SGK1-sensitive renal tubular glucose reabsorption in diabetes

Teresa F. Ackermann,†* Krishna M. Boini,†* Harald Völkl,2 Madhuri Bhandaru,1 Petra M. Bareiss,3 Lothar Just,3 Volker Vallon,4 Kerstin Amann,5 Dietmar Kuhl,6 Yuxi Feng,7* Hans-Peter Hammes,7 and Florian Lang1

1Department of Physiology, University of Tübingen, Tübingen, Germany; 2Department of Physiology and Medical Physics, Innsbruck Medical University, Innsbruck, Austria; 3Department of Anatomy, University of Tübingen, Tübingen, Germany; 4Departments of Medicine and Pharmacology, University of California, San Diego and Veterans Affairs San Diego Healthcare System, San Diego, California; 5Department of Pathology, University of Erlangen, Erlangen; 6Department of Biology, Chemistry, and Pharmacy, Free University Berlin, Berlin; 75th Medical Clinic, Faculty of Clinical Medicine, University of Heidelberg, Mannheim, Germany; and 8Department of Nephrology, Union Hospital, Tongji Medical College, Huaizhong University of Science and Technology, Wuhan, China

Submitted 4 April 2008; accepted in final form 16 January 2009

The kinase has been shown to stimulate Na\(^+\)-coupled glucose transport in vitro and mediate the stimulation of electrogenic intestinal glucose transport by glucocorticoids in vivo. SGK1 expression is confined to glomerula and distal nephron in intact kidneys but may extend to the proximal tubule in diabetic nephorphy. To explore whether SGK1 modifies glucose transport in diabetic kidneys, Akita mice (akita\(^/-\)), which develop spontaneous diabetes, have been crossbred with gene-targeted mice lacking SGK1 on one allele (sgk1\(^/-\)). The data provide the first evidence that SGK1 participates in the stimulation of renal tubular glucose transport in diabetic kidneys.

**HYPERGLYCEMIA INCREASES** the filtered load of glucose, which leads to glucosuria as soon as the maximal renal tubular transport rate is exceeded. Glucose transport in the diabetic kidney is upregulated (52) and thereby blunt the increase of urinary glucose excretion. 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 has been shown to stimulate Na\(^+\)-coupled glucose transport by 10.220.32.247 on October 14, 2017 from http://ajprenal.physiology.org/ Downloaded from

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

* T. F. Ackermann and K. M. Boini contributed equally to this work.

Address for reprint requests and other correspondence: F. Lang, Dept. of Physiology, Univ. of Tübingen, Gmelinstr. 5, D-72076 Tübingen, Germany (e-mail: florian.lang@uni-tuebingen.de).

http://www.ajprenal.org

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 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 specimens, 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 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 (∆PD,Na) 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 NaHPO4. 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. PD,Na 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 pretreatments and incubation 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) 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 mM) within ~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 con-

![Fig. 1. Plasma glucose concentrations in akita+/−/sgk1−/− and akita+/−/sgk1+/− mice. Values are means ± SE for akita+/−/sgk1−/− mice/akita+/−/sgk1+/− mice, n = 3/5, 7/8, 9/11, 7/11, 7/11, 8/9, 6/11, 6/7, 9/9, 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.](http://ajprenal.physiology.org/DownloadedFrom/10.22033.242.247/on October 14, 2017)
concentrations 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 non-diabetic 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 ± 5.7 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

---

Fig. 2. Food and fluid intake, body weight, and plasma aldosterone concentrations in diabetic akita+/−/sgk1−/− and akita+/−/sgk1+/+ mice and in nondiabetic akita+/−/sgk1−/− and akita+/−/sgk1+/+ mice. Values are means ± SE (n = 7–10 for each group) of food intake (A), fluid intake (B), body weight (C), and plasma aldosterone concentrations (D) in mice carrying the Akita mutation (akita+/−) and either lacking (sgk1−/−) or expressing (sgk1+/+) functional SGK1, as well as in nondiabetic mice (akita+/−) either lacking or expressing functional SGK1. *P < 0.05 vs. respective value of akita+/−/sgk1+/+ mice. ###P < 0.001 vs. respective value of akita+/− mice.
The renal cortex but high transcript levels in collecting duct (Fig. 7). In the renal cortex of diabetic mice, SGK1 transcript levels were enhanced, pointing to upregulation of SGK1 transcription in hyperglycemia. As shown in Fig. 8, in some but not all cells, SGK1 colocalizes with SGLT1.

**DISCUSSION**

The present study reveals a novel pathophysiological role of the serum- and glucocorticoid-inducible kinase SGK1. According to our observations, the kinase stimulates proximal tubular 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.
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 and in nondiabetic Akita mice (49), in diabetic Akita+/− mice apparently dependent on the presence of SGK1. Genetic knockout of SGK1 decreases the depolarization by almost 50%, an observation pointing to decreased SGLT1 activity. Accordingly, the glucosuria is significantly enhanced in SGK1-deficient Akita+/− mice, even though plasma glucose concentration was not significantly different.

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.

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.
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, which is a result of impaired SGK1-dependent stimulation of renal tubular Na+ reabsorption (86). Moreover, lack of SGK1 compromises the ability to excrete potassium loads (40) and to enhance salt appetite in response to mineralocorticoid excess (77). Plasma aldosterone concentrations are thus increased 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.

ACKNOWLEDGMENTS

We acknowledge the technical assistance of E. Faber and meticulous preparation of the manuscript by T. Loch and L. Subasic.

GRANTS

This study was supported by Deutsche Forschungsgemeinschaft Grants GRK 1302, DFG GRK 880, and DFG Ha 1755/8-1, National Institutes of Health Grant DK-5628, and National Natural Science Foundation of China NSFC Grant 30270618.

REFERENCES


Regulation of the Na\(^+\)/K\(^+\)-ATPase by the serum and glucocorticoid-inducible kinase SGK1 on the epithelial Na\(^+\) channel (ENaC) and CFTR: implications for cystic fibrosis. Cell Physiol Biochem 289: R395–R401, 2005.


