidism provokes evident long-term alterations in nervous control of heart and blood vessels despite moderate changes in behavior and cognitive activity. Supported by the Russian Science Foundation (Grant N 14-15-00704).

P19 Endothelial dysfunction in small renal arteries as a mechanism of diabetic hyperfiltration

Olga S. Tarasova, G.S. Boleeva, A.A. Borzykh, D.K. Gaynullina, S.I. Sofronova, A.A. Martyanov
Faculty of Biology, M.V. Lomonosov Moscow State University and Laboratory of Exercise Physiology, SRC RF Institute for Biomedical Problems RAS, Moscow, Russian Federation

At the initial stage diabetic kidney pathology appears as hyperfiltration associated with decrease in preglomerular vascular resistance. We aimed at studying endothelium-dependent relaxation in renal arteries of diabetic rats. Type 1 diabetes mellitus was induced in male Wistar rats by a single injection of streptozotocin. Six weeks later the rats demonstrated increases of relative kidney weight (2-fold), diuresis (26-fold), creatinine clearance (1.7-fold) and urine albumin/creatinine ratio (2.2-fold). Interlobar arteries were isolated and studied by wire myography technique. For comparison we studied diabetic alterations in small arteries supplying skin (saphenous), gastrocnemius muscle, diaphragm and intestine. mRNA expression levels were studied by qPCR. All arteries, except renal, showed smaller responses to acetylcholine in diabetic rats compared to controls. Contrarily, renal arteries of diabetic rats demonstrated augmented response to acetylcholine, which was not associated with higher NO production, since the augmentation persisted after NO-synthase inhibition (L-NNA) and arterial sensitivity to NO-donor (DEA-NO) was not changed in comparison to control. However, inhibition of cyclooxygenase (indomethacin) together with NO-synthase eliminated the differences; residual (EDHF-like) component did not differ between the groups. Together with smaller impact of contractile prostanoids renal arteries of diabetic rats compared to controls had higher content of COX1 mRNA, while COX2 and eNOS mRNA levels were not changed. In conclusion, in normal rat kidney contractile influence of prostanoids restricts endothelium-dependent vasorelaxation. Failure of this mechanism may be a factor in glomerular hyperfiltration and nephropathy development.
Supported by the Russian Foundation for Basic Research (grants N13-04-02087 and N14-04-31377).

P20 The role of NA and ATP signaling in perivascular nerves *in vivo*

Alexandra Mazur, V. Matchkov, C. Aalkjær
Department of Biomedicine, Aarhus University, Aarhus, Denmark

Vascular tone is known to be modulated by endothelium and perivascular nerves. Perivascular innervation consists of adrenergic nerves contracting vessels by release of noradrenaline (NA), neuropeptide Y (NPY) and

ATP and sensory nerves relaxing smooth muscles via release of premdominantly calcitonin gene-related peptide (CGRP). In this study we tested the impact of NA and ATP for *in vivo* vasoconstrinction in rat mesenteric arteries in response to electric field stimulation (EFS).
Wistar male rats were anesthetized with ketamin/xylazine. The mesentery was gently pulled out and an arterial segment (perfused with the blood), cleaned of fat and isolated in a small chamber. Inner diameter was recorded during EFS at 2-32Hz. The effects of 1 µM prazosin (an alpha-adrenergic blocker), 7 µM suramin (reversible blocker of P2-purinoceptors) were assessed in the presence of inhibitors of vasodilator substances from the endothelium: 100 µM L-NAME, 50 nM apamin, 1 µM TRAM-34, 1 µM indomethacin. Arterial responses to EFS were fully blocked by tetrodotoxin indicating direct nerve stimulation.
Before application of the inhibitors of endothelial derived substances little tone was seen, but after application of these inhibitors tone developed. EFS elicited frequency dependent tone development. Prazosin was without any significant effect on EFS-induced contraction but fully inhibited the contraction to 10µM of exogenous NA. The combination of prazosin and suramin nearly completely inhibited EFS-induced contraction. The effect of suramin was reversible.
These results suggest a significant role of purinergic signaling for sympathetic vascular responses *in vivo*.

P21 Nicotine exacerbates diabetes-associated cardiac dysfunction

Chris R. Triggle, H. Ding, G. Arunachalam.
Departments of Pharmacology and Medical Education, Weill Cornell Medical College in Qatar, Education City, Doha, Qatar.

Introduction: Excess saturated free fatty acids (FFA) together with high glucose (HG) are causative factors for diabetes and obesity-associated heart failure. Palmitate, a circulating FFA, -induced cardiomyocyte apoptosis contributes to the development of diabetic cardiomyopathy. Cigarette smoke is a major risk factor for both lung cancer and cardiovascular disease (CVD); however, the contribution of nicotine to CVD is unknown. The primary objective of this study was to determine whether nicotine exacerbates cardiac muscle dysfunction in a diabetic milieu.
Experimental approach: Rat cardiomyocytes (H9c2 cells) were cultured under either normoglycaemic (NG, 5.5 mM) or hyperglycaemic (HG, 25 mM) conditions in the presence of palmitate and in in the absence or presence of 100 µM metformin. Cell survival and apoptotic markers were analysed by immuno-blotting. Data is given as Mean±SEM and analyses performed using one-way analysis of variance (ANOVA) with $p<0.05$ to indicate statistical significance.
Results: The western-blot data reveals that palmitate combined with HG results in a significant ($p<0.05$) induction of the transcription factor E2F1 (1.50±0.05 increase vs. NG), a significant ($p<0.05$) reduction in expression of cell survival/anti-apoptotic proteins sirtuin 1 (1.25±0.04 vs. NG), Bcl-2 family proteins and an increase in pro-apoptotic Bim (2.50±0.07 vs. NG). The presence of nicotine enhanced, whereas metformin reversed, the effects of palmitate/HG on cardiomyocyte apoptosis.
Conclusions: These results indicate that in a diabetic milieu nicotine enhances E2F1 activation and results in the down-regulation of sirtuin 1 and anti-apoptotic Bcl2 family proteins. In addition, treatment with metformin attenuated the detrimental effects of palmitate in cardiomyocytes.

P22 Tyrosine kinase inhibitor-induced hypertension in patients diagnosed with renal cell carcinoma is associated with decreased urinary excretion of NO metabolites

Anne R. Tinning[1], N.V. Jensen[2], L. Bastholt[2], B.L. Jensen[1], K. Madsen[1,3]
[1]Department of Cardiovascular and Renal Research, Institute of Molecular Medicine, University of Southern Denmark, Odense, Denmark
[2]Department of Oncology, Odense University Hospital, Odense, Denmark
[3]Department of Pathology, Odense University Hospital, Odense, Denmark

Background: Tyrosine kinase inhibitors (TKIs) are important in the pharmacological treatment of several malignant diseases. A common side effect is hypertension due to a yet unknown mechanism. Most TKIs target the receptors of VEGF, a vascular growth factor known to stimulate NO production. We hypothesize that TKIs increase blood pressure by impaired renal NO bioavailability.
Method & results: 22 patients diagnosed with renal cell carcinoma (RCC) undergoing treatment with the TKI pazopanib were included in the study. Home blood pressure measurements were recorded and blood and urine samples collected at baseline and at two follow-up visits (FU) 4 and 8 weeks after initiation of treatment.
At baseline, mean systolic and diastolic blood pressure was 137±3/76±2 mmHg. After 4 weeks of treatment, both systolic and diastolic blood pressure was significantly increased compared to baseline (150±4/89±2 mmHg, P<0,05) whereas after 8 weeks, systolic but not diastolic blood pressure had returned to baseline values (137±4/81±2 mmHg). During treatment, 5 patients initiated or intensified ongoing antihypertensive treatment before 1st FU and additionally 4 patients before 2nd FU. Urinary protein/creatinine ratio increased significantly after 4 weeks compared to baseline. No significant changes in plasma NO metabolites (NOx) was seen after initiation of pazopanib treatment. However, a significant reduction in urinary NOx excretion normalized to creatinine was seen after both 4 and 8 weeks of treatment.
Conclusion: Pazopanib treatment in RCC patients leads to hypertension associated with decreased urinary excretion of NO metabolites suggesting an important role of reduced renal NO bioavailability in TKI-induced hypertension.

P23 Cancer and melancholia: two "scourge" diseases of the modern world were historically connected

Halmurat Upur[1], Nurmuhammad Amat[1], Aynur Matsidik[1], Ayshamgul Hasim[2], **Batur Mamtimin[2]**, Denis Dubrovin[3]
[1]School of Traditional Uyghur Medicine and Pharmacology, Xinjiang Medical University, Urumqi 830011
[2]Central Laboratory, Xinjiang Medical University, Urumqi 830011, China
[3]1-st Pavlov's Medical University, Sankt-Petersburg 197000, Russia

In the ancient medicine cancer and melancholia were connected by one term – "a black bile" (literally in Greek – Μελανχολία). The "black bile" was considered to be the humor most implicated in cancer. Even if there are no other arguments for correlative relation between cancer and melancholic syndrome, one may be reminded of the strong and intriguing conviction held by Hippocratic school that the that there is a common cause of psychic disorders and cancer, which historically used to be called an 'abnormal black bile'. We selected 240 patients with a pathologically confirmed diagnosis of 20 different types of cancer, and classified them into "melancholic" group and "non-melancholic" group. ^{1}H nuclear magnetic resonance-based metabolomics approach was used to obtain plasma metabolic profiles of their blood samples. The results indicate that cancer patients with melancholic syndrome and those from "non-melancholic" group have similar metabolic changes, but the extent of these changes is different. Concentration of acid metabolites and levels of fat mobilization and glycolysis are higher and the damage to the immune function is greater in "melancholic" group, followed by "non-melancholic" group and healthy controls. At the very least, possible interrelation between psychic

melancholia and cancer is a subject for discussion. It could be illustrated by a lot of evidence from the primitive societies which cultivated folk medicines and where herbal remedies with proved anticancerogenic effects were commonly used. Therefore, it is possible to assume that cancer and "melancholia" may have some common biological foundation.

P24 Common Effects on Follicular Thyroid Cancer Cells Exerted by Simulated Microgravity

Benjamin Svejgaard, S. Kopp, T. J. Corydon, D. Grimm
Department of Biomedicine, Aarhus University, Aarhus, Denmark

This study focuses on gravity-sensitive proteins of two human follicular cancer cell lines (ML-1; RO82-W-1), which were exposed to simulated microgravity (s-µg). After a three (3d)- or seven-day-culture (7d) grown in s-µg, we found both cell types growing threedimensionally within multicellular spheroids (MCS) and also cells remaining adherent (AD) to the culture flask, while 1g- control cultures only formed adherent monolayers.
ML-1 cells grown in s-µg released significantly elevated amounts of IL-6 and MCP-1 into the supernatant when compared to controls. After 3d of s-µg, an accumulation of F-actin around the cellular membrane was detectable in AD cells of both cell lines. IL-6 and IL-8 stimulation of ML-1 cells for 3d and 7d influenced the protein contents of ß1-integrin, talin-1, Ki-67, and beta- actin dose-dependently in adherent cells.The ß1-integrin content was significantly decreased in AD and MCS samples compared with 1g, while talin-1 was higher expressed in MCS than AD populations. The proliferation marker Ki-67 was elevated in AD samples compared with 1g and MCS samples. The ß-actin content of R082-W-1 cells remained unchanged.
In conclusion, s-µg influences the release of cytokines in follicular thyroid cancer cells, and the production of ß1-integrin and talin-1, and predicts an identical effect under real microgravity conditions.

P25 Nitrite-dependent modification of mitochondria protects against oxidative damage in turtles

Amanda Bundgård, A.M. James, E.T. Chouchani, T. Bright, M.P. Murphy, A. Fago
Section for Zoophysiology, Department of Bioscience, Aarhus University, Aarhus, Denmark
MRC Mitochondrial Biology Unit, Cambridge University, Cambridge, United Kingdom

Reintroducing oxygen after a period of anoxia causes oxidative damage to tissues by production of reactive oxygen species (ROS). Recent evidence showed that inhibition of complex I in the electron transport chain by the post---translational modification S--- nitrosation diminishes ROS production and thereby oxidative damage. Metabolites are known to influence the production of ROS, and nitrite has specifically been shown to lead to S---nitrosation of proteins. Red---eared slider turtles (*Trachemys scripta elegans*) can survive anoxia for several months at low temperature, and is one of only few vertebrate species that can endure anoxia without tissue damage following reoxygenation. It has recently been shown that freshwater turtles naturally up---regulate nitrite during anoxia, and in this study I investigated whether the accumulated nitrite during anoxia leads to S---nitrosation of complex I and inhibition of ROS during reoxygenation. We found that turtles acclimated to anoxia had a significantly lower state 3 respiration rate than controls. Anoxic turtles had a lower complex I activity, and H2O2 production showed a tendency to diminish with lower complex I activity. In vitro treatment of isolated mitochondria with nitrite during anoxia lead to similar results, with lower complex I activity and H2O2 production. These results suggest that the high levels of nitrite during anoxia leads to inhibition of complex I in turtles, and that this is protective against oxidative damage.

P26 C57BL/6J mice are resistant to cardiorenal syndrome during high-salt and Angiotensin II treatment compared to Balb/c because of higher oxidative stress.

Sofia Jönsson[1], Mediha Becriovic Agic[1], Maria Tveitarås[2], Trude Skogstrand[2], Tine V. Karlsen[2], Åsa Liden[2], Sabine Leh[3], Bjarne M.Iversen (late)[4], Rolf K. Reed[2], Michael Hultström[1,2,5]
[1]*Department of Medical cellbiology, Uppsala University, Uppsala, Sweden*
[2]*Department of biomedicine, University of Bergen, Bergen, Norway*
[3]*Department of Pathology, Haukeland University Hospital, Bergen, Norway*
[4]*Institute of Medicine, University of Bergen, Bergen, Norway*
[5]*Anaesthesiology and Intensive Care Medicine, Uppsala University, Uppsala, Sweden*

Cardiorenal syndrome is the concurrent failure of heart and kidney function and is associated with high mortality. We investigated C57BL/6J and Balb/c mice after three days of 3%-sodium and 0.5µg/min/kg Angiotensin. C57BL/6J showed lower mortality (RR: 0.20, 95 % CI: 0.05-0.83), while Balb/c developed edema, decreasing blood pressure and urine volumes indicating cardiorenal failure. Ventricular catheterisation and echocardiography confirmed reduced cardiac function in Balb/c (-6.67±0.95 vs. -15.85±1.4 %, $p<0.05$) as well as fluid congestion. C57BL/6J appeared protected because of higher urine excretion during the combination treatment (0.46±0.02 vs. 0.24±0.04 ml/h, $p<0.001$). Microarray of RNA expression identified the antioxidant glutathione-transferase system as enriched (p=4.8e-6). Urinary TBARS confirmed higher oxidative stress in C57Bl/6J (0.019±0.001 vs. 0.009±0.001 nmol/24h, p=1.5e-3). Surprisingly the antioxidant N-Acetylcysteine (NAc, 150 mg/kg/24h) decreased urine excretion (0.32±0.04 vs. 0.15±0.05) and oxidative stress (0.012±0.0015 vs. 0.32±0.04) in C57BL6J during combination treatment but not in Balb/c ($p<0.05$). At the same time NAc increased mortality from 10 % to 60 % in C57BL/6J. In conclusion, combined treatment with high-salt and Angiotensin II is a new model of acute decompensated cardiorenal failure in Balb/c mice, while C57BL/6J are protected because of higher oxidative stress.

P27 Effects of reduced tetanic calcium concentration on the curvature of the force–velocity relationship in isolated rat soleus muscles.

Anders Meldgaard, O.B. Nielsen & K. Overgaard
Department of Sport Science, Aarhus University, Dalgas Avenue 4, DK-8000 Aarhus C, Denmark

Background: Increased curvature of the force-velocity relationship contributes to the loss of power in skeletal muscle during fatigue. The mechanism behind increased curvature has been proposed to be an increase in the proportion of cross bridges in the low force state, which accumulate due to a decrease in tetanic intracellular calcium concentration.
Consequently, we hypothesized that a decrease in tetanic $[Ca^{++}]_i$ would result in an increased curvature in isolated skeletal muscle.
Methods: Contraction force and velocity were measured in freshly isolated rat soleus muscles, in control conditions (incubated at 4 mM $[K^+]$ and stimulated at 60 Hz) and when exposed to three separate interventions which all are known to reduce $[Ca^{++}]$i: 20 mM dantrolene (N=6), 10 mM $[K^+]$ (N=6) and stimulations at 30 Hz (N=4).
For each condition a force-velocity curve was drawn by fitting force and velocity data to the Hill equation. The curvature was measured as a/F_o based on the Hill-equation parameters. A decrease in a/F_o was interpreted as an increased curvature.
Results: Dantrolene and stimulations at 30 Hz increased a/F_o with 39% and 60% respectively, compared to control conditions. However, no significant changes were observed when muscles were exposed to 10 mM [K+] in the fresh muscle.

Conclusion: Contrary to what was expected a reduced $[Ca^{++}]_i$ did not increase the curvature. In fact it seemed to decrease curvature. These results reject a simple causal relation between $[Ca^{++}]_i$ and accumulation of cross bridges in the low force state.

P28 Aggregation of isolated hemoglobins from the turtle, *Trachemys scripta*.

Asbjørn G. Petersen and A. Fago
Zoophysiology, Department of Bioscience, Aarhus University, Aarhus, Denmark

Reports of hemoglobin (Hb) aggregation as in polymers or crystals have been observed in all groups of the higher vertebrates. This aggregation can lead to a phenomenon termed 'supercooperativity' ($n50 > 4$) and in some situations drastically change the shape of red blood cells (RBCs). It is however relatively unknown under what conditions this aggregation occurs.
The aim of this study was to investigate how different conditions (pH, Hb concentration and the presence of an organic phosphate) affected the degree of aggregation in Hbs from the turtle, *Trachemys scripta*. Using fast protein liquid chromatography (FPLC) we separated and isolated the two Hb isoforms, HbA and HbD. We found that the HbD isoform aggregated faster compared to HbA, and that a lower pH and a higher Hb concentration enhanced the processes of polymerization. The aggregation process depended on the addition of ATP. Furthermore, we observed a higher autooxidation of the HbD compared to HbA under the same conditions. This suggests a link between aggregation of Hbs and an accelerated rate of autooxidation which needs further investigation.
This study shows that pH, Hb concentration and ATP do affect aggregation of Hbs. However, to what extent aggregation of Hbs occur in free living turtles still remains to be determined.

P29 Comparison of circulating hydrogen sulfide and nitric oxide metabolites and their potential roles in brown bear hibernation

Inge G. Revsbech[1], X. Shen[2], R. Chakravati[3], F. B. Jensen[4], B. Thiel[5], O. Frøbert[6], D. J. Stuehr[3], C. G. Kevil[2], A. FAGO[1]
[1]*Department of Bioscience, Aarhus University,8000 Aarhus C, Denmark*
[2]*Department of Pathology, Louisiana State University Health Sciences Center, Shreveport, LA71130,USA*
[3]*Department of Pathobiology, Cleveland Clinic Lerner Research Institute, Cleveland, OH 44195, USA*
[4]*Department of Biology, University of Southern Denmark, 5230 Odense M, Denmark*
[5]*Department of Medicine, Case Western Reserve University, Cleveland, OH 44106, USA*
[6]*Department of Cardiology, Örebro University Hospital, 70362 Örebro, Sweden*

Mammalian hibernation is a physiological wonder of temporarily downregulated metabolism, during which the animal does not eat and remains inactive for months. Understanding theunderlying biochemical mechanisms may have great translational medical applications. As ubiquitous inhibitors of mitochondrial metabolism, both nitric oxide (NO) and hydrogen sulfide (H_2S) could in principle play a part in the whole body metabolic depression essential to hibernation. We investigated type and content of blood metabolites of NO and H2S in winter hibernating and summer active, free-ranging brown bears. We found significant changes in composition of sulfide metabolites in plasma, with a decrease in plasma thiosulfate and polysulfides during hibernation, indicating that whilst hibernating, the bear may regenerate H_2S from its oxidation products, thiosulfate and polysulfides. Concurrently, high levels of free sulfide correlated with high levels of cysteine, suggesting that cysteine may be prioritized for glutathione synthesis during hibernation. Thus, this remodeling of sulfide metabolism may work to preserve plasma free cysteine for the generation of glutathione in cells, a central antioxidant also found in high levels in red blood cells during hibernation. For NO, no clear changes could be

measured in circulating nitrite or in the degree of S-nitrosylation of glyceraldehyde-3-phosphante dehydrogenase, although this remains to be investigated further. Our study revealed that circulating H_2S potentially contributes to inhibition of mitochondrial respiration during hibernation.

P30 Pump those ions or you'll wake up dead: Key differences in how chilling affects tropica and temperate *Drosophila* species

Heath A. MacMillan[1], Jonas L. Andersen[1], Shireen A. Davies[2] and Johannes Overgaard[1]
[1]*Department of Bioscience, Aarhus University, Aarhus, Denmark*
[2]*Institute of Molecular, Cell and Systems Biology, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, United Kingdom.*

Environmental temperature is a key predictor of insect distribution and abundance, but we lack and integrative understanding of why exposure to extreme temperatures causes tissue damage and death in insects. Most insect species (including those of the model genus, *Drosophila*) are chill susceptible, meaning they enter a cold-induced coma (chill coma) and succumb to physiological effects of low temperature unrelated to freezing. For such species, cold exposure causes a loss of Na+ and water balance and a progressive rise in extracellular [K+] that is closely correlated with the onset of injury and death. Using five *Drosophila* species with markedly different cold tolerance (and distribution) we clearly demonstrate that disruption of ion and water balance is a primary driver of low temperature injury; chill-tolerant species maintain ion and water homeostasis during a cold exposure while chill susceptible species fail to regulate ion balance, leading to injury and death. These physiological differences at the species-level mirror those induced by thermal acclimation in *D. melanogaster*. To further examine the differences in osmoregulatory performance we measured the activity of the Malpighian tubules (the insect "kidney") in all five species across a range of temperatures, and discuss these findings in relation to their ability maintain ion and water balance in the cold.

P31 Scanning mutagenesis of transmembrane segments M5 and M6 of the mammalian flippase ATP8A2 – evidence for a classic transport pathway?

Stine A. Mikkelsen[1], Anna L. Vestergaard[1], Jonathan A. Coleman[2], Bente Vilsen[1], Robert S. Molday[2], and Jens Peter Andersen[1]
[1]*Dept. of Biomedicine, Aarhus University, DK-8000 Aarhus C, Denmark*
[2]*Dept. Biochemistry and Molecular Biology, University of British Columbia, Vancouver V6T 1Z3 Canada*

P-type ATPases are a large family of membrane proteins that transport substrates uphill across membranes at the expense of ATP. This family constitutes the well characterized P2-type ATPases that transport ions "ion-pumps", such as the Na^+,K^+- and Ca^{2+}-ATPase. Another branch of the family is the P4-type ATPases "flippases" that transport large phospholipids instead of inorganic ions. We know from the ion-pumps that transmembrane segments M5 and M6 contain residues directly involved in coordination and binding of the ions, and are central mechanistic elements. During ATP hydrolysis movements of the cytoplasmic domains are transferred down in the membrane domain, which makes M5 and M6 highly dynamic during the transport of the ions through the membrane. ATP8A2 is a flippase that translocates the aminophospholipids phosphatidylserine and phosphatidylethanolamine from the exoplasmic to the cytoplasmic side of the plasma membrane. We have here investigated the importance of M5 and M6 in ATP8A2. We aligned the residues in ATP8A2 with several of the ion-pumps to locate M5 and M6 of ATP8A2 and replaced all residues of M5 and M6 individually with alanine (alanine scanning) and analyzed the expression level and phosphatidylserine-activated ATP hydrolysis of all the mutants. Two particularly interesting residues, N905 and T909, were further characterized by studying the individual steps of the reaction cycle. These residues are highly conserved among

flippases and align with ion binding residues of the ion-pumps. Our analysis of N905 and T909 mutants showed that these residues are highly important for substrate affinity and activation of dephosphorylation in ATP8A2.

P32 TRP channels function is tightly regulated during early embryo development

Ingrid Carvacho[1,2], H. C. Lee[3], R. A. Fissore[3] and K. Lykke-Hartmann[1,2,4]
[1]Department of Biomedicine, Aarhus University, Denmark.
[2]Centre for Membrane Pumps in Cells and Disease-PUMPKIN, Danish National Research Foundation, Department of Molecular Biology and Genetics, Aarhus University, Denmark.
[3]Department of Veterinary and Animal Sciences, University of Massachusetts, Amherst, MA 01003, USA.
[4]Aarhus Institute of Advanced Studies, Aarhus University, Denmark.

The processes that support the transition of a mature oocyte ready-to-be-fertilized (MII oocyte) to a zygote (fertilized oocyte) are together known as egg activation. In mammals, calcium influx is required during oocyte maturation to refill internal stores and for complete egg activation. The molecular identity and function of the calcium-permeant channel(s) that underlie this influx during maturation and fertilization are not established. Cationic nonselective TRP channels are widely expressed and are modulated by a variety of stimuli and ligands, including G-protein coupled receptors. A member of the TRP channel family, TRPV3, was reported as a mediator of calcium influx in mouse MII oocytes. Selective activation of TRPV3 channels provokes egg activation (parthenogenesis) by mediating massive calcium entry. Here, using biochemistry and calcium imaging assays, we explored the regulation of the expression of TRPV3 channels during oocyte maturation. We also identified a second cationic non-selective channel with differential functional expression during oocyte maturation. Using electrophysiology (patch-clamp, whole cell configuration) we found that the non-selective current is potentiated by high concentrations of 2-Aminoethoxydiphenyl borate (2-APB) and external acidic pH. The channel is blocked by $MgCl_2$ and specific TRP channels blockers. Our results suggest functional expression of more than one member of the TRP channel family in mouse MII oocytes and zygotes. The orchestrated activity of these channels could modulate the filling of the stores during maturation, maintain calcium oscillations during egg activation and support calcium influx post-fertilization

P33 Is the potassium channel ROMK suppressed in patients with proteinuria?

Rikke Ydegaard,[1] P. Svenningsen,[1] J. Stubbe,[1] N. Marcussen,[2] R. Zamani,[3] B.L. Jensen[1]
Department of [1]Renal and Cardiovascular research, Institute of Molecular Medicine, University of Southern Denmark, Odense, Denmark and departments of [2]Clinical Pathology and [3]Urology, Odense university hospital, Odense, Denmark.

Proteinuria is associated with impaired urinary sodium excretion, hypertension and increased activity of the epithelial Na^+ channel (ENaC). Hyperactive ENaC predicts loss of potassium, but hypokalemia is not typically observed. Studies have suggested that proteinuria down-regulates the aldosterone-sensitive K^+-channel, ROMK, in kidneys. We tested the hypotheses that 1) ROMK is stimulated by aldosterone in human kidney; 2) Renal ROMK is suppressed in patients with proteinuria.
ROMK protein level and localization in human kidney was investigated by immunoblotting and immunohistochemistry (Antibodies from NovusBiologicals, Abcam and Alomone were tested). Cancer nephrectomy samples with presurgery high aldosterone (diuretic treatment), low aldosterone (ACEi or ARB treatment) and with proteinuria (dipstick) were compared to tissue from patients given no medication ("control").
With an anti-ROMK antibody from NovusBiologicals, immunoblotting yielded a single protein with kidney homogenate at the predicted molecular mass of ~45 kDa. Densitometry signal increased linearly with loaded

protein. Protein abundance of ROMK was significantly higher in kidneys from patients treated with diuretics (n=4) compared to controls (n=6). There was no significant difference in ROMK protein abundance in tissue from patients treated with ACEi/ARBs compared to controls. In kidney from patients with proteinuria, ROMK abundance was significantly higher than controls. Immunostaining of human kidney sections showed distinct staining in subsets of cortical tubules, predominantly associated with apical membranes, likely of collecting ducts and thick ascending limb of Henles loop.
In conclusion, human kidney cortex ROMK protein abundance is changed in accordance with positive regulation by aldosterone. Proteinuria is not associated with down-regulation of ROMK.

P34 The in vitro NCX1 interactome

Tandekile Lubelwana Hafver[1,2], G.A. De Souza[3], M. Lunde[1,2], M. Martinsen[1,2], O.M. Sejersted[1,2], C.R. Carlson[1,2]
[1]Institute for Experimental Medical Research, Oslo University Hospital and University of Oslo, Norway
[2]KG Jebsen Cardiac Research Center and Center for Heart Failure Research, University of Oslo, Norway [3]Centre for Immune Regulation, Department of Immunology, University of Oslo, Norway

Introduction: The sodium (Na^+) – calcium (Ca^{2+}) exchanger (NCX), is a ubiquitously expressed ion-transporting protein that plays an important role in maintaining cytosolic Ca^{2+} homeostasis. Splice variant NCX 1.1 (referred to as NCX1) is cardiac specific, and is implicated in excitation-contraction coupling in cardiomyocytes. Mammalian NCX1 is organised with 9 transmembrane domains (TM), with a ~ 550 amino acid cytosolic loop between TM5 and TM6 that associates with various cytosolic factors and mediates regulation of the exchanger. In this project we screen for novel NCX1 protein interacting partners, which may serve as targets for therapeutic intervention in pathophysiological conditions.
Methods and results: We used an epitope mapped NCX1 antibody to precipitate NCX1 in left ventricle lysates. As negative control for the immunoprecipitation experiments we used a NCX1 blocking peptide and a non-relevant antibody. The captured protein complexes were further identified by mass spectrometry analysis. In total, we have identified 208 putative NCX1 interaction partners after excluding common contaminants (ribosomal and mitochondrial proteins). The biological processes that the candidate partners are involved in include: cell growth and/or maintenance, metabolism, signal transduction, energy pathways and transport. Several of the putative partners have been cloned and are currently being validated for binding to the NCX1 protein in HEK293 cells. A protein-protein interaction (PPI) map for the NCX1 interactome is under construction.
Conclusion: Identification of new NCX1 interacting partners will extend our understanding of NCX1 regulation, and may suggest novel therapeutic strategies for intervention in pathophysiological conditions.

P35 Modulation of GABAA receptor-mediated synaptic and tonic currents in the rat hippocampus by GLP-1, exendin-4 and diazepam

Sergiy V. Korol, Z. Jin, O. Babateen, **B. Birnir**
Department of Neuroscience, Uppsala University, BMC, Box 593, 751 24, Uppsala, Sweden

Glucagon-like peptide-1 (GLP-1) is an intestinal hormone that stimulates insulin secretion from β-cells in a glucose-dependent manner and decreases glucagon release from α-cells in the islets of Langerhans. GLP-1 receptors are detected not only in peripheral tissues but also in many brain regions including the hippocampus. The hippocampus is known centre for learning and memory. Recently we have showed that GLP-1 and its analogue exendin-4 modulate γ-aminobutyric acid (GABA) signaling in the hippocampal CA3 pyramidal neurons (Korol *et al*, 2015a). We studied how GLP-1, exendin-4 and diazepam, the positive allosteric modulator of the GABAA receptors affected GABAA receptor-mediated synaptic and tonic currents in the cells. Hippocampal

slices from 16–20 days old Wistar rats and whole-cell patch-clamp method were used to register GABAA receptor-mediated currents in the CA3 pyramidalcells. GLP-1 transiently augmented the amplitudes and frequency of the spontaneous inhibitory postsynaptic currents (sIPSCs) as well as enhanced the GABAA receptor-mediated tonic current. Diazepam caused increase in amplitudes and frequency of the sIPSCs and continuous potentiation of GABAA receptor-mediated tonic current. Uninterrupted consecutive co-administration of exendin-4 and diazepam evoked additional enhancement in neither frequency, nor amplitudes of the sIPSCs but transiently increased the tonic current amplitude (Korol *et al*, 2015b). The data show that GLP-1and its analogues enhances IPSCs and subpopulation of extrasynaptic GABAA receptors in hippocampal CA3 pyramidal neurons. These results further imply that metabolic hormones influence hippocampal function.
References Korol SV, Jin Z, Babateen O, Birnir B. *Diabetes*. 2015a;64(1):79-89. Korol SV, Jin Z, Birnir B. *PloS One*. 2015b;10(4):e0124765.

P36 Insulin modulates GABAA receptor-mediated neuronal inhibition in rat hippocampus and amygdala

Zhe Jin, S. V. Korol, Y. Jin, **B. Birnir**
The Division of Molecular Physiology and Neuroscience, Department of Neuroscience, Uppsala University, 75124, Uppsala, Sweden

Insulin, the pancreatic islet hormone, not only regulates blood glucose, but also acts in brain modulating neuronal excitability and memory functions. The main inhibitory neurotransmitter GABA (gamma-aminobutyric acid) activates $GABA_A$ receptors generating phasic and tonic inhibition, which fine-tunes neuronal excitability and network activity. The hippocampus and amygdala, two medial temporal lobe structures, both participate in memory formation. We study the insulin action on $GABA_A$ receptors-mediated neuronal inhibition in the hippocampus and amygdala. Quantitative RT-PCR was run on samples from rat and human post-mortem brain samples. Immunohistochemistry for insulin receptor and electrophysiological recordings were performed on rat brain slices. Our results show that the insulin receptor mRNA is present in both rat and human hippocampal and amygdala samples. Immunostaining of the insulin receptor was observed in rat hippocampal and amygdala neurons. Insulin enhanced the $GABA_A$-mediated tonic conductance in rat hippocampal CA1 neurons by turning on high-affinity $GABA_A$ receptors (Jin *et al*, 2011). In rat amygdala neurons, acute application of insulin increased $GABA_A$-mediated synaptic currents and reduced the action potential firing frequency. These data demonstrate that insulin modulates the inhibitory GABAergic transmission in both the hippocampus and amygdala, and provide a putative cellular mechanism via which insulin improves cognitive function in the brain.
Reference Jin Z, Jin Y, Kumar-Mendu S, Degerman E, Groop L, Birnir B (2011). Insulin reduces neuronal excitability by turning on GABA(A) channels that generate tonic current. *PLoS One* 6(1): e16188.

P37 ComparingATP1A3 mutations causing neurological diseases

Christian Philip Rønn, Vivien R. Schack, Mads Schak Toustrup-Jensen, and Bente Vilsen
Department of Biomedicine, Aarhus University, Denmark

Recent studies have shown that mutations in the ATP1A3 gene, encoding the alpha subunit of the Na+/K+-ATPase, are associated with the severe neurological diseases rapid-onset dystonia-parkinsonism (RDP), alternating hemiplegia of childhood (AHC) and cerebellar ataxia, areflexia, pes cavus, optic atrophy and sensorineural hearing loss (CAPOS) syndrome. The mutations are, with one exception, found to be disease-specific, even though some AHC and RDP mutations affect the very same residues. It has been speculated that, in fact, the diseases are not separate disorders but rather represent a phenotypic continuum of one underlying disease with RDP in the less severe end of the scale and AHC in the other.

Now, having identified the disease-causing mutations, the next step is to functionally characterize the mutations by functional assays.
We have introduced selected RDP, AHC, and CAPOS mutations into mammalian COS-1 cells, in order to allow a functional characterization of the recombinantly expressed mutant enzyme. All of the mutants of our study were successfully expressed transiently in the plasma membrane. However, we find most of the mutants unable to sustain cell growth under ouabain selection pressure, thus indicating that the ion-transporting properties are severely disturbed.

P38 The mRNA expression of GABA-A, GABA-B receptor subunits and chloride transporters in peripheral blood mononuclear cells is influenced by gender, pregnancy and depression.

A. Bhandage[1], C. Hellgren[2], Z. Jin[1], E. Olafsson[1], I. Sundstrom[2] and **B. Birnir[1]**.
[1]Department of Neuroscience, Uppsala University, Sweden;
[2]Department of Women's and Children's Health, Uppsala University, Sweden.

Lymphatic vessels detected in the meninges indicate a direct link between the nervous and the immune system by which immune cells can travel and affect brain. The receptors for γ-aminobutyric acid (GABA), major neuroinhibitory transmitter in CNS, can be expressed by immune cells. GABA is also present in blood where it may act as natural immunomodulator of circulating immune cells. GABA activates GABA-A receptors that are GABA-gated chloride channels in plasma membrane and GABA-B receptors that are G-protein coupled receptors. Here, we studied in human peripheral blood mononuclear cells (PBMCs) if the expression of the 19 GABA-A, 2 GABA-B receptor subunits and 6 chloride transporters was influenced by gender, pregnancy and depression. RT-qPCR was used to determine the mRNA expression level in PBMCs from men, non-pregnant women, healthy and depressed pregnant women. Among GABA-A receptor subunits, ρ2 subunit had highest expression level. In pregnancy, δ and ρ2 subunits were upregulated. The ε subunit was most frequently expressed in healthy pregnant women whereas α6 and γ2 subunits were most frequently expressed in non---pregnant women. Depression in pregnant women altered the expression of β1 and ε subunits. The GABA-B1 receptor was up-regulated by depression in pregnant women, while the transporters NKCC1 and KCC4 were down-regulated in pregnancy. Our results imply participation of GABA receptors in establishing and maintaining tolerance in pregnancy. The correlation of mental health with the expression of specific receptor subunits reveals a connection between the immune cells and the brain. Biomarkers for mental health may be identified in PBMCs.

P39 More severe structural alterations in the cirrhosis phase of the Abnormal Savda syndrome-based hepatocarcinoma

Abuduwyite Reshalaiti[1], B. Sikandeer[2], H. Reshalaiti[2], W. Hamulati[3]
[1]Department of Ultrasound, The First Affiliated Hospital of Xinjiang Medical University, Urumqi, China
[2]Department of Biochemistry and Molecular Biology, School of Basic Medical Sciences, Xinjiang Medical University, Urumqi, China
[3]College of Uyghur Medicine, Xinjiang Medical University, Urumqi, China

Background: Recently, the complementary and alternative medicine has attracted interests in the treatment of the complicated diseases including liver cancer. However, as one of the ethnic traditional medicine in China, the Traditional Uyghur Medicine believes that Abnormal Savda is the foundation for many chronic diseases. In this study, we have examined the effect of Abnormal Savda syndrome on the development of hepatocarcinoma in rat models.

Methods: Abnormal Savda syndrome was established in randomly grouped healthy Wistar rats by following the humoral theory of the Traditional Uyghur Medicine, and then the hepatocarcinoma was induced using diethylnitrosamine (DEN). After sacrifice, the liver tissues from the negative control (normal), positive control (hepatocarcinoma) and Abnormal Savda syndrome---based hepatocarcinoma rats were taken to detect the expression of the target genes using immunohistochemistry together with antibodies.
Results: Expression of the target genes including p53, p21, STAT3 and cyclin D1 in the phase of cirrhosis were significantly up--- regulated in the liver tissues of the Abnormal Savda syndrome---based hepatocarcinoma rats compared with the negative controls ($p<0.01$), and the positive controls ($p<0.05$) respectively.
Conclusion: Abnormal Savda may accelerate the occurrent and the development of the hepatocirrhosis by up---regulating p53, p21, STAT3 and cyclin D1 and effecting on the cell cycle progression.
Financial support: The National Natural Science Foundation of China (project number: 81160545)

P40 Effect of Abnormal Savda Munziq on Hepatocarcinoma rat model

Mahmut·Mijit, Kalbinur·Arken[1], Guo Xia, Halmurat.Upur[2]
Xinjiang Medical University, Department of Pharmacology, College of Preclinical[1]
Faculty of traditional Uyghur medicine, Urumqi 830011, China[2]

This paper aims to study the effect of abnormal savda munziq on the inducing and developing process of hepatocarcinoma, investigate the general manifestation, emotional response or mental stress and observe the pathological change of rat liver after using abnormal savda munziq. We established the hepatocarcinoma rat model using Diethyl nitrosamine (DEN). Samples were prepared in 6 groups, which are blank group; black control group; hepatocarcinoma group; hepatocarcinoma + Abnormal Savda Munziq, with drug intervention groups injecting high, medium and low doses respectively. The general manifestation, emotional response or mental stress, weight differences of each group and pathological changes of the liver were observed dynamically in the process of experiment period. The results show that the rats of disease groups exhibited irritability, loss of appetite, cyanosis, severe weight loss, fur gloss decrease and deteriorated lesion characteristics in the experiment period. Besides, the surface of the liver is rough or slightly granular and slightly dull, also show signs of liver congestion and necrosis of liver cells which manifested as liver cirrhosis or liver cancer. While in abnormal savda munziq intervention group, the symptoms can be ameliorated with different degrees. Besides, weight fluctuation of intervention group is relatively stable and the difference was statistically significance ($P<0.01$). The characteristics and process of hepatocarcinoma which was induced by DEN are similar to human`s. It shows that the model establishment is stable and reliable. Plus, abnormal savda munziq can protect and recover the morphological damage of hepatic tissue in hepatocarcinoma and which also can ameliorate the general manifestation, emotional reactions, mental state or psychological stress with certain degree of efficacy in hepatocarcinoma.

P41 Synergistic Attenuation of Abnormal Savda Munziq Combination with 5-Fluorouracil on Transplantated cervical cancer (U27) Tumor

Tao Yang[1], Halmurat Upur[2], Yusup Abdiryim[2], Omarniyaz Zuhragul[1], Lianlian Shan[1], Ainiwaer Aikemu[3]
[1]Department of Drug Analysis, Faculty of Pharmacy, Xinjiang Medical University, Urumqi Xinjiang, China
[2]Traditional Uighur Medicine and Pharmacology, Xinjiang Medical University, Urumqi, Xinjiang, China
[3]Central Laboratory of Xinjiang Medical University, Urumqi, Xinjiang, China

Abnormal Savda Munziq (ASMq) is a traditional prescription of Uighur Medicine, its treatment of complex diseases such as tumor and asthma has been proved to be effective in Uygur medical clinical practice, this work

is to study the efficacy enhancing and toxicity reducing of ASMq on mice who are treated with 5-fluorouracil on transplantated U27 tumor. Establishment of tumor model for cervical cancer (U27), the transplantated U27 tumor model mice were randomly divided into 6 groups:the normal control group, the tumor control group, the 5-fluorouraeil（5-FU）group, the high dose ASMq + 5-FU group, the middle dose ASMq + 5-FU group, and the low dose ASMq + 5-FU group. The thymus and spleen were collected for determination of the organ index, the changes of T lymphocyte proliferation in spleen were detected by MTT test, Serum interleukin-2(IL-2), interferon-gamma(IFN-γ) levels were measured by ELISA methods. The expression of TNF-α protein was detected by Western blot technology. Observe the pathological changes of the liver. ASMq increased thymus index and spleen index significantly; the serum concentrations of IL-2, IFN-γ were also increased by ASMq. The TNF-α protein expression were increased. The ASMq can improve liver central vein hyperemia and interstitial edema, also can recover the radioactive structure.
Acknowledgments: This investigation was supported by research grants from the National Natural Science Foundation of China (No.81360668) and Jiangxi national traditional medicine collaborative innovation of China (No.JXXT201404001) and Xinjiang Medical University “Tianshan excellence” project（No.Y0382023

P42 Dietary intake of coriander and spinach increases the risk of intensifying the inflammation in the rat models of DNCB---induced ulcerative colitis

Zhang Jingping[1], Y. Kurexi[1], H. Jing-Jing[1], ZH. Li[1], A. Mairipaiti[1], N. Ajiguli[1], M. Gulinisha[1], A. Kasimujiang[1], Y. Buweayixiemu[1], A. Adila[1], A. Halidan[1], A. Abulaiti[1], W. Hamulati[2]
[1]*Department of Biochemistry and Molecular Biology, School of Basic Medical Sciences, Xinjiang Medical University, Urumqi, China*
[2]*College of Uyghur Medicine, Xinjiang Medical University, Urumqi, China*

Background: It has been shown that dietary intake of certain kinds of vegetables can affect on the inflammatory bowel diseases. Here we have investigated the potential effect of coriander and spinach on the 2,4-dinitrochlorobenzene (DNCB)- induced ulcerative colitis of model rats.
Methods: Healthy male Wistar rats were randomly divided as normal (NG), ulcerative colitis (UC) and vegetable-based diet groups - VNG and VUC. Addition to their normal diet, the VNG and VUC groups were supplied with coriander and spinach for 40 days before inducing the UC and VUC models with DNCB. Histological techniques and NMR spectroscopy were used to analyze the target samples.
Results: Histological analysis showed no significant differences between the NG and VNG groups, whereas, the signs of inflammation in the VUC group were more severe compared with the UC group ($P<0.05$). The NMR-based metabolomics analysis of serum showed number of differential metabolites, which are mainly glucose, lipids, energy metabolism related molecules and amino acids. Remarkable changes in the amount (increased or decreased) of these detected metabolites were seen in the treated groups (VNG and VUC).
Conclusion: Our primary results clearly indicate that the coriander and spinach diet can intensify the inflammation in the intestine, but understanding of its underlying mechanisms remains to be further studied.
Financial support: National Natural Science Foundation of China (NNSF, Project number: 81260564)

P43 Healing effect of Xipayi Kui Jie-an on the pathology and ultrastructure of colon tissues in model rats of Abnormal Sapra with ulcerative colitis

Wufuer Hamulati[1], A. Mairipaiti[2], ZH. Jingping[2], ZH. Li[2], H. Jingjing[2], M. Gulinisha[2], N. Ajiguli[2], A. Kasimujiang[2], A. Halidan[2], Y. Buweayixiemu[2], A. Adila[2], A. Abulaiti[2], Y. Kurexi[2]
[1]*College of Uyghur Medicine, Xinjiang Medical University, Urumqi, China*
[2] *Department of Biochemistry and Molecular Biology, School of Basic Medical Sciences, Xinjiang Medical University, Urumqi, China*

Background: Xipayi Kui Jie-an (XKJ) is a kind of herbal compound introduced by the Traditional Uyghur Medicine in western China, it has been used to treat Abnormal Sapra syndrome based chronic inflammatory diseases. The aim of this study was to find structural and ultrastructural evidence for the therapeutic effect of XKJ in the intestinal tissues of bowel disease.
Methods: Animal model of Abnormal Sapra with ulcerative colitis (Abnormal Sapra UC) was established by inducing both Abnormal Sapra syndrome and UC in the healthy male Wistar rats. The procedures were followed in accordance with the humoral theory of Traditional Uyghur Medicine and the descriptions about the DNCN (2,4-dinitrochlorobenzene) induced UC in the literatures. Model Rats were randomly divided and treated with XKJ or distilled water for 20 days by injecting the substances via a clyster to the lower bowel daily. Conventional UC model rats were used as negative controls. Target tissues of sacrificed animals were analyzed using histopathology techniques together with light microscopy and Transmission Electro Microscopy (TEM).
Results: Both H&E stained preparations and the samples analyzed by TEM showed more severe signs of inflammations in the Abnormal Sapra UC compared with the UC group, and after treatment with the XKJ, compared with the negative controls, a significant improvement was seen.
Conclusion: Our results suggested that the Uyghur herbal medicine Xipayi Kui Jie-an has the healing effect on the Abnormal Sapra syndrome based ulcerative colitis. Various studies are needed to further evaluate the effectiveness of the compound.
Financial support: National Natural Science Foundation of China (NNSF, Project number: 81260564)

P44 Effects of Xipayi Kui Jie-an on the serum metabolites of the model rats of Abnormal Sapra with ulcerative colitis

Yunusi Kurexi[1], A. Mairipaiti[1], ZH. Jingping[1], H. Jingjing[1], M. Gulinisha[1], N. Ajiguli[1], A. Kasimujiang[1], A. Halidan[1], Y. Buweayixiemu[1], A. Adila[1], A. Abulaiti[1], W. Hamulati[2]
[1]*Department of Biochemistry and Molecular Biology, School of Basic Medical Sciences, Xinjiang Medical University, Urumqi, China*
[2]*College of Uyghur Medicine, Xinjiang Medical University, Urumqi, China*

Background: The Uyghur herbal compound Xipayi Kui Jie-an (XKJ), in western China, thought to be an effective medicine for the Abnormal Sapra syndrome-based chronic diseases including ulcerative colitis (UC). In this study, we have examined the therapeutic effect of XKJ on the rat models of Abnormal Sapra with UC.
Methods: Rat models of Abnormal Sapra syndrome were established according to the humoral theory of the Traditional Uyghur Medicine and induced ulcerative colitis using the combination of 2,4-dinitrochlorobenzene (DNCB) and acetic acid (AA). XKJ was applied with clyster and injected to lower bowel of the randomly grouped Abnormal Sapra, UC and Abnormal Sapra UC rats for 20 days. Serum was analyzed using NMR---based spectroscopy. Histological approach was applied for detecting inflammatory responses.
Results: More severe inflammation was detected in the colon tissues of the Abnormal Sapra UC rats compared with the Abnormal Sapra and the UC negative control groups. Restoration of the normal histology in the colon

tissues was seen after treatment with XKJ. Significant alterations were seen in more broad ranges of metabolites in the Abnormal Sapra UC rats, and this was improved by the XKJ treatment ($P<0.05$).
Conclusion: For the first time, we have successfully established the rat models of Abnormal Sapra with ulcerative colitis and applied NMR-based metabolomics analysis. Our findings indicate that Xipayi Kui Jie-an has both preventive and therapeutic effect on the Abnormal Sapra with ulcerative colitis. More studies are needed to address how and why XKJ plays such a role in the system.
Financial support: National Natural Science Foundation of China (NNSF, Project number: 81260564)

P45 The diversity of Intestinal microflora in differentiation of persons with different hilits of Traditional Uighur Medicine

Yimiti Delixiati[1], A.Azierguli[1], T.Tuerhung[2], T.Nadire[3], N. Sikandeer[4], M.Zilaiguli[1], CH.Feng[1], S.Yuping[1], W.Hamulati[2]
[1]*Department of Microbiology, School of Basic Medical Sciences, Xinjiang Medical University, Urumqi*
[2]*College of Uyghur Medicine, Xinjiang Medical University, Urumqi, China*
[3]*Department of Ultrasound, First Affiliated Hospital of Xinjiang Medical University, Urumqi, China* [4]*Department of Gastrointestinal Surgery, People's Hospital of Xinjiang, Urumqi, China*

Objective: Analysis of the intestinal microflora diversity to provide microbiological evidence for categorization of healthy subjects into four groups based on the hilit theory of Traditional Uighur Medicine.
Method: We enrolled 206 healthy subjects for this study and divided them into four groups khan, balgham, sawda and sapra, according to the Traditional Uygur Medicine and collected fecal samples. Total genomic DNA extracted from the samples was used for PCR amplification of 16S rRNA V6-V8 regions and analyzed by denaturing gradient gel electrophoresis (DGGE). Based on the DGGE profiles, we compared the intestinal micro flora similarity and the diversity of all subjects representing four hilit groups. We also sequenced the major bands of DGGE analysis to identify bacterial species and generated phylogenetic trees of gut microflora for comparison among groups.
Results: We found differences in the composition of intestinal microflora in each hilit group. There was increased diversity of intestinal microbial communities of khan hilit, and yet there was reduced diversity of intestinal microflora of balgham hilit compared with other groups.
Conclusion: There were differences in the intestinal microbial diversity and composition of persons with different hilit groups. This study established initial phylogenetic trees of intestinal microflora of the healthy subjects representing four different hilits classified according to the Traditional Uighur Medicine and provide microbiological evidence for differentiation of healthy subjects into four different hilit groups.
Financial support: National Natural Science Foundation of China (NNSF, Project number: 81460673)

P46 Sequential Glycan Profiling at Single Cell Level with the Microfluidic Lab-in-a-Trench Platform

Tríona M. O'Connell[1,2], **Damien King[3],** Arnaud Coudray[3,4], Laura Santana González[3,4], Jens Ducrée[3,4], Brendan O'Connor[1,2], Dermot Walls[1,2]
[1]*School of Biotechnology, Dublin City University, Ireland.*
[2]*Irish Science Seperation Cluster (ISSC), Dublin City University, Ireland.*
[3]*School of Physical Sciences, Dublin City University, Ireland.*
[4]*Biomedical Diagnostics Institute (BDI), Dublin City University, Ireland.*

It is now widely recognised that the earliest changes that occur on a cell when it is stressed or becoming diseased are alterations in its surface glycosylation. Techniques for the glycoprofiling of the surfaces of single cells are either limited to the analysis of large cell populations or are unable to handle multiple and / or

sequential probing. Here, we report a novel approach of single live cell glycoprofiling enabled by the microfluidic "Lab-in-a-Trench" (LiaT) platform for performing capture and retention of cells, along with shear-free reagent loading. The significant technical improvement on state-of-the-art is the demonstration of consecutive profiling of glycans on a single cell by sequential elution of the previous lectin probe using their corresponding free sugar.
The merely diffusion-based reagent exchange on the microfluidic "Lab-in-a-Trench" (LiaT) platform permits the observation of cellular changes upon lectin binding and elution in real time. We have used this shear-free micro-confinement environment to perform sequential lectin binding on individual Ramos B-cells. Common microfluidic systems for cell based assays are open or operate in continuous-flow mode. However, by creating a flow-free, 4-nL sub-compartment in the microchannels, the LiaT platform allowed us to handle small cell samples and to deliver reagents to the cell surface while continuously monitoring and tracking individual cells.
We have qualitatively analysed glycan density on the surface of individual cells. This has allowed us to qualitatively co-localise the observed glycans. This approach enables exhaustive glycoprofiling and glycan mapping on the surface of individual live cells with multiple lectins.

P47 Utilising the Microfluidic Lab-in-a-Trench Platform to Enable Single Cell Level Drug Screening of Hepatocytes

Damien King[1], Macdara Glynn[1,2], Sandra Cindric[1,2], Richard Lalor[2], Tríona M. O'Connell[3],4, Jens Ducrée[1,2].
[1]*School of Physical Sciences, Dublin City University, Ireland.*
[2] *Biomedical Diagnostics Institute (BDI), Dublin City University, Ireland.*
[3]*School of Biotechnology, Dublin City University, Ireland.*
[4]*Irish Science Seperation Cluster (ISSC), Dublin City University, Ireland.*

Utilising the diffusion-based reagent exchange on the microfluidic "Lab-in-a-Trench" (LiaT) platform permits the observation of cellular changes upon drug treatment in real time. We use for the first time this shear-free micro-confinement to perform drug screening on individual hepatocytes towards improved treatment of liver cancer. The significant technical advancement on state-of-the-art presented here is the consecutive, real-time drug screen-ing on a cell-by-cell level by sequential reagent exchange to detect apoptosis and cell viability in the treated cell population.
To date, the majority of drug screening tests on cancer cells cultured in well plates and detection methods can only be applied post treatment. Common microfluidic systems for cell culture and drug exposure are open or operate in continuous-flow mode. However, it has been well established that even slight modification of biophysical conditions, including hydrodynamic stress, tends to induce significant alterations in cellular morphology and physiology.
By creating a flow-free, 4-nL sub-compartment in the microchannels the LiaT platform allowed us to handle small cell samples and to deliver low-affinity reagents to the cell surface, both prerequisites for the successful implementation of the developed drug screening method.
Hep-G2 cells are cultured as per standard protocol, captured on the LiaT and then stimulated for necrosis or apoptosis inducement as required. The cells are then characterized by the diffusion based addition of the relevant cell viability or apoptosis detection kit reagents and monitored in real time using fluorescence microscopy methods. The obtained experimental data is also validated against standard colorimetric well plate assays.