A new SGK1 knockout mouse

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THE SERUM AND GLUCOCORTICOID-REGULATED KINASE (SGK1) is a component of the pathway mediating activation of the epithelial sodium channel, ENaC, in the aldosterone-sensitive distal nephron (ASDN). Aldosterone is released when the body needs more sodium or when blood pressure is low, leading to increased SGK1 transcription and translation (7, 23). SGK1 phosphorylates the E3 ubiquitin ligase Nedd4-2 (9, 25), which normally adds ubiquitin onto ENaC at the cell surface, triggering its endocytosis (17). In vitro, phosphorylation of Nedd4-2 by SGK1 leads to a decrease in the interaction between Nedd4-2 and ENaC. This results in increased ENaC at the cell surface, and an increase in sodium transport (9, 25). However, SGK1 can regulate ENaC proteins lacking Nedd4-2-binding (PY) motifs (1, 8). Insulin also activates ENaC through SGK1 (19, 27), and SGK1 contributes to upregulation of α-ENaC transcription in the ASDN (29). SGK1 also regulates other ion channels and transporters (reviewed in Refs. 18 and 20).

Phenotype of SGK1<sup>−/−</sup> mouse. In 2002 Wulff et al. described the phenotype of a SGK1 knockout (SGK1<sup>−/−</sup>) mouse (28). Fejes-Tóth et al. (11) now report a second SGK1 knockout mouse model. Similar to the previously described SGK1<sup>−/−</sup> mice, the new mouse line showed no gross abnormalities under a normal-salt diet. This is in sharp contrast to the severe phenotypes seen in mineralocorticoid receptor (3) and ENaC (2, 16, 22) knockout mouse lines. However, both SGK1 knockout mouse lines did show abnormalities when they were challenged with a low-salt diet.

Both groups report that on a low-salt diet, SGK1<sup>+/+</sup> and SGK1<sup>−/−</sup> mice decreased their urinary salt loss, but whereas SGK1<sup>+/+</sup> mice reduced their sodium excretion to near zero, significant urinary salt wasting occurred in the SGK1<sup>−/−</sup> mice. Salt wasting suggests that lack of SGK1 prevented upregulation of ENaC, so Na<sup>+</sup> was lost in the urine. Measurements were made to ask whether ENaC activity was altered. Wulff et al. (28) reported a lowered amiloride-sensitive transepithelial potential measured in isolated collecting ducts (CD) of SGK1<sup>−/−</sup> mice compared with SGK1<sup>+/+</sup> mice (28). In contrast, Fejes-Tóth et al. (11) report an increased amiloride-sensitive sodium current measured by whole cell patch clamp in isolated collecting ducts of SGK1<sup>−/−</sup> mice compared with SGK1<sup>+/+</sup> mice (11). The reason for this discrepancy is not clear. However, under salt-depleted conditions both mouse lines show hyperaldosteronism, and this may account for the upregulation of ENaC in the new SGK1<sup>−/−</sup> mice, similar to the observation reported for the colon of the first SGK1<sup>−/−</sup> mouse (24).

Both groups measured ENaC activity after the mice had been on a low-salt diet for 2–3 days. Most previous animal studies have focused on a shorter term effect of aldosterone on SGK1 (4); however, these SGK1 knockout papers do not address this time frame. The SGK1<sup>−/−</sup> mice models do suggest that SGK1 is required to prevent renal salt wasting; however, the data of Fejes-Tóth et al. (11) suggest there are ENaC-independent pathways that SGK1 controls to achieve this (also see below). Chronic aldosterone treatment or salt depletion in mice does lead to sustained upregulation of SGK1 transcription, in distal kidney, over a period of at least 6 days (13). New data from Fejes-Tóth et al. (11) show that ENaC activity is upregulated normally with chronic aldosterone treatment of SGK1<sup>−/−</sup> mice.

SGK1 and ENaC processing. The α- and γ-ENaC proteins undergo proteolytic cleavage during and/or after trafficking to the cell surface (5, 6, 15). Increased activity of ENaC is paralleled by increased proteolytic cleavage of the γ-ENaC subunit in cells (5, 14) and in vivo (10, 21, 26). Therefore, it is surprising that in their SGK1<sup>−/−</sup> mice Fejes-Tóth et al. (11) found that the amount of 65-kDa cleaved γ-ENaC protein was significantly reduced in the kidneys of salt-deprived animals when amiloride-sensitive sodium current was increased in the collecting duct. Chronic aldosterone treatment of SGK1<sup>−/−</sup> mice also led to a reduction of 65-kDa γ-ENaC but did not lead to a significant increase in amiloride-sensitive current in the CD (11). The results suggest that SGK1 has a role in ENaC processing and question the role of the 65-kDa γ-ENaC protein in activated ENaC.

SGK1 and other Na<sup>+</sup> transport pathways. Might salt wasting in SGK1<sup>−/−</sup> mice on a low-salt diet be due to loss of SGK1 regulation of an ENaC-independent sodium transport system? Fejes-Tóth et al. (11) report that expression of the Na<sup>+</sup>Cl<sup>−</sup>-cotransporter protein (NCC) is decreased significantly in SGK1<sup>−/−</sup> mice. NCC is normally active in early distal tubules and mediates Na<sup>+</sup> uptake, so this change might account for the salt wasting observed even in the presence of increased ENaC activity. There were no significant changes in the expression levels of type 3 Na<sup>+</sup>/H<sup>+</sup> exchanger or Na<sup>+</sup>-K<sup>+</sup>-2Cl<sup>−</sup> cotransporter (11).

A further possibility is that ENaC is differentially regulated by SGK1 in different regions of the distal nephron. Fejes-Tóth et al. used SGK1’s effect on γ-ENaC proteolytic cleavage as an indirect measure of ENaC changes. Analysis of γ-ENaC proteolytic cleavage in the medulla (CD) and the cortex (connecting tubules and late distal tubules) of SGK1<sup>−/−</sup> mice showed that 65-kDa γ-ENaC was reduced in both regions (11).

Do the aldosterone-insensitive SGK2 or 3 isoforms take over the role of SGK1 in the SGK1<sup>−/−</sup> mice? In a double SGK1/S GK3 knockout mouse, symptoms were not more severe than in the individual knockouts, and SGK2 mRNA was not elevated in the double knockout (12). More investigation is needed on the role of SGK1 in ENaC processing, and in SGK1 control of other renal salt transport pathways to elucidate the pathways by which SGK1 regulates Na<sup>+</sup> homeostasis in the kidney.
REFERENCES


