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ABSTRACTS

CELL DEATH TRIGGERED BY CARDIOTONIC STEROIDS: ROLE OF CELL VOLUME PERTURBATIONS AND α1-NA$^+$,K$^+$-ATPASE SUBUNIT

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This study examine the role of cell volume modulation and Na$^+$,K$^+$-ATPase α-subunits in cell type-specific cytotoxic action of cardiotonic steroids (CTS). Long-term exposure to ouabain caused massive death of MDCK renal epithelial cells expressing a variant of the α1 isoform, CTS-sensitive α1S, documented by their detachment, chromatin cleavage and complete loss of lactate dehydrogenase (LDH) but had no impact on vascular smooth muscle cells (VSMC) from the rat expressing CTS-resistant α1R-Na$^+$,K$^+$-ATPase. Neither MDCK nor VSMC were affected by Na$^+$,K$^+$-ATPase inhibition in K$^+$-free medium. Unlike the distinct impact on cell survival, 2-hr exposure to ouabain and K$^+$-free medium led to the same elevation of the $[Na^+]_i/[K^+]_i$ ratio in both cell types. 5–10 min before the detachment of ouabain-treated MDCK cells, their volume was augmented by ~30–40% whereas massive LDH release from hyposmotically-swollen cells was documented when their volume was increased by ~5-fold. In additional experments, MDCK cells were stably transfected with a cDNA encoding α1R- and a2R-Na$^+$,K$^+$-ATPase, whose expression was confirmed by RT-PCR. At concentration of 10 μM ouabain led to complete inhibition of ^{86}Rb influx both in mock- and α2R-transfected cells, whereas maximal inhibition of ^{86}Rb influx in α1R-transfectd cells was observed at 1,000 μM ouabain. In contrast to massive death of mock- and α2R-transfected cells exposed to 3 μM ouabain, α1R-cells survived after 24-hr incubation with 1000 μM ouabain. We did not observe any effect of extra and intracellular Ca^{2+}-chelators, $[Ca^{2+}]_i$-raising compounds, inhibitors of Ras signaling as well as activators and inhibitors of serine-threonine kinases on the death of ouabain-treated MDCK cells. Thus, our results showed that the rupture of plasma membranes in ouabain-treated MDCK cells was not directly caused by cell swelling mediated by Na$^+$,K$^+$-ATPase inhibition and inversion of the $[Na^+]_i/[K^+]_i$ ratio. Downstream intermediates of $[Na^+]_i/[K^+]_i$-independent signaling triggered by interaction of CTS with a1S- but not with a1R-Na$^+$,K$^+$-ATPase are currently under investigation.

VOLUME-SENSITIVE OUTWARDLY RECTIFYING ANION CHANNELS IN THE DEVELOPING BRAIN

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The volume-sensitive outwardly rectifying (VSOR) anion channel plays a major role in transporting anions across the cell membrane during cell volume regulation. The channel is typically activated by cell swelling in most types of animal cells, thereby facilitating a regulatory volume decrease process and also mediating intercellular communications through release of excitatory amino acids, like glutamate. The channel may also be activated without swelling when certain types of intracellular signaling cascades are elicited by some receptor stimulation. We previously demonstrated that opening of Ca^{2+}-permeable ion channels triggered by G_q protein-coupled receptor activation elicits VSOR channel activation through high concentration regions of intracellular Ca^{2+} in the immediate vicinity of Ca^{2+} channels, so called "Ca^{2+} nanodomains" [1, 2]. This mechanism would provide a basis for local control of cell volume regulation associated with cell shape changes during cell proliferation, differentiation and migration. The roles of the VSOR channel in regulatory and apoptotic volume decreases had been extensively studied so far, especially in tumor cells. But its roles in cell proliferation, differentiation and migration, especially in the neurons in the developing brain, have not been elucidated yet. I have recently begun to examine the VSOR channel in the developing brain, and I would like to report my recent attempts to elucidate the roles of the channel, especially in the context of providing driving forces for cell migration and of communications between neurons during the developing process.

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NA+ IMPORT IS THE RATE-LIMITING STEP OF CELL RE-HYDRATION AFTER CRYO-PRESERVATION

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During cryo-preservation, cells loose considerable amounts of water and they partial regain volumes when brought back from the cold. Here we applied different levels of hypertonic stress as well as cryo-conditions (–50 °C) to perturb the hydration (volume) of HepG2 cells and to define the activation energies (E_as) of aquaporins (AQPs) and HICCs using scanning acoustic microscopy and whole-cell patch-clamp recordings. The E_as of regulatory volume increase (RVI) and HICCs showed a defined osmotic profile, whereas activation of AQPs did not depend on hypertonicity. The E_as of RVI were significantly decreased after cryo-preservation indicative of a higher capability of cells to regain osmolytes and water, very likely through a stimulation of cation uptake. In sharp contrast, the E_as of AQPs were not significantly changed after cryo-preservation, implying a rather permissive role of water channels in the process.

CONTROL OF CYST GROWTH IN AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE (ADPKD): DRUGABLE CHANNEL PATHWAYS

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ADPKD, a common renal genetic disease, occurs with an incidence of 1 : 400–1000. Cyst expansion progresses slowly, and renal function is typically not compromised until the fifth decade but then the decline to renal failure is precipitous. We are exploring regulatory pathways for drug intervention in both early and late stage disease. Based on our observations that PPARγ agonists inhibit CFTR synthesis and that PPARγ agonist treatment ameliorates cyst progression in the slowly progressing PCK rat model, we hypothesized that PPARγ agonists could be used to treat ADPKD. CFTR is one of the epithelial channels responsible for electrolyte-driven fluid secretion into the cyst lumen causing cyst expansion. PPARγ agonists are insulin sensitizing agents used to treat diabetes, albeit with some dose related side effects. Our current data indicate that in the PCK rat after 14 weeks of treatment, both super-pharmacological and sub-pharmacological doses of PPARγ agonists significantly decrease renal cyst burden. In a rapidly progressing model of renal cystic disease, WPK/WPK rats, a 13 day treatment with a PPARγ agonist was only effective when used at a sub-pharmacological dose. These data suggest that very low doses of PPARγ agonists may be effective life-long therapy for PKD patients, particularly if started at time of diagnosis. Studies were also performed to determine factors involved in renal decline during late stage ADPKD when cyst leakage and rupture is likely. Basolateral exposure to cyst fluid stimulates a Cl^- secretory response. The active component in cyst fluid is lysophosphatidic acid. Inhibitor studies in a mouse principal cell line indicate that cyst fluid-stimulated Cl^- secretory responses are due to activation of CFTR as well as a Ca^{2+}-activated Cl^- channel (CaCC). Interestingly, this stimulated Cl^- secretory response is independent of cAMP and is inhibited by BAPTA-AM suggesting a key role for Ca^{2+} as well as a functional interaction between CFTR and CaCC.

THE WARBURG EFFECT AND METABOLIC CONSEQUENCES IN THE DEVELOPMENT OF APOPTOTIC RESISTANCE IN OSMOTICALLY STRESSED LYMPHOID CELLS

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A high rate of glycolysis, in the presence of oxygen, that results in lactic acid fermentation in the cytosol is a common feature in many cancer cells. This observation, known as the Warburg effect, reflects changes in cellular metabolism and has been used clinically to diagnose and monitor cancer patients. We have developed an immature murine T-cell line through multiple generations of osmotic stress and recovery that have gained this feature of aerobic glycolysis. Interestingly, these osmotic stress cells (S49 (OS)) are also resistant to intrinsic apoptotic stimul, including acute osmotic stress, while remaining sensitive to extrinsic agents. Additionally, these S49 (OS) cells have gained an inherent regulatory volume increase (RVI) response, and are exquisitely dependent on glucose as an energy source. Our current studies explore the nature of the Warburg effect in regards to cell death and glucose dependence. Specifically we investigate changes at the level of the mitochondria, and the role that various ions have on the Warburg effect and their overall impact on apoptotic resistance. Furthermore, we will present genetic studies that may influence the signal transduction properties related to the Warburg effect and how this may integrate with the development of apoptotic resistance.

MAPPING OF REDOX STATE OF MITOCHONDRIAL CYTOCHROMES IN LIVE CARDIOMYOCYTES USING RAMAN MICROSPECTROSCOPY

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Cell volume is regulated by multiple interrelated processes taking place in the cell cytoplasm and in the submembrane region. Regulation of the activity of plasma membrane ATPases is one of the important mechanisms for the maintenance of cell volume. This regulation in turn depends on the ATP production of mitochondria. It is known that mitochondrial production of ATP is heavily dependent on the dynamically changing redox status of the electron transport chain (ETC) cytochromes. At present time main methods of mitochondria studies are fluorescent microscopy with small organic dyes or genetically encoded fluorescent proteins or absorption spectroscopy and measurements of O_2 and ETC substrate consumption on isolated ETC complexes. These techniques require cell disruption or affect cell properties. Therefore, for the investigation of mitochondria-related processes of cell volume regulation it is important to develop novel, non-invasive experimental approaches.

We present a non-invasive, label-free approach to study redox state of reduced cytochromes c, c1 and b of complexes II and III in mitochondria of live cardiomyocytes by means of Raman microspectroscopy. For the first time with the proposed approach we perform studies of cardiomyocytes representing different morphological and functional states. Raman mapping reveals that these cardiomyocytes differ in the amounts of reduced cytochromes c, c1 and b. The rod-shaped cardiomyocytes possess uneven distribution of reduced cytochromes c, c1 and b in cell center, and periphery. Moreover, we demonstrated the decrease in the relative amounts of reduced cytochromes c, c1 and b in the rod-shaped cardiomyocytes caused by H_2O_2-induced oxidative stress before any visible morphological changes. Results of Raman mapping and time-dependent study of reduced cytochromes of complexes II and III and cytochrome c in cardiomyocytes are in a good agreement with our fluorescence indicator studies and other published data.

REGULATION OF THE SODIUM PUMP IN INSULIN-SENSITIVE TISSUES

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We have a longstanding interest in the regulation of skeletal muscle sodium pump activity and trafficking in response to metabolic challenges such as Type 2 diabetes, muscle contraction or physical activity/inactivity. Skeletal muscle Na,K-ATPase plays a central role in the clearance of K^+ from the extracellular fluid, thus maintaining blood K^+ concentration. We have shown that impaired skeletal muscle Na,K-ATPase activity in glucose intolerant animals is associated with changes in Na,K-ATPase subunits expression, plasma membrane abundance and altered expression and phosphorylation of the Na,K-ATPase-interacting protein phospholemman (PLM). Importantly, alterations in expression of sodium pump subunits and PLM precede development of insulin resistance. We hypothesize that disturbances in skeletal muscle Na,K-ATPase regulation may contribute to impaired ion homeostasis in insulin-resistant states such as obesity and Type 2 diabetes.

Contraction stimulates Na,K-ATPase activity in skeletal muscle partially via translocation of sodium pump units to the plasma membrane. We have evidence that phosphorylation of PLM plays a critical role in the acute regulation of the Na,K-ATPase response to exercise/muscle contraction. Notably, spinal cord injury leads to rapid reduction of skeletal muscle Na,K-ATPase and PLM abundance in humans. Contraction and metabolic stress are potent activators of AMP-activated protein kinase (AMPK). AMPK activation stimulates Na,K-ATPase activity and increases the sodium pump cell surface abundance in skeletal muscle. AMPK stimulation leads to protein phosphatase 2Ac methylation and de-phosphorylation, which promotes activation of the phosphatase and in turn cause de-phosphorylation of the Na,K-ATPase α_1-subunit at Ser18 which may prevent sodium pump endocytosis. Thus, contrary to the common paradigm we demonstrate an AMPK-dependent activation of an energy consuming ion pumping process. Activation of AMPK may be a potential mechanism mediating exercise- and metabolic stress-induced activation of the sodium pump in skeletal muscle.

QUANTIFICATION OF CELL HYDRATION WITH FLUORESCENCE CORRELATION SPECTROSCOPY AND RAMAN SCANNING MICROSCOPY

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Hydration plays a key role in cell physiology. We defined the hydration profiles of HeLa and HepG2 cells as a function of hypertonicity and temperature, at high time and spatial resolution using Fluorescence Correlation Spectroscopy (FCS) and Raman Scanning Microscopy (RSM) and the underlying activation energies were determined.

With FCS we quantified (1), the diffusion speed and the number of diffusing GFP particles in the cytoplasm and (2), the correlating changes in cell viscosity, under hypertonic conditions. Both parameters were correlated to each other and exhibited a clear dependence on the osmolarity.

RSM, on the other hand, allowed us to monitor directly the absolute amount of cell water and to map the hydration of a probe at high spatial resolution. This was instrumental in verifying the FCS measurements and, furthermore, the actual amount of bound water inside the cell could be computed, in a label-free fashion.

Our measurements prove the robustness of the cell volume regulatory machinery and define the energy profiles of its activation over a wide osmolarity and temperature range.

FUNCTIONAL CONTRIBUTION OF STRETCH-ACTIVATED CHANNELS IN OSMOTIC REACTIONS AND CALCIUM SIGNALING IN MAMMALIAN CELLS

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Mechanically gated ion channels are involved in processes of volume regulation and calcium signaling in living cells. However, functional contribution of stretch-activated cation channels (SACs) into volume-dependent Ca^{2+} signaling remains unclear [1, 2]. Calcium-permeable gadolinium-blocking SACs have been previously characterized in human myeloid leukemia K562 cells [3, 4]. Here, changes in intracellular free ionized Ca^{2+} concentration ($[Ca^{2+}{}_i]$) in K562 cells were monitored in response to reduced bath osmolarity using Fura-2 imaging. Cell swelling induced by decrease of extracellular osmolarity resulted in the elevation of $[Ca^{2+}{}_i]$. Ca^{2+} entry could be blocked by external application of Gd^{3+} ions thus confirming that SACs in plasma membrane mediate swelling-induced Ca^{2+} influx in leukemia K562 cells. In single channel studies, functional impact of membrane cholesterol and actin cytoskeleton on mechanosensitive channel activity in leukemia cells has been analysed [5, 6]. We found that cholesterol depletion-induced suppression of stretch-activated channels was mediated via actin rearrangement. In cell-attached patch clamp experiments on breast cancer MCF7 cells and transformed fibroblasts 3T3B-SV40 we observed typical SAC activity followed by transient activation of K^+ channels (8-10 pS). Importantly, K^+ currents displayed no direct mechanosensitivity. Our results indicate that Ca^{2+}-dependent K^+ channels could be activated by Ca^{2+} influx via colocalized SACs in membrane patch. The observed effect represents the functional coupling between SACs and K^+ channels in plasma membrane. In sum, the data imply that SAC activation underlies putative global and local calcium signaling mechanisms involved in volume regulation in mammalian cells.

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ROLE OF CELL VOLUME DISREGULATION IN CLINICAL PATHOPHYSIOLOGY OF ENDOCRINE DISORDERS

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Cell volume changes may cause some manifestations of the endocrinopathiae or push to their misdiagnosis. Our clinical experience shows that abdominal viscera cell swelling in exacerbation of Addison's disease (Addisonian crises) is regularly displayed with liver enlargement, right side subcostal pain and even imitate acute abdomen. Acute pain in abdominal and liver areas is often accompanied by vomiting, diarrhea and fever. This is diagnostically misleading for physicians not aware of cell volume disorders. Our observation of an Addisonian crisis case is described and discussed. Case was misdiagnosed as acute abdomen and lead to laparotomy with fatal outcome due to lack of glucocorticoid protection [1]. False acute abdomen signs were caused by sodium loss and relative decrease of extracellular fluid tonicity with cell swelling and painful distension of abdominal organs' capsules. Differential signs of Addisonian crises and acute abdomen are discussed. In sub-compensated diabetes mellitus (DM) cell volume of red blood cells (RBC) may vary due to sharp changes of extracellular glucose level and shifts of extracellular fluid tonicity. When RBC loaded with glucose are diluted with isotonic media for electronic counter mean corpuscular volume (MCV) and hematocrit (Ht) check, it may produce false enlargement of MCV and Ht. We checked these parameters with K-800 cell counter in 337 patients with DM type I and 211 patients with DM type II (megaloblastic anemiae were excluded). MCV in DM I appeared to be greater than in DM II ($94,5 \pm 0,7$ vs $90,5 \pm 0,7$; $p < 0,02$). This data correlated to greater Ht and blood glucose level elevated [2]. Similar data also reported by Strauchen, J.A., et al. [3].

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SIGNALING PATHWAYS ASSOCIATED WITH APOPTOTIC RESISTANCE AND REGULATORY VOLUME MECHANISMS IN OSMOTICALLY STRESSED LYMPHOID CELLS

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Apoptosis is a stochastic, physiological mode of cell death characterized by a distinct set of morphological and biochemical properties. The loss of cell volume, termed Apoptotic Volume Decrease (AVD) has been a defining feature of this programmed cell death process. Most cells have inherent volume regulatory mechanisms to combat a change in cell size, however T-cells are devoid of a regulatory volume increase (RVI) response when challenged in a hyperosmotic environment. We have developed a T-cell line, designed S49 (OS) that has gained an inherent RVI response upon acute hyperosmotic exposure. Interestingly, these S49 (OS) cells are resistant to cell death agents that induce the intrinsic apoptotic pathway, while remaining sensitive to extrinsic apoptotic stimuli. We have explored the signaling properties that impact this apoptotic resistance and RVI response in S49 (OS) cells. We show that S49 (OS) cells show a constitutive phosphorylation of AKT upon acute osmotic stress that is absent in the parent cells, and inhibition of this phosphorylation results in sensitivity to apoptosis. Additionally, inhibition of upstream signaling molecules, such as PI3 kinase, also sensitizes S49 (OS) cells to undergo apoptosis. We will present our latest data on the signaling networks that influence not only apoptotic resistance, but also cell volume regulation in lymphoid cells.

THE ROLE OF THE MSCS CYTOPLASMIC VESTIBULUM IN THE CHANNEL ION SELECTIVITY AND REGULATION OF INTRACELLULAR CALCIUM

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The mechanosensitive (MS) channel of small conductance (MscS) has been characterized at both functional and structural levels and plays an integral role in protection of bacterial cells against hypoosmotic shock. In this study we investigated the role that the cytoplasmic domain plays in MscS channel function by investigating ion selectivity and gating properties in solutions containing different mono- and divalent cations. Our results show that MscS preferentially resides in subconducting states at hyperpolarising potentials when Ca^{2+} and Ba^{2+} ions are the major permeant cations. In addition, our results indicate that charged residues proximal to the seven vestibular portals and their electrostatic interactions with permeating cations determine selectivity and regulate conductance of MscS and potentially other channels belonging to the MscS subfamily. We compared the selectivity of several members of the MscS-like channel subfamily, whose ion conducting properties were previously investigated and reported, using them as natural variations of the vestibular primary structure reflected by their ion selectivity. Furthermore, we also mutagenized two carefully chosen charged residues within the cytoplasmic chamber of MscS of *E. coli*. Our choice was based on detailed analysis of the possible effects of different mutations on the electronegative area within the vestibulum of MscS. Together these data provide strong evidence for the role of the C-terminal domain as a selectivity filter in MscS-like channels. Most interestingly of all that on opening MS channels can become not only a passageway for osmolyte exit but also a conduit for the entry of ions including Ca^{2+} suggesting a role for the MS channels in bacterial calcium regulation indicative of functions other than protection against osmolarity changes. In conclusion, our results have important implications for the physiology of bacterial cells.

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IN VIVO VISUALISATION OF THE PARAMETERS OF NORMAL AND CANCER SKIN CELLS USING TWO-PHOTON MICROSCOPY

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Basal cell carcinoma (BCC) is the most frequent type of skin cancer in humans. The standard procedure of BCC diagnostics is a tissue biopsy sampling with subsequent histological examination. This “gold standard” method is highly invasive and time-consuming. Application of quick, non-invasive methods is of high interest for BCC diagnostics. For example, two-photon microscopy (TPM) is a well suited optical method for *in vivo* diagnosis of BCC at high resolution.

In the present study the normal and BCC-affected skin of nine BCC patients were measured noninvasively with TPM prior to surgery. Autofluorescence was provided by TPM under the excitation at 760 nm, where the single cells are clearly visible. The main fluorescent contribution for excitation at 760 nm in the skin is due to NAD(P)H, which is involved in cell mitochondrial activity, molecules of melanin, as well as keratin, elastin and collagen. After the surgery, a histological tissue analysis was additionally performed.

It was found that the nucleus diameter of BCC cells is significantly larger than that of normal cells (granular, spinous and basal cells). The nucleus-to-cytoplasm ratio of BCC cells was found to be significantly lower than that of basal and spinous normal cells. The density of normal cells was found to have increased significantly for granular to basal cell layers, whereas the density of BCC cells is almost equal to that of granular normal cells. It should be taken into consideration that BCC cells were counted in a wide depth range from 10 μm to 200 μm, where their diameter did not significantly change, while the normal granular, spinous and basal cells were counted on average at the depths of (21 ± 7) μm, (39 ± 6) μm and (56 ± 6) μm, respectively.

REGULATION OF NA-(K)-CL COTRANSPORT BY SPAK AND OSR1

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SPAK (Ste20-MST1-related proline alanine rich kinase), OSR1 (Oxidative stress kinase 1) and WNK (With No K-lysine) kinases modulate distal Na^+ transport by affecting the activity of the thick ascending limb of Henle Na-K-2Cl cotransporter and the activity of the distal convoluted tubule Na-Cl cotransporter (NCC). WNK4 affects the cotransporters by regulating their trafficking to the plasma membrane and by phosphorylating and activating SPAK which then phosphorylates and activates the cotransporters. Here we demonstrate that in the presence of Cab39, WNK4 can also directly activate the NaCl cotransporters. We show that when *Xenopus laevis* oocytes are injected with Cab39α or Cab39β cRNA along with WNK4 and NKCC1, we observe a significant increase in the level of K^+ influx. This increase is bumetanide-sensitive indicating that the flux is mediated by the cotransporter. We also show that the stimulation requires the catalytic activity of WNK4 and is not affected by overexpressing dominant negative SPAK. These data suggest that WNK4 is able to phosphorylate and activate the cotransporter in a SPAK-independent manner. When we mutated a phenylalanine residue in a WNK4 domain that resembles the SPAK/OSR1 binding domain, we prevented the Cab39/WNK4 activation of the cotransporter. We further show that residues that are important to SPAK-Cab39 interaction are not involved in the WNK4-Cab39 interaction, indicating different modes of binding. Finally, we demonstrate that while the WNK4 PHAII mutation, E599K, does not affect the ability of WNK4 to activate SPAK, this mutation completely abrogates the activation of NKCC1 by WNK4. These data were reproduced using a chimeric transporter consisting of the N-terminal regulatory tail of NCC fused to NKCC1, indicating that NCC is similarly activated by the Cab39/WNK4 interaction. Our data therefore demonstrate that WNK4 can interact and activate the Na-(K)-Cl cotransporters in the absence of SPAK or OSR1.

THE ROLE OF THYROID HORMONES IN THE REGULATION OF OSMOTIC HOMEOSTASIS

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Cell volume regulation is dependent from the efficiency of renal clearance of excess fluid and osmotically active substances (OAS). Thyroid hormones (TH) – one of the most important regulators of kidney function. In normal conditions excretion of OAS and fluids by kidneys depended from the glomerular filtration rate (GFR) and renal tubular transport. However, information about effects of TH on the GFR and renal excretion OAS is contradictory. In our investigation the effect of hypo- and hyperosmotic NaCl solutions on the GFR and renal excretion OAS in rats with different variants of thyroid status it was studied.

Results. In experiments use 72 Wistar rats. Episodic disturbance of thyroid status induced in rats by a single intragastric administration of thyroxine (T4) 50 mcg per 100 g of body weight. Renal function was investigated after intragastric administration of fluid in amount of 5% of body weight. Concentration of NaCl in the solution is from 0 to 3%. Water load in the rats with episodic disturbance of thyroid status leads to decrease GFR to 375 ± 19 versus control 542 ± 27 l/min ($p < 0,01$). Increased NaCl concentration was accompanied with progressive increase of GFR by the inclusion of renal functional reserve. In rats treated with T4 increase GFR detected only in group in which used 3% solution of NaCl. The level of osmolality of the extracellular fluid, the rate of excretion by the kidneys and the amount of urine OAS excretion in rats with loading by saline solutions had no significant differences between the control and rats treated with T4.

Conclusions. The kidneys of rats with episodic disturbance of thyroid status retain the ability to removal of excessive amounts of fluid and OAS, protecting tissues of internal organs from hypoosmotic state and hyperosmotic stress. In short term dysfunction of thyroid status changes in GFR and OAS tubular transport are reversible

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THE ROLE OF TRPC CHANNELS IN DIFFERENTIATION AND PROLIFERATION – NEW TARGETS FOR CANCER TREATMENT

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The processes involved in the transformation of normal cells to cancer cells and in tumor progression are complex and only partly understood. Chronic infections and inflammation are major risk factors for various types of cancer. Neoplastic transformation is the result of the accumulation of mutations on certain key signaling proteins along with the formation and selection of aggressive cancer subclones. Among these key signaling proteins TRP channels have been identified to modulate a variety of physiological and pathological processes. Beside several TRPV channels e.g. TRPV1, and TRPM channels such as TRPM8 which might be involved in cancer pathology, TRPC6 channel expression was found to be significantly increased in prostate cancer tissue and in glioblastoma. However, several groups as well as our group showed that TRPC6 channels are essential for differentiation of cell types such as keratinocytes. Aberrant keratinocyte differentiation is considered to be a key mechanism in the onset of hyperproliferative dermatological diseases, including basal cell carcinoma (BCC) the most common form of skin cancer. Mainly overexposure to sun light leads to DNA damage. Besides mutations, the local immune system is depressed, possibly decreasing immune surveillance for new tumor cells. Here, we wanted to investigate the interaction the immune system, keratinocyte differentiation and TRPC6 channel function and expression. We used IFNγ, TNFα, IL4 and IL13 and investigated their effect on TRPC6 channel function after 24 h preincubation. Interestingly, all cytokines decreased TRPC6 mediated calcium influx in keratinocytes and also resulted in decreased TRPC6 channels expression. In addition, differentiation and proliferation were also attenuated by the cytokines. These first findings suggest interplay between inflammation and TRPC6 function and expression in keratinocytes. Further experiments need to clarify if these findings also play a role in the pathogenesis of basal cell carcinoma.

DELETION OF 11β-HYDROXYSTEROID DEHYDROGENASE TYPE 2 IN THE NUCLEUS OF THE SOLITARY TRACT CAUSES SALT APPETITE AND HYPERTENSION

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Ablation of the gene encoding 11β-hydroxysteroid dehydrogenase type 2 causes severe hypertension and abnormal renal handling of sodium [1, 2]. *Hsd11b2* heterozygote mice have salt-sensitive blood pressure [3], associated with enhanced activity of renal ENaC [4]. Although these studies suggest that renal sodium retention underpins salt-sensitive blood pressure, 11β-hydroxysteroid dehydrogenase type 2 is also expressed in cardiovascular control centers of the brain. To assess central contributes to salt-sensitivity, Cre-lox technology was used to delete selectively *Hsd11b2* in the nucleus of the solitary tract, leaving renal 11β-hydroxysteroid dehydrogenase type 2 expression unaffected.

Ambulatory blood pressure and renal function were normal but Cre+ mice had an impaired baroreceptor reflex and an innate salt appetite. The hunger for salt was not associated with systemic physiological drivers such as sodium/volume depletion and the increased intake caused a rapid and sustained hypertension. Importantly, matched sodium intake did not increase blood pressure in wild-types. Systemic spironolactone blunted the salt-intake in Cre+ mice.

These studies identify mineralocorticoid receptor activation in the nucleus of the solitary tract as a unifying pathway between sodium intake and blood pressure homeostasis. Targeting of salt appetite is an attractive therapeutic intervention for blood pressure management in cardiovascular disease.

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ACUTE REGULATION OF THE WATER CHANNEL AQUAPORIN-2 BY POSTTRANSLATIONAL MODIFICATION

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The vasopressin-regulated water channel aquaporin-2 (AQP2) is expressed in kidney CNT cells, CD principal cells, and inner medullary collecting duct (IMCD) cells. Apical plasma membrane abundance of AQP2 is the rate-limiting step and controls the reabsorption of water; a result of regulated exocytosis of subapical AQP2 bearing vesicles and regulated AQP2 retrieval from the plasma membrane. Together, these two processes carefully balance the levels of apical membrane AQP2. Several of the cellular functions of AQP2 are regulated by posttranslational modifications. In particular phosphorylation and ubiquitination are mechanisms that regulate AQP2 subcellular sorting and distribution, degradation, and protein interactions. Modifications of AQP2, unlike other channels/transporters, do not appear to affect the transport capacity of the channel. More recent data using AQP2 as a model plasma membrane protein suggests that site specific phosphorylation of AQP2 is able to override the internalization signal mediated by K63-linked polyubiquitination, providing a novel cell biological concept for membrane protein regulation.

REDOX SIGNALING, APOPTOTIC VOLUME DECREASE AND NEURONAL CELL DEATH

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Oxidative stress is involved in the regulation of cell death signaling cascades, but the molecular mechanisms involved remain unclear. GSH is the most abundant low molecular weight thiol antioxidant defense in the cell. We have previously demonstrated that GSH depletion parallels apoptotic volume decrease and precedes the activation of cell death signaling [1–3]. Here, we aim to demonstrate the different mechanisms by which alterations in intracellular GSH homeostasis (transport and metabolism) regulate neuronal cell death progression in experimental Parkinson's disease models. Dopaminergic cell death was induced by environmental/mitochondrial toxins, and overexpression of wild type or mutant (A53T) α-synuclein. Dopaminergic cell death and apoptotic volume decrease were associated with a decrease in total GSH content and an increase oxidative stress. Using novel genetically encoded redox sensors (roGFP) we identified the alterations in redox homeostasis in both mitochondria and cytosolic compartments [4]. GSH depletion was also paralleled by alterations in protein-cysteine-bound GSH (protein glutathionylation), and we demonstrated a signaling role for this oxidative post-translational modification in dopaminergic cell death. Thiol-oxidoreductases exerted protective effects against neuronal cell death by regulating thiol-redox homeostasis within the cell [5]. Our research has contributed significantly to the understanding of the distinct mechanisms by which GSH depletion (transport or metabolism) regulates cell death progression by acting as a redox signaling transducer [6, 7].

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INTRACELLULAR PROTEINS WHICH REGULATE K-CL COTRANSPORTER ACTIVITY MAY SERVE AS NOVEL TARGETS FOR ANTI-CANCER THERAPEUTICS

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The anaplastic and invasiveness nature of high-grade gliomas involves a synchronized reorganization of the cytoskeleton, membrane recycling, and focal adhesion to the extracellular matrix. These cellular processes are aided by the obligatory water movement which occurs when inorganic ions are transported across the plasma membrane. In a previous study, the presence of Na^+-independent K-Cl cotransporter (KCC) isoforms suggested that these membrane proteins had a role in glioma cell motility [1]. Utilizing a rat glioblastoma cell line which shares many characteristics of high-grade gliomas [2], RT-PCR analysis identified RNA expression of: (1) with-no-lysine (WNK) kinases; (2) oxidative stress response (OSR1) kinase; (3) Ste20-related proline-alanine rich kinase (SPAK); (4) protein phosphatase 1 (PP1); and (5) apoptosis-associated tyrosine kinase (AATYK1). Each of these intracellular signaling molecules has been shown to partially regulate cotransporter activity. Although K^+ influx in *Xenopus laevis* oocytes co-injected with KCC1 and AATYK1 cRNA was not different under isosmotic conditions, co-injection of KCC3 and AATYK1 cRNA increased isosmotic K^+ influx to levels observed under hyposmotic conditions. These results suggest that differential expression of specific regulatory proteins might have a role in cotransporter activity, and in turn, possibly glioblastoma cell motility.

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REGULATION OF THE RENAL NACL COTRANSPORTER ACTIVITY BY THE WNK KINASES SIGNALING NETWORK

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The renal NaCl cotransporter NCC is the major salt transport pathway in the distal convoluted tubule of the mammalian nephron. NCC plays a key role in the regulation of arterial blood pressure. Inhibition of NCC with thiazide type diuretics is one of the cornerstones for the treatment of arterial hypertension. Inactivating mutations of NCC in the Gitelman disease results in arterial hypotension accompanied by hypokalemic metabolic alkalosis. In contrast, increased activity of NCC in the Familial Hyperkalemic Hypertension syndrome (FHH), also known as pseudohypoaldosteronism type II, results in hypertension with hyperkalemic metabolic acidosis. Increased activity of NCC in FHHt is the consequence of mutations in regulatory proteins. Four genes have been demonstrated to produce FHHt: two with no lysine kinases, WNK1 and WNK4, and two proteins known as Kelch-3 and Cul3 that together form a RING-type ubiquitin ligase complex. Recent studies suggest that WNK1 and WNK4 are the targets for Kelch-3/Cul3 ubiquitylation system and thus, FHHt due to mutations in Kelch3/Cul3 produce the disease by affecting the expression of WNK1 and WNK4. The mechanisms involved in the regulation of NCC by WNKs are thus a very important pathway to be defined. Initial studies using *in vitro* systems and *in vivo* transgenic mice models suggested that WNK4 is an inhibitor of NCC and that FHHt mutations in WNK4 prevent this inhibition. The model localize WNK1 upstream of WNK4 suggesting that WNK1 inhibits the activity of WNK4 and thus, increased expression of WNK1 in FHHt produces the disease by preventing WNK4 inhibition of NCC. Recent studies in our laboratory provided us with data in both, *in vitro* and *in vivo* systems, to propose a different model: WNK1 and WNK3 are potent activators of NCC, and WNK4 is an inhibitor of WNK1/WNK3. This last effect if prevented by angiotensin II, providing a working model to explain, at lest in part, the angiotensin II induced arterial hypertension.

RECENT PROGRESS IN SINGLE-CELL VOLUME MEASUREMENT TECHNIQUES

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Accurate measurements of rapid cell volume changes in response to different stimuli are essential in the studies examining many aspects of cell physiology and pathology, including cell volume sensor identification. Several technique are used to measure cell volume, including Coulter counter, fluorescent techniques, electronic impedance, and video microscopy. More recent approaches to cell volume measurements utilize atomic force microscopy, ion conductance microscopy, or fluid flow in a rigid system. Each of these techniques has strengths and limitations, differing in accuracy, temporal resolution and applicability to specifically-prepared cells. For example, the Coulter counter technique can rapidly measure cell volume of large cell population, but it can only be used with floating, ideally almost perfectly spherical cells. Here, we will briefly review and compare different currently-available cell volume measurement techniques, focusing on recently developed light microscopy techniques, especially those capable of measuring absolute cell volume in real-time, providing full 3D information on cell shape, and those that could be applied to substrate-attached cells.

THE ROLE OF CYCLIC NUCLEOTIDES IN VOLUME-DEPENDENT REGULATION OF ELECTRICAL AND CONTRACTILE ACTIVITY OF SMOOTH MUSCLE CELLS

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We studied smooth muscle segments of the thoracic aorta of rats and guinea-pig ureter. Amplitude of contractions, recorded as isometric force tension, was measured as percentage of depolarisation-induced vascular smooth muscle cells (SMC) contraction in high-K^+ (30 mM KCl) medium. Iso- and hyperosmotic shrinkage was triggered by SMC transfer from hypo- to isotonic medium and by elevation of medium osmolality with 150 mM sucrose, respectively. To study the electrical and contractile activity was applied the method of the double sucrose bridge.

Activation of cAMP- and cGMP-dependent signaling pathways was caused by forskolin and sodium nitroprusside, respectively. Microfilaments were modulated by cytochalasin B, microtubules by vinblastine, microtubules and microfilaments by colchicine.

Original tension of aortal smooth muscle wasn’t affected by colchicine, however amplitude of contractions caused by potassium chloride was decreased. Vinblastine didn’t affect original tension and value of hyperpotassium contracture in vascular SMC. Cytochalasin B led to decrease of original tension and amplitude of contraction in vascular SMC caused by hyperpotassium solution.

Relaxation of aortic smooth muscle caused by forskolin hasn’t been changed after treatment with colchicine or vinblastine but increased after cytochalasin B. Amplitude and duration of action potential plateau under forskolin as far as of contractions in ureteral SMC caused by depolarized current were decreased. Cytochalasin B depressed electrical and contractile activity in ureteral smooth muscle cells but like colchicine didn’t significantly modified effects of forskolin. Vinblastine increased electrical and contractile activity in ureteral SMC and decreased depressed influence of forskalin toward action potential and contractions.

Thus, cAMP-dependent regulation of contractile activity in aortic SMC of white rats is mediated by microfilaments but in ureteral smooth muscle cells of guinea-pig by microtubules.

ENaC EXPRESSION AND ACTIVITY CORRELATES WITH DEVELOPMENT OF HYPERTENSION IN SPONTANEOUSLY HYPERTENSIVE RATS

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We examined transcriptomic profiles in the whole kidneys of 12-, 40- and 80-week-old spontaneously hypertensive rats and 4 recombinant inbred strains selected for contrasting temporal dynamics of blood pressure elevation. Both microarray results and real-time polymerase chain reaction showed that age-dependent blood pressure increment in males and females of spontaneously hypertensive rats and hypertensive recombinant inbred strains was accompanied by more than 50% heightened expression of epithelial sodium channel β- and γ-subunits. Epithelial sodium channel subunits expression correlated positively with blood pressure but correlated negatively with renin expression. Increased epithelial sodium channel activity was observed in cultured renal epithelial cells isolated from the kidney medulla of 80-week-old spontaneously hypertensive rats but not in age-matched normotensive Wistar Kyoto. This difference remained evident after 24 hour treatment of renal epithelial cells with aldosterone. ^{22}Na uptake in the perfused kidney medulla was increased whereas the urinary Na/K ratio was decreased in old spontaneously hypertensive rats compared to age-matched normotensive controls. The difference was eliminated by the administration of epithelial sodium channel inhibitor benzamil. Unlike spontaneously hypertensive rats, we did not detect any significant elevation of *Scnn1g* and *Scnn1b* expression in HXB17 recombinant inbred strain possessing age-dependent elevation of blood pressure. Our results show that development of hypertension in spontaneously hypertensive rats correlates with renal overexpression of *Scnn1g* and *Scnn1b* subunits and heightened activity of this channel. They also suggest that augmented sodium reabsorption by this channel contributes to blood

pressure elevation via a mechanism that is distinct from the renin-angiotensin-aldosterone system.

CELL VOLUME AND HEPATIC ENCEPHALOPATHY

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Hepatic encephalopathy (HE) defines a reversible neuropsychiatric syndrome frequently associated with acute and chronic liver failure. Current evidence suggests that HE in cirrhotic patients reflects the clinical manifestation of a low grade cerebral edema which exacerbates in response to a variety of precipitating factors (infections, sedatives, bleeding, high protein intake, electrolyte disturbances etc.) after an ammonia-induced exhaustion of the volume-regulatory capacity of astrocytes. Evidence for such low grade cerebral edema in HE came from *in vivo* proton magnetic resonance (^{1}H-MRS) studies on human brain and also by quantitative water mapping of the human brain *in vivo*. The cerebral edema accompanying HE is largely due to astrocyte swelling triggered by an oxidative/nitrosative stress response together with ammonia-induced glutamine accumulation in astrocytes. However, astrocytes not only swell in response to ammonia, but also in response to hyponatremia, benzodiazepines and inflammatory cytokines, all of which induce the rapid formation of reactive oxygen and nitrogen species through *N*-methyl-D-aspartate (NMDA)-receptor- and Ca^{2+}-dependent mechanisms in cultured astrocytes and in rat brain *in vivo*. NMDA receptor activation under these conditions is thought to result from a depolarization-induced removal of a Mg^{2+}-blockade from the receptor and a prostanoid-dependent autocrine amplification of NMDA receptor activity by ammonia-induced astroglial vesicular glutamate release. There is an *auto*-amplificatory signalling loop between astrocyte swelling and oxidative/nitrosative stress: astrocyte swelling induces oxidative/nitrosative stress through NMDA receptor- and Ca^{2+}-dependent mechanisms on the one hand and on the other, NMDA receptor activation and oxidative stress trigger astrocyte swelling. Activation of NADPH oxidase isoforms and of Ca^{2+}/calmodulin-dependent isoforms of nitric oxide synthase in response to hypoosmotic astrocyte swelling or ammonia exposure are the major sources of the early ROS and NO formation.

Functional consequences of the ammonia- and astrocyte swelling-induced oxidative/ nitrosative stress response in HE are protein tyrosine nitration, RNA oxidation and effects on zinc dependent gene transcription. Such phenomena may alter synaptic plasticity and lead to disturbances of oscillatory networks in the brain, which finally trigger HE symptoms. Mobilization of zinc from zinc-sulfur clusters in proteins stimulates Zn^{2+}-dependent gene transcription, which may explain the upregulation of the peripheral benzodiazepine receptor (PBR) in the brain of cirrhotic patients with HE. Upregulation of PBR triggers enhanced formation of neurosteroids, which are not only positive modulators of $GABA_A$ receptors, but also are high-affinity ligands for TGR5 which acts as a neurosteroid receptor in astrocytes, neurons and microglia. Hepatic encephalopathy is also associated with an activation of microglia, but this is not associated with increased synthesis of *pro*-inflammatory cytokine mRNA. This suggests that in HE microglia becomes activated but is not reactive. Most importantly, the above-described hallmarks of HE pathophysiology, such as astrocyte swelling, oxidative/nitrosative stress, protein tyrosine nitration and RNA oxidation have also been identified in human brain.

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ION CHANNELS INVOLVED IN CELL VOLUME REGULATION: ACTIVATION AND PHYSIOLOGICAL ROLES

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Channels involved in regulation of cell volume in EATC and ELA are the K^+ channel TASK-2, the volume regulated anion channel (VRAC) and the Ca^{2+} activated Cl^- channels ANO1 and ANO6. *TASK-2:* Activation of TASK-2 by short term hypotonicity requires a tyrosine phosphorylation of the channel, long term hypotonicity results in a decreased TASK-2 current and a downregulation of TASK-2. TASK-2 is up-regulated in activated T-cells, and we investigate the functional role of TASK-2 in this activation. *VRAC:* The role of VRAC and other Cl^- channels in apoptotic volume decrease (AVD), cell proliferation and migration was studied, and it was investigated if changes in the expression of Cl^- channels are involved in chemotherapy resistance. In an attempt to find the molecular identity of VRAC, we cloned and expressed members of the ANO family of Cl^- channels and investigated their role in cell volume regulation. Studies on ANO knock-downs shows that ANO6 plays a role in cell volume regulation, which is however secondary to a Ca^{2+} influx, in contrast to what is found for VRAC. We investigated the role of the ANO Cl^- channels in cell migration in ELA cells and found that ANO1 and ANO6 knock-down affect the directional migration and the rate of cell migration, respectively. ANO6 is also involved in apoptosis, possibly by contributing to AVD. Moreover we have explored AVD in wild type (wt) and Multidrug Resistant (MDR) EATC. Cisplatin leads to AVD and apoptosis in wt EATC not in MDR cells, and the maximal capacity of VRAC is strongly repressed in MDR EATC. A VRAC inhibitor NS3728 abolishes the differences in AVD and caspase-3 activation between wt and MDR EATC suggesting that impairment of AVD via down regulation of VRAC protects against apoptosis in MDR EATC [1]. We are now comparing the expression of Cl^- channels in wt and MDR EATC as well as in four pancreatic cancer cell lines and a normal pancreatic cell line (HPDE).

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GLYCINE FACILITATES PHAGOCYTOSIS BY A GLYCINE RECEPTOR-INDEPENDENT MECHANISM

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Glycine is involved in cell volume- as well as membrane-potential regulation. Cell shrinkage promotes an intracellular accumulation of glycine, which increases intracellular osmotic pressure and, accordingly, influx of water until normal cell size is regained. Furthermore, binding of glycine to the glycine receptor increases a chloride conductance, whereas activation of system A neutral amino acid transporters (NAATs) increases a Na^+ conductance. Both ions also contribute to cellular volume regulation. In a previous study, we proposed that phagocytosis may be explained by local volume regulatory mechanisms. Specifically, we suggest that formation of engulfment pseudopodia depends on localized perturbation in osmolarity. In the present study we evaluated the impact of glycine on polystyrene microsphere uptake using the murine microglial cell line, BV-2. Glycine facilitated phagocytosis. Because this effect was neither suppressed by the glycine receptor antagonist strychnine, nor mimicked by the glycine receptor agonist taurine, glycine modulated phagocytosis in a glycine receptor (GlyR)-independent manner. Furthermore, mRNA of the glycine receptors GlyR1, GlyR2, GlyR3 or GlyR4 were not detected in BV-2 cells using PCR. However α-(methylamino) isobutyric acid (MeAIB), a specific substrate of NAATs, suppressed glycine-induced increase in phagocytosis. In line with this finding, substitution of Na^+ with choline decreased uptake of microspheres. PCR revealed that BV-2 cell expressed the amino acid transporter Slc38a1, whereas Slc38a2 and Slc38a3 were not detected. Therefore, our find-

ings suggest that glycine increases phagocytosis by activation of NAATs in BV-2 cells.

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NEURONAL AND GLIAL CELL VOLUME DYNAMICS DURING SPREADING DEPOLARIZATION

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Disturbance of brain water homeostasis during stroke and TBI leads to a life-threatening state of brain edema. Spreading depolarizations (SDs) are waves of sustained near-complete neuronal and glial depolarization that actively propagate a collapse of ion gradients through the brain with associated dramatic neuronal and glial swelling that entails *cytotoxic edema*. While short-lasting SDs are withstood in healthy tissue, longer-lasting SDs are harmful in metabolically challenged tissue of the injured brain. In the ischemic penumbra, recurring SDs combine with compromised blood supply to increase the metabolic load, thereby expanding the initial infarct. Similarly to the peri-infarct tissue SDs occurring in the peri-contusional cortex after TBI can be detrimental and contribute to maturation of cortical lesions. The full spectrum from short- to very long-lasting SDs has been recorded in the evolution of stroke and TBI not only in animals but also in the human brain. Moreover, patients with multiple or prolonged SDs have very poor prognoses for recovery pointing to SD as the important *mechanism* in acute human brain injury. Yet, this area of research is still largely neglected. One critical step is to identify how SDs induce damage to neurons, astrocytes and fine synaptic circuitry. Using *in vivo* two-photon laser scanning microscopy and transgenic mouse strains with intrinsic fluorescent neurons and glia we directly distinguish and quantify neuronal and glial components of cytotoxic brain edema during ischemic and traumatic injury in experimental settings relevant to clinical conditions. I will show and discuss data pointing to SD as a specific mechanism that significantly accelerates neuronal and astroglial injury in the metabolically compromised peri-lesional cortex, worsening secondary damage following stroke or TBI.

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DIFFERENT CONFORMATIONS OF NA,K-ATPASE INDUCED BY THE BINDING OF OUABAIN AND MARINOBUFAGENIN

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Na,K-ATPase plays an important role in the regulation of cell volume affecting the activity of different ion-exchangers. Numerous data demonstrate that this enzyme is also receptor for cardiotonic steroids (CTS) that without pump inhibition can activate different signal cascades. CTS ouabain and marinobufagenin are known to inhibit Rb^+ transport providing by Na,K-ATPase in kidney epithelial cells (MDCK) with similar values of IC_{50} equal to about 0,1 μM [1]. Simultaneously ouabain and marinobufagenin induce cell death on the way characterized by mixed sign of necrosis (cell swelling) and apoptosis (activation of caspase-3) with different values of IC_{50} (about 0.05 and 5 μM respectively). The data suggest that different CTS may induce different conformation of Na,K-ATPase that, in turn, may provides the binding to the enzyme different proteins activating signal cascades. Using purified Na,K-ATPase from rabbit kidney we have found that ouabain and marinobufagenin inhibited Na,K-ATPase by similar manner. However, it was shown using Na,K-ATPase labeled with 5-iodoacetamidofluorescein [2] that the conformations of Na,K-ATPase induced by ouabain and marinobufagenin are different. Using isothermal titration calorimetry (ITC) we have found that at 25 °C the binding constants for marinobufagenin and ouabain with ATPase are practically the same (~0.5 μM), but the energy profile for these two reactions is dramatically different. Ouabain binding is enthalpy favorable process, the marinobufagenin binding is entropy driven. Moreover marinobufagenin binds to Na,K-ATPase with two type of sites characterized by Kd 0.5 μM with stoichiometry 1 : 1 mol/mol enzyme (first type) and 4 : 1 mol/mol, Kd 4 μM (second type). The data demonstrate that formation of complex ouabain or marinobufeganin with Na,K-ATPase provides different enzyme conformations.

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BOTH NA^+/K^+-PUMP AND NA^+,K^+, $2CL^-$ COTRANSPORT CONTRIBUTE TO CELL VOLUME CONTROL IN HUMAN LUNG FIBROBLASTS

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This study examined the relative impact of Na^+/K^+-pump and $Na^+,K^+,2Cl^-$ cotransport in the regulation of the volume of human lung fibroblasts. Inhibition of the Na^+/K^+-pump by ouabain or of $Na^+,K^+,2Cl^-$ cotransport by bumetanide for 24 hours attenuated the rate of K^+ (^{86}Rb) influx in human lung fibroblasts from (1537 ± 124) cpm/well in control cells to (695 ± 14) and (522 ± 14) cpm/well in ouabain- or bumetanide-treated cells, respectively, whereas simultaneous addition of both inhibitors decreased the rate of K^+ influx to (180 ± 8) cpm/well. Inhibition of either the Na^+/K^+-pump or of $Na^+,K^+,2Cl^-$ cotransport did not significantly change the volume of human lung fibroblasts, measured as ^{14}C-urea available space, whereas simultaneous blockade of both ion transporters resulted in a 2-fold elevation of cell volume (table). Thus, our results demonstrate for the first time that both Na^+/K^+-pump and $Na^+,K^+,2Cl^-$ cotransport contribute to cell volume control in human lung fibroblasts.

Effect of ouabain and bumetanide on the volume of human lung fibroblasts

+	Cell volume	
	ml per mg protein	pl per cell
None (control)	0.79 ± 0.12	0.23 ± 0.04
Ouabain, 100 nM	0.97 ± 0.14	0.26 ± 0.04
Bumetanide, 10 μM	0.62 ± 0.01	0.22 ± 0.01
Ouabain + bumetanide	1.37 ± 0.13*	0.43 ± 0.04*

* $p < 0.05$ as compare to controls.

BOTH CELL VOLUME CHANGES AND NA^+_i/K^+_i-SENSITIVETRANSCRIPTOME CONTRIBUTE TO INFLAMMATORY AND IMMUNE SYSTEM RESPONSES

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Previously, we reported that cell volume perturbations sharply affect respiratory burst in human neutrophils triggered by different stimuli including zymozan, formyl peptide, Ca^{2+}ionophore and activators of protein kinase C [1]. Under isosmotic conditions cell volume changes are caused by alterated content of monovalent ions and other intracellular osmolytes. Considering this, we hypothesized that inflammatory and immune responses are affected by intracellular $[Na^+]/[K^+]$ ratio. To examine this hypothesis, we identified ubiquitous and tissue-specific $[Na^+]_i/[K^+]_i$-sensitive transcriptomes by comparative analysis of differentially expressed genes in vascular smooth muscle cells from rat aorta(RVSMC), the human adenocarcinoma cell line HeLa, and human umbilical vein endothelial cells (HUVEC). To augment $[Na^+]_i$ and reduce $[K^+]_i$, cells were treated for 3 hrs with the Na^+,K^+-ATPase inhibitor ouabain or placed for the same time in the K^+-free medium. Employing Affymetrix-based technology, we detected changes in expression levels of 684, 737 and 1839 transcripts in HeLa, HUVEC and RVSMC, respectively, that were highly correlated between two treatments ($p < 0.0001$; $R^2 > 0.62$). Among these Na^+_i/K^+_i-sensitive genes, 80 transcripts were common for all three types of cells. To establish if changes in gene expression are dependent on increases in $[Ca^{2+}]_i$, we performed identical experiments in Ca^{2+}-free media supplemented with extracellular and intracellular Ca^{2+} chelators. Surprisingly, this procedure elevated rather than decreased the number of ubiquitous and cell-type specific Na^+_i/K^+_i-sensitive genes. Among the ubiquitous Na^+_i/K^+_i-sensitive genes whose expression was regulated independently of the presence of Ca^{2+}chelators by more than 3-fold, we discovered interleukin-6 (IL6), prostaglandin-endoperoxide synthase 2 (PTGS2) and several other genes playing a key role in inflammatory and immune system responses [2]. The role of elevation of $[Na^+]$i/$[K^+]$i ratio in the triggering of these responses is further confirmed by data showing that the plasma concentration of IL6 increases up to 100-fold during muscular exercise. This increase is followed by the expression of the IL1 receptor agonist (*Il1ra*) and the anti-inflammatory cytokine IL10. Importantly, contracting skeletal muscle rather than the immune cells is the only source of the IL6 in circulation in response to exercise [3]. Importantly, both in humans and experimental animals, intensive exercise increases $[Na^+]_i$ in skeletal muscles by 3-4-fold and decreases $[K^+]_i$ by 15-25% via activation of voltage-gated K^+ and Na^+ channels and partial inactivation of Na^+,K^+-ATPase.

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TRANSCRIPTOMIC CHANGES IN CA^{2+}-DEPLETED CELLS: A MAJOR ROLE OF ELEVATED INTRACELLULAR $[NA^+]_i/[K^+]_i$ RATIO

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It is generally accepted that cell volume alteration affects transcriptome via elevation of ionic strength, i.e. total intracellular concentrations of Na^+, K^+ and Cl^-, that, in turn, activates tonicity enhancer binding protein (TonEBP) via elevation of intracellular Ca^{2+} ($[Ca^{2+}]_i$). To examine the role of Ca^{2+}_i-mediated signaling, we studied the action of Ca^{2+}-deletion triggered by addition of extra (EGTA) and intracellular (BAPTA) Ca^{2+} chelators on gene expression in vascular smooth muscle cells (VSMC) from rat aorta. In 3-hr Ca^{2+}-deletion and Na^+,K^+-ATPase inhibition in K^+-free medium altered expression of 4610 and 3677 transcripts, respectively. Among them we found 1844 genes whose expression was affected by both stimul thus suggesting a key role of elevated $[Na^+]_i/[K^+]_i$ ratio. Indeed, Ca^{2+}-depletion resulted in elevation of $[Na^+]_i$ and attenuation of $[K^+]_i$ by ~3- and 12-fold, respectively. This effect was caused by 3-fold elevation of the permeability of the plasma membrane for Na^+ and K^+ documented by increments of the rate of ^{22}Na and ^{86}Rb influx measured in the presence of ouabain and bumetanide. Among Na^+_i/K^+_i-sensitive genes whose expression was also affected by Ca^{2+} depletion by more than 4-fold we found activating transcription factor *Atf3,* early growth response *Egr1, Egr2* and *Egr3,* regulator of G-protein signaling *Rgs2*, nuclear receptor subfamily 4 group A *Nr4a2* and *Nr4a3*, inositol 1,4,5-trophosphate 3-kinase *Itpkc*. Both elevation of the $[Na^+]_i/[K^+]_i$ ratio and augmented the expression of the above listed genes triggered by Ca^{2+}-deletion or Na^+,K^+-ATPase inhibition were abolished in low-Na^+, high K^+-medium. Thus, we report here for the first time that elevation of the $[Na^+]/[K^+]$ ratio plays a key role in transcriptomic changes triggered by Ca^{2+} depletion. There results have profound implications for the analysis of data obtained in cells subjected to sustained cell volume modulation in the presence of polyvalent cation chelators.

HYPOCHLOROUS ACID EFFECTS ON VOLUME REGULATION OF MIGRATING NEUTROPHILS

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Cell volume regulatory mechanisms participate in different activities of cells including neutrophils migration and ROS generation [1, 2]. The effect of HOCl on the random migration of human neutrophils was studied.

Isolated neutrophils (untreated and HOCl-treated) were placed on glass for 20 min and fixed. The volume (*V*) and projection area (*S*) were calculated for single control ($n = 130$) and treated cells ($n = 160$) using atomic-force microscopy.

Because neutrophils movement consists of repeated cycles including the formation of lamellipodium during cell spreading and a cell retraction, we have identified three cell groups according to its area: 1) $S < 75\ \mu m^2$, inactive cells and hemispherical retractile cells; 2) *S* from 75 to 150 μm^2, cells with growing lamellipodium; 3) $S > 150\ \mu m^2$, fully spreading cells.

The average V of control cells in group 2 increased by 30% as compared to group 1 cells and the values of cell volume varied around a constant mean of 149 μm^3 (from 98 to 225 μm^3). As volume changes associated with lamellipodium growth, as individual size differences of neutrophils may cause this volume fluctuation. With a further area rise above 150 μm^2 the variability of volume values became very low (average *V* 155 μm^3, max *V* 220 μm^3). The rapid increasing of cell area led to marked S/V ratio changes. It varied from 0.4 to 1.8.

The average *V* of exposed to HOCl cells from group 1 was less than control value (96 μm^3). With cell spreading it increased initially by 57%. A slow gradual increase of cell volume was observed for the entire further area growth from 75 μm^2 to the maximum. As a result the average *V* of treated cells reached to control value (group 2) and then overpassed (165 μm^3, group 3). The fluctuation of volume values was greater and persisted in group 3. Because cell area dynamics was similar to the control, the average ratio *S*/*V* of treated cells was lower. Thus, HOCl alters cell volume regulation of random migrating human neutrophils.

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LASER SCANNING MICROSCOPIC *IN VIVO* ANALYSIS OF THE VOLUME OF THE BLOOD CAPILLARIES IN PSORIASIS THERAPY

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Psoriasis is a chronic recurring inflammatory skin disease associated with the development of erythematosquamous patches and plaques and a disturbed skin barrier function. While many etiological aspects have been described in detail, several pathogenetic mechanisms remain unknown. The underlying inflammatory reaction of psoriasis dominated by TH 1 cells entails epidermal hyperproliferation, disturbed differentiation and structural changes in the papillary capillaries.

The blood circulation system of the skin is composed of two horizontally arranged plexi, located parallel to the skin surface. The deep dermal plexus, lying on the border to the subcutis, sends arterioles to the upper plexus lying within the zone of the dermal-epidermal junction. The upper plexus supplies every papilla with a capillary loop.

In clinical practice, the visual inspection of the patient's skin mainly determines the duration of the psoriasis therapy; although it is known that neovascularisations, which persist beyond the healing of the lesions, facilitate the development of recurrences *in loco*.

The capillary loop structure in healthy volunteers was compared to that in psoriatic patients. The diameters of the capillaries and papillae were measured for each group with confocal laser scanning microscopy (CLSM).

All psoriatic patients showed elongated, widened and tortuous microvessels in the papillary dermis, whereas all healthy controls showed a single capillary loop in each dermal papilla. The capillaries of the papillary loop and the dermal papilla were significantly enlarged in the psoriatic skin lesions (diameters (24.39 ± 2.34) μm and (146.46 ± 28.52) μm, respectively) compared to healthy skin (diameters (9.53 ± 1.80) μm and (69.48 ± 17.16) μm, respectively) ($p < 0.001$).

CLSM seems to be a promising non-invasive technique for evaluating dermal capillaries in psoriatic patients and its monitoring. The diameter of the vessels could be seen as a well quantifiable indicator for the state of psoriatic skin.

REGULATION AND FUNCTIONS OF CELL VOLUME SENSITIVE SGK1

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The serum-and-glucocorticoid-inducible-kinase-1 (SGK1) is exquisitely sensitive to cell volume. SGK1 transcription is upregulated by hypertonic and isotonic cell shrinkage. SGK1 transcription is further upregulated by hyperglycemia, ischemia and a variety of hormones including glucocorticoids, mineralocorticoids and TGFß. SGK1 is activated by insulin and growth factors via phosphatidylinositol-3-kinase, 3-phosphoinositide dependent kinase PDK1 and mTOR. SGK1 up-regulates the Na^+/K^+-ATPase,
a variety of carriers (e.g. NCC, NKCC, NHE1, NHE3, SGLT1, several amino acid transporters) and several ion channels (e.g. ENaC, SCN5A, TRPV4-6, Orai1/STIM1, ROMK, KCNE1/KCNQ1, GluR6, CFTR). SGK1 further up-regulates a number of enzymes (e.g. glycogen-synthase-kinase-3, ubiquitin-ligase Nedd4-2), and transcription factors (e.g. forkhead-transcription-factor FOXO3a, ß-catenin, nuclear-factor-kappa-B, NFκB). SGK1 sensitive functions contribute to regulation of epithelial transport, excitability, degranulation, matrix protein deposition, coagulation, platelet aggregation, migration, cell proliferation and apoptosis. Apparently, SGK1 is not required for house keeping functions, as the phenotype of SGK1 knockout mice is mild. However, excessive SGK1 expression and activity participates in the pathophysiology of several disorders including hypertension, obesity, diabetes, thrombosis, stroke, inflammation, autoimmune disease, fibrosis and tumor growth.

NA^+, K^+-ATPASE AND ENDOGENOUS CARDIAC STEROIDS IN DEPRESSIVE DISORDERS

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Depressive disorders, including major depression, dysthymia and bipolar disorder (BD), are a serious and devastating group of diseases that have a major impact on the patient's quality of life. The etiology of depressive disorders remains unclear. It is now recognized that the Monoaminergic hypothesis for the etiology of depressive disorders, which dominated the field for decades, cannot by itself explain the complex etiology of these diseases. Endogenous cardiac steroids (CS) are present in the circulation and in the brain and are considered as hormones synthesized in and released from the adrenal gland. The binding of CS to Na^+, K^+-ATPase inhibits the transport of these ions and leads to the activation of several cell-specific signaling pathways. Recent studies from our and other laboratories have shown that: (1) CS levels in the parietal cortex of BD patients are significantly higher and those in the plasma are lower than in normal subjects; (2) Intra-cerebro-ventricle (i.c.v) injection of anti-ouabain antibodies, which lower brain endogenous CS levels, into Sprague Dawley (SD) rats elicited anti-depressive and anti-manic behaviors in Forced Swimming Test, and amphetamine-induced hyperactivity, respectively; (3) A significant association with BD was observed for six single-nucleotide polymorphisms (SNPs) in the three genes encoding the Na, K-ATPase α isoforms and for a specific polymorphism within the gene encoding the α3 isoform; (4) Acute i.c.v. injection of anti-ouabain antibodies into SD rats caused significant changes in catecholamine levels in brain areas associated with depression; and (5) Injection of a 4-(3'α-15'β-dihydroxy-5'β-estran-17'β-yl) furan-2-methyl alcohol, an antagonistic to CS in several experimental systems, induced an anti-depressive response in SD rats. These results are in accordance with the notion that malfunctioning of the Na^+, K^+-ATPase/CS system may be involved in the manifestation of depressive disorders and BD in particular and identify the system as a target for the treatment of these maladies.

REGULATION OF GARDOS CHANNEL ACTIVITY BY OXYGEN PARTIAL PRESSURE

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Maintenance of optimal volume of erythrocytes is an important condition of normal gas exchange and one of the factors, which determine blood rheology. Regulation of erythrocyte volume is achieved by ion transporters. Ion transporters are usually studied in a medium equilibrated with atmospheric air, i.e. at high oxygen partial pressure. However, activity of ion transport has recently been demonstrated to be dependent on oxygen content in the medium [1]. One of the possible ways of erythrocyte volume regulation is through Ca^{2+}-activated K^+ channels (Gardos channels), which abnormal activity accompanies several diseases and leads to water losses from cells.

Activity of Ca^{2+}- activated K^+ channels was studied by recording pH in suspension of erythrocytes in the presence of protonophore and Ca^{2+} ionophore in the medium with high (equilibrated with atmospheric air) and low oxygen content. Activity of Gardos channels at low oxygen decreased at external calcium concentration of 2.5 μM (which is a threshold of activation of the channels). This effect was controlled by membrane potential: the lower membrane potential, the greater suppression of Gardos channels in the medium with low oxygen. Gardos channels are known to be potential-dependent in the air-equilibrated [2]. On the contrary, at high calcium concentrations in external medium (greatly exceeding the threshold of activation of Garden channels, 400 μM) activity of Ca^{2+}-dependent K^+ transport increased in the low-oxygen medium. These data suggest a hypothesis that lowering of oxygen content in the medium decreases sensitivity of Gardos channel in erythrocytes to Ca^{2+}. However, the rate of K^+ outflux through Gardos channels is getting higher, probably due to conductivity increase in response deoxygenation.

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SALT: IT'S DAMAGING EFFECT ON THE CARDIOVASCULAR SYSTEM

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There is overwhelming evidence that a reduction in our salt intake from the current level in most countries of 9–12 g/day down to 6 g/day i.e. a 50% reduction lowers blood pressure and thereby reduces the number of people dying and suffering from strokes, heart attacks and heart failure – the commonest cause of death and disability in the world. Increasing evidence now suggests that salt has a direct damaging affect on the vascular system by increasing arterial stiffness and impairing endothelial function. These effects may be independent and/or additive to the effect that salt has on blood pressure.

There is now increasing evidence that small changes in plasma sodium could play an important role. For instance, increasing sodium concentration within the physiological range has been shown to increase endothelial stiffness, reduce nitric oxide release as well as having a stimulant effect on some cell cultures.

Clinical studies have shown that every time salt is eaten, even in quite modest amounts, there are changes in plasma sodium in the blood. These are associated with an adverse effect on the vascular system, increasing arterial stiffness and reducing endothelial function.

At the same time, studies in patients on hemodialysis have shown that small changes in the dialysis fluid sodium which result in changes in plasma sodium in the patients, do seem to affect blood pressure at least in the short term. These studies are leading to a better understanding of the mechanism whereby salt could affect not only blood pressure but also have direct effects on the vascular system.

Public health policies to get the food industry to slowly reduce the large amounts of salt they add to food have been successful in Finland, and are being successful in the UK. Every country should now adopt a coherent and workable strategy to reduce salt intake in the whole population. Even a modest reduction in population salt intake will have major beneficial effects on health, along with major cost-savings.

REGULATED EXPRESSION OF NA^+/K^+ PUMP DURING HUMAN BLOOD LYMPHOCYTE PROLIFERATION

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The mitogenic effect of growth factors is mediated by the transcriptional regulation of numerous genes which control cell cycle progression and DNA replication. In addition, in order to realize the mitogenic signal into cell functional response, genes involved in proliferation-related biochemical and physiological processes are co-regulated. In this study we show that in mitogen-stimulated human blood lymphocytes, enhanced ouabain-sensitive $Rb^+(K^+)$ fluxes in the middle/late stage of $G_0/G_1/S$ transit are associated with the increase of the amount of Na^+,K^+ pumps expressed at the cell surface (as determined by the $[^3H]$ouabain binding). Analysis of total RNA (RT-PCR) and protein abundance (Western blotting) showed a 3-fold increase in the level of Na^+,K^+-ATPase α1- and β1-subunits mRNAs and significant increase in the Na^+,K^+-ATPase α1-subunit protein during the first day of mitogen-induced proliferation. In competent T lymphocytes, recombined interleukin-2 (IL-2) was revealed to increase both the transport activity of Na^+/K^+ pump and the content of Na^+,K^+-ATPase α1-protein. The pharmacological inhibition of MEK/ERK or JAK/STAT cascades suppressed the IL-2-induced proliferation and reduced the functional and protein expressions of Na^+,K^+-ATPase. These results suggest that in mitogen-induced human blood lymphocytes (1) the functional expression of Na^+/K^+ pump is closely associated with the cell cycle progression, being dependent on IL-2; (2) the cell cycle-associated enhancement of K^+ transport is a result of increased number of newly synthesized functioning Na^+/K^+ pumps in cell membrane; (3) the cytokine signaling via the IL-2 receptor is necessary for the upregulation of Na^+/K^+ pump during the lymphocyte transition from quiescence to proliferation.

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SHRINKAGE OF ADIPOCYTES AS A POSSIBLE BIOLOGICAL CAUSE FOR WEIGHT REGAIN AFTER WEIGHT LOSS IN OVERWEIGHT/OBESE SUBJECTS

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Adipocytes store energy but also regulate total body energy metabolism by the secretion of adipokines [1, 2]. Preadipocyte differentiation is characterized by an enormous increase in cell volume due to the growing fat droplet. In parallel, the adipokine profile changes and a basal lamina develops as a protective shield [3] and important survival factor [4].

In the obese, adipocytes display hypertrophy linked to a further modification of the adipokine profile, which is possibly involved in type II diabetes and cardiovascular disorders. Several factors may self-limit adipocyte expansion. Overgrowth leads to intracellular hypoxia. Since oxygen is crucial for the modification of collagens, hypoxia may block the growth of the basal lamina. Further, since insulin is the key promoter of collagen maturation [5], insulin resistance may assist in blocking cell expansion. Thirdly, variation of metabolic response may prevent cell growth. In preadipocytes PPARγ stimulates fat storage, but in mature adipocytes it may induce beta-oxidation [6].

Losing 5% of the body weight reduces the risk for complications of overweight/obesity. This 10–15% reduction in adipocyte volume leads to normalization of the adipokine profile. Yet, going from feeding to starvation, molecular pathways do not absolutely revert [7]. Surprisingly, not the fatty acid but the glucose metabolism seems to be the gatekeeper of volume reduction [8].

After weight loss on a low-calorie diet 50% of subjects regain their weight within 1–2 years. Calorie restriction may hamper reconstructing the basal lamina to accommodate the shrinking adipocyte. This mechanical stress may result in an aberrant adipokine profile, including decreased leptin, which influences the eating behavior of the host to drive the refilling of adipocytes [9]. Prevention of weight (re)gain may follow from [1] fixing adipocytes at relatively low volume and blocking adipocyte (over)growth as by the use of resveratrol [10] and from [2] preventing cellular stress during weight loss.

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GLUTATIONYLATION OF α-SUBUNIT OF NA,K-ATPASE FROM RAT HEART RESULTS IN THE ENZYME INHIBITION

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Na,K-ATPase (Na-pump) is known to participate in the regulation of cell volume, therefore the change of Na-pump activity may be important for cell volume adjustment. We focused in this study on the regulation of Na,K-ATPase under hypoxia conditions when concentration of oxidized glutathione is increased. Using specific antibodies we have shown that partially purified preparation of Na,K-ATPase from rat heart contains α1- and α2-isoforms. Both enzyme subunits were found in S-glutathionylated state. Glutathionylation of α1- (but not α2-subunits) is partially removed if purification of Na,K-ATPase was made in the presence of dithiothreitol. Addition of oxidized glutathione *in vitro* inhibits both isoenzymes and increases the level of S-glutathionylation of both subunits. Na,K-ATPase activity of α2-isoform was more sensitive to the action of oxidized glutathione than the activity of α1-isoform. Inhibition of isoenzyme containing α1-subunit is presented by two phases, inhibition constants are equal to 3821 and 246 $M^{-1}min^{-1}$ for fast and slow phases, respectively. Adenylic nucleotides protect Na,K-ATPase activity from the inhibition by oxidized glutathione, their protecting activity falls in the range: ATP, ADP, AMP.

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THE USE OF TRANSMISSION-THROUGH-DYE MICROSCOPY FOR STUDYING APOPTOSIS AND VOLUME CHANGES IN ADHERENT CELLS

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In transmission-through-dye (TTD) microscopy, a nontoxic and cell-impermeant dye Acid Blue 9 is added to the cell culture medium, and the sample is placed in a shallow gap that is just slightly deeper than the cells. In the simplest case, such a gap can be formed between a coverslip and a slide kept apart by a small amount of grease. Alternatively, an attachment to a condenser with a horizontal watertight window at the bottom can be built for an inverted microscope; it is lowered into a Petri dish to bring the window close to the cells, thus creating a temporary narrow gap between the cells and the window. As the cells displace the dye, the depth of the absorbing layer becomes complementary to cell thickness; when such a sample is imaged in transmitted light at the wavelength of maximal dye absorption (630 nm), thicker cells appear brighter. By applying logarithmic transformation to the image and correcting it for the background level, the image contrast can be quantitatively converted to cell thickness and volume [1]. The accuracy of measurements by TTD imaging has been verified using spherical beads and confocal scanning. Dead cells with leaky plasma membranes are darker than the background and are easily distinguishable from intact cells.

TTD can be realized on any widefield microscope, with only a single bandpass filter added anywhere in the optical path, or on a laser scanning microscope in transmission mode. It is well suited for prolonged time-lapse experiments because the cells are not exposed to high-intensity illumination and the results are not affected by possible instability in the light source. TTD is also compatible with fluorescence imaging, making possible simultaneous measurements of the cell volume and various fluorescent markers [2]. We are currently using this method to investigate cell volume changes during apoptosis and the osmotic properties of normal and apoptotic cells.

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CELLULAR SWELLING AND OXIDATIVE STRESS DRIVE PATHOLOGICAL GLUTAMATE RELEASE AND BRAIN DAMAGE IN STROKE

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A number of neurological disorders, including stroke, hyponatremia, epilepsy, and hepatic encephalopathy, are linked to pronounced cellular swelling. Such swelling is largely restricted to glial cells (astrocytes) and the postsynaptic neuronal processes (dendrites). Recent work strongly suggests that cell swelling is a major determinant of brain damage. In brain tissue, increases in cell volume trigger release of the toxic excitatory amino acids, glutamate and aspartate, via the volume-sensitive permeability pathway termed volume-regulated anion channel (VRAC). VRAC blockers strongly reduce ischemic brain damage and alleviate neurological deficits in animal stroke models. Our *in vitro* and *in vivo* studies demonstrated that VRAC is synergistically regulated by swelling and reactive oxygen species (ROS). ROS alone do not cause VRAC opening, but potently stimulate VRAC when combined with even moderate swelling. Given that stroke pathology involves oxidative stress, we tested the hypothesis that antioxidants prevent pathological glutamate release and reduce brain infarction in rodent stroke. With this purpose we explored the neuroprotective properties of two antioxidants, Tempol and edaravone, and tested their effects on pathological amino acid release using a microdialysis approach. Tempol but not edaravone reduced extracellular levels of glutamate and aspartate during a two-hour transient focal ischemia. Consistent with latter data, Tempol but not edaravone decreased brain infarction volumes and improved neurological outcomes. Cellular and biochemical analyses established that the superior protective properties of Tempol are related to its potency in scavenging the ROS superoxide anion. Overall, our work provides new mechanistic insight into the complex relationship between oxidative stress, glutamate release and brain damage in stroke, and creates a novel basis for design and rational selection of novel therapeutic compounds.

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HIGH SALT (NACL) AFFECTS TH17 POLARIZATION

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Current teaching suggests that the “milieu intérieur” bathing all cells is controlled by isosmotic passive equilibration with plasma. Recent evidence has shown this idea needs to be revised. We have the first evidence that a regulatory network between immune cells and the local environment exists in mice and humans. Therefore, we hypothesize that the immune system is influenced by the local electrolyte environment and vice versa. We found that hypertonicity induced by high salt (NaCl) affected cytokine-induced Th17 polarization by a p38MAP kinase, TonEBP, SGK1-dependent mechanism, and that such a salt-driven polarization into a chronic inflammatory state worsens autoimmune disease.

TRPM2-ΔC IS THE HYPERTONICITY-INDUCED CATION CHANNEL (HICC) IN HELA CELLS AND THE ECTO-ENZYME CD38 IS A MEDIATOR OF ITS ACTIVATION

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Hypertonicity-induced cation channels (HICCs) are key-players in proliferation and apoptosis but their molecular correlate remains obscure. Also, the activation pattern of HICCs was not defined yet. We report that in HeLa cells, intracellular adenosine diphosphate ribose (ADPr) and cyclic ADPr, as activators of TRPM2, elicit cation currents that are identical to the osmotic activation of HICCs. Silencing of TRPM2 and of the *ecto*-enzyme CD38 (as the supposed source of ADPr and cADPr) inhibit HICC- and nucleotide-induced currents as well as the osmotic volume response of cells. Quantification of intracellular cADPr and extracellular application of nucleotides reveal, however, that it is the outwardly directed gradient rather than the intracellular activity of ADPr and cADPr triggering TRPM2 and, very likely, this export goes together with a biotransformation of nucleotides. Cloning of TRPM2 identifies the ΔC-splice variant as molecular correlate of the HICC, which is supported by quantification of its Ca^{2+} selectivity. Finally, pull-down and FRET/FLIM experiments reveal a close proximity of TRPM2 and CD38 and we propose a transport related nucleotide export via CD38 as novel mechanism of TRPM2 activation.

AN EMERGING CONCEPT OF VASCULAR SALT SENSITIVITY IN MAN

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For millions of years, daily sodium chloride intake in man was about 1g. Then recently, about 10,000 years ago, salt intake increased by about ten-fold because of the practice of using salt as a food preservative. If salt intake exceeds the kidneys' ability for salt excretion, then salt is deposited in the body which affects heart, blood vessels and kidneys. In this context, the endothelial surface layer facing the blood stream became a focus of interest. This soft layer, termed endothelial glycocalyx, is a negatively charged biopolymer known to preferentially bind sodium. After a salty meal, the translocation of sodium from the blood into the interstitium is delayed by the significant buffering capacity of the endothelial glycocalyx. Excessive sodium intake over long periods damages the glycocalyx and leads to a decrease in its sodium buffering capacity. It has been shown recently that, after mechanical interaction of blood with a damaged endothelial glycocalyx, erythrocyte surfaces also become damaged [1]. This observation led to the conclusion that vascular sodium buffer capacity of an individual could be derived from a blood sample. A test system (Salt-Blood-Test; SBT) was developed based upon the sodium-dependent erythrocyte zeta-potential [2]. Erythrocyte sedimentation velocity was measured in isosmotic, biopolymer-supplemented electrolyte solutions of different sodium concentrations. Erythrocyte sodium sensitivity (ESS), inversely related to erythrocyte sodium buffer capacity, was expressed as the ratio of the erythrocyte sedimentation velocities of 150 over 125 mM Na^+ solutions ($ESS = 150Na^+/125Na^+$). In 61 healthy individuals (mean age: (23.0 ± 0.5) years) ESS ranged between 2 and 8. The mean value was 4.30 ± 0.19. In conclusion, the SBT could serve as an *in vitro* test system for the evaluation of salt sensitivity allowing follow-up measurements in the prevention and treatment of vascular dysfunctions.

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VOLUME-RELATED ION CHANNELS INVOLVED IN CELL SURVIVAL-DEATH SWITCHING

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Cell volume regulation is essential for survival of animal cells. The regulatory volume decrease (RVD) and increase (RVI) are accomplished by water movement driven by KCl efflux and NaCl influx, respectively, mediated by a number of Cl^-, K^+ and cation channels. Persistent cell shrinkage and swelling are major hallmarks of apoptotic and necrotic cell death, respectively, and caused by the apoptotic volume decrease (AVD) and necrotic volume increase (NVI) coupled to impairment of RVI and RVD, respectively. Since the AVD and NVI processes also involve activities of Cl^-, K^+ and cation channels, it is likely that these volume-related ion channels (such as volume-sensitive outwardly rectifying anion channel (VSOR), CFTR, hypertonicity-induced cation channel (HICC), acid-sensitive outwardly rectifying anion channel (ASOR) and TRPM7) play roles in the cell survival-death switching. In fact, our studies *in vitro* showed that apoptotic and necrotic cell death is induced or rescued by controlling activities of these volume-related ion channels under a variety of conditions, including apoptotic stimulation, excitotoxicity, acidotoxicity, lactacidosis and hypoxia-reoxygenation. Also, ischemia-reperfusion-induced neuronal apoptosis and cardiac necrosis *in vivo* were found to be rescued by controlling volume-related anion channel activities. Among these volume-related ion channels, the molecular entities of VSOR and ASOR remain elusive. Although TMEM16F (ANO6) and ClC-3 were proposed as the molecular candidates for VSOR and ASOR, respectively, our recent studies excluded these possibilities and showed that they function as distinct-typed Ca^{2+}-activated and outwardly rectifying chloride channels (CaCC and ORCC), respectively [1–3].

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INTRACELLULAR MONOVALENT CATIONS AS REGULATORS OF CELL VOLUME AND GENE EXPRESSION

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Cells respond to long-term exposure to anisosmotic environment by up- or down-regulation of the expression of gene products involved in the synthesis of compatible organic osmolytes. It is generally accepted that augmented transcription of these genes is caused by elevation of ionic strength, i.e. total intracellular concentrations of Na^+, K^+ and Cl^-, that, in turn, leads to binding of the transcription factor tonicity enhancer binding protein (TonEBP otherwise known as NFAT5) with tonicity enhancer *cis*-element 1 [1]. Importantly, cell volume perturbations in isosmotic environment are caused by alteration of the $[Na^+]_i/[K^+]_i$ ratio rather than ionic strength. Recently, we identified ubiquitous and tissue-specific $[Na^+]_i/[K^+]_i$-sensitive transcriptomes by comparative analysis of differentially expressed genes in vascular smooth muscle cells from rat aorta (RVSMC), the human adenocarcinoma cell line HeLa, and human umbilical vein endothelial cells (HUVEC) [2]. To augment $[Na^+]_i$ and reduce $[K^+]_i$, cells were treated for 3 hrs with the Na^+,K^+-ATPase inhibitor ouabain or placed for the same time in the K^+-free medium. Among ubiquitous Na^+_i/K^+_i-sensitive genes we found ~2-fold increment of NFAT5 and up-to 10 fold elevation of expression of prostaglandin-endoperoxide synthase 2 (PTGS2) and cytochrome P450 CYP1A, i.e. genes that might be involved in cell volume regulation via PLA_2-mediated pathways. Among cell-type specific genes involved in volume regulation of endothelial cells we found up to 3-fold elevation of RNA encoding P2Y2 purinergic receptors, ~2 fold attenuation of mRNA encoding catalytic subunits of PIP 5-kinase (PIP5K1C), up to 5-fold attenuation of regulatory subunits of PI-3-kinase PIK3R4 and PIK3R2, and 10-fold attenuation of expression of a negative regulator of PI-3 kinase activity PI-3-kinase interacting protein 1 (PIK3IP1). The role of these transcriptomic changes in cell volume regulation mediated by polyphosphoinositide signaling is currently investigated.

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COMPLEX METHOD FOR THE STUDY OF VOLUME-DEPENDENT RED BLOOD CELL BEHAVIOR

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The investigations of processes following cell volume changes require a complex of methods to reveal and visualize structural alteration of cell membrane and cytoplasm. In our lab we developed combination of approaches that allows us to investigate red blood cells under change of media osmolarity, as a rule accompanied by changes of cell volume [1–3].

Laser interference microscopy (LIM) technique makes possible to investigate cytoplasmic alteration in living cells. LIM method in combination with a direct method for cell volume measurement may be employed for estimation of morphological changes and for assessment of redistribution of cell substances in the cytoplasm evoked by diverse stimuli including osmotic stress.

Micro Raman spectroscopy allows estimating conformation of hemoglobin in erythrocyte and atomic force microscopy (AFM) gives precise information about cell geometric dimensions and physical properties of cell surface. We have showed that the combination of these methods provide detailed information about the distribution of cytosolic hemoglobin inside RBC.

Red blood cell surface relief studied by AFM demonstrated volume-dependent alterations under change of media osmolarity. Results propose an implication of a two-dimensional membrane carcass in volume sensing and/or signal transduction.

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ACID-EXTRUDING TRANSPORTERS IN MAMMARY AND PANCREATIC ADENOCARCINOMA: REGULATION AND ROLES IN CELL MOTILITY

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A fundamental property of solid tumors is an altered pH-profile compared to normal tissues. This at least in part reflects increased glycolytic metabolism, necessitating increased acid extrusion to maintain survival, and in turn stimulating cancer cell motility [1, 2]. Acid extruding transporters are therefore interesting potential targets in cancer. The overall aim of these studies was to explore the regulation and roles of acid extruding transporters in human mammary and pancreatic adenocarcinomas.

In MCF-7 mammary cancer cells, expression of a constitutively active ErbB2 receptor (ΔNErbB2) greatly increased pH regulatory capacity, mediated by the Na^+/H^+ exchanger NHE1 and the Na^+, HCO_3^- cotransporter NBCn1 [3]. ΔNErbB2 expression or stimulation of wt ErbB receptors greatly increased NBCn1 expression and induced NHE1 phosphorylation at Ser703 [3, 4]. NHE1 was strongly expressed in invadopodial rosettes and, surprisingly, negatively regulated adhesion and 2D motility of ΔNErbB2-MCF-7 cells [5]. Finally, NHE1 and NBCn1 were upregulated in primary carcinomas and lymph node metastases of mammary cancer patients and Na^+,HCO_3^- cotransport was a major determinant of pH_i regulation in freshly dissected human mammary tumors.

In PDAC, we screened a panel of 4 human PDAC cell lines (AsPC-1, BxPC-3, Panc-1, MIAPaCa-2) against normal human pancreatic ductal cells (HPDE) by qPCR, immunoblotting and immunocytochemistry, and found major changes in the expression of NHE1, -2, V-type H^+-ATPase subunit a3, Na^+-HCO_3^- transporters SLC4A7 and -A8, anion exchangers SLC4A2 and -3, and H^+/lactate cotransporters MCT1 and -4.

In conclusion, expression and regulation of NHE1 and NBCn1 are markedly altered in breast cancer, resulting in altered pH regulation and cell motility. Also PDAC cells exhibit major changes in the expression pattern of acid extruding transporters. Ongoing experiments are addressing the functional relevance of this in PDAC development.

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THERMODYNAMICS OF ADENINE NUCLEOTIDES BINDING TO DUCK NA,K-ATPASE SUGGESTS THE ROLES OF β-PHOSPHATE IN COMPLEX FORMATION AND γ-PHOSPHATE IN CONFORMATIONAL CHANGES

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In all animal cells, active transport of sodium and potassium ions across the plasma membrane is facilitated by Na,K-ATPase affecting the cell volume regulation. The transport is accomplished by enzyme conformational changes between two main states, E1 and E2. Na,K-ATPase in the E1 conformation has a high affinity to ATP and Na^+, while enzyme in the E2 conformation has a low affinity to ATP and high affinity to K^+. It is not clear if binding of ATP to E1 conformation induces some structural changes and what is a role of phosphate groups of ATP in this process. We have used isothermal titration calorimetry to estimate the equilibrium constants, along with the enthalpic and entropic components for AMP, ADP and ATP binding to Na,K-ATPase from duck salt glands at different temperatures. We have found that β-phosphate of ADP involved in complex formation by increasing the affinity to Na,K-ATPase one order of magnitude, while the γ-phosphate of ATP has not effect on the affinity of the enzyme. Furthermore, we demonstrated that ATP, but not ADP, binding to Na,K-ATPase in E1 state generates a conformational change of the enzyme, which is compatible with the movement of 2350 ± 450 $Å^2$ of molecular surface area from a solvent exposed to a solvent-protected state. We propose that this structural change is caused by the locking of the P domain of Na,K-ATPase to the γ-phosphate of ATP, thereby reducing the exposed surface area of the protein.

Study was supported by RFBR grant №12-04-00103a and State Agreement № 16.512.11.2280.

TEMPERATURE DEPENDENCE OF VOLUME CHANGES TRIGGERED BY ANISOSMOTIC MEDIA IN INTACT AND PERMEABILIZED A549 CELLS

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Differing strikingly from aqueous solution, the gel-like cytoplasm of all cells shows enhanced viscosity, slowed diffusion and an ability to retain water. However, the gel-like character of the cytoplasm has not been fully documented for mammalian cells, and its impact on cellular processes, such as volume sensing and regulation, remains unclear. Previously, we employed the dual-image, surface reconstruction (DISUR) technique based on phase-contrast, digital video microscopy [1] and revealed that gentle perforation of the surface membrane with digitonin preserves the gel-like character of the whole-cell cytoplasm and cytoplasm of permeabilized cells swells or shrinks in response to alterations of external osmolarity [2]. In mammalian erythrocytes, regulation of volume-sensitive ion transporter such as Na^+,K^+,$2Cl^-$ cotransport, Na^+/H^+ exchanger and K^+,Cl^- cotransport by anisosmotic medium was completely abolished by 10 min incubation at 48–50 °C [3, 4]. Keeping this in mind, we compared cell volume adjustment of intact and permeabilized A549 cells subjected to 10-min preincubation at 37 °C, 44 °C and 48 °C. In intact cells, 2-fold decrease of medium osmolality resulted in transient elevation of cell volume by 50% followed by it almost complete restoration in 30 min via regulatory volume decrease (RVD). Consistently with previous reports, shrinkage evoked by 30 min elevation of medium osmolality was not accompanied by regulatory volume increase (RVI). As predicted, plasma membrane permeabilization completely abolished RVD. Ten min preincubation of intact, as well as permeabilized cells at 48 °C but not at 44 °C, completely blocked volume changes triggered by cell exposure to anisosmotic medium. Our results strongly suggest that temperature-dependent inhibition of volume-sensitive ion carries documented in early studies [3] is caused by inactivation of osmosensing behaviour of cytoplasmic biogel rather than biochemical signalling involved in RVD and RVI.

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VOLUME CHANGES TRIGGERED BY ANISOSMOTIC MEDIA IN INTACT AND PERMEABILIZED A549 CELLS: ROLE OF CYTOSKELETON NETWORK

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Differing strikingly from aqueous solution, the gel-like cytoplasm of all cells shows enhanced viscosity, slowed diffusion and an ability to retain water. However, the gel-like character of the cytoplasm has not been fully documented for mammalian cells, and its impact on cellular processes, such as volume sensing and regulation, remains unclear. Previously, we employed 3D imaging light microscopy developed in our laboratory [1] and revealed that gentle perforation of the surface membrane with digitonin preserves the gel-like character of the whole-cell cytoplasm. Moreover, we demonstrated that the cytoplasm of substrate-attached permeabilized cells swells or shrinks in response to alterations of external osmolarity [2]. Numerous studies demonstrated important role of cytoskeleton in cell volume adjustment. In this study, we compared cell volume adjustment of intact and permeabilized A549 cells subjected to disruption of microtubules by vinblastine (VB) and microfilament disassembly by cytochalasin B (CB). Both CB and VB decreased the maximal amplitude of cell swelling in hyposmotic medium and completely abolished cell volume increment triggered by hyposmotic medium in permeabilized cells. Consistently with previous reports, cell swelling of A549 cells in hyposmotic medium was accompanied by rapid regulatory volume decrease (RVD) that was absent in permeabilized cells. Neither CB nor VB abolished RVD seen in intact cells. In contrast to swelling, 30-min shrinkage of A549 cells resulted in negligible regulatory volume increase. Permeabilization did not significantly affect the amplitude of shrinkage evoked by hyperosmotic medium. Both in intact and permeabilized cells, the rates of shrinkage were sharply attenuated by CB. Viewed collectively, our results demonstrate that cytoskeleton network has a minor impact on intracellular signalling involved in RVD but plays a key role in the behaviour of cytoplasmic biogel as an osmosensor.

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MECHANISMS BY WHICH HYPERTONICITY INHIBITS TRANSEPITHELIAL NA^+ TRANSPORT ACROSS HUMAN BRONCHIAL EPITHELIAL CELLS FROM CYSTIC FIBROSIS DONORS

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Hyperosmotic challenge (HC) saline inhalation benefits Cystic Fibrosis (CF) patients. Surprisingly, these benefits are long-lasting and amiloride diminishes them. Our aim was to explain these effects. Human bronchial epithelial (HBE) cells from CF donors were grown in inserts and were used to measure amiloride-sensitive short circuit currents (I_{Na}), and transepithelial conductance (G_T) and capacitance (C_T). HC solutions were prepared by either adding additional NaCl or mannitol to the isosmotic buffer or to a buffer containing a low (6 mM) Na^+ concentration (6Na-HC). Our main results are:

1) The HC-induced inhibition of I_{Na} was protracted and required 60 minutes of re-exposure to the isosmotic solution to recover up to 75%;

2) The time of exposure to HC required to inhibit I_{Na} was < 2.5 minutes;

3) Exposure to HC-NaCl or 6Na-HC- solutions inhibited I_{Na} but the inhibition produced by the former solutions was significantly larger than the one observed by the latter solutions at the same osmolalities;

4) Amiloride significantly accelerated the recovery of I_{Na} following exposure to HC-NaCl but this effect was absent in low Na^+ HC solutions, i.e., 6Na-HC;

5) Both apical and basolateral exposure to HC solutions inhibited I_{Na};

6) Apical membrane permeabilization with nystatin simultaneously enhanced I_{SC}, completely inhibited the amiloride sensitive current but did not preclude the HC-induced inhibition of I_{SC};

7) Basolateral membrane permeabilization increased I_{SC}, precluded ouabain-sensitive inhibition of I_{SC} but did not obliterate the HC-induced inhibition of I_{SC};

8) Imaging of HBE membranes labeled with membrane fluorophores (i.e,. FM 4-64 and FM 4-64FX) suggests that exposure to HC induces membrane endocytosis.

Conclusions: 1) Exposure to HC inhibits HBE I_{Na} probably by producing an increase in the intracellular Na^+ concentration ($[Na^+]_i$) and inducing endocytosis of apical ENaC and basolateral Na/K ATPases; 2) Amiloride may produce its unexpected effects on CF patients by "protecting" ENaC from being inhibited by a HC-induced increase in $[Na^+]_i$.

THE POSSIBLE ROLE OF THE NON-GASTRIC H^+/K^+-ATPASE ATP12A (ATP1AL1) IN APOPTOSIS

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The non-gastric H^+/K^+-ATPase ATP12A (ATP1AL1) is expressed in various tissues. We found by RTPCR and/or western blotting, intracellular pH measurements, electron microprobe analysis, cell volume (CV) measurements and flow cytometry that ATP12A is expressed in human myelomonocytic HL60 cells, rat insulinoma Ins-1E cells, human pancreatic islets, the prostate cancer cell lines LNCaP, PC3 and DU-145 as well as in normal and cancerous human prostate tissue. Treatment of HL60 cells with low (1mM) concentrations of butyrate leads to monocyte-directed differentiation whereas higher (5-10mM) concentrations induce apoptosis as assessed by flow cytometric determination of CD86 expression, CV, cell granularity, caspase activity, phosphatidylserine exposure on the outer cell membrane leaflet, cell cycle analysis and cell proliferation. Ins-1E cells undergo apoptosis upon treatment with dexamethasone, the polyphenol resveratrol or by glucose starvation.Transcriptional up-regulation of ATP12A is evident during apoptosis in HL60 and Ins-1E cells and both cell types exhibit apparent apoptotic volume decrease (AVD). The inhibitor of the H^+/K^+-ATPase SCH28080 leads per se to induction of apoptosis in HL60 cells and Ins-1E cells and accelerates the time course of induced apoptosis. Moreover ATP12A expression is altered in tissue from benign hyperplasia of human prostate and in prostate cancer. In summary it is shown that ATP12A is functionally active, plays a role during apoptosis in HL60 cells and Ins-1E cells and is differently expressed in normal and pathological prostate tissue.

THE INVOLVEMENT OF GLUTATHIONE SYSTEM IN THE REGULATION OF THE FUNCTIONAL ACTIVITY OF NA^+/K^+-ATPASE PROTEINS IN OXIDATIVE STRESS

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The change in condition of glutathione system in oxidative stress is considered as one of the regulation mechanisms of cell volume. In formation of oxidative stress, the attack of active forms of oxygen is primarily directed not to the lipids, but the redox-sensitive amino acids in the proteins of cytosolic membranes. Therefore, the role of oxidative modification of proteins in disruption of plasmalemma can be put into the forefront. Glutathione and enzymatic redox-proteins of thiol-disulfide system associated with it ensure the process of S-thiolation/dethiolation of active protein nuclei, protecting them from irreversible oxidative modification and inactivation, which contributes to regulation of functional activity of the cells. It is known that the cell volume depends on functional activity of ion-transporting systems, including Na^+/K^+-ATPase. Providing catalytic functions and transitions between conformational states of Na^+/K^+-ATPase depends on lipid-protein interactions. A number of molecular mechanisms of damage lead to oxidation of SH-groups of the enzyme.

The simulation of oxidative stress was carried out in blood lymphocytes by adding H_2O_2 into incubation medium at a final concentration of 5 mM; the concentration of hydroxyl radical and protein-bound glutathione was estimated. It is shown that in incubating blood lymphocytes in the presence of 5 mM of H_2O_2, there occurred a fairly significant increase of the fraction of protein-bound glutathione (by 1.43 times) and hydroxyl radical (by 2.01 times), as compared with intact cells. In the formation of oxidative stress, the process of glutathionylation of cell proteins is being activated, and the activity of Na^+/K^+-ATPase increases, which contributes to maintaining the optimum lymphocyte volume for functioning.

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DIFFERENTIAL EFFECTS OF OXLDL COMPONENTS ON ENDOTHELIAL BIOMECHANICAL PROPERTIES

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Endothelial biomechanical properties have been suggested to play important roles in multiple endothelial functions. Our earlier studies have shown that endothelial stiffness is significantly increased by oxidized modifications of low-density lipoproteins (oxLDL). The goal of the current study was to identify the bioactive oxLDL components that are responsible for this effect. To address this goal, we systematically tested all the lipid fractions and the major bioactive lipid components of oxLDL for their effects on endothelial stiffness. We show here that the two fractions that contribute to the increase in endothelial stiffness are oxidized phospholipids (oxPC) and oxysterols. Furthermore, we show that oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphocholine (oxPAPC), a major oxPC component, and 7-ketocholesterol and 7α-hydroxycholesterol, the two major oxysterol components of oxLDL, play the key roles in endothelial stiffening. In addition, 27-hydroxycholesterol, a minor component of oxLDL that is abundant in atherosclerotic lesions also induces an increase in endothelial stiffness. We also find that oxPAPC- and oxysterol-induced endothelial stiffening is fully reversible by cholesterol supplement suggesting that this effect should be attributed to changes in the lipid composition of the membrane. Indeed, we show that significant amounts of oxidized PC products and oxysterols, specifically, 7-ketocholesterol accumulate in the membranes of endothelial cells exposed to oxLDL.

PARTICIPATION OF P38 MAPK IN APOPTOTIC VOLUME DECREASE IN JURKAT CELLS TREATED BY GAS TRANSMITTERS

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It has been shown that the volume decrease is an early essential component of apoptotic cell death. Activation of stress-responsive MAPKs (JNK and p38) was reported to mediate activation of volume-sensitive outwardly rectifying anion channels in several cell types [1–4]. Participation of gaseous transmitters (nitric oxide, hydrogen sulfide, carbon monoxide) in apoptosis regulation is one of the intriguing field of up-to-day science.

Contribution of p38 MAPK in apoptosis development will be investigated in this work. p38 MAPK participation in apoptosis regulation in cells treated by gas transmitters could be the evidence of this kinase role in functioning of volume-sensitive outwardly rectifying anion channels in above mentioned conditions.

We have shown that p38 MAPK acted proapoptotically in the cases of intracellular increase of nitric oxide and hydrogen sulfide and antiapoptotically in carbon monoxide-treated cells. As the role of p38 MAPK could vary from proapoptotic to antiapoptotic in the case of different gas transmitters action this molecule could have activating as well as inhibiting influence on volume-sensitive outwardly rectifying anion channels in the case of nitric oxide, hydrogen sulfide and carbon monoxide intracellular level increase.

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THE ROLE OF UBIQUITYLATION IN THE CONTROL OF NA^+ HOMEOSTASIS AND BLOOD PRESSURE CONTROL

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The kidneys play a major role in Na^+ homeostasis and consequently in blood volume and pressure regulation. This is highlighted by the genetic linkage of numerous genetic diseases causing either salt-sensitive hypo- or hypertension to mutations affecting the regulation of ion transporters or channels in the kidney (e.g. Liddle's, Familial Hyperkalemic Hypertension (FHHt), or Gitelman's syndrome). It is also well established that ubiquitylation (i.e. the post-translational modification of proteins with ubiquitin-polypeptides) is crucially involved in these processes. As an example, the intrinsic activity and cell surface expression of the epithelial Na^+ channel ENaC is negatively regulated by the ubiquitin-protein ligase NEDD4-2. In Liddle's syndrome, characterized by hypertension, hypokalemic metabolic alkalosis and elevated ENaC activity, the regulation by NEDD4-2 is impaired by mutations on ENaC that inactivate the interaction site with ENaC. Recently, we have shown both *in vitro* and *in vivo* that NEDD4-2 is also involved in the control of the thiazide-sensitive Na^+,Cl^--cotransporter NCC. Indeed, NEDD4-2 co-immunoprecipitates with NCC induces its ubiquitylation and downregulates NCC at the cell surface *in vitro*. Moreover, in inducible, nephron-specific NEDD4-2 KO mice NCC is strongly upregulated. It has been shown that NEDD4-2 is regulated by kinases such as the aldosterone-induced SGK1 kinase, or the WNK kinases. Certain kindred of FHHt, characterized by hypertension, hyperkalemia and metabolic acidosis and increased NCC activity, are linked to mutations in KLHL3 or CULLIN3, two subunits of ubiquitin-protein ligase complex, further highlighting the importance of the ubiquitin system in salt regulation and blood pressure control. We will discuss recent progress in the understanding of the role of ubiquitin in this field.

CELL SWELLING-INDUCED HORMONE SECRETION – PATHOPHYSIOLOGICAL IMPLICATIONS

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Cell swelling induces exocytosis of protein and peptide hormones after exposure of cells to relative hyposmolarity or permeant agents. Phenomenon utilizes unique signaling pathway; resistance to endogenous inhibitors is its frequent attribute, secretion involves also secretory vesicles not involved in conventional stimulation due to participation of sequential exocytosis as dominating mechanism. Hyposmosis-induced secretion is more sensitive to high cellular cholesterol than conventional one. These features determine also different behavior of hormone secretion at some pathological situations. Cell swelling-induced exocytosis possesses limited selectivity, cells specifically involved in water and salt regulation retain their specific response to osmotic stimuli; e.g. neurons of the hypothalamic supraoptic and paraventricular nuclei release oxytocin, vasopressin and angiotensin II and III in response to hyperosmotic stimulation. This specific response could be obviated by $GdCl_3$ and at these conditions general unspecific response (exocytosis) to hyposmotic stimuli emerges. This phenomenon could play a role in the syndrome of inappropriate secretion of antidiuretic hormone – SIADH when specific reception of osmolarity is disturbed. Clinical experience indicates that unexpected, frequent, and prolonged hypoglycemia is a substantial problem after alcohol ingestion in diabetic subjects. Ethanol in clinically relevant concentration in isosmotic medium induced dose-dependent release of insulin *in vitro*. Ethanol as permeant enters also neurons and pituitary cells and induces swelling and release of peptides from neurons and pituitary. It is likely that release of beta-endorphin from pituitary cells induced by hypotonicity could be induced also by ethanol. Swelling-induced products could be mediators of ischemic preconditioning involved also in protection of diabetic heart. Supported by VEGA SR 2/0132/12 and 2/0054/11, APVV 486-10 and Chicago Diabetes Project.

NA^+-K^+-2CL^- COTRANSPORTER IN CEREBRAL ISCHEMIA

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Na^+-K^+-2Cl^- cotransporter isoform 1 (NKCC1) transports 1Na^+, 1K^+, and 2Cl^- ions into cells and is important in regulation of intracellular Na^+ and Cl^-, cell volume, and K^+ uptake in the central nervous system under physiological conditions. Under ischemic conditions, NKCC1 activation causes intracellular Na^+ and Cl^- overload in astrocytes and neurons. The intracellular Na^+ overload subsequently stimulates the reverse mode operation of Na^+/Ca^{2+} exchange and leads to a delayed, secondary cytosolic Ca^{2+} rise and Ca^{2+} dysregulation in ER and mitochondria. Most importantly, either pharmacological inhibition or genetic ablation of NKCC1 shows significant neuroprotective effects in *in vivo* focal ischemia model and *in vitro* ischemia model. Thus, over-stimulation of NKCC1 activity contributes to cerebral ischemic damage. Better understanding of NKCC1 regulation and function will benefit for developing more effective stroke therapy.

FACE MODULATES TRANSEPITHELIAL WATER RESORPTION IN THE ALVEOLUS

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Tight regulation of the alveolar fluid layer is essential for lung function. Impaired water transport across lung epithelia results in severe health problems ranging from impairment of mucociliary clearance to edema. Yet, the mechanisms and the role of alveolar epithelial cells for maintaining alveolar fluid homeostasis are still controversial. We have recently described that exocytosis of lamellar bodies (LBs) in primary alveolar type II (ATII) epithelial cells results in "fusion-activated cation-entry" (FACE) via $P2X_4$ receptors on LBs. Fusion of LBs with the apical plasma membrane (PM) and subsequent cation entry via $P2X_4$ receptors offers a new potential route for apical to basolateral water movement in the alveolus.

Initially, we looked at the I_{sc} of ATII cell monolayers, and the role for agonist induced activation of $P2X_4$ following LB fusion. While both ATP and UTP can stimulate fusion of LBs with the PM, only ATP can activate FACE. While stimulation with both resulted in an increase in I_{sc} and fusion, an additional increase in I_{sc}, indicating FACE, was only seen when an initial stimulation via UTP was followed by ATP. Over-expressing dominant-negative $P2X_4$ abrogated this effect by ~50%, whereas potentiating $P2X_4$ lead to ~80% increase in I_{sc}. Using fluorescently labeled dextran loaded apically on ATII cell monolayers grown at air-liquid interphase, we were able to directly establish a role for $P2X_4$ receptors in water transport from the alveolar surface fluid (ASF) across the alveolar epithelium. Fluid transport was seen only in the event of LB fusions that were followed by FACE. Again, monolayers transfected with dominant-negative $P2X_4$ reduced the water resportion from the ASF.

Finally, the link between these two studies of cation movement via the $P2X_4$ receptor and water transport in the whole lung was studied in *in situ* experiments looking at changes in lung compliance upon activation of FACE at time of LB fusion. Results from the *in situ* experiments confirmed the idea that FACE directly couples surfactant secretion and transepithelial water transport in the lung.

HOMEOSTATIC IMMUNE FUNCTION: MACROPHAGES REGULATE INTERSTITIAL ELECTROLYTE STORAGE AND BLOOD PRESSURE

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Renal control of blood composition by urinary electrolyte and water excretion is considered sufficient for maintaining the internal environment of the interstitial space. We have found that the interstitium of the skin comprises a separate, locally-regulated compartment, where cells of the mononuclear phagocyte system (MPS) sense local interstitial hypertonicity and actively modulate the internal environment by expressing vascular endothelial growth factor C (VEGF-C) in response to local osmotic stress. Interfering with this MPS/VEGF-C-driven homeostatic response by genetic deletion of TonEBP in MPS cells prevents VEGF-C driven lymph capillary hyperplasia in skin, resulting in skin Cl^- accumulation and salt-sensitive hypertension. Whether MPS cells exert their blood pressure regulatory activity via VEGF-C/VEGFR-2-mediated increases in eNOS expression, or whether VEGF-C/VEGFR-3-driven hyperplasia of the skin lymph capillary network is mechanistically involved, remained unclear. Selective blockade of MPS-driven VEGF-C/VEGFR-3-mediated lymph capillary hyperplasia in the skin resulted in salt-sensitive hypertension, despite increased eNOS expression via the intact VEGF-C/VEGFR-2 regulatory pathway. This salt-sensitive hypertension in response to experimental blockade of physiologic lymph capillary hyperplasia again was paralleled by increased Cl^- retention in the skin. We conclude that MPS cells deploy homeostatic and blood pressure regulatory activity in response to local hypertonic electrolyte accumulation in the skin. The cutaneous immune cells apparently organize local interstitial Cl^- clearance from the skin via VEGF-C/VEGFR-3-driven hyperplasia of cutaneous lymph vessels. It is unclear whether or how skin electrolyte accumulation is functionally coupled with blood pressure increase.

ORAI CA^{2+} SELECTIVE CHANNELS AND CANCER

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The Ca^{2+} sensor stromal interacting molecule 1 (STIM1) and the Ca^{2+} channel ORAI1 mediate the ubiquitous store-operated Ca^{2+} entry (SOCE) pathway activated by depletion of internal Ca^{2+} stores and mediated through the highly Ca^{2+}-selective, Ca^{2+} release-activated Ca^{2+} (CRAC) current. Furthermore, STIM1 and ORAI1, along with ORAI3, encode store-independent Ca^{2+} currents regulated by either arachidonate or its metabolite, leukotriene C_4. ORAI channels are emerging as important contributors to numerous cell functions, including proliferation, migration, differentiation, and apoptosis. Recent studies suggest critical involvement of STIM. ORAI proteins are controlling the development of several cancers including malignancies of the breast, brain, prosexpressiontate, and cervix. Here, we will present data from our group regarding the involvement of different isoforms of ORAI in native Ca^{2+} entry pathways in cancer cells and their contribution to malignancy.

IMMUNE EFFECTS OF THE STRESS EXPOSURES AND SUSCEPTIBILITY TO GLUCOCORTICOIDS IN INHERITED STRESS-INDUCED ARTERIAL HYPERTENSION RAT STRAIN WITH STRESS-SENSITIVE ARTERIAL HYPERTENSION

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Activation of the Hypothalamic–Pituitary–Adrenal (HPA) axis is regarded for one mechanism for the regulation of cell volume. Chronic or long-term stress as well as endogenous glucocorticoids at pharmacologic concentrations, and synthetic glucocorticoids can suppress immunity by decreasing immune cell numbers. Glucocorticoids decrease the immune cell numbers via induction of apoptosis especially in thymus. Male Wistar rats and male ISIAH (Inherited Stress-Induced Arterial Hypertension) rats were used in the study. Animals were exposed to four periods of repeated stress exposures by immobilization to a board. In the next experiments, stressed and unstressed rats were subcutaneously injected by glucocorticoid drug Kenalog (dose 2 mg/kg) 24 hours after repeated stress exposures cessation. Stress exposures caused significant elevation of the percentage of the apoptotic cell nuclei and decrease the percentage of thymocytes in the $S/G_2/M$ phase cells only in the ISIAH rats. Whereas in the Wistar rats were no significant differences in the percentage of the cells in the subdiploid, diploid and tetraploid DNA peaks. Previously stress exposures are completely preserve involution of thymus, apoptosis enhancement and the arrest of the thymocytes in the S/G2/M phase cells after Kenalog administration in ISIAH rats. In conclusion, we have shown that stress-mediated conditioning of the glucocorticoid secretion and responsiveness might represent a previously unrecognized mechanism that is involved in shaping immune response.

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THE USE OF SODIUM-FREE LITHIUM SOLUTION TO MODEL A CELL WITHOUT THE NA,K-ATPASE PUMP

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Lithium can cross the cell membrane via almost every sodium route except the Na,K-ATPase pump. Cells in a sodium-free lithium medium rapidly lose internal sodium and thereafter behave as if without the pump, which can counteract accumulation of cations dominating the external solution. Therefore the ion and water balance in a cell as a double Donnan system is upset. It is known that it takes time for cell swelling to develop after the pump is inactivated. During the first stage, the external cations replace intracellular potassium; cell chloride and water content begin to increase later.

Modeling the monovalent ion redistribution is a rather difficult problem: it has been solved only for some special cases. We created a program for simulation of the monovalent ion redistribution following a balance disturbance for the cell model that includes the sodium pump, electroconductive ion channels of the Goldman type, and cotransporters NKCC, KCC and Na-Cl. The results of simulation were compared with the experimental data obtained on lymphoid U937 cells. It appears that kinetic parameters found for the balanced state give satisfactorily results for non-stationary processes after the sodium pump stops. The similarity between the ion redistribution processes in cells treated with ouabain and with sodium-free lithium solution has been confirmed both in experiment and in simulation. The difference is that the ion disturbance in the lithium medium can be reverted (at least after 3 h). Simulation shows how the process of ion redistribution following pump inhibition depends on the cell type, which can be characterized by two parameters: the intracellular K^+/Na^+ ratio and the membrane potential. Simulation shows that the equivalent Li^+/Na^+ exchange in a sodium-free lithium solution can occur without specialized Li^+/Na^+ counter-transporters. The "lithium cell" model can be useful in understanding of the roles of sodium and potassium in the cell. It has been found, for instance, that in "lithium" U937 cells placed in hyperosmotic solutions the RVI response disappears whereas AVD persists. *The study was supported by the RFBR grants no. 12-04-01669-a 09-04-00301a and by the Program №7 of the Presidium of RAS.*

REGULATION OF CELL VOLUME IN THE LENS: THE ROLE OF CATION CHLORIDE COTRANSPORTERS AND THEIR PHOSPHO-REGULATORS IN MAINTAINING LENS TRANSPARENCY

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Lens transparency is dependent on strict cell volume regulation to maintain its highly ordered tissue architecture. In diabetic cataract, cell swelling causes tissue liquefaction and light scattering, resulting in cortical cataract. Elucidating the mechanisms leading to cell volume disruption has identified that members of the Cation Chloride Cotransporter (CCC) family that include the K-Cl-cotransporter (KCC), the Na-K-Cl-cotransporter (NKCC) and the Na-Cl-cotransporter (NCC) play a key role in lens volume regulation [1]. Pharmacological inhibition of KCC, NKCC and activity by DIOA, bumetanide respectively, revealed specific lens damage phenotypes consistent with a role for these transporters in maintaining lens volume regulation under steady state conditions. In other tissues, KCCs and NKCCs are reciprocally regulated by a group of kinases and phosphatases [2–4]. In this study, we utilised RT-PCR, Western blotting and immunohistochemistry to show that the regulatory kinases, With no Lysine Kinase (WNK1, 3, 4), Ste-20 like Proline/Alanine rich Kinase (SPAK) and Oxidative Stress Response Kinase 1 (OSR1) were identified in the rat lens at the transcript and protein level and confirmed the expression of Protein Phosphatases PP1 and PP2A in the rat lens. We also show that these phospho-regulatory components are differentially expressed throughout the lens, with the majority expressed in all regions as peripheral proteins. Furthermore, inhibition of PP1 and PP2A by Okadaic Acid and Calyculin A resulted in cell volume disruption in the lens. Taken together this data reveals that the CCCs are functional in the lens and that their key phospho-regulators are expressed and localised in close proximity to the CCCs whereby they can interact to regulate their activity. As such, this study represents the first link in identifying the cellular mechanisms by which a dysfunction in cell volume regulation may lead to diabetic cataract.

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