S 21-2

HUMAN DISEASES AND KNOCKOUT MICE TELL THE ROLES AND REGULATION MECHANISMS OF AQUAPORINS

Sei Sasaki

Tokyo Medical and Dental University

Total 13 aquaporins (AQPs) have been identified in mammals. They distribute in all organs in the body and contribute to water transport and other functions. Knockout mice have been generated for all AQPs except AQP10, which is a pseudo gene in mouse. A few hereditary human diseases have been linked to gene mutations of AQPs. It is important and helpful to carefully analyze the phenotype of knockout mice and inherited AQP diseases for understanding of the roles and regulation mechanisms of AQPs. AQP7 is present in the apical membrane of kidney proximal tubules, testis, adipocytes, and others. Generated AQP7 knockout mice showed a minimal urine concentration defect but showed a significant urinary loss of glycerol, indicating that AQP7 works as a route of glycerol reabsorption in proximal tubules. Adult AQP7 knockout mice had 3.7-fold increased body fat mass and this was due to 3-fold decrease of glycerol permeability in adipocyte, indicating an important role of AQP7 in glycerol/fat metabolism. AQP11 is a new member of AQP family and it is unique in that it has atypical NPA box and localizes at intracellular vesicles. AQP11 mice were born and viable but most of them died before weaning because of renal failure with polycystic kidney. The cysts were derived from endoplasmic reticulum of proximal tubules and filled with water. The mechanisms whereby AQP11 deficiency leads to cyst formation are unknown, but we expect further analysis will show a new function of AQP. AQP2 is the vasopressin-regulated water channel of collecting duct and its gene mutations cause human nephrogenic diabetes insipidus (NDI), a disease manifested by inability to concentrate urine. We have analyzed 53 families of NDI and found AQP2 mutations in 7 families. Among them 4 mutations were found in the C-terminal and they showed dominant inheritance. Mutant AQP2 protein expressed in MDCK cells was misrouted to the basolateral membrane instead of proper apical membrane. Knockin mice expressing this mutation confirmed this missorting. These results further led us to isolate two proteins that directly bind to AQP2; SPA-1, a GTPase-activating protein (GAP) for Rap1, and cytoskeletal protein actin. A large scale proteomic analysis of rat renal medulla extract identified further 11 binding proteins, and most of them have ability to interact with actin. We speculate that these proteins make a multiprotein complex and dynamics of the complex regulates the trafficking of AQP2.

Key Words: aquaporin, knockout, hereditary disease, urine concentration

S 21-3

REGULATION OF THE Na,K-ATPASE IN RENAL CELLS

Mary L. Taub, Keikantse Matlhagela and Trivikram Rajkhowa

State University of New York at Buffalo, Buffalo, NY, USA

Prostaglandins are biologically potent products of arachidonic acid metabolism by cyclooxygenase, which play significant roles in blood pressure regulation. The kidney is a major site both of prostaglandin production, and of prostaglandin action. Included amongst the renal processes modulated by prostaglandins are ion transport systems, blood flow, glomerular filtration and renin release. Of particular interest to this report are the affects of prostaglandins on renal Na,K-ATPase activity. Prostaglandins have both acute and chronic affects on renal Na,K-ATPase. Acute affects modulate the integration of the Na,K-ATPase into the plasma membrane, whereas chronic affects modulate Na,K-ATPase gene expression. An in vitro model system that is particularly amenable for studies concerning such prostaglandin affects is the Madin Darby Canine Kidney (MDCK) cell line. The MDCK cell line is a well characterized distal tubule model system in which the role of the Na,K-ATPase in transepithelial solute has been clearly defined. In addition, MDCK cells are particularly amenable for studies concerning prostaglandin affects due to the availability of a hormonally defined serum free medium. Previously, we reported that PGE_1 and 8-Bromocyclic AMP stimulate the activity of the Na,K-ATPase in MDCK cells in such a serum free medium. The stimulatory effects of PGE1 and 8-Bromocyclic AMP on Na,K-ATPase activity were explained by an increase in the level of expression of the Na,K-ATPase. The Na,K-ATPase consists of both an alpha subunit, responsible for the catalytic activity, and a beta subunit, required for the integration of the Na,K-ATPase into the plasma membrane. PGE_1 was observed to increase beta subunit mRNA levels up to 10 fold, and to affect alpha subunit mRNA levels to a smaller extent. As beta subunit levels limit alpha/beta heterodimer formation, regulation of beta1 subunit transcription was examined in greater detail. Transient transfection studies were conducted with a human Na,K-ATPase beta1 promoter/luciferase construct, pHbeta1-1141. The results indicated that PGE1 stimulates Na,K-ATPase beta1 subunit gene transcription by both the cAMP and calcium signaling pathways. Studies with deletion mutants led to the identification of a region in the human Na,K-ATPase beta1 subunit promoter (-167 to -72) that was required in order to elicit a PGE_1 response. We have identified a Prostaglandin-Responsive Element (a PGRE) in this region. In this report we further analyze the receptor signaling pathways involved in mediating the prostaglandin response, as well as the role of PGREs in regulating the transcription of the Na,K-ATPase beta1 subunit gene.

Key Words: kidney, transport, prostaglandin, Na,K-ATPase