Title
Regulation of the epithelial Na+ channel by the mTORC2/SGK1 pathway

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Full Review

Regulation of the epithelial Na$^+$ channel by the mTORC2/SGK1 pathway

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ABSTRACT

The epithelial Na$^+$ channel (ENaC) is decisive for sodium reabsorption by the aldosterone-sensitive distal nephron (ASDN) of the kidney. ENaC is regulated by the serum- and glucocorticoid-inducible kinase 1 (SGK1), a kinase genomically upregulated by several hormones including glucocorticoids and mineralocorticoids. SGK1 is activated by the serine/threonine kinase mammalian target of rapamycin (mTOR) isoform mTORC2. SGK1 knockout ($sgk1^{-/-}$ mice) impairs renal Na$^+$ retention during salt depletion. The mTOR catalytic site inhibitor, PP242, but not mTORC1 inhibitor rapamycin, inhibits ENaC, decreases Na$^+$ flux in isolated perfused tubules and induces natriuresis in wild-type mice. PP242 does not lead to further impairment of Na$^+$ reabsorption in $sgk1^{-/-}$ mice. The mTORC2/SGK1 sensitive renal Na$^+$ retention leads to extracellular volume expansion with increase of blood pressure. A SGK1 gene variant (prevalence ∼3–5% in Caucasians, ∼10% in Africans) predisposes to hypertension, stroke, obesity and type 2 diabetes. Future studies will be required to define the role of mTORC2 in the regulation of further SGK1 sensitive transport proteins, such as further ion channels, carriers and the Na$^+$/K$^+$-ATPase. Moreover, studies are required disclosing the impact of mTORC2 on SGK1 sensitive disorders, such as hypertension, obesity, diabetes, thrombosis, stroke, inflammation, autoimmune disease, fibrosis and tumour growth.

Keywords: aldosterone, epithelial Na$^+$ channel ENaC, glucocorticoids, mammalian target of rapamycin mTOR, renal Na$^+$ excretion

INTRODUCTION

Serum- and glucocorticoid-inducible kinase 1 (SGK1) has been identified as a serum and glucocorticoid sensitive gene but was later found to be genomically up-regulated by several other hormones including mineralocorticoids, 1,25-dyhydroxyvitamin D$_3$ (1,25(OH)$_2$D$_3$), erythropoietin, transforming growth factor β (TGFβ), interleukin 6, fibroblast growth factor, platelet-derived growth factor, thrombin and endothelin [1, 2]. SGK1 expression is further upregulated by advanced glycation end products (AGE), activators of peroxisome proliferator-activated receptor γ, cell shrinkage, excessive glucose concentrations, oxidative stress, heat shock, radiation, DNA damage, and ischaemia [1, 3].

Translation of SGK1 transcripts into protein is stimulated by phosphphoinositide 3-kinase (PI3-kinase) signalling and requires actin polymerization [1]. SGK1 protein is activated by several hormones including growth factors, insulin, thrombin and glucocorticoids [1]. Mechanisms invoked in the activation of SGK1 include PI3-kinase and 3-phosphoinositide (PIP3)-dependent kinase PDK1 [1], WNK1 (with no lysine kinase 1), and mammalian target of rapamycin mTOR complex-2 (mTORC2) comprising mTOR, Rictor (rapamycin-insensitive companion of mTOR), Sin1 (stress-activated protein kinase-interacting protein 1), mLST8 and Protor-1 [4]. These mediators, particularly PI3-kinase and mTOR mediate the effects of various hormones and growth factors to activate SGK 1 through a kinase cascade [5].

SGK1 is degraded by ubiquitinylation involving NEDD4-2 (neuronal precursor cells expressed developmentally downregulated) and Rictor/Cullin-1, as well as the ER-associated

ubiquitin ligases, Chip and HRD1 [6]. Several SGK1 inhibitors have been identified [7–9].

SGK1 participates in the regulation of a wide variety of transport proteins including the Na^+/K^+-ATPase, several carriers (the NaCl transporter NCC, the $Na^+,K^+,2Cl^-$ cotransporter NKCC, the Na^+/H^+ exchangers NHE1 and NHE3, the Na^+,glucose cotransporter SGLT1, diverse amino acid transporters) and several ion channels (e.g. the epithelial Na^+ channel ENaC, the voltage gated Na^+ channel SCN5A, the transient receptor potential channels TRPV4-6, the Ca^{2+} release activated Ca^{2+} channel Orai1/STIM1, the renal outer medullary K^+ channel ROMK, the voltage gated K^+ channel KCNE1/KCNQ1, the glutamate/kainate receptor GluR6, and the cystic fibrosis transmembrane regulator CFTR) [5].

The present brief review focusses on the role of mTORC2/SGK1 in the regulation of ENaC. In view of the limited number of references allowed, recent reviews are cited quoting the earlier original publications [1, 2].

REGULATION OF ENaC BY mTORC2/SGK1

The first channel shown to be stimulated by SGK1 is the epithelial Na^+ channel ENaC [10], which, together with Na^+/K^+ ATPase in the basolateral cell membrane of principal cells accomplishes Na^+ transport in the aldosterone-sensitive distal nephron [2]. ENaC is expressed in the apical cell membrane and mediates Na^+ flux from lumen into the cell [2]. The resulting depolarization of the apical cell membrane drives K^+ movement from the cell into the lumen via the renal outer medullary K^+ channel ROMK [2]. ENaC is composed of the structurally related subunits αENaC, βENaC and γENaC [11]. The proteins are integrated in a large multiprotein ENaC regulatory complex (ERC), which includes SGK1, Nedd4-2, the complex stabilizing aldosterone-induced chaperone (GILZ1) and the PDZ containing scaffold protein connector enhancer of kinase suppressor of Ras isoform 3 (CNK3) [2, 12, 13].

The specific mTOR catalytic site inhibitor PP242 decreases ENaC channels in patch clamp studies, abrogates Na^+ reabsorption in isolated perfused tubules and enhances urinary Na^+ excretion in clearance studies [14]. In contrast, the mTORC1 inhibitor rapamycin fails to modify Na^+ flux in isolated perfused tubules or urinary Na^+ excretion. PP242 does not affect ROMK [14], which associates with the scaffolding protein Na^+/H^+ exchange regulating factor NHERF2 [2]. PP242 fails to inhibit ENaC in SGK1 knockout mice, indicating that ENaC regulation by mTORC2 depends on SGK1 [14]. A schematic depiction of mTORC2 regulation of ENaC and other targets through SGK1 is shown in Figure 1.

MECHANISMS OF SGK1-DEPENDENT REGULATION OF ENaC

SGK1 may modify ENaC activity by direct phosphorylation of the channel protein [1, 15, 16]. The SGK1 phosphorylation consensus sequence is R-X-R-X-X-(S/T)-phi (X = any amino acid, R = arginine, phi = hydrophobic amino acid) [1].

SGK1 up-regulates ENaC further indirectly by phosphorylating Nedd4-2 [1, 17, 18]. The phosphorylation by SGK1 fosters interaction of Nedd4-2 with the chaperone protein 14-3-3 thus precluding Nedd4-2-dependent target ubiquitination and subsequent degradation of the channel protein [1].

SGK1 further regulates ENaC by modifying the activity of other ENaC regulating kinases. SGK1 phosphorylates and thus inhibits the serine/threonine kinase WNK (with no lysine) 4 WNK4 [7, 19], a kinase, which in turn inhibits ENaC [1] and ROMK [20]. SGK1 further phosphorylates PIP2 forming phosphatidylinositol-3-phosphate-5-kinase PIKfyve [21], B-Raf kinase, glycogen synthase kinase-3 GSK-3, extracellular signal-regulated kinase ERK2 [1], mitogen-activated protein kinase/ERK kinase kinase 3 MEKK3, and stress-activated kinase SEK1 [7]. Whether or not any of those kinases impact on ENaC activity remains to be seen.

SGK1 may further enhance ENaC activity by down-regulation of the inducible nitric oxide synthase, which in turn inhibits ENaC by formation of nitric oxide [1].

SGK1 may influence ENaC transcription by phosphorylation and thus inhibition of the putative transcription factor ALL1-fused gene from chromosome 9 Af9, a suppressor of ENaCα expression [1]. SGK1 influences expression of other channels by upregulating the transcription factor nuclear factor kappa B (NFκB) [1], which, however, decreases ENaC activity by down-regulation of SGK1 [22]. Conversely, SGK1 activates N-myc downregulated gene NDRG1, which in turn down-regulates NFκB signalling [23]. SGK1 influences gene expression by modifying several further transcription factors, including p53 [1], cAMP responsive element binding protein (CREB) [24], activator protein-1 [24], and forkhead transcription factor FKHR-L1 (FOXO3a) [1, 7, 25]. Whether or not those transcription factors participate in SGK1-sensitive regulation of ENaC, remains to be seen.

IMPACT OF mTORC2/SGK1 SENSITIVE ENaC REGULATION ON RENAL TUBULAR Na^+ RETENTION, BLOOD PRESSURE AND POLYCYSTIC KIDNEY DISEASE

Up-regulation of ENaC by mTORC2/SGK1 is expected to cause renal Na^+ retention, extracellular volume expansion, enhancement of cardiac output and thus increase of blood pressure [26–31]. As a matter of fact, several SGK1 gene variants have been identified to impact on blood pressure [29] including combined polymorphisms in intron 6 [I6CC] and exon 8 [E8CC/CT] [7]. The I6CC/E8CC/CT gene variant is common in Caucasians (3–5%) and even more common in Africans (10%) [7]. Gene targeted mice lacking functional SGK1 maintain a normal blood pressure at regular diet, as they compensate for the loss of SGK1 by up-regulation of aldosterone release [5]. During dietary salt depletion, the mice are, despite increased aldosterone release, unable to appropriately retain Na^+ [5]. The salt-deficient diet thus leads to extracellular volume contraction and decrease of blood pressure in SGK1-deficient mice in contrast to their wild-type littermates. Conversely, the induction of hypertension by glucocorticoid excess is blunted in SGK1-deficient mice [7].
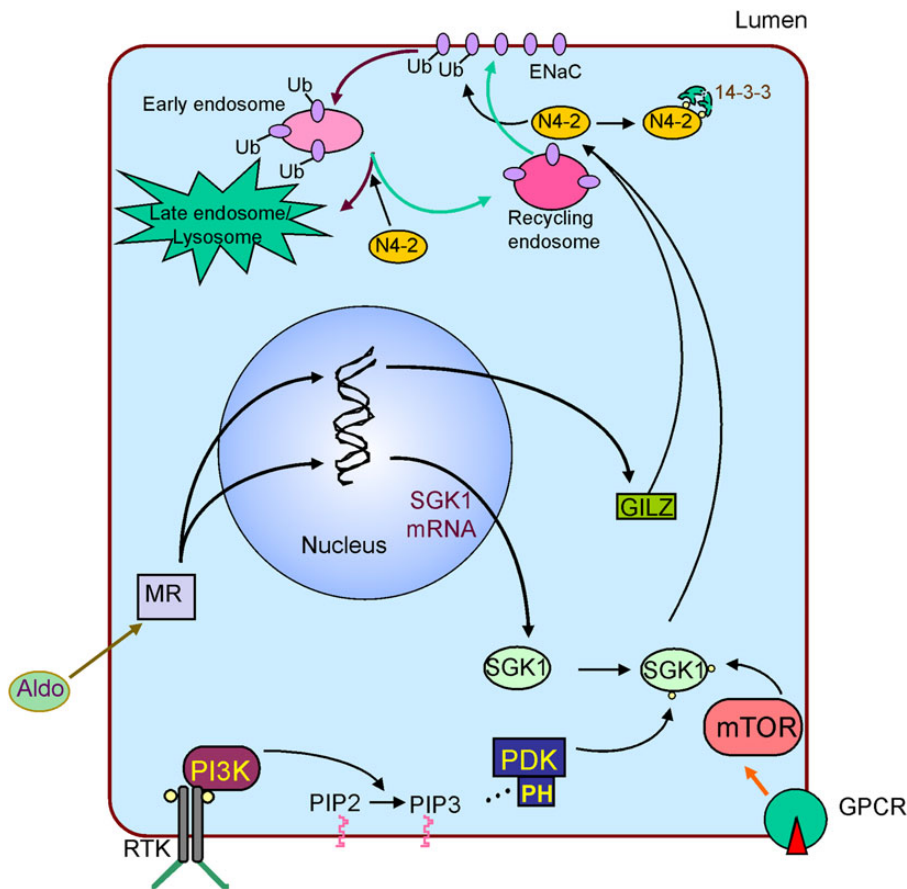
FIGURE 1: Ion transport regulation by mTORC2 and SGK1 in renal principal cells. Principal cells respond to a variety of hormonal and non-hormonal stimuli to control Na^+ and K^+ transport. Aldosterone acts through the mineralocorticoid receptor (MR) to alter expression of various targets that modulate ion transport, notably the serum and glucocorticoid inducible kinase SGK1 and the small chaperone GILZ1 (Glucocorticoid-inducible leucine zipper protein 1). Other factors, such as insulin and angiotensin II act through the master kinases phosphatidylinositide 3-kinase (PI3K) and mTOR complex 2 (mTORC2) to stimulate SGK1 activity. mTORC2 directly phosphorylates SGK1 in its hydrophobic motif, while PI3K acts through PI3K-dependent kinase to phosphorylate SGK1 in its activation loop. Only when phosphorylated at both these sites is SGK1 fully active. SGK1 phosphorylates a variety of proteins, notably the ubiquitin ligase, Nedd4-2, which is an ENaC inhibitor. SGK1 phosphorylation triggers interaction of Nedd4-2 with 14-3-3 proteins, hence reducing ENaC internalization and degradation. ENaC surface expression and activity are governed by a multiprotein ENaC Regulatory Complex (ERC, not shown for simplicity), whose assembly at the plasma membrane is orchestrated by the scaffold protein CNK3 (Connector Enhancer of Kinase Suppressor of Ras Isoform 3), which is also MR-regulated. Electrogenic Na^+ reabsorption *via* ENaC is balanced by K^+ secretion and Cl^- reabsorption through multiple pathways (not shown). N4-2: Nedd4-2; PDK1: phosphoinositide-dependent kinase-1; PIP2: Phosphatidylinositol-4,5 bisphosphate; PIP3: phosphatidylinositol-3,4,5 trisphosphate; PH: pleckstrin homology domain; Ub: ubiquitin; RTK: receptor tyrosine kinase; GPCR: G-protein coupled receptor; Yellow circles: phosphate groups; other abbreviations as in text.

Moreover, generation of hyperinsulinism with high-fructose diet or high-fat diet leads to hypertension in wild-type mice, but not in SGK1-deficient mice [5]. Pharmacological inhibition of SGK1 abrogates the hypertension of hyperinsulinemic mice but does not affect blood pressure in normoinsulinemic mice [5]. Accordingly, hyperinsulinism leads to hypertension presumably by SGK1-dependent stimulation of renal tubular salt reabsorption [7]. It must be kept in mind, though, that the effect of SGK1 on blood pressure is not necessarily due to mTORC2/SGK1 sensitivity of ENaC. SGK1 further stimulates NKCC, NCC, NHE3 and Na^+/K^+ ATPase, all transport systems contributing to renal tubular salt reabsorption [1, 7, 30, 32, 33]. As a matter of fact, renal tubular salt loss of SGK1-deficient mice may be due to decreased NCC activity [34, 35]. However, fractional fluid delivery to distal tubules is decreased in SGK1-deficient mice [36],

an observation pointing to compensatory increase of salt reabsorption in the nephron segments proximal to the puncture site. This observation suggests that defective ENaC and Na^+/K^+ ATPase activities rather than decreased NHE3, NKCC and NCC activity account for the salt loss of SGK1-deficient mice. Whether or not the effect of SGK1 on transport systems other than ENaC is dependent on mTORC2 sensitive regulation of SGK1 activity, remains to be seen. In this regard, it is interesting to note that the electrolyte excretion pattern induced by blockade of mTOR is distinct in WT versus SGK1 KO mice: in WT, the urinary Na^+/K^+ ratio reflects largely inhibition of ENaC, whereas in SGK1 KO mice the pattern resembles NCC inhibition. It seems likely that the role of mTOR in controlling NCC is uncovered with loss of SGK1. Thus, the effects of mTOR on ENaC appear to be largely or entirely SGK1-dependent. However, it appears

F. Lang and D. Pearce

that mTOR (specifically, mTORC2) also regulates NCC in an SGK1-independent fashion.

SGK1 sensitivity of blood pressure could, at least in theory, result from an influence of SGK1 on extrarenal targets. SGK1 mediates the stimulation of salt appetite and thus salt intake by aldosterone [1, 37], an effect expected to impact on extracellular fluid volume and blood pressure. SGK1 and ENaC are further expressed in endothelial cells [38]. Aldosterone upregulates ENaC abundance in the cell membrane of endothelial cells thus leading to Na$^+$ entry, cell swelling and later stiffening of those cells [39]. Moreover, aldosterone augments endothelial stiffening following excess Na$^+$ intake [39]. Enhanced endothelial cell stiffness decreases the triggering of endothelial nitric oxide (NO) formation following shear stress and thus blunts the endothelial induced vasodilation [39]. Endothelial stiffening is thus expected to increase blood pressure. Whether or not those effects of aldosterone on endothelial NO synthesis are mediated by mTORC2/SGK1 signalling, remains, however, elusive.

There is growing recognition that ENaC subunits are expressed in vascular smooth muscle cells, and likely play a role in vasoconstriction [40]. SGK1 and mTORC2 are expressed in these cells; however, a direct connection between these kinases and ENaC regulation in this context has not been established.

The hypertension following mineralocorticoid excess may result from additional effects of aldosterone on the cardiovascular system [41], such as triggering of vascular inflammation [42]. Mineralocorticoid excess leads to SGK1 sensitive cardiac and renal fibrosis [43]. SGK1 upregulates and the SGK1 phosphorylation target N-myc downstream regulated gene 1 (NDRG1) down-regulates the nuclear factor NFκB [5, 44]. The transcription factor triggers in turn the expression of several genes relevant for induction of fibrosis including connective tissue growth factor CTGF [43]. Genetic knockout of SGK1 virtually abrogates the fibrosing effect of mineralocorticoids and salt excess in kidney and heart [43]. The fibrosing effect of mineralocorticoids may eventually contribute to the increase of blood pressure following sustained mineralocorticoid excess [43]. SGK1-dependent up-regulation of NFκB is involved in the stimulation of monocyte/macrophage migration and plays a decisive role in vascular inflammation during atherogenesis [45]. SGK1-dependent NFκB activity is further involved in the regulation of platelet function [46–48] as well as in the regulation of tissue factor synthesis and thus the stimulation of coagulation [43]. In none of those effects, has the impact of mTORC2 been tested.

Relatively little is known about the role of mTORC2 in either BP regulation or renal Na$^+$ handling in humans. The use of active site mTOR inhibitors, which inhibit both mTORC1 and mTORC2, has considerable potential toxicity and has been limited in humans to treatment of malignancies [49]. To our knowledge, there are no published data addressing the effects on blood pressure or renal electrolyte handling. It is well known that rapamycin has less hypertension-causing effects than cyclosporine. Chronic treatment with either an mTORC1 inhibitor like rapamycin or active site inhibitor would be predicted to have complex effects on cardiovascular health, including blood pressure and Na$^+$ handling. For example, there is increasing evidence that effects of over-nutrition are in part mediated by mTOR (likely predominantly mTORC1), and that mTOR inhibition has a protective effect [50].

The mTOR plays a role in the pathogenesis of polycystic kidney disease (PKD), as first described by Shillingford et al. [51]. Rapamycin, at high doses was shown to inhibit cyst progression in several animal models of PKD [52]. Based on these and other findings, several clinical trials were started, with mixed results [53, 54]. A major difference between the animal experiments and human trials in PKD was the dose of rapamycin used, which was much lower in humans. Importantly, prolonged rapamycin at high doses inhibits both mTORC1 and mTORC2, and hence it may indeed be mTORC2 that is implicated in PKD pathogenesis. This possibility was supported by studies in which a global mTOR inhibitor, PP242, was shown to block progression of PKD in the Han:SPRD rat [55]. Similar observations were made in the V/V mouse, which has a PKD-causing mutation in the PKD1 gene [56], and D.P.'s unpublished results. Whether the canonical mTORC2-SGK1 pathway is implicated in PKD pathogenesis is unknown at this time.

(PATHO)PHYSIOLOGICAL ROLE OF mTORC2/SGK1 SENSITIVE REGULATION OF ENaC IN FUNCTIONS UNRELATED TO KIDNEY AND BLOOD PRESSURE

SGK1 expression could be induced in virtually all cells tested and is thus expected to participate in the regulation of ENaC activity in additional tissues. SGK1 sensitivity of ENaC has been shown in respiratory epithelial cells and SGK1-dependent stimulation of ENaC contributes to the regulation of fluid clearance from lung tissue. SGK1 expression is excessive in cystic fibrosis and the SGK1 sensitive stimulation of ENaC may contribute to the impaired net fluid secretion in this genetic disease [5].

SGK1 and ENaC are further coexpressed in neurons. SGK1 is considered to be a powerful regulator of several neuronal functions, such as memory consolidation and fear retention [43]. Moreover, deranged SGK1 sensitive regulation may contribute to the pathophysiology of several cerebral diseases, such as Parkinson's disease, schizophrenia, depression, Alzheimer's disease and consequences of ischaemia [43, 57]. The impact of SGK1 on physiology and pathophysiology of the brain results, however, presumably from SGK1 sensitive regulation of channels and transporters other than neuronal ENaC [43].

SGK1 and ENaC are coexpressed in epithelial cells of the inner ear and in human adrenocortical cells. The functional significance of SK1-dependent regulation of ENaC in those tissues remains, however, elusive.

SGK1 and ENaC are further expressed in endometrium, and both may influence embryo implantation and thus fertility [58, 59]. Embryo implantation appears to be compromised by both excessive SGK1 activity [59] and decreased ENaC activity [58]. It must be kept in mind that SGK1 may influence implantation by mechanisms other than up-regulation of ENaC. Clearly, additional experimental effort is required to fully understand how SGK1-sensitive ENaC activity interferes with embryo implantation.

CONCLUSIONS

SGK1 is activated by mTORC2, and mTORC2/SGK1 signalling is a powerful stimulator of renal ENaC activity. SGK1 contributes to glucocorticoid induced hypertension and fully accounts for the hypertension during hyperinsulinism. Gain of function gene polymorphisms/mutations of both SGK1 and ENaC are associated with increased blood pressure. Whether mTORC2/SGK1 dependent renal tubular ENaC activity accounts for SGK1 induced hypertension remains to be seen, however. At least in theory, SGK1 may modify other renal transport systems, such as NHE3, NKCC2, NCC, and Na^+/K^+ ATPase as well as extrarenal mechanisms, influencing blood pressure, such as salt appetite, endothelial NO release, and vascular smooth muscle cell reactivity. Future experiments are required exploring whether those transport systems and mechanisms are similarly sensitive to mTORC2. Interestingly, mTORC2 appears to have an effect on NCC, which is SGK1-independent [14]. However, the significance of this effect is uncertain. Moreover, future experiments may uncover a role of mTORC2/SGK1 sensitive ENaC activity in the regulation of functions unrelated to renal tubular salt transport and blood pressure regulation.

CONFLICT OF INTEREST STATEMENT

None declared.

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F. Lang and D. Pearce

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