Nephrology Dialysis Transplantation 29 (Supplement 3): iii13–iii15, 2014
doi:10.1093/ndt/gfu113

WATER AND SALT

SO030 A MOUSE MODEL OF SALT WASTING, HYPERCALCIURIA AND KIDNEY STONES

Manoocher Soleimani[1], Sharon Barone[1], Jie Xu[1] and Kamyar Zahedi[1]
[1]*University of Cincinnati, Cincinnati, OH*

Introduction and Aims: Calcium nephrolithiasis is a complex disease with multiple pathogenetic mechanisms. The role of salt wasting and the subsequent volume depletion in the pathogenesis of hypercalciuria and kidney stone formation remains speculative. The only known monogenic disorder associated with salt wasting/volume depletion, hypercalciuria and nephrolithiasis/ nephrocalcinosis is Bartter Syndrome. We have developed a model of distal tubule salt wasting, which is caused by the simultaneous deletion of the Cl^-/HCO_3exchanger pendrin and the Na-Cl co-transporter NCC (PNAS 2012). These animals become severely volume depleted and have nephrogenic DI (PNAS 2012). We hypothesized that salt wasting followed by volume depletion, irrespective of the etiology or the nephron segment(s) involved, activates a cascade of events that lead to increased generation of the arachidonic acid metabolites Prostaglandin E2 (PGE2) and 20-hydroxyeicosatetraenoic acid (20-HETE) that impair salt and calcium reabsorption in the thick ascending limb and the proximal tubule, and block the action of AVP and aldosterone on water and salt reabsorption in the collecting duct, leading to the worsening of volume depletion and calcium wasting. We hypothesize that this self-propagating disadvantageous cycle can lead to super-saturation of calcium crystals in the urine and result in nephrocalcinosis and/or nephrolithiasis.
Methods: DNA microarray, northern hybridization, western blotting, immunofluorescence labeling, H and E staining, Von Kossa stain, functional studies and appropriate treatment with various chemicals were performed on kidneys of wt and double pendrin/NCC KO mice.
Results: DNA microarray demonstrated the activation of arachidonic acid metabolites PGE2 and 20-HETE-generating cytochrome p450 isoforms (Cyp4a12a and 12b) in kidneys of dKO mice. The 24 hr urine collection indicated significant increases in PGE2 and 20-HETE excretion in dKO mice ($p<0.01$ vs WT or single KO mice). In addition to profound salt wasting, the 24 hr urine analysis showed a 3-fold increase in calcium excretion in dKO mice. The histological analysis of kidneys demonstrated multiple calcium stones in the medullary collecting ducts in dKO mice but not in pendrin or NCC single KO mice. The stones were comprised of calcium based on strong staining with Von Kossa stain. Phosphate excretion increased by 2 folds in dKO mice and correlated with a significant reduction in the expression of NaPi-IIa, the major phosphate absorbing transporter in the proximal tubule. Serum calcium and phosphate levels were mildly reduced in dKO mice but remained normal in single KO or WT mice. Blood levels for PTH, 1,25 Vitamin D and FGF23 in dKO mice were comparable to WT or single KO mice. dKO mice had low urine osmolality despite severe volume depletion and had impaired response to exogenous dDAVP, consistent with the presence of nephrogenic DI. Treatment with the PG inhibitor indomethacin for 3 days significantly decreased PGE2 excretion and calcium wasting and caused a significant decrease in urine output and an increase in urine osmolality in dKO mice.
Conclusions: We conclude that salt wasting followed by volume depletion can trigger a cascade of events that includes the activation of arachidonic acid metabolites PGE2 and 20-HETE, which in turn exacerbate salt wasting in multiple nephron segments and cause hypercalciuria, which in the context of volume depletion can result in the precipitation of calcium crystals and calcium stone formation.

SO031 MECHANISMS AND RELEVANCE OF ENAC REGULATION BY EGF: ROLE IN THE DEVELOPMENT OF SALT-SENSITIVE HYPERTENSION AND PKD

Tengis Pavlov[1], Vladislav Levchenko[1], Daria Ilatovskaya[1], Oleg Palygin[1] and Alexander Staruschenko[1]
[1]*Medical College of Wisconsin, Milwaukee, WI*

Introduction and Aims: Epidermal Growth Factor (EGF) is a potent multifunctional hormone involved in many processes including ion channels regulation. Particularly, it was identified that EGF regulates the epithelial Na+ channel (ENaC) in the distal nephron. Dysfunction and aberrant regulation of ENaC leads to a spectrum of diseases associated with improper renal Na+ conservation and wasting. The goal of our study was to explore regulation of ENaC by EGF in animal models of salt-sensitive hypertension and autosomal recessive polycystic kidney disease (ARPKD).
Methods: Dahl salt-sensitive (SS) rat, which exhibit high blood pressure when fed a high salt diet and PCK rat, a model of ARPKD, were used here to assess role of ENaC in development of hypertension and PKD. A combination of electrophysiological, immunohistochemical, biochemical, microscopy and chronic studies in vivo, ex vivo and in vitro was used here to provide mechanistic insights on how ENaC is regulated by EGF in these models.
Results: We demonstrated that developing of hypertension in SS rats was accompanied by increased ENaC activity in the cortical collecting ducts (CCDs) compared to SS rats fed a low salt diet or salt-resistant consomic SS.13BN rats. Treatment with ENaC inhibitor benzamil precluded the development of salt-sensitive hypertension. EGF concentration in the kidney cortex of SS rats fed a high salt diet was significantly lower compared to control rats. To directly evaluate EGF effect on the development of hypertension and ENaC activity, EGF was i/v infused and blood pressure was monitored continuously. Administration of EGF increased Na+ excretion and prevented the development of hypertension in SS rats fed a high salt. At the end of experiments, these rats were used for patch clamp analysis. Chronic infusion of EGF decreased ENaC activity in isolated CCDs compared to the control group. Furthermore, histological analysis revealed that EGF attenuated renal glomerular and tubular damage.Since EGF chronically downregulates sodium transport in CCDs and it has also been reported that EGF concentrations are increased in cystic fluid and that EGF receptor is overexpressed and mislocalized to the apical membranes in the cystic epithelia of animals models and in human with PKD, we tested whether ENaC plays a role in the cyst development in PCK rats. 4 and 12 weeks treatment with benzamil caused more severe cyst formation in PCK rats whereas thiazide diuretic furosemide had no effect on cyst progression. Patch clamp studies of ENaC in the freshly isolated cysts and immunohistochemical analysis confirmed impaired ENaC expression and activity in the freshly isolated cysts of PCK compared to noncystic collecting duct. Abnormal EGF signaling leads to inappropriately low ENaC activity which contributes to cyst formation in PCK rats.
Conclusions: We conclude that deficiency of renal cortical EGF increases ENaC activity and contributes to salt-sensitive hypertension. Furthermore, the disruption of the EGF-EGFR axis reported in ARPKD is linked to suppression of normal Na+ reabsorption, fluid accumulation in cysts and further progress of this kidney disease. Thus, EGF and other members of the EGF-family are important signaling molecules in the maintaining electrolyte homeostasis in the kidney and EGF pathway abnormalities and consequent ENaC dysfunction are critical in the development of salt-sensitive hypertension and polycystic kidney diseases.

SO032 RENAL RESPONSE TO DIURETICS IN WILD TYPE AND CALBINDIN-D28K KNOCKOUT MICE

Chien-Te Lee[1], Hwee-Yeong Ng[1], Yueh-Ting Lee[1], Yeong-Hau Lien[2], Li-Wen Lai[3] and Terry Chiou[1]
[1]*Kaohsiung Chang-Gung Memorial Hospital, Kaohsiung, Taiwan,* [2]*Arizona Kidney Disease and Heart Center, Tucson, AZ,* [3]*University of Arizona, Tucson, AZ*

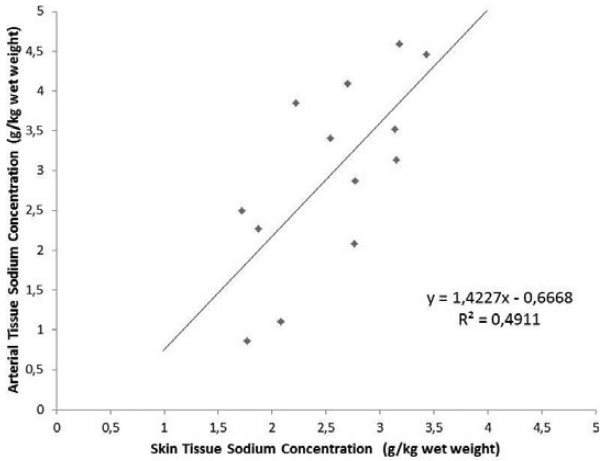
Introduction and Aims: Renal calcium (Ca) handling is complex and modulated by multiple factors. The distal tubule is a critical nephron and considered as the fine tune of Ca transport. In this segment, Ca transport is tightly regulated through Ca transport machinery, including TRPV5/6, calbindin-D (CBD) 28k and Ca pumps. In addition to calcitropic hormone, a variety of physiological and pharmacological factors can influence Ca homeostasis by acting on distal tubule. Previous study has demonstrated increased urinary Ca excretion in CBD-28k knockout mice. Whether defect in distal tubule Ca transport affects Ca handling during diuretics administration is not clear. In the present study, we aimed to investigate renal response to chlorothiazide (CTZ) and furosemide (FSM) in CBD-28k knockout mice.
Methods: Both CTZ and FSM were adminsitered to wild type mice and CBD-28k knockout mice for 3 days. The urine samples were collected to calculate urinary Ca excretion (urinary Ca: creatinine ratio: Ca/Cr) at the end of experiment. Their blood samples were also collected to determine serum creatinine and Ca level.
Results: CTZ (50 mg/kg/day) administration for 3 days was associated with reduced urinary Ca excretion (Ca/Cr: 0.08 ± 0.04 *vs.* 0.19 ± 0.03, $p < 0.05$) and FSM treatment (15 mg/kg, BID for 3 days) caused significant calciuria in wild type mice (0.32± 0.05 *vs.* 0.19 ± 0.03, $p < 0.05$). There was no significant change in urinary Ca excretion after CTZ injection in CKD-28k knockout mice (0.35±0.08 *vs.* 0.28±0.03, both $p > 0.05$). FSM administration further enhanced Ca excretion in CKD-28k knockout mice (0.54 ±0.02 *vs.* 0.28±0.03, both $p < 0.05$). No significant changes in serum creatinine and Ca levels were noted after diuretics administration in both wild and CBD-28k knockout mice. Immunofluorescence staining study revealed a 2-fold increase in TRPV5 and CBD-28k in CTZ-treated wild type mice (TRPV5: 183±4%; CBD-28k: 239±5%, both $p < 0.05$), but there was no significant change in KO mice (TRPV5: 92±5%, $p > 0.05$). FSM treatment induced incremental change in TRPV5 and CBD-28k (TRPV5: 227± 5%; CBD-28k: 248±6%, both $p < 0.05$) in wild type mice. The abundance of TRPV5 was not affected by FSM in knockout mice (TRPV5: 108±6%, $p > 0.05$).
Conclusions: It is concluded that the CBD-28k knockout mice did not manifest hypo-and hypercalciuric response to CTZ and FSM respectively. Our study supports a central role of CBD-28k in distal tubule Ca transport.

SO033 TISSUE SODIUM CONTENT VARIES INTERINDIVIDUALLY AND CORRELATES WITH GLYCOSAMINOGLYCAN EXPRESSION

Michael Fischereder[1], Bernhard Michalke[2], Elisa Schmoeckel[1], Antje Habicht[1], Bernadett Szabados[1], Peter J Nelson[1] and Manfred Stangl[1]

[1]Klinikum Der LMU, Munich, Germany, [2]Helmholtz Zentrum München– Deutsches Forschungszentrum für Gesundheit und Umwelt GmbH, Neuherberg/Munich, Germany

Introduction and Aims: Experimental studies in rats exposed to exogenously administered mineralocorticoids suggest the possibility of water-free storage of sodium via incorporation into glycosaminoglycans (GAG). Recently, water-free sodium-storage has been proposed for hypertensive and even healthy humans. We hypothesized that patients on dialysis exhibit a decreased capacity to excrete sodium resulting in increased tissue sodium concentrations, GAG content and expression of XYLT-1, the enzyme initiating GAG synthesis.
Methods: We studied 15 patients on dialysis undergoing renal transplantation from a live donor. Donors served as healthy controls. All patients were free of clinically detectable edema. During transplant surgery, abdominal skin, muscle and arteries were biopsied. Sodium concentration was determined by inductively coupled plasma - optical emission spectrometry after microwave digestion, semiquantitative GAG content with Alcian stain and XYLT-1 expression by real-time PCR.
Results: Adequate samples for analysis were available from 11 recipients and 2 healthy donors. Recipients and donors were comparable with respect to age (56.1 vs. 62.7 years), BSA (1.86 vs. 2.00 m^2), systolic blood pressure (135 vs. 137 mmHg) and serum sodium (140 vs. 139 mmol/l). Donors tended to have a higher BMI (27.7 vs. 23.6 kg/m^2, p=0.04). Tissue sodium concentration was significantly higher in arteries of recipients with 3.26 ± 1.03 vs. 1.47 ± 0.86 g/kg wet weight (p=0.04). Despite clinical euvolemia, tissue sodium concentrations of both, arteries and skin, were ranging between 0.86 and 4.59 g/kg wet weight. Skin sodium concentrations of individual patients were significantly correlated with their respective sodium concentrations in muscle and arterial tissue (figure, R^2=0.49, p=0.008). Blinded semiquantitative analysis of GAG staining correlated significantly with tissue sodium content (p=0.01, R^2=0.483). XYLT-1 expression in muscles and arteries was also correlated with tissue sodium content (p<0.001, R^2=0.624) .
Conclusions: Our data confirm the observation of highly variable skin sodium concentrations in humans and extend this observation to tissue sodium concentrations in human arteries and muscles. These data support the hypothesis of water independent sodium storage via GAG synthesis in human tissues, including arteries. This mechanism may represent the link between sodium-loading and arteriolar angiopathy and deserves further study.



SO033

SO034 DEFICIENCY IN HYALURONIDASES HYAL1 OR HYAL2 IN MICE LEADS TO IMPAIRED RENAL WATER HANDLING

Vanessa Colombaro[1], Anne-Emilie Declèves[1,2], Inès Jadot[1], Virginie Voisin[1], Laetitia Giordano[1], Isabelle Habsch[1], Bruno Flamion[1] and Nathalie Caron[1]

[1]University of Namur, Namur, Belgium, [2]Université Libre de Bruxelles, Bruxelles, Belgium

Introduction and Aims: Hyaluronan (HA) is a glycosaminoglycan involved in many biological processes. In the kidney, HA is thickly concentrated in the inner medulla where it plays a role in water handling but is poorly present in the cortex and outer medulla. Its degradation occurs through the action of hyaluronidases, HYAL1 and HYAL2. In view to these points, our study aimed to characterize the renal excretory capacities and renal water handling in response to water deprivation or acute water loading in Hyal1 -/- and Hyal2 -/- mice compared to their reference littermates.
Methods: In order to evaluate their responses to changes in water balance, male Hyal1-/- and Hyal2-/- mice were compared to the respective wild-type (WT) mice. In the first experiment, 24h-urine collection was obtained at baseline after appropriate acclimatation, as well as after 24h of water deprivation. In the second experiment, after baseline measurements, the capacity to excrete a water load was tested on an hourly basis during the 6h following i.p. injection of 2 ml of sterile water. Urinary osmolarity as well as Na+ and K+ urinary excretion were measured. HA content was measured either by ELISA or histochemistry in kidney samples.
Results: In baseline conditions, Hyal1-/- mice, compared to the WT mice, are characterized by a lower diuresis (890 ± 79 vs 1159 ± 60 µl/24h, P<0.05) associated with a higher urine osmolarity (5015 ± 339 vs 4037 ± 304 mOsm/l, P<0.05). In contrast, Hyal2-/- mice did not present any difference regarding these parameters in comparison with the WT mice (1059 ± 61 vs 1312 ± 160 µl/24h and 3153 ± 164 vs 3357 ± 287 mOsm/l, NS). After 24h water deprivation, Hyal1-/- mice were characterized by an impaired ability to concentrate urine in comparison with WT mice (5088 ± 345 vs 5716 ± 281 mOsm/l, P<0.05), while no differences were noticed in Hyal2-/- mice. Moreover, Hyal1-/- and Hyal2-/- mice demonstrated a significant delay in the diuretic response induced by an acute water loading (Fig. 1 & 2). Nevertheless, at the end of the 6h urine collection period, the total amount of excreted water was similar in both groups. Regarding intrarenal HA in baseline conditions, both Hyal1-/- and Hyal2-/- mice present a higher HA content in comparison with WT mice. After

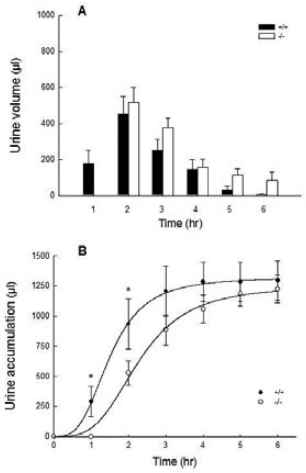


Figure 1. Diuretic response to an acute water loading (2 ml of intraperitoneally sterile water) in six pairs of mice. (A) Hourly urine volume; (B) Urine volume accumulation. Data are means ± SEM. Statistical analysis: * P < 0.05 between *Hyal1*$^{+/+}$ and *Hyal1*$^{-/-}$ mice (Student t test).

SO034

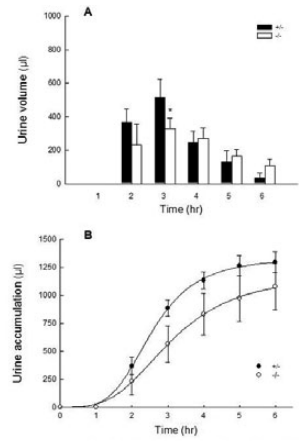


Figure 2. Diuretic response to an acute water loading (2 ml of intraperitoneally sterile water) in six pairs of mice. (A) Hourly urine volume; (B) Urine volume accumulation. Data are means ± SEM. Statistical analysis: * P < 0.05 between *Hyal2*$^{+/+}$ and *Hyal2*$^{-/-}$ mice (Student t test).

SO034

24h water deprivation, HA content was significantly decreased in both WT mice. It was also the case in Hyal1-/- and Hyal2-/- mice, but HA content in Hyal2-/- mice nevertheless remained higher than in WT mice. Two hours after acute water loading, HA content tended to increase but there were no differences between KO and WT mice.

Conclusions: Taking together, our data demonstrated that the mechanism of urine concentration was impaired in Hyal1-/- and Hyal2-/- mice, thereby demonstrating the importance of intrarenal HA dynamics in renal water handling, that remains to be further investigated.