The upregulation of glucose transport is considered to result at the kidney is upregulated (52) and thereby blunts the glucosuria. HYPERGLYCEMIA INCREASES the filtered load of glucose, which enters the proximal tubule and is then actively reabsorbed. The mechanisms accounting for the increase of renal tubular glucose transport have remained ill-defined. A candidate is the serum- and glucocorticoid-inducible kinase SGK1. The kinase was originally cloned as a glucocorticoid-sensitive gene (34) but later shown to be similarly regulated by growth factors (25, 47, 79), fibroblast and platelet-derived growth factor (56), thrombin (4), transforming growth factor-β (44, 83), interleukin-6 (54), fibroblast and platelet-derived growth factor (56), thrombin (4), and endothelin (85), as well as other cytokines (25, 47, 79). SGK1 transcription has further been shown to be stimulated by cell shrinkage (82), cell swelling (65), heat shock, UV radiation, and oxidative stress (51). SGK1 is activated by insulin (43) through a signaling cascade involving phosphatidylinositol-3-kinase (PI3 kinase) and 3-phosphoinositide-dependent protein kinase-1 (PDK1) (6, 43, 62).

Beyond its effect on nutrient transporters, SGK1 is a potent regulator of renal electrolyte transporters, such as the epithelial Na⁺ channel ENaC (2, 17, 23, 26, 32, 57, 73, 81, 84), the renal K⁺ channels ROMK (60, 87, 91) and KCNE1/KCNQ1 (22, 30), the epithelial Ca²⁺ channel TRPV5 (31, 58), the Na⁺/H⁺ exchanger NHE3 (89, 90), the Na⁺³-K⁺-2Cl⁻ cotransporter NKCC2, and the Na⁺³-K⁺-ATPase (39, 69, 92). SGK1 contributes to the effect of aldosterone, IGF-1, and insulin on ENaC activity and thus renal salt reabsorption (7–10, 84). A gene variant of the SGK1 gene is associated with increased blood pressure (21, 22, 80) and obesity (28). Therefore, SGK1 has been suggested as a candidate for the development of metabolic syndrome (46).

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The present study explored whether glucosuria in the diabetic kidney is affected by the presence of SGK1. To this end, Akita mice spontaneously developing early onset diabetes (20, 88) were crossed with gene-targeted mice lacking SGK1 on one allele (sgk1+/−). Comparison was made between akita+/−/sgk1+/− mice and akita+/−/sgk1+/+ mice.

METHODS

All animal experiments were conducted according to the guidelines of the American Physiological Society as well as German law for the welfare of animals and were approved by local authorities.

Akita mice that spontaneously develop diabetes were crossed with heterozygous SGK1 knockout mice (sgk1+/−). Comparison was made between akita+/−/sgk1+/− mice and akita+/−/sgk1+/+ mice. Mice of similar age, sex, and background were used. Before the mice were included in the study, the diabetic phenotype was assured by determination of glucose levels. Glucose levels >200 mg/dl were considered as diabetic. The mice were fed a standard rodent diet (1314; Altromin, Heidenau, Germany) and allowed free access to tap drinking water.

For evaluation of renal excretion, mice were placed individually in metabolic cages (Techniplast, Hohenpeissenberg, Germany) for a 24-h urine collection (74). They were allowed a 3-day habituation period during which body weight, food intake, water intake, and urinary flow rate were recorded daily to ascertain that the mice had adapted to the new environment. Subsequently, a 24-h-collection of urine was performed for 2 consecutive days to obtain the basal urinary parameters. To ensure quantitative urine collection, the inner wall of the metabolic cages was siliconized and the urine was collected under water-saturated oil.

To obtain blood specimen, animals were lightly anesthetized with diethylether (Roth, Karlsruhe, Germany), and ~200 μl of blood were withdrawn into heparinized capillaries by puncturing the retro-orbital plexus. Plasma and urinary concentrations of Na+ and K+ were measured by flame photometry (AFM 5051; Eppendorf, Germany). Plasma glucose concentrations were determined using a glucometer (Accutrend; Roche, Mannheim, Germany). Creatinine concentrations were measured in serum using an enzymatic colorimetric method (creatinine PAP; Lehmann, Berlin, Germany) and in urine using the Jaffe reaction (Sigma, St. Louis, MO) according to the manufacturers’ instructions. Plasma aldosterone concentrations were determined using a commercial RIA kit (Demeditec, Kiel, Germany). Urinary glucose concentrations were similarly determined using a commercial enzymatic kit (gluco-quant; Roche Diagnostics, Mannheim, Germany).

The potential difference across the basolateral cell membrane (ΔPDn0) in isolated perfused mid to late proximal tubules was determined with and without glucose in the perfusate to stimulate electrogenic absorption as previously described (75, 76). The bath and luminal perfusates were composed of (in mM) 110 NaCl, 5 KCl, 20 NaHCO3, 1.3 CaCl2, 1 MgCl2, and 2 NaH2PO4. In the bath, 1 mM glucose, 2 mM glutamine, 1 mM Na-lactate, and 20 mM mannitol, and in the lumen, 25 mM mannitol, were added. Where indicated, 20 mM mannitol was replaced by 20 mM glucose in the luminal perfusate. PDn0 was measured by a high-impedance electrometer (FD223; WPI Science Trading, Frankfurt, Germany) connected with the electrode via a Ag-AgCl half cell. The Ag-AgCl reference electrode was connected with the bath. Entry of positive charge by electrogenic transport is expected to depolarize the basolateral cell membrane. The magnitude of the depolarization depends on the magnitude of the induced current, on one hand, and on the resistances of cell membranes and shunt on the other.

In situ hybridization for SGK1 mRNA was performed on cryosections from adult murine kidneys as previously described (37). The hybridized digoxigenin-labeled RNA was detected by an alkaline phosphatase-conjugated anti-digoxigenin antibody (Roche) and visualized with nitroblue tetrazolium salt-5-bromo-4-chloro-3-indolyl phosphate substrate solution (Roche).

For SGLT1 immunohistochemistry, hybridized sections were heat treated with citrate buffer (100 mM citric acid, pH 6.0). The following pretreatment, incubation, and washing steps were carried out as previously described (55). The primary SGLT1 antibody (rabbit anti-SGLT1, 1:800; Abcam, Cambridge, UK) was incubated overnight at 4°C. After being washed in Tris-buffered saline (TBS) buffer, the sections were incubated with the biotinylated secondary antibody (swine anti-rabbit IgG, 1:400; DAKO, Hamburg, Germany) for 30 min at room temperature. Detection of biotinylated antibody was accomplished with the avidin-biotin-peroxidase system (Dako) and visualized with 3,3'-diaminobenzidine substrate solution (DAB; Sigma, Munich, Germany) and hydrogen peroxide (Sigma). After additional washing steps, stained sections were coveredslipped with Kaiser’s glycerol gelatin (Merck, Darmstadt, Germany).

Data are means ± SE, and n represents the number of independent experiments. All data were tested for significance using a paired or unpaired Student t-test, and only results with P < 0.05 were considered statistically significant.

RESULTS

Cross breeding of Akita mice with gene-targeted mice lacking SGK1 on one allele (sgk1+/−) eventually led to the generation of heterozygous Akita mice lacking SGK1 (akita+/−/sgk1−/−) or expressing SGK1 (akita+/−/sgk1+/−). As shown in Fig. 1, both akita+/−/sgk1−/− and akita+/−/sgk1+/+ mice developed profound hyperglycemia (>20 mg/dl) in urine during ~6 wk. No significant difference was observed in plasma glucose concentrations between the genotypes.

Food intake was significantly higher in akita+/−/sgk1−/− than in akita+/−/sgk1+/− mice. Accordingly, lack of SGK1 enhanced food intake in diabetic mice. Food intake was significantly lower in nondiabetic controls irrespective of the sgk1 genotype (Fig. 2A). Thus food intake was enhanced as a consequence of the induction of diabetes. Fluid intake tended to be higher in akita+/−/sgk1−/− than in akita+/−/sgk1+/+ mice. The fluid intake was significantly lower in nondiabetic controls (Fig. 2B). Accordingly, diabetes increased fluid intake in both genotypes. No differences were observed between the genotypes in body weight (Fig. 2C). Plasma aldosterone concentration was higher in akita+/−/sgk1−/− compared to akita+/−/sgk1+/− mice. Values are means ± SE for akita+/−/sgk1−/− mice/lakita+/−/sgk1+/− mice, n = 3/5, 7/8, 9/11, 7/11, 8/9, 6/11, 67/99, and 7/10 for weeks 5–14, respectively of plasma glucose concentrations in fed mice carrying the Akita mutation (akita+/−) and either lacking (sgk1−/−) or expressing (sgk1+/−) functional SGK1. Blood glucose concentrations were determined regularly between ages 5 and 14 wk.

Fig. 1. Plasma glucose concentrations in akita+/−/sgk1−/− and akita+/−/sgk1+/− mice. Values are means ± SE (for akita+/−/sgk1−/− mice/lakita+/−/sgk1+/− mice, n = 3/5, 7/8, 9/11, 7/11, 8/9, 6/11, 67/99, and 7/10 for weeks 5–14, respectively) of plasma glucose concentrations in fed mice carrying the Akita mutation (akita+/−) and either lacking (sgk1−/−) or expressing (sgk1+/−) functional SGK1. Blood glucose concentrations were determined regularly between ages 5 and 14 wk.
centrations were significantly higher in sgk1−/− than in sgk1+/+ mice irrespective of the Akita genotype (Fig. 2D).

No significant differences were observed in plasma Na+ and K+ concentrations between akita+/−/sgk1+/− and akita+/+/sgk1+/+ mice. However, akita+/+/sgk1−/− mice had significantly higher plasma Na+ concentrations than akita+/+/sgk1+/− mice (Fig. 3A), and akita+/+/sgk1+/− mice had significantly lower plasma K+ concentration than akita+/+/sgk1+/+ mice (Fig. 3B). Among nondiabetic controls, sgk1−/− mice had significantly higher plasma K+ concentration than sgk1+/+ mice (Fig. 3B).

Creatinine clearance tended to be higher in akita+/+/sgk1−/− mice than in akita+/+/sgk1+/− mice. However, the difference did not reach statistical significance. Nondiabetic controls showed a significantly lower creatinine clearance irrespective of the sgk1 genotype (Fig. 4A). Urinary flow rate was significantly higher in akita+/+/sgk1−/− than in akita+/+/sgk1+/− mice. Nondiabetic controls excreted significantly lower urine volume irrespective of the sgk1 genotype. Thus diabetic mice had higher urinary volumes than nondiabetic mice (Fig. 4B).

Urinary excretions of both Na+ and K+ were significantly higher in akita+/+/sgk1−/− than in akita+/+/sgk1+/− mice. In nondiabetic controls lacking sgk1, the Na+ excretion was significantly lower than in diabetic mice lacking sgk1. Furthermore, non-diabetic controls had a lower K+ excretion compared with diabetic mice irrespective of the sgk1 genotype (Fig. 4, C and D).

As shown in Fig. 5, urinary glucose excretion was significantly higher in akita+/+/sgk1−/− than in akita+/+/sgk1+/− mice. Nondiabetic mice did not excrete appreciable amounts of glucose irrespective of the sgk1 genotype.

Isolated tubules were perfused to determine whether the enhanced renal glucose excretion in akita+/+/sgk1−/− mice was the result of decreased electrogenic proximal renal tubular glucose transport. The basolateral cell membrane potential tended to be higher in akita+/+/sgk1−/− mice (−64.6 ± 2.9 mV, n = 7) than in akita+/+/sgk1+/− mice (−62.1 ± 2.1 mV, n = 6). Luminal application of K+ channel blocker barium (2 mM) depolarized the basolateral cell membrane potential in both akita+/+/sgk1−/− (to −58.1 ± 2.0 mV, n = 6) and akita+/+/sgk1+/− mice (to −45.8 ± 5.8 mV, n = 5). The effect was significantly blunted in akita+/+/sgk1−/− mice, and thus the basolateral cell membrane potential in the presence of luminal barium was significantly higher in akita+/+/sgk1−/− than in akita+/+/sgk1+/− mice. Application of 20 mM glucose to the luminal perfusate depolarized the basolateral cell membrane, an effect significantly blunted in akita+/+/sgk1−/− compared with akita+/+/sgk1+/− mice (Fig. 6). In nondiabetic controls, no difference was observed between sgk1−/− and sgk1+/+ mice. The responses to glucose in both the absence and presence of barium provided evidence that the electrogenic glucose uptake in the luminal membrane of proximal tubules is blunted in akita+/+/sgk1−/− mice.

In situ hybridization of SGK1 mRNA abundance in kidneys from normal mice disclosed very low SGK1 transcript levels in
reabsorption of glucose in diabetic animals. Diabetes has been
induced by crossing with Akita (akita+/−) mice. The akita+−
mice spontaneously develop diabetes due to death of pancreatic
β-cells.

**DISCUSSION**

The present study reveals a novel pathophysiological role of
the serum- and glucocorticoid-inducible kinase SGK1. Accord-
ing to our observations, the kinase stimulates proximal tubular
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**DISCUSSION**

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the serum- and glucocorticoid-inducible kinase SGK1. Accord-
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As shown earlier (48), excessive extracellular glucose concentrations stimulate the transcription of SGK1. Accordingly, the SGK1 transcript levels were enhanced in the renal cortex of diabetic mice (Fig. 7). A summary of hyperglycemia, the filtered load exceeds the maximal tubular transport rate of glucose and thereby leads to glucosuria. The glucose-induced increase of plasma glucose levels. Presumably, extrarenal mechanisms compensated for the enhanced renal loss of glucose, such as enhanced levels of hormones increasing plasma glucose concentrations.

Both in vitro (28, 59, 72) and in vivo (18, 37, 59) data demonstrate the ability of SGK1 to stimulate glucose transporters. In addition, SGK1 can activate the potassium channel KCNE1/KCNQ1 (30), which is expressed in the apical membrane of the proximal tubule and serves to repolarize the cell membrane during electrogenic glucose reabsorption (75, 76). Addition of barium to the luminal perfusate abrogates electrogenic K+ exit across the apical cell membrane, an effect expected to increase the glucose induced depolarization. By the same token, barium depolarizes the apical cell membrane, thus

The enhanced renal loss of glucose in hyperglycemic mice lacking SGK1 (akita+/−/sgk1−/−) did not result in a significant decrease of plasma glucose levels. Presumably, extrarenal mechanisms compensated for the enhanced renal loss of glucose, such as enhanced levels of hormones increasing plasma glucose concentrations.
decreasing electrogenic glucose transport. Apparently, in the present experiments the opposing effects largely cancelled each other, and thus barium had little influence on the glucose-induced depolarization. In earlier experiments utilizing lower luminal glucose concentrations, apical barium or genetic knockout of KCNQ1 enhanced the depolarizing effect of glucose (75, 76). In any case, according to the present study, the differences in electrogenic proximal tubular glucose transport between akita+/−/sgk1−/− and akita+/−/sgk1+/+ mice are not abrogated by luminal application of the K+ channel blocker barium. This observation does not rule out a participation of KCNE1/KCNQ1 in the SGK1-sensitive regulation of SGLT1 activity but clarifies that altered activity of those channels does not fully account for the differences between akita+/−/sgk1−/− and akita+/−/sgk1+/+ mice.

Electrogenic glucose transport in intestine was not significantly different between SGK1 knockout mice (sgk1−/−) and their wild-type littermates (sgk1+/+) under basal conditions but was significantly stimulated by the glucocorticoid dexamethasone only in sgk1+/+ mice, not in sgk1−/− mice (37).

Glucose transport in renal proximal tubules was not considered to be regulated by SGK1, since in healthy humans and intact animals SGK1 is expressed in the aldosterone-sensitive distal nephron rather than in the proximal tubule (35, 46, 53, 78). In the absence of hyperglycemia, SGK1 deficiency leads to impaired Na+ reabsorption in the aldosterone sensitive nephron segments with a compensatory increase in proximal tubular Na+ reabsorption (86). Hyperglycemia, however, markedly upregulates SGK1 expression (33, 44). Accordingly, excessive SGK1 expression has been observed throughout the kidney in diabetic nephropathy (44, 48). In isolated perfused proximal tubules, the glucose-induced current tended to be higher in diabetic akita+/−/sgk1+/+ mice than in nondiabetic akita+/−/sgk1+/+ mice, a difference, however, that did not reach statistical significance. Thus electrogenic glucose transport may not be increased in diabetic animals. In any case, in diabetic, but not nondiabetic animals, electrogenic glucose transport is partially dependent on the presence of SGK1.

Impaired upregulation of proximal tubular glucose reabsorption augments glucosuria. The nonreabsorbed glucose leads to osmotic diuresis and thus increases urinary output and renal excretion of Na+ and K+ (45). Accordingly, urinary flow rate and urinary excretions of Na+ and K+ were increased to significantly greater extent in akita+/−/sgk1−/− mice than in akita+/−/sgk1+/+ mice. The renal loss of Na+ is expected to cause volume depletion, which should stimulate Na+ intake and aldosterone release. Plasma aldosterone levels are indeed significantly higher in akita+/−/sgk1−/− than in akita+/−/sgk1+/+ mice. Even in nondiabetic conditions, plasma aldosterone concentrations are enhanced in sgk1+/− mice even under control conditions and partially compensate for the lack of SGK1.

In conclusion, the present observations disclose that SGK1 contributes to the upregulation of renal tubular glucose reabsorption in the diabetic kidney.

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