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# Blunted hypertensive effect of combined fructose and high-salt diet in gene-targeted mice lacking functional serum- and glucocorticoid-inducible kinase SGK1

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**Huang, Dan Yang, Krishna M. Boini, Björn Friedrich, Marco Metzger, Lothar Just, Hartmut Osswald, Peer Wulff, Dietmar Kuhl, Volker Vallon, and Florian Lang.** Blunted hypertensive effect of combined fructose and high-salt diet in gene-targeted mice lacking functional serum- and glucocorticoid-inducible kinase SGK1. *Am J Physiol Regul Integr Comp Physiol* 290: R935–R944, 2006. First published November 10, 2005; doi:10.1152/ajpregu.00382.2005.—Serum- and glucocorticoid-inducible kinase (SGK1) is transcriptionally upregulated by mineralocorticoids and activated by insulin. The kinase stimulates the renal epithelial $Na^+$ channel and may thus participate in blood pressure regulation. Hyperinsulinemia is triggered by dietary fructose, which sensitizes blood pressure for salt intake. The role of SGK1 in hypertensive effects of combined fructose and high-salt intake was thus explored in SGK1 knockout mice ($sgk1^{-/-}$) and their wild-type littermates ($sgk1^{+/+}$). Renal SGK1 transcript levels of $sgk1^{+/+}$ mice were significantly elevated after fructose diet. Under control diet, fluid intake, urinary flow rate, urinary $Na^+$, $K^+$, and $Cl^-$ excretion, and blood pressure were similar in $sgk1^{-/-}$ and $sgk1^{+/+}$ mice. Addition of 10% fructose to drinking water increased fluid intake and urinary flow rate in both genotypes, and did not significantly alter urinary $Na^+$, $K^+$, and $Cl^-$ output in either genotype. Additional high NaCl diet (4% NaCl) did not significantly alter fluid intake and urine volume but markedly increased urinary output of $Na^+$ and $Cl^-$, approaching values significantly ($P < 0.05$) larger in $sgk1^{-/-}$ than in $sgk1^{+/+}$ mice ($Na^+$: 2,572 ± 462 vs. 1,428 ± 236; $Cl^-$: 2,364 ± 388 vs. 1,379 ± 225 μmol/24 h). Blood pressure was similar in $sgk1^{+/+}$ and $sgk1^{-/-}$ mice at control diet or fructose alone but increased only in $sgk1^{+/+}$ mice (115 ± 1 vs. 103 ± 0.7 mmHg, $P < 0.05$) after combined fructose and high-salt intake. Acute intravenous insulin infusion (during glucose clamp) caused antinatriuresis in $sgk1^{+/+}$ mice, an effect significantly blunted in $sgk1^{-/-}$ mice. The observations reveal a pivotal role of SGK1 in insulin-mediated sodium retention and the salt-sensitizing hypertensive effect of high fructose intake.

insulin; blood pressure; salt sensitivity; epithelial sodium channel; kidney

DIETARY FRUCTOSE LEADS TO hyperglycemia, hyperinsulinemia, and an increase in blood pressure (19, 35, 50, 60, 62, 79). Because insulin was observed to be antinatriuretic in rats, dogs, and humans, a role of insulin in the development of salt-sensitive hypertension has been suggested (22, 32, 49). The antinatriuretic effect of insulin appears to result from direct action on tubular transport in distinct nephron segments (18, 26, 33, 63). Insulin stimulates ENaC and may thus lead to renal retention of NaCl (6, 63, 78), which in turn favors the development of hypertension (44, 46). The effect of insulin on epithelial $Na^+$ channel (ENaC) requires activation of the Phosphatidylinositide-3 (PI3)-kinase (9, 12, 64).

The regulation of ENaC activity by insulin may involve the serum- and glucocorticoid-inducible kinase SGK1 (31). Expression of SGK1 has previously been shown to be stimulated by mineralocorticoids (5, 14, 20, 25, 45, 51, 56, 59, 71). SGK1 is activated by insulin (40) through a signaling cascade involving PI3-kinase and phosphoinositide-dependent kinase 1 (PDK1) (7, 40, 55). Targets of SGK1 action include ENaC (1, 2, 13, 20, 21, 23, 29, 43, 51, 61, 72, 73). SGK1 is expressed in the aldosterone-sensitive distal nephron (1, 45) and presumably contributes to the effect of aldosterone, insulin-like growth factor-I, and insulin on ENaC activity and thus renal salt reabsorption (10–12, 73). Beyond that, SGK1 stimulates further renal transport molecules, including the $K^+$ channels ROMK (54, 76) and KCNE1/KCNQ1 (17, 27), the $Na^+$-$K^+$-ATPase (34, 58, 77), the $Na^+$-$K^+$-$2Cl^-$ cotransporter NKCC2 (43), the epithelial $Ca^{2+}$ channel TRPV5 (28, 53), the $Na^+/H^+$ exchanger NHE3 (75, 76), and the $Na^+$-coupled glucose transporter SGLT1 (24).

Urinary $Na^+$ excretion is normal in SGK1 knockout mice under normal salt intake but cannot be adequately decreased after exposure to salt-deficient diet (74). Thus dietary salt deficiency leads to a marked decrease of glomerular filtration rate (GFR) and blood pressure in those mice (74). Conversely, enhanced SGK1 activity may be expected to increase blood pressure by stimulation of both renal $Na^+$ reabsorption (74) and salt appetite (67). As a matter of fact, a certain variant of the SGK1 gene has been associated with increased blood pressure (15, 16).

Chronic fructose feeding causes arterial hypertension, which is associated with insulin resistance and hyperinsulinemia in rats (18, 38, 70). The present study aimed to explore the role of SGK1 in the hypertensive effect of combined treatment with

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fructose and salt load. Thus renal electrolyte excretion and blood pressure were determined in SGK1 knockout mice ($sgk1^{-/-}$) and their wild-type littermates ($sgk1^{+/+}$) before and during oral fructose administration with and without additional high-salt diet.

## METHODS

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

Mice deficient in SGK1 ($sgk1^{-/-}$) on 129Sv background were generated and bred as previously described (36, 74). The C57BL/6J strain is commonly chosen to induce diabetes in mice and exhibits defects in glucose tolerance independent of obesity (39, 41). Thus gene-targeted mice with mixed Sv129 and C57BL/6J background were backcrossed for up to three generations in C57BL/6J mouse strains (Charles River, Sulzfeld, Germany), and heterozygous mice were intercrossed to produce homozygous mice. Male and female SGK1 knockout ($sgk1^{-/-}$) mice and their wild-type littermates ($sgk1^{+/+}$) were fed a control diet (1314; 0.2% $Na^+$, 0.4% $Cl^-$, and 0.7% $K^+$; Altromin, Heidenau, Germany) and had free access to tap drinking water. After a control period, tap drinking water was replaced by 10% fructose for 3 wk. Consequently, the control diet was replaced by a high-salt diet (1324; 4% NaCl; Altromin), and 10% fructose was maintained as the drinking solution for a further 17 days. In another series of experiments, $sgk1^{+/+}$ and $sgk1^{-/-}$ mice were kept on a high-salt diet for 18 days after a control period. For evaluation of renal excretion, both, $sgk1^{-/-}$ and $sgk1^{+/+}$ mice were placed individually in metabolic cages (Techniplast Hohenpeissenberg, Germany) for 24-h urine collection (65) during *1*) control diet plus tap water (basal); *2*) 18 days on high-salt diet plus tap water (high-salt diet); *3*) 3 wk on control diet plus 10% fructose drinking fluid (fructose); or *4*) 17 days on a high-salt diet plus 10% fructose drinking fluid (fructose + high-salt diet). The inner wall of the metabolic cages was siliconized, and urine was collected under water-saturated oil. To obtain blood specimens, animals were lightly anesthetized with isoflurane (Abbott, Wiesbaden, Germany), and ~200 μl of blood were withdrawn in heparinized capillaries by puncturing the retroorbital plexus. For determination of fasted blood glucose levels, food was withheld overnight from 7:00 PM until 9:00 AM the next morning when blood samples were taken. For measurement of nonfasted plasma insulin concentrations, blood was drawn at 9:00 AM in the morning and centrifuged immediately after the collection.

To further investigate the role of SGK1 in the antinatriuretic action of insulin, male $sgk1^{+/+}$ and $sgk1^{-/-}$ mice were subjected to renal clearance experiments, as described previously (36, 67). Briefly, mice were anesthetized using 100 mg/kg ip Inactin (Sigma-Aldrich Chemie, Steinheim, Germany) and 100 mg/kg im ketamine (CuraMED Pharma, Karlsruhe, Germany). Animals were then placed on a temperature-controlled operating table to keep rectal temperature at 37°C. Tracheostomy was performed, and the right jugular vein was cannulated for continuous infusion. Arterial blood pressure was recorded, and blood samples were taken via a catheter inserted in the left femoral artery. A catheter was placed in the urinary bladder for timed urine collection. After the surgery, all mice received a bolus of 0.85% NaCl in an amount equal to 5% body weight. Continuous infusion was maintained at a rate of 450 $\mu l \cdot h^{-1} \cdot 30$ g body $wt^{-1}$, and [$^3$H]inulin was added in the infusion for evaluation of whole kidney GFR. After stabilization of the animals for 30 min, 30-min timed urine collections were performed for determination of basal urinary flow rate and [$^3$H]inulin and sodium concentrations. Blood was obtained in the middle of the basal period for measurement of blood glucose and [$^3$H]inulin and sodium concentrations. After the basal period, all mice received a loading dose of 20 mU/kg body wt insulin (Novo Nordisk Pharma, Mainz, Germany) intravenously followed by a continuous infusion of 2 $mU \cdot kg^{-1} \cdot min^{-1}$ for 60 min. This insulin regimen has previously been shown to produce antinatriuresis in rats (32). At the onset of the insulin infusion, a 25% glucose infusion was started at the rate of 3 μl/min. Blood glucose was determined at 10-min intervals. The rate of glucose infusion was adjusted to maintain the blood glucose concentrations at basal levels. Under those conditions, urine and blood were collected as described above. Plasma and urinary concentrations of $Na^+$ and $K^+$ were measured by flame photometry (ELEX 6361). $Cl^-$ concentrations were analyzed by electrometric titration (Chloridometer 6610). Creatinine concentrations were assessed by a commercial enzymatic kit (Labor+Technik, Berlin, Germany). Concentrations of [$^3$H]inulin in plasma and urine were measured by liquid phase scintillation counting. Plasma concentrations of insulin were determined using an enzyme immunoassay kit (Mercodia, Uppsala, Sweden). Plasma aldosterone concentrations were analyzed using a commercial RIA kit (Beckman Coulter, Krefeld, Germany).

Systolic arterial blood pressure was determined by application of the tail-cuff method. As reviewed recently (47), the tail cuff approach to determine arterial blood pressure requires certain precautions to reduce the stress of the animals, including appropriate training of the mice over multiple days, prewarming to an ambient temperature of 29°C, measurement in a quiet and semidarkened and clean environment, and performance of the measurements by one person and during a defined day time, when blood pressure is stable (between 1:00 and 3:00 PM). All these precautions were taken in the present study. Tail-cuff measurements were performed according to the user's manual for the blood pressure analyzer (IITC model 179; Hugo Sachs Elektronik). Blood pressure recording was considered to be successful if the mouse did not move and a clear initial pulse could be seen (Fig. 1). Five tail-cuff measurement were made in a session. The blood pressure for the session was accepted when the deviations of the five blood pressure readings were <5 mmHg (Fig. 1).

In another series, $sgk1^{+/+}$ wild-type mice were divided into four groups (5 mice/group) and treated separately with *1*) control diet; *2*) 10% fructose for 3 wk; *3*) 4% NaCl diet for 2 wk; and *4*) 10% fructose for 3 wk plus 4% NaCl diet for 2 wk. Mice were anesthetized with ketamine plus xylazine. Kidneys were immediately taken out and shock-frozen in liquid nitrogen. Tissue RNA was isolated using the Qiagen Mini kit and 2 μg of RNA transcribed into cDNA using RT Moloney murine leukemia virus (Roche Diagnostics, Roche Applied Science, Mannheim, Germany). An aliquot of cDNA, corresponding to the amount of RNA as indicated in each experiment, was used for quantification of mRNA. Primers used for SGK1 mRNA quantification were sense primer 5′-TGTCTTGGGGCTGTCCTGTATG-3′ and antisense primer: 5′-GCTTCTGCT GCTTCCTTCACAC-3′. mRNA was quantified using a light cycler system (Roche Diagnostics, Roche Applied Science). PCR reactions were performed in a final volume of 20 μl containing 2 μl cDNA, 3 mmol/l $MgCl_2$, 0.5 μmol/l of each primer, and 2 μl cDNA Master Sybr Green I mix (Roche Molecular Biochemicals, Mannheim, Germany). The transcript levels of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase of each sample were taken as reference using a commercial primer kit (Search LC, Heidelberg, Germany). Amplification of the target DNA was performed during 35 cycles, each 10 s at 95°C, 10 s at 68°C, and 16 s at 72°C. Melting curve and agarose gel analyses confirmed the specificity of amplified products. Results were calculated as a ratio of the target vs. housekeeping gene transcripts.

For in situ hybridization of SGK1 mRNA, control and fructose-treated (10% fructose for 3 wk) mice were killed by carbon dioxide incubation. Kidneys were removed, immediately frozen in −25°C cold isopentane, and sliced on a freezing microtome at 12 μm thickness. Sections were subsequently mounted on silane-coated slides (2% 3-aminopropyltriethoxy-silane; Sigma, Taufkirchen, Germany) in acetone, dried at 60°C for 30 s, and fixed with 4% phosphate-buffered paraformaldehyde for 20 min. After three washes with PBS (0.1 M, pH 7.4), slides were incubated with 100 mM Tris

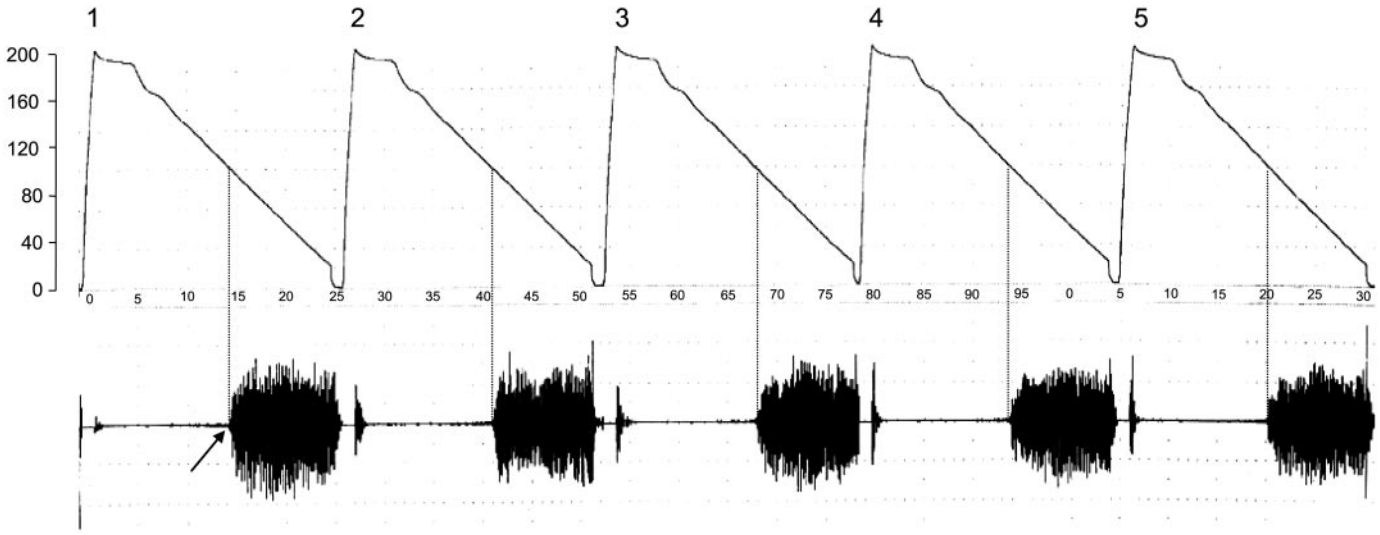


Fig. 1. Representative original blood pressure tracing measured by the tail-cuff method. Precautions for tail-cuff pressure measurement were taken in the present study (see METHODS). Blood pressure recording was considered to be successful if the mouse did not move and a clear initial pulse (arrow) could be seen. Five tail-cuff recordings were obtained in each session. The blood pressure for the session was accepted when the deviations of the 5 blood pressure readings were <5 mmHg.

and 50 mM EDTA (pH 8) containing 2 μg/ml proteinase K for 10 min at room temperature and rinsed again three times with PBS. To reduce nonspecific background, slides were acetylated with 0.1 M triethanolamine (pH 8.0) containing 0.25% (vol/vol) acetic anhydride (Sigma) two times for 5 min. After prehybridization with hybridization buffer [50% formamide (Sigma), 10% dextran sulfate, 5 mM EDTA, 20 mM Tris, pH 8, 10 mM dithiothreitol, 1× Denhardt's solution, 0.05% tRNA, and 300 mM NaCl] for 1 h at 62°C, sections were incubated with fresh hybridization buffer containing the denatured digitonin (DIG)-labeled sense or antisense probe (200 ng/ml) overnight at 63°C. After hybridization, slides were briefly rinsed in 2× saline-sodium citrate (SSC) at room temperature and three times in 0.1× SSC for 15 min at 63°C. Detection of DIG-labeled RNA probe was performed according to the protocol of the DIG nucleic acid detection kit (Roche). The tissues were blocked for 30 min with blocking buffer (1% blocking reagent; Roche) in maleic acid buffer (0.1 M maleic acid, 0.15 M NaCl, pH 7.5) and then incubated with alkaline phosphatase-conjugated antibody solution (anti-DIG antibody, 1:2,500; Roche) in blocking buffer containing 0.1% Triton X-100 for 1 h. After four washes with maleic acid buffer for 15 min, slides were equilibrated for 5 min in Tris buffer, pH 9.5 (0.1 M Tris, pH 9.5, 0.1 M NaCl, and 50 mM $MgCl_2$). The color development was carried out with freshly prepared substrate solution [nitroblue tetrazolium salt and 5-bromo-4-chloro-3-indolyl phosphate (Roche) in Tris buffer, pH 9.5]. After three washes with PBS, slides were rinsed in distilled water, dried, and coverslipped with Kaiser's solution (Merck, Darmstadt, Germany).

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

## RESULTS

Under control diet, blood glucose levels and plasma insulin concentrations were similar in *sgk1*$^{-/-}$ and *sgk1*$^{+/+}$ mice. As reported previously (74), plasma aldosterone concentrations were significantly higher in *sgk1*$^{-/-}$ than in *sgk1*$^{+/+}$ mice (Fig. 2). As shown in Fig. 2, high-salt diet alone did not significantly alter blood glucose levels or plasma insulin concentrations, whereas plasma aldosterone concentrations were suppressed by a high-salt diet in both genotypes. As were expected from previous studies, replacement of plain tap water with 10% fructose solution as drinking fluid led to a marked increase of blood glucose levels and plasma insulin concentrations in both genotypes (Fig. 2). In contrast, plasma aldosterone concentrations were not consistently altered by an oral fructose load. The additional high-salt diet did not significantly affect blood glucose levels or plasma insulin concentrations. In both *sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ mice, plasma aldosterone concentrations were reduced significantly by an additional high-salt diet (Fig. 2).

Packed cell volume and plasma $Na^+$ concentrations were similar in *sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ mice fed a standard diet, whereas plasma $K^+$ concentrations in *sgk1*$^{-/-}$ mice were significantly higher than in *sgk1*$^{+/+}$ mice (Fig. 3). After a high-salt diet, packed cell volume was significantly smaller in *sgk1*$^{-/-}$ than in *sgk1*$^{+/+}$ mice. High-salt diet significantly increased plasma $K^+$ and $Cl^-$ concentrations in both genotypes. Fructose load alone did not significantly alter packed cell volume or plasma $Na^+$, $K^+$, and $Cl^-$ concentrations (Fig. 3). Under combined fructose and high-salt diet, plasma $K^+$ and $Cl^-$ concentrations were modestly elevated in both genotypes. Plasma $Na^+$ concentrations were slightly but significantly lower in *sgk1*$^{-/-}$ than in *sgk1*$^{+/+}$ mice treated with combined fructose and high-salt diet (Fig. 3).

Under control diet, fluid intake, urinary flow rate, creatinine clearance, and urinary $Na^+$, $K^+$, and $Cl^-$ excretions were similar in *sgk1*$^{-/-}$ and *sgk1*$^{+/+}$ mice (Fig. 4, *A* and *B*). In both *sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ mice, high-salt diet alone increased fluid intake, urinary flow rate, and urinary $Na^+$, $K^+$, and $Cl^-$ excretions (Fig. 4, *A* and *B*). The addition of 10% fructose to the drinking water (for 3 wk) led to a similar increase of fluid intake and urinary flow rate in both genotypes and did not significantly alter creatinine clearance or urinary $Na^+$, $K^+$, and $Cl^-$ output in either genotype (Fig. 4, *A* and *B*). The further addition of dietary NaCl (4%) did not significantly alter fluid

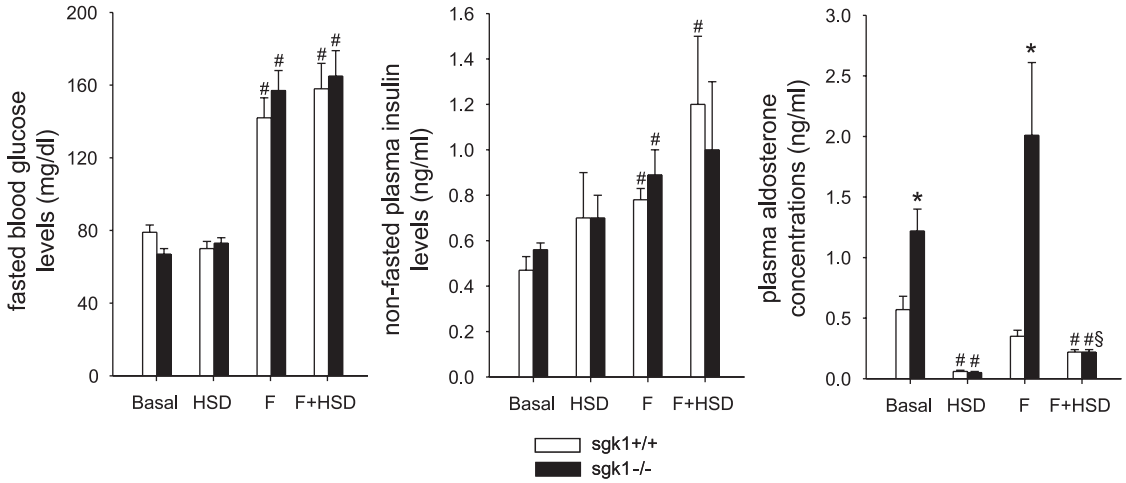


Fig. 2. Plasma glucose, insulin, and aldosterone concentrations in serum- and glucocorticoid-inducible kinase (SGK1) wild-type mice ($sgk1^{+/+}$) and knockout mice ($sgk1^{-/-}$) on standard diet (basal), high-salt diet (HSD), fructose load (F), and fructose load + high-salt diet (F+HSD). Data are arithmetic means ± SE ($n = 7$ mice in each group) in $sgk1^{-/-}$ (filled bars) and $sgk1^{+/+}$ (open bars). $P < 0.05$ vs. respective value under standard diet (#), vs. respective value under fructose load (§), and vs. $sgk1^{+/+}$ (*).

intake and urine volume but markedly increased urinary output of $Na^+$ and $Cl^-$ in $sgk1^{+/+}$ mice, an effect that was enhanced in $sgk1^{-/-}$ mice. Thus urinary $Na^+$, $Cl^-$, and $K^+$ excretions were higher in $sgk1^{-/-}$ than in $sgk1^{+/+}$ mice ($Na^+$: 2,572 ± 462 vs. 1,428 ± 236; $Cl^-$: 2,364 ± 388 vs. 1,379 ± 225; $K^+$: 679 ± 115 vs. 370 ± 60 μmol/24 h, $P < 0.05$ for all) after treatment with combined fructose and high-salt treatment. Experiments in additional mice treated with combined fructose and high-salt diet revealed that food intake and thus NaCl and $K^+$ intake were higher in $sgk1^{-/-}$ than in $sgk1^{+/+}$ mice (3.3 ± 0.4 vs. 2.2 ± 0.2 g/24 h, $n = 6$/group, $P < 0.05$). Additional high-salt diet caused a similar decrease of creatinine clearance in $sgk1^{+/+}$ and $sgk1^{-/-}$ mice on high fructose (Fig. 4*A*).

Blood pressure was similar in $sgk1^{+/+}$ and $sgk1^{-/-}$ mice during the control diet (98.4 ± 1.9 vs. 98.2 ± 1.1 mmHg) or after administration of fructose alone for 3 wk (99.7 ± 0.7 vs. 99.6 ± 0.7 mmHg), but significantly increased only in $sgk1^{+/+}$ after combined treatment with fructose and high-salt intake (115 ± 1 vs. 103 ± 0.7 mmHg, $P < 0.05$; Fig. 5*A* and Table 1). In contrast, without previous fructose load, high-salt diet

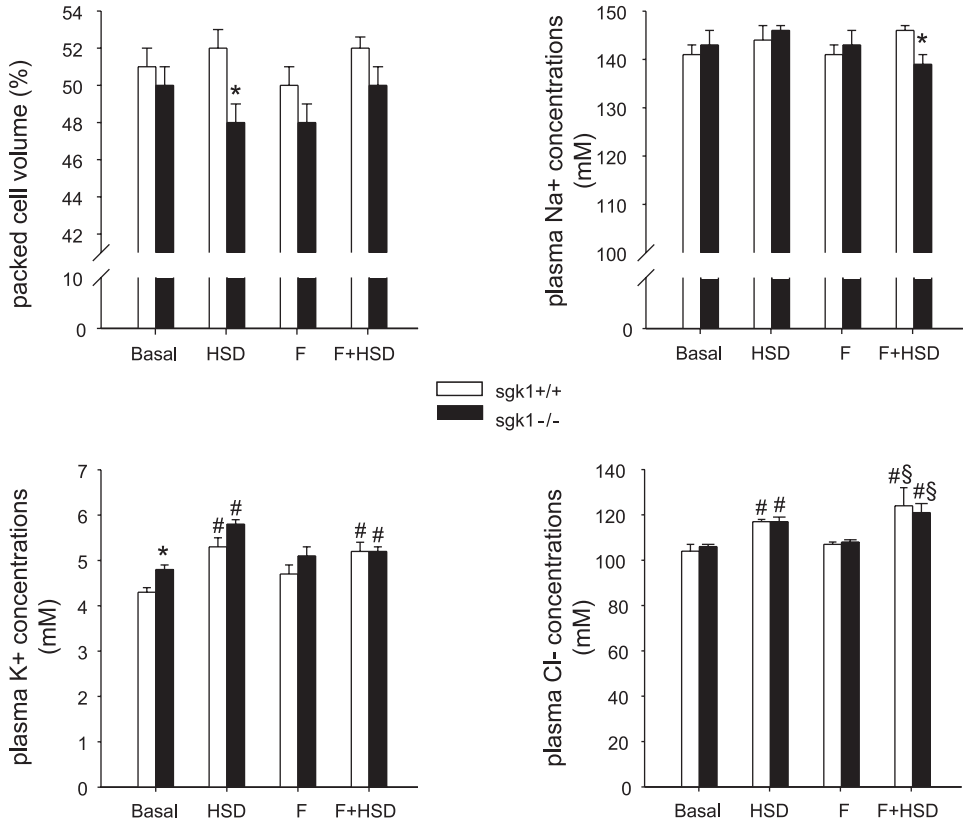


Fig. 3. Packed cell volume and plasma $Na^+$, $K^+$, and $Cl^-$ concentrations in $sgk1^{+/+}$ and $sgk1^{-/-}$ mice on standard diet, high-salt diet, fructose load, and fructose load + high-salt diet. Data are arithmetic means ± SE ($n = 7$ each group) in $sgk1^{-/-}$ mice (filled bars) and $sgk1^{+/+}$ mice (open bars). $P < 0.05$ vs. respective value under standard diet (#), vs. respective value under fructose load (§), and vs. $sgk1^{+/+}$ (*).

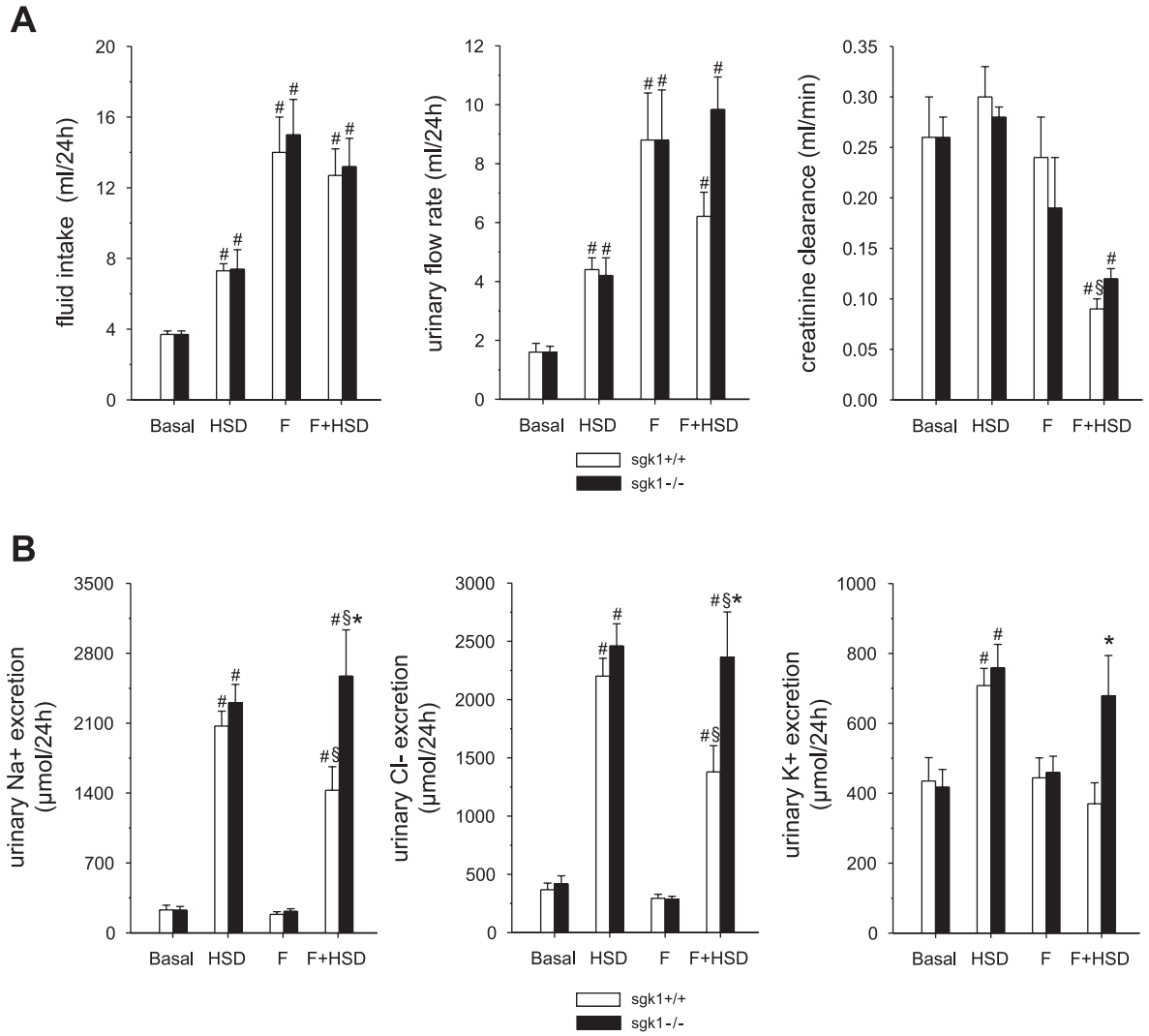


Fig. 4. Fluid intake, urinary flow rate, and creatinine clearance (*A*) and urinary excretion of $Na^+$, $K^+$, and $Cl^-$ (*B*) in $sgk1^{+/+}$ and $sgk1^{-/-}$ mice on standard diet, high-salt diet, fructose load, and fructose load + high-salt diet. Data are arithmetic means ± SE ($n = 7$ each group) in $sgk1^{-/-}$ (filled bars) and $sgk1^{+/+}$ (open bars). $P < 0.05$ vs. respective value under standard diet (#), vs. respective value under fructose load (§), and vs. $sgk1^{+/+}$ (*).

alone did not significantly affect systolic blood pressure in either genotype (Fig. 5*B*).

To further investigate the role of SGK1 in the antinatriuretic action of insulin, renal effects of intravenous insulin were determined in clearance experiments under anesthesia. As shown in Table 2, baseline blood pressure, GFR, blood glucose levels, and fractional urinary $Na^+$ excretion were similar in $sgk1^{+/+}$ and $sgk1^{-/-}$ mice. Acute insulin infusion reduced fractional urinary $Na^+$ excretion in $sgk1^{+/+}$ mice, an effect significantly blunted in $sgk1^{-/-}$ mice (Table 2). Thus fractional urinary $Na^+$ excretion was significantly higher in $sgk1^{-/-}$ than in $sgk1^{+/+}$ mice during the insulin administration. Acute intravenous application of insulin did not significantly affect blood pressure or GFR in either genotype (Table 2).

As shown in Fig. 6, fructose diet significantly increased the SGK1 transcript levels in kidneys from $sgk1^{+/+}$ mice. Additional treatment with high-salt diet led to a decrease of SGK1 transcript levels, which, however, tended to remain higher than under control conditions, despite a marked decrease of plasma aldosterone concentration (Fig. 2). In situ hybridization of SGK1 mRNA identified the glomeruli, cortical tubular structures, and collecting ducts as the principal sites of SGK1 expression (Fig. 7). Fructose diet again caused a significant increase in the SGK1 expression.

## DISCUSSION

As reported earlier (36), the plasma $K^+$ concentrations and/or plasma aldosterone concentrations were higher in $sgk1^{-/-}$ than in $sgk1^{+/+}$, pointing to a deficiency in renal $K^+$ excretion. As shown earlier (36), renal $K^+$ excretion is indeed impaired in $sgk1^{-/-}$ mice, an effect presumably the result of deranged $K^+$ channel regulation (54, 76) and decreased electrical driving force resulting from decreased ENaC activity (36). Under both basal conditions and high-fructose intake, the administration of 4% NaCl decreased the plasma aldosterone levels in both $sgk1^{-/-}$ and $sgk1^{+/+}$ mice and abolished the difference between the genotypes. The decrease of plasma aldosterone concentrations prevents excessive extracellular

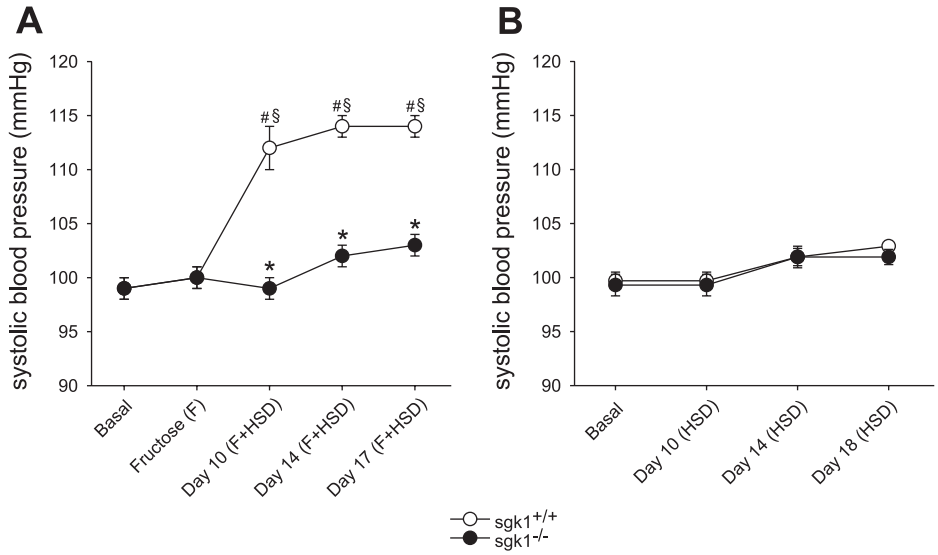


Fig. 5. Change of systolic blood pressure in $sgk1^{+/+}$ and $sgk1^{-/-}$ mice on a standard diet, fructose load for 3 wk (fructose), and 10, 14, and 17 days of fructose load + high-salt diet (*A*) and on a standard diet and after 10, 14, and 18 days of a high-salt diet (*B*). Data are arithmetic means ± SE ($n = 6$ mice in each group) of systolic blood pressure in $sgk1^{-/-}$ mice (●) and $sgk1^{+/+}$ (○). $P < 0.05$ vs. respective value under standard diet (#), vs. respective value under 3 wk of fructose load (§), and vs. $sgk1^{+/+}$ (*).

volume expansion during high-salt intake (5, 14, 20, 25, 45, 51, 56, 59, 71). Notably, despite the marked decrease of plasma aldosterone concentration, renal SGK1 transcript levels tended to remain higher during combined fructose and high-salt intake than during control conditions.

The present observations further confirm the previous observations in $sgk1^{-/-}$ mice on 129Sv background (74) that, under standard diet, urinary salt excretion and blood pressure are similar in $sgk1^{-/-}$ and $sgk1^{+/+}$ mice. However, the elevated aldosterone plasma concentrations in $sgk1^{-/-}$ mice also point to the functional significance of SGK1-dependent regulation of renal salt conservation, even under those dietary conditions. The enhanced plasma aldosterone concentration overrides 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 (74). Accordingly, the phenotype of the SGK1 knockout mouse is by far less severe than that of mice lacking functional mineralocorticoid receptors (4) or mice lacking functional ENaC (37). The mineralocorticoid receptor knockout mouse suffers from severe renal salt wasting (4), and the ENaC knockout mouse is not viable (37). The phenotype of the SGK1 knockout mouse became only apparent after exposure of the mice to a salt-depleted diet. NaCl excretion decreased under NaCl depletion in both $sgk1^{-/-}$ and $sgk1^{+/+}$ mice. However, the renal NaCl loss was significantly larger in

Table 1. *Raw data of systolic blood pressure in the $sgk1^{+/+}$ and $sgk1^{-/-}$ mice fed on standard diet, fructose load for 3 wk, and consequent 10, 14, and 17 days of fructose load + high-salt diet measured by tail-cuff method*

| | $sgk1^{+/+}$ | | | | |
|---|---|---|---|---|---|
| | | | Fructose + 4%NaCl | | |
| | Basal *Days 1–3* | Fructose *Days 1–3* | *Day 10* | *Day 14* | *Day 17* |
| *Mouse 1* | 93.06 | 99 | 109.6 | 112 | 119.6 |
| *Mouse 2* | 101.73 | 98.4 | 107 | 108 | 113 |
| *Mouse 3* | 99.13 | 101.6 | 110.4 | 116 | 116 |
| *Mouse 4* | 103.20 | 96.3 | 108.8 | 111 | 112.8 |
| *Mouse 5* | 92.13 | 102.1 | 113.6 | 117.6 | 115 |
| *Mouse 6* | 101.06 | 100.7 | 118.4 | 117 | 114 |
| Means ± SE | 98.4 ± 1.9 | 99.7 ± 0.9 | 111.3 ± 1.7 | 113.6 ± 1.6 | 115.1 ± 1.0 |
| | $sgk^{-/-}$ | | | | |
| | | | Fructose + 4% NaCl | | |
| | Basal *Days 1–3* | Fructose *Days 1–3* | *Day 10* | *Day 14* | *Day 17* |
| *Mouse 1* | 97.83 | 100.2 | 99 | 107 | 103.2 |
| *Mouse 2* | 98.93 | 97.3 | 99.2 | 101.6 | 102.7 |
| *Mouse 3* | 96.26 | 100.3 | 96.8 | 104.8 | 104 |
| *Mouse 4* | 103.4 | 102 | 96 | 103 | 102.4 |
| *Mouse 5* | 97.06 | 98.1 | 98 | 100 | 104.8 |
| *Mouse 6* | 95.60 | 99.5 | 99 | 97 | 100 |
| Means ± SE | 98.2 ± 1.1 | 99.6 ± 0.7 | 98.0 ± 0.5 | 102.2 ± 1.4 | 102.9 ± 0.7 |

Basal, standard diet; fructose, fructose load for 3 wk; fructose + 4% NaCl, fructose load + high-salt diet; $sgk^{+/+}$, serum- and glucocorticoid-inducible kinase (SKG1) wild type; $sgk^{-/-}$, SGK1 knockout.

Table 2. *MAP, GFR, Fr-$U_{Na}$ V, and blood glucose levels in the $sgk1^{+/+}$ and $sgk1^{-/-}$ mice before and during insulin clamp*

| | MAP, mmHg | | GFR, μl/min | | Fr-$U_{Na}$ V, % | | Blood Glucose (mg/dl) | |
|---|---|---|---|---|---|---|---|---|
| | Basal | Insulin | Basal | Insulin | Basal | Insulin | Basal | Insulin |
| $sgk1^{+/+}$ ($n$ = 6) | 111±3 | 110±2 | 433±36 | 431±68 | 7.1±1.3 | 2.6±0.6* | 121±19 | 107±13 |
| $sgk1^{-/-}$ ($n$ = 6) | 115±5 | 114±4 | 404±88 | 350±32 | 9.6±2.5 | 6.8±1.7† | 103±13 | 104±15 |

Data are arithmetic means ± SE; *n,* no. of mice. MAP, mean arterial pressure; GFR, glomerular filtration rate; Fr-$U_{Na}$ V, fractional urinary sodium excretion. $P < 0.05$ vs. respective values under basal (*) and vs. $sgk1^{+/+}$ (†).

$sgk1^{-/-}$ mice than in $sgk1^{+/+}$ mice, despite an exaggerated increase of plasma aldosterone concentrations, decrease of blood pressure, decrease of GFR, and enhanced proximal tubular $Na^+$ reabsorption in the $sgk1^{-/-}$ mice (74). Thus lack of SGK1 limits the ability to reabsorb $Na^+$. Those observations do not necessarily indicate that lack of SGK1 protects from an increase of blood pressure during salt excess.

In contrast to animals of either genotype under control diet, the blood pressure of $sgk1^{+/+}$ mice loaded with fructose is sensitive to additional salt intake. Dietary fructose correlates with peripheral insulin resistance, abnormal glucose metabolism, and hyperinsulinemia; the latter favors the development of salt-sensitive hypertension by increasing salt and water reabsorption in different nephron segments (18, 26, 33, 63). Indeed, our present observations demonstrate that acute intravenous application of insulin at a superphysiological dose significantly reduced fractional urinary sodium excretion without affecting blood pressure and GFR in $sgk1^{+/+}$ mice. More importantly, the insulin-induced antinatriuresis was blunted in $sgk1^{-/-}$ mice, implying a role of SGK1 in insulin-induced renal sodium retention. Presumably because of its stimulating effect on the renal ENaC (1, 2, 13, 20, 21, 23, 29, 43, 51, 61, 72, 73) and the $Na^+$-$K^+$-ATPase (34, 58, 77), the kinase contributes to the antinatriuretic effect of insulin (6, 63, 78). Thus SGK1 may be the primary kinase mediating the PI3-kinase-dependent effects of insulin on renal tubular $Na^+$ transport (9, 12, 64). The upregulation of SGK1 expression by mineralocorticoids (5, 14, 20, 25, 45, 51, 56, 59, 71) is expected to sensitize the distal nephron for the antinatriuretic effects of insulin. Because expressed SGK1 requires activation through PI3-kinase and PDK1 (7, 40, 55), aldosterone may have smaller effects on renal $Na^+$ transport at low insulin levels.

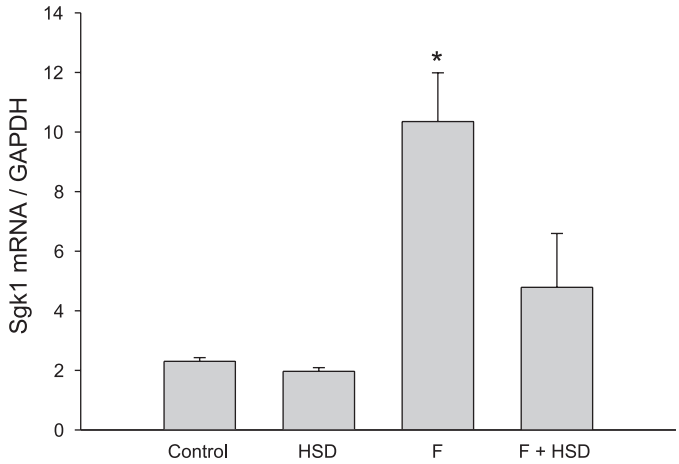


Fig. 6. Effects of control diet, fructose load (F), 4% NaCl diet (HSD), and combined fructose + high-salt diet on renal SGK1 mRNA expression in wild-type mice. Data are arithmetic means ± SE ($n$ = 5 mice in each group). *$P < 0.05$ vs. control group.

Notably, fructose alone is not sufficient to elicit an SGK1-dependent blood pressure increase. It has been shown that an increase of arterial blood pressure induced by fructose feeding depends on dietary salt intake (52). Our data similarly show that high fructose intake alone does not significantly alter blood pressure in mice on a normal salt diet. However, the additional salt load increases blood pressure in $sgk1^{+/+}$ but not in $sgk1^{-/-}$ mice, an observation disclosing the role of SGK1 in hypertension during combined excess of dietary fructose and salt. Notably, the different responses in blood pressure occurred despite a greater food intake and thus salt load in $sgk1^{-/-}$ than in $sgk1^{+/+}$ mice. High fructose diet leads to increased insulin plasma concentrations that in turn are expected to stimulate SGK1 and subsequently ENaC-mediated $Na^+$ retention in distal nephrons (10–12, 29, 73). A high-salt diet unmasks the fructose-induced SGK1-dependent renal $Na^+$ retention, and body salt balance requires an increase of blood pressure. Our data demonstrate that fructose diet significantly increases renal SGK1 transcription levels, and additional high-salt diet failed to suppress SGK1 mRNA expression to control levels. Besides ENaC, activation of SGK1 could increase renal $Na^+$ reabsorption by affecting further transport systems, including NHE3 and KCNQ1/KCNE1 in proximal tubules or BSC-1 in thick ascending limbs. Finally, our study cannot exclude the possibility that SGK1 in addition influences blood pressure through further effects on the cardiovascular system.

The fall in creatinine clearance in response to a high-salt diet in mice treated with fructose diet may reflect changes in GFR. A decrease in GFR in response to a high-salt diet, however, would be counterintuitive with regard to salt balance, implying a "salt paradox" that has been observed previously in rats (68, 69) and patients (48) with type I diabetes mellitus. According to micropuncture experiments, diabetes sensitizes proximal tubular reabsorption to dietary salt, and thus via respective changes in the luminal signal of the tubuloglomerular feedback elicits the paradoxical effect of dietary salt on GFR (66). The mechanisms involved are not understood but are independent of renal nerves (8) and ANG II receptor activation (69). Further studies are required on the effect of salt intake on GFR under conditions of high fructose intake/hyperinsulinemia.

The present observations demonstrate that SGK1 is not only important for the prevention of hypotension during salt depletion but may as well contribute to hypertension during salt excess. Along those lines, enhanced SGK1 expression has been observed in the salt-sensitive Dahl rats (30). In addition,

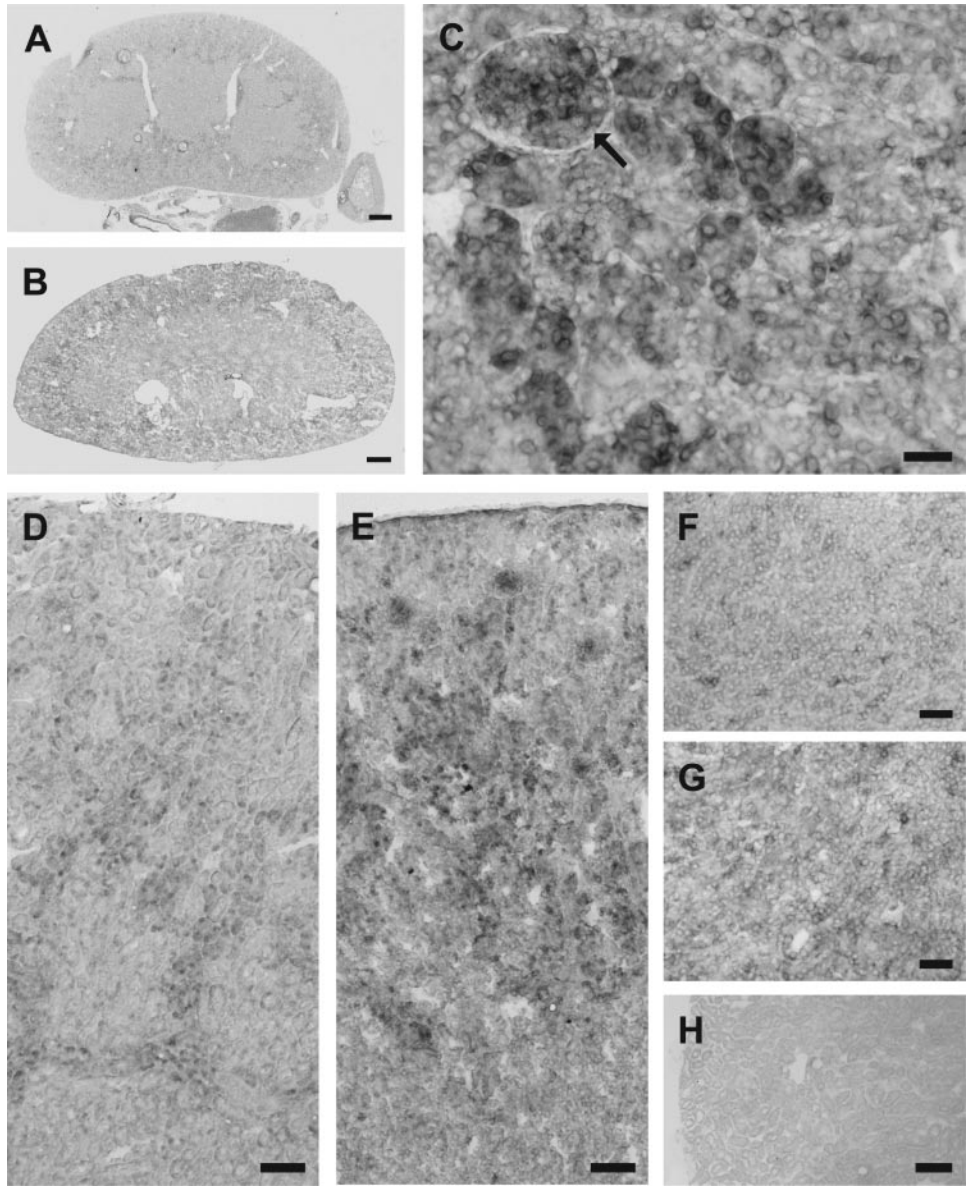


Fig. 7. In situ hybridization of SGK1 mRNA on kidney cryostat sections of SGK1 wild-type mice with and without fructose load. *A* and *B*: overview of SGK1 mRNA expression in the kidneys of untreated (*A*) and fructose-treated (*B*) mice. *D* and *E*: magnification photomicrographs of nephrogenic zones from untreated (*D*) and fructose-treated (*E*) mice. In the renal cortex of fructose-treated mice, SGK1 mRNA expression was enhanced significantly. *C*: higher magnification of the outer cortex of fructose-treated mouse. Glomerula (arrow), tubular structures, and collecting ducts were strongly stained. *F* and *G*: SGK1 expression pattern in the medulla of untreated (*F*) and fructose-treated (*G*) mice. *H*: in situ hybridization on cryostat section of kidney with SGK1 sense probe. Scale bars: *A* and *B* = 600 μm; *C* = 25 μm; *D* and *E* = 100 μm; *F* and *G* = 50 μm; *H* = 100 μm.

moderately enhanced blood pressure is observed in individuals carrying a variant of the SGK1 gene, affecting as many as 5% of unselected Caucasians (15). In the same individuals, increased body mass index (24) and a shortening of the Q-T interval (15, 16) have been observed. The increased body mass index may be partially because of enhanced stimulation of the intestinal glucose transporter SGLT1 (24) and the accelerated cardiac repolarization resulting from enhanced activation of the cardiac $K^+$ channel KCNE1 (17, 27). Thus altered regulation of carriers and channels by SGK1 could account for the coincidence of obesity, hypertension, and altered cardiac action potential (42).

The present observations provide insight into prerequisites for the SGK1-dependent increase of blood pressure and thus may provide a clue to the increased blood pressure in the 5% of the common population carrying the SGK1 gene variant. The observations suggest that SGK1 plays a critical role in the hypertensive effect of hyperinsulinemia. Because a gain of function gene variant of SGK1 could simultaneously increase blood pressure and body mass index, SGK1 may indeed be one of the signaling molecules contributing to the metabolic syndrome or syndrome X, a condition characterized by the coincidence of several disorders, including hypertension, obesity, insulin resistance, and hyperinsulinemia (57). Metabolic syndrome shares several attributes of Cushing's syndrome but does not require increased plasma cortisol levels (3). Instead, the disorder may be caused by inappropriate activity of downstream signaling elements that could well include the serum- and glucocorticoid-inducible kinase SGK1.

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