Photolysis at the apical PM resulted in a robust increase in K^+ and Cl^- currents. A localized reduction in $[Ca^{2+}]$ at the apical PM following photolysis of Diazo-2, a caged-Ca^{2+} chelator, resulted in a decrease in both K^+ and Cl^- currents. The K^+ currents evoked by apical photolysis were partially blocked by both paxilline and TRAM-34, specific blockers of BK and IK respectively and almost abolished by incubation with both antagonists. Apical TRAM-34 sensitive K^+ currents were also observed in BK *null* parotid acini. In contrast, when the $[Ca^{2+}]$ was increased at the basal PM no increase in either K^+ or Cl^- currents was evoked. These data provide strong evidence that K^+- and Cl^--channels are similarly distributed in the apical PM. Furthermore, both IK and BK channels are present in this domain and that the density of these channels appears higher in the apical vs. basal PM. In total, this study provides support for a model in which fluid secretion is optimized following expression of K^+-channels specifically in the apical PM.

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EGF Deficiency in the Renal Cortex Contributes to Salt-Sensitive Hypertension via Upregulation of ENaC Activity

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Dahl salt-sensitive (SS) rats fed a high salt diet exhibit increased blood pressure and renal damage. Epithelial sodium channel (ENaC), is responsible for the fine tuning of Na^+ absorption in the kidney. We and others have shown recently that members of the epidermal growth factor (EGF) family are important for maintaining transepithelial Na^+ transport and that EGF biphasically modulates sodium transport in principal cells. A combination of electrophysiological, immunohistochemical, biochemical, microscopy and chronic studies *in vivo* and *in vitro* was used here to provide mechanistic insights on how ENaC is regulated by EGF and how changes in this pathway contributes to salt induced hypertension in the SS rats. Western blotting and immunohistochemistry analyses demonstrate that expression of ENaC subunits are significantly increased in SS rats fed a high salt diet and in SS versus consomic, salt-resistant SS-13^{BN} rats. Patch clamp analysis of ENaC activity in split opened tubules also demonstrated upregulation of ENaC activity in SS rats fed a high salt diet. As measured by ELISA, EGF concentration in the kidney cortex of SS rats fed a high salt diet was significantly lower compared to rats on a low salt diet. To directly evaluate EGF effect on the development of hypertension and ENaC activity, EGF was intravenously infused and MAP was monitored continuously. Supplementation of EGF prevented the development of hypertension in SS rats and attenuated renal glomerular and tubular damage. Moreover, chronic infusion of EGF decreased ENaC activity in isolated distal nephron. Thus, EGF and related growth factors are decreased under conditions of an elevated dietary NaCl intake leading to decreased activation of the EGF receptor and thus resulting in the insertion of enhanced ENaC in the apical membrane of the principal cells.

3305-Pos Board B166

In Search of ENaC Sodium Sensors

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The epithelial Na^+ channel (ENaC), which constitutes the limiting conduit for transepithelial Na^+ transport in several tissues, is inhibited by extracellular Na^+. Extracellular Na^+ likely inhibits ENaC allosterically by binding at low affinity sites in the large extracellular regions of ENaC subunits. These sites remain unidentified. Examination of a recent comparative model of the ENaC α subunit revealed an acidic cleft, analogous to the acidic region observed in the resolved structure of acid sensing ion channel 1 (ASIC1). We hypothesized that this cleft may contain the putative low affinity Na^+ effector sites. We mutated acidic residues within the defined region of the α subunit, and measured the effect of mutation on Na^+ self-inhibition. We identified one site in the peripheral finger domain at which mutation reduced Na^+ self-inhibition. Testing the analogous sites in the β and γ subunits produced a similar result. Inspection of our α ENaC model suggested that this site is near a poorly conserved loop emanating from a β-sheet from the central core of the structure. Scanning mutagenesis of the loop revealed marked effects on Na^+ self-inhibition. Introducing cys to both the site on the finger domain and the nearby loop led to channels that were strongly stimulated by dithiothreitol. Our results suggest that conformational changes in the acidic cleft are associated with channel gating, making it a candidate for a Na^+ sensor site on ENaC.

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Stimulation of ENaC by Thiasolidinediones

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Thiazolidinediones (TZDs) are peroxisome proliferator-activated receptor gamma (PPARγ) agonists used to treat type II diabetes. Recent studies have shown that TZDs can cause fluid retention and oedema by increasing sodium reabsorption in the renal collecting duct. Those side effects may be caused by the up-regulation of the epithelial Na^+ channel (ENaC) and Na^+/K^+-ATPase. However, the mechanisms involved are not clearly understood. Our goal is to study the effects and the mechanisms involved in rosiglitazone action on the expression and function of ENaC. To do so, we performed two electrode voltage clamp studies (TEVC) in *Xenopus laevis* oocytes expressing PPARγ receptor, wild type (wt) and mutant ENaC channels. We have shown that a 48h treatment with 10 μM RGZ produced a 2-fold increase of ENaC activity and this activation is blocked by GW9662, a PPARγ antagonist. We have also generated a mutation in a potential SGK1 (serum- and glucocorticoid-regulated kinase) binding site in the ENaC subunit and expressed the mutant channel together with the PPARγ receptor in *Xenopus* oocytes. RGZ-induced activation was similar in both wt and mutant channels, suggesting that direct phosphorylation of ENaC by SGK1 is not involved in this regulation. SGK1 is also known to inhibit ENaC internalization through Nedd4-2 phosphorylation and subsequent inactivation. ENaC lacking Nedd4-2 binding motifs (Liddle's mutation) are not stimulated by RGZ. Those results suggest RGZ treatment have increased ENaC expression and activity through Nedd4-2 inhibition mediated by SGK1. In accordance with these results, RGZ increase the activity of ENaC by enhancing its cell surface expression, most probably indirectly, through the increase of SGK1 expression. Western blot analysis and confocal microscopy experiments have confirmed that the RGZ-induced ENaC activity and expression via the SGK1/Nedd4-2 pathway in our model.

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Bile Salts Activate BLINaC - An Epithelial Na^+ Channel in the Liver

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Brain Liver Intestine Na^+ Channel (BLINaC) is an ion channel of the DEG/ENaC gene family of unknown function. Expression of rat BLINaC (rBLINaC) in Xenopus oocytes leads to small unselective currents that are only weakly sensitive to amiloride. rBLINaC is potently inhibited by micromolar concentrations of extracellular Ca^{2+} and removal of Ca^{2+} leads to robust currents and increases Na^+ selectivity. Recently we identified the fenamate flufenamic acid (FFA) and related compounds as agonists of rBLINaC. Application of millimolar concentrations of FFA to rBLINaC expressing oocytes induces a robust, Na^+-selective current, which is partially blocked by amiloride. The identification of FFA as an artificial activator for BLINaC suggests the presence of an endogenous ligand.

In rodents, the blinac mRNA is expressed mainly in brain, liver and intestine; in humans, it is mainly expressed in the small intestine. Immunhistochemical stainings of mouse liver revealed prominent expression of the BLINaC protein in the apical membrane of cholangiocytes lining the bile duct of mice, potentially implicating BLINaC in the generation of secondary bile. Guided by this localisation we tested whether the channel is affected by bile. The application of diluted bile to Xenopus oocytes expressing rBLINaC indeed induced a strong and reversible amiloride-sensitive unselective current. We identified bile salts as the BLINaC-activating molecules in bile and are currently trying to unravel the mechanistic basis of this activation. Bile salts are amphiphatic molecules that affect the structure, curvature and fluidity of membranes and might thus be activating BLINaC indirectly via a membrane dependent mechanism. Using a chimeric approach with ASIC1a, a related but bile salt-insensitive channel, we are currently defining the domains of BLINaC that are crucial for sensing membrane modulation induced by bile salts.

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Reconciling Aquaporin Knockout Studies with Epithelial Permeability: Insights from Modelling the Salivary Gland

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The salivary gland is an example of an epithelial tissue that transports fluid isotonically, and at a (relatively) high overall rate. Aquaporins are integral membrane proteins that are usually thought to play a crucial role in enhancing membrane water permeability by an amount sufficient to achieve these