Original Article

Serum- and Glucocorticoid-Inducible Kinase 1 Mediates Salt Sensitivity of Glucose Tolerance

Krishna M. Boini,[1,2] Anita M. Hennige,[3] Dan Yang Huang,[2] Björn Friedrich,[3] Monica Palmada,[1] Christoph Boehmer,[1] Florian Grahammer,[1] Ferruh Artunc,[1] Susanne Ullrich,[1] Diana Avram,[1] Hartmut Osswald,[2] Peer Wulff,[4] Dietmar Kuhl,[5] Volker Vallon,[2,6] Hans-Ulrich Häring,[3] and Florian Lang[1]

Excess salt intake decreases peripheral glucose uptake, thus impairing glucose tolerance. Stimulation of cellular glucose uptake involves phosphatidylinositide-3-kinase (PI-3K)–dependent activation of protein kinase B/Akt. A further kinase downstream of PI-3K is serum- and glucocorticoid-inducible kinase (SGK)1, which is upregulated by mineralocorticoids and, thus, downregulated by salt intake. To explore the role of SGK1 in salt-dependent glucose uptake, SGK1 knockout mice (*sgk1*$^{-/-}$) and their wild-type littermates (*sgk1*$^{+/+}$) were allowed free access to either tap water (control) or 1% saline (high salt). According to Western blotting, high salt decreased and deoxycorticosterone acetate (DOCA; 35 mg/kg body wt) increased SGK1 protein abundance in skeletal muscle and fat tissue of *sgk1*$^{+/+}$ mice. Intraperitoneal injection of glucose (3 g/kg body wt) into *sgk1*$^{+/+}$ mice transiently increased plasma glucose concentration approaching significantly higher values ([glucose]p,max) in high salt (281 ± 39 mg/dl) than in control (164 ± 23 mg/dl) animals. DOCA did not significantly modify [glucose]p,max in control *sgk1*$^{+/+}$ mice but significantly decreased [glucose]p,max in high-salt *sgk1*$^{+/+}$ mice, an effect reversed by spironolactone (50 mg/kg body wt). [Glucose]p,max was in *sgk1*$^{-/-}$ mice insensitive to high salt and significantly higher than in control *sgk1*$^{+/+}$ mice. Uptake of 2-deoxy-D-[1,2-^{3}H]glucose into skeletal muscle and fat tissue was significantly smaller in *sgk1*$^{-/-}$ mice than in *sgk1*$^{+/+}$ mice and decreased by high salt in *sgk1*$^{+/+}$ mice. Transfection of HEK-293 cells with active ^{S422D}SGK1, but not inactive ^{K127N}SGK, stimulated phloretin-sensitive glucose uptake. In conclusion, high salt decreases SGK1-dependent cellular glucose uptake. SGK1 thus participates in the link between salt intake and glucose tolerance. *Diabetes* 55: 2059–2066, 2006

From the [1]Department of Physiology, University of Tübingen, Tübingen, Germany; the [2]Department of Pharmacology and Toxicology, University of Tübingen, Tübingen, Germany; the [3]Department of Internal Medicine 4, University of Tübingen, Tübingen, Germany; the [4]Department of Clinical Neurobiology, University Hospital of Neurology, Heidelberg, Germany; the [5]Department of Biology, Chemistry, and Pharmacy, Free University Berlin, Berlin, Germany; and the [6]Departments of Medicine and Pharmacology, University of California San Diego and Veterans' Administration San Diego Health Care System, San Diego, California.

Address correspondence and reprint requests to Florian Lang, Department of Physiology, University of Tübingen, Gmelinstr 5, D-72076 Tübingen, Germany. E-mail: florian.lang@uni-tuebingen.de.

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K.M.B. and A.M.H. contributed equally to this work.

2-DOG, 2-deoxy-D-[1,2-^{3}H]glucose; DOCA, deoxycorticosterone acetate; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PI-3K, phosphatidylinositide-3-kinase; PKB, protein kinase B; SGK, serum- and glucocorticoid-inducible kinase.

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Excessive salt intake may impede cellular glucose uptake in peripheral tissues and, thus, lead to delayed decrease of plasma glucose concentrations following a glucose load (1–5). Mechanisms accounting for the salt sensitivity of peripheral glucose uptake have remained ill defined. Glucose transport into insulin-responsive cells is accomplished in large part by insertion of the Na^+-independent glucose carriers, GLUT1 (SLC2A1) and GLUT4 (SLC2A4), into the cell membrane (6–8). The stimulation of the transporters by insulin requires phosphatidylinositide-3-kinase (PI-3K) and is abrogated by pharmacological inhibitors (wortmannin and LY294002) or genetic knockout (PI-3K dominant-negative mutants) of the kinase (9–14). Downstream elements of PI-3K include the phosphoinositide-dependent kinase PDK-1, which in turn phosphorylates and thus activates the serine/threonine kinase Akt/protein kinase B (PKB) (15–17). The effect of PI-3K on GLUT4 trafficking is mediated by PKB (18,19). PKB is, however, at least in some cells, not required for the PI-3K–dependent trafficking of GLUT1 (18). Thus, some other PI-3K–dependent protein kinase is presumably involved in the regulation of GLUT1.

A further downstream molecule in the PI-3K signaling cascade is the serum- and glucocorticoid-inducible kinase (SGK)1 (20–22), which has previously been shown to stimulate several transport proteins (23), including the Na^+-coupled glucose transporter SGLT1 (24). SGK1 was originally cloned as a glucocorticoid-sensitive gene from rat mammary tumor cells (25,26) and later as a human cell volume–regulated gene (27). SGK1 is strongly upregulated by mineralocorticoids (28–36) and participates in the stimulation of the renal epithelial Na^+ channel ENaC by mineralocorticoids (30,33–38) and insulin (39–42).

The present study has been performed to explore whether SGK1 is involved in the link between salt intake and glucose tolerance. To this end, experiments were performed in wild-type animals, in gene-targeted mice lacking SGK1 (*sgk1*$^{-/-}$), and in their wild-type littermates (*sgk1*$^{+/+}$). Glucose tolerance and peripheral glucose uptake have been determined in animals with or without excessive salt intake. The results demonstrate that the effect of excessive salt intake on peripheral glucose uptake

and alterations of plasma glucose concentration are dependent on, and mediated by, SGK1.

RESEARCH DESIGN AND METHODS

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

Mice deficient in SGK1 ($sgk1^{-/-}$) were generated and bred as previously described (43). In brief, a conditional targeting vector was generated from a 7-kb fragment encompassing the entire transcribed region on 12 exons. The neomycin resistance cassette was flanked by two loxP sites and inserted into intron 11. Exons 4–11, which code for the sgk1 kinase domain, were "floxed" by inserting a third loxP site into intron 3. A clone with a recombination between the first and third loxP site (type I recombination) was injected into C57BL/6 blastocytes. Male chimeras were bred to C57BL/6 and 129/SvJ females. Heterozygous SGK1-deficient mice were backcrossed to 129/SvJ wild-type mice (Charles River, Sulzfeld, Germany) for 10 generations and then intercrossed to generate homozygous SGK1 knockout mice ($sgk1^{-/-}$) and their wild-type littermates ($sgk1^{+/+}$). The genetic background of the animals was thus 129/SvJ. For the study, 4-month-old male SGK1 knockout ($sgk1^{-/-}$) mice and their wild-type littermates ($sgk1^{+/+}$) were selected. Where indicated, female SGK1 knockout ($sgk1^{-/-}$) mice and their wild-type littermates ($sgk1^{+/+}$) and unrelated (not littermate) wild-type male 129/SvJ mice were studied.

The mice were fed a control diet (1310/1314; Altromin, Heidenau, Germany) (44,45) and allowed free access to tap water or to 1% NaCl solution.

For determination of glucose tolerance, mice were starved overnight and glucose (3 g/kg body wt i.p.) was injected. Then, a drop of blood was drawn from the tail into a test strip of a glucometer (Accutrend; Roche, Mannheim, Germany) for measuring the blood glucose levels before and 15, 30, 45, 60, 75, 90, 120, 150, or 180 min after the injection.

In a further series of experiments, long-acting insulin (Novo Nordisk, Mainz, Germany) was injected (0.15 units/kg body wt i.p.) and plasma glucose concentrations were determined at 0, 15, 30, 45, 60, and 90 min as described above.

In vivo tissue glucose uptake during a glucose tolerance test was determined in male mice that have been starved for 16 h. 2-deoxy-D-[1,2-^{3}H]glucose (2-DOG) was mixed with 20% of regular glucose (10 μCi/mouse, 3 g/kg body wt) and injected intraperitoneally. Blood glucose levels were determined to ensure adequate injection. After 120 min, the mice were killed and 100 mg of tissues were homogenized in 1 ml of water. Seven-percent ice-cold perchloric acid (800 μl) was added to homogenate (800 μl). The sample was then cleared by centrifugation, and 1 ml of the supernatant was neutralized for 30 min with 2.2 mol/l $KHCO_3$. The precipitate was removed by centrifugation, and 500 μl of the supernatant was used to determine total ^{3}H radioactivity.

To explore whether salt loading decreases SGK1 protein abundance, $sgk1^{+/+}$ mice were divided into four groups (five mice per group) and treated separately with *1*) control diet for 2 weeks, *2*) control diet for 2 weeks plus 1% NaCl in drinking water for 2 weeks, *3*) control diet for 2 weeks plus deoxycorticosterone acetate (DOCA; 35 mg/kg body wt s.c.) treatment for 2 days, and *4*) control diet for 2 weeks plus DOCA (35 mg/kg body wt s.c.) and spironolactone (50 mg/kg body wt s.c.) treatment for 2 days. Mice were anesthetized with ketamine (60 mg/kg body wt i.p.) plus xylazine (10 mg/kg body wt i.p.). Skeletal muscle and fat tissues were removed and immediately shock frozen in liquid nitrogen. For Western blot analysis, the tissues were homogenized by using a homogenizer (Labortechnik, Mullheim, Germany). The muscle tissues were homogenized in lysis buffer containing 50 mmol/l Tris-HCl, 50 mmol/l NaF, pH 7, 50 mmol/l β-glycerophosphate, 10 mmol/l potassium phosphate, 10 mmol/l $MgCl_2$, 1 mmol/l EDTA, 1 mmol/l EGTA, 1 mmol/l sodium vanadate, 0.1% Tween 20, and a protease inhibitor cocktail tablet (Complete mini EDTA-free; Roche). The fat tissues were homogenized in lysis buffer containing 20 mmol/l Tris, pH 7.4, 2 mmol/l EDTA, 137 mmol/l sodium chloride, 1% NP-40, 10% glycerol, 12 mmol/l β-glycerol phosphate, and a protease inhibitor cocktail tablet (Complete mini EDTA-free; Roche). The homogenates were centrifuged at 10,000*g*, 4°C for 30 min, and the supernatant was removed and used for Western blotting. Total proteins were measured using the Bradford assay. Total proteins (100 μg) were separated by SDS-PAGE (10% SDS Tris-Glycine), transferred to nitrocellulose membranes, blocked overnight in blocking buffer (5% fat-free milk in PBS containing 0.1% Tween) at 4°C, and incubated 1 h with a polyclonal anti-SGK1 antibody (46) diluted 1:1,000 in blocking buffer. The antibody was kindly provided by Nicola Perrotti, Catanzaro, Italy. After incubation with a horseradish peroxidase–conjugated anti-rabbit secondary antibody (diluted 1:1,000 in blocking buffer; Amersham, Freiburg, Germany), the bands were visualized with enhanced chemoluminescence (Amersham) according to the manufacturer's instructions. Homogenates were also probed with a primary glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Santa Cruz, Heidelberg, Germany) antibody as a loading control. Densitometric analysis of SGK1 protein abundance was performed by using Quantity One software (1998 version; Bio-Rad Laboratories, Hercules, CA) and normalized using GAPDH.

To further explore whether SGK1 increases glucose transport, HEK-293 cells were seeded on six-well plates at 0.2×10^6 cells/well, and 24 h later, cells were transfected with 2 μg constitutively active ^{S422D}SGK1, inactive ^{K127N}SGK1, or empty vector (as a control) by using Lipofectamine (Invitrogen, Karlsruhe, Germany) according to the manufacturer's instructions. For determination of transport, 2-DOG was used as the glucose analog. 2-DOG uptake was measured in HEK-293 cells 2 days after transfection by incubating the cells at 37°C for 5 min (linear range of uptake) in glucose-free Krebs Ringer HEPES buffer containing 0.3 mmol/l unlabeled 2-DOG and 0.1 μCi/well [^{3}H]2-DOG with or without 100 μmol/l phloretin. Uptake was stopped by rapid aspiration of uptake solution and washing four times with ice-cold PBS containing 50 mmol/l unlabeled 2-DOG. Thereafter, cells were lysed with 10 mmol/l NaOH/0.1% Triton X-100 and radioactivity measured with a liquid scintillation counter. Protein concentration was determined by the Bradford method.

Data are provided as arithmetic means ± SE. Where indicated, n represents the number of independent experiments. All data were tested for significance using ANOVA or paired or unpaired Student's t test, as applicable. Results with a P value <0.05 were considered statistically significant.

RESULTS

Glucose tolerance tests were performed in mice lacking SGK1 ($sgk1^{-/-}$) and in their wild-type littermates ($sgk1^{+/+}$). Before the experiments, the animals were fed a standard diet and allowed free access to either plain tap water (control) or 1% saline solution (high salt). As illustrated in Fig. 1, intraperitoneal injection of glucose (3 g/kg body wt) led to a transient increase of plasma glucose concentration approaching significantly higher values in high salt than in control $sgk1^{+/+}$ mice. The delayed decrease of the plasma glucose concentrations after a glucose load points to impaired cellular uptake of glucose in saline-drinking animals. The effect of saline was observed in both male (Fig. 1*A*) and female (Fig. 1*B*) mice.

The increase of plasma glucose concentration was significantly larger in tap water–drinking $sgk1^{-/-}$ mice than in tap water–drinking $sgk1^{+/+}$ mice and similar in tap–water drinking $sgk1^{-/-}$ mice and saline-drinking $sgk1^{+/+}$ mice (Fig. 1*A* and *B*). The difference between $sgk1^{-/-}$ and $sgk1^{+/+}$ mice was again apparent in both male (Fig. 1*A*) and female (Fig. 1*B*) mice. In $sgk1^{-/-}$ mice, the increase of plasma glucose concentration was not significantly different between animals drinking tap water and animals drinking saline. Thus, in contrast to $sgk1^{+/+}$ mice, salt excess did not further affect glucose tolerance in $sgk1^{-/-}$ mice. As illustrated in Fig. 2, the decline of plasma glucose concentrations following intraperitoneal injection of insulin (0.15 units/kg body wt) was similarly blunted by salt excess in $sgk1^{+/+}$ mice. Again, insulin sensitivity of plasma glucose concentration was significantly smaller in tap water–drinking $sgk1^{-/-}$ mice than in tap water–drinking $sgk1^{+/+}$ mice. The salt sensitivity of the insulin-induced decrease of plasma glucose concentration was significantly smaller in $sgk1^{-/-}$ mice than in $sgk1^{+/+}$ mice. Those observations strongly suggest that SGK1 mediates part of the insulin-induced cellular uptake of glucose and substantially contributes to, or even accounts for, the salt sensitivity of glucose tolerance.

As high salt intake decreases the plasma concentration of aldosterone, a known stimulator of SGK1 expression, we explored in $sgk1^{+/+}$ mice, whether the effect of high salt intake on glucose tolerance was due to decreased mineralocorticoid action. Animals were treated with subcutaneous injection of the mineralocorticoid DOCA (dissolved in soybean oil, 35 mg/kg body wt) 4 h before the

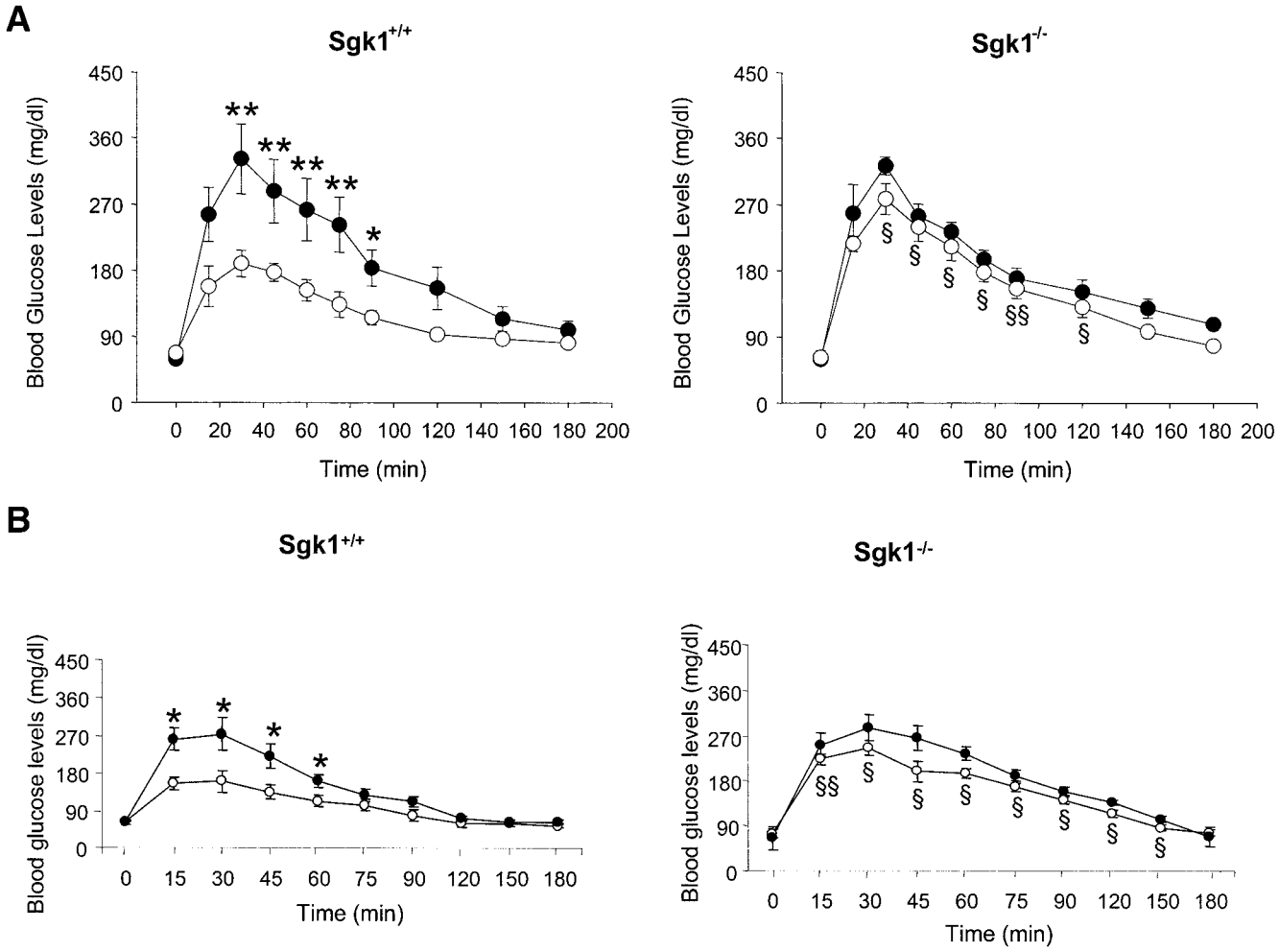

FIG. 1. Plasma glucose concentrations following intraperitoneal glucose injection in *sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ male (*A*) and female (*B*) mice. Arithmetic means ± SE of plasma glucose concentrations following intraperitoneal injection of 3 g/kg body wt glucose in SGK1 knockout mice (*sgk1*$^{-/-}$, *right panels*) and in wild-type littermates (*sgk1*$^{+/+}$, *left panels*). Experiments were performed in mice drinking tap water (○) and in mice drinking 1% NaCl (●) for 14 days. *A*: $n = 7$ for tap water–drinking male mice and $n = 6$ for 1% NaCl-drinking male mice. *B*: $n = 5$ for both tap water–drinking and 1% NaCl-drinking female mice. *$P < 0.05$, **$P < 0.01$ vs. tap water–drinking animals; §$P < 0.05$, §§$P < 0.01$ vs. tap water–drinking *sgk1*$^{+/+}$ mice.

glucose tolerance test. The DOCA treatment did not significantly modify the increase of plasma glucose concentrations in control *sgk1*$^{+/+}$ mice but significantly blunted the increase of plasma glucose concentrations in saline-drinking *sgk1*$^{+/+}$ mice (Fig. 3). After treatment with DOCA, the increase of plasma glucose concentrations was similar in saline-drinking *sgk1*$^{+/+}$mice as in *sgk1*$^{+/+}$ mice drinking tap water (Fig. 3).

The subcutaneous injection of the mineralocorticoid receptor antagonist spironolactone (dissolved in soybean oil, 50 mg/kg body wt) did not significantly modify the increase of plasma glucose concentrations following glucose injection in control *sgk1*$^{+/+}$ mice but reversed the effect of DOCA in *sgk1*$^{+/+}$ mice on a high-salt diet. The increase of plasma glucose concentration was not significantly different in saline-drinking *sgk1*$^{+/+}$ mice treated with DOCA plus spironolactone and saline-drinking *sgk1*$^{+/+}$ mice without pharmacological treatment. The values were, however, significantly higher than the values of untreated tap water–drinking *sgk1*$^{+/+}$ mice (Fig. 3).

Further experiments were performed on glucose uptake to identify the tissues accounting for the delayed cellular uptake of plasma glucose in salt-loaded wild-type animals or in animals lacking SGK1. To this end, radiolabeled [H^3]2-DOG was injected into the peritoneal cavity and the radioactive tracer was determined in several tissues. As illustrated in Fig. 4, glucose uptake in muscle and fat tissue was significantly lower in saline-drinking *sgk1*$^{+/+}$ mice compared with tap water–drinking *sgk1*$^{+/+}$ mice. Moreover, during free access to tap water, glucose uptake into muscle, liver, and fat tissue was significantly smaller in *sgk1*$^{-/-}$ mice than in *sgk1*$^{+/+}$ mice. Conversely, glucose uptake into kidney was significantly enhanced in *sgk1*$^{-/-}$ mice, an effect possibly due to enhanced plasma glucose concentrations.

Western blot analysis was performed to determine whether the decrease of 2-DOG uptake into skeletal muscle and fat tissue was indeed paralleled by a decrease of SGK1 protein expression following salt load. As illustrated in Fig. 5, SGK1 protein abundance was indeed significantly decreased by high-salt intake. Treatment of tap water–drinking mice with DOCA treatment significantly enhanced the SGK1 protein expression, an effect reversed by spironolactone (Fig. 5).

Those experiments pointed to the ability of SGK1 to stimulate cellular glucose uptake. To further test this possibility, constitutively active ^{S422D}SGK1 or, for comparison, the inactive mutant ^{K127N}SGK1, were expressed in

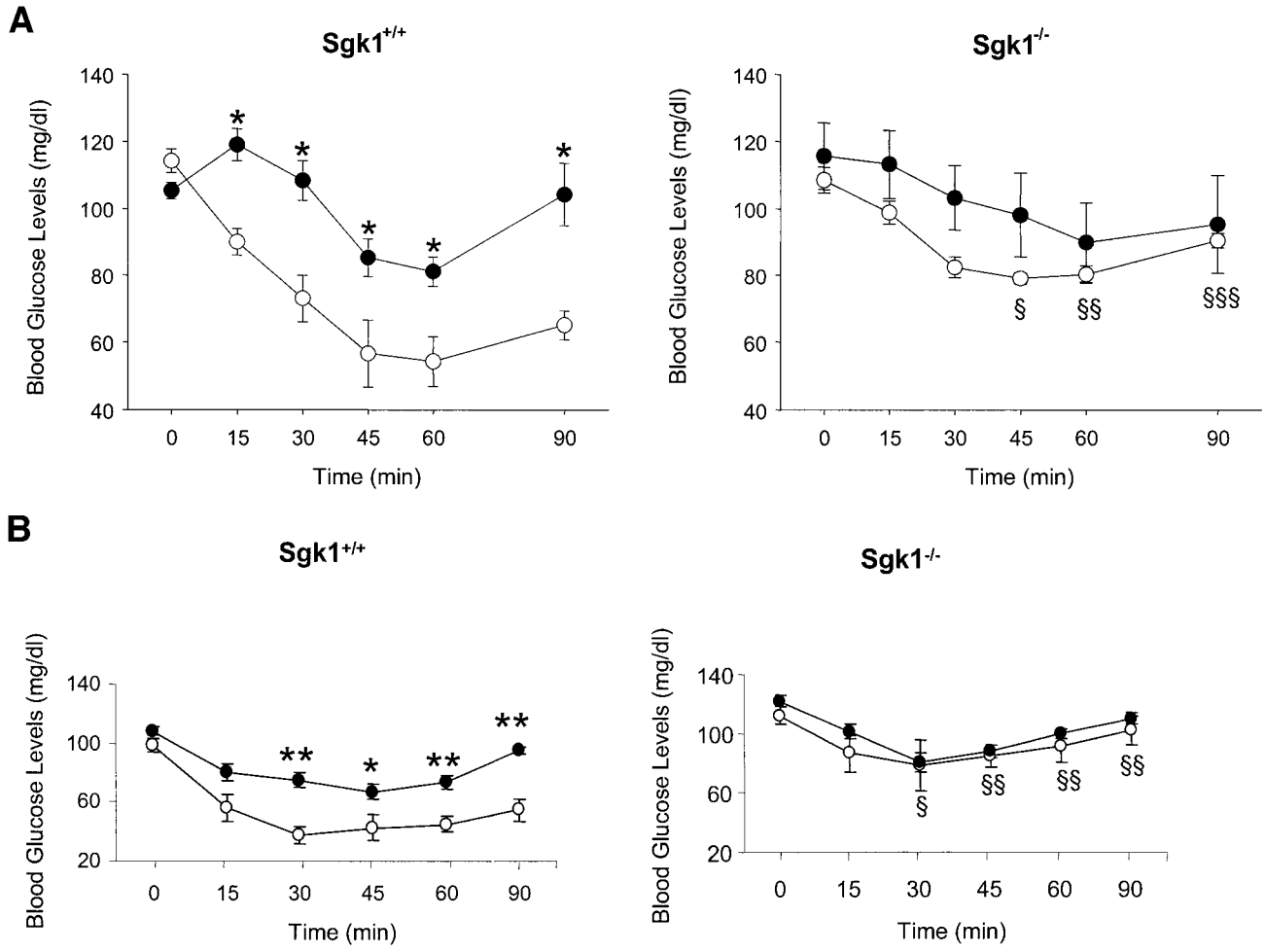

FIG. 2. Plasma glucose concentrations following intraperitoneal insulin injection in *sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ male (*A*) and female (*B*) mice. Arithmetic means ± SE of plasma glucose concentrations following intraperitoneal injection of 0.15 units/kg body wt insulin in SGK1 knockout mice (*sgk1*$^{-/-}$, *right panel*) and wild-type littermates (*sgk1*$^{+/+}$, *left panel*). Experiments were performed in mice drinking tap water (○) and in mice drinking 1% NaCl (●) for 14 days. *A*: $n = 7$ for tap water–drinking male mice and $n = 6$ for 1% NaCl-drinking male mice. *B*: $n = 5$ for both tap water–drinking and 1% NaCl-drinking female mice. *$P < 0.05$, **$P < 0.01$ vs. tap–water drinking animals; §$P < 0.05$, §§$P < 0.01$, §§§$P < 0.005$ vs. tap water–drinking *sgk1*$^{+/+}$ mice.

HEK-293 cells and 2-DOG uptake determined. As illustrated in Fig. 6, the transfection with active ^{S422D}SGK1 led to a marked increase of 2-DOG uptake. In contrast, transfection with inactive ^{K127N}SGK1 decreased 2-DOG uptake. In the presence of phloretin, 2-DOG uptake was markedly reduced and no more sensitive to transfection with

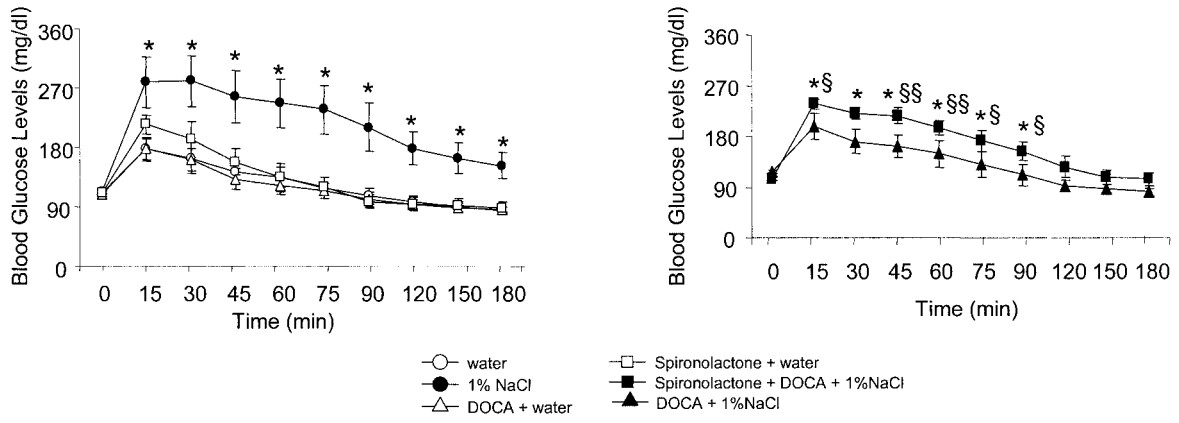

FIG. 3. Plasma glucose concentrations following intraperitoneal glucose injection in tap water–drinking and 1% NaCl saline–drinking wild-type mice with and without prior treatment with DOCA and/or spironolactone. Arithmetic means ± SE ($n = 6$ each group) of plasma glucose concentrations following intraperitoneal injection of glucose (3 g/kg body wt) in separately bred SV129 wild-type mice drinking tap water (open symbols) and/or 1% NaCl (closed symbols) for 14 days. Animals receiving no drug treatment are labeled by circles, animals receiving DOCA before the glucose tolerance test are indicated by triangles, and animals receiving spironolactone before the glucose tolerance tests are indicated by squares. *$P < 0.05$ vs. tap water–drinking animals; §$P < 0.05$, §§$P < 0.01$ vs. DOCA + 1% NaCl–drinking animals.

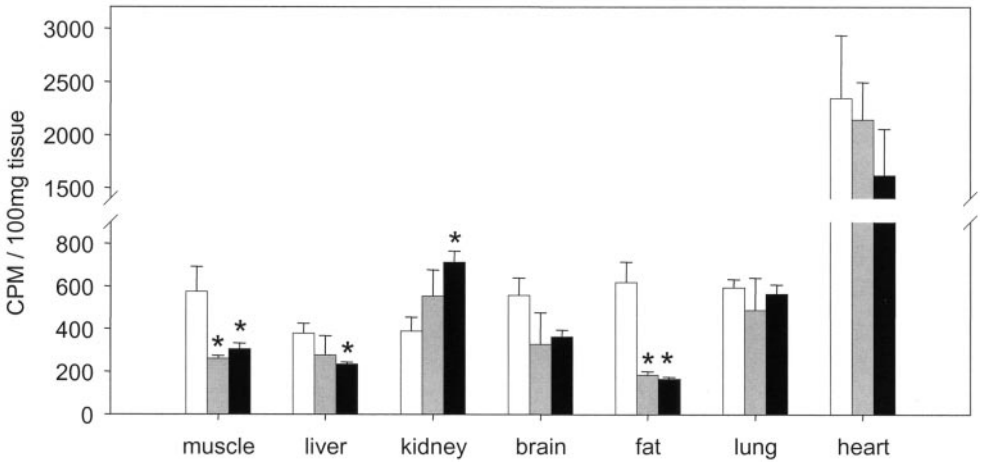

FIG. 4. 2-DOG uptake into tissues following intraperitoneal glucose injection in tap water–drinking and saline-drinking *sgk1*$^{+/+}$ mice and in *sgk1*$^{-/-}$ mice. Arithmetic means ± SE (n = 8 each group) of tissue radioactivity following intraperitoneal injection of radioactively labeled 2-DOG in glucose (3 g/kg body wt). Glucose uptake has been determined in wild-type mice drinking tap water (□), wild-type mice drinking saline for 14 days (▨), and tap water–drinking *sgk1*$^{-/-}$ (■). *P < 0.05 vs. tap water–drinking wild-type animals.

^{S422D}SGK1 or ^{K127N}SGK1. HEK-293 cells mainly express GLUT1, which is sensitive to phloretin (47).

DISCUSSION

Excessive salt intake increases blood pressure, an effect paralleled by insulin resistance and/or glucose intolerance in salt-sensitive subjects (3,48). Our present data demonstrate that high salt intake leads to impaired glucose tolerance and impaired insulin sensitivity following an intraperitoneal glucose load. More importantly, the observations provide a cellular mechanism accounting for this pathophysiologically relevant phenomenon. A high-salt diet decreases SGK1 expression, thus disrupting SGK1-dependent glucose uptake into several tissues, including liver, fat, and skeletal muscle. The transfection experiments indeed confirm the ability of SGK1 to profoundly stimulate glucose uptake.

This observation discloses a novel function of SGK1, including the participation in the stimulation of cellular glucose uptake by insulin. Accordingly, SGK1 does not only integrate the effects of mineralocorticoids and insulin on renal tubular Na^+ transport (39–42) but similarly affects glucose transport.

The contribution of SGK1 to the regulation of renal tubular Na^+ transport is modest, and the SGK1 knockout mouse is seemingly normal and able to maintain normal renal salt excretion under standard diet. However, the elevated plasma aldosterone concentration in *sgk1*$^{-/-}$ mice points to the functional significance of SGK1-dependent regulation of renal salt conservation even under those

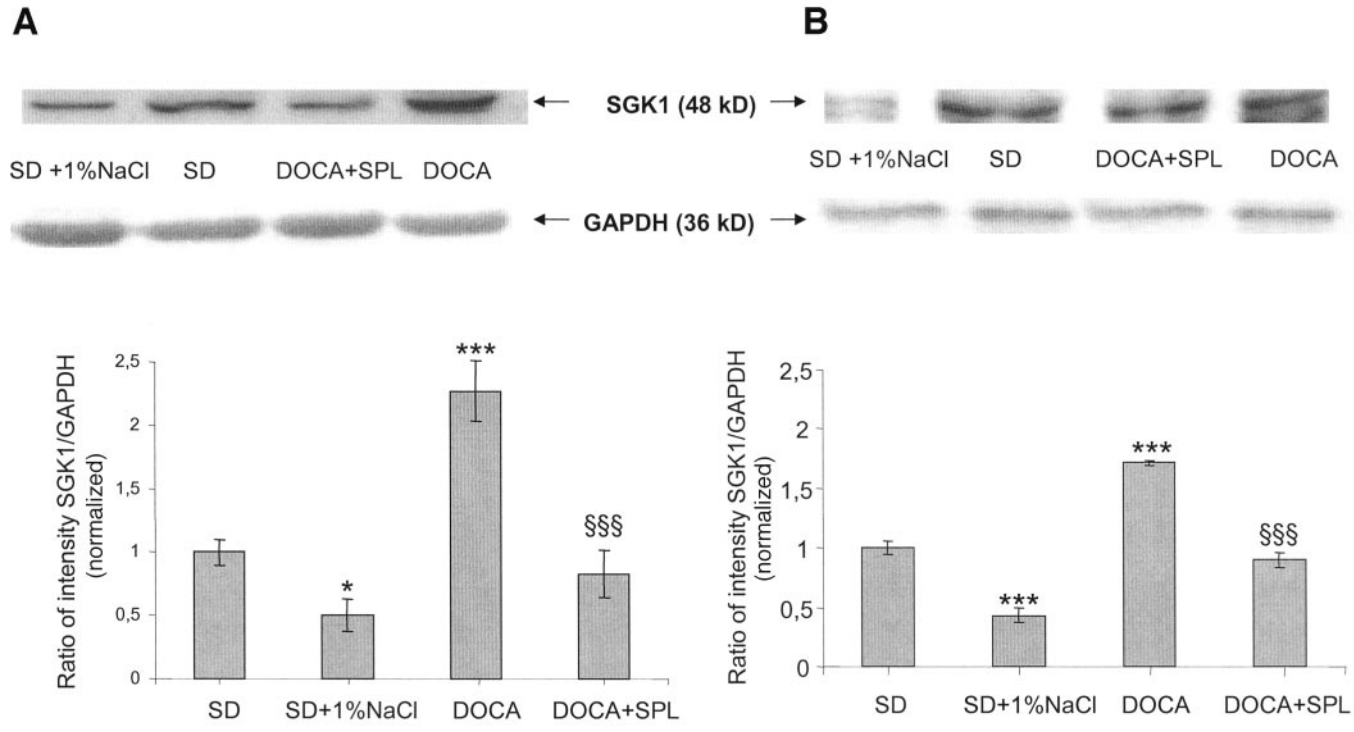

FIG. 5. Effects of control diet, 1% NaCl, DOCA, and combined DOCA + spironolactone on skeletal muscle (*A*) and fat tissue (*B*) SGK1 protein abundance in *sgk1*$^{+/+}$ mice. Effects of control diet (SD), control diet +1% NaCl (SD +1% NaCl), control diet + DOCA (DOCA), and control diet + DOCA + spironolactone (DOCA+SPL) on skeletal muscle (*left panel*) and fat tissue (*right panel*) SGK1 protein abundance in SGK1 wild-type mice. Arithmetic means ± SE (n = 5 each group). For Western blotting, SGK1/GAPDH band intensities from five independent experiments were normalized in each group to the mean value of SGK1/GAPDH band intensity of skeletal muscle and fat tissue from control group (*sgk1*$^{+/+}$ mice). *P < 0.05, ***P < 0.005 vs. control group; §§§P < 0.005 vs. DOCA-treated mice.

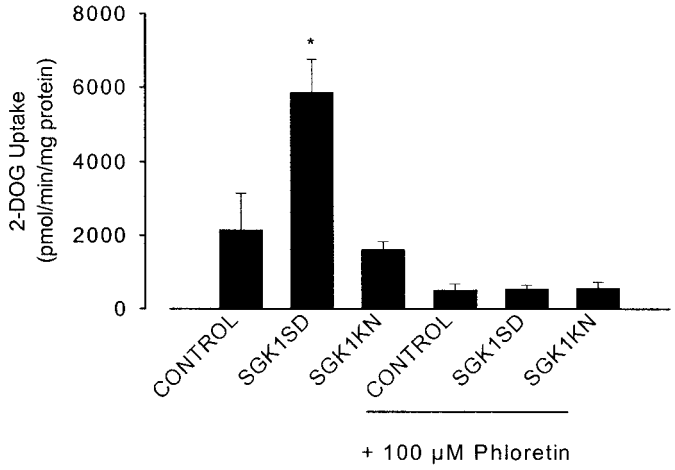

FIG. 6. Constitutively active, but not inactive, SGK1 stimulates 2-DOG transport. HEK-293 cells were transfected with active ^{S422D}SGK1, inactive ^{K127N}SGK1, or empty vector. Labeled 2-DOG uptake was studied 2 days after transfection in the presence and absence of phloretin (100 μmol/l). 2-DOG uptake was stimulated by ^{S422D}SGK1 (SGK1SD), whereas transport remained unaffected upon expression of the inactive ^{K127N}SGK1 (SGK1KN). Arithmetic means ± SE (n = 4). *P < 0.05 vs. transfection with empty vector (control).

dietary conditions. The enhanced plasma aldosterone concentration compensates for the lack of SGK1 and allows the maintenance of normal blood pressure. The maintenance of blood pressure in *sgk1*$^{-/-}$ mice is consistent with SGK1-independent regulation of renal Na^+ reabsorption (43). In contrast, severe salt wasting is observed in the mineralocorticoid receptor knockout mice (49). The ENaC knockout mice survive only a few days after delivery (50). The contribution of SGK1 to renal Na^+ reabsorption becomes crucial during salt depletion. The ability of mice lacking functional SGK1 to lower urinary NaCl excretion during salt restriction is impaired, leading to severe, and eventually lethal, volume loss despite decrease of glomerular filtration rate and increase of proximal tubular reabsorption (43).

Saline tended to decrease the insulin sensitivity of blood glucose levels in *sgk1*$^{-/-}$ mice. Even though the difference between tap water and saline-drinking *sgk1*$^{-/-}$ mice was not statistically significant, in view of the scatter of the data, the present experiments do not rule out additional SGK1-independent mechanisms leading to insulin resistance during sodium excess. Possible candidates include the other SGK or PKB/Akt isoforms. Clearly, the contribution of SGK1 to the regulation of cellular glucose uptake is modest and does not match the glucose transport stimulating effect of PKB (18,51,52). Particularly, PKB/Akt2 plays a key role in the stimulating effects of insulin on cellular glucose uptake (53–56). Recently, PKB/Akt2 has been shown to be required for the upregulation of skeletal muscle glucose transport during calorie restriction (56). However, cellular glucose uptake is not disrupted in PKB/Akt2-deficient mice, pointing to additional kinases serving a similar function. One of those kinases could well be SGK1, which may fully account for the deranged glucose tolerance during excessive salt intake.

Unlike the expression of PKB, the expression of SGK1 could be upregulated by a variety of hormones, mediators, and other regulators (16). Most importantly, SGK1 is under strong transcriptional control of mineralocorticoids (28–36). Moreover, SGK1 expression is markedly upregulated by cerebral ischemia (57) and may contribute to the enhancement of cellular glucose uptake in ischemic tissues. Along those lines, the enhanced cerebral SGK1 expression in enriched environment (58) may serve to adjust glucose uptake to the enhanced demand following stimulation of neuronal activity. As suggested in Fig. 4, the tissues with SGK1-sensitive glucose uptake may include the brain. Considering the virtually ubiquitous expression of SGK1 (27), it is likely that SGK1 participates in the regulation of nutrient uptake in a variety of further tissues.

Salt loading has previously been shown to decrease SGK1 expression (59), an effect at least partially mediated by a decrease of aldosterone concentration (28–34,36). Accordingly, the effect of salt excess on plasma glucose concentrations following a glucose load can be reversed by replacing the endogenous mineralocorticoids with exogenous DOCA. Moreover, the effect of DOCA is abrogated by the aldosterone receptor antagonist spironolactone. However, the increase of plasma glucose concentration following a glucose load is not significantly affected by spironolactone in the absence of DOCA. Possibly, treatment of tap water–drinking mice with spironolactone leads to volume depletion, which may lead in turn to mineralocorticoid independent stimulation of SGK1 expression (e.g., by increase of angiotensin II).

The present observations do not address the mechanisms of SGK1-dependent regulation of cellular glucose uptake. Clearly, expression of SGK1, but not of the inactive mutant, enhances glucose uptake into HEK-293 cells, indeed confirming the ability of SGK1 to stimulate glucose transport. Most recent in vitro studies (60) revealed the ability of SGK1 to enhance phloretin-sensitive glucose uptake into adipocytes.

The stimulating effect of SGK1 on glucose uptake into fat tissue would be expected to favor the development of obesity. As a matter of fact, a "gain-of-function" gene variant of SGK1 affecting as many as some 5% of a Caucasian population is associated with obesity (24). The obesity may eventually decrease insulin sensitivity despite the stimulating effect of SGK1 on glucose uptake. Moreover, the same gene variant is associated with increased blood pressure (61) presumably due to the salt-retaining property of SGK1 (43). The salt excess would again favor insulin resistance. Thus, SGK1 may participate in the pathophysiology of metabolic syndrome, a condition characterized by a variety of disorders including hypertension, obesity, and insulin resistance (62). Along those lines, metabolic syndrome shares several features with glucocorticoid excess (63), which should upregulate SGK1 expression (25). According to the present data, upregulation of SGK1 may blunt the peripheral insulin resistance caused by glucocorticoids. On the other hand, SGK1 mediates the inhibition of insulin release by glucocorticoids (64). Clearly, additional experiments are required to elucidate the role of SGK1 in glucose metabolism.

In conclusion, excessive salt intake impairs glucose tolerance and insulin-induced glucose uptake. The effect is reversed by application of mineralocorticoids and abrogated in SGK1 knockout mice. The observations point to a novel mechanism participating in the regulation of cellular glucose transport.

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REFERENCES

1. Cheng ZJ, Vaskonen T, Tikkanen I, Nurminen K, Ruskoaho H, Vapaatalo H, Muller D, Park JK, Luft FC, Mervaala EM: Endothelial dysfunction and salt-sensitive hypertension in spontaneously diabetic Goto-Kakizaki rats. *Hypertension* 37:433–439, 2001
2. Gaboury CL, Simonson DC, Seely EW, Hollenberg NK, Williams GH: Relation of pressor responsiveness to angiotensin II and insulin resistance in hypertension. *J Clin Invest* 94:2295–2300, 1994
3. Giner V, Coca A, de la Sierra A: Increased insulin resistance in salt sensitive essential hypertension. *J Hum Hypertens* 15:481–485, 2001
4. ter Maaten JC, Voordouw JJ, Bakker SJ, Donker AJ, Gans RO: Salt sensitivity correlates positively with insulin sensitivity in healthy volunteers. *Eur J Clin Invest* 29:189–195, 1999
5. Vicent D, Ilany J, Kondo T, Naruse K, Fisher SJ, Kisanuki YY, Bursell S, Yanagisawa M, King GL, Kahn CR: The role of endothelial insulin signaling in the regulation of vascular tone and insulin resistance. *J Clin Invest* 111:1373–1380, 2003
6. Cushman SW, Wardzala LJ: Potential mechanism of insulin action on glucose transport in the isolated rat adipose cell: apparent translocation of intracellular transport systems to the plasma membrane. *J Biol Chem* 255:4758–4762, 1980
7. James DE, Pilch PF: Fractionation of endocytic vesicles and glucose-transporter-containing vesicles in rat adipocytes. *Biochem J* 256:725–732, 1988
8. Suzuki K, Kono T: Evidence that insulin causes translocation of glucose transport activity to the plasma membrane from an intracellular storage site. *Proc Natl Acad Sci U S A* 77:2542–2545, 1980
9. Bentley J, Itchayanan D, Barnes K, McIntosh E, Tang X, Downes CP, Holman GD, Whetton AD, Owen-Lynch PJ, Baldwin SA: Interleukin-3-mediated cell survival signals include phosphatidylinositol 3-kinase-dependent translocation of the glucose transporter GLUT1 to the cell surface. *J Biol Chem* 278:39337–39348, 2003
10. Cheatham B, Vlahos CJ, Cheatham L, Wang L, Blenis J, Kahn CR: Phosphatidylinositol 3-kinase activation is required for insulin stimulation of pp70 S6 kinase, DNA synthesis, and glucose transporter translocation. *Mol Cell Biol* 14:4902–4911, 1994
11. Haruta T, Morris AJ, Rose DW, Nelson JG, Mueckler M, Olefsky JM: Insulin-stimulated GLUT4 translocation is mediated by a divergent intracellular signaling pathway. *J Biol Chem* 270:27991–27994, 1995
12. Kotani K, Carozzi AJ, Sakaue H, Hara K, Robinson LJ, Clark SF, Yonezawa K, James DE, Kasuga M: Requirement for phosphoinositide 3-kinase in insulin-stimulated GLUT4 translocation in 3T3–L1 adipocytes. *Biochem Biophys Res Commun* 209:343–348, 1995
13. Okada T, Kawano Y, Sakakibara T, Hazeki O, Ui M: Essential role of phosphatidylinositol 3-kinase in insulin-induced glucose transport and antilipolysis in rat adipocytes: studies with a selective inhibitor wortmannin. *J Biol Chem* 269:3568–3573, 1994
14. Perrini S, Natalicchio A, Laviola L, Belsanti G, Montrone C, Cignarelli A, Minielli V, Grano M, De Pergola G, Giorgino R, Giorgino F: Dehydroepiandrosterone stimulates glucose uptake in human and murine adipocytes by inducing GLUT1 and GLUT4 translocation to the plasma membrane. *Diabetes* 53:41–52, 2004
15. Khan AH, Pessin JE: Insulin regulation of glucose uptake: a complex interplay of intracellular signalling pathways. *Diabetologia* 45:1475–1483, 2002
16. Lang F, Cohen P: Regulation and physiological roles of serum- and glucocorticoid-induced protein kinase isoforms (Review). *Sci STKE* 2001: RE17, 2001
17. Virkamaki A, Ueki K, Kahn CR: Protein-protein interaction in insulin signaling and the molecular mechanisms of insulin resistance (Review). *J Clin Invest* 103:931–943, 1999
18. Foran PG, Fletcher LM, Oatey PB, Mohammed N, Dolly JO, Tavare JM: Protein kinase B stimulates the translocation of GLUT4 but not GLUT1 or transferrin receptors in 3T3–L1 adipocytes by a pathway involving SNAP-23, synaptobrevin-2, and/or cellubrevin. *J Biol Chem* 274:28087–28095, 1999
19. Zhou QL, Park JG, Jiang ZY, Holik JJ, Mitra P, Semiz S, Guilherme A, Powelka AM, Tang X, Virbasius J, Czech MP: Analysis of insulin signalling by RNAi-based gene silencing. *Biochem Soc Trans* 32:817–821, 2004
20. Biondi RM, Kieloch A, Currie RA, Deak M, Alessi DR: The PIF-binding pocket in PDK1 is essential for activation of S6K and SGK, but not PKB. *EMBO J* 20:4380–4390, 2001
21. Kobayashi T, Cohen P: Activation of serum- and glucocorticoid-regulated protein kinase by agonists that activate phosphatidylinositide 3-kinase is mediated by 3-phosphoinositide-dependent protein kinase-1 (PDK1) and PDK2. *Biochem J* 339:319–328, 1999
22. Park J, Leong ML, Buse P, Maiyar AC, Firestone GL, Hemmings BA: Serum and glucocorticoid-inducible kinase (SGK) is a target of the PI 3-kinase-stimulated signaling pathway. *EMBO J* 18:3024–3033, 1999
23. Lang F, Henke G, Embark HM, Waldegger S, Palmada M, Bohmer C, Vallon V: Regulation of channels by the serum and glucocorticoid-inducible kinase - implications for transport, excitability and cell proliferation. *Cell Physiol Biochem* 13:41–50, 2003
24. Dieter M, Palmada M, Rajamanickam J, Aydin A, Busjahn A, Boehmer C, Luft FC, Lang F: Regulation of glucose transporter SGLT1 by ubiquitin ligase Nedd4–2 and kinases SGK1, SGK3, and PKB. *Obes Res* 12:862–870, 2004
25. Firestone GL, Giampaolo JR, O'Keeffe BA: Stimulus-dependent regulation of the serum and glucocorticoid inducible protein kinase (Sgk) transcription, subcellular localization and enzymatic activity. *Cell Physiol Biochem* 13:1–12, 2003
26. Webster MK, Goya L, Ge Y, Maiyar AC, Firestone GL: Characterization of sgk, a novel member of the serine/threonine protein kinase gene family which is transcriptionally induced by glucocorticoids and serum. *Mol Cell Biol* 13:2031–2040, 1993
27. Waldegger S, Barth P, Raber G, Lang F: Cloning and characterization of a putative human serine/threonine protein kinase transcriptionally modified during anisotonic and isotonic alterations of cell volume. *Proc Natl Acad Sci U S A* 94:4440–4445, 1997
28. Bhargava A, Fullerton MJ, Myles K, Purdy TM, Funder JW, Pearce D, Cole TJ: The serum- and glucocorticoid-induced kinase is a physiological mediator of aldosterone action. *Endocrinology* 142:1587–1594, 2001
29. Brennan FE, Fuller PJ: Rapid upregulation of serum and glucocorticoid-regulated kinase (sgk) gene expression by corticosteroids in vivo. *Mol Cell Endocrinol* 166:129–136, 2000
30. Chen SY, Bhargava A, Mastroberardino L, Meijer OC, Wang J, Buse P, Firestone GL, Verrey F, Pearce D: Epithelial sodium channel regulated by aldosterone-induced protein sgk. *Proc Natl Acad Sci U S A* 96:2514–2519, 1999
31. Djelidi S, Beggah A, Courtois-Coutry N, Fay M, Cluzeaud F, Viengchareun S, Bonvalet JP, Farman N, Blot-Chabaud M: Basolateral translocation by vasopressin of the aldosterone-induced pool of latent Na-K-ATPases is accompanied by alpha1 subunit dephosphorylation: study in a new aldosterone-sensitive rat cortical collecting duct cell line. *J Am Soc Nephrol* 12:1805–1818, 2001
32. Loffing J, Zecevic M, Feraille E, Kaissling B, Asher C, Rossier BC, Firestone GL, Pearce D, Verrey F: Aldosterone induces rapid apical translocation of ENaC in early portion of renal collecting system: possible role of SGK. *Am J Physiol Renal Physiol* 280:F675–F682, 2001
33. Naray-Fejes-Toth A, Canessa C, Cleaveland ES, Aldrich G, Fejes-Toth G: sgk is an aldosterone-induced kinase in the renal collecting duct: effects on epithelial na+ channels. *J Biol Chem* 274:16973–16978, 1999
34. Pearce D: SGK1 regulation of epithelial sodium transport. *Cell Physiol Biochem* 13:13–20, 2003
35. Shigaev A, Asher C, Latter H, Garty H, Reuveny E: Regulation of sgk by aldosterone and its effects on the epithelial Na(+) channel. *Am J Physiol Renal Physiol* 278:F613–F619, 2000
36. Verrey F, Loffing J, Zecevic M, Heitzmann D, Staub O: SGK1: aldosterone-induced relay of Na^+ transport regulation in distal kidney nephron cells. *Cell Physiol Biochem* 13:21–28, 2003
37. Böhmer C, Wagner CA, Beck S, Moschen I, Melzig J, Werner A, Lin JT, Lang F, Wehner F: The shrinkage-activated Na(+) conductance of rat hepatocytes and its possible correlation to rENaC. *Cell Physiol Biochem* 10:187–194, 2000
38. Lang F, Klingel K, Wagner CA, Stegen C, Warntges S, Friedrich B, Lanzendorfer M, Melzig J, Moschen I, Steuer S, Waldegger S, Sauter M, Paulmichl M, Gerke V, Risler T, Gamba G, Capasso G, Kandolf R, Hebert SC, Massry SG, Broer S: Deranged transcriptional regulation of cell-volume-sensitive kinase hSGK in diabetic nephropathy. *Proc Natl Acad Sci U S A* 97:8157–8162, 2000
39. Blazer-Yost BL, Liu X, Helman SI: Hormonal regulation of ENaCs: insulin and aldosterone. *Am J Physiol* 274:C1373–C1379, 1998
40. Blazer-Yost BL, Paunescu TG, Helman SI, Lee KD, Vlahos CJ: Phosphoino-

sitide 3-kinase is required for aldosterone-regulated sodium reabsorption. *Am J Physiol* 277:C531–C536, 1999
41. Blazer-Yost BL, Vahle JC, Byars JM, Bacallao RL: Real-time three-dimensional imaging of lipid signal transduction: apical membrane insertion of epithelial Na(+) channels. *Am J Physiol Cell Physiol* 287:C1569–C1576, 2004
42. Wang J, Barbry P, Maiyar AC, Rozansky DJ, Bhargava A, Leong M, Firestone GL, Pearce D: SGK integrates insulin and mineralocorticoid regulation of epithelial sodium transport. *Am J Physiol Renal Physiol* 280:F303–F313, 2001
43. Wulff P, Vallon V, Huang DY, Volkl H, Yu F, Richter K, Jansen M, Schlunz M, Klingel K, Loffing J, Kauselmann G, Bosl MR, Lang F, Kuhl D: Impaired renal Na(+) retention in the sgk1-knockout mouse. *J Clin Invest* 110:1263–1268, 2002
44. Huang DY, Wulff P, Volkl H, Loffing J, Richter K, Kuhl D, Lang F, Vallon V: Impaired regulation of renal K+ elimination in the sgk1-knockout mouse. *J Am Soc Nephrol* 15:885–891, 2004
45. Vallon V: In vivo studies of the genetically modified mouse kidney. *Nephron Physiol* 94:1–5, 2003
46. Menniti M, Iuliano R, Amato R, Boito R, Corea M, Le Pera I, Gulletta E, Fuiano G, Perrotti N: Serum and glucocorticoid-regulated kinase Sgk1 inhibits insulin-dependent activation of phosphomannomutase 2 in transfected COS-7 cells. *Am J Physiol Cell Physiol* 288:C148–C155, 2005
47. Cidad P, Almeida A, Bolanos JP: Inhibition of mitochondrial respiration by nitric oxide rapidly stimulates cytoprotective GLUT3-mediated glucose uptake through 5′-AMP-activated protein kinase. *Biochem J* 384:629–636, 2004
48. Ogihara T, Asano T, Fujita T: Contribution of salt intake to insulin resistance associated with hypertension. *Life Sci* 73:509–523, 2003
49. Berger S, Bleich M, Schmid W, Cole TJ, Peters J, Watanabe H, Kriz W, Warth R, Greger R, Schutz G: Mineralocorticoid receptor knockout mice: pathophysiology of Na+ metabolism. *Proc Natl Acad Sci U S A* 95:9424–9429, 1998
50. Hummler E, Barker P, Gatzy J, Beermann F, Verdumo C, Schmidt A, Boucher R, Rossier BC: Early death due to defective neonatal lung liquid clearance in alpha-ENaC-deficient mice. *Nat Genet* 12:325–328, 1996
51. Rathmell JC, Fox CJ, Plas DR, Hammerman PS, Cinalli RM, Thompson CB: Akt-directed glucose metabolism can prevent Bax conformation change and promote growth factor-independent survival. *Mol Cell Biol* 23:7315–7328, 2003
52. Taha C, Liu Z, Jin J, Al Hasani H, Sonenberg N, Klip A: Opposite translational control of GLUT1 and GLUT4 glucose transporter mRNAs in response to insulin: role of mammalian target of rapamycin, protein kinase b, and phosphatidylinositol 3-kinase in GLUT1 mRNA translation. *J Biol Chem* 274:33085–33091, 1999
53. Bae SS, Cho H, Mu J, Birnbaum MJ: Isoform-specific regulation of insulin-dependent glucose uptake by Akt/protein kinase B. *J Biol Chem* 278:49530–49536, 2003
54. Jiang ZY, Zhou QL, Coleman KA, Chouinard M, Boese Q, Czech MP: Insulin signaling through Akt/protein kinase B analyzed by small interfering RNA-mediated gene silencing. *Proc Natl Acad Sci U S A* 100:7569–7574, 2003
55. Kohn AD, Summers SA, Birnbaum MJ, Roth RA: Expression of a constitutively active Akt Ser/Thr kinase in 3T3–L1 adipocytes stimulates glucose uptake and glucose transporter 4 translocation. *J Biol Chem* 271:31372–31378, 1996
56. McCurdy CE, Cartee GD: Akt2 is essential for the full effect of calorie restriction on insulin-stimulated glucose uptake in skeletal muscle. *Diabetes* 54:1349–1356, 2005
57. Nishida Y, Nagata T, Takahashi Y, Sugahara-Kobayashi M, Murata A, Asai S: Alteration of serum/glucocorticoid regulated kinase-1 (sgk-1) gene expression in rat hippocampus after transient global ischemia. *Brain Res Mol Brain Res* 123:121–125, 2004
58. Lee EH, Hsu WL, Ma YL, Lee PJ, Chao CC: Enrichment enhances the expression of sgk, a glucocorticoid-induced gene, and facilitates spatial learning through glutamate AMPA receptor mediation. *Eur J Neurosci* 18:2842–2852, 2003
59. Farjah M, Roxas BP, Geenen DL, Danziger RS: Dietary salt regulates renal SGK1 abundance: relevance to salt sensitivity in the Dahl rat. *Hypertension* 41:874–878, 2003
60. Palmada M, Boehmer C, Akel A, Rajamanickam J, Jeyaraj S, Keller K, Lang F: SGK1 kinase upregulates GLUT1 activity and plasma membrane expression. *Diabetes* 55:421–427, 2006
61. Busjahn A, Aydin A, Uhlmann R, Krasko C, Bahring S, Szelestei T, Feng Y, Dahm S, Sharma AM, Luft FC, Lang F: Serum- and glucocorticoid-regulated kinase (SGK1) gene and blood pressure. *Hypertension* 40:256–260, 2002
62. Roth JL, Mobarhan S, Clohisy M: The metabolic syndrome: where are we and where do we go? *Nutr Rev* 60:335–337, 2002
63. Bahr V, Pfeiffer AF, Diederich S: The metabolic syndrome X and peripheral cortisol synthesis. *Exp Clin Endocrinol Diabetes* 110:313–318, 2002
64. Ullrich S, Berchtold S, Ranta F, Seebohm G, Henke G, Lupescu A, Mack AF, Chao CM, Su J, Nitschke R, Alexander D, Friedrich B, Wulff P, Kuhl D, Lang F: Serum- and glucocorticoid-inducible kinase 1 (SGK1) mediates glucocorticoid-induced inhibition of insulin secretion. *Diabetes* 54:1090–1099, 2005