SGK1 as a determinant of kidney function and salt intake in response to mineralocorticoid excess

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Vallon, Volker, Dan Yang Huang, Florian Grahammer, Amanda W. Wyatt, Hartmut Osswald, Peer Wulf, Dietmar Kuhl, and Florian Lang. SGK1 as a determinant of kidney function and salt intake in response to mineralocorticoid excess. Am J Physiol Regul Integr Comp Physiol 289: R395–R401, 2005; doi:10.1152/ajpregu.00731.2004.—Mineralocorticoids modify salt balance by both stimulating salt intake and inhibiting salt loss. Renal salt retention is accomplished by upregulation of reabsorption, an effect partially mediated by serum- and glucocorticoid-inducible kinase 1 (SGK1). The present study explored the contribution of SGK1 to the regulation of renal function, salt intake, and blood pressure during mineralocorticoid excess. DOCA/1% NaCl treatment increased blood pressure and creatinine clearance to a similar extent in SGK1-deficient sgk1−/− and wild-type sgk1+/+ mice but led to more pronounced increase of proteinuria in sgk1−/− mice (by 474 ± 89%) than in sgk1−/− mice (by 154 ± 31%). DOCA/1% NaCl treatment led to significant increase of kidney weight (by 24%) and to hypokalemia (0.9 to 16.5 mEq/l) only in sgk1−/− mice (by 474 ± 89%) than in sgk1−/− mice (by 154 ± 31%). DOCA/1% NaCl treatment led to significant increase of kidney weight (by 24%) and to hypokalemia (0.9 to 16.5 mEq/l) only in sgk1−/− mice (by 474 ± 89%) than in sgk1−/− mice (by 154 ± 31%). DOCA/1% NaCl treatment led to significant increase of kidney weight (by 24%) and to hypokalemia (0.9 to 16.5 mEq/l) only in sgk1−/− mice (by 474 ± 89%) than in sgk1−/− mice (by 154 ± 31%). DOCA/1% NaCl treatment led to significant increase of kidney weight (by 24%) and to hypokalemia (0.9 to 16.5 mEq/l) only in sgk1−/− mice (by 474 ± 89%) than in sgk1−/− mice (by 154 ± 31%).

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 as previously described (63). SGK1 knockout (sgk1−/−) mice and their wild-type littermates (sgk1+/+) were implanted with a 21-day-release 50-mg DOCA pellet (Innovative Research of America, Sarasota, FL) in the neck area during anesthesia (intraperitoneal 0.5 mg/kg medetomidine + 5 mg/kg midazolam + 0.05 mg/kg fentanyl, which was reversed by subcutaneous 2.5 mg/kg atipamezol + 0.5 mg/kg flumazenil + 1.2 mg/kg naloxon). The use of the pellets was described previously (19, 22). After implantation, mice were switched from tap water to a 1% NaCl solution. One day before implantation of DOCA pellets, sgk1−/− and sgk1+/+ mice were weighed and placed individually in metabolic cages (Techniplast, Hohenpeissenberg, Germany) for basal 24-h urine collection with free access to a standard mouse diet (0.2% Na+, 0.4% Cl−, 0.7% K+, Altromin 1314; Heidenau, Germany) (58). The inner wall of the metabolic cages was siliconized, and urine was collected under water-saturated oil. In the absence of animals, the recovery in metabolic cages after addition of 0.5 ml of isotonic NaCl solution (from the grid the animals are sitting on) was 93 ± 2 and 92 ± 4% for water and Na+, respectively (means ± SE, n = 5).

Systolic arterial blood pressure was determined using the tail-cuff method before and on days 1, 2, 4, 6, 10, 14, and 18 of DOCA/1% NaCl treatment. As reviewed recently (37), the tail-cuff approach can be used to determine arterial blood pressure if certain precautions are taken to reduce the stress of the animals. These precautions include appropriate training of the mice over multiple days, prewarming to an ambient temperature of just about 29°C, measurement in a quiet, semidarkened, clean (free from foreign scent) environment, and per-
formance of the measurements by one person during a fixed time period of the day when blood pressure is stable (between 1:00 and 3:00 PM). All these precautions were taken in the present study.

On day 19 of DOCA/high-salt treatment, 24-h urine and body weight were again determined. Animals were then anesthetized (intraperitoneal ketamine and xylazine), and 200 μL of blood were withdrawn into heparinized capillaries by puncturing the retroorbital plexus. Plasma and urinary concentrations of Na⁺ and K⁺ were measured using flame photometry (ELEX 6361; Eppendorf, Hamburg, Germany). CT⁺ concentrations were determined using electrometric titration (Chloridometer 6610; Eppendorf), and creatinine was assessed with a commercial enzymatic kit (Sigma, Munich, Germany). Creatinine clearance and apparent fractional electrolyte excretion (electrolyte clearance/creatinine clearance) were calculated using a standard formula. Creatinine clearance previously has been used as an estimate of glomerular filtration rate in mice (12, 30, 39). Urinary albumin concentrations were analyzed using a commercial fluorometric kit (Progen Biotechnik, Heidelberg, Germany).

Kidneys were removed, and the kidney weight was determined. For comparison, kidney weight was obtained from untreated, age-matched animals. To assess salt appetite, we placed sglk₁⁻/⁻ and sglk₁⁺/+ mice in metabolic cages with access to two drinking bottles and made daily measurements of fluid intake from each bottle (8). Bottle 1 always contained tap water. On day 4, bottle 2 was switched from tap water to 1% NaCl, and on day 10, a DOCA pellet was implanted as described above. On day 20, bottle 2 was reversed to tap water.

Data are provided as means ± SE; n represents the number of independent experiments. All data have been tested for significance using paired or unpaired Student’s t-test, and only results with P < 0.05 are considered statistically significant.

RESULTS

Before DOCA/high-salt treatment of mice, both body and kidney weights were similar in SGK1 knockout sglk₁⁻/⁻ mice and their wild-type sglk₁⁺/+ littermates. DOCA/high-salt treatment significantly increased the weight of both left and right kidneys in sglk₁⁻/⁻ mice, an effect that was lacking in sglk₁⁺/+ mice (Fig. 1). Accordingly, during DOCA/high-salt treatment, kidney weight was significantly higher in sglk₁⁻/⁻ than in sglk₁⁺/+ mice (Fig. 1).

Creatinine clearance was similar in untreated sglk₁⁻/⁻ and sglk₁⁺/+ mice (Fig. 2). DOCA/high-salt treatment increased
creatinine clearance to a similar extent in \( sgk1^{-/-} \) and \( sgk1^{+/+} \) mice. Despite a similar increase in creatinine clearance, DOCA/high-salt treatment led to a significantly more pronounced increase in proteinuria in \( sgk1^{+/+} \) compared with \( sgk1^{-/-} \) mice (Fig. 3). Urinary albumin excretion during DOCA/high-salt treatment showed a tendency to be higher in \( sgk1^{+/+} \) mice \((0.065 \pm 0.038, n = 6)\) compared with \( sgk1^{-/-} \) mice \((0.011 \pm 0.003 \text{ mg} \cdot 24 \text{ h}^{-1} \cdot \text{g body wt}^{-1}, n = 6)\); however, this tendency was not statistically significant \((P = 0.18)\). DOCA/high-salt treatment increased urinary flow rate to

![Graph showing changes in plasma concentrations and urinary excretions during DOCA/high-salt treatment](image-url)
A greater extent in sgk1+/+ than in sgk1−/− mice (Fig. 2). DOCA/high-salt treatment was followed by a significant increase in plasma Na+ concentration in sgk1+/+ but not in sgk1−/− mice (Fig. 3). Plasma Na+ concentration was increased in sgk1+/+ mice despite significant increases in absolute and apparent fractional urinary Na+ excretion, effects significantly blunted in the sgk1−/− mouse (Fig. 3).

Plasma K+ concentration was significantly higher in sgk1−/− mice before DOCA/high-salt treatment (Fig. 3). This enhanced basal plasma K+ concentration could not be explained by increased food consumption in the sgk1+/+ mice, because food intake was comparable between sgk1+/+ and sgk1−/− mice during basal measurements (3.7 ± 0.2 vs. 3.5 ± 0.1 g/day). DOCA/high-salt treatment did not significantly alter food intake in either genotype (3.5 ± 0.2 vs. 3.5 ± 0.1 g/day), but it significantly decreased plasma K+ concentration in sgk1+/+ mice but not in sgk1−/− mice. Accordingly, plasma K+ concentration was higher in sgk1−/− mice during DOCA/high-salt treatment (Fig. 3).

Blood pressure was similar in untreated sgk1−/− and sgk1+/+ mice. In both genotypes, DOCA/high-salt treatment led, within 2 days, to statistically significant increases in blood pressure, which remained similarly elevated in both genotypes throughout the experiments (up to 18 days). No significant difference in blood pressure was observed between sgk1−/− and sgk1+/+ mice during DOCA/high-salt treatment (Fig. 4).

The increase in renal Na+ excretion during DOCA/high-salt treatment suggested a potential increase in salt intake, because the renal effects of DOCA/high-salt treatment were expected to enhance renal salt reabsorption and thus to decrease renal salt elimination. To explore the influence of DOCA on salt appetite, we placed mice in individual cages with access to two drinking bottles, one of which (bottle 1) always contained tap water. Switching bottle 2 from tap water to 1% NaCl did not significantly alter water intake from bottle 1 in sgk1+/+ or sgk1−/− mice. Before DOCA treatment, the intake was not significantly different between sgk1+/+ and sgk1−/− mice. In sgk1+/+ mice, implantation of the DOCA pellet was followed by a pronounced increase in salt intake from bottle 2, consistent with DOCA-induced salt appetite (Fig. 5). This response was significantly attenuated in sgk1−/− mice. A significant difference in fluid intake from bottle 2 persisted between sgk1+/+ and sgk1−/− mice even after tap water was restored in bottle 2, indicating that sgk1+/+ mice maintained an enhanced search for salt in comparison with sgk1−/− mice (Fig. 5).

During extended exposure (19 days) to 1% NaCl in drinking water, the high salt intake continued to be significantly higher in sgk1+/+ mice (24.6 ± 5.1 ml/day, n = 7) than in sgk1−/− mice (11.0 ± 0.9 ml/day, n = 7; P < 0.05 vs. sgk1+/+).

**DISCUSSION**

As previously reported (63), under normal salt diet, creatinine clearance and renal salt excretion are similar in mice lacking SGK1 (sgk1−/−) and their wild-type littermates (sgk1+/+), implying that SGK1 is not required for the maintenance of renal tubular Na+ reabsorption. Moreover, DOCA/high-salt treatment is followed by increases in blood pressure and creatinine clearance in both sgk1−/− and sgk1+/+ mice. Mineralocorticoid treatment previously has been shown to enhance glomerular filtration rate (35) and blood pressure (14, 64), effects resulting at least in part from extracellular volume expansion caused by April NaCl retention (21).

The increase in both creatinine clearance and blood pressure of sgk1−/− mice is consistent with significant upregulation of renal Na+ reabsorption independent of SGK1 (63). Clearly, mineralocorticoids increase renal NaCl reabsorption largely by mechanisms not requiring SGK1. Therefore, the functional
abnormalities of SGK1 knockout mice are far less severe than those of mice lacking functional mineralocorticoid receptors (1) or mice lacking functional ENaC (27). In contrast to the seemingly normal SGK1 knockout mouse (63), the mineralocorticoid receptor knockout mouse suffers from severe renal salt loss (1), and the ENaC knockout mouse is not viable (27). The role of SGK1 in renal NaCl reabsorption, however, is disclosed by exposing the mice to a salt-depleted diet. Even though NaCl excretion decreases substantially under NaCl depletion in both sgk1−/− and sgk1+/+ mice, the renal NaCl loss remains significantly larger in sgk1−/− mice compared with sgk1+/+ mice, despite an exaggerated increase in plasma aldosterone concentration, a decrease in blood pressure, decreased glomerular filtration rate, and enhanced proximal tubular Na+ reabsorption in the sgk1−/− mice (63).

As shown in the present study, the SGK1-independent up-regulation of renal salt reabsorption by DOCA-mediated activation of mineralocorticoid receptors is apparently sufficient to induce net renal NaCl retention and thus to increase blood pressure. It is worth noting that the increase in blood pressure in sgk1−/− mice was similar to that in sgk1+/+ mice, even though a lack of SGK1 was expected to blunt the effect of DOCA. Possibly in both sgk1−/− and sgk1+/+ mice, pressure-natriuresis may have prevented any further acute increases in blood pressure. Moreover, the excessive stimulation of mineralocorticoid receptors during DOCA treatment may have enhanced ENaC expression to an extent overriding the lack of SGK1. Nevertheless, we cannot rule out a participation of SGK1 in mechanisms limiting blood pressure increase. Moreover, the present observations do not rule out differences in blood pressure at prolonged exposure to high-salt diet or under different experimental conditions.

Despite an identical increase in blood pressure and creatinine clearance, the effects of DOCA/high salt on kidney growth and proteinuria were significantly blunted in the sgk1−/− mouse. Thus SGK1 could participate in the regulation of kidney growth and glomerular permeability. SGK1 is activated by IGF-I, a hormone implicated in the regulation of cell proliferation and renal growth (4, 13, 16, 17, 23, 25, 38, 55, 60). Moreover, excessive SGK1 transcription has been observed in diabetic nephropathy (31, 32) and glomerulonephritis (15). In both conditions, abundant SGK1 transcript levels have been observed in glomerula. However, the mechanisms linking SGK1 expression to renal growth and glomerular function have remained elusive.

DOCA induced a significant hypokalemia in sgk1+/+ but not in sgk1−/− mice, implicating a role for SGK1 in mineralocorticoid-regulated renal K+ excretion. SGK1 regulates the renal outer medullary K+ channel ROMK1 (65). Furthermore, stimulation of ENaC by mineralocorticoids would be expected to depolarize the apical cell membrane of principal cells, thus favoring K+ secretion. The impaired stimulation of ENaC in sgk1−/− mice is thus expected to decrease renal K+ excretion (63). Impaired renal potassium elimination by the sgk1−/− mice has indeed been reported recently (26).

The DOCA/high salt-induced increases in plasma Na+ concentration, urinary flow rate, and NaCl excretion were significantly attenuated in sgk1−/− compared with sgk1+/+ mice. In view of the defective stimulation of renal Na+ reabsorption in the sgk1−/− mice (63), the opposite, i.e., enhanced rather than decreased urinary NaCl output in those mice, was expected. We thus hypothesized that SGK1 may contribute to mineralocorticoid-induced salt appetite. The differences of salt intake by sgk1−/− and sgk1+/+ mice led to respective alterations of renal salt excretion concealing subtle differences of renal tubular Na+ transport regulation.

The experiments with free access to salt-containing and salt-free fluid confirmed the stimulation of salt appetite by DOCA. Regulation of salt appetite by mineralocorticoids has been shown previously in a variety of animals (10, 52), including baboons (49), hamsters (8), rats (19, 33, 34, 36, 46, 57), and mice (56). The effects of mineralocorticoids are apparently mediated by central mineralocorticoid receptors (43, 57). The present study demonstrates that in mice this effect is significantly blunted in the absence of functional SGK1. Thus SGK1 is required for the full stimulatory effect of DOCA on salt appetite.

The mechanisms by which SGK1 regulates salt appetite are currently unknown. Nevertheless, it is worth noting that the stimulation of salt appetite by aldosterone was paralleled by upregulation of the expression of Na+-K+-ATPase in several structures of the brain, including the amygdala (19), an area implicated in the regulation of salt appetite by mineralocorticoids (6, 28, 36, 44, 47, 66). In vitro experiments have indeed demonstrated the ability of SGK1 to upregulate the Na+-K+-ATPase (24, 48, 61, 62). Mineralocorticoids have further been shown to upregulate Fos (41, 42), angiotensin receptors (51), oxytocin receptors (51), vasopressin (20), and the vasopressin V1a receptor (41, 42). Vasopressin (11) and angiotensin II (9, 29, 45, 50, 51) in turn stimulate, whereas oxytocin (53) inhibits, salt intake. Interestingly, the effect of mineralocorticoids or salt depletion on salt appetite may be modified by glucocorticoids (18, 34, 50) and amphetamine (5). Moreover, salt appetite has been shown to be dependent on the age of the animal (54). It would be interesting to explore whether SGK1 participates in the modulation of salt appetite by other stimuli and by age.

Our data provide the first evidence that SGK1 plays a dual role in mineralocorticoid-regulated Na+ homeostasis involving not only inhibition of output by stimulating renal Na+ reabsorption (63) but also stimulation of uptake by mineralocorticoid-induced salt appetite. SGK1 may similarly participate in the regulation of salt appetite by glucocorticoids (10), and a SGK1-dependent increase of salt intake may contribute to the enhanced extracellular fluid volume and blood pressure during stress conditions (2). Moreover, enhanced salt appetite and subsequently increased salt uptake and extracellular fluid expansion may contribute to the higher blood pressure values of individuals carrying a common polymorphism within the SGK1 gene, affecting as many as 5% of unselected Caucasians (3).

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REFERENCES


SGK1 AND SALT APPETITE


5. Fitzsimons JT, Flynn FW, Kirchner TR, and Clinton ME. R400 SGK1 AND SALT APPETITE


7. Firestone GL, Giampaolo JR, and O’Keeffe BA. Physiological, biochemical and pathophysiological roles of aldosterone.


