**Commentary**

Title: SGK and disturbed renal sodium transport in diabetes

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**Abstract**

Diabetes is associated with a number of side effects including retinopathy, neuropathy, nephropathy and hypertension. Recent evidence has shown that serum and glucocorticoid inducible kinase-1 (SGK1) is increased in models of diabetic nephropathy. Whilst clearly identified as glucocorticoid responsive, SGK1 has also been shown to be acutely regulated by a variety of other factors. These include insulin, hypertonicity, glucose, increased intracellular calcium and transforming growth factor-β, all of which have been shown to be increased in type II diabetes. The principal role of SGK1 is to mediate sodium reabsorption via its actions on the epithelial sodium channel (ENaC). Small alterations in the sodium resorptive capacity of the renal epithelia may have dramatic consequences for fluid volume regulation and SGK1 maybe responsible for the development of hypertension associated with diabetes. This short commentary considers the evidence that supports the involvement of SGK1 in diabetic hypertension, but also discusses how aberrant sodium reabsorption may account for the cellular changes seen in the nephron.

Diabetic nephropathy is a leading cause of chronic kidney disease (CKD) and end-stage renal disease in the US and Europe (reviewed in Ritz 1999). This condition includes both structural and functional alterations in the kidney of the diabetic patient (Reeves and Andreoli, 2000). Structural changes include renal hypertrophy, thickening of the glomerular basement membrane and increased extracellular matrix accumulation in the glomeruli, whilst functional disturbances include increased glomerular filtration rate, glomerular hypertension, proteinuria, systemic hypertension and finally renal failure. Increases in the $Na^+$ resorptive capacity of the renal epithelia probably contributes to the pathogenesis of hypertension associated with diabetes. However, small alterations in $Na^+$ absorption by renal cells may be responsible for some of the changes seen in cellular function in diabetic nephropathy. One of the key regulators of $Na^+$ reabsorption in the nephron is the serum and glucocorticoid induced kinase-1 (SGK1). This short commentary looks at SGK1 pathophysiology and discusses the consequences of disturbed SGK1-mediated $Na^+$ reabsorption in diabetes in relation to the development of diabetic nephropathy.

**SGK1**

The serum and glucocorticoid kinase was originally isolated as a glucocorticoid responsive gene from rat mammary tumour cells and was termed SGK1 to reflect its transcriptional regulation by both serum and glucocorticoids (Webster *et al.* 1993). Following its initial cloning, two additional, closely related isoforms (SGK2 and SGK3), have been identified (Kobayashi *et al*. 1999). SGK1 is expressed in a variety of tissues including kidney, eye, liver, ovary, heart, pancreas, skeletal muscle, intestine, and lung and brain (reviewed in Loffing *et al*. 2006). In the kidney SGK1 is predominantly expressed in the thick ascending limb of the loop of Henle, distal convoluted tubules and the cortical collecting duct (Alvarez de la Rosa *et*

*al*. 2003). The subcellular localisation of SGK1 is less clear. It has been found in the cytosol (Loffing *et al*. 2001; Hills *et al*. 2006a), associated with the $Na^+$,$K^+$-ATPase in the basolateral membrane (Alvarez de la Rosa *et al*. 2003) and colocalised with mitochondria (Cordas *et al*. 2007). Furthermore, treatment with serum or glucose causes translocation to the nucleus (Buse *et al.* 1999; Hills *et al*. 2006a). In the kidney SGK1 is a transcriptional target of aldosterone and functions as an important regulator of transepithelial sodium transport in the principal cells of the cortical collecting duct through its actions on the apical ENaC (reviewed in McCormick *et al*. 2005; Pearce, 2003) and the $Na^+$,$K^+$-ATPase (Alvarez de la Rosea *et al*. 2006; Henke *et al*. 2002; Setiawan *et al*. 2002; Zecevic *et al*. 2004). However, in addition to regulating ENaC activity, SGK1 is involved in controlling a wide variety of cellular processes including apoptosis, ion transport and cellular differentiation (reviewed in Lang *et al.* 2006). SGK1 expression is regulated through gene transcription and regulated protein degradation, while kinase activity is dependant on phosphatidylinositol-3-kinase (PI3-K) activity and subcellular localisation (reviewed in Lang *et al.* 2006). These various mechanisms allow SGK1 activity to adapt to different roles within the cell, dependant on the nature of the stimuli present (Firestone *et al*. 2003). In addition, three SGK1 splice variants have been identified recently and it is possible that these confer cellular functions (Simon *et al.* 2007).

**SGK1 and ENaC in the development of hypertension**

Sodium reabsorption occurs throughout the nephron by a number of apical transporters. Key in this process are the thiazide-sensitive NaCl co-transporter and the amiloride sensitive epithelial sodium channel (ENaC) (Capasso *et al*. 2005). Expressed throughout the aldosterone-sensitive distal nephron (Loffing *et al.* 2001) and in the apical membrane of the

principal cells in the cortical collecting duct (Hager *et al*. 2001), ENaCs promote $Na^+$ reabsorption from the glomerular filtrate. The driving force for this $Na^+$ reabsorption is maintained by the basolateral $Na^+$,$K^+$-ATPase (Vinciguerra *et al*. 2004).

The ENaC is a member of the ENaC/degenerin gene family (Kellenberger & Schild 2002). Five ENaC subunits have been cloned namely α-, β-, γ-, δ-, and ε-ENaC (Ji *et al.* 2006). Although it appears that not all subunits are necessary to form a functional channel (Bonny *et al*. 1999), studies suggest that the α-, β- and γ- subunits are required. Proposed stoichiometries include either 2α/1β/1γ or alternatively, 3α/3β/3γ (Kosari *et al*. 1998; Snyder *et al*. 1998). Recent studies have highlighted additional potential interactions with the δ-subunit (Ji *et al*. 2006) and suggest that ENaC is a trimeric channel (Jasti *et al*. 2007).

The development of some forms of hypertension are clearly linked to increased ENaC-mediated $Na^+$ reabsorption (reviewed in Pratt 2005). Activating mutations in the β- and γ-subunits of ENaC are responsible for Liddle's syndrome, a severe form of low-renin, low-aldosterone hypertension (Shimkets *et al*. 1994; Hansson *et al*. 1995; Liddle *et al*. 1963). Likewise amiloride and spironolactone (ENaC and mineralocorticoid receptor antagonists) are effective in reducing blood pressure (Saha *et al*. 2005). However, the effect of SGK1 on salt wasting and blood pressure are not as severe as seen in either mineralocorticoid or ENaC mutants (Berger *et al*. 1998; Hummler *et al*. 1996), although it is interesting to note, that inactivation of αENaC in the cortical collecting duct alone, does not alter impair sodium balance (Rubera *et al*. 2003). In the salt sensitive Dahl rat (a model of salt-sensitive hypertension) SGK1 expression is increased (Farjah *et al*. 2003). Likewise genetic variants of the SGK1 gene correlate with slightly increased blood pressure (Busjahn *et al*. 2002; von Wowern *et al*. 2005). However the picture in mice lacking SGK1 is less clear and studies

indicate that SGK1 is not solely responsible for ENaC mediated changes in blood pressure (reviewed in Lang *et al.* 2006). Lack of SGK1 has little effect on salt or fluid retention under normal dietary conditions, but under low salt diets the $SGK1^{-/-}$ mice are unable to adequately retain $Na^+$ and so fail to maintain their blood pressure (Wulff *et al*. 2002). Likewise, a high salt diet in $SGK1^{-/-}$ mice did not increase blood pressure (Huang *et al*. 2000a; 2006b), whilst in mice fed DOAC (deoxycorticosterone acetate) on a high salt diet, blood pressure significantly increased in both wild type and SGK1 knockout animals (Artunc *et al*. 2006; Vallon *et al*. 2006). Interestingly, after 7 weeks of treatment the $SGK1^{-/-}$ mice failed to show any further induction in blood pressure and did not develop renal scarring suggesting that a lack of SGK1 was protective against a DOAC/high salt diet (Artunc *et al*. 2006). SGK3 knockout mice also display a mild phenotype with normal sodium handling and glucose tolerance (McCormick *et al*. 2004). It appears that SGK1 and SGK3 are not replacing each other as double knockout mice (SGK1 and SGK3) do not have significantly different phenotypes from the single isoform knockouts (Grahammer *et al*. 2006).

SGK1 has been shown to increase ENaC-mediated $Na^+$ transport by a number of mechanisms including increased apical membrane localisation of the ENaC, inhibition of ENaC degradation (Debonneville *et al*. 2001) and stimulation of ENaC transcription (Boyd and Naray-Fejes-Toth, 2004). Studies examining the mechanism of SGK1 mediated modification of ENaC function have implicated the neural precursor cell-expressed, developmentally downregulated gene 4 isoform (Nedd4-2) as a negative regulator of ENaC cell surface expression (Kamynina & Staub, 2002). Nedd4-2 is an ubiquitin ligase that directs proteasome mediated degradation of ENaCs (Malik *et al*. 2005). Activation of SGK1 via PI3-K leads to sequential phosphorylation of SGK1 at the Serine 422 and Threonine 256 residues via the two downstream 3-phosphoinositide (PIP3)-dependent kinases PDK2 and PDK1 respectively

(Park *et al*. 1999; Kobayashi & Cohen, 1999). Following activation by aldosterone, SGK1 binds to and phosphorylates Nedd4-2, impairing formation of the ENaC-Nedd4-2 complex and promoting $Na^+$ transport (Debonneville *et al*. 2001; Flores *et al*. 2005). Interestingly, phosphorylation of Nedd4-2 induces ubiquitination and degradation of SGK1 suggesting that SGK1 and Nedd4-2 are able to regulate each other (Zhou & Snyder, 2005). Thus, it has been suggested that in the absence of SGK1, the physical association between Nedd4-2 and ENaC results in ubiquitination of ENaC subunits inducing channel retrieval from the plasma membrane and subsequent proteosomal degradation (reviewed by Staub & Verrey, 2005). However, studies have indicated that SGK1-Nedd4-2 interaction and Nedd4-2 phosphorylation are not the sole regulators of ENaC function, with aldosterone increasing SGK1-mediated Nedd4-2 phosphorylation, albeit to a lesser extent than SGK1 phosphorylation (Flores *et al*. 2005). Nedd4-2 protein expression is also reduced by aldosterone and a low salt diet (Loffing-Cueni *et al*. 2006). Additionally, studies in *Xenopus* oocytes have shown direct regulation of ENaC open probability by Nedd4-2 (Michlig *et al*. 2005). Likewise, it appears that SGK1 can stimulate ENaC activity independent of Nedd4-2 interaction (Diakov and Korbmacher, 2004).

**SGK1 in cell volume regulation**

Renal epithelial cells are exposed to constant fluctuations in filtrate flow and osmolality. Consequently, tubule cells have developed a number of mechanisms to compensate for alterations in filtrate flow rates and to regulate osmotically induced changes in cell volume. However, the mechanisms by which renal epithelial cells detect and subsequently respond to flow and osmotic changes require further clarification. Two potential, complementary, regulatory mechanisms include SGK1 and the mechano-sensitive transient receptor potential

channel (TRPV4), a $Ca^{2+}$ permeable action channel, which is proposed to respond to numerous stimuli including increased flow rates and cell swelling (Cohen, 2005; Wu *et al*. 2007). In addition to it's identification as a glucocorticoid responsive gene, SGK1 was also cloned from human liver cells, as one of the principal volume-regulated protein kinases that serve to restore cell volume upon exposure to hypertonicity (Waldegger *et al.* 1997). Often referred to as hSGK in the literature, it is virtually identical to SGK1 and in mammals its expression is markedly increased following hypertonic cell shrinkage, an effect mediated via the p38 mitogen activated protein kinase (MAPK) (Bell *et al*. 2000; Waldegger *et al*. 2000). However, in A6 cells, which are derived from the freshwater African claw-toed frog, SGK1 is stimulated by hypotonicity (Rozansky *et al*, 2002). The reason behind this difference is not yet apparent. However, it is interesting to note that in A6 cells hypotonicity results in increased intracellular calcium concentration (Rozansky *et al*, 2002; Taruno *et al*. 2008), which we have shown, in human renal cells, causes increased SGK1 expression (Hills *et al*. 2006a).

**SGK1 in diabetic nephropathy**

Poorly controlled type 2 diabetes results in hyperinsulinaemia and hyperglycaemia, which is thought to be the predominant factor involved in the development of diabetic nephropathy. Studies have shown that SGK1 is increased in models of diabetic nephropathy where insulin and glucose have shown to stimulate SGK1 expression and phosphorylation via PI3-K (Kumar *et al*. 1999 Lang *et al.* 2000; Perrotti *et al*. 2002; Wang *et al*. 2005) and SGK1 polymorphisms are associated with type 2 diabetes (Schwab *et al*. 2008). Furthermore, signalling molecules located upstream of SGK1 including TGF-β1, protein kinase C (PKC), diacylglycerol (DAG) and $Ca^{2+}$ all show increased expression in models of type 2 diabetes.

Likewise, ENaC expression is induced by infusion of insulin in streptozotocin-induced diabetes (Song *et al*. 2003; 2006), whilst mineralocorticoid receptor antagonists have been shown to reduce renal injury in models of type 1 and type 2 diabetes mellitus (Guo *et al*. 2006). These changes clearly have the potential to induce the hypertension that is seen so often in diabetics. However, whilst we have considered hyperglycaemic evoked changes in SGK1 expression and the resultant effect that this may have over ENaC mediated $Na^+$ transport, it is vital that we also consider the direct effect of glucose on the collecting duct. Glucosuria, a consequence of hyperglycaemia, results in an osmotic diuresis leading to high urine flow rates and fluctuations in urine osmolality. These changes in urine composition and flow characteristics are able to modify SGK1-mediated $Na^+$ reabsorption either directly or indirectly via changes in cell volume.

Hyperosmotic urine will facilitate osmotically induced cell shrinkage in the renal epithelial cells. This in turn will activate SGK1 (Bell *et al*. 2000) increasing both ENaC-mediated $Na^+$ and water uptake thereby inducing a regulatory cell volume increase. However, SGK1 has been shown to alter expression and insertion of the glucose transporters GLUT1 and SGLT1 into the cell membrane (Dieter *et al*. 2004; Palmada *et al*. 2006). As a result, glucose is able to enter the hexoamine pathway or the polyol pathway. In the polyol pathway glucose, in the presence of aldose reductase, is reduced to sorbitol, an organic osmolyte, which increases intracellular osmolarity leading to cell swelling (Schüttert *et al*. 2002). This would instigate a regulatory cell volume decrease, mediated most likely by increased TRPV4 activity and a concomitant reduction in SGK1 activity. Evidence suggests that urinary sorbitol excretion is increased in diabetic rats, indicating increased conversion of glucose to sorbitol. Administration of the aldose reductase inhibitor, epalrestat, reduced both total body and urinary sorbitol levels (Tsugawa *et al*. 2004).

Increased urine flow rates may also regulate ENaC mediated $Na^+$ transport (Satlin *et al*. 2001; Morimoto *et al*. 2006) through direct modulation of the ENaC, or indirect effects on cell signalling. Numerous studies have reported elevated levels of cytosolic calcium in patients with diabetes (reviewed in Symonia *et al*. 1998), an effect linked to hyperglycaemia in both proximal and distal tubule cells of the kidney (Symonia *et al*. 1998; Hills *et al*. 2006a). Cell swelling in the proximal tubule is also associated with increased $[Ca^{2+}]_i$ and this is linked to activation of PLC, the generation of $IP_3$ and activation of PKC (O'Neil & Leng 1997). We have demonstrated that cells in the human collecting duct (HCD cells) are sensitive to touch (a surrogate for cell membrane stretch and cell volume expansion) and that this is associated with a TRPV4 mediated rise in $[Ca^{2+}]_i$ (Hills *et al*. 2006a). In HCD cells this increase in $[Ca^{2+}]_i$ rapidly propagates to adjacent cells via the gap junction protein connexin-43 and it is this $Ca^{2+}$ induced signal that is thought to aid cell volume recovery through activation of $K^+$ and $Cl^-$ channels subsequently restoring cell volume. However, constitutive activation of TRPV4 under pathological conditions, in those cells exposed to an increased flow rate, may result in a constant state of cell shrinkage and high $[Ca^{2+}]_i$ levels. In an attempt to respond and counteract the effects of TRPV4, SGK1 expression will be induced, a response stimulated further by the increased $[Ca^{2+}]_i$ levels generated in response to TRPV4 activation. Whilst there to aid the cell volume recovery process, a rise in $[Ca^{2+}]_i$ will induce both SGK1 and αENaC expression thus further exacerbating the state of aberrant renal $Na^+$ handling.

It is also interesting to consider the role of SGK1 in fibrosis, as deposition of extracellular matrix is a hallmark of diabetic nephropathy (Mason and Wahab, 2003). TGF-β1 is thought to be key in this process (reviewed in Reeves and Andreoli, 2000) and is increased by glucose in renal cells (Di Paolo *et al*. 1996; Hoffman *et al*. 1998; Hills *et al*. 2006a). Whilst the

downstream targets of TGF-β1 mediating the underlying pathophysiology of diabetic nephropathy remain largely elusive, cell hypertrophy and increased intracellular $Na^+$ observed in response to elevated TGF-β1 levels may in part be mediated by increased SGK1 activity. SGK1 is up-regulated by TGF-β1 in a number of cell types (Waldegger *et al*. 1999; Lang *et al.* 2000; Hills *et al.* 2006a). In addition, glucose induced changes in SGK1 mediate fibronectin formation in diabetic mice (Feng *et al*. 2005). It is interesting to consider the cross talk between the TGF-β1 and MAPK signalling pathway highlighting utilisation of the same signalling pathway as that initiated in response to osmotic stress. TGF-β1 formation together with osmotically-driven increases in SGK1 provide a link between poorly controlled plasma glucose and the development of excess ENaC-mediated $Na^+$-resorption that underlies secondary hypertension and nephron damage seen in people with diabetes.

**Concluding Comments**

Recent studies have demonstrated that diabetes is associated with enhanced SGK1 expression and/or function and it is likely that changes in SGK1-mediated sodium transport are responsible for the development of diabetic hypertension. However, subtle changes in sodium transport will also instigate a number of downstream signals that can influence both cell volume and integrity, which may result in the loss of nephron function seen in diabetic nephropathy. In physiological conditions, the ENaC, the $Na^+$,$K^+$-ATPase, TRPV4 and SGK1 are likely to work together to maintain a constant intracellular [$Na^+$] and so cell volume. However, in diabetes the epithelial cells of the distal nephron are exposed to a number of stimuli capable of increasing SGK1-mediated $Na^+$ transport (figure 1). Hyperinsulinaemia and hyperglycaemia will both induce SGK1 signalling. Glycosuria causes an osmotic diuresis, which may lead to osmotic cell shrinkage, activation of TRPV4 and a consequent increase in

SGK1 activity. Of particular interest though, is the demonstration of the glucose transporters SGLT1 (Suzuki *et al*. 1996) and GLUT12 (Linden *et al*. 2006) on the apical membrane of distal tubules and the collecting duct. It is usually assumed that these cells would not normally be exposed to glucose on this surface, but this is clearly not the case in diabetes. Apical expression of SGLT1 and GLUT12 suggests that glucose may have direct effects both at the basolateral and apical surface, but few *in vitro* studies have attempted to delineate between the cell surface responding. Therefore, in diabetes continuous exposure to increased high glucose may see constitutive activation of SGK1 signalling leading to excessive $Na^+$ reabsorption further exacerbating both the hypertension and the cellular damage seen in diabetes (figure 1).

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**Figure legend.**

Model for the regulation of SGK1 dependant $Na^+$ transport in Type II diabetes. Phosphorylated SGK1 allows for insertion and retention of ENaCs into the apical cell membrane, promoting ENaC-mediated $Na^+$ reabsorption from the lumen of the cortical collecting duct. SGK1 also stimulates the $Na^+$,$K^+$-ATPase. TRPV4 receptors may be activated either by the high urine flow rates that are associated with polyuria or as a result of osmotically induced cell swelling. This will be compensated for by a regulatory cell volume decrease leading to cell shrinkage. Similarly glycosuria may initiate cell shrinkage as water is lost to the lumen. Cell shrinkage is a key trigger for SGK1 activation. Insulin and glucose are also able to modify SGK1 activity. Insulin induces SGK1 phosphorylation via PI3-K, while glucose increases SGK1 expression via TGF-β1 and PKC. We suggest that all aspects of Type II diabetes promote an increase in ENaC-mediated $Na^+$ reabsorption. This will result in increased $Na^+$ and water retention, an imbalance which may predispose the development of hypertension that is associated with diabetes. Furthermore, these changes may contribute towards the loss of cell function and nephron damage that is associated with diabetic nephropathy.