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Genomic and non-genomic effects of a new sodium sensing network in renal and lung epithelia

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ABSTRACT

Sodium and water homeostasis is crucial for cell function, survival and health of the individual. The kidney is responsible for sodium regulation in the body, balancing sodium secretion and reabsorption, and thereby also regulating the blood pressure, primarily via the Na^+,K^+-ATPase. The Na^+,K^+-ATPase is situated in the basolateral part of the polarized cell membrane and drives the vectorial transport of Na^+ over the epithelium and water follows the ionic gradient. Vectorial sodium transport is important also in the lung alveoli, where the epithelium needs to be free from excess fluid for efficient gas exchange. Stimulation of the Na^+,K^+-ATPase has been shown to increase lung edema clearance necessary for patient survival. The aim of this thesis is to investigate the existence of a cell sodium-sensing network in sodium-transporting epithelia, and the potential role of such network for cell and organ physiology.

In Article I a new network for activation of the Na^+,K^+-ATPase in response to increased intracellular sodium was discovered. In a cell line originating from the proximal tubules of Opossum kidney (OK), we found that Salt Inducible Kinase 1 (SIK1) was responsible for activating the Na^+,K^+-ATPase in response to increased intracellular sodium. Briefly, when $[Na^+]_i$ increases, $[Ca^{2+}]_i$ increases via the Na^+/C^{2+}-exchanger which in turn activates calcium-calmodulin kinase 1 (CaMK1). CaMK1 phosphorylates SIK1 that becomes active and subsequently phosphorylates protein phosphatase methylesterase-1 (PME-1). Unphosphorylated PME-1 binds to and inhibits protein phosphatase 2A (PP2A). PME-1 leaves PP2A in its active state and PP2A dephosphorylates the Na^+,K^+-ATPase that become active and removes the excess Na^+ from the intracellular space. Increased sodium reabsorption via SIK1 activating the Na^+,K^+-ATPase may cause hypertension.

In Article II we were able to detect all three isoforms of SIK, SIK 1-3, in lung cells from mouse and rat, as well as in cell models from mouse and human. The β-adrenergic receptor (β-AR) agonist Isoproterenol (Iso) has been shown to increase Na^+,K^+-ATPase activity in lung cells by increasing the number of active Na^+,K^+-ATPase at the plasma membrane and to increase lung edema clearance. By depletion of SIK1 kinase activity, Na^+,K^+-ATPase activity did not increase in response to Iso and the transport of Na^+,K^+-ATPase to the plasma membrane was disrupted. SIK1 may therefor play an important role for lung edema clearance.

In Article III we show that reduction of SIK1 in MLE-12 cells lead to inhibited expression of E-cadherin, the hallmark protein for polarity. This was confirmed in lung tissue from *sik1*$^{-/-}$ mice, and supported further in cells with higher SIK1 levels where more E-cadherin was expressed. We disclosed a regulatory mechanism where SIK1 reduction increased the activity of CREB, leading to increased levels of the transcription factors Snail2 and Twist, which in turn repressed the transcription of E-cadherin. The disruption of E-cadherin expression led to impaired sodium transport and integrity of the polarized epithelium.

In summary, SIK1 is needed for Na^+,K^+-ATPase activation both in response to increases in $[Na^+]_i$ in the kidney and in response to β-AR stimulation in lung cells, important for blood pressure control and lung edema clearance, respectively. SIK1 contributes to cell polarity and function via regulation of E-cadherin gene expression.

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